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# Flattened cortisol rhythms in metastatic breast cancer patients

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## KEYWORDS

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**Summary** Allostatic load, the physiological accumulation of the effects of chronic stressors, has been associated with multiple adverse health outcomes. Flattened diurnal cortisol rhythmicity is one of the prototypes of allostatic load, and has been shown to predict shorter survival among women with metastatic breast cancer. The current study compared diurnal cortisol slope in 17 breast cancer patients and 31 controls, and tested associations with variables previously found to be related to cortisol regulation, i.e. abdominal adiposity, perceived stress, social support, and explicit memory. Women with metastatic breast cancer had significantly flatter diurnal cortisol rhythms than did healthy controls. Patients with greater disease severity showed higher mean cortisol levels, smaller waist circumference, and a tendency toward flatter diurnal cortisol rhythms. There were no relations between cortisol slope and psychological or cognitive functioning among patients. In contrast, controls with flatter rhythms showed the expected allostatic load profile of larger waist circumference, poorer performance on explicit memory tasks, lower perceived social support, and a tendency toward higher perceived stress. These findings suggest that the cortisol diurnal slope may have important but different correlates in healthy women versus those with breast cancer.

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## 1. Introduction

In most healthy individuals, cortisol typically shows marked diurnal variation, peaking in early morning, and declining throughout the day (Stone et al., 2001). The health consequences of flattened

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cortisol rhythms or aberrant peaks and troughs have not yet been precisely delineated, but alteration in rhythmicity of cortisol has been associated with various negative outcomes, including tumor growth, early mortality in cancer, (Sapolsky and Donnelly, 1985; Sephton et al., 2000; Filipinski et al., 2002) and obesity and disrupted glucose metabolism (e.g., Rosmond et al., 1998). Although women with metastatic breast cancer have been shown to maintain circadian cycling of cortisol (Touitou et al., 1995; Haus et al., 2001), data in previous studies were not compared to control groups to determine whether the rhythms were as pronounced as in healthy individuals. Furthermore, Touitou and colleagues (1995) showed that breast cancer patients with the most severe metastases (in the liver) showed flattened rhythms of several bioactive substances, including cortisol. Thus, women with severe metastatic breast cancer appear to have disrupted circadian rhythmicity. Circadian abnormalities also appear to have prognostic value in regard to the initial occurrence of breast cancer. Patients at high risk show abnormal circadian patterns among an array of hormones including cortisol (Ticher et al., 1996). In addition, the circadian rhythmicity of cortisol appears to have long-term prognostic value for women already diagnosed with metastatic disease. Our laboratory reported that loss of normal diurnal variation in cortisol predicts early mortality in metastatic breast cancer for at least seven years later. The divergence in survival as a function of cortisol rhythm emerged approximately one year after cortisol assessment and extended at least six years after (Sephton et al., 2000).

A limitation of our prior study examining the relation between diurnal cortisol rhythm and mortality in breast cancer was the lack of a healthy control group. Thus, it was impossible to determine whether the patient group as a whole showed abnormal cortisol rhythmicity compared to healthy individuals. In order to address this issue, we conducted this cross-sectional study on a separate sample.

### 1.1. Cortisol rhythmicity, allostatic load, and psychological functioning

We were also interested in relations between cortisol rhythmicity and other aspects of physiological as well as psychological functioning. In a review of the human stress literature, McEwen and Seeman (1999) document the adverse health effects of cumulative stressors which can lead to failure of the body to effectively terminate stress responses. "Allostatic load" occurs when external demands and/or adaptation efforts are excessive, leading to dysregulation of the HPA axis, impairing

negative feedback and diurnal rhythmicity, and eventually leading to dysregulation across multiple physiological systems—including the immune and cardiovascular systems, and metabolic regulation of energy balance and fat deposition.

HPA axis dysregulation is hypothesized to be an early indicator of allostatic load, and is sensitive to psychosocial factors such as stress and social support (Chrousos and Gold, 1998; Turner-Cobb et al., 2000; Gunnar and Vazquez, 2001). Cortisol is also known to have direct effects on memory (e.g., Abercrombie et al., 2003), and chronic cortisol dysregulation causes hippocampal dysfunction and memory impairment (cf. Lupien and McEwen, 1997; Lupien and Lepage, 2001), although no studies have yet linked declarative memory performance to aberrations in diurnal rhythmicity.

Dysregulation in the HPA axis can also disrupt glucose homeostasis and energy balance, resulting in greater overall or abdominal adiposity. Bjorntorp and colleagues have linked a flattened diurnal rhythm to aspects of the Metabolic Syndrome (Rosmond et al., 1998, 2000), which is a cluster of inter-related factors, including abdominal and general obesity, insulin resistance, glucose intolerance, hyperlipidemia, and hypertension, and strongly predicts cardiovascular disease (CVD) and type II diabetes (Lapidus et al., 1984; Kissebah and Krakower, 1994). Newer studies have linked indices of the Metabolic Syndrome, such as increased abdominal fat distribution (Ballard-Barbash and Swanson, 1996; Kaaks et al., 1998) as well as high levels of circulating insulin (independent of obesity) (Del Giudice et al., 1998), to incidence of breast cancer (Kaaks et al., 1998). However, the relationship between adiposity and health status in breast cancer patients is complex. Whereas obesity is a risk factor for breast cancer incidence and more rapid progression (Zumoff et al., 1982; Stoll, 1996; Chlebowski et al., 2002), advancing cancer is often associated with wasting. Furthermore, not all studies support the connection between obesity and cancer progression (Rock and Demark-Wahnefried, 2002; Dignam et al., 2003), and one study even shows that low body mass index is a predictor of local recurrence (Marret and Perrotin, 2001). Therefore, it is unclear whether a flattened diurnal rhythm would be linked with excess adiposity or weight loss in metastatic breast cancer patients, although stage of disease may be critical.

In order to better understand the salience to breast cancer of the syndromal aspects of disrupted rhythmicity of cortisol, it is important to examine both physiological and psychological correlates of altered rhythms. We therefore hypothesized that

1) metastatic breast cancer patients would show flattened diurnal slope of cortisol compared to controls, and that the patients with the most severe metastatic disease would show the flattest rhythms, and 2) relations would be apparent between diurnal slope of cortisol and adiposity and memory functioning, as well as psychosocial factors—perceived stress and social support.

## 2. Method

### 2.1. Participants

Participants included 17 metastatic breast cancer patients and 31 healthy female controls. Nine other breast cancer patients were excluded on the basis of exclusionary criteria (see below) or unwillingness to complete the protocol. Likewise, nine other potential control subjects were excluded because unwillingness to initiate or complete the protocol. Patients were recruited by letter of request through breast cancer clinics at Stanford University, and healthy controls were recruited through advertisements or through breast cancer patients in the study who were invited to refer a friend or relative. All participants were above the age of 30, were not currently pregnant, were not diagnosed with any form of psychopathology, and were not being treated with systemic corticosteroids. Controls had no prior history of cancer. See Table 1 for subject characteristics. The patients and controls were similar on age,  $t(46) = 0.45$ , n.s., but differed on other demographic variables, including years of education, income, and marital status (See Table 1). Written informed consent was obtained in accordance with Stanford University Medical School Human Subjects Committee guidelines.

### 2.2. Disease severity

Progression of cancer was indexed by reported sites of metastasis. Patients were assigned a number, increasing in level of severity, based on the type of tissue to which the cancer had metastasized (1 = soft tissue, 2 = bone, and 3 = organ).

### 2.3. Psychological self-report measures

Self-reported levels of stress were measured with the Perceived Stress Scale (PSS; Cohen et al., 1983), which assesses the degree to which participants perceived their lives as uncontrollable, unpredictable, or overwhelming over the last month. The size of the participants' perceived social network (tangible supports) was assessed with the Single Item Measure of Social Supports (SIMSS; Blake and McKay, 1986). Although only a

**Table 1** Sample characteristics

	Controls	Patients
<i>n</i>	31	17
Age, mean (SD)	56.0 (12.98)	57.6 (10.76)
<i>Level of education, % of sample</i>		
Less than college degree	19.4	41.2
Bachelor's degree	25.8	35.3
Graduate education	54.8	23.5
<i>Household income before taxes, % of sample</i>		
Less than \$40,000	16.1	41.2
\$40,000–79,999	25.8	17.6
\$80,000 or above	45.2	35.3
Decline to answer	12.9	5.9
<i>Ethnicity, % of sample</i>		
European	93.5	94.1
American/White		
African	6.5	0
American/Black		
Mexican	0	5.9
American/Chicano		
<i>Marital Status, % of sample</i>		
Married or living as married	45.1	62.5
Never married (single)	16.1	12.5
Divorced or Separated	19.3	25.0
Widowed	19.4	0

single item, this measure of social support has been found to be a good measure of morbidity for women (Blake and McKay, 1986).

### 2.4. Memory

The Rey Auditory-Verbal Learning Test (AVLT) was used to assess both short- and long-term retention of a 15-word list (Rey, 1964; Lezak, 1995). The AVLT consists of 5 consecutive trials in which the experimenter reads a list of 15 words to the participant at the rate of 1 word per second. On each trial, immediate word recall was assessed after presentation of the list. After an interference trial and a delay of 30 minutes, delayed recall was assessed. Immediate recall (i.e., average immediate recall for Trials 1–5) and delayed recall were analyzed.

### 2.5. Biological measures

#### 2.5.1. Waist circumference

Adiposity was estimated with measurements of waist circumference using the narrowest point around the waist (Lohman et al., 1988). Measures of body mass index [BMI; weight in kilograms ÷ (height in meters)<sup>2</sup>] were also computed. In the current sample, BMI and waist circumference

were largely redundant measures ( $r = 0.89$ ,  $p < 0.001$ ) and had virtually identical correlates. Thus, correlations will be presented for waist circumference only.

### 2.5.2. Salivary cortisol

The measurement of cortisol in saliva reliably reflects physiologically active free cortisol levels in blood, since unbound plasma cortisol diffuses easily from blood to saliva (Kirschbaum and Hellhammer, 1994). Saliva collection was requested on three consecutive days at 4 time points: waking, 1200h, 1700h, and 2100h. Twelve pre-labeled "Salivette" devices (Sarstedt, Inc., Newton, NC) were provided. Mean (and SD) self-reported collection times were 0717h (0:57), 1215h (0:25), 1717h (0:20), 2128h (0:40). Data were examined for outliers with respect to sample times. Five data points associated with sample times that were  $>4$  SD from the mean time for the respective time point were excluded. There were no differences between patients and controls for average sample time for any time point (all  $p$ 's  $> 0.27$ ), and no differences in variance of sample times for patients and controls (all  $p$ 's  $> 0.49$ ), confirming that patients and controls did not differ significantly in compliance, as measured by deviations from the average collection times.

Cortisol kits were refrigerated until returned in person or retrieved by a research assistant. Samples were stored at  $-70^{\circ}\text{C}$  until assayed using EIA kits from Salimetrics, Inc. (State College, PA). The intra-assay coefficients of variation were 4.3 and 0.7% for low and high controls, and inter-assay values were 3.04 and 1.6%, respectively. Assay sensitivity was  $0.007\ \mu\text{g}/\text{dl}$ . Cortisol values were examined for outliers. Because all raw values were in the physiological range ( $0.01$ – $2.54\ \mu\text{g}/\text{dl}$ ) no data were excluded from the analyses on this basis.

## 2.6. Computation of cortisol dependent measures

In our original study (Sephton et al., 2000), we found that it was specifically diurnal variation in cortisol as represented by the slope (or, rate of decay) during the day that predicted survival time, but not mean cortisol levels or area under the curve. Therefore, we hypothesized that cortisol slope in particular would distinguish breast cancer patients from healthy controls. Because other previous research has used mean cortisol levels rather than diurnal slope of cortisol, we include cortisol data for both the diurnal slope and the mean of all the values across the sampling

days (hereafter referred to as "mean cortisol levels").

The slope of diurnal change in cortisol values was calculated to estimate how each patient fit the normal (i.e., descending) profile (Smyth et al., 1997). Since the distribution of raw cortisol values is typically skewed and the normal diurnal profile may be approximated by an exponential curve, raw values were log transformed. Mean cortisol was calculated using all 12 log-transformed values.<sup>1</sup> The diurnal slope was calculated using the regression of the 12 cortisol values on the time of sample collection.<sup>2</sup> Steeper slopes are represented by smaller  $\beta$  values for the slope of the regression, which indicate cortisol declining more rapidly. Flatter slopes (larger  $\beta$  values) indicate slower declines, abnormally timed peaks, or increasing levels during the day.

This approach to quantifying the diurnal rhythm was chosen over Random Coefficient Modeling (i.e., "mixed models"), which has been advocated by some researchers (e.g., Smyth et al., 1998). Rogosa and Saner (1995) advocate the use of simpler regression models and demonstrate equivalence with the more complex Random Coefficient Modeling. We chose to use the simpler model for calculating diurnal slope (Gibbons et al., 1993) because it replicates the long-range prognostic variable identified in our prior research (Sephton et al., 2000).

## 2.7. Data analysis

T-tests were used to test for group differences in diurnal cortisol slope and mean cortisol levels. Because of group differences in demographic variables (See Table 1), significant effects of group

<sup>1</sup>Few participants had missing cortisol samples, with only one participant missing an entire day of samples. This participant was included in the analyses as she provided 2 entire days worth of samples. Two additional participants were missing 2 samples on 3rd day of the sampling, but provided samples across the day during the prior days. Five additional participants were missing at most one sample on one or two of the sampling days. Missing samples were distributed evenly across patients and controls.

<sup>2</sup>Examination of within subject stability of diurnal slope of cortisol showed moderate stability, with intraclass correlations across the three days of measurement for patients of 0.48 and controls of 0.46 (Shrout and Fleiss, 1979). Previous research has shown the importance of aggregating across multiple assessments of physiological measures to increase reliability of measurement, especially for measures reflecting both variation in concurrent state and relatively stable individual differences (e.g., Kirschbaum and Hellhammer, 1989; Tomarken et al., 1992; Smyth et al., 1997). Diurnal slope of cortisol was thus computed by pooling values across all 3 days of measurement.

were followed up with additional analyses. Blocking was used to assess the effects of education<sup>3</sup> and marital status, and whether these demographic variables interacted with the effects of group. For level of education, participants were blocked into three categories (less than college degree, bachelor's degree, and graduate education) with adequate representation of both groups at each level (minimum cell size = 4 participants). ANOVA was performed with group and level of education as factors. The existence of a group X education interaction would suggest that the effects of group depended on the level of education of the participants. Identical procedures were used to assess the effects of marital status, using 2 categories (married & unmarried) because of the small number of unmarried breast cancer patients (minimum cell size = 6 participants).

Correlations were tested between psychological variables and biological indicators separately for patients and controls. Furthermore, to test whether diurnal cortisol slope and waist circumference can be regarded as reflecting the unitary construct of "allostatic load," correlations were tested between diurnal cortisol slope and waist circumference. For breast cancer patients, correlations between disease severity and biological indicators were also tested. All tests were two-tailed.

### 3. Results

#### 3.1. Comparison of patients vs. controls on biological indicators

Patients had flatter diurnal slope of cortisol than controls,  $t(46) = -2.19$ ,  $p < 0.05$ . (See Table 2 for means, and see Fig. 1 for log cortisol by time point.) The effect size for the group difference in diurnal slope was 0.67, which is a medium effect size (Cohen, 1977). The groups did not differ on mean cortisol levels,  $t(46) = 0.4$ , n.s. Furthermore, no group differences emerged in cortisol levels for any particular time of day, all  $t$ 's  $< 1.06$ ,  $p$ 's  $> 0.29$ . Thus, the significant group difference in cortisol emerges only when the entire diurnal rhythm is examined. Neither education,  $F(2,42) = 1.8$ , n.s., nor marital status  $F(1,43) = 0.78$ , n.s. showed a main effect on diurnal slope of cortisol. Additionally, the effect of group on diurnal slope of cortisol did not depend on either

education [group X education,  $F(2,42) = 0.16$ , n.s.] or marital status [group X marital status,  $F(1,43) = 0.01$ , n.s.].<sup>4</sup>

No group difference was found for waist circumference,  $t(46) = 0.09$ , n.s. (See Table 2.) In the control group, larger waist circumference was related to flatter diurnal slope of cortisol,  $r = 0.51$ ,  $p < 0.005$ , which is consistent with the hypothesis that cortisol diurnal slope and waist circumference vary together, potentially reflecting "allostatic load." However, in patients, waist circumference and diurnal slope of cortisol were not significantly correlated,  $r = -0.30$ , n.s. Mean cortisol levels were not related to waist circumference in either group,  $p$ 's  $> 0.45$ .

#### 3.2. Correlational analyses: Patients

Table 3 lists correlations between diurnal slope of cortisol and other variables separately for patients and controls.<sup>5</sup> For patients, no correlations emerged between psychological measures and diurnal cortisol slope or mean cortisol levels, all  $p$ 's  $> 0.32$ . However, more severe disease status (as indexed by severity of metastatic spread) was associated with higher mean cortisol levels,  $r = 0.50$ ,  $p < 0.05$ , and was positively but not significantly related to flatter diurnal slopes of cortisol,  $r = 0.45$ ,  $p = 0.07$ . Conversely, greater disease severity was associated with smaller waist circumference,  $r = -0.56$ ,  $p < 0.02$ .

#### 3.3. Correlational analyses: Controls

For controls, stress measured with the PSS was positively but not significantly correlated with diurnal slope of cortisol (See Table 3 for  $r$ -values), reflecting a tendency for an association between flatter slopes of cortisol and greater perceived stress. Lower perceived social support was associated with flatter diurnal cortisol slope. In addition, flatter diurnal slope of cortisol was associated with poorer immediate and delayed recall on the AVLT. Because previous research has shown relations among age, memory, and cortisol functioning (e.g., Lupien et al., 1999), we also examined correlations for memory after first adjusting for variance in age. After adjusting for age, the correlation between delayed memory and cortisol slope remained significant (semi-partial  $R^2 = 0.17$ ,

<sup>3</sup>Because Level of Education and Income Level redundantly represent Social Economic Status, and because 5 participants declined to provide their income level, only the effects of Level of Education were tested.

<sup>4</sup>Consistent with the effect of group on cortisol slope found using the t-test, both the two-way ANOVAs assessing the effects of demographic variables (i.e., education level and marital status) revealed a main effect of group on diurnal cortisol slope ( $F$ 's  $> 4.3$ ,  $p$ 's  $< 0.05$ ).

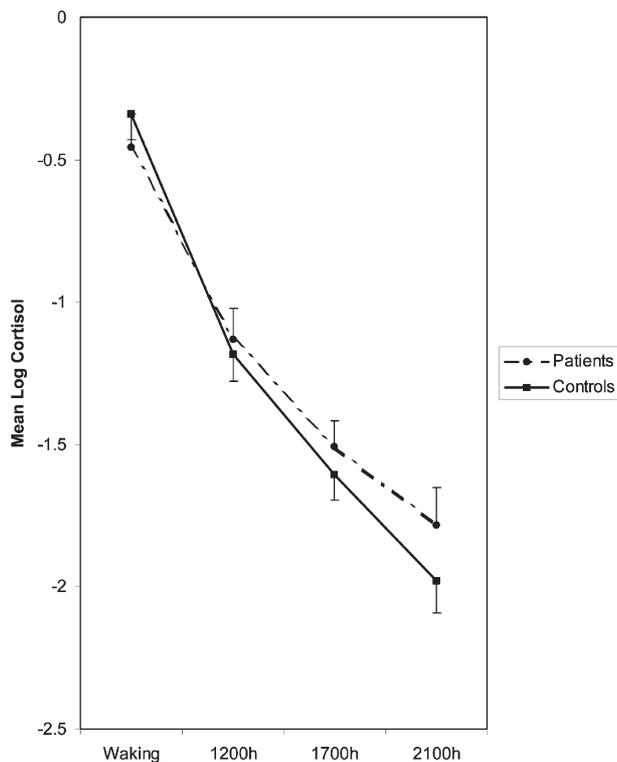
<sup>5</sup>Examination of all scatter plots confirmed that correlations were not due to outliers.

**Table 2** Mean (SD) by group for study variables

	Controls	Patients	<i>p</i>
Diurnal slope of cortisol	−0.113 (0.030)	−0.092 (0.033)	<0.05
<i>Mean cortisol levels</i>			
Log cortisol	−1.27 (0.46)	−1.22 (0.39)	n.s.
Raw cortisol in µg/dL	0.39 (0.16)	0.38 (0.13)	N/A
BMI (kg/m <sup>2</sup> )	26.18 (6.7)	26.10 (5.04)	n.s.
Waist circumference in cm	83.7 (16.4)	84.7 (13.5)	n.s.
<i>Psychological measures</i>			
Perceived stress scale	21.9 (3.6)	21.5 (2.8)	n.s.
Social support	3.5 (1.0)	3.7 (1.1)	n.s.
<i>Memory</i>			
AVLT-immediate	52.5 (9.4)	55.9 (9.4)	n.s.
AVLT-delay	10.8 (2.9)	12.0 (3.3)	n.s.

$p < 0.03$ ), but the correlation between immediate memory and cortisol slope was no longer significant (semi-partial  $R^2 = 0.10$ ,  $p = 0.08$ ). No signifi-

cant correlations emerged between mean cortisol levels and psychological measures within the control group, all  $p$ 's > 0.20.



**Fig. 1.** Mean log cortisol for each time point averaged across 3 days of at-home saliva sampling in patients with metastatic breast cancer compared to controls. Error bars represent standard error of the mean. No significant differences emerged for cortisol levels at any single time point ( $p$ 's > 0.29). However, compared to controls, patients had flatter diurnal slope of cortisol ( $p < 0.05$ ), which represents the rate of decline in cortisol levels over the course of the day. See Table 2 for mean slope values for patients vs. controls.

#### 4. Discussion

In the current study diurnal cortisol rhythms were flattened in metastatic breast cancer patients compared to healthy controls. We also found that within the group of breast cancer patients, individuals with more severe metastatic spread showed higher mean cortisol levels and a tendency toward flatter diurnal slopes of cortisol. In addition, we previously found in a separate sample that flatter diurnal slope of cortisol was predictive of earlier death in women with metastatic breast cancer (Sephton et al., 2000). The inclusion of a cancer-free control group in the current study augments such findings from previous studies showing associations between disrupted cortisol rhythmicity

**Table 3** Pearson *r*-values for correlations between diurnal slope of cortisol and other measures

	Controls ( <i>n</i> = 31)	Patients ( <i>n</i> = 17)
Disease severity (i.e., metastatic spread)	N/A	0.45 <sup>a</sup>
Waist circumference	0.51*	−0.30
<i>Psychological variables</i>		
Perceived stress scale	0.32 <sup>a</sup>	−0.02
Social support	−0.40*	0.26
<i>Memory</i>		
AVLT-immediate recall	−0.36*	0.14
AVLT-delayed recall	−0.43*	−0.12

\* $p < 0.05$ .

<sup>a</sup> $p = 0.07$ .

and cancer prognosis or cancer severity (Touitou et al., 1995; Ticher et al., 1996; Sephton et al., 2000). Together, these studies indicate a robust relation between dysregulated circadian rhythmicity of cortisol and incidence, severity, and prognosis of metastatic breast cancer.

Many causal pathways may account for the relation between aberrant cortisol rhythms and breast cancer. For instance, genes that are controlled by the circadian clock and irregular circadian cycles have important effects on tumor suppression (Fu et al., 2002; also see Sephton & Spiegel, 2003 for extensive discussion of circadian disruption in cancer). Furthermore, cortisol dysregulation may have direct effects on tumor progression or may indirectly affect cancer via effects on the immune system (see discussion below). Alternatively, disrupted rhythms may be a marker of advancing cancer, resulting from the progressing illness (cf. Mormont and Levi, 1997).

Although several causal pathways may be operating, ample evidence exists suggesting that cortisol dysregulation may play a causal role in progression of cancer. Animal studies have shown that stress-induced elevations in cortisol (Sapolsky and Donnelly, 1985) and disrupted cortisol rhythms (Filipski et al., 2002) are associated with more rapid growth of implanted tumors, suggesting that cortisol dysregulation may cause more rapid progression of cancer. In addition, disrupted cortisol regulation is known to suppress aspects of immune function relevant to tumor defense, such as natural killer cell numbers (Levy et al., 1991; Munck and Guyre, 1991; Whiteside and Herberman, 1995; Sephton et al., 2000). Furthermore, because of differential effects of cortisol on metabolic processes in tumor versus healthy cells, energy may be directed to the tumor and away from normal cells when the HPA axis is not adequately regulated (Romero et al., 1992; also see studies reviewed by Turner-Cobb et al., 2000). Thus, dysregulation of cortisol may play an important contributory role in the progression of breast cancer (see Sephton and Spiegel, 2003 for more extensive discussion of these issues).

#### 4.1. Adiposity and cortisol rhythm in breast cancer

In the current study, patients did not differ from controls on waist circumference, but more severe metastatic spread was associated with smaller waist circumference. Thus, the patients with the most severe disease were the thinnest. No significant relation was found between waist circumference and slope of cortisol in patients. In contrast, previous research has shown that abdominal obes-

ity is positively related to altered cortisol rhythmicity, hyperandrogenic states, insulin resistance, and greater bioavailable estrogens. This underlying endocrine milieu may influence carcinogenic growth of breast tissue (Stoll, 1996). This may help explain links between greater abdominal obesity and higher incidence of breast cancer shown in previous research (e.g., Kaaks et al., 1998). However, advancing cancer is often associated with catabolic processes, wasting, and weight loss. Indeed, one study showed that lean body mass predicts recurrence of breast cancer (Marret and Perrotin, 2001). Furthermore, while hormonal treatments frequently used with breast cancer patients such as megestrol and tamoxifen often cause weight gain, cytotoxic chemotherapy agents such as cyclophosphamide, methotrexate, 5-fluorouracil, taxol, and adriamycin induce nausea and vomiting and may therefore reduce weight. Thus, greater waist girth and BMI would be hypothesized to predict breast cancer incidence, but as the disease progresses, these measures are more likely to reflect than predict the stage of disease and its treatment. Furthermore, the increase in distress, anxiety, and depression that may accompany seriously advancing disease (Butler et al., 2003) may also lead to a decrease in appetite and weight loss.

In summary, while more dysregulated cortisol was associated with more severe cancer in our study, *smaller* waist circumference was correlated with greater severity of cancer, and no significant relation was found between waist circumference and slope of cortisol in patients. The lack of a positive relationship between cortisol dysregulation and waist circumference in cancer highlights the fact that, likely because of disease-related processes that occur as cancer progresses, "allostatic load" measurement may become more complex in an ill sample. In controls in our study, larger waist circumference was associated with flatter slope of cortisol, as expected based on prior research (Brindley and Rolland, 1989; Bjorn- torp, 1990). For disease free women, cortisol dysregulation and fat deposition may vary together comprising aspects of the syndrome of "allostatic load" (McEwen and Seeman, 1999).

#### 4.2. Psychological functioning and cortisol rhythm in breast cancer

Studies in physically healthy individuals have shown relations between disruption of HPA axis rhythms and affective mental illness and chronic stress (Ockenfels et al., 1995; Yehuda et al., 1996; Deuschle et al., 1997; Chrousos and Gold,

1998). The HPA axis is a physiological system exquisitely sensitive to environmental and psychological factors (e.g., Levine, 2000), and is therefore a prime system for studying the role of psychological factors in the progression of cancer. However, in patients in our study, cortisol was not related to psychological measures. It appears that the disease-related variance overwhelms any variance in cortisol functioning that is potentially related to psychological measures. It should be noted though, that because of the small sample sizes, the null hypothesis of no relation between psychological factors and cortisol rhythmicity should not be accepted. With a larger sample size, relations between psychological and biological measures may have emerged, as in previous studies (e.g., Turner-Cobb et al., 2000). Although cortisol dysregulation may be a consequence of disease-related processes, chronic psychological stress may also contribute to flattened rhythms in breast cancer patients. Prior research has shown that changes in psychological state induced by stress can have an adverse effect on breast cancer incidence (Geyer, 1991, 1993) and relapse (Ramirez et al., 1989), although not all studies support this conclusion (e.g., Barraclough et al., 1992, 1993; Graham et al., 2002). Future research is needed to precisely determine whether cortisol dysregulation related to psychological stress contributes to cancer incidence and/or progression.

In controls, flatter diurnal slope of cortisol predicted worse memory functioning, lower perceived social support, and a tendency towards greater perceived stress. These findings are consistent with previous literature indicating how psychological factors relate to cumulative wear and tear on physiological systems represented as allostatic load (McEwen and Seeman, 1999). The memory findings are important because they are the first to show in a normal sample an association between flattened diurnal slope of cortisol and worse memory performance. Prior animal and human research suggests that glucocorticoid exposure and stress can adversely affect hippocampal volume and functioning, including performance on memory tasks (McEwen & Sapolsky, 1995; Kirschbaum et al., 1996; Lupien and McEwen, 1997). Flattened diurnal cortisol slope may reflect chronic cortisol dysregulation and/or aberrant stress responsivity of cortisol, which both have neuropsychological significance.

#### 4.3. Limitations and future directions

The cross-sectional and correlational nature of the study precludes causal statements about effects of cortisol dysregulation on outcomes. It should be noted that among cancer patients with disrupted cortisol rhythms, other circadian fluc-

tuations are also abnormal (Touitou et al., 1995). The effects of a generalized circadian dysregulation may be as important as, or more important than, aberrant cortisol rhythms, per se, in cancer outcomes. This notion is supported by the results of studies with colorectal cancer in which rest-activity rhythms had prognostic value, while cortisol rhythms did not (Mormont et al., 2000, 2002). Future research is needed in order to further establish which aspects of circadian perturbation and cortisol dysregulation are causally implicated in breast cancer outcomes.

The current study is also limited by a small patient sample and the ensuing lack of power to detect relations between psychological variables and biological indicators. Our sample was further limited by differences between the groups in demographic variables, although these factors do not appear to account for our findings. Markers of socioeconomic status tended to be lower in patients than controls, and could potentially account for HPA axis dysregulation through increasing life stress. However, patients and controls were similar in perceived stress, making this less likely, and findings remained after blocking on SES.

Previous research has revealed relations between poor sleep and dysregulation in immune and endocrine factors associated with cancer progression (Vgontzas and Chrousos, 2002). However, sleep quality and quantity were not measured in the current study, and should be assessed in future research. Furthermore, the current study methods precluded measurement of intra-abdominal fat, which should be more closely tied to aberrant cortisol rhythms than overall adiposity. Although patients and controls had similar average BMI and waist circumference, patients may have had greater abdominal fat distribution relative to peripheral fat. Other markers of the metabolic syndrome that we did not measure, such as insulin resistance, may be more prognostic of disease progression. Our current longitudinal research is further examining the prognostic value of adiposity and insulin resistance. The current study would have been strengthened by including actual measures of insulin resistance, which may be equal in importance to flattened cortisol rhythm in promoting cancer progression, as recent work by Goodwin and colleagues (2002) suggests. Future research must combine larger samples, longitudinal designs, and experimental pharmacological examination of HPA axis functioning to more precisely determine aspects of HPA dysregulation that either contribute to or result from cancer.

## 5. Summary

We found that women with metastatic breast cancer had significantly flatter diurnal cortisol rhythms than did healthy controls. Patients with greater disease severity showed higher mean cortisol levels, smaller waist circumference, and a tendency toward flatter diurnal cortisol rhythms. Thus, we found that metastatic disease is associated with dysregulated cortisol functioning, and the most ill, advanced patients have greatest cortisol dysregulation and most progressive cachexia.

No relations were found between psychological measures and biological indicators in patients. However, controls with flatter cortisol rhythms showed larger waist circumference, poorer performance on explicit memory tasks, lower perceived social support, and a tendency toward higher perceived stress. These findings add to the expanding literature on cortisol diurnal rhythm and psychological functioning. Future research must continue to address the relation between psychological functioning and stress-related physiological functioning in cancer incidence and progression.

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## References

- Abercrombie, H.C., Thurow, M.E., Rosenkranz, M.A., Kalin, N.H., Davidson, R.J., 2003. Cortisol variation in humans affects memory for emotionally-laden and neutral information. *Behav. Neurosci.* 117, 505–516.
- Ballard-Barbash, R., Swanson, C., 1996. Body weight: Estimation of risk for breast and endometrial cancers. *Am. J. Clin. Nutr.* 63, 437S–441S.
- Barraclough, J.P., Osmond, C., Taylor, I., Perry, M., Collins, P., 1993. Life events and breast cancer prognosis (letter comment). *Br. Med. J.* 307, 325.
- Barraclough, J., Pinder, P., Cruddas, M., Osmond, C., Taylor, I., Perry, M., 1992. Life events and breast cancer prognosis [see comments]. *Br. Med. J.* 304, 1078–1081.
- Bjorntorp, P., 1990. "Portal" adipose tissue as a generator of risk factors for cardiovascular disease and diabetes. *Atherosclerosis* 10, 493–496.
- Blake, R.L., McKay, D.A., 1986. A single-item measure of social supports as a predictor of morbidity. *J. Fam. Pract.* 22, 82–84.
- Brindley, D.N., Rolland, Y., 1989. Possible connections between stress, diabetes, obesity, hypertension and altered lipoprotein metabolism that may result in atherosclerosis. *Clin. Sci.* 77, 453–461.
- Butler, L.D., Koopman, C., Cordova, M.J., 2003. Psychological distress and pain significantly increase before death in metastatic breast cancer patients. *Psychosom. Med.* 65, 416–426.
- Chlebowski, R.T., Aiello, E., McTiernan, A., 2002. Weight loss in breast cancer patient management. *J. Clin. Oncol.* 20, 1128–1143.
- Chrousos, G., Gold, P., 1998. A healthy body in a healthy mind—and vice versa—The damaging power of uncontrollable stress. *Clin. Endocrinol. Metab.* 83, 1842–1845.
- Cohen, J., 1977. *Statistical Power Analysis for the Behavioral Sciences*. Academic Press, New York.
- Cohen, S., Kamarck, T., Mermelstein, R., 1983. A global measure of perceived stress. *J. Health Soc. Behav.* 24, 385–396.
- Del Giudice, M., Fantus, I., Ezzat, S., McKeown-Eyssen, G., Page, G., Goodwin, P., 1998. Insulin and related factors in premenopausal breast cancer risk. *Breast Cancer Res. Treat.* 47, 111–120.
- Deuschle, M., Schweiger, U., Weber, B., Gotthardt, U., Korner, A., Schmider, J., 1997. Diurnal activity and pulsatility of the hypothalamus-pituitary-adrenal system in male depressed patients and healthy controls. *Clin. Endocrinol. Metab.* 82, 234–238.
- Dignam, J.J., Wieand, K., Johnson, K.A., Fisher, B., Xu, L., Mamounas, E.P., 2003. Obesity, tamoxifen use, and outcomes in women with estrogen receptor-positive early-stage breast cancer. *J. Natl. Cancer Inst.* 95, 1467–1476.
- Filipski, E., King, V.M., Li, X., Granda, T.G., Mormont, M.C., Liu, X., Claustrat, B., Hastings, M.H., Levi, F., 2002. Host circadian clock as a control point in tumor progression. *J. Natl. Cancer Inst.* 94, 690–697.
- Fu, L., Pelicano, H., Liu, J., Huang, P., Lee, C., 2002. The circadian gene *Period2* plays an important role in tumor suppression and DNA damage response in vivo. *Cell* 111, 41–50.
- Geyer, S., 1991. Life events prior to manifestation of breast cancer: a limited prospective study covering eight years before diagnosis. *J. Psychosom. Res.* 35, 355–363.
- Geyer, S., 1993. Life events, chronic difficulties and vulnerability factors preceding breast cancer [see comments]. *Soc. Sci. Med.* 37, 1545–1555.
- Gibbons, R.D., Hedeker, D., Waternaux, C., Kraemer, H.C., Greenhouse, J.B., 1993. Some conceptual and statistical issues in the analysis of longitudinal psychiatric data. *Arch. Gen. Psychiatry* 50, 730–750.
- Goodwin, P.J., Ennis, M., Pritchard, K.I., Trudeau, M.E., Koo, J., Madarnas, Y., Hartwick, W., Hoffman, B., Hood, N., 2002. Fasting insulin and outcome in early-stage breast cancer: results of a prospective cohort study. *J. Clin. Oncol.* 20, 42–51.
- Graham, J., Ramirez, A., Love, S., 2002. Stressful life experiences and risk of relapse of breast cancer: observational cohort study. *Br. Med. J.* 324, 1420–1422.

- Gunnar, M.R., Vazquez, D.M., 2001. Low cortisol and a flattening of expected daytime rhythm: potential indices of risk in human development. *Dev. Psychopathol.* 13, 515–538.
- Haus, E., Dumitriu, L., Nicolau, G.Y., Bologa, S., Sackett-Lundeen, L., 2001. Circadian rhythms of basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), insulin-like growth factor (IGF-1), insulin-like growth factor binding protein-3 (IGFBP-3), cortisol, and melatonin in women with breast cancer. *Chronobiol. Int.* 18, 709–727.
- Kaaks, R., Van Noord, P., Den Tonkelaar, I., Peeters, P., Riboli, E., Grobbee, D., 1998. Breast-cancer incidence in relation to height, weight, and body-fat distribution in the Dutch “DOM” cohort. *Int. J. Cancer.* 76, 647–651.
- Kirschbaum, C., Hellhammer, D.H., 1989. Salivary cortisol in psychobiological research: An overview. *Neuropsychobiology* 22, 150–169.
- Kirschbaum, C., Hellhammer, D.H., 1994. Salivary cortisol in psychoneuroendocrine research: Recent developments and applications. *Psychoneuroendocrinology* 19, 313–333.
- Kirschbaum, C., Wolf, O.T., May, M., Wippich, W., Hellhammer, D.H., 1996. Stress- and treatment-induced elevations of cortisol levels associated with impaired declarative memory in healthy adults. *Life Sci.* 58, 1475–1483.
- Kissebah, A.H., Krakower, G., 1994. Regional adiposity and morbidity. *Physiol. Rev.* 74, 761–811.
- Lapidus, L., Bengtsson, T., Hallstrom, T., Bjorntorp, P., 1984. Distribution of adipose tissue and risk of cardiovascular disease and death: A 12-year follow-up of participants in the population study of women in Gothenburg, Sweden. *Br. Med. J.* 289, 1257–1261.
- Levine, S., 2000. Influence of psychological variables on the activity of the hypothalamic-pituitary-adrenal axis. *Eur. J. Pharmacol.* 405, 149–160.
- Levy, S.M., Herberman, R.B., Lippman, M., 1991. Immunological and psychosocial predictors of disease recurrence in patients with early-stage breast cancer. *Behav. Med.* 17, 67–75.
- Lezak, M.D., 1995. *Neuropsychological Assessment*, 3rd Ed. . Oxford University Press, New York.
- Lohman, T.G., Roche, A.F., Martorell, R., 1988. *Anthropometric Standardization Reference Manual*. . Human Kinetics Books, Champaign, IL.
- Lupien, S.J., Lepage, M., 2001. Stress, memory, and the hippocampus: can't live with it, can't live without it. *Behav. Brain Res.* 127, 137–158.
- Lupien, S.J., McEwen, B.S., 1997. The acute effects of corticosteroids on cognition: Integration of animal and human model studies. *Brain Res. Rev.* 24, 1–27.
- Lupien, S.J., Nair, N.P., Briere, S., Maheu, F., Tu, M.T., Lemay, M., McEwen, B.S., Meaney, M.J., 1999. Increased cortisol levels and impaired cognition in human aging: implication for depression and dementia in later life. *Rev. Neurosci.* 10, 117–139.
- Marret, H., Perrotin, F., 2001. Low body mass index is an independent predictive factor of local recurrence after conservative treatment for breast cancer. *Breast Cancer Res. Treat.* 66, 17–23.
- McEwen, B.S., Sapolsky, R.M., 1995. Stress and cognitive function. *Curr. Opin. Neurobiol.* 5, 205–216.
- McEwen, B.S., Seeman, T., 1999. Protective and damaging effects of mediators of stress. Elaborating and testing the concepts of allostasis and allostatic load. *Ann. N Y Acad. Sci.* 896, 30–47.
- Mormont, M.C., Bogdan, A., Cormont, S., Touitou, Y., Levi, F., 2002. Cortisol diurnal variation in blood and saliva of patients with metastatic colorectal cancer: relevance for clinical outcome. *Anticancer Res.* 22, 1243–1249.
- Mormont, M.C., Lévi, F., 1997. Circadian-system alterations during cancer processes: A review. *Int. J. Cancer* 70, 241–247.
- Mormont, M.C., Waterhouse, J., Bleuzen, P., Giacchetti, S., Jami, A., Bogdan, A., Lellouch, J., Misset, J.L., Touitou, Y., Levi, F., 2000. Marked 24-h rest/activity rhythms are associated with better quality of life, better response, and longer survival in patients with metastatic colorectal cancer and good performance status. *Clin. Cancer Res.* 6, 3038–3045.
- Munck, A., Guyre, P., 1991. Glucocorticoids and immune function. In: Ader, R., Fetton, D., Cohen, N. (Eds.), *Psychoneuroimmunology*. Academic Press, San Diego.
- Ockenfels, M.C., Porter, L., Smyth, J., Kirschbaum, C., Hellhammer, D.H., Stone, A.A., 1995. Effect of chronic stress associated with unemployment on salivary cortisol: overall cortisol levels, diurnal rhythm, and acute stress reactivity. *Psychosom. Med.* 57, 460–467.
- Ramirez, A.J., Craig, T.K., Watson, J.P., Fentiman, I.S., North, W.R., Rubens, R.D., 1989. Stress and relapse of breast cancer. *Br. Med. J.* 298, 291–293.
- Rey, A., 1964. *L'examen Clinique en Psychologie*. . Presses Universitaires de France, Paris.
- Rock, C.L., Demark-Wahnefried, W., 2002. Nutrition and survival after the diagnosis of breast cancer: a review of the evidence. *J. Clin. Oncol.* 20, 3302–3316.
- Rogosa, D., Saner, H., 1995. Longitudinal data analysis examples with random coefficient models. *J. Educ. Behav. Stat.* 20, 149–170.
- Romero, L.M., Raley-Susman, K.M., Redish, D.M., Brooke, S.M., Horner, H.C., Sapolsky, R.M., 1992. Possible mechanism by which stress accelerates growth of virally derived tumors. *Proc. Natl. Acad. Sci.* 89, 11084–11087.
- Rosmond, R., Dallman, M., Bjorntorp, P., 1998. Stress related cortisol secretion in men: Relationships with abdominal obesity and endocrine, metabolic, and hemodynamic abnormalities. *J. Clin. Endocrinol. Metab.* 83, 1853–1859.
- Rosmond, R., Holm, G., Bjorntorp, P., 2000. Food-induced cortisol secretion in relation to anthropometric, metabolic and haemodynamic variables in men. *Int. J. Obes. Relat. Metab. Disord.* 24, 416–422.
- Sapolsky, R.M., Donnelly, T.M., 1985. Vulnerability to stress-induced tumor growth increases with age in rats: role of glucocorticoids. *Endocrinology* 117, 662–666.
- Sephton, S., Sapolsky, R., Kraemer, H., Spiegel, D., 2000. Diurnal cortisol rhythm as a predictor of breast cancer survival. *J. Natl. Cancer Inst.* 92, 994–1000.
- Sephton, S., Spiegel, D., 2003. Circadian disruption in cancer: A neuroendocrine-immune pathway from stress to disease? *Brain Behav. Immun.* 17, 321–328.
- Shrout, P.E., Fleiss, J.L., 1979. Intraclass correlations: Uses in assessing rater reliability. *Psychol. Bull.* 86, 420–428.
- Smyth, J.M., Ockenfels, M.C., Gorin, A.A., Catley, D., Porter, L.S., Kirschbaum, C., Hellhammer, D.H., Stone, A.A., 1997. Individual differences in the diurnal cycle of cortisol. *Psychoneuroendocrinology* 22, 89–105.
- Smyth, J., Ockenfels, M.C., Porter, L., Kirschbaum, C., Hellhammer, D.H., Stone, A.A., 1998. Stressors and mood measured on a momentary basis are associated with salivary cortisol secretion. *Psychoneuroendocrinology* 23, 353–370.
- Stoll, B.A., 1996. Obesity and breast cancer. *Int. J. Obes. Relat. Metab. Disord.* 20, 389–392.
- Stone, A.A., Schwartz, J.E., Smyth, J., Kirschbaum, C., Cohen, S., Hellhammer, D., Grossman, S., 2001. Individual differences in the diurnal cycle of salivary free cortisol: a replication of flattened cycles for some individuals. *Psychoneuroendocrinology* 26, 295–306.

- Ticher, A., Haus, E., Ron, I.G., Sackett-Lundeen, L., 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.
- Tomarken, A.J., Davidson, R.J., Wheeler, R.E., Kinney, L., 1992. Psychometric properties of resting anterior EEG asymmetry: temporal stability and internal consistency. *Psychophysiology* 29, 576–592.
- Touitou, Y., Levi, F., Bogdan, A., Benavides, M., Bailleul, F., Misset, J.L., 1995. Rhythm alteration in patients with metastatic breast cancer and poor prognostic factors. *J. Cancer Res. Clin. Oncol.* 121, 181–188.
- Turner-Cobb, J.M., Sephton, S.E., Koopman, C., Blake-Mortimer, J., Spiegel, D., 2000. Social support and salivary cortisol in women with metastatic breast cancer. *Psychosom. Med.* 62, 337–345.
- Vgontzas, A.N., Chrousos, G.P., 2002. Sleep, the hypothalamic-pituitary-adrenal axis and cytokines: multiple interactions and disturbances in sleep disorders. *Endocrinol. Metab. Clin. North Am* 31, 15–36.
- Whiteside, T.L., Herberman, R.B., 1995. The role of natural killer cells in immune surveillance of cancer. *Curr. Opin. Immunol.* 7, 704–710.
- Yehuda, R., Teicher, M.H., Trestman, R.L., Levengood, R.A., Siever, L.J., 1996. Cortisol regulation in posttraumatic stress disorder and major depression: a chronobiological analysis. *Biol. Psychiatry* 40, 79–88.
- Zumoff, B., Gorzynski, J.G., Katz, J.L., Weiner, H., Levin, J., Holland, J., Fukushima, D.K., 1982. Nonobesity at the time of mastectomy is highly predictive of 10-year disease-free survival in women with breast cancer. *Anticancer Res.* 2, 59–62.