# Circadian Rhythms and Diurnal Profiles of Salivary Alpha Amylase in Women with Breast Cancer

Christy Albert, BS[1], Trevor Stantliff[1], Elizabeth Cash, PhD[1,2,3], and Sandra E. Sephton, PhD[1,2]

[1]Department of Psychological and Brain Sciences, University of Louisville, Louisville, KY [2]James Graham Brown Cancer Center, University of Louisville, Louisville, KY
[3]Department of Otolaryngology-HNS & Communicative Disorders, University of Louisville School of Medicine, Louisville, KY

## Introduction

**PURPOSE:**
This study aims to examine the relationship between circadian activity rhythms and the diurnal profile of salivary alpha amylase in pre-surgical breast cancer women.

**BACKGROUND:**
Approximately 1 in 8 U.S. women will develop an invasive breast tumor during the course of her life. Numerous biological processes, including those responsible for tumor suppression, are organized into a hierarchy of phase coupled genetic oscillators incorporating auto-regulatory transcription-translation feedback loops. *Disruptions in this hierarchy result in tumor promoting environments[1].*

The suprachiasmatic nucleus (SCN), at the top of the hierarchy, follows an endogenous 24 hour cycle syncing itself to light/dark via photic information from the retina. Additionally, efferent and afferent pathways to many brain regions results in phase locking of downstream oscillators, regulating not only biological rhythms but also behavioral circadian rhythms such as the sleep/wake cycle and rest/activity rhythms**.** *Disruptions in these behavioral rhythms have associations with biomarkers of tumor progression[2].*

The diurnal profile of cortisol secretion, its connection to rest/activity rhythms[3], and cortisol's ability to reach peripheral organs suggests the HPA axis as a potential communicator between the SCN, diverse brain regions, and peripheral cell oscillators. Moreover, *disruptions of HPA rhythms, which can be caused by chronic stress, are prognostic for early mortality in breast cancer patients[4].*

The sympathetic nervous-adrenomedullary (SAM) system is also activated in response to stress and has the ability to signal peripheral organs. The SAM may be another mechanism by which the SCN coordinataes peripheral cell oscillators. Salivary alpha amylase (sAA), a biomarker for norepinephrine release via the SAM system, follows a diurnal profile.

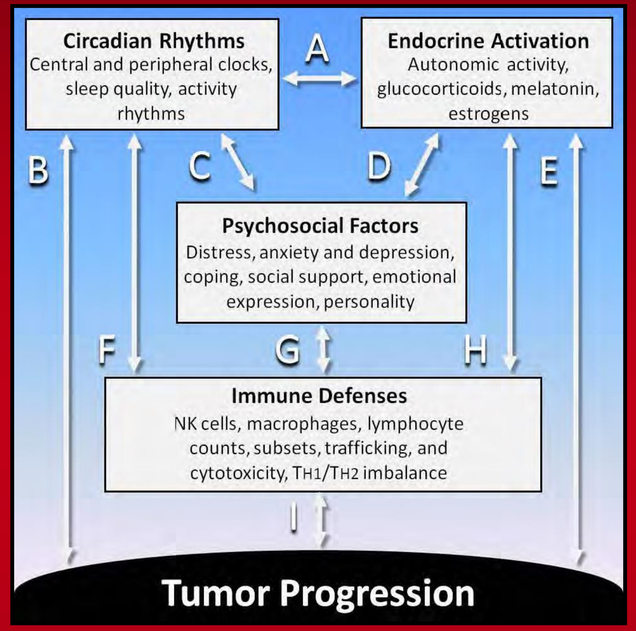
Our laboratory's model (Figure 1) illustrates circadian effects in psychoneuroendocrine and immune pathways related to tumor progression (Eismann, et al., 2010). Circadian rhythms and autonomic activity influence tumor progression directly (pathways B and E) and/or indirectly (multiple pathways). This study investigates how they may influence (relationships to) each other (pathway A).

**RATIONALE:**
The non-invasive collection nature of sAA makes it a good candidate for studying the relationship between circadian activity rhythms and sympathetic activation in cancer patients.

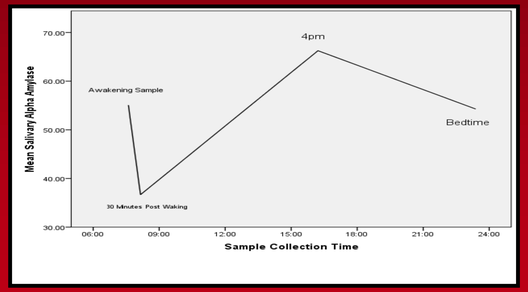
While research investigating the HPA axis with regard to circadian activity rhythms has led to effective interventions ameliorating the effects of chronic stress on tumor progressions and cancer outcomes, research into sympathetic activation with regard to circadian regulation has been lacking.

Research here could shed new light on the mechanisms integrating biorhythms and behavior, resulting in more integrative approaches to cancer treatment.

**Our laboratory's model of circadian effects in psychoneuroendocrine and immune pathways related to tumor progression (Eismann, et al., 2010)**



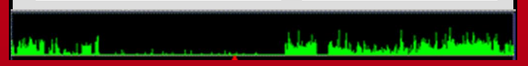

Mean sAA levels in our group exhibit a diurnal profile with a dip in concentration 30 minutes after waking, a steady increase during the day, and a dip in concentration from 4pm to bed.




24 Hour Autocorrelation Coefficient Distribution

| | | |
|---|---|---|
| N | Valid | 47 |
| Mean | | .279 |
| Std. Deviation | | .158 |
| Range | | .738 |
| Minimum | | -.074 |
| Maximum | | .721 |
| Variance | | .025 |

Actigraphy watches measure activity levels in one minute epochs. Auto-correlation is a technique used to show how one minute epochs on day 1 correlate with one minute epochs on subsequent days. Higher values are representative of stronger daily rhythms[5].



## Sample (N = 50)

**race**

| | | Frequancy | Percent | Valid Percent |
|---|---|---|---|---|
| Valid | Asian | 1 | 2.0 | 2.0 |
| | Black | 18 | 36.0 | 36.7 |
| | Native American | 2 | 4.0 | 4.1 |
| | White/Caucasian | 28 | 56.0 | 57.1 |
| | Total | 49 | 98.0 | 100.0 |
| Missing | System | 1 | 2.0 | |
| Total | | 50 | 100.0 | |

**income**

| | | Frequency | Percent | Valid Percent |
|---|---|---|---|---|
| Valid | Less than $20,000 | 18 | 36.0 | 40.0 |
| | $20,000-39,999 | 12 | 24.0 | 26.7 |
| | $40,000-59,999 | 4 | 8.0 | 8.9 |
| | 60,000-79,999 | 4 | 8.0 | 8.9 |
| | $80,000-$99,999 | 3 | 6.0 | 6.7 |
| | 100,000 and above | 4 | 8.0 | 8.9 |
| | Total | 45 | 90.0 | 100.0 |
| Missing | System | 5 | 10.0 | |
| Total | | 50 | 100.0 | |

**stage**

| | | Frequency | Percent | Valid Percent |
|---|---|---|---|---|
| Valid | DCIS | 4 | 8.0 | 8.0 |
| | stage 1 | 23 | 46.0 | 46.0 |
| | stage 2A | 5 | 10.0 | 10.0 |
| | stage 2B | 4 | 8.0 | 8.0 |
| | stage 3A | 7 | 14.0 | 14.0 |
| | stage 3B | 1 | 2.0 | 2.0 |
| | stage 3C | 2 | 4.0 | 4.0 |
| | stage 4 | 4 | 8.0 | 8.0 |
| | Total | 50 | 100.0 | 100.0 |

**Descriptive Statistics**

| | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| age at diagnosis | 47 | 21 | 79 | 52.00 | 13.568 |
| Valid N (listwise) | 47 | | | | |

## Methods

This study falls within the scope of a larger study in which sixty breast cancer patients awaiting surgery were recruited. None of the recruits were currently taking contraceptives or hormone replacement therapy. Saliva samples were collected from participants at awakening, 30 minutes post waking, 4pm, and bedtime over a collection period of three days. Participants also wore an actigraphy watch (ambulatory monitoring) during the collection period.

Salivary alpha amylase samples were quantified using kinetic assay technique (Salimetrics) which employs a 2-chloro-*p*-nitrophenol substrate linked to maltrose. Enzymatic activity of alpha amylase on this substrate yields 2-chloro-*p*-nitrophenol which can be spectrophotometrically measured at 405nm using a standard optical density plate reader. Raw amylase values were log transformed before slopes (waking to 30 minutes post waking; waking, 4pm, and bed; and 30 minutes post waking to 4pm) were calculated.

Actigraphy data was translated into rest/activity rhythm variables (24hr autocorrelation coefficient) using Action 4 software.

One participant was excluded due to shift work. Additional variances in N value reflect insufficient quantity of saliva samples (samples were first analysed for salivary cortisol), samples with collection times outside the proposed times, or other collection errors that invalidated the sample.

## Results

Hierarchical linear regression models showed no significance (p>.05) between circadian activity rhythms (autocorrelation coefficient) and salivary alpha amylase diurnal profile slopes (waking, 4pm, and bed; waking to 4pm; post waking, 4pm, and bed; post waking to 4pm; and waking to 30 minutes post waking (morning response).

After controlling for age, stage, and income; models with circadian activity rhythms as a predictor showed no significance (p>.05) while models with amylase slope as a predictor became significant, with income as the only significant predictor to these models. Models with slope (Waking, 4pm, bed) as a predictor became significant (p=.02,$R^2$=.277) with income as the significant predictor to the model (p=.001,β=.511), models with amylase slope (waking to 4pm) became significant (p=.005, $R^2$=.346) with income as the significant predictor to the model (p=.001,β=.50), models with amylase slope (post waking, 4pm, bed) became significant (p=.022,$R^2$=.272) with income as the significant predictor to the model (p=.001,β=.51), models with amylase slope (post waking to 4pm) became significant (p=.021,$R^2$=.283) with income as the significant predictor to the model (p=.001,β=.52) and models with amylase slope (morning response) as a predictor became significant (p=.025,$R^2$=.272) with income as a significant predictor to the model (p=.002,β=.50)

## Conclusions

Our study found no significance between circadian activity rhythms and the diurnal profile of salivary alpha amylase in this sample. However, significant relationships between circadian activity rhythms and the diurnal profile of cortisol were found from this same sample[3].

This may indicate that the oscillatory phase coupling mechanisms resulting in diurnal patterns of sympathetic activation are more robust against behavioral influences and exogenous signals compared to the HPA axis, or it could point to other mediators influencing the interplay between circadian activity rhythms and SNS activation. Additional research investigating these possible mediators could shed new light on this intricate interplay of biorhythms and behavior, leading to more integrative approaches to cancer treatment.

Additionally, income as a predictor reveals potential moderating effects of socioeconomic factors in these outcome variables, and illustrates the need for future studies to frame cancer research within a socioeconomic context.

## Acknowledgements

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