

Project 0 Report: Bios6624

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Introduction

This study assessed the use of a novel saliva collection device for investigating daily patterns of the stress system hormones, cortisol and DHEA. 31 healthy control subjects collected saliva samples and reported the time of sample collection four times per day for three days for a total of 372 samples. One primary outcome of interest was the accuracy of subject self-reporting for sample collection times compared to an electronic monitoring device in the cap of the bottle containing the sampling equipment. The second primary outcome of interest was the subject adherence to the intended sampling regime of subject wake, 30 minutes after waking, before lunch, and 600 minutes after waking. As wake up time and lunch time are not verifiable with the data collected, analysis was limited to the time points at 30 and 600 minutes after waking. Additionally, the daily pattern of cortisol and DHEA levels was a secondary outcome of interest.

The purpose of this report is to answer the following questions of interest:

1. Are the subject's recordings of sampling times biased compared to the times recorded by the electronic monitoring cap?
2. What proportion of subjects were within 7.5 or 15 minutes of the required sampling times?
3. How do cortisol and DHEA levels change over time?

Methods

Dataset and Data Cleaning

372 observations were collected from 31 subjects. N = 87 observations with missing time points for the booklet or cap were dropped as imputation was not considered feasible due to the limited number of covariates, resulting in 337 observations for analysis. N = 4 additional observations were removed from the analysis of cortisol and NHEA as they were missing both outcome variables. Observations of cortisol over 80 nmol/L were considered to be lab errors and excluded. Observations of DHEA that reached the upper detection limit of 5.205 nmol/L were not measurable and were excluded. Additionally, any patients with multiple measures at 5.205 were excluded as this likely indicated underlying health problems that interfere with the normal pattern of DHEA. Using the exclusion criteria, one cortisol observation was removed. One subject was excluded from hormone analysis for having multiple observations at 5.205. 2 additional observations were also removed based on this criteria. This resulted in a total of 321 observations for analysis.

Data Analysis

The agreement between the time recorded in the booklet and the time recorded by the cap was examined using a linear mixed effects model with booklet time as a predictor for cap time, with subject ID as a random effect. The intercept of the model can be considered to represent overall bias and the slope represents drift in measurements over time. If the measurements are unbiased, the intercept will be 0 and the slope will be

1. The true intercept and slope were compared to these values using a t-test. $P < 0.05$ will be considered significant.

The proportion of subjects who were within 7.5 and 15 minutes of the desired sample intervals of 30 minutes after waking and 600 minutes after waking were quantified.

The patterns of cortisol and DHEA were each assessed using a piecewise linear mixed effects model. The explanatory variable was the amount of time since the subject woke up, with subject ID as a random effect. No covariates were used in the model. Assumptions of linearity, homoscedasticity, and normality will be confirmed using diagnostic plots. Cortisol was log transformed to adjust for a right skew in the data. Based on prior research into patterns of cortisol and DHEA levels, the breakpoint was at 30 minutes after waking.

Analyses were run using R version 4.4.3.

Results

Sample characteristics are summarized in table 1.

Table 1: Descriptive Summary of Sample

	Overall	1	2	3	4
n	337	88	87	82	80
Time Difference (minutes) (mean (SD))	-7.71 (32.09)	-4.00 (8.75)	-16.05 (35.87)	-6.18 (42.66)	-5.40 (29.92)
Cortisol (nmol/L) (mean (SD))	6.03 (7.04)	7.33 (6.14)	8.73 (4.59)	3.33 (2.27)	4.39 (10.97)
DHEA (nmol/L) (mean (SD))	1.01 (1.04)	1.77 (1.30)	1.13 (0.94)	0.55 (0.47)	0.50 (0.65)

The subject recordings were not biased relative to the cap times. The intercept and slope were not significantly different from the null hypothesis of 0 and 1 (table 2).

Table 2: Summary of Association Model Fixed Effects

Term	Hypothesis	Estimate	95% CI	t-value	p-value
Intercept	beta = 0	-3.95	(-14.187, 6.29)	-0.76	0.448
Slope	beta = 1	0.99	(0.981, 1.008)	-0.83	0.407

The proportion of subjects within 7.5 and 15 minutes of the required sampling time for both the booklet and cap recordings is given in table 3.

Table 3: Proportion of Subjects Within Desired Sampling Margins

	Booklet	Cap
Within 15 Minutes	0.76	0.66
Within 7.5 Minutes	0.62	0.50

Model coefficients for the effect of time since wakeup on $\log(\text{cortisol})$ are given in table 4. There was not a significant association between time since wakeup and $\log(\text{cortisol})$ within the first 30 minutes of waking. The change at 30 minutes was significant ($p = 0.03$). After 30 minutes, cortisol decreased 0.22% per minute after wakeup (t-test, 95% CI: 0.19%, 0.26%, $p < 0.001$). The predicted $\log(\text{cortisol})$ level for an average subject is given in figure 2.

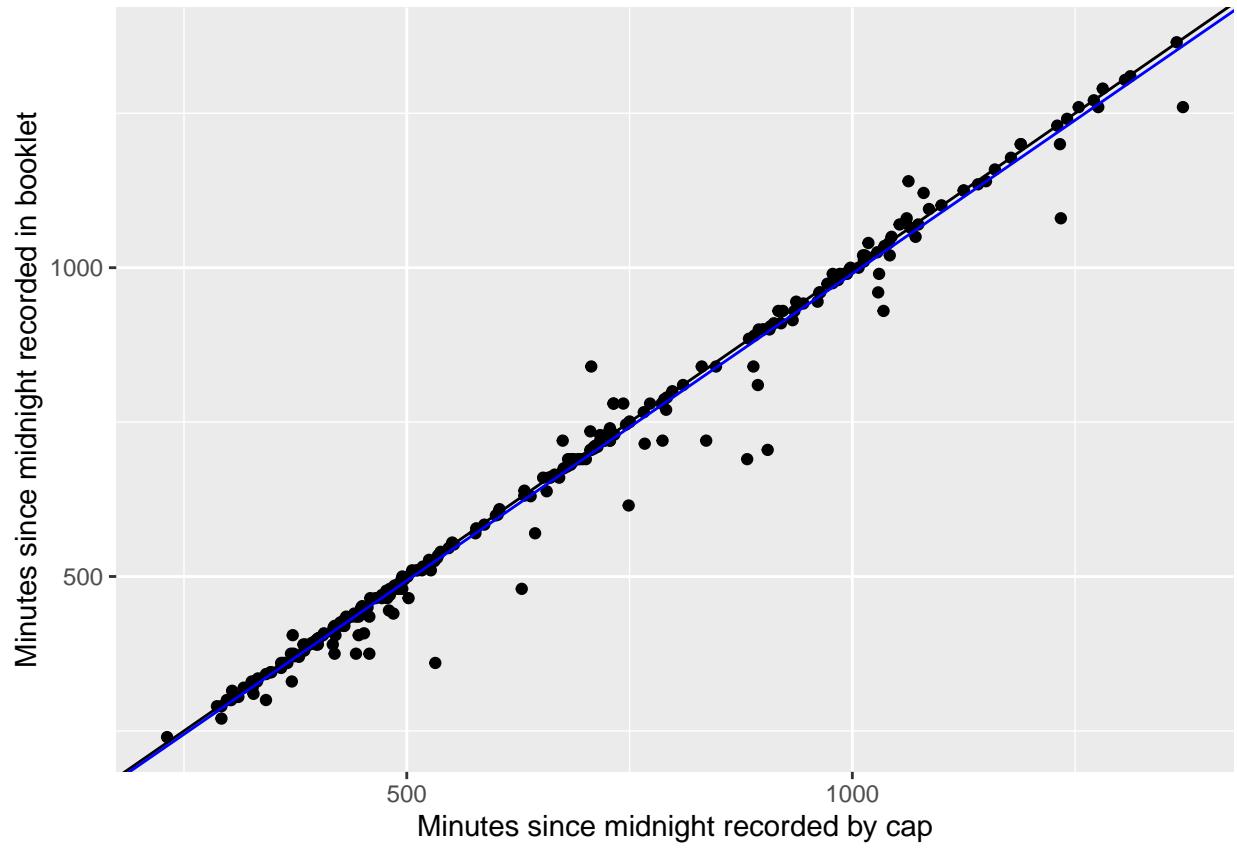
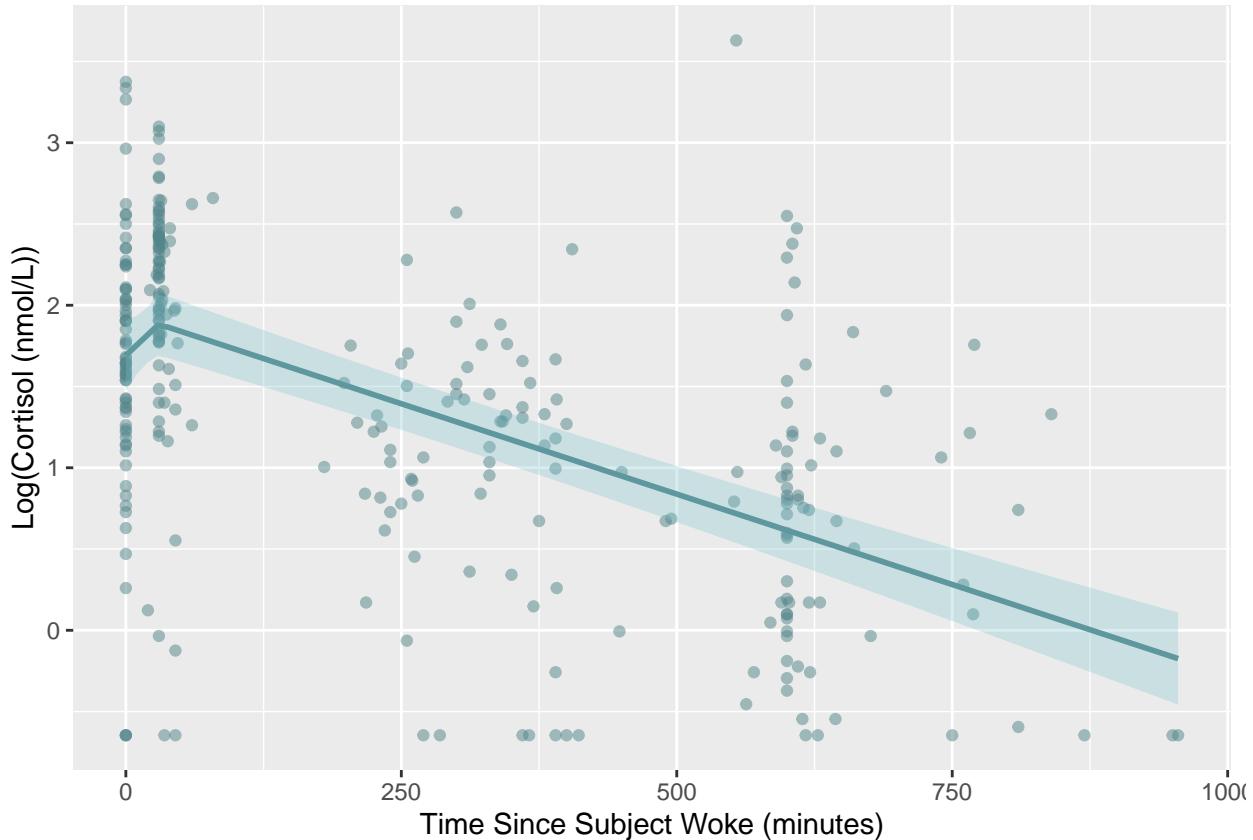


Figure 1: Agreement between the time recorded in the booklet and the time recorded by the electronic cap. The black line represents perfect agreement and the blue line is the model prediction.

Table 4: Summary of Log(Cortisol) Model Fixed Effects

Term	Estimate	95% CI	t-value	p-value
Intercept	1.69	(1.47, 1.913)	15.15	<0.001
Time since subject woke (minutes)	0.01	(-0.001, 0.014)	1.65	0.101
Change in slope after 30 minutes post wakeup	-0.01	(-0.016, -0.001)	-2.17	0.031



Model coefficients for the effect of time since wakeup on DHEA are given in table 4. DHEA decreased at a rate of -0.023 nmol/L per minute for the first 30 minutes (t-test, $p < 0.001$, 95% CI: -0.029, -0.017). The change at 30 minutes was significant ($p < 0.001$). After 30 minutes it decreased at a rate of -0.0009 nmol/L per minute (t-test, $p < 0.001$, 95% CI: -0.0012, -0.0006). The predicted DHEA level for an average subject is given in figure 3.

Table 5: Summary of DHEA Model Fixed Effects

Term	Estimate	95% CI	t-value	p-value
Intercept	1.632	(1.418, 1.846)	15.20	<0.001
Time since subject woke (minutes)	-0.023	(-0.029, -0.017)	-7.21	<0.001
Change in slope after 30 minutes post wakeup	0.022	(0.016, 0.028)	6.77	<0.001

Discussion and Summary

The recordings of the booklet were not biased compared to the booklet recordings. Self-reported times generally agreed with the cap time. This indicates that the booklet is a promising tool for future research.

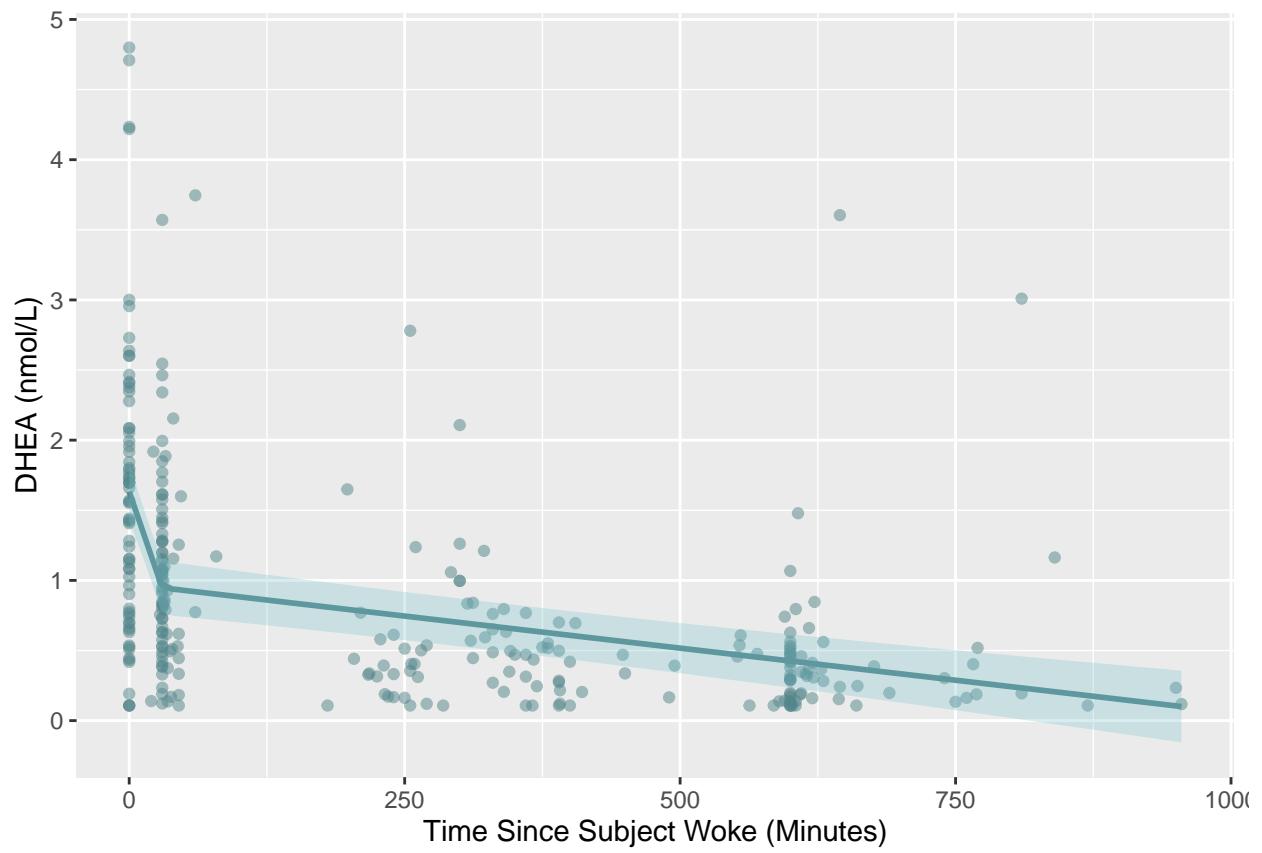


Figure 2: Relationship between time since the subject woke and DHEA levels. Model prediction with 95% confidence interval given.

The proportion of subjects that were within the desired sampling timeframe was lower when recorded by the cap than when recorded by the subject. This may indicate that the subjects were rounding the numbers that they recorded. It could also indicate subjects recorded values that showed them as adhering when they were not. However, as the booklet and cap recordings were not biased, this may be random chance. Overall, adherence was moderate. Measures to improve adherence may be beneficial.

Findings for the pattern of daily hormone change was concordant with previous research. Levels were highest within 30 minutes after waking and a significant change in trend occurred at 30 minutes after wakeup. However, due to the study design there were gaps in sampling between 40 and 200 minutes and 400 and 600 minutes. This results in less reliable estimations of trend. Additionally, a sample size of only 30 subjects is not very high, despite the relatively high number of total measures. Generalizing these results is difficult without more knowledge of subject selection and demographics.