

Project 0

Joseph Nagel

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Introduction

Cortisol and dehydroepiandrosterone (DHEA) are hormones released by the endocrine system that help regulate physiological responses to stress. These hormones exhibit pronounced diurnal patterns, meaning their levels change systematically across the day. Understanding these patterns is important as disruptions of these rhythms may indicate altered regulation of the stress response system.

The data analyzed in this report come from a study designed to evaluate a novel at-home saliva collection method for characterizing diurnal hormone profiles. Specifically, the investigator tested the Saliva Procurement and Integrated Testing (SPIT) booklet, which collects saliva on filter paper strips, and compared timing information recorded by the booklet to a Medication Event Monitoring System (MEMS) cap on the storage bottle. The dataset included 31 subjects who collected saliva samples four times per day for three consecutive days. Sampling was scheduled at waking, 30 minutes post-waking, before lunch, and 600 minutes post-waking. Subjects were allowed to define their own waking time and before lunch timing. The goals of the study were to determine the agreement between the booklet and MEMS cap samples times, measure the adherence to the study protocol's sampling

times, and characterize the diurnal patterns of cortisol and DHEA. These goals were assessed with the following statistical hypotheses:

1. Measurement Method Agreement: Is there significant additive or proportional bias between the booklet and MEMS measuring cap sample times?
2. Sample Timing Adherence: What proportion of samples fell within ± 7.5 minutes (good) and ± 15 minutes (adequate) of the target times for the 30-min and 10-hour post-waking collections?
3. Diurnal Hormone Patterns: What is the change in hormone levels from waking to 30 minutes post waking? What is the change per hour in hormone levels after 30 minutes post waking?

Methods

The key measures in the dataset (subject's sleep diary wake time, booklet sample time, MEMS cap sample time, cortisol (nmol/l), and DHEA (nmol/l)) were assessed for missingness and outliers. Outliers were defined as cortisol ≥ 80 nmol/l and DHEA ≥ 5.205 nmol/l (the assay upper detection limit for DHEA). Any samples exceeding these limits and all samples for subjects with multiple readings at the DHEA assay limit were excluded from the diurnal pattern analysis. Cortisol and DHEA were log transformed to reduce skewness and mitigate the violation of regression assumptions. The time between sleep diary waking time and sample collection was calculated for both the booklet and MEMS cap samples times. A table of descriptive statistics was created for the primary variables in the analysis. Prior to analysis for research question 3, outlier values of cortisol and DHEA were set to missing and one patient (subject 3037) was removed due to multiple DHEA readings at the assay limit.

All analysis were performed using a type one error rate (α) of 0.05. All models and figures

were created with R version 4.5.1. ChatGPT version 5.2 was used to debug code, outline the report, and copy-edit the report.

Measurement Method Agreement

To address the agreement and bias between timing methods, we estimated the additive and proportional bias between the MEMS cap and booklet sample times. Additive bias represents a systematic difference in the reported times independent of time of day while proportional bias represents a relationship in the difference between the measurements that changes throughout the day. A linear mixed-effects model was fit with MEMS cap sample time since wake as the outcome and booklet sample time since wake as the predictor with a subject-specific random intercept to account for repeated measures. Additive bias was evaluated by testing whether the fixed effect intercept was different from 0 and proportional bias was evaluated by testing whether the fixed effect slope was different from 1. This relationship was also visualized.

Sample Timing Adherence

To evaluate adherence to the study protocol's collection schedule, we assessed the proportion of samples that fell within ± 7.5 minutes (good adherence) and ± 15 minutes (adequate adherence) of the target collection time for the 30-minute and 10-hour post-waking collections. For each sample, we calculated the absolute difference between the observed collection time and the target collection time and created indicator variables for each of the adherence windows (± 7.5 and ± 15 minutes). The outcome was defined as the proportion of observed (nonmissing) samples that fell within the window. This was performed for both the booklet and MEMS cap times.

Diurnal Hormone Patterns

To characterize diurnal patterns in cortisol and DHEA, we estimated changes over two intervals: waking to 30 minutes post waking and after 30 minutes post waking. The booklet sample time was used to define measurement time. This was decided based on the agreement and bias analysis as well as missingness. As described above, hormone concentrations were log transformed to reduce right skewness prior to modeling. Piecewise linear mixed-effects models were fit with time since waking as the predictor and hormone level as the outcome, including a subject-specific random intercept to account for repeated measures. Models included a single knot at 30 minutes (0.5 hours) to allow for distinct trends before and after the knot. The relative (multiplicative) change from waking to 30 minutes post waking and the relative change per hour after 30 minutes were estimated from the fitted models.

Results

Data availability and missingness are summarized in **Table 1**. Notably, the booklet provided more complete timing data (9.4% missing) compared to the MEMS cap system (16% missing). Both hormone measurements had similar levels of missingness (~5% missing).

Table 1: Data Availability and Missingness

Characteristic	Expected	Observed	Missing	Missingness
Booklet Recording Time	372	337	35	9.4%
MEMS Cap Recording Time	372	311	61	16%
Both Time Recordings	372	285	87	23%
Sleep Diary Wake Time	372	372	0	0%
Cortisol (nmol/L)	372	354	18	4.8%
DHEA (nmol/L)	372	353	19	5.1%

¹ n

² %

Measurement Method Agreement

The linear mixed-effect model comparing the booklet sample times to the MEMS cap sample times (**Table 2**) revealed significant attative bias between the measurements. The model's slope was not significantly different from 1 (p-value = 0.2) indicating no proportional bias (bias did not change with time) between the measurement methods. However, the intercept was significantly different from 0 (p-value < 0.001) indicating addative bias. At waking, MEMS cap sample times were on average 10.2 minutes (95% CI: 4.4, 15.9) minutes later than booklet sample times. The relationship is visualized in **Figure 1**. While the model showed slight additive bias between the measurements, the plot shows high agreement between the two methods, with the fitted and perfect agreement ($y = x$) lines almost completely overlapping.

Table 2: Agreement Model Fixed Effects

Term	Estimate	95% CI	Hypothesis	t-value	P-value
Intercept	0.170	(0.074, 0.265)	beta = 0	3.53	<0.001
Slope	0.991	(0.977, 1.005)	beta = 1	-1.28	0.2

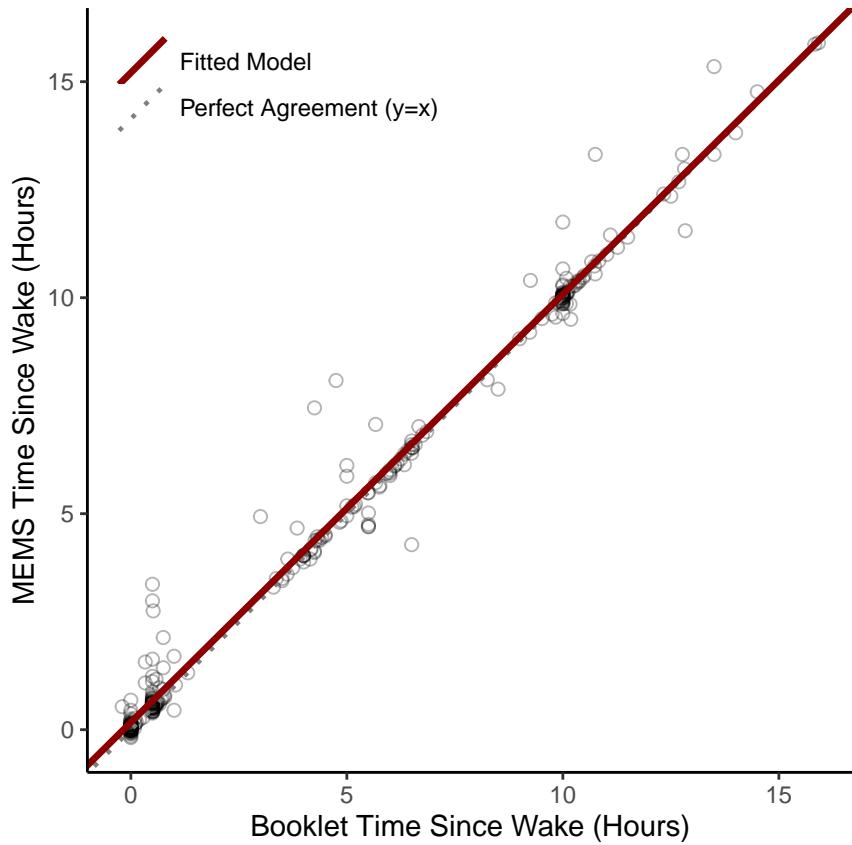


Figure 1: Plot of Booklet vs MEMS Cap Times with LMM Fit

Sample Timing Adherence

Adherence to the study protocol varied by recording method (**Table 3**). For the good adherence threshold (± 7.5 minutes), only 41.8% of the MEMS cap samples were within the window, compared to 62.9% reported in the booklet. A similar pattern was observed for the adequate adherence threshold (± 15 minutes), where the booklet-reported adherence (73.7%) was substantially higher than the MEMS cap adherence (54.2%).

Table 3: Adherence Rates by Recording Method

Recording Method	Adherence Thresholds	
	15-Minute Window	30-Minute Window
MEMS Cap	41.8%	54.2%
Booklet	62.9%	73.7%

Diurnal Hormone Patterns

Table 4: Relative Changes in Cortisol and DHEA

Hormone	Period	% Change	95% CI	p-value
Cortisol	0-30 min (total change)	20.20	(-3.08, 49.09)	0.09
Cortisol	> 30 min (change/hour)	-12.53	(-14.42, -10.6)	0.00
DHEA	0-30 min (total change)	-41.30	(-50.99, -29.71)	0.00
DHEA	> 30 min (change/hour)	-8.82	(-10.46, -7.15)	0.00

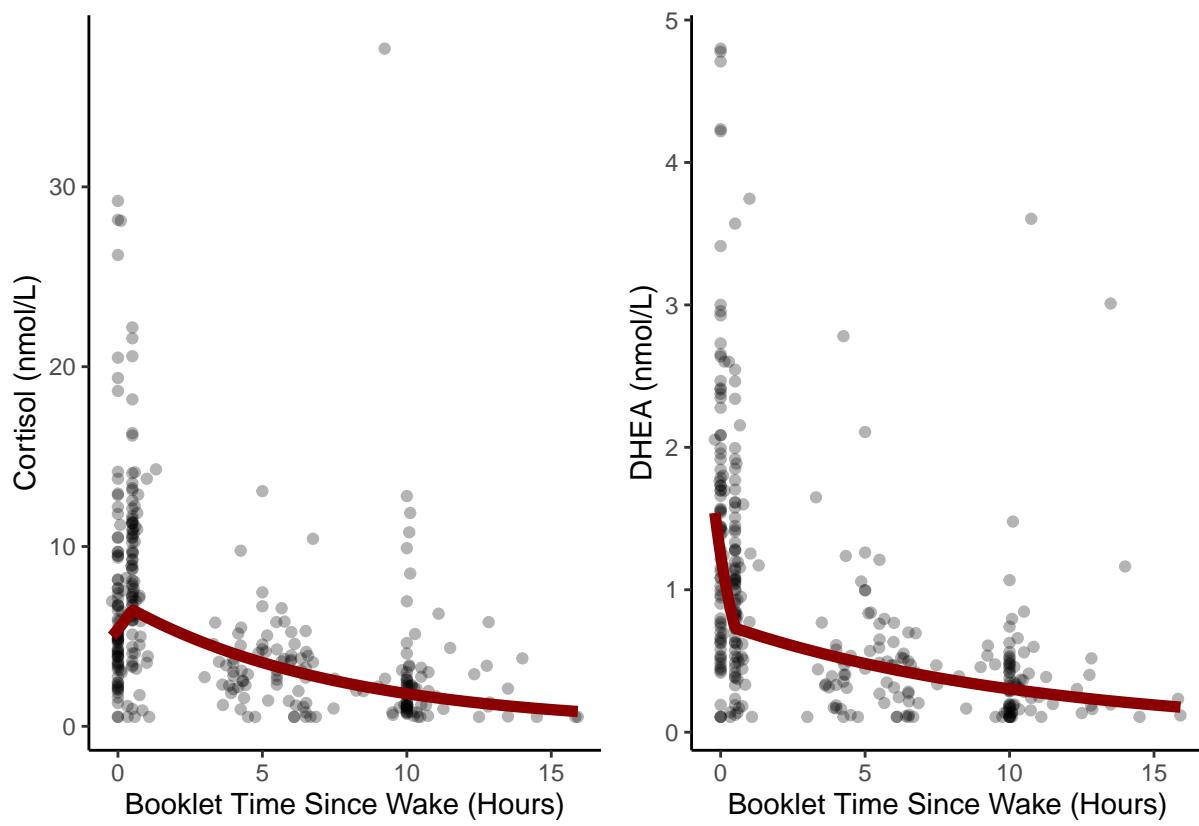


Figure 2: Fitted Diurnal Trajectories of Cortisol and DHEA

Discussion