Feasibility and Timing Accuracy of a Novel Filter Paper–Based Saliva Collection Method for Assessing Diurnal Cortisol and DHEA

Mahfuza Haque Mahi

February 4, 2026

Research Question

Scientific objective

- Evaluate the feasibility and timing accuracy of the Saliva Procurement and Integrated Testing (SPIT) collection booklet for assessing diurnal cortisol and DHEA.

Statistical questions

- Do booklet-recorded sampling times agree with electronically recorded (MEMS) times?
- Are participants adherent to the protocol-specified +30-minute and +10-hour sampling windows?
- Does the SPIT method capture expected diurnal patterns of cortisol and DHEA?

Data Overview

Study Design and Sample:

- Feasibility study of **31 healthy adults**
- Saliva collected over **3 consecutive days**
- Up to 4 samples per day (waking, +30 min, lunch, +10 hours)
- Total scheduled samples: 360 (90 per timepoint)

Data Cleaning and Management:

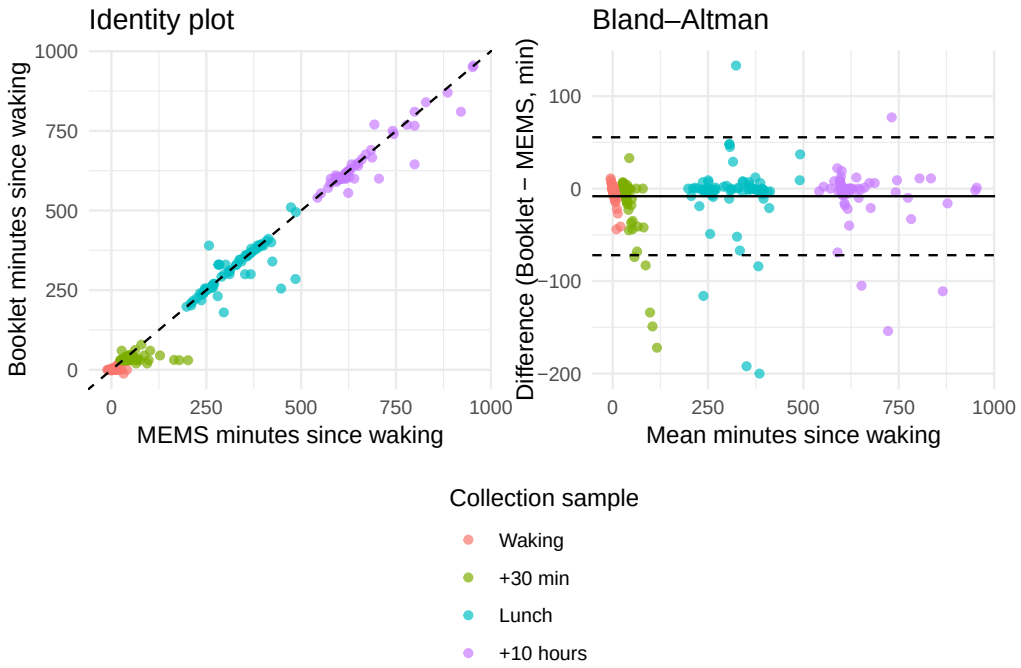
- Excluded alternative hormone units to avoid redundancy
- Cortisol values > 80 nmol/L treated as implausible (set to missing)
- DHEA measurements at detection limit (5.205 nmol/L) excluded
- Derived variables: minutes since waking (booklet and MEMS sources)

Data Quality:

- Booklet times available: 90.3% of samples
- MEMS cap times available: 83.3% of samples
- Cortisol and DHEA missing: $< 3\%$ across all timepoints

Methods & Results: Agreement Between Timing Methods

Figure 1: Agreement between booklet− and MEMS−recorded sampling times



Identity plot and Bland–Altman plot comparing booklet- and MEMS-recorded minutes since waking.

Mixed-effects regression results

- Outcome: booklet minutes since waking
- Predictor: MEMS minutes since waking
- Random intercept for subject

Key estimates

- Slope $= 0.99$ (95% CI: 0.98, 1.01), $p < 0.001$
- Intercept $= -6.7$ minutes (95% CI: -12.6, -0.8)

Near-unity slope indicates strong proportional agreement between methods, with a small systematic tendency for booklet times to be recorded slightly earlier than MEMS times.

Methods & Results: Protocol Adherence to Scheduled Sampling Times

Definition

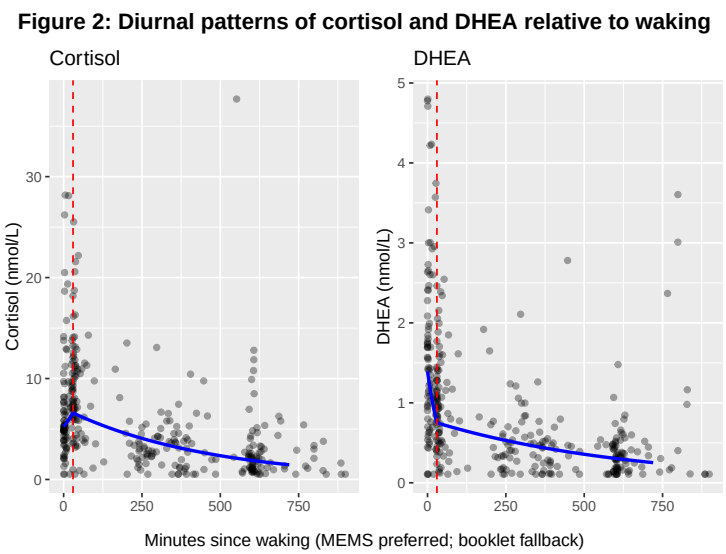
- Adherence defined as collection within:
  - ±7.5 minutes
  - ±15 minutes
- Reference: sleep diary–reported wake time

Key findings (MEMS-based)

- **+30-minute sample**
  - Booklet: 78.6% within ±7.5 min
  - Booklet: 90.5% within ±15 min
  - MEMS: 52.9% within ±7.5 min
  - MEMS: 70.6% within ±15 min
- **+10-hour sample**
  - Booklet: 48.1% within ±7.5 min
  - Booklet: 55.8% within ±15 min
  - MEMS: 33.8% within ±7.5 min
  - MEMS: 41.2% within ±15 min

Booklet-recorded times consistently overestimated adherence relative to electronically monitored MEMS times, particularly for the late-day collection.

Results: Diurnal Patterns of Cortisol and DHEA



Observed hormone concentrations over time since waking with population-level mixed-effects model predictions.

Model

- Log-transformed hormone concentrations
- Piecewise linear mixed-effects models with knot at 30 minutes post-waking
- **Cortisol**
  - Waking $\rightarrow$ 30 min: $+25.9\%$ (95% CI: -2.3, 62.2)
  - After 30 min: -12.3% per hour ($p < 0.001$)
- **DHEA**
  - Waking $\rightarrow$ 30 min: -46.2% ($p < 0.001$)
  - After 30 min: -9.0% per hour ($p < 0.001$)

The SPIT method captured biologically plausible diurnal patterns consistent with known hormone dynamics.

Discussion

Key conclusions

- Good agreement between booklet and MEMS sampling times with minimal systematic bias
- Moderate protocol adherence, particularly strong for early post-waking (+30 min) collections
- SPIT method captures expected diurnal patterns of cortisol and DHEA
- Results support feasibility of the SPIT booklet for future studies

Limitations

- Modest sample size and healthy-only population
- Missing timing and hormone data
- Reliance on self-reported wake time