



Stressed from Head to Toe: Opportunities and Challenges of Steroid and Endocannabinoid Analysis in Keratinized Matrices

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Steroid hormones and endocannabinoids (eCBs) are key regulators of the human neuroendocrine stress response, which can be activated by psychological, physiological, or biochemical stressors. Keratinized matrices such as hair and nails enable long-term monitoring of stress biomarkers as it allows retrospective monitoring over extended time periods. This work highlights both the opportunities and challenges of steroid and eCB analysis in keratinized matrices.

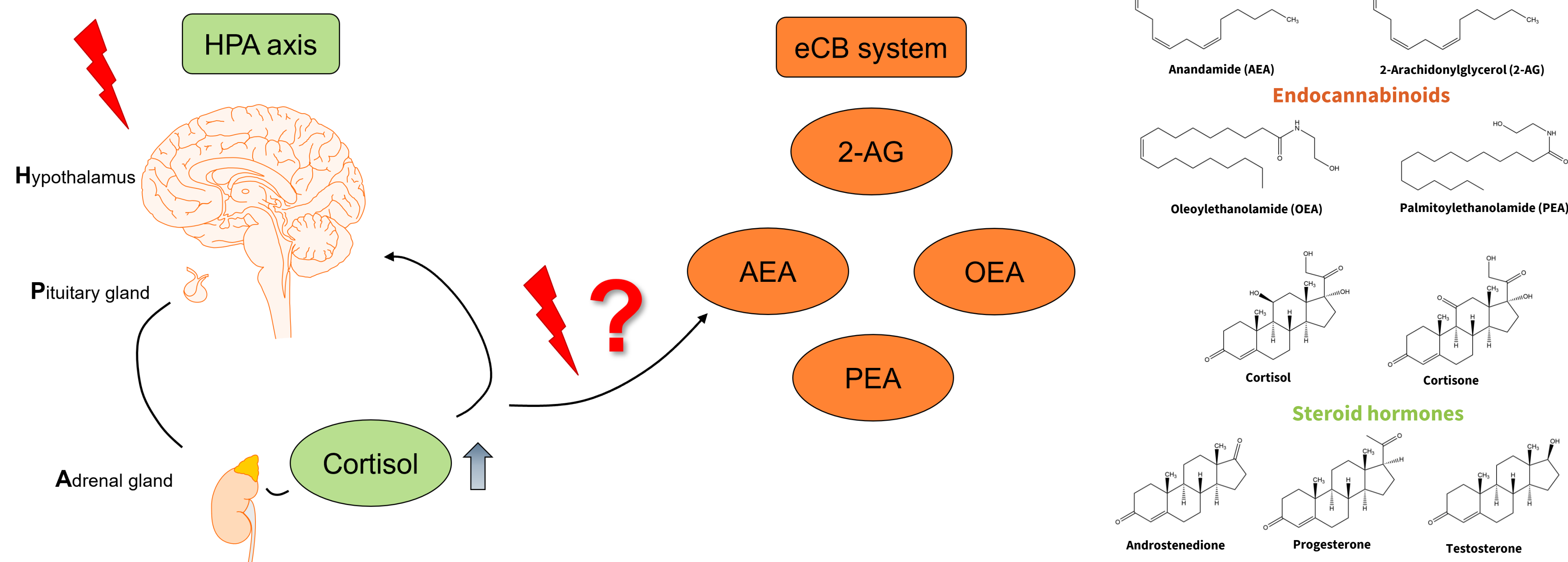
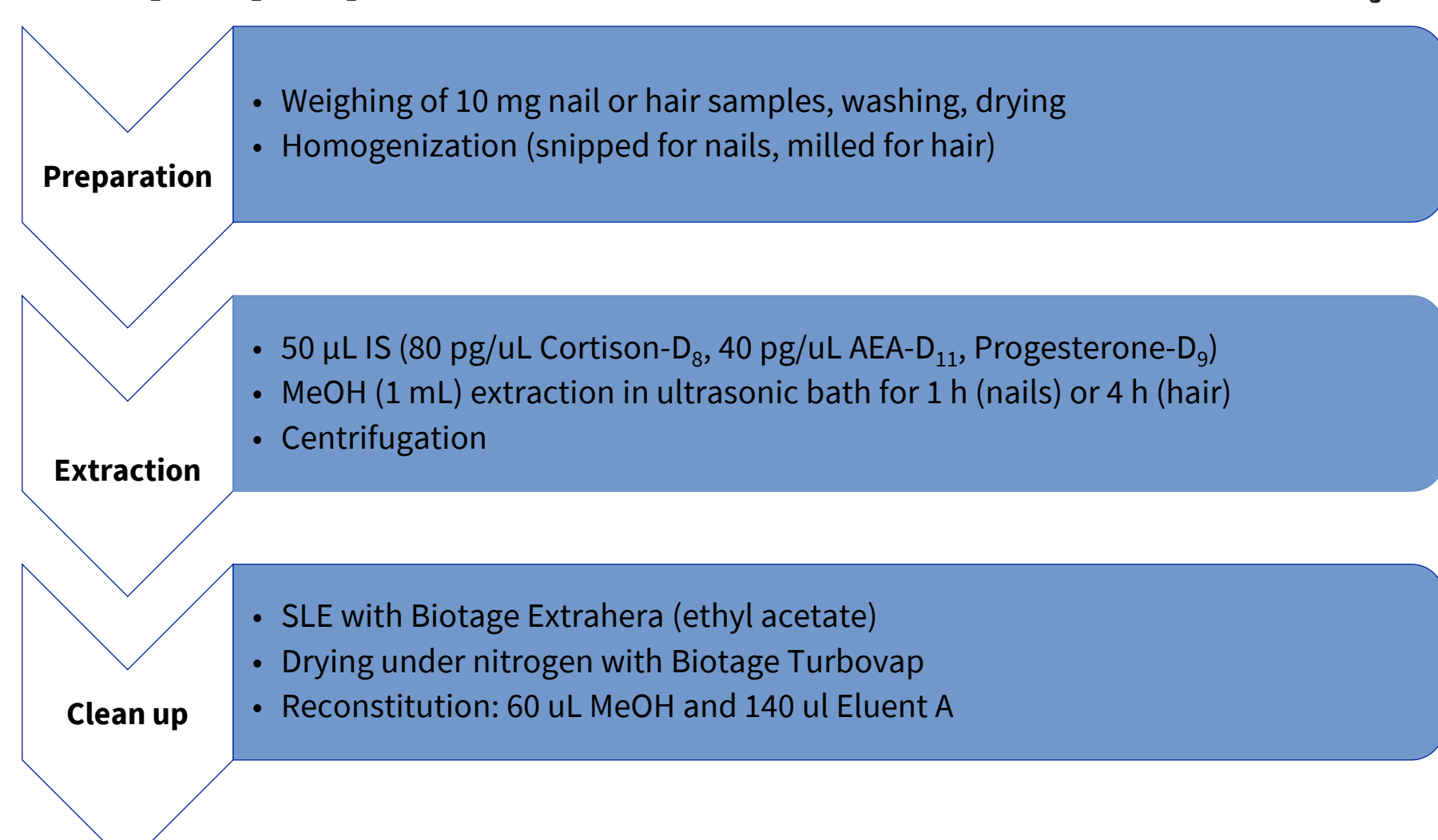


Fig. 1: The hormonal response to stress is regulated by the hypothalamic-pituitary-adrenocortical (HPA) axis. First, corticotropin-releasing hormone is secreted, which induces the release of adreno-corticotrophic hormone (ACTH). In the adrenal cortex, glucocorticoid synthesis and secretion takes place. This can be measured by increased cortisol levels in serum. HPA axis homeostasis is maintained by the endocannabinoid system. Most explanatory models are suggesting that glucocorticoid secretion alters endocannabinoid signaling, which leads to a negative feedback mechanism.

Analytical challenges



Sample preparation workflow:



- Column: Phenomenex® Kinetex® XB-C₁₈ (2.6 µm, 50 x 2.10 mm)
- Eluent A: 0.2 mM NH₄F in water/MeOH 97/3 v/v
- Eluent B: 0.2 mM NH₄F in water/MeOH 3/97 v/v
- MS: QTRAP® 7500 linear ion trap quadrupole mass spectrometer (Sciex)
- ESI positive, MRM scheduled
- The following parameters were validated using surrogate analyte method (D_x-labeled for eCBs and ¹³C₃-labeled for steroids): selectivity, response factor, limit of detection (LOD), limit of quantification (LOQ), linearity, matrix effect, recovery, accuracy, precision and robustness

Surrogate analyte approach

- The following surrogate analytes were used: (D₄-AEA (Fig. 2), D₅-2-AG, D₄-OEA, D₄-PEA, ¹³C₃-cortisol, ¹³C₃-cortisone, ¹³C₃-androstenedione, ¹³C₃-progesterone, and ¹³C₃-testosterone). The response factor was determined for every surrogate and authentic analyte.

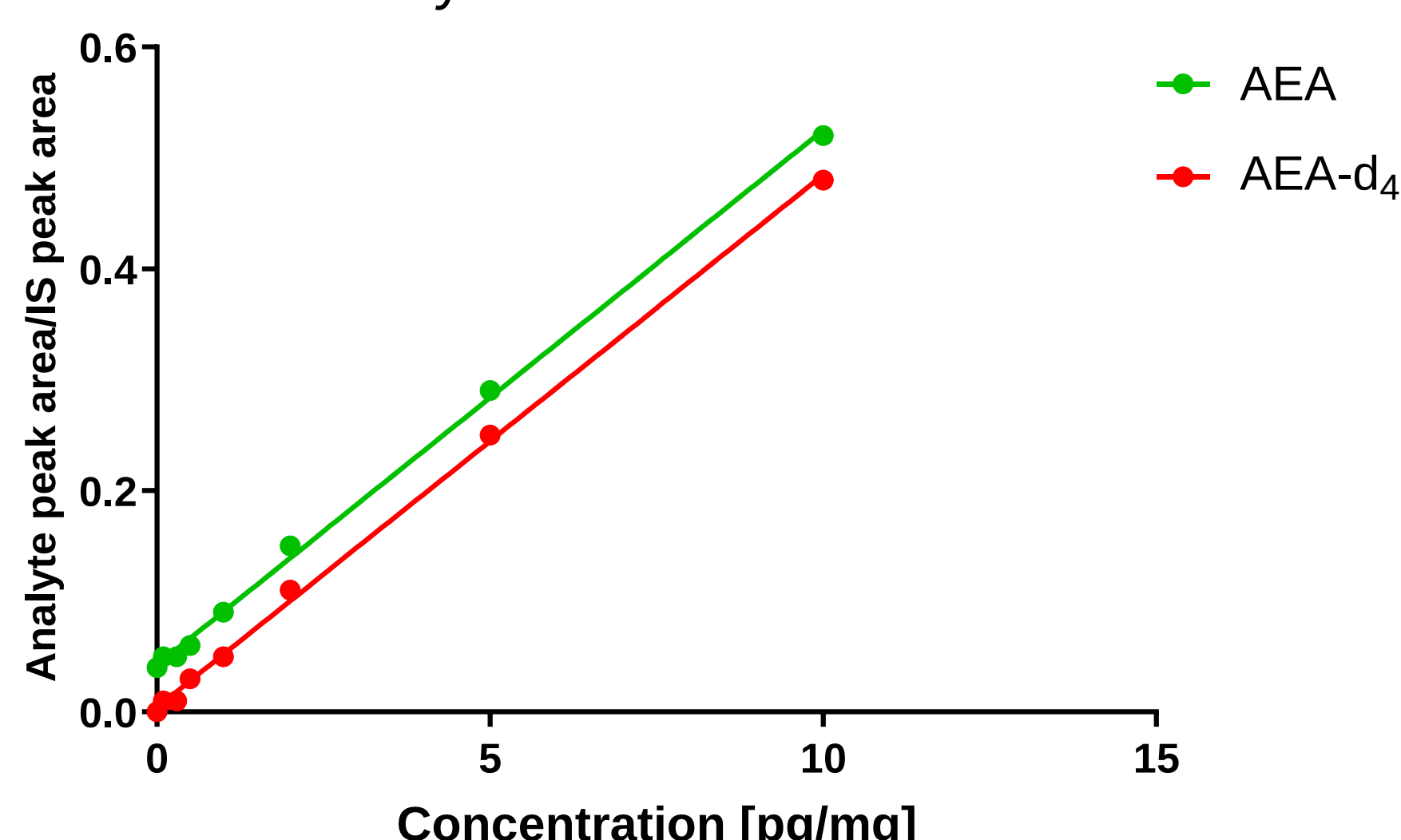


Fig. 2: Calibration curve for AEA and AEA-D₄ in nails

Study I: Monitoring of steroids and eCBs in nails of pregnant women

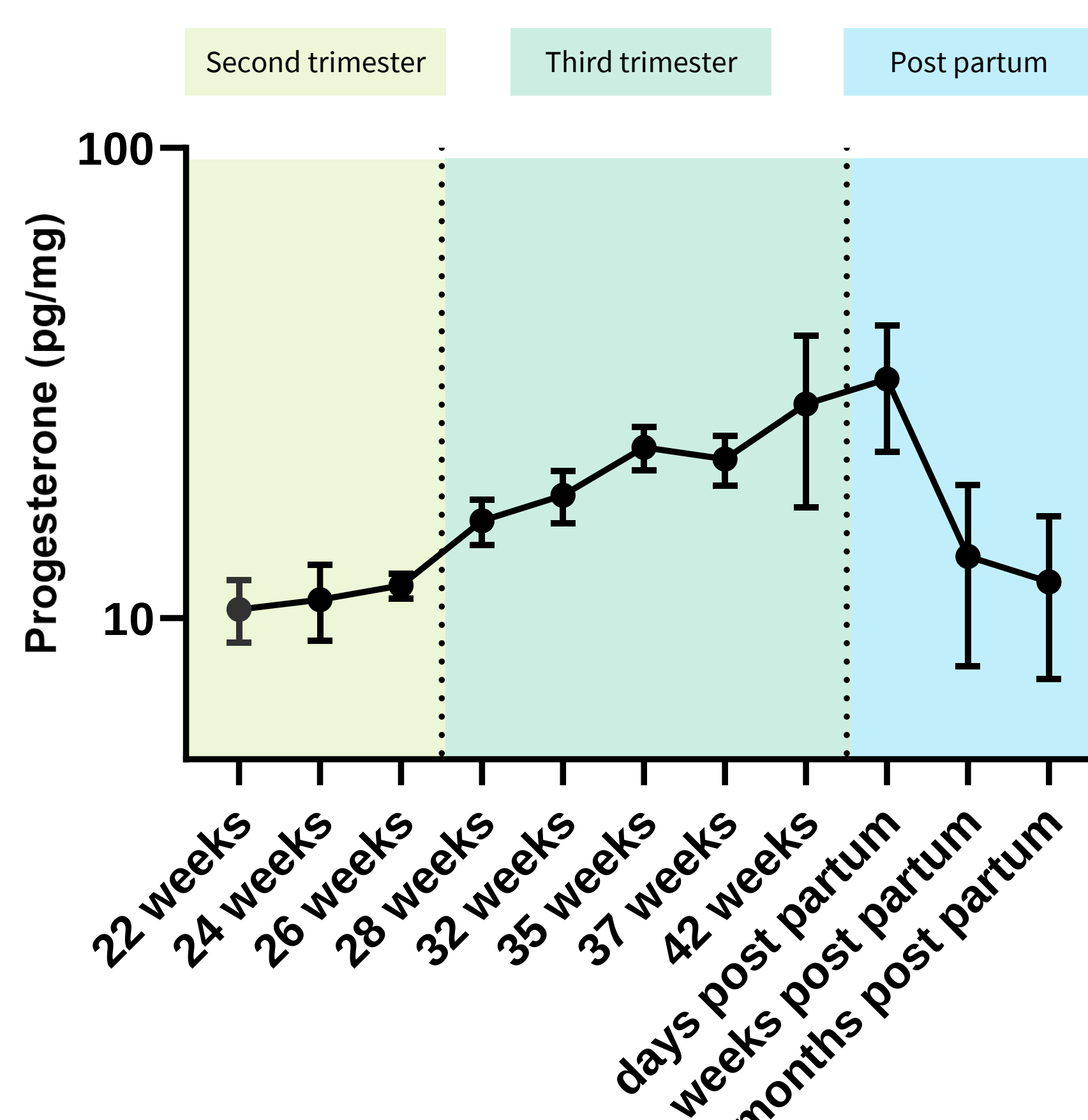


Fig. 3: Time course of progesterone concentrations in maternal (n= 5) fingernail samples during pregnancy and postpartum. Dots represent the arithmetic mean and bars represent the standard deviation. The concentrations are shown in logarithmic transformation.

- Pilot study: **five mothers** collected **fingernails** during and after **pregnancy**.
- **Maternal progesterone** and **cortisone** was significantly **higher** in the **third trimester** compared to the second (Wilcoxon test, p<0.05) (Fig. 3).
- **AEA** and **OEA** levels were significantly **lower** in the **third trimester** compared to the second (Wilcoxon test, p<0.05).
- This non-invasive method could serve as a valuable tool for **long-term hormonal monitoring** with promising implications for maternal-fetal health research.

Conclusion

Challenges such as low endogenous concentration levels and the difficulty of quantifying these compounds in solid matrices were overcome by the use of surrogate analytes, enabling the simultaneous quantification of steroids and eCBs in human hair and nail samples. Overall, the findings demonstrate that the analysis of steroid and eCB concentrations in hair and nail samples represents a highly promising and reliable approach for the retrospective assessment of biomarkers associated with chronic stress.

Study II: Alterations of glucocorticoids and eCBs in hair of chronic cocaine users

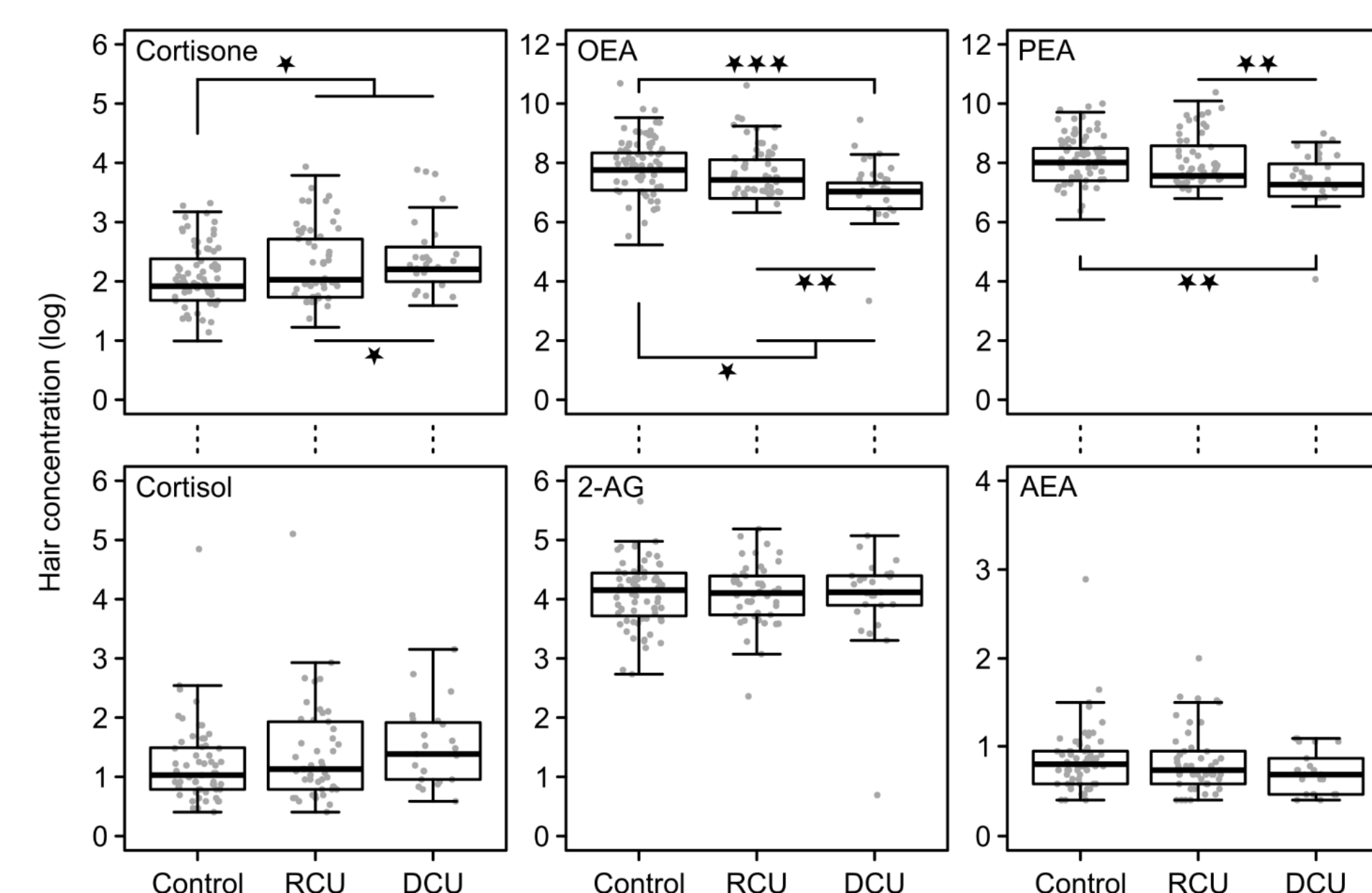


Fig. 4: General linear regression models showed significant higher concentrations of hair cortisone in chronic cocaine users compared to controls, which was mainly driven by the dependent cocaine user (DCU) group. Hair concentration of OEA and PEA was the lowest in the DCU group compared to controls and recreational user group (RCU).

- **140 hair samples** (3 cm proximal hair strands): 67 cocaine-naïve control participants, 48 recreational cocaine users (RCU), 25 dependent cocaine users (DCU)
- Within **cocaine users**, **elevated cocaine hair concentration** was as a significant predictor for **increased glucocorticoids** and **decreased OEA** (Fig. 4). **Increased years of cocaine use** in self-reports significantly predicted **lower PEA** levels.
- Our results corroborate previous findings of an **elevated chronic physiological stress** burden and establish novel findings of a **reduced eCB signaling system in cocaine users**.
- This suggests a **link** between **cocaine addiction**, the **HPA-axis**, and the **eCB system**.

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