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New insights into hair cortisol measurement for long-term stress monitoring in chimpanzees (*Pan troglodytes*)

Hair cortisol concentrations (HCC) are increasingly recognized as useful objective measures of long-term stress in mammals, but there is still a large gap in knowledge about the practical significance and the underlying mechanisms of confounding factors. Three problems stand out: the effect of body region, waning effects, and the reliability of HCC measures for inter-individual comparisons of stress levels. Significant differences between certain body regions (chest > shoulder blade > back = forearm) were found using hair from semi-wild and zoo living chimpanzees. However, strong correlations between regions ($r \ge 0.6$, p < 0.001) and results from a factor analysis suggest that the HCC values of all body regions mainly reflect one process and provide a similar biological signal. Comparisons with thermal images further suggest that intra-individual differences in skin blood flow may be the underlying mechanism of the body region effect. Concerning the waning effect, HCC along the hair shaft showed a strong, systematic decline towards distal segments in semi-wild living chimpanzees but was numerically negligible in zoo-chimpanzees. Correlations between HCC and the amount of rain, that hair was exposed to, suggest that other factors than rain are more likely to cause the strong waning effect in semiwild living chimpanzees. Finally, we found that HCC levels were biologically meaningful at individual levels: our results revealed a strong correlation with large effect size between HCC and animal keeper-ranked stress levels for 38 chimpanzees $(pr_s=0.6, p<0.001)$. In conclusion, HCC levels in chimpanzees are biologically meaningful if body region and waning effect are considered.

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