Measuring Handling Stress at Multiple Time Scales in the Chronically Lead-exposed California Condor

Z Kuspa[1], C Tubbs[2], D R Smith[1], and M Finkelstein[1]

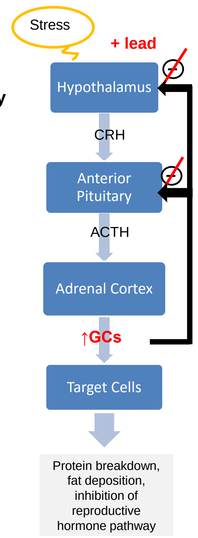
[1]Department of Microbiology and Environmental Toxicology, University of California, Santa Cruz, CA

[2] Institute for Conservation Research, San Diego Zoo Global

Introduction

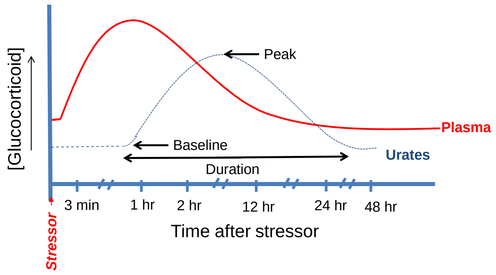
Why study stress in California condors?

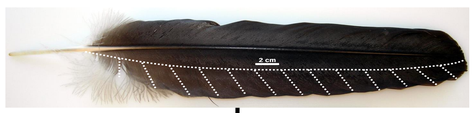
- **Altered stress responses can reduce avian reproduction and survival.**
- **California condors are chronically lead exposed** from ingesting lead-based ammunition in their food sources. 60 – 70% of wild condors experience subclinical lead exposure while 20 - 30% require clinical treatment per year.
- **Lead exposure elevates levels of glucocorticoid stress hormones (GCs) released in response to a stressor.**
- The **stress induced by frequent trapping and handling** of California condors as well as the clinical management for lead poisoning is unknown.



Why are multiple sample types necessary to assess condor stress response?

- **Measuring baseline (pre-stress) levels of GCs in plasma is not feasible in wild species.**
- Measuring GC metabolites in urates and feather allows for non-invasive measurement of baseline GC concentrations in a wild condors, as well as quantifying stress at multiple time scales:





Condor primary feathers: Growth rate ~0.5 cm/day. [GC] of 2cm feather sections represent 4-5 day integration of plasma hormone levels.

Why is careful validation of existing hormone measurement methods necessary for condor samples?

- **Most immunoassay kits are optimized for use with plasma only,** although they advertise kit compatibility with other biological fluids.
- **The hormonal stress response varies between species** in magnitude, duration, and dominant stress hormones. Hormone metabolism and sample matrix is also species-specific.
- Thus, **we must first validate a hormone measurement method** for condors before we can assess the interactions of lead poisoning and clinical management (e.g., capture and handling) on the stress response.

Study Objectives

1. **Evaluate precision and accuracy of immunoassay methods for measurement of corticosterone and corticosterone metabolite concentrations in California condor plasma, urates, and feathers**
2. **Biologically validate method for detecting changes in corticosterone release in a live condor**
3. **Validate experimental stressor for comparing stress responses between individual condors**

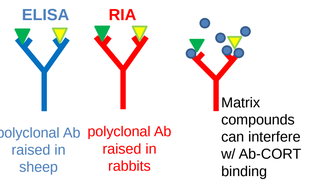
Methods

Hormone Measurement

Primary GC in birds: **Corticosterone***

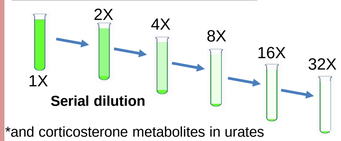
Kits Tested: Two immunoassays optimized to measure corticosterone in plasma

1) **Enzyme-linked Immunoassay (ELISA) (Enzo Life Sciences)**
2) **Radioimmunoassay (RIA) (MP Biomedicals)**



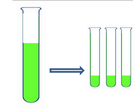
Method Validation

1. Matrix/dilution effects:



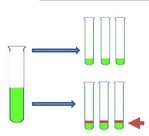
*and corticosterone metabolites in urates

2. Precision:



Pooled plasma or sample extract run in triplicate

3. Accuracy:



Spike Recovery
Known quantity of corticosterone

Sample Collection for Biological Validation of Method

Samples are collected opportunistically during a health check:

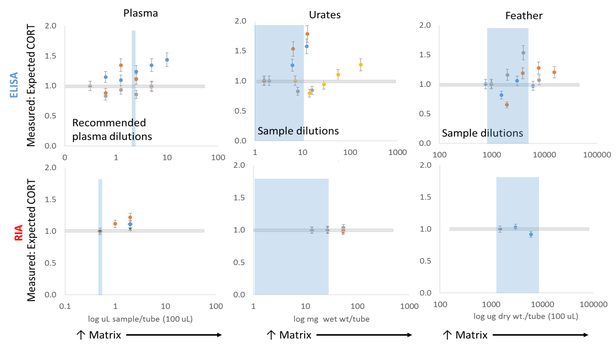
1) Trap condor by hoop net
2) Restrain condor (15-30 minutes)
3) Kennel (1-6 hours) for urate sampling. Collect urate at ~15 min. intervals. Urate corticosterone metabolite concentrations stable 30 minutes before freezing.

Conclusions

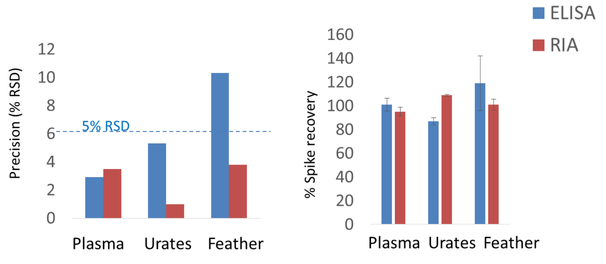
- **RIA better suited for measurement and comparison of corticosterone and corticosterone metabolites across all sample types in study: plasma, urates, and feather**
- **Both RIA and ELISA return accurate and precise corticosterone measurements for condor plasma**
- **My results highlight the need to validate immunoassays for novel sample types**
- **Capture and handling elicits an increase in corticosterone release, measureable in urates and feathers**
- **Corticosterone responses to capture and handling stressor varies widely among individual condors (2-11 fold over baseline)**
- **Next Steps: Determine lead effect on stress response in condors with different lead exposure histories**

Results

1. Corticosterone measurements by RIA more consistent across sample type and sample dilution range than ELISA

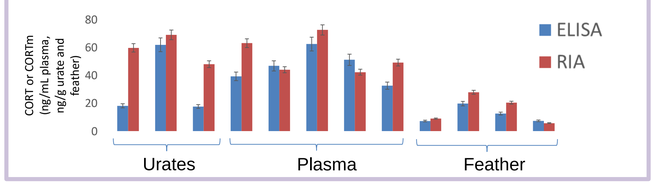


2. Both methods perform well for condor plasma, but RIA more reliable than ELISA for urate and feather

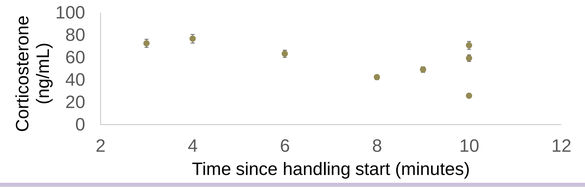


3. Different methods will yield different corticosterone* levels in the same samples

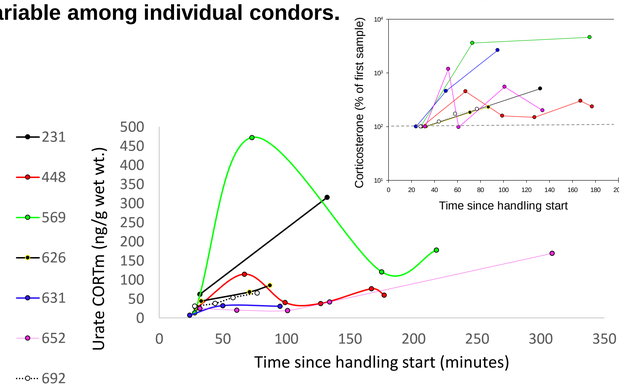
*and corticosterone metabolites (CORTm) in urates



4. Plasma corticosterone values vary among individual condors tested but does not appear related to time after handling start



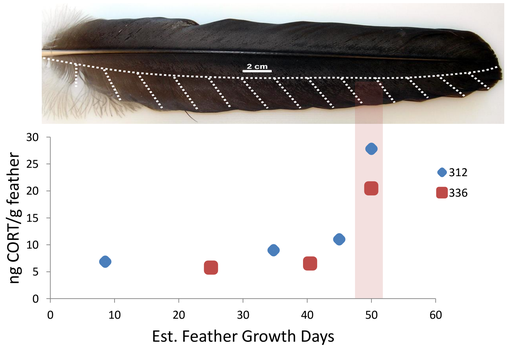
5. Urates from 7 condors show significant increase in corticosterone metabolites after capture and handling stresso (p=0.0026). Additionally corticosterone stress responses variable among individual condors.



Magnitude of measured corticosterone metabolites excreted in response to stressor across avian species

Species	Stressor	Tissue Sample	Average corticosterone* peak (% baseline)	Method	Authors	Year
Golden Eagle (Aquila chrysaetos)	Saline Injection	feces	111%	RIA	Staley et al.	2007
Greater Rhea (Rhea americana)	Saline Injection	feces	464%	RIA	Lèche et al.	2011
Budgerigar (Melopsittacus undulatus)	Saline Injection	feces	400%	RIA	Young and Hallford	2013
California condor (Gymnogyps californianus)	Capture and restraint	urates	653%	RIA	Kuspa et al.	unpub.

6. Feather grown during capture and handling stressor has higher corticosterone concentration than feather grown before stressor



Feathers from condors 312 and 336. Red arrows indicate size and location of sections on primary feather. Red shading indicates estimated time of handling event. Each section represents 4-5 days of feather growth.

Acknowledgements

We would like to acknowledge the Los Angeles Zoo condor keepers and the field staff at Pinnacles National Park and Ventana Wildlife Society for their help with sample collection. Without them this study would not be possible. We would also like to thank Dr. Christopher Tubbs and Alan Fetter from the San Diego Zoo for providing lab space, RIA expertise, and mentorship. Thanks also to the UCSC METX department and members of the Smith lab who provided their help and technical support.