Individual variation in natural or manipulated corticosterone is not related to variation in circulating glucose in a wild bird.

Conor C. Taff1,2\*, Cedric Zimmer1, Thomas A. Ryan1, David Chang van Oordt1, Maren Vitousek1,2

1 Department of Ecology & Evolutionary Biology, Cornell University

2 Lab of Ornithology, Cornell University

\* Correspondence: Conor C. Taff, [cct63@cornell.edu](mailto:cct63@cornell.edu) 518-332-3983

**SUPPLEMENTARY METHODS**

To ensure that Cortrosyn® had the desired effect in elevating the corticosterone response, we conducted two validation studies on a separate set of nestlings and adults that were not part of the main study presented here. Once reconstituted, Cortrosyn is not stable at room temperature. Therefore, rather than delivering exact doses based on individual mass, we reconstituted vials of lyophilized Cortrosyn (Amphastar Pharmaceutical Incorporated, Item #054881) and prepared aliquots pre-measured into syringes based on the average mass in our population (on day 15 nestlings weigh approximately the same amount as full grown adults and the same dose was used for both adults and nestlings). Aliquoted Cortrosyn doses were stored frozen for < 2 weeks and thawed immediately before injection.

In 2018, we carried out a validation experiment on 15-day old nestlings from 9 nests. At each nest, individual nestlings were alternately assigned to a Cortrosyn injection group (n = 23 nestlings) or a control group that received a saline injection (n = 20 nestlings). For all nestlings, a baseline blood sample (< 30 μl) was collected within 3 minutes of disturbance and then nestlings were immediately injected with either 50 μl of saline or 50 μl of 0.1 microgram per μl freshly thawed Cortrosyn. Following injection, two additional blood samples (< 30 μl) were collected 15 and 30 minutes after injection.

In 2020, we carried out a separate validation experiment on adult females captured during incubation that were not part of the main study presented here. For each female, we collected a baseline blood sample (< 70 μl) within 3 minutes of disturbance. All females received a saline injection 50 μl immediately after this baseline sample was collected and then had a second blood sample (< 30 μl) taken 30 minutes later. Immediately after this second sample, females were injected with either an additional saline dose of the same volume (n = 9) or a dose of Cortrosyn (n = 9; 50 μl at 0.1 microgram per μl). Thirty minutes after this second injection, a final blood sample (< 30 μl) was collected. For both adults and nestlings in these validation experiments, blood samples were processed and corticosterone measured exactly as described in the main text.

To compare corticosterone between treatment groups, we fit a single model for each dataset (adults and nestlings). For nestlings, we fit a linear mixed model with corticosterone measurement as the response, an interaction between treatment and sampling time point (fit as a factor) as predictors, and individual identity as a random effect. Because multiple nestlings were sampled from the same nest, this model included a random effect for nest of origin. For adults, we fit a similar model, except that there was no need for a random effect for nest since only one female was sampled at each nest. We used the full models to compare circulating corticosterone in saline versus Cortrosyn injected birds at each of the three timepoints. Significance in these mixed models was assessed with p-values based on the Satterthwaite's Method implemented by the `lmerTest` package in R (Kuznetsova et al., 2017).

**SUPPLEMENTARY RESULTS**

Injection with Cortrosyn led to a clear increase in circulating corticosterone in both nestlings (Figure S1) and adults (Figure S2). For nestlings, corticosterone increased in both groups from baseline to 15 minutes but increased significantly more in the Cortrosyn injected group when compared to saline injection. From 15 to 30 minutes, saline injected nestlings declined in circulating corticosterone, but Cotrosyn injected nestlings continued to rise, resulting in an even larger difference in circulating corticosterone between the two groups at 30 minutes (Table S2, P < 0.003).

Adult females similarly increased circulating corticosterone from baseline to 30 minutes. The two treatment groups did not differ at the first or second timepoint (before Cortrosyn was injected). However, by the final timepoint (30 minutes after injection), the Cortrosyn injected group had significantly higher circulating corticosterone. This difference was driven by a continued increase in corticosterone from 30 to 60 minutes in the Cortrosyn group coupled with a stable circulating level from 30 to 60 minutes in the saline injected group (Table S2, P < 0.001).

**SUPPLEMENTARY TABLES & FIGURES**

*Chart

Description automatically generated*

**Figure S1.** Circulating corticosterone before and after Cortrosyn or saline injection in adult females.

*Chart

Description automatically generated*

**Figure S2.** Circulating corticosterone before and after Cortrosyn or saline injection in 15 day old nestling tree swallows.

**Table S1.** Model details for the effect of Cortrosyn injection on nestling and adult circulating corticosterone level. Timepoints 2 and 3 are at 15- and 30-minutes post injection for nestlings and at 30 and 60 minutes for adults.

**Table

Description automatically generated**

**Table S2.** Total sample sizes for glucose measurements by year, location, and sample type.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | Adults | | | Nestlings | | |
| State | Year | Base | Induced | Post-Cortrosyn | Base | Induced | Post-Cortrosyn |
| NY | 2016 | 244 | 178 | - | - | - | - |
| NY | 2017 | 140 | 138 | - | - | - | - |
| NY | 2018 | 188 | 123 | - | - | - | - |
| NY | 2019 | 204 | 147 | 45 | 185 | 177 | 159 |
| AK | 2016 | 117 | 84 | - | - | - | - |
| AK | 2017 | 134 | 100 | - | - | - | - |
| TN | 2018 | 228 | 167 | - | - | - | - |
| WY | 2018 | 236 | 160 | - | - | - | - |

**Table S3.** Results of linear mixed models with glucose or corticosterone as the response and sample type as a categorical predictor.

**Table

Description automatically generated**

**Table S4.** Results of linear mixed models on New York adults with glucose as the response variable and corticosterone, mass, a corticosterone by mass interaction, and sex as predictors. In each case, the corticosterone measure included spans the same interval as the glucose response variable. Unsupported interactions were dropped. Corticosterone and mass are scaled to a mean of zero and standard deviation of one to make effect sizes easier to interpret.

Table

Description automatically generated

**Table S5.** Results of linear mixed models on New York nestlings with glucose as the response variable and corticosterone, mass, a corticosterone by mass interaction, and sex as predictors. In each case, the corticosterone measure included spans the same interval as the glucose response variable. Unsupported interactions were dropped. Corticosterone and mass are scaled to a mean of zero and standard deviation of one to make effect sizes easier to interpret.

**Table

Description automatically generated**

**Table S6.** Results of linear mixed models with baseline glucose as the response and corticosterone, mass, and their interaction as predictors in each of the four studied populations. Only adult females are included. Predictors are scaled to a mean of zero and standard deviation of one.

**Table

Description automatically generated**

**Table S7.** Results of linear mixed models with induced - baseline glucose as the response and induced – baseline corticosterone, mass, and their interaction as predictors in each of the four studied populations. Only adult females are included. Predictors are scaled to a mean of zero and standard deviation of one.

Table

Description automatically generated

**SUPPLEMENTARY REFERENCES**

Kuznetsova, A., Brockhoff, P. B. and Christensen, R. H. B. (2017). “lmerTest” Package: Tests in Linear Mixed Effects Models. *J. Stat. Soft.* 82,.