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DO HEALTHY COQUEREL'S SIFAKAS IN CAPTIVITY HAVE UNUSUALLY LOW CIRCULATING CORTISOL?

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Abstract: Cortisol, and other glucocorticoids, are routinely used as markers of physiological stress in wildlife. Typically, stress activates the hypothalamic–pituitary–adrenal (HPA) axis, with adrenocorticotropic hormone (ACTH) signaling the adrenal glands to release cortisol. Nevertheless, recent anecdotes in captive Coquerel's sifakas (*Propithecus coquereli*), strepsirrhine primates that are difficult to maintain under human care, may challenge the assumption that physiological stress universally increases circulating cortisol. Here, the authors ask if low circulating cortisol and minimal response to adrenal stimulation might be hallmarks of outwardly healthy sifakas in captivity. Comparative ACTH stimulation or control tests were performed in 10 Coquerel's sifakas and six ring-tailed lemurs (*Lemur catta*) at the Duke Lemur Center (DLC). At baseline, sifakas had average cortisol concentrations of just 0.67 μg/dl, whereas those of ring-tailed lemurs averaged 12.53 μg/dl. Stressful pre-experiment procedures, including kenneling and handling, activated the HPA axis in ring-tailed lemurs, masking further cortisol release from ACTH administration; however, neither these procedures nor exogenous ACTH raised cortisol concentrations in sifakas. Additionally, cortisol in dozens of serum samples from DLC sifakas banked over 17 yr was assayed. Across samples, cortisol concentrations averaged just 0.49 μg/dl and did not vary by animal sex, age, or housing condition. Comparable samples from two individual sifakas in sepsis at the end of life (4.28 and 21.88 μg/dl) indicate that the assay does capture meaningful variation in cortisol in captive sifakas, although robust biological validation is needed. Currently there is a lack of comparative data from wild Coquerel's sifakas that might determine if these unusual endocrine patterns are characteristic of the species or a function of captivity. If the latter, chronic stress in captivity could lead to a downregulated HPA axis, with persistent hypocortisolism perhaps contributing to the Coquerel's sifaka's susceptibility to infection under human management.

INTRODUCTION

Chronic stress is a major health concern for wildlife in anthropogenic landscapes, including captivity. Many studies, including of diverse primates, measure glucocorticoids or their metabolites as proxies for monitoring physiological stress,[1,3,4,26,39,46] with the expectation that greater stress equates to greater glucocorticoid concentrations. This expectation comes from an understanding of the neuroendocrine response to stress coordinated along the hypothalamic–pituitary–adrenal (HPA) axis. The HPA axis is a complex system of feedback loops and hormone cascades that mediates physiological homeostasis.[49] Under typical conditions, stress stimulates the hypothalamus to produce and release corticotropin-releasing hormone (CRH), which stimulates the anterior pituitary to release adrenocorticotropic hormone (ACTH) into the bloodstream, which stimulates the adrenal cortex to release glucocorticoids, such as cortisol, into the bloodstream. Circulating cortisol organizes key aspects of the metabolic stress response.[55] The CRH–ACTH–cortisol hormone cascade works via a negative feedback loop, with elevated concentrations of cortisol ultimately dampening continued HPA axis activity.[49]

The expectation that stress universally raises cortisol cannot necessarily capture more nuanced dynamics along the HPA axis, and might not be equally applicable to all wildlife species.[2,30,42] The present team recently became interested in HPA-axis dynamics, and especially cortisol, in captive Coquerel's sifakas (*Propithecus coquereli*). The Coquerel's sifaka is a critically endangered strepsirrhine primate endemic to northwestern Madagascar.[29] The species is one of the few folivorous lemurs to survive and breed under human management.[20] Currently, all Coquerel's sifakas outside of Madagascar are owned and managed by

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the Duke Lemur Center (DLC) in Durham, NC, although some members of the population are housed at other Association of Zoos and Aquariums (AZA) and European Association of Zoos and Aquaria (EAZA) accredited institutions.

Interest in cortisol in captive Coquerel's sifakas was sparked by two recent anecdotes. First, a 17-yr-old female at the Maryland Zoo presenting with nondescript symptoms was diagnosed with Addison's disease,[32] based partially on low circulating cortisol (0.36 μg/dl). Second, a 5-yr-old female at the DLC with similar nondescript symptoms, along with two healthy, age-matched females, were administered an ACTH stimulation test to rule out Addison's disease. All three sifakas had surprisingly low cortisol at baseline (mean = 1.27 μg/dl) and all showed little increase in circulating cortisol after exogenous ACTH administration (mean = 1.74 μg/dl). The sick individual was later successfully treated for a *Bartonella* infection (DLC in-house records).

Here, the authors expand on these anecdotes to ask if low baseline cortisol, and a blunted response to adrenal stimulation, are perhaps hallmarks of seemingly healthy sifakas in captivity. Circulating cortisol was measured in conjunction with ACTH stimulation tests in 10 clinically healthy Coquerel's sifakas and six clinically healthy ring-tailed lemurs (*Lemur catta*) housed under identical conditions at the DLC. The inclusion of ring-tailed lemurs allowed the authors to ask if another lemur species shows similar patterns of HPA axis activity under human management. Compared to ring-tailed lemurs, it was expected that sifakas would show reduced circulating cortisol and a dampened response in cortisol release in response to exogenous ACTH administration.

Second, banked blood samples from dozens of outwardly healthy sifakas at the DLC across 17 yr were analyzed. Consistent with anecdotal evidence, it was expected that all sifakas would show consistently low circulating cortisol regardless of sex, age, or housing condition. A small set of banked serum samples from individuals in renal failure and sepsis at or near the end of life was used to investigate whether sick individuals show different cortisol patterns, and to ensure that the present endocrine assay appropriately captures meaningful biological variation in circulating cortisol in this population.

MATERIALS AND METHODS

ACTH stimulation, sampling, and statistics

ACTH or control stimulation tests were performed on 10 Coquerel's sifakas (three female [F], seven male [M]) between 2 and 14 yr of age that resided in five social groups, and six ring-tailed lemurs (3 F, 3 M) between 8 and 20 yr of age that resided in four social groups. All the animals were at a healthy weight at the time of study and were clinically normal, as assessed by serum chemistry and CBC panels, and DLC veterinary exam. At the time of study, all lemurs were socially housed with conspecifics in the DLC's standard indoor/outdoor housing units. DLC sifakas and ring-tailed lemurs are fed species-specific diets. Sifakas receive a mix of fresh vegetables and leafy greens, nuts, beans, local browse, and a high-fiber manufactured primate biscuit (Mazuri 5672 Leaf Eater Mini Biscuit); ring-tailed lemurs receive a mix of fresh fruits and vegetables and a manufactured primate biscuit with moderate fiber levels (Mazuri 5MA2 Primate Maintenance Biscuit). Water is always freely available. Structural and/or sensory enrichment is provided daily.

ACTH and control stimulation tests were performed on two subjects/day from 8 February to 8 March 2022. In the morning (~8:30 am), DLC staff placed lemurs into individual transport kennels and hand-carried them to the veterinary clinic. Clinical procedures began around 9:00 am. Lemurs were manually restrained, administered Telazol (5–15 mg/kg, IM), and placed in transport kennels until fully sedated. Once the animals were sedated, a baseline blood sample was collected from the external saphenous vein and the ACTH challenge (Cosyntropin: 125 μg in 0.5 ml, IV) was administered to seven sifakas and four ring-tailed lemurs, or the control challenge was administered (sterile isotonic fluids: 0.5 ml, IV) to the remaining three sifakas and two ring-tailed lemurs. Additional blood samples were collected from the saphenous vein at 30, 60, and 90 min postchallenge. Animals were returned to their home enclosures either the same afternoon or the following morning, depending on how swiftly they recovered from anesthesia. This study was approved by the DLC's Research Committee (BSM-12-21-2) and Duke University's Institutional Animal Care and Use Committee (Protocol A235-21-12).

Baseline blood samples were aliquoted into serum separator tubes and into EDTA-lined tubes; samples taken at additional time points were placed solely into serum separator tubes. All blood samples were processed within 5 min of collection. From the baseline blood draw, aliquots of refrigerated serum and whole blood were shipped on the day of sampling to IDEXX

Table 1. Results of serum chemistries and CBCs on sifakas and ring-tailed lemurs in the study.

Analyte (units)	Coquerel's sifakas		Ring-tailed lemurs		Wilcoxon tests	
	Mean ± SD	Min–Max	Mean ± SD	Min–Max	*P*	Relationship
Sodium (mmol/L)	145.5 ± 1.7	142–147	145.2 ± 2.1	143–149	0.47	NA
Potassium (mmol/L)	3.1 ± 0.5	2.3–4.1	4.3 ± 0.4	3.7–4.8	0.002	Sifakas < ring tails
Na:K ratio	47.6 ± 7.5	35–63	34.3 ± 3.1	30–39	0.004	Sifakas > ring tails
Chloride (mmol/L)	104 ± 4.0	94–108	105 ± 3.2	99–108	0.51	NA
Total CO_2 (mmol/L)	26.3 ± 5.1	22–39	21.7 ± 4.2	16–26	0.17	NA
Anion gap (mmol/L)	18.2 ± 3.0	14–24	22.8 ± 5.8	16–29	0.14	NA
Calcium (mg/dl)	10.3 ± 1.0	8.3–11.7	9.8 ± 0.5	9.3–10.4	0.17	NA
Phosphorus (mg/dl)	4.0 ± 1.4	1.5–6.3	3.7 ± 1.1	2.4–5.3	0.70	NA
Glucose (mg/dl)	128.5 ± 19.6	102–165	156.2 ± 29.8	126–200	0.04	Sifakas < ring tails
Blood urea nitrogen (mg/dl)	23.8 ± 6.4	15–39	27.5 ± 15.1	19–58	0.78	NA
Creatinine (mg/dl)	0.7 ± 0.1	0.6–0.8	1.0 ± 0.1	0.8–1.2	0.003	Sifakas < ring tails
Alanine transaminase (U/L)	50.8 ± 13.7	35–71	115.5 ± 42.3	65–189	0.001	Sifakas < ring tails
Aspartate aminotransferase (U/L)	18.2 ± 5.7	11–29	26 ± 14.9	16–55	0.30	NA
Alkaline phosphatase (U/L)	174 ± 82.8	91–299	278 ± 101.4	118–394	0.39	NA
Gamma-glutamyl transferase (U/L)	10.6 ± 3.0	6–15	37.2 ± 10.6	28–57	0.001	Sifakas < ring tails
Amylase (U/L)	147 ± 48	72–229	2,796 ± 524	2,110–3,480	<0.001	Sifakas < ring tails
Lipase (U/L)	10.3 ± 3.1	5–15	24.3 ± 20.8	10–66	0.02	Sifakas < ring tails
Creatine kinase (U/L)	376 ± 337	130–1,219	1,267 ± 829	683–2,838	0.005	Sifakas < ring tails
Total bilirubin (mg/dl)	0.2 ± 0.06	0.1–0.3	0.5 ± 0.2	0.3–0.7	0.001	Sifakas < ring tails
Total protein (g/dl)	7.1 ± 0.5	6.3–7.9	6.9 ± 0.7	5.9–7.7	0.55	NA
Albumin (g/dl)	4.5 ± 0.2	4.2–4.9	5.3 ± 0.7	4.2–5.9	0.04	Sifakas < ring tails
Globulin (g/dl)	2.6 ± 0.4	2.0–3.0	1.6 ± 0.2	1.4–1.8	0.001	Sifakas > ring tails
Cholesterol (mg/dl)	141.6 ± 49.0	90–250	77.3 ± 12.5	62–94	<0.001	Sifakas > ring tails
RBCs (cells × 10^6/μl)	9.0 ± 4.2	8.4–9.6	8.2 ± 9.2	6.8–9.2	0.09	NA
WBCs (cells × 10^3/μl)	8.2 ± 1.6	6.0–10.8	6.3 ± 2.2	3.6–10.1	0.05	Sifakas > ring tails
Lymphocytes (%)	36.3 ± 10.9	22–53	41.3 ± 10.1	29–57	0.32	NA
Neutrophils (%)	58.2 ± 10.7	43–71	48.8 ± 8.7	40–61	0.12	NA
Monocytes (%)	4.2 ± 2.3	1–9	3.7 ± 2.3	0–6	0.82	NA
Eosinophils (%)	1.3 ± 2.1	0–6	6.2 ± 2.2	3–8	0.004	Sifakas < ring tails
Basophils (%)	0	0	0	0	NA	NA

Laboratories, Inc (Westbrook, ME, USA) for a standard serum chemistry panel and CBC. Remaining samples were spun to serum or plasma, frozen at −80°C until analysis, and shipped on ice packs to the Michigan State University Veterinary Diagnostic Laboratory (Lansing, MI, USA) for testing. Serum samples from the four time points (baseline and 30, 60, and 90 min postchallenge) were analyzed for circulating cortisol (see the following for assay details and validation). The plasma samples from the baseline draw were analyzed for endogenous ACTH (see supplementary material, including Supplemental Figs. 1 and 2).

For the serum chemistries and CBCs, species' averages and standard deviations for each analyte were calculated (Table 1). Significant differences between sifakas and ring-tailed lemurs were evaluated using nonparametric Wilcoxon signed-rank tests, implemented in RStudio (version 1.3.959)[37] using R software (version 4.0.2).[36] The clinicians on the team (LNE and CVW) also evaluated baseline values and determined that no subject should be excluded from the study.

Wilcoxon-signed rank tests in RStudio were used for statistical analyses of cortisol. First, concentrations at baseline between sifakas and ring-tailed lemurs were compared. Next, the investigators asked if baseline cortisol was significantly different between control and treated subjects within species. The area under the curve (AUC) per individual was then calculated in GraphPad Prism (version 10.1.1) to determine patterns of cortisol release across all sampling timepoints. AUC differences between control subjects and ACTH-challenged subjects were evaluated. Lastly, the maximum increase in cortisol between baseline and any other sampling time point for each individual lemur was determined. Simple summary statistics and Wilcoxon tests were used to determine if treated versus control subjects showed differences in their maximal hormonal response.

Adding additional banked samples

The authors next asked if captive sifakas show variation in circulating cortisol relative to age, sex, or housing condition by analyzing samples

from the DLC's curated repository. In total, 62 serum samples from outwardly healthy sifakas were identified for cortisol testing, including the 10 baseline samples from the ACTH stimulation tests conducted in 2022, and 52 samples that were similarly collected and banked from 2007 to 2024 that stemmed from routine physical exams, quarantine exit exams, or that were unused aliquots from prior research projects. All samples were collected, processed, stored, and submitted for cortisol testing using similar protocols. These samples stem from 43 individual sifakas (19 F, 24 M) that ranged in age from 1 to 27 yr of age at the time of sampling. Whereas 28 sifakas provided a single sample, 11 individuals provided two samples, and four individuals provided three samples. At the DLC, some sifaka groups gain access to large, multiacre forest enclosures during the warmer months, typically April through October, in which they roam and forage ad libitum.[19] Otherwise, groups are housed in standard, indoor/outdoor stalls. The data set included 19 samples from sifakas housed in forest enclosures at the time of sampling and 44 samples from sifakas housed in standard stall caging at the time of sampling.

Because most sifakas were only represented once in the data set, cortisol concentrations were compared using analysis of variance (ANOVA) implemented in RStudio. Cortisol concentrations were the dependent variable and sex (two categories: male or female), age (continuous, in years), and housing (two categories: forest enclosure or stall cages) the dependent variables. Log transformation did not improve model fit as assessed by Akaike information criterion (AIC) scores.

Cortisol assay validation

A commercially available solid-phase, competitive chemiluminescent enzyme immunoassay (Veterinary Cortisol, Immulite® 2000XPi, Siemens Healthcare Diagnostics Inc., Glyn Rhonwy Llanberis, Caernarfon LL55 4EL, United Kingdom) was used to detect circulating concentrations of cortisol in lemur serum, at the Michigan State University Veterinary Diagnostic Laboratory.[18] This assay was validated for both laboratory reliability and biological activity.

The assay's manufacturer reported the following cross-reactivities: 62% with prednisolone, 22% with methylprednisolone, 6.1% with prednisone, and <1.7% with other steroids tested. The manufacturer also reported an analytical sensitivity of 0.20 μg/dl. One investigator (SIJ) tested for assay repeatability (using 10 replicates) in pooled lemur serum with low, medium, and high cortisol concentrations of 0.20, 2.27 and 9.81 μg/dl. Intra-assay coefficients of variation (% CV) were found to be 13.9, 3.8, and 2.7%, respectively for the low, medium, and high pools. The interassay repeatability, as determined by analysis of 10 replicates of each pool run on 10 consecutive days, yielded % CVs of 20.6, 5.9, and 6.4%, respectively, for the low, medium, and high sample pools. Next, these pools were mixed in respective volume concentrations of 1:9, 1:3, 1:1, 3:1, and 9:1 to assess parallelism. For the low and high pool mixtures, observed/expected recovery rates of 91.4, 111.6, 103.7, 96.3, and 96.8% were found. The low and medium pool mixtures yielded observed/expected recovery rates of 99.9, 110.8, 102.4, 108.1, and 109.7%. It was thus confirmed that this cortisol assay performs as expected for lemur serum.

The cortisol assay for ring-tailed lemurs and sifakas was biologically validated separately, using banked samples. For ring-tailed lemurs, banked serum from 13 adults (7 F, 6 M) subjected to rapid sampling for a prior research project (i.e., the time between the initial stressor of capture and blood draw was less than 3 min) was submitted. These samples were compared to the baseline samples collected in conjunction with ACTH stimulation tests from six adults (3 F, 3 M) that underwent routine sampling (i.e., the time between the initial stressor of capture and blood draw was greater than 30 min). Cortisol concentrations between samples collected using rapid and routine methods were compared with a nonparametric Wilcoxon signed-rank test implemented in RStudio.

For sifakas, cortisol concentrations from the 62 samples from outwardly healthy sifakas were compared with five banked samples taken from sick sifakas at or near the end of life, including two sifakas in renal failure (1 F, 1 M), two animals in sepsis (1 M, 1 F), and one male for which cause of death remains unidentified. At the DLC, humane euthanasia is typically performed via exsanguination, followed by injection of potassium chloride, to enable collection and banking of blood samples for research. Given the small number of samples in this data set, we compare cortisol concentrations from healthy and sick sifakas using descriptive statistics only.

Ring-tailed lemurs subjected to rapid blood draws had cortisol concentrations that ranged from 1.16 to 4.20 μg/dl (median = 2.10 μg/dl; average = 2.36 μg/dl; SD = 0.90). By contrast, ring-tailed lemurs that underwent routine blood

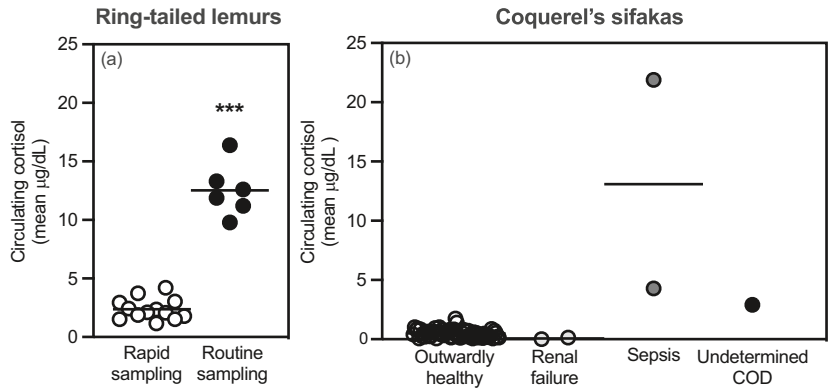

Figure 1. Biological validation of the cortisol assay for captive (a) ring-tailed lemurs and (b) Coquerel's sifakas at the Duke Lemur Center. Depicted are cortisol concentrations per sample (dots) and group averages (lines) for (a) ring-tailed lemurs that underwent rapid sampling (white) and routine sampling (black), as well as for Coquerel's sifakas that were outwardly healthy at the time of sampling (white), in renal failure (light gray) or sepsis (dark gray) at or near the end of life, or that died from an unknown cause (black). *** $P \leq 0.001$.

sampling had cortisol concentrations that ranged from 9.79 to 16.38 µg/dl (median = 12.25 µg/dl average = 12.53 µg/dl; SD = 2.24). This difference was statistically significant (W = 78, $P < 0.001$; Fig. 1a). The cortisol assay therefore detected a ~sixfold increase in circulating cortisol collected from ring-tailed lemurs before and after experiencing an acute physiological stressor.

Cortisol concentrations in samples from outwardly healthy sifakas ranged from 0.07 to 1.74 µg/dl (median = 0.44 µg/dl; average = 0.49 µg/dl; SD = 0.34). The two sifakas in renal failure likewise had low circulating cortisol concentrations of 0.0 and 0.14 µg/dl. In contrast, the two sifakas in sepsis had elevated cortisol concentrations of 4.28 and 21.88 µg/dl. The male who died from an unidentified cause also had marginally elevated cortisol of 2.90 µg/dl. This cortisol assay thus detected a ~27-fold increase in average concentrations between healthy sifakas and those in sepsis, but not in renal failure (Fig. 1b).

RESULTS

ACTH stimulation, serum chemistries, and CBCs

All lemurs that underwent ACTH or control stimulation had serum chemistry and CBC values that fell within the standard reference intervals for their species in captivity (Table 1). Numerous differences between species were noted, with ring-tailed lemurs showing greater concentrations of circulating potassium, glucose, creatinine, alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), amylase, lipase, creatine kinase, total bilirubin, and albumin, and sifakas having greater concentrations of circulating globulin and cholesterol, and a greater ratio of sodium to potassium. Ring-tailed lemurs had a greater proportion of eosinophils, but sifakas had greater abundances of total white blood cells.

Baseline cortisol was significantly greater in ring-tailed lemurs versus sifakas (W = 60, $P = 0.001$; Fig. 2a). In ring-tailed lemurs, baseline cortisol ranged from 9.79 to 16.38 µg/dl (median = 12.25 µg/dl average = 12.53 µg/dl; SD = 2.24); in sifakas, baseline cortisol ranged from 0.22 to 1.01 µg/dl (median = 0.67 µg/dl; average = 0.67 µg/dl; SD = 0.23). Neither the ring-tailed lemurs nor sifakas showed a typical response to ACTH stimulation with Cosyntropin (Fig. 2b). After confirming that baseline cortisol was not significantly different between control and treated subjects within ring-tailed lemurs (W = 1, $P = 0.267$) and sifakas (W = 7, $P = 0.493$), no significant difference was found in the area under the curve (AUC) between control and treated subjects for cortisol in ring-tailed lemurs (W = 2, $P = 0.53$) and sifakas (W = 18, $P = 0.12$).

For ring-tailed lemurs given exogenous ACTH, the maximum individual difference in cortisol concentrations from baseline, that is, the cortisol spike, averaged 6.47 µg/dl (range = 3.52–9.21 µg/dl; SD = 2.37). Ring-tailed lemurs given control tests saw a maximum rise in cortisol of 5.81 µg/dl on average (range = 3.3–8.31 µg/dl; SD = 5.81). For sifakas given exogenous ACTH, the maximum

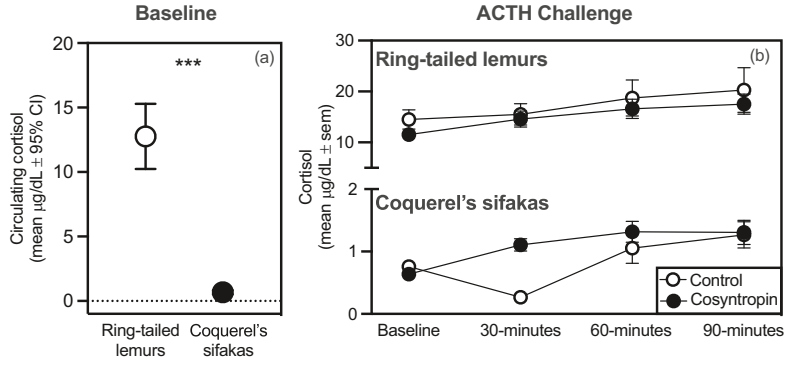

Figure 2. Cortisol concentrations measured during ACTH stimulation tests. Depicted here are concentrations of cortisol (a) from the baseline blood draw in ring-tailed lemurs (white) and Coquerel's sifakas (black); and (b) at baseline and 30, 60, and 90 min after adrenal stimulation in ring-tailed lemurs (top) and Coquerel's sifakas (bottom) that received Cosyntropin (black) or the control test (white). *** $P \leq 0.001$.

increase in cortisol concentrations from baseline averaged 0.83 µg/dl (range = 0.21–1.34 µg/dl; SD = 0.44). Sifakas given control tests saw a maximum cortisol increase of 0.52 µg/dl on average (range = 0.04–0.8 µg/dl; SD = 0.42). The difference in cortisol increase between control and treated subjects was not statistically significant for either ring-tailed lemurs (W = 5, $P = 0.80$) or sifakas (W = 15, $P = 0.38$).

Summary of banked samples

Cortisol concentrations in outwardly healthy, captive Coquerel's sifakas sampled between 2007 and 2024 were similarly low in concentration, ranging from 0.07 to 1.74 µg/dl (median = 0.44 µg/dl; average = 0.49 µg/dl; SD = 0.34). Curiously, the two samples with the greatest circulating cortisol, respectively, stemmed from the only founder in the study that was 27 yr old at the time of sampling (1.74 µg/dl) and from a healthy yearling who had required consistent supplemental feeding and veterinary support during early life (1.41 µg/dl). This same individual, later sampled at 12 yr of age, had circulating cortisol of 0.47 µg/dl. All other samples had concentrations at or below 1.01 µg/dl. No statistically significant differences in circulating cortisol between sexes ($F_{1,58} = 0.331$, $P = 0.568$; Fig. 3a), with increasing age ($F_{1,58} = 0.365$, $P = 0.548$; Fig. 3b), or between housing conditions ($F_{1,58} = 1.969$, $P = 0.166$; Fig. 3c) were detected.

DISCUSSION

This study found preliminary evidence that clinically and outwardly healthy Coquerel's sifakas in captivity have consistently low circulating cortisol and show minimal cortisol response to acute stress or adrenal stimulation. Although more robust assay validation is needed, circulating concentrations of cortisol in sifakas averaged just 0.49 µg/dl, and some values were as low as 0.07 µg/dl. Sifakas and ring-tailed lemurs both showed atypical, but different endocrine responses to ACTH stimulation tests. For ring-tailed lemurs, strongly elevated cortisol at "baseline" compared to values from samples collected using rapid sampling techniques indicates that pre-experiment stressors, such as kenneling and handling, activated the HPA-axis prior to exogenous ACTH administration, thus masking the ability to stimulate adrenal cortisol release further.

For sifakas, however, neither the pre-experiment conditions nor exogenous ACTH administration seemingly stimulated adrenal cortisol release. This result, coupled with the lesser baseline glucocorticoid values, might point to Addison's disease; however, other clues, including the sheer consistency of the endocrine patterns across conspecifics, lend support to the theory that low cortisol characterizes captive sifakas and derives from a different etiology. Captive sifakas lack several classic signs of Addison's disease. Concentrations of circulating sodium and potassium, and the ratio between the two, fell within standard reference intervals[52] and the animals showed no signs or symptoms of mineralocorticoid insufficiency[35] (although see[5,27,28] for discussion of rare Addisonian cases with only insufficient glucocorticoids). Outside of this study, the present clinicians have not identified adrenal abnormalities on necropsy in DLC sifakas

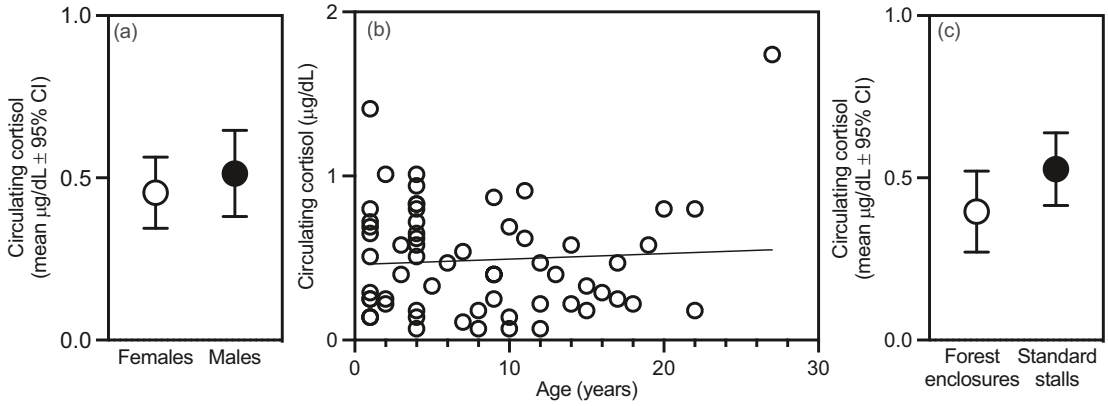

Figure 3. Variation in circulating cortisol in 62 samples from 43 individual sifakas collected between 2007 and 2024 relative to animal (a) sex, (b) age, and (c) housing in forest enclosures versus standard indoor/outdoor stalls. Dots in (b) depict individual samples; the line shows the regression line ($Y = 0.003x + 0.461$; $R^2 = 0.004$; $P = 0.622$).

that had low cortisol at the end of life, histopathology results that would be expected in untreated Addisonian individuals.

Critically, the results for sifakas in this study cannot be fully interpreted without knowing whether low baseline cortisol and a blunted response to exogenous ACTH typifies wild individuals in Madagascar. Currently no comparative data from wild Coquerel's sifakas are available to test this explicitly, although there is some evidence to suggest that captive sifakas excrete lower concentrations of fecal glucocorticoids than do wild sifakas in Madagascar.[48] Moreover, emerging clues indicate that sifakas are biologically capable of increasing cortisol production in response to stress. The two captive sifakas that were humanely euthanized when in sepsis had marginally or strongly elevated cortisol concentrations. Moreover, wild sifakas (including Coquerel's sifakas) show clear variation in fecal glucocorticoids across conditions and seasons,[6,11,16,31,44,45,54] and in response to ACTH stimulation.[16] Unfortunately, published data on circulating cortisol in wild sifakas are rare. This is likely because most (if not all) populations must be darted for blood sampling, leading to the reasonable expectation that stressful capture procedures would mask the ability to measure basal cortisol.[56] Future studies could beneficially measure circulating cortisol and longitudinally track fecal glucocorticoids in multiple populations of Coquerel's sifakas living in habitats of varying quality and anthropogenic influence. Examination of other glucocorticoids, like corticosterone and cortisone, as well as the actions of corticosteroid transport proteins, enzymes, and receptors, could clarify the major steroid hormones in this system, and their modes of action and metabolism.[10,14,21,43]

If baseline cortisol turns out to be abnormally low in healthy captive Coquerel's sifakas relative to their healthy wild kin in Madagascar, a second question would regard an underlying mechanism. Stress is presumed to increase circulating cortisol; however, there is accruing evidence for the paradoxical situation wherein chronic stress can ultimately dampen cortisol production.[17,25,55] Under this paradox, low circulating cortisol or "hypocortisolism" can occur in the absence of hypoadrenocorticism. The concept of stress-induced hypocortisolism is extended from the general adaptation syndrome and posits that chronic stress ultimately leads the HPA axis to transition from overresponsive to underresponsive.[17,47] At the extreme, hypocortisolism in the human literature has been coined as adrenal "fatigue" or "burnout" and has received significant attention and criticism over its validity as a clinical diagnosis.[7,13] Nevertheless, the long-term health effects of subtle and chronic hypocortisolism are well established and can include generalized symptoms of fatigue and malaise, increased autoimmune disease, and decreased humoral immunity that potentially contributes to pathogen susceptibility.[13,55]

Two lines of evidence point to stress-induced hypocortisolism as an important hypothesis to frame continued research in sifakas. First, sifakas in the present study showed baseline cortisol of 0.49 µg/dl after experiencing an acute

stressor of kenneling and handling. These patterns contrast those of the ring-tailed lemurs that had average baseline cortisol of 2.36 μg/dl prior to an acute stressor and 12.53 μg/dl after an acute stressor. Study sifakas also showed a blunted response to adrenal stimulation with exogenous ACTH, results that are consistent with an underresponsive HPA axis. Second, captive sifakas experience health concerns that could be consistent with those linked to hypocortisolism.[8,9] Indeed, despite decades of husbandry optimization,[20] sifakas in captivity remain notoriously fragile. Reproductive success is challenged by high infant and juvenile mortality[8,58] and captive individuals[58] do not have greater longevity than their wild counterparts.[41,57] Compared to other captive lemurs, sifakas face more frequent gastrointestinal distress, dehydration, and intestinal pathogens (particularly *Cryptosporidium* and *Listeria*) and more frequently succumb to infection, often sepsis.[8,9] Susceptibility to such pathogens echoes patterns of immunocompromised humans.[22,50]

Subtle differences in blood markers between the target species may help explain species differences in health and immunity. Notably, compared to captive ring-tailed lemurs, captive sifakas have reduced albumin and increased globulin, patterns that reflect differences in immune function and match species differences for wild sifakas and ring-tailed lemurs.[12,23,24,33,34,40] On average, captive sifakas appear to show lower albumin and greater globulin concentrations relative to wild sifakas.[23,24,34,40] The relationship between albumin and globulin is a critical marker of inflammation in captive sifakas: A precipitous drop in albumin and rise in globulin is one of the first signs of illness, with white-blood-cell counts spiking 1–2 days later (DLC in-house records). The age disparity between the ring-tailed lemur and sifaka subjects could have driven some of the differences in health markers,[51] but it is possible that captive sifakas have persistent low-grade inflammation, linked to persistently low cortisol, with resultant increases in inflammatory markers.

Lastly, there is some evidence that stress-induced hypocortisolism may be characteristic of sifakas and lemurs facing environmental challenge in Madagascar. In a study of wild diademed sifakas (*P. diadema*), fecal glucocorticoids increased during the lean season when high-quality foods were scarce; however, this pattern was accentuated for groups living in continuous forests, whereas those in fragmented habitats showed a more muted seasonal change.[54] The authors posited that sifakas in degraded habitats show downregulated adrenal activity. In red-bellied lemurs (*Eulemur rubriventer*), similarly muted seasonal fluctuations in cortisol were reported for animals in disturbed versus undisturbed habitats.[53] In ring-tailed lemurs, patterns of hypocortisolism were reported following an extreme cyclone, but not a drought.[15] Evolutionarily, a downregulated or underresponsive HPA axis might function to counter the long-term consequences of persistently elevated cortisol, and thereby support more robust immune responses when infection is more likely.[17,38] That sifakas, relative to other lemurs, may show signs of hypocortisolism in captivity could indicate that they are particularly sensitive to the environmental, social, and/or nutritional stress associated with human management.

Future research is strongly warranted to confirm the presence and manifestation of hypocortisolism in captive sifakas. If it is a consistent finding, downregulation of the HPA axis due to chronic stress may provide a mechanism through which to understand species-specific susceptibility to environmental stress and anthropogenic influence.

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