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Environmental Toxicology and Chemistry, Vol. 28, No. 5, pp. 982–989, 2009

Printed in the USA
0730-7268/09 $12.00 + .00

SUBLETHAL EFFECTS OF METHYLMERCURY ON FECAL METABOLITES OF TESTOSTERONE, ESTRADIOL, AND CORTICOSTERONE IN CAPTIVE JUVENILE WHITE IBISES (*EUDOCIMUS ALBUS*)

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(*Received* 28 *May* 2008; *Accepted* 5 *November* 2008)

Abstract—Methylmercury (MeHg) is a globally distributed neurotoxin, endocrine disruptor, and teratogen, and its effects on birds are poorly understood, especially within an environmentally relevant exposure range. In an effort to understand the potential causal relationship between MeHg exposure and endocrine development, we established four dietary exposure groups (0 [control], 0.05, 0.1, and 0.3 mg/kg wet wt/d of MeHg) of postfledging white ibises (*Eudocimus albus*) in a divided, free-flight aviary that spanned the estimated range of environmental exposure for this species. Fecal samples were collected from individually identified ibises over six months in 2005 and processed for hormone evaluation. Significant sex-related differences in fecal estradiol concentrations, though unpredicted in direction, suggest that this steroid could be related to juvenile development in this species. Using repeated-measures general linear models, we tested a set of candidate models to explain variation in endocrine expression. We found that MeHg exposure led to significant differences in fecal estradiol concentrations between the control and medium-dose groups, whereas differences in fecal corticosterone concentrations were observed between the control and both the low- and high-dose groups. These results suggest highly nonlinear dose–response patterns for MeHg. Many endocrine-disrupting contaminants are theorized to affect multiple endpoints in a nonlinear manner, making results difficult to interpret using a traditional toxicological approach. The evidence presented here suggests that endocrine effects of MeHg exposure could behave similarly.

Keywords—Methylmercury White ibis Endocrine disruption Fecal metabolites

INTRODUCTION

Methylmercury (MeHg) is a globally distributed contaminant that has a wide range of endocrine-disrupting, neurological, and developmental effects in animals [1,2], with apparently large effect threshold differences among avian species [3]. The effects of endocrine-disrupting contaminants (EDCs) are not often studied in birds at risk of exposure—especially within an environmentally relevant exposure range and in an experimental setting. Mercury contamination has been demonstrated to affect egg viability [3], parental behavior [4], and foraging behavior [5] and to have multigenerational effects [6], and it decreases fledging rates and high-energy behaviors of free-living birds [7]. These effects could have severe consequences on reproductive success in wild birds, though it is uncertain whether endocrine disruption plays a role in producing these effects.

Heath and Frederick [8] showed that MeHg in wild breeding white ibises (*Eudocimus albus*) was correlated with a decrease in plasma estradiol-17β concentrations in females and an increase in plasma testosterone concentrations in males when controlling for timing during the breeding cycle. In addition, variation in feather MeHg of long-legged wading birds over the past 30 years has been correlated with variation in numbers of breeding white ibises in the Florida Everglades, USA [8]. In common loons, Evers et al. [9] demonstrated a positive association between blood mercury concentrations and blood corticosterone concentrations. Methylmercury has been suggested to contribute to the decline of the common loon (*Gavia immer*) in the northern United States; however, ecological variables confounded with MeHg exposure limit inference in the contaminated Great Lakes region [10]. Thaxton et al. [11] showed a decrease in plasma corticosterone concentration with increasing MeHg in chickens (*Gallus domesticus*), but Heath and Frederick [8] found no relationship between mercury exposure and plasma corticosterone concentration in breeding white ibises. These studies strongly suggest a link between MeHg exposure and breeding success or breeding population size via the mechanism of endocrine expression. Given the wide range of sensitivities to most mercury effects across avian species [1–3], large, dose-dependent differences in endocrine responses to contamination should be expected.

Although limited evidence suggests that EDC exposure can affect sex steroid and glucocorticoid hormone concentrations in avian species [8,12], causal evidence linking MeHg exposure with endocrine disruption is lacking. Based on studies of birds [12], fish [13,14], and mammals [15], endocrine effects seem to be most likely or powerful when exposure occurs during the embryonic stage. Mercury has its most potent effects on embryotoxicity and teratogenesis during the embryonic stage, [3], but shortly thereafter, nestlings can be relatively well-protected from even high exposure to mercury while feathers are growing [16]. Thus, the period of development after feather growth stops—that is, the juvenile stage—could be the most sensitive life stage to endocrine disruption. Hormone-regulated secondary sexual differentiation [17] and endocrine activity during the nonbreeding period [18] represents potential mechanisms by which EDC exposure may alter individual fitness during a juvenile or nonbreeding period.

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Published on the Web 12/1/2008.

The available literature suggests that MeHg exposure in birds could alter hormone synthesis and expression, which are integral to successful development and reproduction [19,20]. Here, we report on a study examining the effects of MeHg on endocrine function in a juvenile, long-legged wading bird, the white ibis, using a controlled experimental approach. We chose to study this species because considerable information is available regarding its breeding ecology [21–24], effects of MeHg on endocrine function have been demonstrated in adult birds, and exposure to low levels of the contaminant is continuing in the wild [8]. Estradiol, testosterone, and corticosterone metabolites were measured in fecal samples from chronically exposed birds over an environmentally relevant range of experimental mercury exposures. We hypothesized that increasing exposure to MeHg would significantly alter fecal steroid hormone concentrations in a direct, dose-dependent fashion.

MATERIALS AND METHODS

We raised wild-caught white ibis nestlings in a large, free-flight aviary on four diets that differed only in MeHg concentration. Ibises were collected as nestlings (age, 25–32 d) in April 2005 from the northern Everglades (Water Conservation Area 3, Broward County, FL, USA; 26°11.179′N, 80°31.431′W) and from a colony near White Springs (Hamilton County, FL, USA; 30°19.900′N, 82°45.367′W). We sexed, weighed, measured, banded, and removed approximately four scapular feathers for mercury analysis from all 210 nestlings within 20 h of capture before randomly distributing ibises to their respective enclosures.

Ibises were raised in a large, circular aviary (radius, 21 m; height, 10 m) divided into equal quadrants by net walls. The interior of the aviary contained numerous perches, artificial nest cups, and a feeding/wading pool. We fed all birds in each enclosure on a different diet containing MeHg: Control or 0.05, 0.1, and 0.3 mg/kg of MeHg (all values in diet are wet wt). The flooring was impermeable polyvinyl chloride sheeting that drained toward a common central drain.

Methylmercury was administered via diet beginning at 90 d of age by dissolving MeHgCl into corn oil and spraying the mixture onto Flamingo diet pelletized feed (Mazuri Company). A 50-ml dose of corn oil was sprayed onto a rotating mass (11.3-kg batches) of pelletized food within a portable cement mixer. Each dose group had a complete set of glassware and mixing devices, including a cement mixer dedicated solely to that dose regime. Appropriate stock solution concentrations of MeHg were confirmed before the onset of feeding by using direct measurement of mercury concentrations in the food. Differences and consistency of dose regimes also were verified by determining the resulting mercury concentrations of scapular feathers [25] in January of 2006 and 2007 using standard cold-vapor techniques based on U.S. Environmental Protection Agency Method 245.1 (minimum detection level, 0.5 ppb; practical quantification level, 1.5 ppb) [26] (Fig. 1).

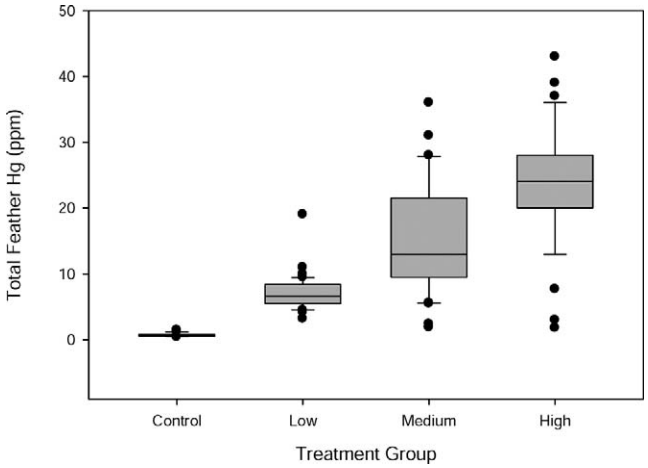

Fig. 1. Feather mercury levels for individual birds in each exposure group in January 2006. Data are presented in box plots, with outliers represented as dots, the gray box showing where 50% of the data lie, and the mean represented by the horizontal line within the box.

Fecal hormone sampling technique, storage, and extraction

Sex steroid and corticosteroid hormones are most commonly measured in the blood plasma of animals. A number of recent studies [27–29], however, have shown that useful hormone concentrations also can be obtained from fecal samples. The latter sampling method is noninvasive, thus avoiding the need to correct for the short-term stress response in some hormones. In addition, hormone concentrations in feces represent an integration of fluctuating hormone concentrations over the gut passage time, which allows an analogous alternative measurement of baseline hormone concentrations. Potential difficulties with the method include validating the relationship between blood hormones and fecal metabolites and developing effective extraction techniques [30].

We collected fecal samples on clean, black plastic sheeting placed below perching structures, and we identified feces of individual ibises by directly observing excretion of individually banded birds. The location, time, and band number of each excretion was recorded. On collection days, we collected feces from any dose group for two 1-h periods, with 1 h between them (typically between 11:00 AM and 12:00 PM and between 1:00 PM and 2:00 PM). During collection bouts, we removed samples from the plastic every 10 min. Two observers usually watched during each collection bout, and at each 10-min interval, both observers approached the plastic sheets at the same time and collected fecal samples that were visually marked. Unidentified samples were marked on the plastic sheet to avoid confusion. If unidentified samples were in very close proximity to target samples (within 0.5 m), the sample was considered to be contaminated and was not collected. We estimated bird through-put time (i.e., time from ingestion to excretion) at 2 to 3 h by using food containing colored plastic beads.

Four fecal sample collection periods were used during the present study. The first was early June 2005, immediately before mercury exposure began (age, ~90 d). The second was late June, immediately after the initiation of dosing (age, ~110 d). The third was late July 2005 (age, ~140 d). The fourth was in late December 2005 and early January 2006 (age, ~290 d). Ibises retain nonadult plumage through the first year of life and are believed to breed after 20 months of age [19], although all treatment groups in this experiment bred at 10 months of age.

Individual fecal samples were collected in 2-ml cryotubes and placed in an ice-filled cooler for no more than 3 h until they could be stored at −20°C; all samples were analyzed for hormone concentrations in May to July of 2006. Total numbers of samples per treatment group (with total number of individuals sampled in parentheses) were 64 (39), 76 (35), 62 (34), and 72 (33) from the control, low, medium, and high group,

Table 1. Average extraction efficiencies for each hormone by percentage ethanol used for extraction

% Ethanol used	Extraction efficiency (%)		
	Estradiol	Testosterone	Corticosterone
80	77	64	90
90	72	62	98
100	38	80	102

respectively. Fecal samples were individually lyophilized, after which the dried and stabilized samples were then weighed and placed into glass extraction vials. Samples that contained more than 0.05 g of sample were homogenized and subsampled, whereas samples that contained 0.05 g or less were used in their entirety. Samples less than 0.02 g were not used, because the relationship between sample mass and hormone concentration became nonlinear below that level.

We used ethanol diluted by deionized water to extract hormone metabolites from the sample [31,32]. Ethanol was combined with a measured amount of fecal sample in a capped glass culture tube, and we used a multitube vortexer to shake the mixture for 30 min, cycling the vortexer on for 1 min and off for the next. Culture tubes were then spun in a refrigerated, superspeed centrifuge at 3,000 rpm for 20 min. The resulting ethanol supernatant was decanted into clean culture tubes and stored at −80°C. We compared the extraction efficiency of 80% (20% deionized water), 90% (10% deionized water), and 100% ethanol solutions by adding a known amount of radiolabeled hormone to standardized desiccated fecal matrix and then performing the extraction procedure. Eighty percent ethanol was selected because its extraction efficiency was highest when considering all hormone tests (Table 1). All results were adjusted for mean extraction efficiency (estradiol, 77%; testosterone, 64%; and corticosterone, 90%).

Radioimmunoassay

We tested each sample extract for estradiol metabolites using Estradiol ^{125}I Coat-a-Count radioimmunoassay (RIA) kits (Diagnostic Products). All samples were incubated at room temperature for 3 h. The manufacturer's protocol was followed, with 100 μl of extract used initially and dilutions performed when needed. This kit has shown high accuracy and dependability in previous avian fecal hormone studies [32]. The manufacturer's reported antibody cross-reactivities were 10% for estrone, 4.4% for δ-equilenin, 1.8% for estrone-β-δ-glucuronide, and less than 1% for all other tested steroids.

We tested for testosterone metabolites using a Testosterone ^{125}I Double-Antibody RIA kit (MP Biomedicals). The manufacturer's protocol was followed, with 50 μl of extract used initially and dilutions performed as needed. This kit has been validated previously for fecal metabolites in birds [31] and other vertebrates [32]. The manufacturer's reported cross-reactivities were 3.4% for 5α-dihydrotestosterone, 2.2% for 5α-androstane-3β,17β-diol, 2% for 11-oxotestosterone, and less than 1% for all other tested steroids.

We tested for corticosterone metabolites using a Corticosterone ^{125}I Double-Antibody RIA kit (MP Biomedicals). As with the other steroid assays, the manufacturer's protocol was followed, with 50 μl of extract initially required and dilutions performed as needed. This and similar kits have been validated in several avian fecal glucocorticoid studies [31,33]. The manufacturer's reported cross-reactivates were 34% for deoxycorticosterone, 10% for testosterone, 5% for cortisol, 3% for aldosterone, 2% for progesterone, 1% for androstenedione, 1% for 5α-dihydrotestosterone, and less than 1% for all other tested steroids and glucocorticoids.

All kits were validated by running a set of internal standards into standard hormone extract and hormone-stripped extract using the manufacturer's standard curve. To determine whether the extraction matrix would interfere with the accuracy of the assay, we tested for differences between all curves for all kits and found none to be significantly different (analysis of covariance, $p > 0.22$). Thus, we found our assay to be internally valid for each hormone.

Statistical analysis

We tested for differences in hormone concentration resulting from treatment using a repeated-measures analysis of covariance. Sampling was not uniform across sampling periods (i.e., certain individuals were not represented in some sampling periods), so traditional repeated-measures methods could not be used. All samples collected before the initiation of dosing (early June 2005 sample) were analyzed to determine if preexisting differences were present between treatment groups (analysis of variance, $p > 0.05$). Treatment group is a categorical variable in this analysis; all experimental groups (i.e., the groups being exposed to MeHg) are only compared to the control group, never to each other. In any analysis conducted here, the control group will not have a parameter estimate, because it is the benchmark to which all experimental groups are compared and the means by which we determine significance. After averaging hormone concentrations from each individual for each collection period, we took the natural log of concentrations of estradiol, testosterone, and corticosterone. Estradiol and testosterone data were analyzed by collection period, and corticosterone was analyzed on a daily basis because of differences in the roles that each hormone plays in the nonbreeding season. A compound symmetry structure to the covariance matrix was used to link individuals (nested within treatment) over time in a repeated-measures framework (SAS® Ver 9.1 PROC MIXED; SAS Institute). Kenward-Rogers–calculated degrees of freedom were used, because the technique minimizes type I errors in repeated-measures studies that have gaps in the data as a result of sampling inequity [34].

Using treatment group, time (grouped by collection period as above), and sex as main effects, we developed a set of 21 biologically relevant a priori models based on our predictions. These 21 models were selected because of our hypothesis that treatment group, sex, collection period, and all potential interactions of such terms were biologically plausible models. Time of day was incorporated into early models, but it was not found to be a significant component. We included all possible combinations of the three terms (time, treatment, and sex) for each hormone analysis up to the most complicated model, which included the time × treatment × sex term. We also included a time × time interaction that allowed nonlinear changes in hormone concentration over time, but we did not allow this term to interact with other main effects. We included one term a posteriori—namely, a categorical grouping that compared the control group against all experimental groups. Model selection was based on sample size–corrected Akaike's Information Criterion (AIC_c) [35], and models were ranked by Akaike weight (w_i). Akaike's Information Criterion is only used for model selection in this application; on selection, independent variables in the model are evaluated in the analysis-

Table 2. Akaike weight ranking of various models explaining variation in hormone metabolites[a]

Hormone	Model	AIC_c	ΔAIC_c	Akaike wt (w_i)
Estradiol				
	time, sex, treatment, time × time, treatment × time	167.6	0	0.52
	time, sex, treatment, time × time, sex × time, treatment × time	168.9	1.3	0.27
	time, sex, time × time	171.2	3.6	0.09
	time, sex, treatment, time × time	172.8	5.2	0.04
	time, sex, treatment, time × time, time × treatment, sex × treatment	173.9	6.3	0.02
	time, sex, treatment, time × time, sex × time	174.3	6.7	0.02
	time, sex, treatment, time × time, sex × time, treatment × time, sex × treatment	175	7.4	0.01
	time, time × time	175.5	7.9	0.01
	time, sex, treatment, treatment × time	176.8	9.2	0.01
Testosterone				
	time, time × time	111.8	0	0.29
	time, control, time × time	112.8	1	0.17
	time, sex, time × time	113.4	1.6	0.13
	time, treatment, time × time	113.5	1.7	0.12
	time, sex, treatment, time × time	114.7	2.9	0.07
	time	114.7	2.9	0.07
	time, treatment	115.3	3.5	0.05
	time, sex	116.2	4.4	0.03
	time, sex, treatment, time × time, sex × time	116.7	4.9	0.02
	time, sex, treatment, time × time, sex × treatment	118.6	6.8	0.01
	time, sex, treatment, time × time, time × treatment	119.7	7.9	0.01
Corticosterone				
	time, sex, treatment, time × time, treatment × time	270.4	0	0.67
	time, sex, treatment, time × time, sex × time, treatment × time	272.6	2.2	0.22
	time, sex, treatment, time × time, treatment × time, sex × treatment	276.1	5.7	0.04
	time, treatment, time × time	276.7	6.3	0.03
	time, sex, treatment, time × time	277.8	7.4	0.02
	time, sex, treatment, time × time, sex × time, treatment × time, sex × treatment	278.4	8	0.01
	time, sex, treatment, time × time, sex × time	279.8	9.4	0.01

[a] Only models with a weight of greater than 0.01 were included. Akaike's information criterion corrected for sample size (AIC_c) and the difference between AIC_c values from a given model and the best model (ΔAIC_c) also are listed.

of-variance framework described above. The AIC_c method is invalid when used with data generated via restricted maximum-likelihood methods that are default in PROC MIXED; standard maximum-likelihood methods were used instead.

Finally, to examine possible effects because of individual variation in MeHg exposure within groups (Fig. 1), we categorized each individual as having either high or low MeHg exposure within its exposure group based on the median feather mercury quantity for each exposure group. We then removed the a priori treatment factor (control, low, medium, and high) from the model and replaced it with seven new categories (control, low-low, high-low, low-medium, high-medium, low-high, and high-high) using only the best model as previously selected by AIC_c. The new parameter estimates are indicators of differences between each new group and the control. Alpha was set at 0.05.

Table 3. Estradiol parameter estimates from the best model selected that included treatment with respective standard errors of the estimate[a]

Model parameter	Parameter estimate (β)	SE	*p*
Time	−0.5287	0.1347	0.0001
Time × time	0.04372	0.01268	0.0007
Sex			
Male			
Female	−0.1500	0.0538	0.0063
Treatment			
Control			
Low	−0.1968	0.1529	0.1998
Medium	−0.3408	0.1619	0.0367
High	0.08553	0.1518	0.5739
Time × treatment			
Control			
Low	−0.04606	0.03197	0.1515
Medium	0.07704	0.03723	0.0400
High	0.001684	0.03273	0.9590

[a] The *p*-value was determined using an *F* test. Note that treatment and sex effects are categorical, and control and male groups, respectively, act as a reference. All others are estimated relative to that group. SE = standard error of the test.

RESULTS

The models that best explained estradiol, testosterone, and corticosterone data were identical for estradiol and corticosterone, whereas the model that best described the testosterone data was appreciably different from the former (Table 2). The best models for both estradiol and corticosterone included all main effects (sex, time, and treatment) and two higher-level terms that allowed nonlinear effects over time (time × time) and linear effects of treatment over time (treatment × time). No single model was best for testosterone. Four models were found to be important; however, only one had the treatment term. These results alone imply that estradiol and corticosterone fecal hormone concentrations were influenced by MeHg treatment, whereas fecal testosterone concentrations were less so.

The best estradiol model was significantly better than most competing models (w_i = 0.52), and though the second-rated model was moderately close (ΔAIC_c = 1.3), it was less parsimonious than the top-rated model and extremely similar, with both having the treatment × time term. Both the time and sex factors were significant in the top model, but females had significantly less estradiol than males (Table 3). In the top

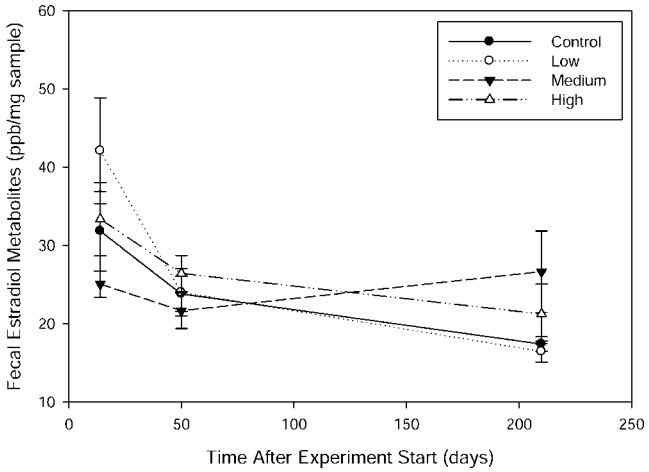

Fig. 2. Mean estradiol concentration by treatment group for each collection period. Error bars represent the standard error of the calculated mean and do not consider intracollection dependency issues as our statistical models do.

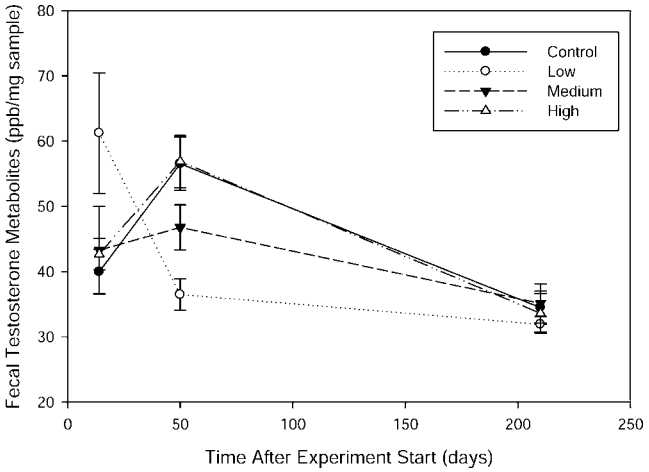

Fig. 3. Mean testosterone concentration by treatment group for each collection period. Error bars represent the standard error of the calculated mean for each collection period and do not consider intracollection dependency issues as our statistical models do.

model, only the medium-exposure group was significantly different than the control and showed a significantly higher rate of increase in estradiol over time ($p = 0.04$) (Fig. 2). This result directly contradicted our prediction of a linear dose–response relationship.

No one model clearly explained the testosterone data. The first model that included the treatment term received limited support ($w_i = 0.12$), and on further analysis, the treatment term was not significant in the analysis of covariance (Table 4). Testosterone varied significantly over time (Fig. 3). Low significance of independent variables in all these models suggests that none of these models fits the data well. Overall, these data provided almost no support for our initial hypothesis.

Corticosterone data were best described by a single model that include all three base factors along with the time × time and time × treatment factors (Table 2). This model was appreciably better than all others that were run ($w_i = 0.67$), and it is the only one we discuss here. No sex-related differences were found in corticosterone expression ($p = 0.3912$), and all values showed an increase over time ($p = 0.0004$), with a concave curvature ($p < 0.0001$) (Table 5). The two middle-exposure groups (low and medium) were more similar to the control than the high-exposure group (Fig. 4). The low-exposure group showed a borderline significant decrease over time ($p = 0.0599$). The medium-exposure group showed a marginally significant increase ($p = 0.0713$), whereas the high-exposure group showed consistently high values for the whole period ($p = 0.0476$). These results suggest an effect of MeHg, but a nonlinear relationship exists between dose and response.

The analysis of within-group variance in the responses of all hormones to MeHg was designed to look for similarities in individuals with high overlap in MeHg exposure (e.g., the high-medium group and low-high group). The results of this analysis indicate that similarly dosed individuals between treatment groups were not responding similarly, but this varied among the dose groups and hormones measured (Table 6). We found considerable variance in response within individual treatment groups. Variance in the MeHg effect appeared to increase with increasing MeHg exposure. Whereas the low-exposure group was relatively consistent in direction and magnitude of effect, the parameter estimates for medium- and high-exposure groups showed larger differences between subgroups, particularly in the corticosterone response.

Table 4. Testosterone parameter estimates from the best model selected that included treatment with respective standard errors of the estimate[a]

Model parameter	Parameter estimate (β)	SE	*p*
Time	0.1752	0.1090	0.1103
Time × time	−0.02261	0.01046	0.0322

[a] The *p*-value was determined using an *F* test. Note that treatment and sex effects are categorical, and control and male groups, respectively, act as a reference. All others are estimated relative to that group. SE = standard error of the test.

Table 5. Corticosterone parameter estimates from the best model selected that included treatment group with respective standard errors of the estimate[a]

Model parameter	Parameter estimate (β)	SE	*p*
Time	0.01143	0.003141	0.0004
Time × time	−0.00005	0.000013	<0.0001
Sex			
Male			
Female	−0.06792	0.07884	0.3912
Treatment			
Control			
Low	0.05888	0.1394	0.6733
Medium	−0.2376	0.1429	0.0981
High	0.2653	0.1330	0.0476
Treatment × time			
Control			
Low	−0.00214	0.001132	0.0599
Medium	0.002425	0.001337	0.0713
High	−0.00095	0.001140	0.4068

[a] The *p*-value was determined using an *F* test. Note that treatment and sex effects are categorical, and control and male groups, respectively, act as a reference. All others are estimated relative to that group. SE = standard error of the test.

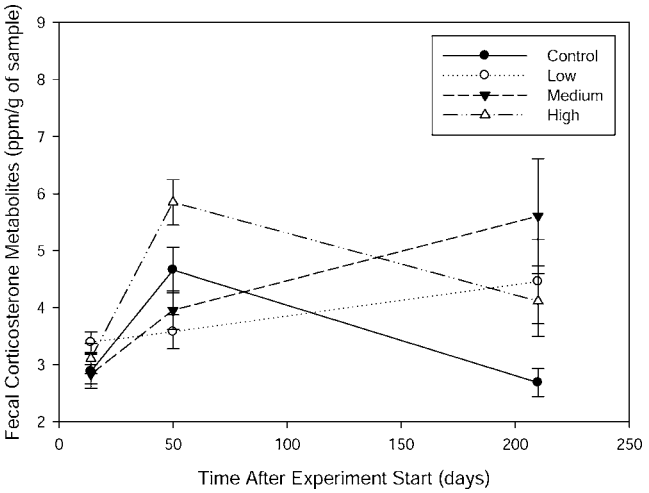

Fig. 4. Mean corticosterone concentration by treatment group for each collection period. Error bars represent the standard error of the calculated mean and do not consider intracollection dependency issues as our statistical models do.

DISCUSSION

Our predictions about the relationship between MeHg and the hormones studied were not generally supported by our findings. The most consistently violated prediction was that responses would be linearly (or incrementally) related to MeHg exposure. Methylmercury exposure significantly altered estradiol, although this result appeared to be driven by large differences between the medium-exposure group and all other groups. Testosterone showed no significant dose–response relationship with all a priori tests of MeHg exposure and only a weak trend over time. Corticosterone varied significantly with MeHg exposure, but in an unpredicted fashion—namely, the control and medium-exposure groups were similar, whereas the low- and high-exposure groups were on their respective extremes. Our prediction that concentrations of steroid and glucocorticoid hormones would vary by sex was not well-supported overall, and males showed unexpectedly greater amounts of estradiol than females. The juvenile life stage of white ibises remains poorly understood, but some differences in sex steroid expression throughout this period suggest that sexual development could be continuing. Finally, our analysis of variation in intragroup MeHg exposure did not aid in explaining these nonlinear, or at least nonincremental, dose–response patterns—similarly dosed individuals between groups did not appear to be responding similarly.

It is possible that the levels of mercury exposure were simply not high enough to have a consistent effect and/or that effects of mercury were overwhelmed by random or confounding effects. Few experimental studies have been conducted within these MeHg exposure ranges on any species, and to our knowledge, none has been conducted on birds. We observed complex, nonlinear responses in our hormone data that have been seen in many physiological/endocrine studies where various factors (e.g., biological processes) influence the response [36,37]. For example, fecal hormone concentrations not only are an integration of time (gut passage rate) but also represent an integration of gonadal/adrenal synthesis, variable storage time of the hormones in the blood bound to various plasma proteins, and hepatic biotransformation and clearance [38]. Thus, for example, if MeHg affects a hepatic enzyme function, such as hormone biotransformation (e.g., aromatization of androgens to estrogens) and clearance, fecal con-

Table 6. Summary of tests for differences in mercury response within dose groups[a]

Hormone	Treatment group	Parameter estimate (β)	SE	95% CI	
				Minimum	Maximum
Estradiol					
	Control				
	Low-low	−0.03490	0.03843	−0.11022	0.040423
	High-low	−0.05383	0.03699	−0.12633	0.01867
	Low-medium	0.1419	0.05431	0.035452	0.248348
	High-medium	0.03699	0.04262	−0.04655	0.120525
	Low-high	−0.039420	0.04236	−0.12245	0.043606
	High-high	0.02611	0.03636	−0.04516	0.097376
Testosterone					
	Control				
	Low-low	−0.1229	0.08581	−0.29109	0.045288
	High-low	−0.05164	0.08942	−0.2269	0.123623
	Low-medium	−0.1211	0.09162	−0.30068	0.058475
	High-medium	−0.1431	0.08942	−0.31836	0.032163
	Low-high	−0.03724	0.08656	−0.2069	0.132418
	High-high	0.02922	0.07778	−0.12323	0.181669
Corticosterone					
	Control				
	Low-low	−0.00171	0.001318	−0.00429	0.000873
	High-low	−0.00248	0.001342	−0.00511	0.00015
	Low-medium	0.004959	0.002012	0.001015	0.008903
	High-medium	0.001405	0.001514	−0.00156	0.004372
	Low-high	−0.00015	0.001479	−0.00305	0.002749
	High-high	−0.00104	0.001259	−0.00351	0.001428

[a] Each treatment group was subdivided by median feather mercury quantities (see Fig. 1) for each hormone. The treatment grouping was added to the best a priori model selected by Akaike's information criterion. The parameter estimate is for the highest-order term that includes treatment as an effect; this was treatment × time for estradiol, treatment for testosterone, and treatment for corticosterone. All parameter estimates are relative to the control group. The control group lacks an estimate, because it is the reference to which all others are compared. Thus, its estimate would be zero for all accounts. CI = confidence interval; SE = standard error of the test.

centrations could be either increased or decreased, depending on current physiological conditions.

If the biological phenomena in the steroid hormone synthesis and clearance pathway have differing sensitivities to the effects of MeHg, then one would predict nonlinear and complex responses. For example, we saw a complex pattern of response in corticosterone, with low MeHg exposure decreasing concentrations in the feces and high exposure increasing concentrations, whereas intermediate exposure had no apparent effect. Varying exposure levels to MeHg likely would alter adrenal synthesis and hepatic clearance of corticosterone, transforming fecal concentrations. Similar complex responses associated with the synthesis, transport, biotransformation, and clearance of androgens and estrogens could explain the patterns we observed. Interestingly, one would predict that such subtle but important variations in responsiveness at various points in this pathway would not be detected by high-dose studies, which would overwhelm the threshold of responsiveness for all these biological mechanisms [36]. The important observation here is that several models clearly support the hypothesis that exposure to MeHg alters endocrine parameters as measured by fecal hormone concentrations. These patterns are neither linear nor biologically simple, however, because numerous processes are involved that are all potential targets of alteration with potentially different thresholds of response. Future studies need to examine whether such differences in threshold responsiveness exist in the steroid synthesis and clearance responses to MeHg exposure.

An alternative biological explanation for the nonlinear dose–response relationships in corticosterone could be that MeHg exposure acts as a physiological stressor, thus causing both downregulation and upregulation of the hormone. Corticosterone values have been found to vary nonlinearly with EDC exposure in other avifauna [39], and this may be caused by the way that birds respond to toxic stress. It is possible that high, chronic levels of MeHg simulate high levels of physiological stress or disease, producing an increase in baseline corticosterone. The lower mercury exposure levels could be causing a decrease in baseline corticosterone, either by altering hormone synthesis or by acting as a repeated, minor physiological stressor, which has been suggested to lower baseline corticosterone in some cases (e.g., hormesis [40]).

In sum, MeHg affects the expression of estradiol and corticosterone in a nonlinear pattern in juvenile ibises at low, chronic doses. There does not appear to be an effect of MeHg dose on testosterone, but it is possible that a threshold effect occurs at higher levels or that sex steroid hormone expression generally is insensitive to mercury at the juvenile stage. Although the earlier work of Heath and Frederick [8] strongly suggests an effect of mercury on expression of these hormones in wild breeding adults, we see a dramatically reduced effect on juvenile birds in this experimental study. Because the dose levels in the present study spanned the range of exposures estimated in the wild birds studied by Heath and Frederick, the more probable explanation appears to be that endocrine expression in young birds is less sensitive to mercury at that stage of life. Whereas the pattern of corticosterone response was not predicted, a reasonable case can be made for a nonlinear dose–response relationship. Nonlinear effects of MeHg have been detected in similar studies with behavioral endpoints [5], and the present results demonstrate the variation in the sensitivities and dose–response relationships of endpoints to low and chronic MeHg exposure.

Acknowledgement—The authors would like to thank Mary Christman for statistical assistance, John Simon and Eric Trum for field assistance, and the entire Guillette laboratory for technical assistance with the hormone assays. This research has been supported by the Florida Department of Environmental Protection, U.S. Fish and Wildlife Service, and U.S. Army Corps of Engineers and by logistical help from the U.S. Department of Agriculture Animal and Plant Health Inspection Services Gainesville field station.

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