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Corticosterone Alters Body Weight, but Not Metabolites, during Chronic Stress

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ABSTRACT

The stress response is partially mediated by increased levels of circulating glucocorticoids. While the stress response may be adaptive in the short term, chronically elevated levels of glucocorticoids can be pathological. We aimed to verify that chronic stress causes metabolic dysregulation via increased corticosterone (Cort) exposure by monitoring free fatty acid (FFA) concentrations (evidence of fat breakdown), uric acid concentrations (evidence of protein breakdown), and organ weights (furcular fat, abdominal fat, liver, and pectoralis muscle) in chronically stressed juvenile house sparrows (*Passer domesticus*). The sparrows were chronically stressed for 3 wk by applying a series of rotating mild psychological stressors. One group of birds received injections of a glucocorticoid steroidogenesis inhibitor (mitotane) and a second group received injections of a glucocorticoid receptor antagonist (RU486) halfway through the chronic stress period to test whether glucocorticoids are responsible for protein and fat catabolism during chronic stress. Toward the end of the chronic stress period, mitotane birds increased weight compared to control and RU486 birds. Contrary to expectations, we saw no differences in FFA or uric acid levels between control and mitotane birds, but RU486 temporarily decreased stress-induced uric acid levels. Neither mitotane nor RU486 significantly altered organ weights at the end of the 3 wk. In conclusion, Cort does appear to negatively affect body weight, but the mechanism does not appear to involve increased protein or lipid metabolism.

Keywords: corticosterone, fat metabolism, protein metabolism, stress, birds, reactive scope.

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Introduction

Reactive scope posits that the symptoms of homeostatic overload, or chronic stress, derive from physiological mediators of the stress response shifting from beneficial to pathological effects (Romero et al. 2009). Although it is still unknown when this transition takes place (Romero and Wingfield 2016), it likely occurs at different timescales for different stressors and mediators (Gormally et al. 2018, 2019*a*). Perhaps the best-studied mediators of the stress response are glucocorticoids, and one of the preeminent physiological effects of glucocorticoids is the regulation of energy metabolism. Glucocorticoid regulation of energy is thought to be critical to survival in the short term (Wingfield et al. 1998); however, the extended elevation of glucocorticoids can lead to energy dysregulation (e.g., Dallman and Bhatnagar 2001; Korte et al. 2005), and chronic stress can be a major risk factor for metabolic syndrome (e.g. Depke et al. 2008). Consequently, reducing glucocorticoid levels or blocking the actions of glucocorticoids during chronic stress should ameliorate any dysregulation of metabolism.

In birds, metabolic changes occur as a result of stress (Fokidis et al. 2012). Although the high-affinity mineralocorticoid (MR) receptor for corticosterone (Cort), which is the primary glucocorticoid in birds, is thought to regulate energy at basal Cort levels (Romero and Wingfield 2016), the low-affinity glucocorticoid receptor (GR) is implicated in energy regulation in birds during stress (Landys et al. 2004*a*). While glucose is the preeminent molecule for energy usage in mammals, glucose regulation in house sparrows differs from that in mammals, with glucose transport into skeletal muscles being lower for birds than for mammals (Sweazea and Braun 2005, 2006). In addition, chronic stress, applied in the same manner as in this study, did not alter plasma glucose in starlings (Cyr et al. 2007). Consequently, we focused this study on lipid and protein metabolism by measuring free fatty acids (FFAs) and uric acid, respectively. We do note, however, that uric acid levels are not solely a marker of protein breakdown and may be indicative of other processes, such as antioxidant capacity (Cohen et al. 2008; Haskins et al. 2017). We were also interested in whether any changes in lipid or protein metabolism would be reflected in the weights of furcular fat, abdominal fat, liver, or pectoralis muscle.

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We used captive juvenile house sparrows (*Passer domesticus*) to test the hypothesis that metabolic dysregulation during chronic stress is caused by increased exposure to circulating glucocorticoids. In order to measure the potential impact of Cort on metabolism during chronic stress, we decreased Cort release via an injection of mitotane, a glucocorticoid steroidogenesis inhibitor (Breuner et al. 2000). Chronic stress exposure generally causes body weight loss in birds (DuRant et al. 2016; Gormally et al. 2019*a*); however, because our juvenile birds were still growing, we anticipated a reduction in body weight gain in control birds versus mitotane birds. We predicted that this reduction in body weight gain would be accompanied by increased catabolism of lipids and proteins, which would perhaps be substantial enough to decrease organ weights. To test the relative importance of GR activation on these metabolic processes, we also injected a separate group of birds with RU486, a selective GR inhibitor (Landys et al. 2004*b*). If GR activation is responsible for metabolic dysregulation during chronic stress, then birds injected with RU486 would have increased weight gain, lower levels of circulating lipids and proteins, and heavier organs compared to control birds.

Methods

Animals and Housing

Twenty-six wild house sparrows (*Passer domesticus*) were captured with Potter traps in the northeastern US from March 10 to March 17, 2012. The birds were taken to indoor facilities and housed in mixed-sex pairs in 45 × 37 × 33-cm cages. All cages were housed in the same room. The sparrows were held on a 12L : 12D light cycle at 22°C with food and water available ad lib. The birds were allowed at least 10 d to acclimate to captivity before the experiment was started. The birds were fed a mixed-seed diet (Supreme Wild Bird Mix, Lebanon Seaboard) and were also provided grit. All experiments were approved by the Institutional Animal Care and Use Committee at our university and were carried out according to the guidelines for the use of wild birds in research (Fair et al. 2010).

Chronic Stress in Juvenile Sparrows

Our experiment consisted of two stages: control (days −6 to 0) and chronic stress (days 1 to 20). During the control stage, the birds were not exposed to stressors. During the chronic stress stage, the birds were subjected to four different 30-min stressors per day. The order of stressors was randomized; however, no type of stressor was used twice in a row or more than twice a day. The time of day for each stressor was also chosen randomly; however, stressors occurred only during lights-on, and there were always at least 2 h between stressors. In our lab, the birds were exposed to four different stressors taken from previous chronic stress protocols (Rich and Romero 2005; Cyr et al. 2007; Lattin and Romero 2014; Gormally et al. 2019*b*), including exposure to a loud radio, cage tapping, a novel human voice (reading out loud), and placing cages on a cart and then rolling the cart.

After 1 wk into the chronic stress stage (day 8), the birds were placed into three groups: control ($n = 8$), mitotane ($n = 10$), or RU486 ($n = 8$). Cagemates received the same treatment. Every other day, the birds received a 100-μL subcutaneous injection of peanut oil (control), peanut oil plus mitotane (0.045 mg/μL), or peanut oil plus RU486 (0.014 mg/μL). Previous studies of white-crowned sparrows (*Zonotrichia leucophrys*) used similar doses of mitotane (Breuner et al. 2000) and RU486 (Landys et al. 2004*b*). The last injection was given the day before the end of the experiment. We used sonication to incorporate mitotane (Bristol-Myers Squibb, Wallingford, CT) and RU486 (Tocris Bioscience, Ellisville, MO) into the solution. Because glucocorticoids help animals mobilize glucose during energetic challenges, chemically adrenalectomized animals are more likely to become hypoglycemic during stressors and thus risk physiological failure and death (Breuner et al. 2000). To prevent this, we added chopped apples to all food dishes so the birds could behaviorally upregulate their glucose levels.

Throughout the entire experiment, baseline and stress-induced blood samples were taken at least once a week so we could measure concentrations of Cort and blood metabolites. We also tested negative feedback efficacy via dexamethasone (DEX) injection at the beginning of the control stage and at the end of the chronic stress stage. During blood sampling and injections, the birds were also weighed to the nearest 0.5 g. The birds were also weighed on some mornings when no blood sampling or injections occurred so that all birds were weighed at least three times per week.

Every night of the experiment, 30 min before or after lights-out, we removed the food dishes from all cages and swept up any spilled food on the cage bottom. Food was returned approximately 30 min after lights-on. Food was removed during the night to ensure that the birds would not eat before the next morning's blood sampling, as this could affect the levels of metabolites. If food was removed after lights-out, a blue light would be used to prevent the birds from being photostimulated. Water and grit were always available ad lib.

At the conclusion of the experiment, all birds were euthanized, and the liver and right pectoralis muscle were removed and weighed. We also fully dissected out and weighed the abdominal and furcular fat depots. The birds were weighed and euthanized one at a time, and organs were weighed immediately to prevent any desiccation. Because we analyzed all organ masses relative to body mass, this should have controlled for potential treatment-induced differences in osmotic balance. All organ harvesting took place on the same day, and the order of birds was randomly determined.

Sampling Procedure and Assay

To control for diel Cort rhythms (Rich and Romero 2001), baseline and stress-induced blood samples were always taken within 30 min of lights-on. Blood samples were obtained from the brachial vein and were collected in heparinized capillary tubes. Baseline samples (~60 μL) were always taken within 3 min of entering the room because Cort levels begin to increase 2–3 min after disturbance (Romero and Reed 2005). The birds were then placed in opaque cloth bags, and stress-induced blood samples

(~30 μL) were taken 30 min later. Restraining birds in cloth bags is a standard acute stressor (Nephew et al. 2003). After taking stress-induced blood samples, the birds were then injected intramuscularly with a 1 mg/kg body weight dose of DEX (Vedco, St. Joseph, MO). The birds were put back into cloth bags, and a blood sample (~60 μL) was taken 90 min later (Lattin et al. 2012). All blood samples were placed on ice until centrifugation. Samples were centrifuged at ~600 *g* within 4 h of collection, and plasma was stored at −20°C until further analysis. All samples were then measured for Cort via radioimmunoassay (Wingfield et al. 1992). The intra- and interassay variations were 3.8% and 9.2%, respectively. Uric acid (BioAssay Systems, QuantiChrom uric acid assay kit, Hayward, CA) and FFA (BioAssay Systems, EnzyChrom free fatty acid assay kit, Hayward, CA) concentrations were measured through colorimetric assay according to the manufacturer's directions.

Statistical Analysis

Because we could not sample all 26 birds for baseline blood samples on the same day, even with multiple researchers taking blood samples, the blood samples were taken over the course of 2–3 d and aggregated into a single time point. For all statistical analyses, we used the middle day as our time point when blood collection took 3 d and the first day as our time point when blood collection took 2 d. Therefore, we have five different time points for Cort and metabolite data: days −5, 0, 6, 13, and 19. To reiterate, chronic stress began on day 1, and treatment injections began on day 8. Because of sample loss during collection or centrifugation, we do not have Cort or metabolite data at every time point for every bird. Therefore, sample sizes differ slightly for each measured variable. For organs, we corrected for body size by dividing organ weight by body weight.

To determine whether chronic stress affected the Cort response, we used repeated-measures linear mixed models to test whether control birds' baseline and stress-induced Cort changed over time (days −5, 0, 6, 13, and 19 and days −5, 6, 13, and 19, respectively). We also examined whether negative feedback and integrated Cort significantly changed from the beginning (day −6) to the end (day 19) of the experiment. Negative feedback was calculated as the relative decrease from stress-induced Cort levels to those 90 min after DEX injection: (stress-induced Cort − post-DEX Cort)/(stress-induced Cort) × 100 (Lattin et al. 2012). If post-DEX Cort levels were higher than stress-induced levels, a negative feedback value of zero was assigned, as there is little biological relevance of having a negative feedback response that is worse than no response. Integrated Cort was calculated as the total amount of Cort an individual was exposed to during an acute stressor and the 90 min after DEX injection (i.e., the area under the curve: (baseline Cort × 30) + [(stress-induced Cort − baseline Cort) × 15] + [(stress-induced Cort − post-DEX Cort) × 45] + (post-DEX × 90)). Using an independent-samples *t*-test, we also compared the control and mitotane integrated Cort at the end of the experiment (day 19) to validate that mitotane treatment had significantly decreased Cort exposure.

To determine whether chronic stress affected body weight, baseline and stress-induced uric acid levels, and baseline FFA levels, we used repeated-measures linear mixed models to test whether these values significantly changed over time in control birds (days −5, 0, 6, 13, and 19 for all variables except stress-induced uric acid and body weight, which were measured on days −5, 6, 13, and 19 and days −5, −2, 0, 3, 6, 8, 10, 12, 14, 16, 18, 19, and 22, respectively). We also used repeated-measures linear mixed models to examine whether uric acid levels significantly changed during acute stress. To determine whether decreased Cort exposure and GR activation mediated these effects of chronic stress, we used repeated-measures mixed models to test whether the mitotane and RU486 treatments affected body weight, baseline and stress-induced uric acid levels, and baseline FFA levels (days 6, 13, and 19 for all variables except body weight, which was measured on days 8, 10, 12, 14, 16, 18, 19, and 22). For significant interactive effects between time and treatment, we ran mixed models on each treatment group separately. We also used one-way ANOVA to test whether treatment significantly affected abdominal fat, furcular fat, pectoralis muscle, and liver mass at the end of the experiment.

All data were analyzed in SPSS (ver. 24). For significant treatment or time effects, we used least squares means post hoc analyses to test for pairwise differences. Baseline Cort, stress-induced Cort, and negative feedback were analyzed separately, as studies have shown these variables to be independently regulated (Romero 2004).

Results

Effects of Chronic Stress on Cort Response (Control Birds)

Baseline Cort levels did not significantly change in control birds over the course of the experiment (fig. 1*a*; mixed model, $F_{4, 31.4} = 1.29$, $P = 0.30$). Stress-induced Cort also did not significantly change over the experimental period in control birds (fig. 1*b*; mixed model, $F_{3, 23.3} = 2.07$, $P = 0.13$). The negative feedback efficacy for control birds was similar before (day −5) and after (day 19) the chronic stress period (fig. 1*c*; mixed model, $F_{1, 7.2} = 0.21$, $P = 0.66$). When calculating the total amount of Cort exposure the birds experienced during an acute stressor, however, we found a trend for integrated Cort to be lower after the chronic stress period (fig. 1*d*; mixed model, $F_{1, 14.5} = 3.98$, $P = 0.06$).

Validation of Mitotane Treatment

At the end of the treatment period (day 19), integrated Cort was significantly lower in mitotane birds compared to that in control birds (fig. 1*d*; two-tailed *t*-test, $t_{14} = 2.15$, $P = 0.04$).

Body Weight

Control birds significantly increased in weight over the chronic stress period (fig. 2; mixed model, $F_{12, 96} = 2.26$, $P = 0.01$; LSD post hoc, days 5–12 < days 18–19; all $P < 0.05$). After 1 wk of chronic stress, body weight significantly increased for all birds (fig. 2; mixed model, time: $F_{12, 312} = 9.97$, $P < 0.001$; LSD post

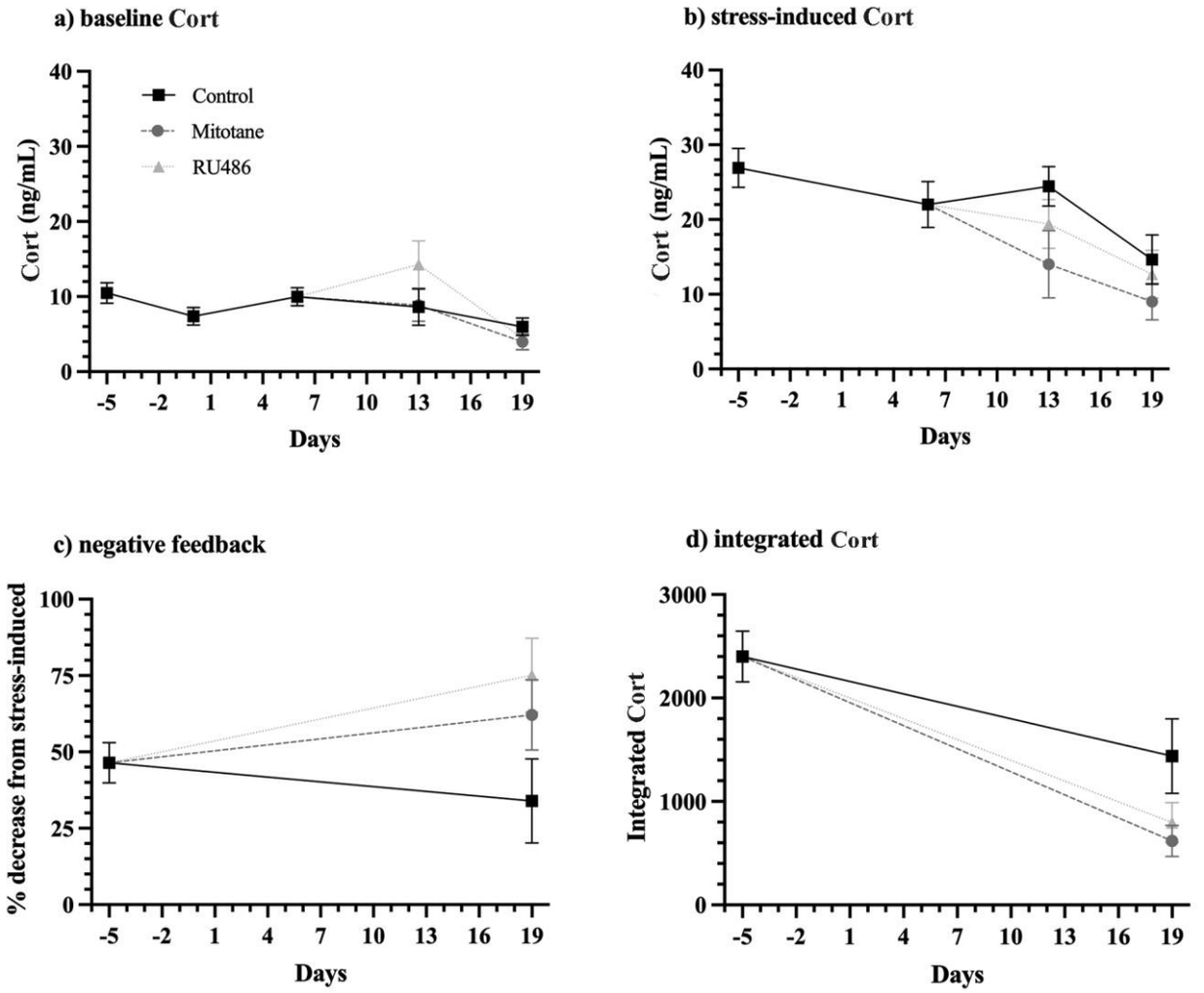

Figure 1. Mean ± SE of baseline corticosterone (Cort; *a*), stress-induced Cort (*b*), negative feedback efficacy (*c*), and integrated Cort (*d*) in control house sparrows ($n = 8$), mitotane-treated house sparrows ($n = 10$), and RU486-treated house sparrows ($n = 8$), *Passer domesticus*. The chronic stress period was from days 1 to 20, and injections started on day 8. Because there was no difference in treatment before injections, all birds were considered control birds until day 8. Negative feedback was calculated as the percentage decrease from stress-induced Cort levels to those 90 min after dexamethasone injection. Integrated Cort was calculated as the total amount of Cort an individual was exposed to during an acute stressor and the 90 min after dexamethasone injection.

hoc, days 8–10 < days 14–22; all $P < 0.05$). While there was no significant effect of treatment on body weight (mixed model, treatment: $F_{2, 26} = 0.11, P = 0.90$), the interactive effect of time and treatment was significant (time × treatment, $F_{24, 312} = 3.25$, $P < 0.001$). When we analyzed body weight from the beginning of the injection period (day 8) to the end of the experiment (day 22), we found that mitotane birds had gained significantly more weight than both control and RU486 birds (fig. 2; mixed model, $F_{2, 23} = 12.01$, $P < 0.001$; LSD post hoc all $P < 0.01$).

Uric Acid Levels

Chronic stress did not significantly affect baseline uric acid levels in control birds (fig. 3*a*; mixed model, $F_{4, 32.1} = 0.27, P = 0.90$). After treatments were applied, baseline uric acid levels did not significantly differ between control, mitotane, and RU486 birds (mixed model, $F_{2, 26.4} = 0.13$, $P = 0.88$) nor was there a significant interaction between time and treatment (mixed model, $F_{4, 51.5} = 0.50$, $P = 0.73$).

The stress-induced uric acid levels were significantly lower than baseline levels (fig. 3; mixed model, $F_{1, 87} = 38.70$, $P < 0.01$). In control birds, there was a trend of stress-induced uric acid levels decreasing over the course of the experiment (fig. 3*b*; mixed model, $F_{3, 19.9} = 2.45$, $P = 0.09$). After several days of chronic stress, stress-induced uric acid levels did not significantly differ over time for all birds or between treatments (fig. 3*b*; mixed model, time: $F_{2, 43.6} = 2.07$, $P = 0.14$; treatment: $F_{2, 22.9} = 0.53$, $P = 0.59$). However, the interactive effect of time and treatment was significant (time × treatment, $F_{4, 43.5} = 4.30$, $P = 0.01$). After running each treatment separately, only RU486 had

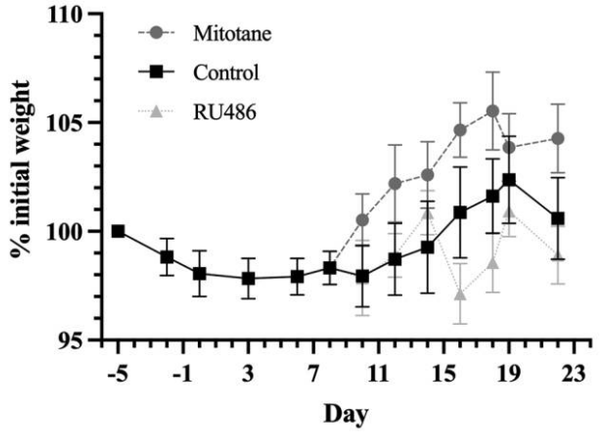

Figure 2. Mean ± SE of percentage of initial body weight of control house sparrows ($n = 8$), mitotane-treated house sparrows ($n = 10$), and RU486-treated house sparrows ($n = 8$), *Passer domesticus*. The chronic stress period was from days 1 to 20, and injections started on day 8. Because there was no difference in treatment before injections, all birds were considered control birds until day 8.

stress-induced uric acid significantly change over time, where levels decreased a few days after RU486 administration began but then returned to pretreatment levels by the end of the experiment (mixed model, time: $F_{2, 12.8} = 8.15$, $P = 0.01$; LSD post hoc, days 6 and 19 < day 13; all $P < 0.05$).

Free Fatty Acid Levels

Chronic stress did not significantly affect FFA levels in control birds (fig. 4; mixed model, $F_{4, 30.4} = 1.65$, $P = 0.19$). After several days of chronic stress, there was no significant effect of time, treatment, or the interaction between time and treatment on FFA levels for all birds (fig. 4; mixed model, time: $F_{2, 49.7} = 0.59$, $P = 0.56$; treatment: $F_{2, 26.3} = 0.54$, $P = 0.59$; time × treatment: $F_{4, 49.6} = 0.80$, $P = 0.53$).

Organ and Fat Weights

RU486 birds tended to have less abdominal fat than control and mitotane birds (fig. 5*a*; one-way ANOVA, $F_{2, 23} = 2.97$, $P = 0.07$), although furcular fat mass did not significantly differ among treatment groups (fig. 5*b*; one-way ANOVA, $F_{2, 23} = 0.97$, $P = 0.39$). Pectoralis (fig. 5*c*; $F_{2, 23} = 0.41$, $P = 0.67$) and liver (fig. 5*d*; $F_{2, 23} = 1.20$, $P = 0.32$) mass also did not significantly differ among the three treatments.

Discussion

This study examined whether increased exposure to circulating Cort levels causes metabolic dysregulation during periods of chronic stress; our results partially support this hypothesis. We reduced Cort exposure and GR-mediated signaling by injecting juvenile house sparrows (*Passer domesticus*) with mitotane or RU486, respectively, during a period of chronic stress. Mitotane treatment did significantly reduce circulating Cort levels, and as expected, mitotane birds weighed more than control birds by the end of the experiment; however, this was not accompanied by lower uric acid levels, lower FFA levels, or increased organ weights. Also contrary to expectations were results from RU486 birds, as this treatment group did not have higher body weights, lower uric acid levels, lower FFA levels, or increased organ weights as predicted. These results suggest that Cort levels are partially responsible for body weight regulation during stress but that the mechanism does not appear to involve increased protein or lipid metabolism. Additionally, GR does not appear to be the primary mediator of Cort's negative effects on body weight.

Manipulating Cort had divergent effects on body weight. Note that these juvenile birds were lighter (about 24 g) than typical adults (27–30 g) and that control birds continued to gain weight

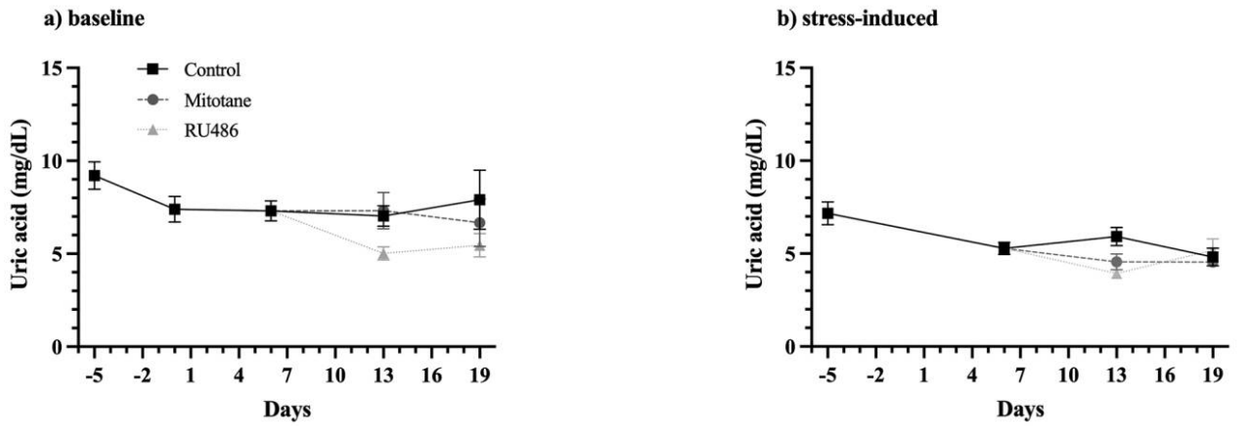

Figure 3. Mean ± SE of baseline (*a*) and stress-induced (*b*) uric acid levels in control house sparrows ($n = 8$), mitotane-treated house sparrows ($n = 10$), and RU486-treated house sparrows ($n = 8$), *Passer domesticus*. The chronic stress period was from days 1 to 20, and injections started on day 8. Because there was no difference in treatment before injections, all birds were considered control birds until day 8.

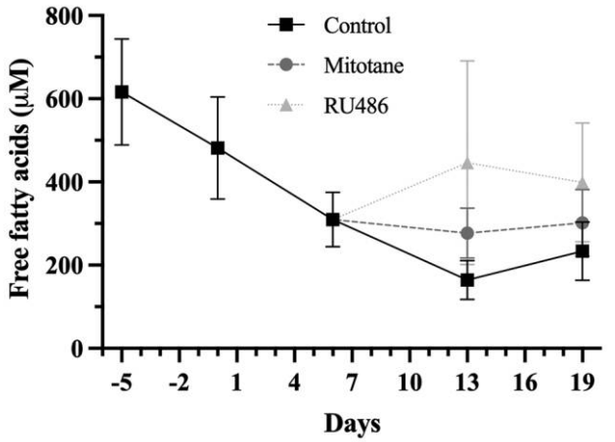

Figure 4. Mean ± SE of free fatty acid concentration in control house sparrows ($n = 8$), mitotane-treated house sparrows ($n = 10$), and RU486-treated house sparrows ($n = 8$), *Passer domesticus*. The chronic stress period was from days 1 to 20, and injections started on day 8. Because there was no difference in treatment before injections, all birds were considered control birds until day 8.

despite the chronic stress. Compared to control birds, decreasing the impact of Cort via RU486 did not alter weight. This is similar to a study of red knots (*Calidris canutus islandica*) preparing for migration where RU486 had no impact on weight, although that study did not examine the impact of stress (Landys et al. 2004*a*). In contrast, we found that mitotane accentuated the increase in weight gain experienced by control birds. This supports earlier evidence that blocking Cort release via mitotane prevented weight loss in adult birds when using the chronic stress protocol used here (DuRant et al. 2016). However, the effect may be stressor or time dependent. When chronic stress was induced by 1 wk of captivity in freshly caught house sparrows, rather than by 3 wk of rotating stressors, mitotane had no effect on body weight (Fischer and Romero 2020). In summary, our findings suggest that elevated Cort levels prevent weight gain, as mitotane birds gained more weight compared to control birds over the chronic stress period, but that this is not mediated via GR binding, as RU486 birds did not differ from control birds.

Acute stress resulted in lower stress-induced uric acid levels (cf. fig. 3*b* and 3*a*). The stress-induced decrease was not surprising. Acute stress generally (Jenni-Eiermann and Jenni 2001; Davies et al. 2013; Deviche et al. 2014, 2016*a*; Cooper et al. 2020), but not always (Viblanc et al. 2018), decreases uric acid levels. However, the connection between stress-induced decreases and Cort is not established. Whereas a stress-induced decrease in uric acid is associated with elevated Cort levels in rufous-winged sparrows (*Peucaea carpalis*; Deviche et al. 2016*b*), it is not in northern cardinals (*Cardinalis cardinalis*; Haskins et al. 2017). Our findings do, however, provide indirect evidence that Cort modulates uric acid levels during acute stress. Blocking Cort signaling via RU486 significantly decreased stress-induced uric acid levels temporarily (fig. 3*b*), suggesting that stress would induce a strong decrease in uric acid that would be counteracted by Cort activating GR. Interestingly, mitotane did not have the same effect as RU486 on stress-induced uric acid levels, which may suggest that MR also plays an important role in uric acid regulation. If Cort does inhibit a reduction in uric acid, it is not clear whether the result is a net positive or negative. Whereas the lack of uric acid decreases may indicate that proteins are not being broken down (Romero and Wingfield 2016), it may also indicate that uric acid is not being consumed as an antioxidant to prevent oxidative damage (Cohen et al. 2008; Haskins et al. 2017). It is also possible that stress could impact plasma uric acid levels via changes in uric acid secretion into avian proximal tubules in the kidney. However, we find this unlikely, as Bataille et al. (2011) found that cellular stress decreases uric acid secretion, which would lead to increased plasma uric acid levels, and is therefore opposite of both acute and chronic stress uric acid patterns in this study.

Baseline uric acid levels did not significantly differ between treatment groups during the chronic stress period; therefore, this suggests that differential exposure to Cort has little to no impact on uric acid levels. This result is supported by Awerman and

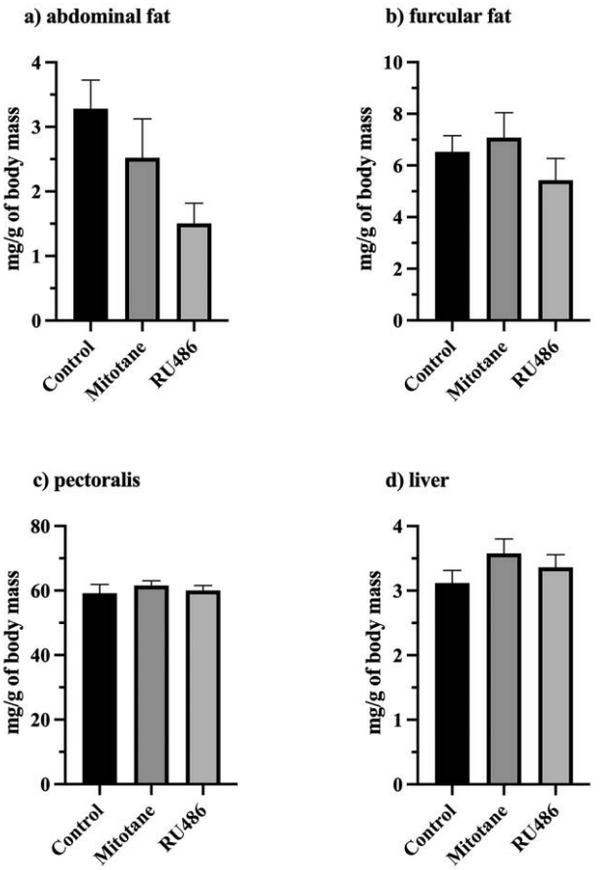

Figure 5. Mean ± SE of each organ mass as by corrected body weight of abdominal fat (*a*), furcular fat (*b*), pectoralis (*c*), and liver (*d*) in control house sparrows ($n = 8$), mitotane-treated house sparrows ($n = 10$), and RU486-treated house sparrows ($n = 8$), *Passer domesticus*, at the end of the experiment.

Romero (2010), who found that European starlings exposed to the same chronic stress protocol used here also did not alter baseline uric acid levels. In contrast, earlier studies in house sparrows using a modified version of this chronic stress protocol had mixed results, with one showing a decrease (Gormally et al. 2018) and another showing an increase (Gormally et al. 2019*a*) in baseline uric acid levels. Data are similarly mixed for other protocols for inducing chronic stress. Overnight exposure to low temperatures while in captivity increased baseline uric acid levels in house sparrows in one study (Cohen et al. 2008), whereas long-term Cort administration in Abert's towhees (*Melozone aberti*), mimicking chronic Cort exposure, showed no impact on baseline uric acid levels (Davies et al. 2013). Similar to baseline uric acid results, we also found that stress-induced uric acid levels did not significantly differ between treatment groups. This finding is supported by similar studies in house sparrows, where exposure to low temperatures (Liknes et al. 2014) and mitotane treatment (Lattin et al. 2015) did not affect stress-induced uric acid levels. Similarly, Davies et al. (2013) found that long-term Cort administration did not significantly impact stress-induced uric acid levels in captive Abert's towhees. Consequently, it remains unclear how or whether Cort exposure alters uric acid levels.

Neither reducing Cort exposure via mitotane administration nor reducing GR activation via RU486 treatment significantly altered FFA levels in juvenile house sparrows. Few studies have examined the effects of Cort exposure on FFAs. Several species of birds reacted to the stress of capture during a migratory bout with decreases in FFA levels (Jenni-Eiermann and Jenni 2001), but acute stress in king penguins (*Aptenodytes patagonicus*) increased fatty acid levels (Viblanc et al. 2018). In addition, house sparrow nestlings exposed to acute stress decrease triglyceride levels (Butler et al. 2020). RU486 also did not alter uric acid or FFAs in red knots (*Calidris canutus islandica*) preparing for migration (Landys et al. 2004*a*) but did decrease FFAs in white-crowned sparrows (*Zonotrichia leucophrys gambelii*) preparing for migration (Landys et al. 2004*b*). However, neither the red knots nor the white-crowned sparrows were stressed in those experiments. While it is possible that subcutaneous injections of our vehicle (peanut oil) may have affected FFA levels in this experiment, we doubt that this is the case, as FFA concentrations tended to decrease after injections started on day 8 and all blood samples were collected at least 23 h or longer after injection. The conclusion from the present experiment is that the house sparrows were not utilizing substantial FFAs for fuel during the chronic stress period, and there is little support for Cort modulating this response.

Finally, consistent with the lack of substantial responses with the metabolites, manipulating Cort during chronic stress had little impact on organ weights. Unfortunately, our protocol measured only organ weight end points, as we did not assess organ weights at different time points of our study. Three previous studies in house sparrows, however, have examined how organ weights change over a period of stress. Using factorial designs, 3 wk of captivity decreased liver mass (Love et al. 2017), but providing a small skin wound had no impact on liver or pectoralis mass during healing (Lattin et al. 2015). In contrast, when we used computed tomography in a repeated-measures design, wild house sparrows brought into captivity (i.e., a significant stressor) and measured 2 wk later had increases in fat volumes and a decrease in pectoralis muscle density, but while similar to the present results, those changes were not altered by mitotane treatment (Lattin et al. 2017). Interestingly, chronic stress alters the distribution of GR in different tissues in the body. After exposing house sparrows to the same chronic stress protocol used here, GR was increased in pectoralis muscle but not in liver or either abdominal fat or furcular fat (Lattin and Romero 2014). This suggests that RU486 might have a different impact on metabolism in the pectoralis muscle during chronic stress, although it did not alter pectoralis mass in the present study. Overall, we found that reducing Cort exposure via mitotane or inhibiting Cort signaling via RU486 had no significant impact on organ weights, even though body mass was significantly higher in mitotane birds when compared to that in control and RU486 birds at the end of the study. This increase in body mass may be due to small increases in several body tissues scattered throughout the body, so that differences were not seen at the individual organ level.

In conclusion, our findings partially support the hypothesis that metabolic dysregulation during chronic stress is caused by increased exposure to glucocorticoids. It is clear that Cort plays a major role in regulating body weight during chronic stress, as blocking Cort release results in substantial weight gain even in the midst of chronic stress. Cort's role, however, is unlikely to be from mobilizing fat or protein stores from fat, muscle, or liver. Blocking Cort's release or action via GR had minimal effects on FFAs, uric acid, or organ weights. Further studies are needed to examine other potential mechanisms, such as Cort binding to MR or synergies between carbohydrate, fat, and protein metabolism during chronic stress.

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