

Corticosterone and insulin interact to regulate glucose and triglyceride levels during stress in a bird

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Remage-Healey, Luke, and L. Michael Romero. Corticosterone and insulin interact to regulate glucose and triglyceride levels during stress in a bird. *Am J Physiol Regulatory Integrative Comp Physiol* 281: R994–R1003, 2001.—Captive European starlings (*Sturnus vulgaris*) were exposed to the stress of handling and restraint while corticosterone, glucose, and triglyceride concentrations were monitored in blood plasma. In saline-injected controls, basal samples were taken within 3 min of disturbance with subsequent samples taken at 40, 70, and 150 min. This was repeated at two times during the daily cycle (day and night) on two different photoperiods: short and long days. During both photoperiods, corticosterone concentrations approximately tripled (compared with a sixfold increase in free-living starlings) and triglyceride concentrations decreased 25–45% in response to stress at both times of the day, whereas an ~25% stress-induced hyperglycemia occurred only at night. Exogenous corticosterone (200 μ g), 1.0 or 4.0 IU/kg of insulin, or a combination of corticosterone with each insulin dose was then separately administered to alter the above responses. Insulin did not affect corticosterone or triglyceride concentrations but resulted in a dose-dependent hypoglycemia of 10–40%. Injected corticosterone resulted in supraphysiological corticosterone concentrations (three- to fivefold higher than normal), yet it did not affect the already altered plasma glucose or triglyceride concentrations. This suggests that glucose output and triglyceride decreases were already maximal in response to handling and restraint. However, the low glucose concentrations resulting from exogenous insulin returned to basal quicker with exogenous corticosterone but only during the day. No response to either hormone showed photoperiodic differences. These data suggest that corticosterone's role in metabolism changes to meet varying energetic demands throughout the day.

daily rhythms; seasonal rhythms; hypoglycemia; energy metabolism

GLUCOCORTICOID RELEASE in response to stress has been well characterized in a variety of species (e.g., 44). The most common glucocorticoid in birds is corticosterone (Cort; Ref. 19), although the exact function of Cort in the avian stress response remains unclear.

One associated action of Cort in birds is the mobilization of energy substrates, especially during stressful situations. Glucocorticoids classically have been shown to inhibit glucose uptake into tissues of the gut and

periphery (29). The majority of glucose mobilization resulting from glucocorticoid stimulation takes place in hepatocytes (24). Adipose tissue is also a site of glucocorticoid-promoted lipolysis, producing an increase in plasma free fatty acids (16, 25, 37). In this way, glucocorticoids appear to release energy stores to help an animal cope with a stressful stimulus, recover from a period of acute stress, or prepare for an upcoming stressor.

Cort has also been proposed as a major antagonistic hormone to the actions of insulin, the primary hormone responsible for glucose uptake throughout the body (14). Glucocorticoids and insulin have been widely shown to work in opposition on energy balance in traditional mammalian models. In rats, for example, glucocorticoids administered centrally stimulate hyperphagia, whereas insulin inhibits feeding (40). Perhaps more important, however, is evidence that glucocorticoids antagonize the effects of insulin at tissues where insulin performs its major storing actions (hepatocytes, adipocytes, and muscle tissue) by exerting opposite, gluconeogenic effects to increase plasma glucose levels (14, 20, 25, 40). Still, little is known about whether this insulin/Cort antagonism on plasma glucose exists in birds.

There are indications that lipids may be a more significant source of energy than glucose in birds, especially during migration and fasting (1, 3, 21, 32, 42). In white-crowned sparrows, feeding decreases when plasma lipids are elevated, but feeding is insensitive to changes in plasma glucose levels (5). Because fat yields twice as much energy and water per gram than does carbohydrate or protein (15, 21), this preference for lipids as the primary energy substrate is far more economical for an organism adapted for flight.

Glucocorticoids released during stress mobilize lipids from adipose tissue, which supports gluconeogenesis. Stored triglycerides are broken down into non-esterified fatty acids (NEFAs), which then can be processed by the liver and other tissues for synthesis of ATP (29). In this way, an increase in plasma NEFAs produces lower plasma triglyceride levels. Stress decreases plasma triglycerides in rats (18, 39), and exogenous ACTH administered to the domestic fowl ele-

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vates Cort levels and leads to increases in plasma NEFAs (17). In Japanese quail, both ACTH and dexamethasone (a synthetic glucocorticoid) administration caused an increase in plasma NEFAs (6). Thus the evidence for stress-induced hyperlipidemia is compelling, although lipid mobilization in response to stress remains relatively unstudied in passerines.

We recently showed that stress elicits both a robust Cort response and a marked hyperglycemic response in a common passerine, the European starling (*Sturnus vulgaris*; Refs. 33, 35). These two studies also demonstrated distinct daily (circadian) and seasonal (circannual) rhythms in both plasma Cort and glucose levels. These results led us to focus on the following three questions: 1) What is the result of the antagonism between insulin and Cort on stress-induced hyperglycemia in starlings? 2) Do plasma triglycerides respond to stress and in what way does the insulin/Cort antagonism affect stress-induced hyperlipidemia? 3) Do these responses vary on either a daily basis or in response to changes in photoperiod? We continue to use starlings so results can be compared with our earlier studies on Cort and glucose.

MATERIALS AND METHODS

Birds. Wild European starlings were captured using mist nets during the winter in Eastern Massachusetts. Juveniles were not captured for use in this study, but some birds may have been hatch-year or second-year birds (31), giving a mixture of young and older birds. Birds were housed communally for several months in large indoor flight aviaries on an 11:13-h light-dark cycle, and 10 birds were transferred to individual cages for 2 wk before injections were initiated ($n = 10$). All rooms were climate controlled and maintained at 25°C for the duration of the study. Light cycles were adjusted throughout the experiment to induce seasonal changes in the birds. Birds were initially maintained on short days (11:13-h light-dark cycle), and once all injections had been completed, birds were shifted to long days (19:5-h light-dark cycle) as described previously (33). Birds were allowed 2 wk to acclimate to the new photoperiod before injections were resumed. Food and water were provided ad libitum. Captive starlings undergo a predictable daily variation in body weight (33). Because body weight is correlated with food intake (27), we can assume that the basal nutritional state of each bird is similar when samples are taken at the same time of the day over multiple days. All experiments were performed according to American Association for Accreditation of Laboratory Animal Care guidelines and approved by the Institutional Animal Care and Use Committee at Tufts University.

Stress/sampling protocols. Initial blood samples were taken within 3 min of entering the room. These were considered basal samples and pooled for statistical purposes as Cort does not significantly respond to stress until after ~3 min (45) and stress-induced hyperglycemia is not evident until 15 to 20 min following acute stress (7, 11, 12, 33, 43). Control birds were administered a saline injection immediately following blood sampling. Basal samples are reported as 0 min; however, injections were administered following basal sampling. Because this is a within-subjects study, and because each bird was bled at exactly the same time of day for each trial, there should be little change in the nutritional state of each bird before each experiment. Consequently, basal samples were only taken for the saline-injected controls.

In separate trials, birds were injected with insulin, Cort, or a combination of insulin and Cort immediately upon entering the room. Administering the injections constituted a period of handling stress. Birds were held in opaque cloth bags after injection for a period of restraint stress, and subsequent samples were taken at 40, 70, and 150 min postinjection. Accordingly, saline injection served as the control for the stress of handling (which included the injection itself) and restraint.

Birds were allowed 48 h between experiments to replenish blood volumes and recover between stressful stimuli. Experiments were conducted at 1100 and 2300 during both photoperiods, because these times approximate the peaks and troughs of both the Cort and glucose daily cycle (33, 35). At each time point, the alar vein was punctured, ~60 μ l of blood were collected into microhematocrit capillary tubes, and cotton stanching blood flow. During lights-off, blue light was used for illumination during sampling, because it is less likely to stimulate photoreceptors and reset circadian rhythms (30).

Seven free-living starlings were captured in the winter to assess Cort responses to stress without the confounding captivity. Birds were subjected to a 45-min period of restraint as described above, with blood samples collected within 2 min of capture and at 15, 30, and 45 min postcapture.

Injections. Within 5 min of entering the experimental chamber, birds received subcutaneous injections in the dorsal neck region of either insulin (human recombinant expressed in *Escherichia coli*; Sigma Chemical) or Cort (dissolved in peanut oil; Sigma Chemical) or a combination of the two hormones. Two doses of insulin were used (1.0 and 4.0 IU/kg), as well as one dose of Cort (200 μ g), and 0.85% saline served as the vehicle control. These combined to give six treatments: saline; 1.0 IU insulin; 4.0 IU insulin; saline + 200 μ g Cort; 1.0 IU insulin + 200 μ g Cort; 4.0 IU insulin + 200 μ g Cort. Each bird was given all six treatments at two times a day, with the individual treatment order randomized. Body weight of the birds in this study ranged from 70 to 85 g.

Justification of doses. The normal range of plasma glucose in birds is ~10–20 mmol/l (22, 23), so the target glucose levels we sought for the highest dose of injected insulin ranged from 6 to 12 mmol/l. Additionally, we sought a dose of insulin that would show biological activity for 2.5 h or more, because postmeal insulin (endogenous) takes ~3 h to store ingested carbohydrates in birds and mammals (29, 42). One study of insulin injections in a passerine, the white-crowned sparrow, demonstrated that low doses of human recombinant insulin (0.5 and 1.0 IU/kg) became ineffective at changing food intake by 30 min postinjection, whereas higher doses (2.0 and 4.0 IU/kg) remained biologically active up to 120 min following injection (4). We determined an optimal dosage range and time course for insulin injection into starlings during a pilot study (Fig. 1A). Two doses of insulin, 1.0 and 4.0 IU/kg, injected subcutaneously were found sufficient to show a dose response of plasma glucose levels to insulin administration over the course of 2.5 h.

In a previous study, we demonstrated that stress-induced Cort levels ranged from 40 to 50 ng/ml in starlings (35), and maximal responses in individual birds did not exceed 100 ng/ml (unpublished observations). We sought to extend this Cort response to mimic the 2.5-h time course in insulin action by administering exogenous Cort at varying doses in a second pilot study (Fig. 1B). This was necessary because negative feedback can rapidly inhibit endogenous Cort release (13). From these data, we concluded that an intermediate dose of 200 μ g Cort would be sufficient to obtain a maximal (although supraphysiological) dose of plasma Cort at 30 min

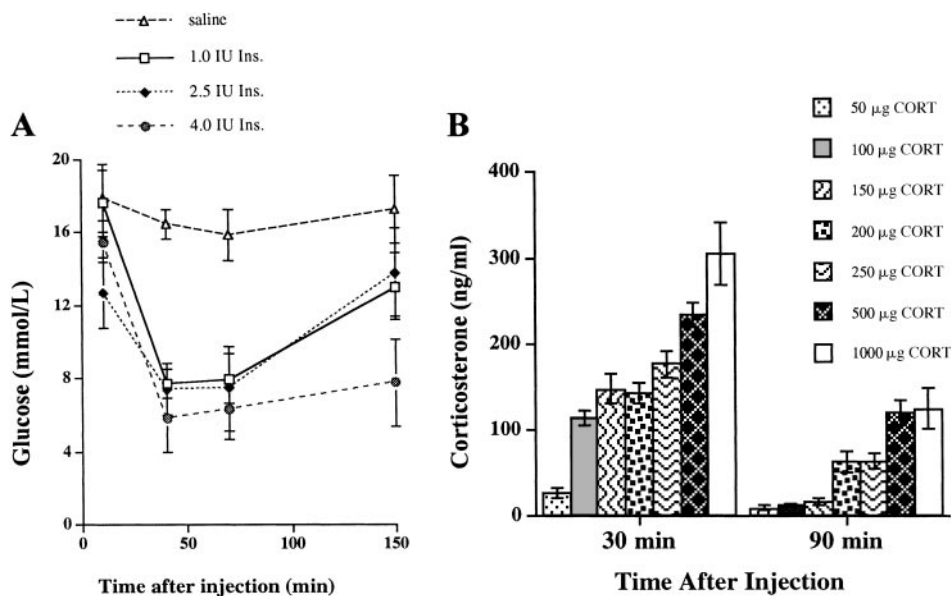


Fig. 1. Plasma glucose (A) and corticosterone (Cort; B) responses to stress under influence of graded doses of exogenous insulin (Ins.) or Cort, respectively, in birds held on short days at 1100.

and return to high physiological (stress induced) Cort levels by 90 min postinjection. This 200- μ g dose was a compromise between high initial levels of Cort and extension of elevated Cort over 1.5 h. This ensured that Cort would remain at normal stress-induced levels or higher for most of the 2.5 h of insulin action.

Sample processing and assays. Hematocrit tubes were sealed with clay at one end and centrifuged at \sim 400 *g* for 5 min. Plasma was removed and frozen until analyzed. Cort was extracted from plasma with dichloromethane and analyzed using a radioimmunoassay as described previously (47). Glucose levels were determined using a hexokinase reagent (Sigma Chemical) combined with spectrophotometry as described previously (33). Plasma triglyceride levels were measured using a lipoprotein lipase/ESPA assay combined with colorimetric spectrophotometry (GPO Trinder, Sigma Chemical). Interassay variability for Cort, glucose, and triglyceride assays was 9.3, 8.5, and 5.9%, respectively. Intra-assay variability for Cort was 6.8%.

Statistics. Data were analyzed using repeated-measures ANOVA for the effects of injection, time of day, and photoperiod on basal and stress-induced (40, 70, and 150 min) levels of Cort, glucose, and triglycerides. *P* values were adjusted to control for performing multiple ANOVAs (38). In addition, the effect of stress on Cort release was analyzed by comparing basal and 40-min plasma Cort levels using paired *t*-tests.

RESULTS

Basic response to stress. Basal levels of Cort and glucose showed distinct circadian variation for birds held on short days (Fig. 2A). Cort levels were higher at 2300 than 1100 [$F(1,18) = 9.14$, $P < 0.01$], but, in contrast, basal glucose concentrations were elevated at 1100 [$F(1,16) = 9.07$, $P < 0.01$]. Triglycerides, on the other hand, showed no circadian variation. Plasma Cort levels significantly increased after saline injection between 0 and 40 min at 1100 ($t = 4.42$, $df = 9$, $P < 0.002$) and at 2300 ($t = 3.83$, $df = 9$, $P < 0.005$). At 1100, Cort levels remained at stress-induced concentrations for the duration of sampling, whereas at 2300, Cort began

to approach basal concentrations by the end of the sampling period. Although plasma glucose did not change after saline injection at 1100, there was a significant hyperglycemic stress response at 2300 through 40 min, after which glucose levels return to baseline [$F(3,12) = 4.98$, $P < 0.02$]. Saline injection also produced a decrease in plasma triglycerides at both 1100 [$F(3,15) = 37.92$, $P < 0.0001$] and 2300 [$F(3,15) = 14.25$, $P < 0.0001$].

The concentrations and responses of Cort, glucose, and triglycerides were slightly different for birds held on long days (Fig. 2B). Although Cort concentrations did not show circadian variation [$F(1,16) = 4.046$, $P = 0.06$] as in short-day birds, basal glucose concentrations were elevated at 1100 [$F(1,16) = 5.286$, $P < 0.04$]. Also, as with birds held on short days, triglycerides show no circadian variation. Cort also increased after saline injection on this photoperiod, with Cort concentrations significantly elevated by 40 min at 1100 [$F(3,24) = 4.437$, $P < 0.02$] and also at 2300 [$F(1,8) = 12.577$, $P < 0.008$]. On long days, Cort approaches basal concentrations by 150 min at both 1100 and 2300. Again, similar to short days, birds held on long days exhibit a hyperglycemic response to stress only at 2300, and the levels are significantly elevated through the entire duration of sampling [$F(3,51) = 3.315$, $P < 0.03$]. Furthermore, triglyceride concentrations decrease in response to stress at both 1100 [$F(3,15) = 30.49$, $P < 0.0001$] and at 2300 [$F(3,15) = 23.77$, $P < 0.0001$], matching patterns on short days.

The Cort response to stress in captive starlings is in the range of the Cort response to stress in free-living starlings (Fig. 2C). Cort concentrations significantly increase throughout the 45 min of restraint [$F(3,18) = 17.93$, $P < 0.0001$] and peak at \sim 43 ng/ml.

Responses to insulin. Although there were indications that Cort concentrations tended to be increased at 70 and 150 min following the high-dose insulin

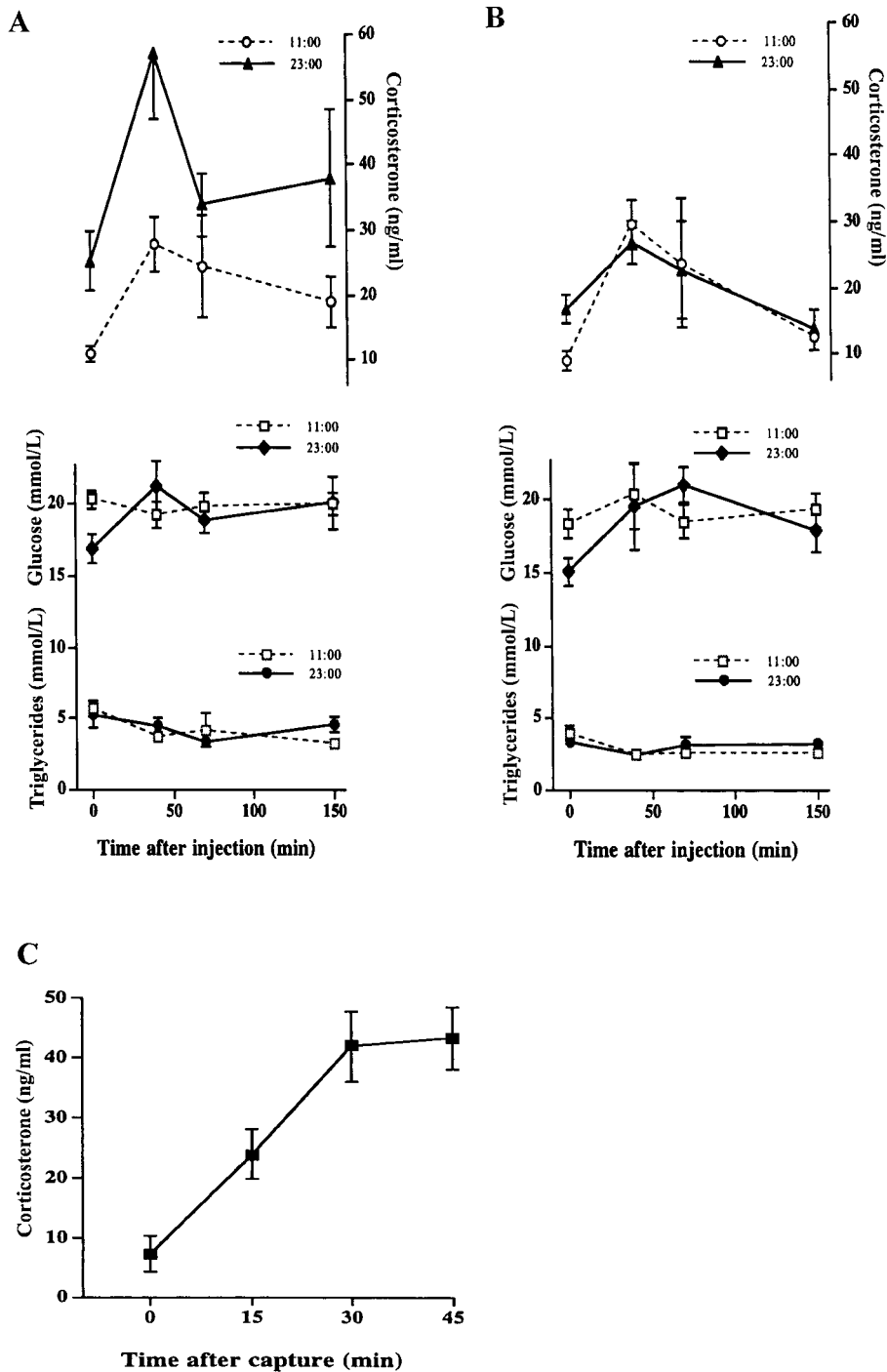


Fig. 2. Glucose, Cort, and triglyceride responses to stress at 1100 or 2300 in birds held on short (A) or long days (B) and Cort response to restraint in free-living starlings during short days (C). Points represent means \pm SE for $n = 10$ for captives and $n = 7$ for free-living starlings. Note differences in scale for Cort graphs.

injection (4.0 IU), this response was not statistically significant (Fig. 3) [although the increase during long days was nearly significant, with $F(1,15) = 5.00$, $P > 0.05$ after adjusting for multiple comparisons]. In contrast, injections of insulin reduced plasma glucose levels (Fig. 4). There was an overall dose-dependent treatment effect as insulin suppressed glucose concentrations at 1100 on both the short-day [$F(2,27) = 39.16$, $P < 0.0001$; Fig. 4A] and long-day [$F(2,23) = 9.15$, $P < 0.002$; Fig. 4C] photoperiods. Insulin also prevented the stress-induced hyperglycemia at 2300 on both short [$F(2,27) =$

20.21, $P < 0.0001$; Fig. 4B] and long days [$F(2,22) = 10.32$, $P < 0.001$; Fig. 4D]. Plasma triglyceride concentrations, however, did not change in response to insulin regardless of photoperiod, time of day, or dose (data not shown).

Responses to Cort. Injections of Cort produced supraphysiological levels of Cort (Fig. 5). In birds held on short days (Fig. 5A), Cort was at maximum 40 min after the injection at both 1100 [$F(3,81) = 39.03$, $P < 0.0001$] and 2300 [$F(3,66) = 59.12$, $P < 0.0001$]. Similarly, in birds held on long days (Fig. 5B), Cort

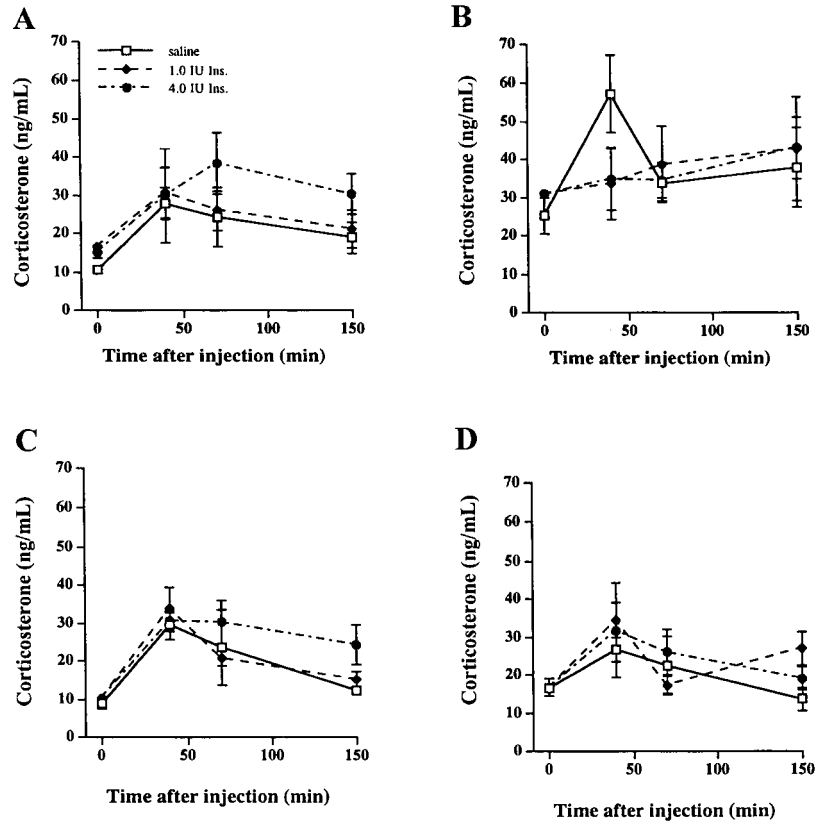


Fig. 3. Cort responses to stress following insulin injection in birds held on short days at 1100 (A), short days at 2300 (B), long days at 1100 (C), or long days at 2300 (D). Points represent means \pm SE for $n = 10$. Saline-injected controls are repeated from Fig. 2.

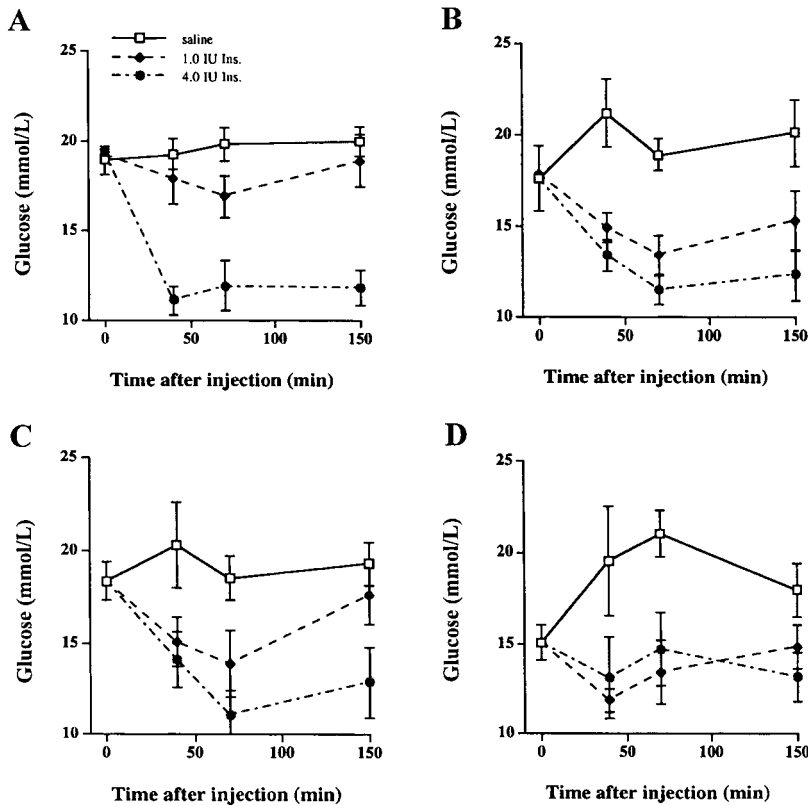


Fig. 4. Glucose responses to stress following insulin injection in birds held on short days at 1100 (A), short days at 2300 (B), long days at 1100 (C), or long days at 2300 (D). Points represent means \pm SE for $n = 10$. Saline-injected controls are repeated from Fig. 2.

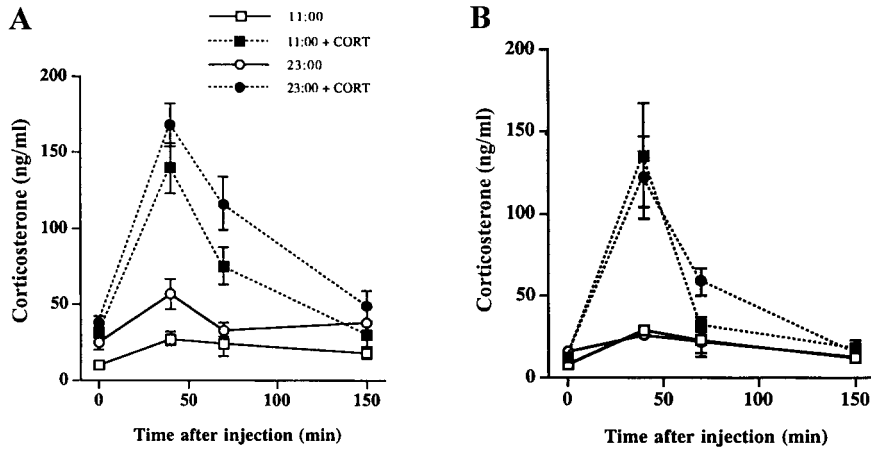


Fig. 5. Cort responses to stress following injection of saline (unmarked) or 200 μg Cort (+Cort) at 1100 or 2300 in birds held on short (A) or long days (B). Points represent means \pm SE for $n = 10$. Saline-injected controls are repeated from Fig. 2.

peaked at 40 min at both 1100 [$F(3,42) = 50.54$, $P < 0.0001$] and 2300 [$F(3,40) = 22.60$, $P < 0.0001$] in response to Cort injection. Exogenous Cort did not alter the glucose response at either time of day or during either photoperiod (Fig. 6) nor did it alter plasma triglyceride concentrations at 1100 in either season (Fig. 7). However, at 2300 on both photoperi-

ods, triglyceride levels appeared to recover after 150 min, and Cort administration seemed to counteract this recovery (Fig. 7). Unfortunately, there was insufficient statistical power to conclusively draw this conclusion [for short day, $F(1,16) = 1.66$, $P = 0.22$ for Cort effect; $F(3,54) = 0.77$, $P = 0.51$ for the interaction of Cort and sampling time; for long day,

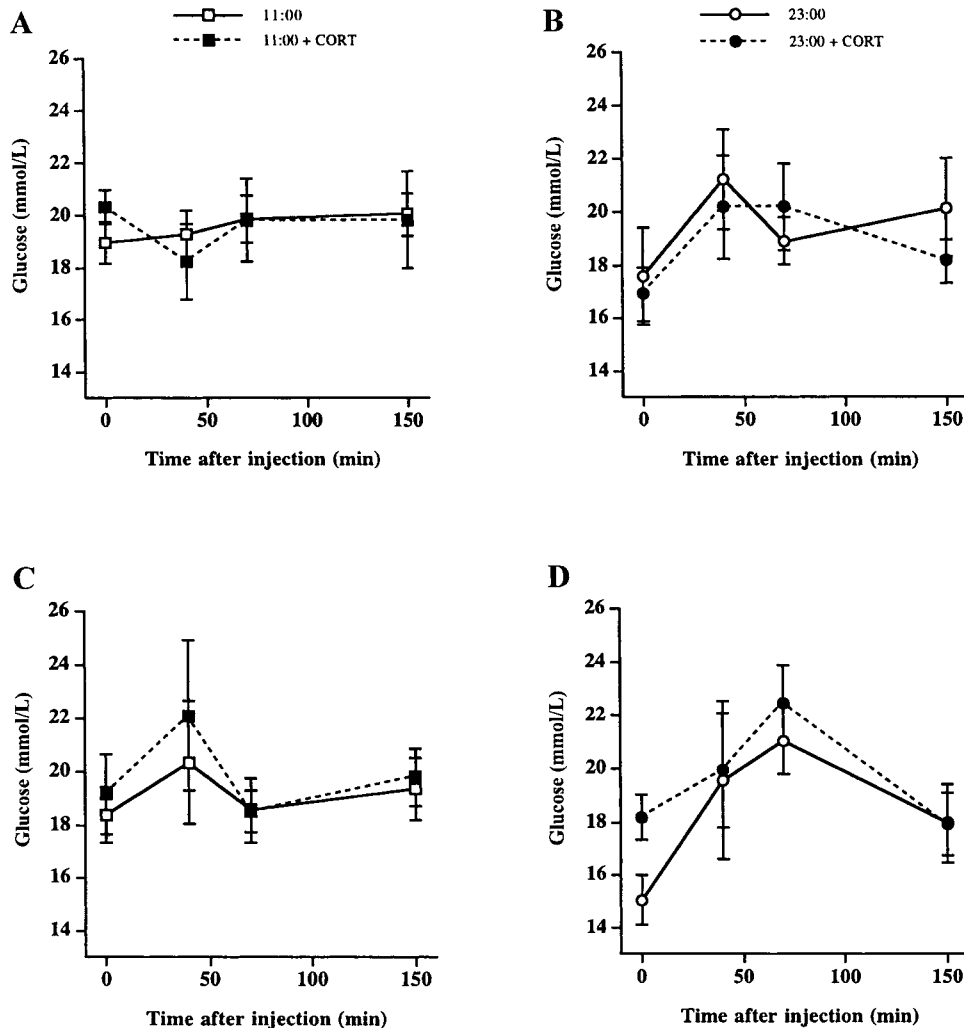


Fig. 6. Glucose responses to stress following injection of saline (unmarked) or 200 μg Cort (+Cort) in birds held on short days at 1100 (A), short days at 2300 (B), long days at 1100 (C), or long days at 2300 (D). Points represent means \pm SE for $n = 10$. Saline-injected controls are repeated from Fig. 2.

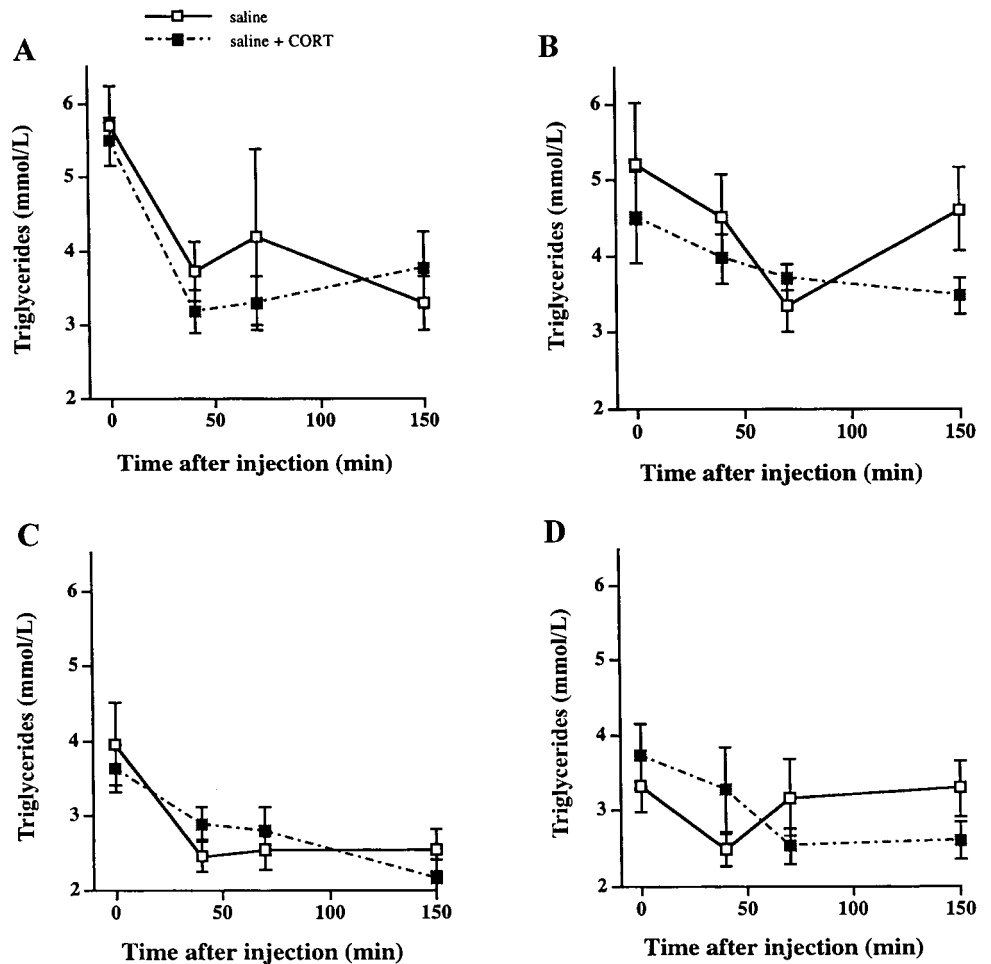


Fig. 7. Triglyceride responses to stress following saline or saline + Cort injection in birds held on short days at 1100 (A), short days at 2300 (B), long days at 1100 (C), or long days at 2300 (D). Points represent means \pm SE for $n = 10$. Saline-injected controls are repeated from Fig. 2.

$F(1,16) = 0.013$, $P = 0.91$ for Cort effect; $F(3,50) = 2.206$, $P = 0.1$ for the interaction of Cort and sampling time].

Interaction between insulin and Cort. Insulin did not alter Cort concentrations in response to exogenous Cort injections, and insulin and Cort did not interact to alter the triglyceride response to stress (data not shown). Exogenous Cort, however, had a limited effect on glucose concentrations by hastening

plasma glucose recovery from insulin-induced hypoglycemia (Fig. 8). This effect was only apparent during the day and did not occur at night during either photoperiod (Fig. 8, A and B). By 150 min at 1100, glucose concentrations in insulin + Cort birds were higher than concentrations in birds that received insulin alone and in birds maintained on short [$F(3,32) = 5.54$, $P < 0.005$] and long days [$F(3,27) = 8.30$, $P < 0.0005$].

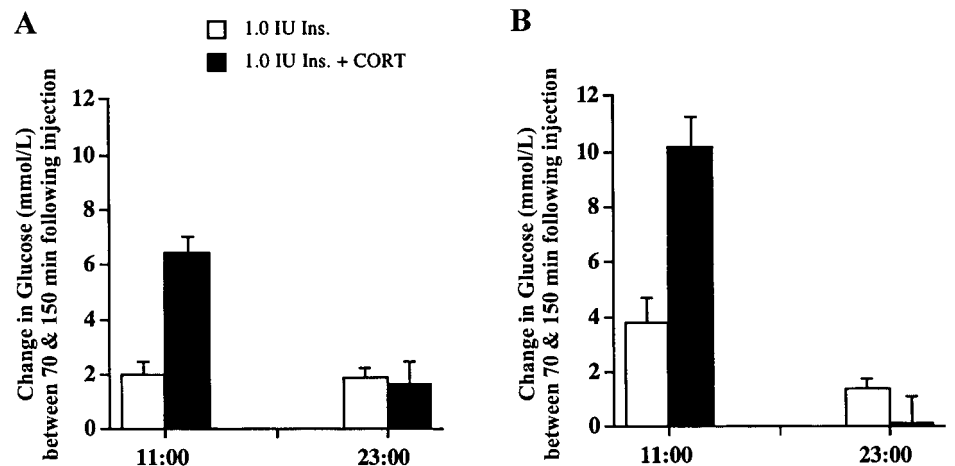


Fig. 8. Change in glucose recovery from insulin injection (1.0 IU/kg) in birds receiving insulin alone (Ins.) or insulin + Cort (Ins. + Cort) on short (A) or long days (B). Points represent means \pm SE of difference between glucose levels at 70 vs. 150 min for $n = 10$. Some data are repeated from earlier figures.

DISCUSSION

Cort. Acute handling and restraint stress caused plasma Cort levels to increase in both photoperiods and at both times of the day. This verifies findings from a previous study in starlings (35) and adds to the growing body of comparative data on stress in wild and captive birds (e.g., 36, 46). This study has shown that stress-induced Cort levels begin to attenuate at 70 min and approach basal (nonstressed) levels by 150 min following initiation of an acute stressful stimulus. To our knowledge, this is the first measure of Cort responses to stress for this length of time in a wild or captive bird. These findings suggest a window of importance for Cort release in response to stress in birds, because after ~2.5 h following a stressor, circulating Cort begins to approach basal levels. Thus even though the birds were repeatedly exposed to the stress of handling and restraint throughout the duration of sampling, it is likely that negative feedback on the hypothalamic-pituitary-adrenal axis (e.g., 13) has reduced the Cort stress response dramatically by 2.5 h after the initial stressor.

This study also supports previous findings of daily and photoperiodic Cort rhythms in starlings. Previous work showed that the daily rhythm in circulating Cort peaked during the scotophase (lights-off) and was at nadir in the middle of the photophase (lights-on, Ref. 35). The present study also shows that birds held on short days have higher Cort levels at night (2300) than in the middle of the day (1100). The exact function of this nighttime peak in Cort remains unclear, but a previous study suggested that Cort treatment induces nocturnal restfulness in passerines (10). Although not statistically significant, a similar trend occurred in birds held on long days. The lack of elevated levels during the night on long days may result from birds on short days being held in darkness for longer at the time of sampling (short-day scotophase begins at 1800) than birds held on long days (long-day scotophase begins at 2200). Because daily Cort levels peak in mid-to-late scotophase (9, 35), perhaps sampling at 2300 in birds held on long days was not close enough to the Cort maximum to contrast nighttime and mid-day Cort concentrations. Absence of a photoperiod difference in basal Cort concentrations between short and long days was expected from a previous study in starlings (35).

The present study used an exogenous dose of Cort in starlings that produced a maximum in pharmacological Cort concentrations for at least 40 min following injection. Cort levels then attenuated rapidly to reach high physiological levels by 70 min, and they approached basal levels by 150 min. Whether this dose (2.67 mg Cort/kg body wt) is appropriate for other passerines remains to be tested (for other methods, see Ref. 8). Additionally, this study showed an indication that insulin-induced hypoglycemia can elevate circulating Cort concentrations. Although not a significant result, the highest dose of insulin (4.0 IU) tended to produce a slight increase in Cort release in both seasons at 1100. This insulin-induced stress response has

been documented in rats (e.g., Ref. 34) and other models. A higher insulin dose may have elicited a significant Cort increase, because human insulin may not be as effective as native starling insulin. However, starling insulin is not available, and we chose human insulin because it has been used in other studies in passerines (e.g., Ref. 4).

Glucose. Plasma glucose levels changed in response to handling and restraint stress only during scotophase sampling under both photoperiods. This result supported earlier findings in the same species (33) where stress elevated glucose only at night (not during the day) in birds held on short days, long days, and during a prebasic molt. This earlier study also showed a circadian rhythm in plasma glucose, with levels highest in mid-day and lowest at night. The present study supported these results, as basal glucose levels were elevated at 1100 over 2300 in both photoperiods. One reason proposed for the lack of a mid-day hyperglycemic response to stress is the variability associated with food intake through the active period (33). Because the birds are feeding throughout the photophase, perhaps it is difficult to turn on a strong hyperglycemic response while plasma glucose levels are elevated at mid-day. The release of insulin to clear postmeal surges in glucose levels may prevent a bird from mobilizing a hyperglycemic stress response until after the storing activity of insulin has subsided. However, we chose to perform these studies on fed, rather than fasted, birds to more closely mimic natural conditions.

The injection of the potent hypoglycemic hormone insulin during mid-day and at night allowed us to address these questions. In this study, insulin administration caused a dose-dependent hypoglycemic response during the day and also completely shut off the stress-induced hyperglycemic response at night in both photoperiods. We then used a Cort injection to see if the hyperglycemic response could be restored by administering pharmacological doses of glucocorticoid alongside the insulin injection. Interestingly, even 1.0 IU insulin (which has been used as a low dose in other passerine injection studies; e.g., Ref. 4) caused a hypoglycemic response even in the presence of supraphysiological doses of Cort. This effect was independent of photoperiod or time of day, but it was only present for approximately the first hour postinjection. In other words, the effects of insulin vs. insulin + Cort injections were statistically similar only through the 70-min sample. By 150 min, injected Cort appeared to hasten the recovery of plasma glucose levels from the effects of insulin at 1100 in both photoperiods. This result supports one role that glucocorticoids are thought to play in the stress response by exerting permissive effects on the hyperglycemic actions of epinephrine and glucagon (12, 26). Because Cort, as a steroid hormone, primarily affects the rates of transcription in target cells (29), the permissive effects on glucose levels may take a longer time to develop. In this study, at 1100 in both seasons, Cort concentrations in response to Cort injection are approaching basal levels by 150 min, yet this is the only time point that glucose levels show the effects of

injected Cort. This supports the role of Cort as a permissive agent of hyperglycemia in birds.

The attenuation of insulin-induced hypoglycemia by Cort was not evident in either season at 2300. This suggests that the hormones Cort permissively acts on to cause hyperglycemia may not be as effective during the scotophase. A more complete understanding of this system calls for further study of the disparity between mid-day and nighttime roles of Cort in hyperglycemia.

Triglycerides. Plasma triglyceride levels decreased in response to stress in all groups, independent of photoperiod, time of day, or injection. This result was expected in light of previous studies in mammals (18, 39). Whether this is an effect of glucocorticoids or another system responsive to stress (e.g., catecholamine release) remains to be tested. Glucocorticoids are known to inhibit synthesis of triglycerides from NEFAs (2), but epinephrine has also been shown to increase triglyceride lipase activity (29).

Circulating triglycerides did not exhibit circadian variation in basal levels, indicating that plasma lipids may remain at relatively constant levels throughout the daily cycle in this species. Whether this pattern is true of basal lipids throughout the 24-h cycle is unknown.

Recent evidence has shown that small birds exhaust their glycogen reserves in the first few hours of an overnight fast and that NEFAs derived from stored triglycerides become the primary fuel for the remainder of the scotophase (21). In light of this, perhaps falling triglyceride levels in response to stress are less tolerable at night, and regulatory mechanisms that bring levels back to basal are activated faster during scotophase than photophase. This may prevent triglycerides from falling below critical levels at times when they are needed most. A post hoc inspection of the present data suggested that triglycerides recovered from stress-induced depletion at night and that this recovery was eliminated by Cort injection. However, the power of this test was not sufficient to draw significant conclusions. Nonetheless, because lipids appear to be the primary source of energy for small birds (1, 3, 21, 32, 41), these results are intriguing and warrant further study.

This study showed that injections of Cort were unable to stimulate a further reduction in plasma triglycerides below stress-induced levels. This suggests that lipid levels are tightly regulated in birds and will not fall below a precise range, even with further stimulation by increased Cort. Support for this idea comes from feeding behavior changes in white-crowned sparrows in response to altered triglyceride levels (4).

Surprisingly, insulin injection produced no effect on triglyceride levels at either dose. In light of previous studies where insulin increased NEFA levels in white-crowned sparrows (5) and domestic hens (17) and also increased triglyceride levels in geese (28), it is unclear what the relationship is between insulin and plasma lipids in birds. As with the effects of Cort, the present study suggests that plasma lipid levels are tightly

regulated in birds and may not deviate from a critical range, despite insulin action.

Perspectives

This study aims to further an understanding of the adaptive function of glucocorticoid release during stress. We have long known that Cort increases in response to stress and that Cort can alter glucose levels, yet we still have only a rudimentary idea of how this helps animals survive stressful periods. This information is especially lacking in taxa such as birds. This study has three main results that can help address this problem. First, there appears to be a window of importance for Cort release in birds, because stress-induced Cort concentrations begin to approach basal levels ~2.5 h following acute stress. Second, the adaptive function of hormone release during stress changes to meet varying energetic demands throughout the day. Consequently, the hormones released during stress can exert very different effects over the 24-h daily cycle. For example, stress-induced hyperglycemia only occurs at night, and Cort can only counteract insulin's actions during the day. Third, this study shows that plasma lipid levels are responsive to stress and suggests that lipids are insensitive to manipulations of both Cort and insulin concentrations in a bird. Because lipids are an important energy substrate in birds, an understanding of lipid regulation during stress in birds is fundamental to appreciate the adaptive significance of the vertebrate stress response.

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