ELSEVIER

Plasma corticosterone increases during migratory restlessness in the captive white-crowned sparrow *Zonotrichia leucophrys gambelli*

Mēta M. Landys*, John C. Wingfield, Marilyn Ramenofsky

Department of Biology, University of Washington, Seattle, WA 98195, USA

Abstract

Plasma corticosterone increases during the period of spring migration in a variety of bird species. Long-distance migrants show elevations in corticosterone specifically in association with the stage of flight, suggesting that corticosterone may support flight-related processes, for example, locomotor activity and/or energy mobilization. The pattern of corticosterone secretion as it relates to migratory flight has hitherto not been clearly described in migrants that frequently interrupt flight to refuel, for example, the Gambel's white-crowned sparrow (*Zonotrichia leucophrys gambelii*). The Gambel's white-crowned sparrow fuels by day and expresses peak migratory activity during the first few hours of night. To determine if plasma corticosterone increases in association with the stage of migratory flight also in this short-bout migrant, we induced captive white-crowned sparrows to enter into the migratory condition by placing photosensitive birds on long days (16L:8D) and then evaluated birds for plasma corticosterone and locomotor activity during four time points of the day. Patterns found in long-day birds were compared to those observed in short-day controls (8L:16D). Differences in energy metabolism as determined from plasma metabolites were also evaluated. We found that locomotor activity and corticosterone were significantly elevated at the onset of the dark period, but only in long-day birds. Plasma β-hydroxybutyrate (a ketone body) was also elevated. Thus, findings suggest that plasma corticosterone and ketogenesis increase in association with migratory restlessness in a short-bout migrant. In fact, corticosterone may play a regulatory role, because it shows a trend to increase already before night-time activity.
© 2004 Elsevier Inc. All rights reserved.

Introduction

Migrating birds must precisely regulate behavior and physiology if travel from wintering sites to breeding grounds is to be completed successfully. Glucocorticoid hormones may support the expression of migratory processes because plasma levels of corticosterone increase in association with spring migration in a variety of avian species (e.g., Holberton, 1999; Holberton et al., 1996; Romero et al., 1997). Moreover, elevations occur specifically in association with migratory flight, suggesting the

regulation of flight-associated behavior and physiology. For instance, migrating bar-tailed godwits (*Limosa lapponica)* show elevated plasma levels of corticosterone at the conclusion of a 2-day nonstop flight (Landys-Ciannelli et al., 2002). In addition, captive red knots (*Calidris canutus*) increase plasma corticosterone after having attained a spring body mass similar to that of migrating conspecifics preparing to initiate long-distance flight (Piersma et al., 2000) and free-living red knots display elevated corticosterone as they arrive onto their breeding grounds in the North American arctic (Reneerkens et al., 2002).

Because corticosterone promotes the mobilization of stored lipids (Landys et al., 2004; Nagra et al., 1963), the observed pattern of corticosterone secretion in long-bout migrants suggests that elevations in corticosterone may

---

* Corresponding author. Department of Biology, University of Oslo, PO Box 1050, N-0316 Oslo, Norway. Fax: +47 22854605.
E-mail address: meta.landys@bio.uio.no (M.M. Landys).

assist animals in meeting the energetic demands associated with extended activity. Corticosterone may also support the direct expression of migratory activity; that is, corticosterone treatment promotes restlessness during the period of migration (Dolnik and Blyumental, 1967; Meier and Martin, 1971). Corticosterone treatment in birds has also been shown to affect general locomotion (Astheimer et al., 1992; Bruener et al., 1998), and individuals held on long days appear to be especially sensitive to such effects (Breuner and Wingfield, 2000).

In contrast to long-bout migrants, short-bout migrants undertake flights that last only a few hours each day. Such a migration strategy is common when travel is conducted over hospitable terrain and can be freely interrupted. Although short bouts of flight are not as energetically demanding, evidence suggests that corticosterone levels also increase in association with short-bout migration. For example, Holberton (1999) described the annual pattern of corticosterone secretion in the captive yellow-rumped warbler (*Dendrioca coronata*) and found that plasma corticosterone was elevated in association with the migratory peak in body mass, when birds typically initiate departure activities. Furthermore, Schwabl et al. (1991) showed that migratory activity affected the diel corticosterone pattern in the garden warbler (*Sylvia borin*). Finally, Ramenofsky et al. (1999) demonstrated that dark-eyed juncos (*Junco hyemalis*) display higher plasma levels of corticosterone during nights marked by migratory restlessness. Such correlations between corticosterone and migratory activity suggest that corticosterone may play an important role also in the support of short-bout migration.

The Gambel's white-crowned sparrow (*Zonotrichia leucophrys gambelii*) is a classic short-bout migrant—this species fuels by day and expresses peak migratory activity during the first few hours of night (King and Farner, 1963; Ramenofsky et al., 2003). In addition, white-crowned sparrows display a quiescent period (a period of inactivity) immediately before onset of night-time restlessness, during which time the gut is emptied and food is metabolized to reduce excess weight (Morton, 1967). Past research has demonstrated that corticosterone levels in the white-crowned sparrow are elevated during the general period of spring migration (Romero et al., 1997). Moreover, Breuner et al. (1999) has shown that photosensitive white-crowned sparrows placed on long days (20L:4D) show elevated corticosterone levels during the dark phase, which is the period typically marked by migratory restlessness. However, data from this latter study do not indicate the precise relationship between plasma corticosterone and migratory activity. Breuner et al. (1999) did not record locomotor activity of birds, and birds were sampled up to 6 weeks after photostimulation—when migratory intensity is usually on the decline.

To expand on previous findings and to determine the specific relationship between corticosterone and migratory restlessness in a short-bout migrant, we stimulated photo-

sensitive Gambel's white-crowned sparrows to enter into peak migratory condition by placing them on long days (16L:8D) and then examined birds for plasma corticosterone and locomotor activity. We hypothesized that during the period of spring migration, plasma corticosterone would peak specifically during early night, when white-crowned sparrows show intense activity in relation to migration. In contrast, we predicted that short-day controls (8L:16D) would display low plasma corticosterone at this time, as previously determined by Breuner et al. (1999). We also measured potential metabolic fuels in the blood plasma to determine whether changes in plasma corticosterone levels correlate with the mobilization of stored energy depots. We predicted that the early-night period would be characterized by an increased mobilization of fat stores—the primary fuel source used during active migration (Jenni-Eiermann and Jenni, 1991, 1992).

Methods

Animals

Gambel's white-crowned sparrows were captured during fall migration in Sunnyside, Washington, in the months of September and October 2002. After capture, sparrows were housed in outdoor flight aviaries at the University of Washington. In January 2003, nine wintering birds were placed into an environmental chamber kept on short days (8L:16D). We allowed animals to acclimate to new surroundings for 3 weeks before initiating experiments. Nine birds that were to represent long-day birds were placed into a second environmental chamber and were exposed to a long-day photoperiod (16L:8D) for 3 weeks. This stimulated entry into a migratory state that was characterized by night-time restlessness (King and Farner, 1963; Ramenofsky et al., 2003). Experiments were initiated at this time, and were run for a total of 2 weeks. We discontinued experiments after this time to ensure that data were collected only when birds were in peak migratory condition (Ramenofsky et al., 2003). Animal use was approved by the Animal Care Committee at the University of Washington: protocol 2212-29.

Temperature in both environmental chambers was set to vary between 20 and 12°C during the day and night, respectively. Birds were provided with ad libitum bird chow and wild bird seed mix at all times. A nightlight (<1 lx) was provided to simulate moonlight/starlight, without which nocturnal migrants such as the white-crowned sparrow typically do not express migratory behavior (M. Ramenofsky, unpublished observations).

Data collection

Body mass, fat score and daily food intake were determined for each bird on the first day of the experimental period (21 days after photostimulation for long-day

birds) to verify that long-day birds and short-day controls were in the migratory and wintering conditions, respectively (King and Farner, 1963). Fat depots were scored in the furcular fossa and in the abdominal cavity on an arbitrary scale ranging from 0 to 5 (Wingfield and Farner, 1978). A score of 0 represents no fat, and a score of 5 indicates the presence of bulging fat bodies. Food intake was determined by weighing food trays with bird food mix before and after a 24-h feeding period, and is reported here as grams of food consumed per day. Food spillage from trays was minimized by allowing only a small opening in trays for food access. Differences in body mass between long-day birds and short-day controls were compared with a Student's *t* test. Differences in fat score and daily food intake were compared with nonparametric Mann–Whitney Rank Sum Tests because data did not satisfy normality requirements.

To determine whether plasma corticosterone increases in association with night-time migratory restlessness, we sampled individual birds repeatedly during different time points of the day. Because birds remain in peak migratory condition for only 2 weeks, and at least 3–4 days should be allowed for recovery between blood sampling procedures, we were able to sample each bird only four times. Past research on the white-crowned sparrow indicates that plasma corticosterone remains uniformly low throughout the photophase both under long days (20L:4D) and short days (8L:16D), and begins to increase only with the onset of the scotophase (the dark period) (Breuner et al., 1999). In addition, Gwinner (1967) has shown that migratory restlessness in nighttime migrants is stimulated by the onset of darkness. Therefore, as we were limited in the number of time points that we could sample, and as previous research has shown that plasma corticosterone does not markedly change during the photophase (Breuner et al., 1999), we chose to sample birds in reference to onset of the scotophase. We were especially interested in investigating the correlation between plasma corticosterone and activity during (1) the transitional quiescent period that preceeds the onset of darkness and (2) the period of early night, when birds display peak locomotor activity in association with the migration condition. We also sampled birds at the beginning and end of the photophase.

We determined locomotor activity (hops/min) in long-day birds and short-day controls during the following four half-hour periods: morning (1.5–2 h after lights-on), evening (1.5–1 h before lights-off), early night (0.5–1 h after lights-off), and predawn (1.5–1 h before lights-on). The four periods were sampled in random order. Locomotor activity was recorded in registration cages equipped with motion detectors (Radio Shack) (Sperry et al., 2003; Wikelski et al., 1999). When birds crossed an infrared beam of light running parallel to and above their perch, the electric signal was transmitted to a computer and catalogued as an instance of activity by Labview Program (National Instruments). Differences in activity among the four periods

were evaluated with a two-way repeated-measures ANOVA. Photoperiod (long days or short days) was included in the model as a factor. Activity data was normalized through log_{10}-transformation.

Blood sampling

Blood samples were collected at the conclusion of each of the four activity periods for the determination of associated plasma levels of corticosterone. Birds bled during dark periods were exposed to short pulses of dim blue light (30 lx) to illuminate chambers while collecting samples. We verified that these light pulses did not result in photo-stimulation of short-day birds by measuring gonad size 4 weeks after the conclusion of experiments. In contrast to the large gonads (>2 mm) found in long-day birds, the gonads of all short-day birds were small (<1 mm), suggesting that these birds were not photo-stimulated.

Blood samples were obtained by puncturing the alar wing vein with a sterile 26-gauge needle. Blood droplets were collected into heparinized microhematocrit capillary tubes within 3 min of entry into chambers. Corticosterone levels as determined from these samples reflect undisturbed corticosterone concentrations (Wingfield et al., 1982) and are henceforth referred to as baseline levels. To examine corticosterone levels in response to an unpredictable perturbation, we subjected birds to a standardized handling-stress protocol (Wingfield et al., 1995) and collected a blood sample 30 min following initial disturbance. Birds were held in individual cloth bags between the baseline and 30-min bleeds. Differences in both baseline and handling-induced levels of corticosterone among the four examined periods were tested with two-way repeated-measures ANOVAs. Photoperiod was included in the test as a factor. All corticosterone data were normalized with log_{10}-transformation before use in statistical tests.

Hematocrit (packed red cell volume) was determined from blood collected during the first bleed only, that is, 21 days after long-day birds were photo-stimulated. Blood was collected into heparinized microhematocrit capillary tubes and was spun for 5 min in a microhematocrit centrifuge. Hematocrit was then measured as the percent cellular fraction of total blood volume. Differences in hematocrit between long-day birds and short-day controls were compared with a Student's *t* test.

Blood collected during the morning and early-night periods was also evaluated for plasma concentrations of five metabolites: triglycerides, free fatty acids, uric acid, β-hydroxybutyrate, and glucose. Triglycerides are the storage form of lipids. They are synthesized in the liver and are transported to peripheral tissues for deposition into fat bodies, but can also enter the blood via dietary absorption (Robinson, 1970). Free fatty acids are released when triglycerides are hydrolyzed and indicate lipid mobilization from adipose tissue (Elia et al., 1987; Hurley et al., 1986; Scow and Chernick, 1970). Uric acid results

from the breakdown of protein that originates from body tissue or the diet (Lindgård et al., 1992; Mori and George, 1978; Robin et al., 1987). β-Hydroxybutyrate (a ketone body) is synthesized from free-fatty acids and substitutes for glucose in the heart and in nervous tissue during energy shortage (Goldstein et al., 1993; Robinson and Williamson, 1980). Plasma concentrations of each of these five metabolites were compared between the morning and early-night periods with a two-way repeated-measures ANOVA. Photoperiod was included in the model as a factor. Metabolite concentrations were $\log_{10}$-transformed to normalize data for use in statistical analyses.

Plasma for the determination of corticosterone and metabolites was stored in microcentrifuge tubes at −20 and at −80°C, respectively. Corticosterone concentrations were determined by direct radioimmunoassay (RIA) exactly as described in Landys et al. (2004). All samples were run within a single assay. Intraassay variation as determined from 10 known standards run in a separate assay was 8.7%.

Metabolite concentrations in blood plasma were determined on a microplate spectrophotometer (BIO-TEK Instruments) exactly as described in Landys et al. (2004).

Results

We compared body mass, fat score, daily food intake, and hematocrit between long-day birds and short-day controls. Body mass ($t = -6.338$, $P < 0.001$) and fat score ($t = 45.000$ and $P < 0.001$) were significantly higher under long days (Fig. 1). Long-day birds displayed greater daily food intake than short-day birds ($t = 60.000$, $P = 0.027$), and also showed a trend for higher hematocrit ($t = -1.881$, $P = 0.078$).

Average locomotor activity was not different between long-day birds and short-day controls ($F_{1,33} = 2.247$, $P = 0.162$) (Fig. 2A). Additionally, locomotor activity did not vary among examined time periods ($F_{3,33} = 1.816$, $P = 0.163$). However, a significant interaction between photoperiod regime and time period suggests that the pattern of activity over the course of the day was different between long-day birds and short-day controls ($F_{3,33} = 3.629$, $P = 0.023$). Data indicate that long-day birds showed higher activity levels than short-day birds during the early-night period (Fisher's LSD post hoc test: $P = 0.005$). Activity was similar between long-day birds and short-day controls during the other examined periods: morning ($P = 0.501$), evening ($P = 0.269$) and predawn ($P = 0.437$) (see Fig. 2A).

Average baseline levels of corticosterone were higher in long-day birds than in short-day controls ($F_{1,48} = 5.923$, $P = 0.027$) (Fig. 2B) and changed significantly across the four examined time periods ($F_{3,48} = 21.217$, $P < 0.001$). In addition, the interaction between photoperiod regime and time period was significant ($F_{3,48} = 4.735$, $P = 0.006$), suggesting that the pattern of corticosterone secretion changed differently between birds held on different photo-
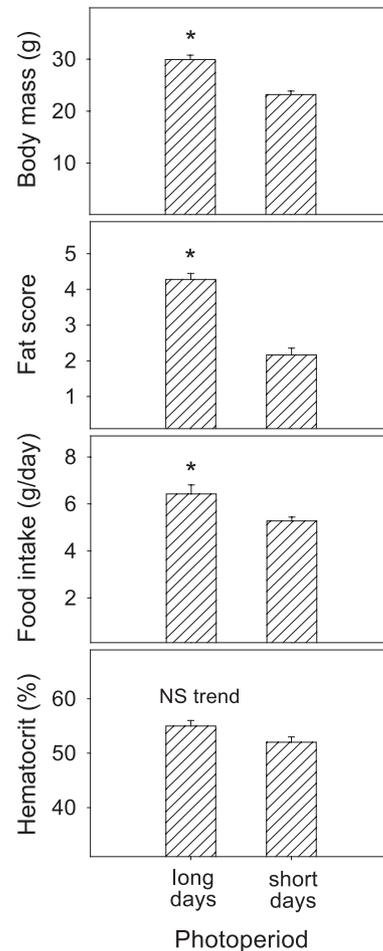

Fig. 1. Body mass (g), fat score, daily food intake (g/day), and hematocrit (%) of Gambel's white-crowned sparrows held under long days (16L:8D) or short days (8L:16D). Fat was scored on an arbitrary scale ranging from 0 to 5: a score of 0 represents no fat, and a score of 5 indicates the presence of bulging fat bodies. Long-day birds were photo-stimulated 21 days before data collection. Long-day and short-day conditions are each represented by nine birds. Asterisks indicate significant differences between life-history stages. Error bars represent standard errors of the mean.

periods. Specifically, baseline plasma corticosterone was higher during the early-night period in long-day birds than in short-day controls (Fisher's LSD post hoc test: $P = 0.006$). Long-day birds also showed a trend for higher baseline levels in the evening ($P = 0.059$). Baseline plasma corticosterone was similar between photoperiod groups during the morning ($P = 0.384$) and predawn periods ($P = 0.212$) (see Fig. 2B).

Handling-induced plasma levels of corticosterone were not different between long-day birds and short-day controls ($F_{1,48} = 1.090$, $P = 0.312$) (Fig. 3). However, levels were significantly different among the four time periods examined ($F_{3,48} = 17.097$, $P < 0.001$) (see Fig. 3). The interaction between photoperiod regime and time period was not significant ($F_{3,48} = 0.840$, $P = 0.479$).

Photoperiod regime significantly affected concentrations of plasma metabolites. Plasma β-hydroxybutyrate levels were comparatively higher in long-day birds than in short-
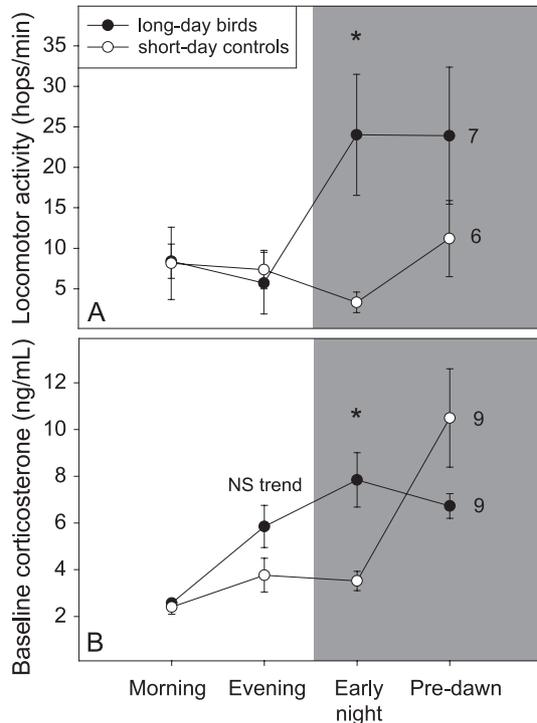
Fig. 2. (A) Average locomotor activity (hops/min) of long-day birds and short-day controls during four half-hour time periods: morning, evening, early night, and predawn. Long-day birds displayed significantly elevated activity in comparison to short-day controls during the early-night period. (B) Plasma corticosterone levels (ng/ml) of long-day birds and short-day controls at the conclusion of the same four half-hour time periods. Blood samples for the determination of baseline corticosterone levels were collected within 3 min of disturbance. In comparison to short-day controls, long-day birds showed significantly elevated baseline levels during the early-night period. Long-day birds also displayed a trend for higher baseline levels in the evening. Sample size is indicated. Error bars represent standard errors of the mean. Light/dark cycles for long-day birds and short-day controls were 16L:8D and 8L:16D, respectively. The shaded area denotes samples that were collected during the dark phase. Asterisks indicate significant differences between photoperiod regimes. This figure is not meant to depict a diel rhythm in locomotor activity or plasma corticosterone.

day controls ($F_{1,16} = 12.931$, $P = 0.002$), whereas plasma triglycerides ($F_{1,16} = 8.182$, $P = 0.011$) and plasma uric acid levels ($F_{1,16} = 32.056$, $P < 0.001$) were comparatively higher in short-day birds (Fig. 4). Plasma levels of free-fatty acids ($F_{1,16} = 0.421$, $P = 0.526$) and glucose ($F_{1,16} = 2.801$, $P = 0.114$) did not change with photoperiod.

Plasma metabolites varied significantly also according to time period. Plasma triglycerides ($F_{1,16} = 36.711$, $P < 0.001$) and plasma uric acid ($F_{1,16} = 16.946$, $P < 0.001$) were higher during the morning than during early night, both in long-day birds and short-day controls (interaction terms: $F_{1,16} = 0.397$, $P = 0.537$ and $F_{1,16} = 0.965$, $P = 0.340$, respectively; Fig. 4). In contrast, plasma levels of β-hydroxybutyrate were higher during the early-night period ($F_{1,16} = 9.284$, $P = 0.008$), but only in long-day birds (interaction term: $F_{1,16} = 36.523$, $P < 0.001$). Plasma levels of free-fatty acids ($F_{1,16} = 0.094$, $P = 0.763$) and glucose ($F_{1,16} = 0.510$, $P = 0.485$) did not change with time period.

Discussion

Past studies have demonstrated that the Gambel's white-crowned sparrow displays increased fat stores and body mass in association with the period of spring migration (King and Farner, 1963; Romero et al., 1997; Wingfield et al., 1996). In this study, we verified that photostimulation led to an increase in body mass and fat score, as well as to an increase in food intake. In addition, there was a trend for hematocrit—another attribute that characteristically increases in association with migration (DeGraw et al., 1979; Morton, 1994), to be elevated under long days. Thus, we are confident that the long-day photoconditions employed in this study were effective in promoting birds to enter into a migratory state.

To investigate the pattern of corticosterone secretion in the migratory Gambel's white-crowned sparrow, we determined plasma corticosterone levels in relation to the daily expression of migratory activity. We found that birds in the migratory condition showed elevated corticosterone levels during the period of early night—the time at which migratory restlessness is maximal (King and Farner, 1963; Ramenofsky et al., 2003) (see Fig. 2). In contrast, and as already shown by Breuner et al. (1999), birds held on short days displayed low plasma corticosterone during early night, and were inactive. These data suggest that corticosterone levels increase specifically in association with periods of migratory activity, and thus, that corticosterone may participate in the support of correlated migratory behaviors and physiological processes. It is interesting to note that although plasma corticosterone in short-day birds was elevated before the onset of day, this increase was not
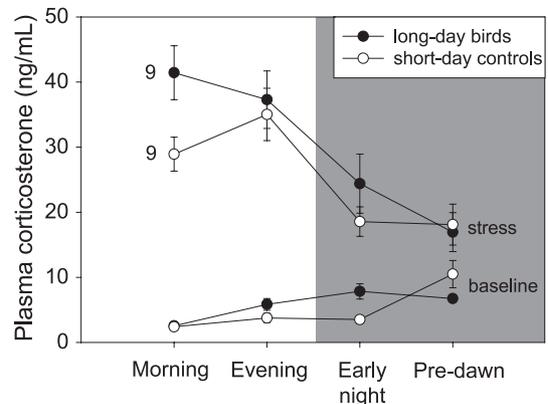

Fig. 3. Handling-induced plasma levels of corticosterone (ng/ml) in long-day birds and short-day controls during four time periods: morning, evening, early night, and predawn. Samples were evaluated for plasma corticosterone after 30 min of handling stress. Handling-induced levels of corticosterone were similar between photoperiod regimes. Sample size is indicated. Error bars represent standard errors of the mean. Light:dark cycles for long-day birds and short-day controls were 16L:8D and 8L:16D, respectively. The shaded area denotes samples that were collected during the dark phase. For purposes of comparison, baseline levels of corticosterone (taken from Fig. 2B) are also shown. This figure is not meant to depict a diel rhythm in plasma corticosterone.
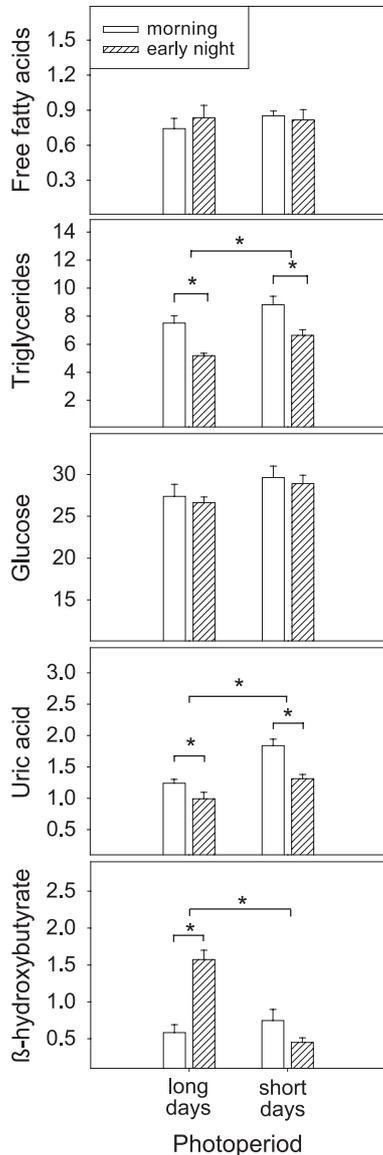
Fig. 4. Plasma metabolite concentrations (mmol/l) of long-day birds and short-day controls during the periods of morning and early night. Long-day and short-day conditions are each represented by nine birds. Error bars represent standard errors of the mean. Asterisks indicate significant differences between time periods and/or between photoperiod regimes.

associated with a significant increase in locomotor behavior. Activity levels of birds held on short days appear to be less sensitive to increased corticosterone (Breuner and Wingfield, 2000), possibly as a consequence of relatively lower glucocorticoid receptor numbers (Breuner and Orchinik, 2001; Landys et al., 2004).

We found that plasma corticosterone in long-day birds was elevated not only in association with migratory restlessness, but also showed a trend to increase during the transitional quiescent period that precedes onset of dark (see Fig. 2). Such data suggest that migrants may show elevations in corticosterone already in anticipation of migratory activity. A preemptive pattern of corticosterone secretion has previously been hypothesized by Landys-

Ciannelli et al. (2002), who showed elevated corticosterone levels in godwits that were preparing to depart a stopover site. Commensurate with this hypothesis, Ramenofsky et al. (1999) found that during the final stages of daytime feeding, night-active migratory juncos increased plasma levels of corticosterone. Thus, much in the same way as diel corticosterone elevations in most vertebrates anticipate ensuing demands of the active period (Dallman et al., 1993), preflight elevations in corticosterone may help migrants to prepare for the impending energetic demands of migratory activity.

Although baseline corticosterone was elevated in association with migratory restlessness, levels did not approach maximal concentrations: at all sampled time points, baseline levels were at least half of those induced by 30 min of handling stress (see Fig. 3). This suggests that migration in itself does not constitute an unpredictable perturbation, but is a predictably demanding period within an animal's life history that may be in part supported by an increase in circulating corticosteroids (Wingfield et al., 1998). Furthermore, peak levels of corticosterone occurred during periods marked by the nadir in baseline corticosterone (see Fig. 3). Such a pattern in corticosterone stress-responsiveness confirms past work conducted both on this species (Breuner et al., 1999) and in mammals (e.g., Bradbury et al., 1991; Dallman et al., 1992), and has been attributed to neural mechanisms—baseline corticosterone levels do not affect feedback signals in the rat (Bradbury et al., 1991).

We evaluated plasma levels of five metabolites in long-day birds and short-day controls to determine how energy metabolism correlates with the expression of migratory restlessness. Under long days, birds showed elevated plasma β-hydroxybutyrate during the early-night period (see Fig. 4), suggesting an increase in ketone body production from fats specifically in association with migratory activity. Such an effect may be stimulated by corticosterone: hypercortisolemia has been shown to increase plasma levels of β-hydroxybutyrate in the dog (Goldstein et al., 1993). Past research on various bird species confirms that plasma β-hydroxybutyrate increases in association with periods of energetic demand (Jenni-Eiermann and Jenni, 1991, 1994; Jenni and Schwilch, 2001; Schwilch et al., 1996). Because β-hydroxybutyrate may serve as a glucose-sparing mechanism in the brain and the heart (Robinson and Williamson, 1980), ketogenesis may be particularly important for migrating birds, which do not store glycogen in preparation for migration (King et al., 1963).

Although fats are a major fuel source during migration (Jenni-Eiermann and Jenni, 1991, 1992), captive white-crowned sparrows did not show elevated free-fatty acids in association with the period of migratory restlessness (when corticosterone is elevated) (see Fig. 4). This result is somewhat surprising because corticosteroids are strongly lipolytic (reviewed in Landys, 2003). However, studies are beginning to suggest that endogenous glucocorticoids do not induce lipid breakdown in the absence of stress or

energetic challenge. For example, antagonism of the glucocorticoid receptor in birds does not affect plasma free-fatty acids unless animals are subjected to the energetic stress of fasting (Landys et al., 2004). Thus, lack of change in free-fatty acids in the present study suggests that captive migrants may not undergo the energetic demands typical of wild populations. The fact that glucose levels were also unaffected further suggests that energetic demands were low. In free-living birds, plasma glucose decreases with migratory flight (Jenni-Eiermann and Jenni, 1991).

Plasma levels of triglycerides and uric acid were higher during active daytime feeding than during the night (see Fig. 4). This suggests an anabolic state during daylight hours—a high rate of fat storage and dietary protein breakdown, respectively. Interestingly, levels of both triglycerides and uric acid were higher in short-day controls than in long-day birds. This suggests a comparatively increased energy acquisition rate in the wintering condition (Jenni and Schwilch, 2001; Jenni-Eiermann and Jenni, 1994) and may be due to limited foraging time under short days (white-crowned sparrows do not feed at night; Morton, 1967). Indeed, we found that short-day controls consumed food at a rate of 0.66 ± 0.02 g/h of daylight, while long-day birds consumed food at a rate of only 0.40 ± 0.02 g/h of daylight (Student's t test: $t = 8.178$, $P < 0.001$).

In summary, we have demonstrated that in association with the period of spring migration, Gambel's white-crowned sparrows show an increase in corticosterone specifically during the early-night period, when migratory restlessness is maximally expressed. Data suggest that corticosterone may even increase before migratory activity, to perhaps regulate lipolysis and glucose sparing. For example, plasma β-hydroxybutyrate was significantly elevated in conjunction with the increase in plasma corticosterone. Corticosterone may also be involved in the support of migratory activity, because administration of corticosterone equivalents increases migratory restlessness in various passerine species (Dolnik and Blyumental, 1967; Meier and Martin, 1971). In short, corticosterone may function as a key endocrine element to ensure that behavior and energy metabolism are appropriately regulated to meet perceived and/or anticipated demands during the period of migration.

References

Astheimer, L.B., Buttemer, W.A., Wingfield, J.C., 1992. Interactions of corticosterone with feeding, activity and metabolism in passerine birds. Ornis Scand. 23, 355–365.

Bradbury, M.J., Cascio, C.S., Scribner, K.A., Dallman, M.F., 1991. Stress-induced adrenocorticotropin secretion: diurnal responses and decreases during stress in the evening are not dependent on corticosterone. Endocrinology 128, 680–688.

Breuner, C.W., Orchinik, M., 2001. Seasonal regulation of membrane and intracellular corticosteroid receptors in the house sparrow brain. J. Neuroendocrinol. 13, 412–420.

Breuner, C.W., Wingfield, J.C., 2000. Rapid behavioral response to corticosterone varies with photoperiod and dose. Horm. Behav. 37, 23–30.

Breuner, C.W., Greenberg, A.L., Wingfield, J.C., 1998. Noninvasive corticosterone treatment rapidly increases activity in Gambel's white-crowned sparrows (*Zonotrichia leukophrys gambelli*). Gen. Comp. Endocrinol. 111, 386–394.

Breuner, C.W., Wingfield, J.C., Romero, L.M., 1999. Diel rhythms of basal and stress-induced corticosterone in a wild, seasonal vertebrate, Gambel's white-crowned sparrow. J. Exp. Zool. 284, 334–342.

Dallman, M.F., Akana, S.F., Scribner, K.A., Bradbury, M.J., Walker, C.D., Strack, A.M., Cascio, C.S., 1992. Stress, feedback and facilitation in the hypothalamo–pituitary–adrenal axis. J. Neuroendocrinol. 4, 517–526.

Dallman, M.F., Strack, A.M., Akana, S.F., Bradbury, M.J., Hanson, E.S., Scribner, K.A., Smith, M., 1993. Feast and famine: critical role of glucocorticoids with insulin in daily energy flow. Front. Neuroendocrinol. 14, 303–347.

DeGraw, W.A., Kern, M.D., King, J.R., 1979. Seasonal changes in the blood composition of captive and free-living white-crowned sparrows. J. Comp. Physiol., B 129, 151–162.

Dolnik, V.R., Blyumental, T.I., 1967. Autumnal premigratory and migratory periods in the chaffinch (*Fringilla coelebs coelebs*) and some other temperate zone passerine birds. Condor 69, 435–468.

Elia, M., Zed, C., Neale, G., Livesey, G., 1987. The energy cost of tryglyceride-fatty acid recycling in non-obese subjects after an overnight fast and four days of starvation. Metabolism 36, 251–255.

Goldstein, R.E., Wasserman, D.H., McGuinness, O.P., Lacy, D.B., Cherrington, A.D., Abumbad, N.N., 1993. Effects of chronic elevation in plasma cortisol on hepatic carbohydrate metabolism. Am. J. Physiol. 264, E119–E127.

Gwinner, E., 1967. Wirkung des Mondlichtes auf die Nachtaktivität von Zugvögeln. Lotsenversuch an Rotkehlchen, *Erithacus rubecula*, und Gartenrotschwänzen, *Phoenicurus phoenicurus*. Experientia 23, 227.

Holberton, R.L., 1999. Changes in patterns of corticosterone secretion concurrent with migratory fattening in a Neotropical migratory bird. Gen. Comp. Endocrinol. 116, 49–58.

Holberton, R.L., Parrish, J.D., Wingfield, J.C., 1996. Modulation of the adrenocortical stress response in Neotropical migrants during autumn migration. Auk 113, 558–564.

Hurley, B.F., Nemeth, P.M., Marcin III, W.H., Hagberg, J.M., Dalsky, G.P., Holloszy, J.O., 1986. Muscle triglyceride utilization during exercise: effect of training. J. Appl. Physiol. 60, 562–567.

Jenni, L., Schwilch, R., 2001. Plasma metabolite levels indicate change in body mass in reed warblers *Acrocephalus scirpaceus*. Avian Sci. 1, 55–65.

Jenni-Eiermann, S., Jenni, L., 1991. Metabolic responses to flight and fasting in night-migrating passerines. J. Comp. Physiol., B 161, 465–474.

Jenni-Eiermann, S., Jenni, L., 1992. High plasma triglyceride levels in small birds during migratory flight: a new pathway for fuel supply during endurance locomotion at very high mass-specific metabolic rates? Physiol. Zool. 65, 112–123.

Jenni-Eiermann, S., Jenni, L., 1994. Plasma metabolite levels predict individual body-mass changes in a small long-distance migrant, the garden warbler. Auk 111, 888–899.

King, J.R., Farner, D.S., 1963. The relationship of fat deposition to Zugunruhe and migration. Condor 65, 200–233.

King, J.R., Barker, S., Farner, D.S., 1963. A comparison of energy reserves during autumnal and vernal migratory periods in the white-crowned sparrow, *Zonotrichia leucophrys gambelli*. Ecology 44, 513–521.

Landys, M.M., 2003. The role of baseline corticosterone in the regulation of avian migration. PhD thesis, University of Washington, Seattle, USA.

Landys-Ciannelli, M.M., Ramenosky, M., Piersma, T., Castricum Ringing Group, Wingfield, J.C., 2002. Baseline and stress-induced plasma corticosterone during long-distance migration in the bar-tailed godwit, *Limosa lapponica*. Physiol. Biochem. Zool. 75, 101–110.

Landys, M.M., Ramenofsky, M., Guglielmo, C., Wingfield, J.C., 2004. The low-affinity glucocorticoid receptor regulates feeding and lipid break-down in the migratory Gambel's white-crowned sparrow *Zonotrichia leucophrys gambelii*. J. Exp. Biol. 207, 143–154.

Lindgård, K., Stokkan, K.A., Le Maho, Y., Groscolas, R., 1992. Protein utilization during starvation in fat and lean Svalbard ptarmigan (*Lagopus mutus hyperboreus*). J. Comp. Physiol., B 162, 607–613.

Meier, A.H., Martin, D.D., 1971. Temporal synergism of corticosterone and prolactin controlling fat storage in the white-throated Sparrow, *Zonotrichia albicollis*. Gen. Comp. Endocrinol. 17, 311–318.

Mori, J.G., George, J.C., 1978. Seasonal changes in serum levels of certain metabolites, uric acid and calcium in the migratory Canada goose (*Branta canadensis interior*). Comp. Biochem. Physiol., B 59, 263–269.

Morton, M.L., 1967. Diurnal feeding patterns in white-crowned sparrows (*Zonotrichia leucophrys gambelii*). Condor 69, 491–512.

Morton, M.L., 1994. Hematocrits in montane sparrows in relation to reproductive schedule. Condor 96, 119–126.

Nagra, B., Breitenbach, R.P., Meyer, R.K., 1963. Influence of hormones on food intake and lipid deposition in castrated pheasants. Poult. Sci. 42, 770–775.

Piersma, T., Reneerkens, J., Ramenofsky, M., 2000. Baseline corticosterone peaks in shorebirds with maximal energy stores for migration: a general preparatory mechanism for rapid behavioral and metabolic transitions? Gen. Comp. Endocrinol. 120, 118–126.

Ramenofsky, M., Savard, R., Greenwood, M.R.C., 1999. Seasonal and diel transitions in physiology and behavior in the migratory dark-eyed junco. Comp. Biochem. Physiol., A 122, 385–397.

Ramenofsky, M., Agatsuma, R., Barga, M., Cameron, R., Harm, J., Landys, M., Ramfar, T., 2003. Migratory behavior: new insights from captive studies. In: Bertold, P., Gwinner, E. (Eds.), Avian Migration. Springer Verlag, Heidelberg, pp. 97–111.

Reneerkens, J., Morrison, R.I.G., Ramenofsky, M., Piersma, T., Wingfield, J.C., 2002. Baseline and stress-induced levels of corticosterone during different life cycle substages in a shorebird on the high arctic breeding grounds. Physiol. Biochem. Zool. 75, 200–208.

Robin, J.-P., Cherel, Y., Girard, H., Géloen, A., Le Maho, Y., 1987. Uric acid and urea in relation to protein catabolism in long-term fasting geese. J. Comp. Physiol., B 157, 491–499.

Robinson, D.S., 1970. The function of the plasma triglycerides in fatty acid transport. In: Florkin, M., Stotz, E.H. (Eds.), Comprehensive Bio-chemistry, vol. 18. Elsevier, Amsterdam, pp. 51–105.

Robinson, A.M., Williamson, D.H., 1980. Physiological roles of ketone bodies as substrates and signals in mammalian tissues. Physiol. Rev. 60, 143–187.

Romero, L.M., Ramenofsky, M., Wingfield, J.C., 1997. Season and migration alters the corticosterone response to capture and handling in an arctic migrant, the white-crowned sparrow (*Zonotrichia leucophrys gambelli*). Comp. Biochem. Physiol., C 116, 171–177.

Schwabl, H., Bairlein, F., Gwinner, E., 1991. Basal and stress-induced corticosterone levels of garden warblers, *Sylvia borin*, during migration. J. Comp. Physiol., B 161, 576–580.

Schwilch, R., Jenni, L., Jenni-Eiermann, S., 1996. Metabolic responses of homing pigeons to flight and subsequent recovery. J. Comp. Physiol., B 166, 77–87.

Scow, R.O., Chernick, S.S., 1970. Mobilization, transport, and utilization of free fatty acids. In: Florkin, M. (Eds.) Comprehensive Biochemistry vol. 18. Elsevier, Amsterdam, pp. 19–50.

Sperry, T.S., Thompson, C.K., Wingfield, J.C., 2003. Effects of acute treatment with 8-OH-DPAT and fluoxetine on aggressive behavior in male song sparrows (*Melospiza melodia morphna*). J. Neuroendocrinol. 15, 150–160.

Wikelski, M., Lynn, S., Breuner, C., Wingfield, J.C., Kenagy, G.J., 1999. Energy metabolism, testosterone and corticosterone in white-crowned sparrows. J. Comp. Physiol., A 185, 463–470.

Wingfield, J.C., Farner, D.S., 1978. The endocrinology of a natural breeding population of the white-crowned sparrow (*Zonotrichia leucophrys pugetensis*). Physiol. Zool. 51, 188–205.

Wingfield, J.C., Smith, J.P., Farner, D.S., 1982. Endocrine response of white-crowned sparrows to environmental stress. Condor 84, 399–409.

Wingfield, J.C., O'Reilly, K.M., Astheimer, L.B., 1995. Modulation of the adrenocortical responses to acute stress in arctic birds: a possible ecological basis. Am. Zool. 35, 285–294.

Wingfield, J.C., Hahn, T.P., Wada, M., Astheimer, L.B., 1996. Interrelationship of day length and temperature on the control of gonadal development, body mass, and fat score in white-crowned sparrows, *Zonotrichia leucophrys gambelii*. Gen. Comp. Endocrinol. 101, 242–255.

Wingfield, J.C, Maney, D.L., Breuner, C.W., Jacobs, J.D., Lynn, S., Ramenofsky, M., Richardson, R.D., 1998. Ecological bases of hormone–behavior interactions: the "emergency life history stage". Am. Zool. 38, 191–206.