Comparative Biochemistry and Physiology, Part A 152 (2009) 46–52

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Exogenous and endogenous corticosterone alter feather quality

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ARTICLE INFO

Article history:
Received 9 July 2008
Received in revised form 21 August 2008
Accepted 21 August 2008
Available online 5 September 2008

Keywords:
Birds
Protein
Molt
Corticosterone
Feather quality
Feathers
Stress

ABSTRACT

We investigated how exogenous and endogenous glucocorticoids affect feather replacement in European starlings (*Sturnus vulgaris*) after approximately 56% of flight feathers were removed. We hypothesized that corticosterone would retard feather regrowth and decrease feather quality. After feather regrowth began, birds were treated with exogenous corticosterone or sham implants, or endogenous corticosterone by applying psychological or physical (food restriction) stressors. Exogenous corticosterone had no impact on feather length and vane area, but rectrices were lighter than controls. Exogenous corticosterone also decreased inter-barb distance for all feathers and increased barbule number for secondaries and rectrices. Although exogenous corticosterone had no affect on rachis tensile strength and stiffness, barbicel hooking strength was reduced. Finally, exogenous corticosterone did not alter the ability of *Bacillus licheniformis* to degrade feathers or affect the number of feathers that failed to regrow. In contrast, endogenous corticosterone via food restriction resulted in greater inter-barb distances in primaries and secondaries, and acute and chronic stress resulted in greater inter-barb distances in rectrices. Food-restricted birds had significantly fewer barbules in primaries than chronic stress birds and weaker feathers compared to controls. We conclude that, although exogenous and endogenous corticosterone had slightly different effects, some flight feathers grown in the presence of high circulating corticosterone are lighter, potentially weaker, and with altered feather micro-structure.

1. Introduction

One hallmark of the stress response is the production of glucocorticoids (Romero, 2004). Glucocorticoids are produced in response to stressors, whether anthropogenic (e.g., restraint, disturbance) or natural (e.g., predator attacks, storms), and are thought to serve as one of the central regulators that orchestrates successful physiological and behavioral responses to perturbations (Sapolsky et al., 2000; Wingfield and Romero, 2001). The dominant glucocorticoid in birds is corticosterone, which elevates plasma glucose concentrations via increased protein breakdown and decreased peripheral glucose utilization (Eigler et al., 1979; Sapolsky et al., 2000). Plasma corticosterone concentrations are modulated seasonally in many birds, with concentrations lowest during the prebasic molt, when all feathers are replaced in many species (Romero and Wingfield, 1999; Romero, 2002; but see Heath et al., 2003).

Avian molt replaces worn feathers and is seasonal, with most passerines molting once or twice each year. Feather production is energetically costly, increasing basal metabolic rate 9–111% in some species (King, 1974; Lindström et al., 1993), and requires significant protein sequestration (Murphy, 1996). During molt, a large amount of protein is shed and replaced; depending on the species feathers can be 4–12% of an individual's body mass (Murphy, 1996). The quality of a bird's feathers is central to its fitness for many reasons, including predator escape (e.g., Swaddle et al., 1996; Swaddle et al., 1999) and influencing mate selection (e.g., Fitzpatrick, 1998; Ferns and Lang, 2003; Pryke and Andersson, 2005). Growth bars in feathers are wider when a bird has good nutrition, so they can indicate individual quality in both males and females. Growth bars have been correlated with reproductive success in some species (e.g., Takaki et al., 2001), and they also might be an indirect indicator of territory quality (Witter and Lee, 1995).

Feather quality and molting efficiency can influence individual energetic expenditures associated with aerodynamic efficiency (e.g., Dawson et al., 2000). Feather gaps in wings during molt decrease flight efficiency and reduce escape success from predators (e.g., Swaddle et al., 1996, 1999; Hedenström, 2003), and poor quality feathers can break, creating gaps that are not replaced immediately through molt. For instance, Tucker (1991) reported that when primaries are molted in Harris's Hawk (*Parabuteo unicinctus*), gliding performance decreases by 40%. The effects on efficiency, however, are not equal across all flight feathers. Hedenström and Sunada (1999) modeled different patterns of molt gaps and found that gaps in secondary feathers had a greater impact on flight performance than did gaps in primaries. Similar reductions in flight performance due to wing gaps have been reported in European Starlings (*Sturnus vulgaris*)

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doi:10.1016/j.cbpa.2008.08.034

(Swaddle et al., 1999; Williams and Swaddle, 2003) and Ruby-throated Hummingbirds (*Archilochus colubris*) (Chai, 1997).

Thermoregulation is another component of avian energetics that is influenced by feather quality. Nilsson and Svensson (1996) experimentally demonstrated that constraining the amount of time and energy Blue Tits (*Parus caeruleus*) have for fall molt results in lower over-winter survival due to thermoregulatory energy expenditure caused by lower quality feathers. Additionally, in the subsequent season, experimental birds delayed breeding and had smaller clutches compared to controls, demonstrating the potential long-term effects of poor feather quality on survival and fitness.

We proposed, therefore, that the reason seasonal corticosterone concentrations are lowest during prebasic molt is that birds down-regulate corticosterone release to avoid corticosterone's degradative effects on proteins and its inhibition in protein synthesis during feather growth. Corticosterone's proteolytic properties could have profound impacts on feathers that are 95% protein (Murphy, 1996), and feather proteins are more costly to synthesize than are muscle proteins (Lindström et al., 1993). We tested this hypothesis by measuring the effects of elevated exogenous corticosterone on feather mass as well as using a number of novel tests of morphological characters related to feather structure and strength. Although these morphological features have not, to our knowledge, been empirically demonstrated to impact feather performance, we propose that they might be related to fitness and survival of individuals through thermoregulatory and flight-performance associated costs. We then compared our results to effects from elevated endogenous corticosterone resulting from different types of stressors. Recent data suggests that endogenous corticosterone influences feather quality differently than exogenous sources (Strochlic and Romero, 2008). European starlings were selected for this study because high corticosterone levels have been shown to decrease the growth rates of feathers in this species (Romero et al., 2005).

Finally, we also tested whether exogenous corticosterone altered the regrown feathers' resistance to wear by *Bacillus licheniformis*, a feather degrading bacterium. *B. licheniformis* has been shown to breakdown feathers by using keratin as its only supply of carbon and sulfur (Burtt and Ichida, 1999). This organism and other keratinolytic bacteria are readily found on wild birds and in the environment (Wood, 1995; Lucas et al., 2003). The discovery of the anti-microbial properties of preen oil (Shawkey et al., 2003) further supports the hypothesis that these bacteria can negatively affect feather quality in birds.

2. Methods

2.1. Exogenous experiments

To simulate molt, we plucked 26 flight feathers (about 56% of the total) from 21 birds: four primaries and three secondaries on each wing, and all tail feathers (rectrices) (for details see Strochlic and Romero, 2008). The day feathers were plucked is referenced as day 0. On day 14, we subcutaneously implanted silastic capsules between the shoulder blades of each bird. Nine birds were implanted with capsules containing crystalline corticosterone (Sigma Chemical Co.). Each implant was 20 mm in length (inner diameter of 1.47 mm) with one end sealed with silicone-based glue and the other end left open to facilitate corticosterone diffusion. The remaining 12 birds received empty capsules. On day 19, we removed the implants. The exogenous corticosterone was only applied for five days for two reasons. First, that is approximately as long as we see increases in corticosterone with our implants (Romero et al., 2005). Second, we were attempting not to push the corticosterone concentrations too much into the pharmacological range, so that the duration was a compromise between a sustained increase in corticosterone while minimizing the pharmacological consequences. To determine if corticosterone implants were effective, we collected blood samples three times during the experiment: before implantation and three and five days after implantation. All surgical and blood sampling procedures follow Romero et al. (2005), and implant success was assessed via radio-immunoassay, after Wingfield et al. (1992). Once feather replacement was complete, new feathers were plucked. For each feather we determined (1) feather mass (an index of protein content because feathers are almost entirely protein), (2) rachis length, (3) vane area, (4) distance between barbs, (5) number of barbules, and for a subset of feathers (6) tensile strength and (7) stiffness of the rachis (measures of how well feathers resist breaking), and (8) hooking strength of barbicels. We also noted if (9) new feathers failed to replace plucked feathers and (10) relative breakdown of feathers due to bacterial degradation. For each bird, we used a mean value for each feather type (primary, secondary, and rectrix) for each measurement for analyses.

Rachis length and vane area were measured using Scion Image (4.0.3.2; http://www.scioncorp.com/pages/scion_image_windows.htm). Barbule numbers and inter-barb distances were measured on images taken using an Imaging Retiga 1300 digital camera mounted onto a Zeiss Stemi SVII dissecting scope. Barbule numbers were counted in a fixed area (0.13 mm^2) of each image and the distance between barbs was measured between the two center-most barbs in each image.

To determine rachis tensile strength, we used an Instron model 3366 materials tester. Individual feather rachis were glued across small card frames using a cyanoacrylate adhesive and later secured between two testing grips separated by a 15 mm gauge length, as reported elsewhere (Bonser and Dawson, 1999). The samples were pulled apart at a rate of 0.27 mm/s (Fig. 1), similar to methods reported by MacLeod (1980). All material testing data were collected and exported using Bluehill Software Ver. 2.0. Data were later analyzed by traditional mechanical testing techniques: force was normalized to measured values of the rachis cross-sectional area for a calculation of stress, while the specimen extension was normalized against the sample gauge length for calculation of strain. This normalization allowed for measurements of the intrinsic material response (i.e. microstructural behavior), rather than the contribution due to feather size. The highest stress was reported as the sample "tensile strength" while the slope of the linear portion of each stress/strain curve prior to sample failure was reported as the sample "stiffness". Feathers used in the Instron were not used in any other manipulation test.

We used air pressure to measure barbicel hooking strength in primaries and rectrices. A stream of pressurized nitrogen gas was projected through the trailing vane of each feather, and hooking strength was determined as the amount of air pressure required to disrupt the interlocking barbicels and break through the vane (Fig. 2). Air pressure was measured using a manometer.

For bacterial degradation we used the technique described by Williams et al. (1990). Briefly, 0.03 g of each feather was prepared (with duplicates for each bird and feather type) in a sterilized feather

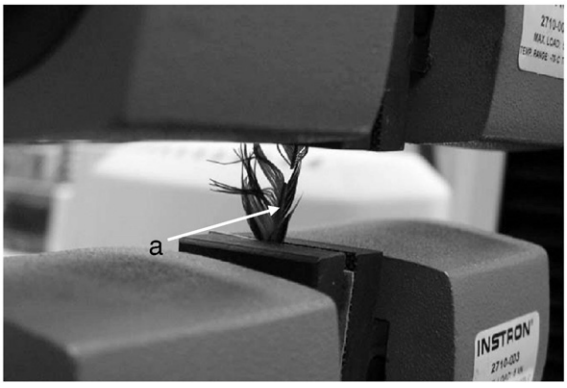

Fig. 1. Instron Model 3369 materials tester, with feather mounted between the two support grips; (a) feather rachis.

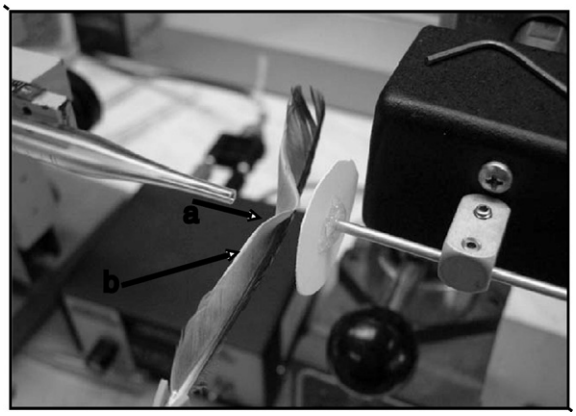

Fig. 2. Set up for the barbicel hooking strength test; (a) direction of pressurized air; (b) feather vane.

medium, designed to support the growth of *B. licheniformis* (Williams et al., 1990). Inoculated media were incubated for 7 days at 37 °C. Spectrophotometry (at λ=220 nm) was used to analyze the concentration of oligopeptide fragments released by bacterial degradation of keratin.

2.2. Endogenous experiments

To test the effects of endogenous corticosterone on feather quality we performed assays on a separate set of European Starling feathers from a different set of birds that were growing during a nearly three week period of psychological or physical stress. Other data from this experiment (e.g. feather mass and corticosterone concentrations) have been previously published (Strochlic and Romero, 2008). See the earlier paper for full experimental details. Briefly, Strochlic and Romero (2008) plucked flight feathers from study birds on day 0 and applied psychological stressors daily from days 5–25, and food-restricted birds were subjected to a daily fast from days 2–25. Psychological stress (21 days) was induced in two ways. First, birds were exposed to an acute, single 30 min restraint stressor every second day. Second, we induced chronic stress by presenting four different stressors each day for 30 min, with each stressor selected randomly from five different stressors. Chronic stressors included restraint, cage disturbance, music, movement on a rolling cart, and voice. Physical stress (24 days) was induced by removing food for 4 h each day, with the time of removal randomly varying between midmorning, midday, and evening. Using the feathers regrown during the Strochlic and Romero (2008) study, we compared how acute and chronic stress and food restriction affect the (1) distance between barbs, (2) number of barbules, (3) tensile strength and (4) stiffness of the rachis, and (5) hooking strength of barbicels. We performed these assays following identical protocols to the exogenous corticosterone experiment.

Radioimmunoassay data were analyzed with repeated measures ANOVA. Statistical analysis of treatment effects on primaries, secondaries, and rectrices for feather quality variables was done using MANOVAs. We averaged all tensile strength and stiffness values across feathers within treatment groups so we would not lose any missing values during analysis and analyzed these in a separate MANOVA for the effects of exogenous corticosterone analysis. There was a missing value for the comparison of the effect of exogenous corticosterone on barbicel hooking strength in primaries, so we analyzed these data using a separate ANOVA. There also was a missing value for the comparison of the effect of endogenous corticosterone on feather quality of rectrices, so we analyzed these data using separate ANOVAs. We used Tukey's *post hoc* test after each MANOVA to test for differences in pairwise comparisons; *p*-values <0.05 were considered statistically significant. Based on previous research looking at the effect of corticosterone on molt in starlings (Romero et al., 2005; Strochlic and Romero, 2008) we predicted *a priori* that implantation with corticosterone would result in lighter and shorter feathers, with smaller vane areas that would break more easily and have barbules that failed to maintain the integrity of the vane. As a result, we performed one-tailed tests for these comparisons. However, we had no *a priori* predictions for how corticosterone would influence barbule number or the distance between barbs, so we report two-tailed results. Analyses were performed using SAS (version 9.1).

3. Results

Birds with corticosterone implants showed higher levels of circulating corticosterone than did controls (Fig. 3, $F_{2,\ 61}=18.16$, $p<0.01$). The first MANOVA included feather mass, rachis length, vane area, inter-barb distance, barbule number, and barbicel hooking strength of rectrices, and was statistically significant ($F_{16,\ 4}=8.70$, $p<0.05$). Specifically, feather mass of corticosterone implanted birds was significantly lower than sham implanted birds for rectrices but not for primaries or secondaries (Fig. 4a). Inter-barb distances were shorter for all feather types for birds with corticosterone implants (Fig. 4b). Barbule number was greater in corticosterone implanted birds for secondaries and rectrices and marginally greater for primaries (Fig. 4c). We also found barbicel hooking strength to be significantly weaker for rectrices in birds with corticosterone (Fig. 4d), and we found the same result for primaries in a separate ANOVA ($F_{1,\ 18}=6.78$, $p<0.02$). See Table 1 for all non-significant pairwise comparisons.

Finally, the number of incomplete feathers or feathers that failed to regrow did not differ significantly between treatments (treatment, $n=20$, mean (s.d.)=2.2 (2.0) feathers per bird; control, $n=33$ mean (s.d.)=2.75 (2.4) feathers per bird; $t_{19}=-0.54$, $p=0.30$). In a related study using the same experimental protocol, we found feather breakage during regrowth was marginally higher in corticosterone treated birds than in control birds (treatment, $n=7$, mean (s.d.)=2.86 (1.1) broken feathers per bird; control, $n=9$, mean (s.d.)=1.67 (1.3) broken feathers per bird; $t_{14}=1.34$, $p=0.07$) (unpubl. data). Finally, there was no difference between treatments for degradation rates of primary feathers by *B. licheniformis* and only a marginal effect of corticosterone on bacterial degradation of rectrices (Table 1).

Endogenous corticosterone concentrations resulting from psychological and physical stressors are reported in Strochlic and Romero (2008). In brief, there were no differences in baseline corticosterone with any psychological stressor, but elevated concentrations with physical stress. In contrast, stress-levels of endogenous corticosterone showed a more complicated pattern. Concentrations were higher in birds subjected to chronic psychological stress, but only during the

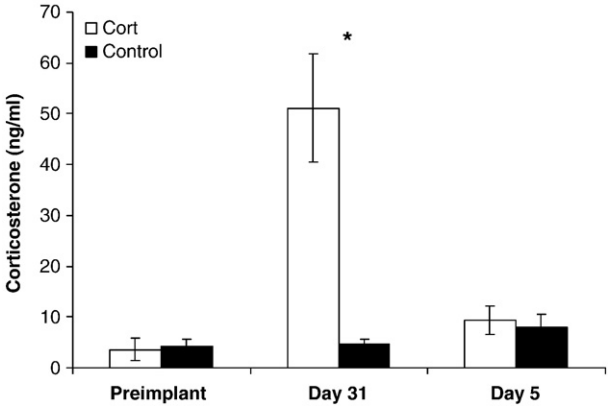

Fig. 3. Mean (±SE) baseline corticosterone levels of corticosterone treated (Cort, $n=9$) and control birds ($n=12$) before implantation, three days and five days after implantation. *=$p<0.01$.

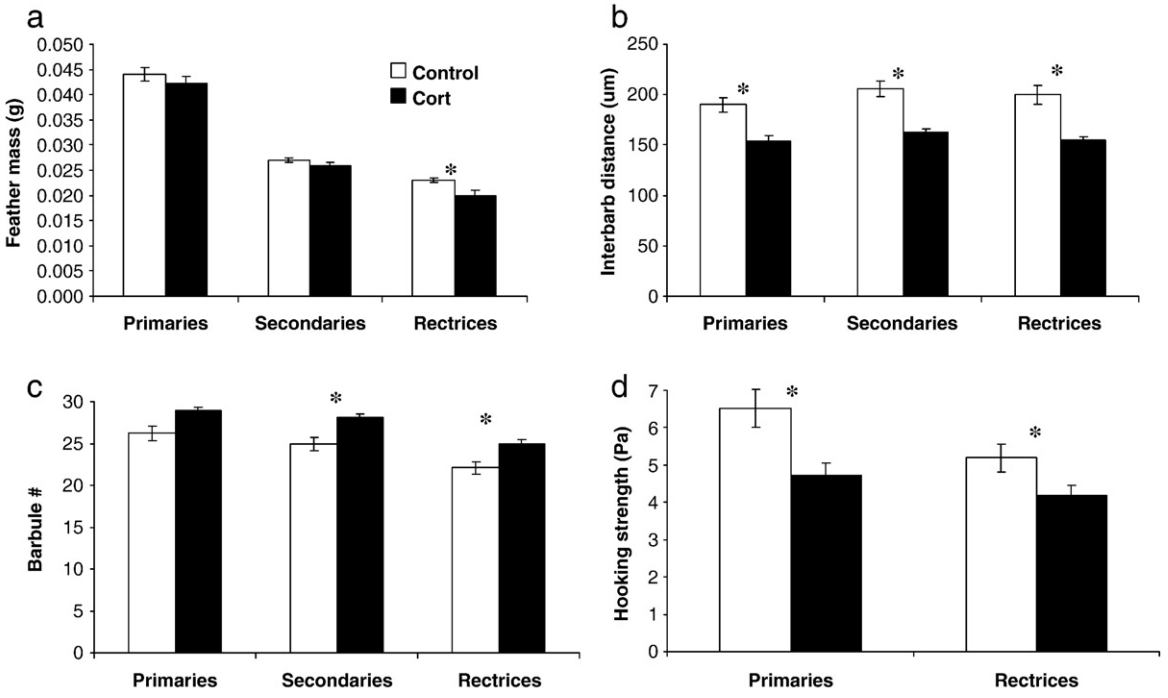

Fig. 4. Mean (±SE) (a) feather mass, (b) inter-barb distance, (c) barbule number, and (d) barbicel hooking strength of feathers from corticosterone Cort-treated ($n=9$) and control birds ($n=12$). Bars represent mean pooled values of each feather type per bird per treatment. *=$p<0.05$ (Tukey's *post hoc* test).

middle of the experimental period, whereas acute stress showed an immediate elevation, but no long-term differences compared to controls. Like baseline concentrations, physical stress resulted in long-term elevations of stress-induced corticosterone compared to controls. Additionally, stress-levels of corticosterone in the psychological and physical stress treatments decreased over time. The MANOVA we used to test for an effect of endogenous corticosterone included inter-barb distance, barbule number, and barbicel hooking strength of primaries and secondaries and tensile strength and stiffness of the rachis and was statistically significant ($F_{24,\ 58.6}=2.80$, $p<0.01$). Specifically, inter-barb distances in primaries and secondaries of food-restricted birds were significantly greater than the control, acute and chronic stress groups (Fig. 5a). Food-restricted birds also had significantly greater inter-barb distances in rectrices compared to the chronic stress group (ANOVA: $F_{3,\ 26}=4.38$, $p<0.05$) (Fig. 5a). Primaries of food-restricted birds had significantly fewer barbules than chronic stress birds (Fig. 5b) but there were no other differences in barbule number (ANOVA for rectrices: $F_{3,\ 26}=0.2$, $p=0.9$). Control birds had feathers with significantly higher tensile strength than food-restricted birds (Fig. 5c), but stiffness and barbicel hooking strength did not differ between any of the treatments (Table 2). Additionally, Strochlic and Romero (2008) observed that rectrices of physically-stressed birds were significantly lighter than those of psychologically-stressed birds. Primary rachis length of physically-stressed individuals was significantly shorter than non-stress controls. Finally, vane area of primaries and rectrices was significantly smaller in food-restricted birds compared to controls.

Table 1
Results for all non-significant *post hoc* Tukey's tests for feather quality tests comparing corticosterone implanted (CORT) European Starlings (*Sturnus vulgaris*) with sham implanted birds (controls)

Independent variable	Feather type	CORT birds	Controls
		Mean (SD)	Mean (SD)
Vane area (cm^2)	Primary	6.41 (0.79)	6.96 (0.75)
	Secondary	6.11 (0.98)	5.99 (0.64)
	Rectrix	4.42 (0.90)	4.84 (0.87)
Rachis length (cm)	Primary	9.22 (0.66)	9.44 (0.52)
	Secondary	7.33 (0.57)	7.32 (0.30)
	Rectrix	6.60 (0.69)	6.86 (0.57)
Rachis tensile strength (kPa)[a]	Combined	82499 (21945)	73268 (24570)
Rachis stiffness (kPa)[a]	Combined	1310358 (152563)	1198687 (305666)
Bacterial degradation	Primary[b]	0.85 (0.16)	0.78 (0.23)
	Rectrix[c]	0.57 (0.12)	0.42 (0.21)

[a] MANOVA: $F_{2,\ 16}=0.83$, $p=0.45$.
[b] *t*-test: $t_{13}=0.64$, $p=0.27$.
[c] *t*-test: $t_{13}=1.69$, $p=0.06$.

4. Discussion

4.1. Increases in exogenous corticosterone

We found that the quality of some flight feathers in European starlings was significantly impacted by the presence of high concentrations of exogenous circulating corticosterone. In the presence of high corticosterone, rectrices were lighter, all feather types had shorter inter-barb distances and had more barbules, and primaries and rectrices had weaker barbicel hooking strength. These data are consistent with the hypothesis that corticosterone is down-regulated during molt to increase protein availability for feather production (Romero, 2002).

Decreases in feather quality could have significant implications for individual survival. For example, the lower mass of the rectrices could increase the rate of feather abrasion (Dawson et al., 2000) and lead to a decrease in flight performance because rectrices are critical for stable flight over different speeds, maneuverability, and lift (Thomas and Balmford, 1995). Also, feathers with weaker barbicel hooking strength could compromise the integrity of the feather vane. Since hooking strength is essential for maintaining the aerodynamic surface of the feather and wing, it stands to reason that decreases in hooking strength would make more likely for the aerodynamic surface to fail (as shown with our test in Fig. 2). Although there are as yet no empirical data that we are aware of linking hooking strength to flight dynamics, there is a strong potential that weaker hooking strengths could lead to gaps in the wings, and feather gaps in the secondaries

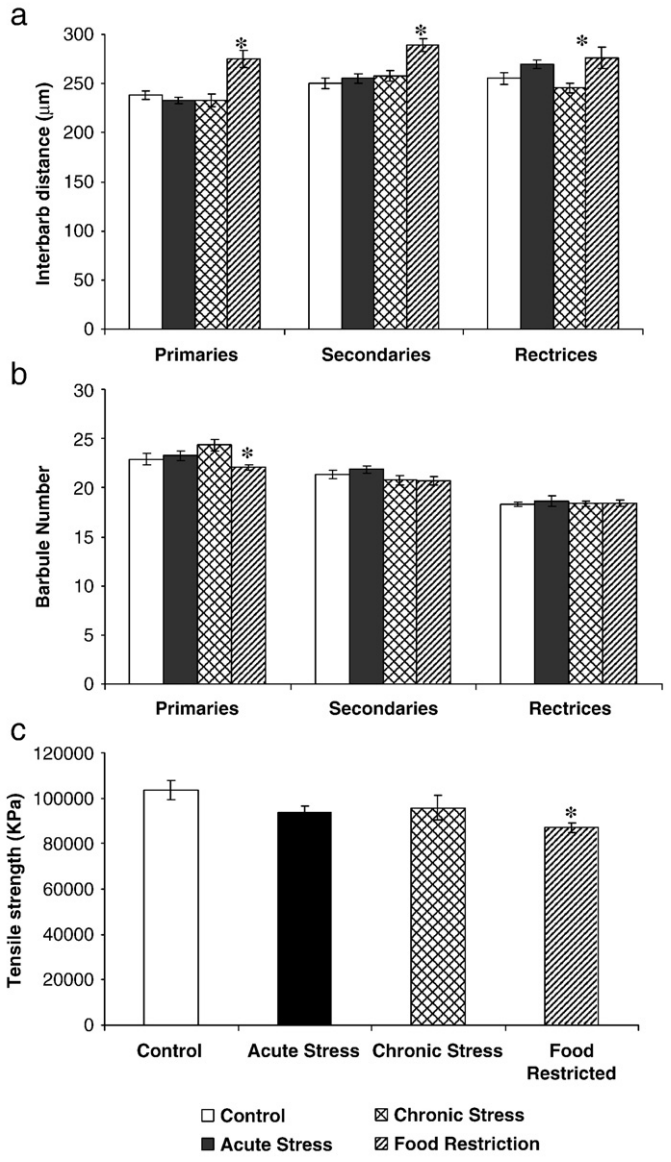

Fig. 5. Mean (±SE) (a) inter-barb distance, (b) barbule number, and (c) tensile strength of feathers from control (n=7), acute stress (n=8), chronic stress (n=8), and food-restricted birds (n=8). Bars represent mean pooled values of each feather type per bird per treatment. *=p<0.05 (Tukey's *post hoc* test).

(i.e., resulting from molt) have been shown to impact flight performance (Hedenström and Sunada, 1999). Failure of the barbicels to maintain vane integrity may also result in thermoregulatory costs associated with heat loss. For example, if barbicel hooking strength is caused by a lack of barbicels or weaker barbicels, then birds may experience increased feather abrasion rates. As a result, feathers may retain less heat (Dawson et al., 2000). All of these indices of feather quality appear to be compromised by corticosterone. Consequently, even though corticosterone release is thought to be vital to surviving stressors, there appear to be substantial potential trade-offs in terms of feather quality.

Inter-barb distances were also greater for control birds (indicating fewer barbs overall), and barbule numbers were higher for feathers of corticosterone treated birds. These results are potentially inconsistent with the observation that physiological stress can increase the frequency of fault bars in feathers, where decreased protein availability reduces barbule density (Machmer et al., 1992; Negro et al., 1994). However, the previous studies did not determine barbule density in the vane outside of fault bars, allowing the possibility that physiological stress manifests itself unevenly in structural elements across a feather. This notion is further supported by the observation that fault bars of varying intensity can occur across a single feather (Sarasola and Jovani, 2006). It is unclear why treatment birds had greater barbule density. Increased barbule numbers would be expected to result in an increase in the number of barbicels as well, and therefore increased hooking strength. In fact, we found the opposite, a decrease in hooking strength. Since we did not measure barbicel number, we could not verify that barbule and barbicel number are indeed linked. Furthermore, our findings that rectrices were both lighter and had weaker barbicel hooking strength despite having more barbules, suggests that there were fewer barbicels. It is possible that bird with exogenous corticosterone differentially allocate protein between barbules and barbicels because differential protein allocation during feather growth is well-documented in a variety of other species (e.g., Jovani and Blas, 2004).

Despite the decrease in some aspects of feather quality, we did not see differences in rachis length or vane area between the two treatment groups. This suggests that these two components are critical for survival because decreased take-off speed and reduced flight performance occur in European Starlings with shorter primaries (Swaddle et al., 1996) and smaller wing areas (Williams and Swaddle, 2003). Additionally, feather strength and flexibility are central to proper feather function, so if corticosterone affects the mechanical properties of feathers it could significantly reduce individual fitness. However, we did not find a difference in tensile strength or stiffness between control and corticosterone treated birds which was inconsistent with our predictions. Additionally, we found no difference in the number of incomplete feathers and feathers that failed to regrow between the two treatments. It is unclear why control birds would exhibit so many failed feathers because exogenous corticosterone is known to slow feather growth (Romero et al., 2005).

There was only a marginal effect of corticosterone on degradation of rectrices by *B. licheniformis*. These results are similar to those reported by Cristol et al. (2005) where they inoculated wing feathers of live birds with *B. licheniformis* and found no significant degradation compared with controls.

4.2. Increases in endogenous corticosterone

We increased endogenous corticosterone in three ways: through an acute stressor every other day, through chronic stress over three

Table 2
Results for all non-significant *post hoc* Tukey's tests for feather quality tests comparing the effect of acute and chronic stress and food restriction on feather quality in European Starlings (*Sturnus vulgaris*)

Independent variable	Feather type	Control	Acute stress	Chronic stress	Food restriction
		Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Rachis stiffness (kPa)	Combined	1,620,643 (346,788)	1,668,694 (203,328)	1,770,791 (284,050)	1,607,896 (201,983)
Barbicel hooking strength (Pa)	Primary	6.61 (1.78)	6.48 (1.21)	6.57 (1.32)	5.08 (1.57)
	Secondary	3.85 (0.88)	3.87 (0.92)	3.55 (0.73)	2.86 (0.40)
	Rectrix[a]	5.02 (1.49)	5.56 (1.31)	4.72 (1.34)	3.98 (0.92)

[a] ANOVA: $F_{3,\ 26}$=1.96, p=0.14.

weeks, and through unpredictable food restriction. In contrast to the effects of exogenous corticosterone on feather quality, only food restriction had any impact on feather quality. This highlights that corticosterone implants are not equivalent to chronic stress. Furthermore, if endogenous release ever reached concentrations equivalent to our exogenous concentrations, there would be an impact on feather quality. Since endogenous concentrations never reach this level (although they do at other times of the year, Romero and Remage-Healey, 2000), it suggests that starlings are adapted to never release that much corticosterone during molt (Strochlic and Romero, 2008), thereby further supporting the hypothesis that corticosterone concentrations are decreased to avoid interfering with molt.

Unpredictable food restriction, on the other hand, did alter feather structure. However, food restriction likely impacted feather quality in two ways. First, this protocol for food restriction results in significantly augmented corticosterone concentrations (Strochlic and Romero, 2008). Second, feather quality could have been altered due to the decreased amount of food availability during feather regrowth, although we did not measure food consumption in this study. The combination had a profound effect. There were greater inter-barb distances in both primaries and secondaries in food-restricted birds compared to the other groups, as well as greater inter-barb distances in rectrices compared to the chronic stress group. This is not too surprising because these same birds also had shorter and lighter feathers than other treatment groups including controls (Strochlic and Romero, 2008). Lower barbule number in primaries of food-restricted birds compared to chronic stress birds further supports the idea that they may be protein-limited.

Food-restricted birds also had weaker feathers compared to control birds. This means that it takes less force to break a feather, which is likely due to microstructural changes (i.e. protein content, crosslinking, packing, etc), which might affect maneuverability during flight (cf. Tubaro, 2003). Another potential cause of decreased tensile strength might be due to an increased frequency of fault bars, which are structural defects in feathers caused by reduced protein deposition during growth (Riddle, 1908). Strochlic and Romero (2008) found greater spontaneous feather breakage during regrowth (i.e. feathers not tested for tensile strength) in food-restricted birds, although data were too limited to compare breakage rates among flight feather types. Serrano and Jovani (2005), however, found no difference in fault bar frequency between primaries and secondaries in Barn Swallows (*Hirundo rustica*).

Our results partially support the hypothesis that birds with high levels of circulating exogenous corticosterone were more likely to have feathers of inferior quality, and provide insight as to how feathers are affected. We found high corticosterone associated with lower mass in some feathers, and some evidence of changes in micro-structure. Rachis length, vane area, mechanical properties, feather regrowth, and bacterial degradation were largely unaffected, however. Additionally, endogenous sources of corticosterone also influenced feather micro-structure and breakage, but only in food-restricted birds. Interestingly, there were no differences in our measures of feather quality between the acute and chronic stress groups compared to controls. This suggests that exogenous sources of corticosterone may artificially inflate the physiological consequences of the stress response (e.g. decreasing feather growth rates, Romero et al., 2005; Strochlic and Romero, 2008). In concordance with this idea, Strochlic and Romero (2008) argue that corticosterone release is so finely regulated that natural increases due to stress are prevented from ever reaching concentrations where they would compromise molt. Regardless of the scenario, the hypothesis that corticosterone artificially inflates the physiological consequences of stress bears further investigation. Our results provide experimental evidence for the importance of down-regulating the stress response during the molt period in birds.

Acknowledgments

We thank D. Kaplan for helping us perform the mechanical properties tests with the Instron materials tester; K. McLaughlin allowed us access to the Imaging Retiga 1300 digital camera and Zeiss Stemi SVII dissecting scope for photographing feathers; B. Trimmer and his lab provided technical support for the barbicel hooking strength test; M. Dickens helped count barbules; N. Cyr, B. Shrestha, and D. Strochlic helped bleed birds. This study was supported by grants IBN-0235044 and IOB-0542099 (to LMR) and 0243668 (a Research Experience for Undergraduates grant awarded to C. Orians at Tufts University) from the U.S. National Science Foundation (NSF), P41 grant EB002520 (to the Tissues Engineering Resource Center at Tufts University) from the National Institute of Biomedical Imaging and Bioengineering.

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