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RESEARCH ARTICLE

Does immune suppression during stress occur to promote physical performance?

Lynn B. Martin*, Amber J. Brace, Alexandra Urban, Courtney A. C. Coon and Andrea L. Liebl

University of South Florida, Department of Integrative Biology, SCA 110, Tampa, FL 33620, USA

*Author for correspondence (lbmartin@usf.edu)

SUMMARY

Two adaptationist hypotheses have been proposed to explain why stress, particularly elevation of stress hormones (i.e. glucocorticoids), tends to suppress immune functions. One is that immune suppression represents efforts to minimize autoimmune responses to self-antigens released as organisms cope with stressors (i.e. the autoimmune-avoidance hypothesis). The other is that immune suppression occurs to promote a shunting of resources to life processes more conducive to survival of the stressor (i.e. the re-allocation hypothesis). Here in wild-caught house sparrows (*Passer domesticus*), we tested the second hypothesis, asking whether sustained elevation of baseline glucocorticoids, due to captivity, caused a greater rate of decline in immune functions than flight performance. A greater decline in immune functions than flight performance would support the re-allocation hypothesis. As in previous studies, we found that captivity tended to alter baseline corticosterone, suggesting that house sparrows experience captivity as a stressor. Captivity also affected several constitutive and induced innate immune metrics: bacterial (*Escherichia coli*) killing activity of blood and oxidative burst of leukocytes both changed in a manner consistent with immune disregulation. In contrast, breast muscle size and vertical flight (hovering) duration improved over captivity. Collectively, these changes provide indirect support for the re-allocation hypothesis, although within individuals, changes in immune and physical performance were unrelated.

Key words: *Passer domesticus*, house sparrow, trade-off, captivity, corticosterone, stress.

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INTRODUCTION

Immune defenses should be beneficial to all organisms at all times, so why are they so commonly altered in response to stressors? To date, two adaptationist hypotheses have been proposed. The first, the re-allocation hypothesis, invokes physiological trade-offs, specifically that immune suppression is less detrimental to survival of most stressors than decrements in physical performance (Sapolsky et al., 2000). According to this hypothesis, during acute stress responses (e.g. predation events), limited resources (i.e. calories or critical amino acids) should be shunted to muscles over lymphoid cells and tissues to foster escape from or survival of the stressor (Sapolsky et al., 2000; Sternberg, 2006). The second (non-exclusive), autoimmune-avoidance hypothesis, recognizes that novel self-antigens will often be revealed to the immune system during stress responses (Råberg et al., 1998). Free-radical degradation of self-tissues in response to stressors could expose antigen-presenting and -processing leukocytes to peptides they would otherwise rarely experience. Without attenuation of immune cell activities (*via* stress hormones), individuals might be prone to autoimmune damage.

Tests of these hypotheses are rare (Bourgeon et al., 2009), with most relevant support coming from domesticated species. However, domesticated species probably experience far fewer and less intense stressors than wild animals (Morgan and Tromborg, 2007); indeed, the domestication process itself profoundly changes the regulation of stress hormones, including the glucocorticoids that have such profound effects on immune functions (Trut et al., 2009). For instance, domesticated guinea pigs exhibited lower glucocorticoid release in response to a stressor than wild cavies (*Cavia porcellus*) and even cavies bred for 30 generations in captivity (Künzl et al., 2003). These confounding factors, plus the relative paucity of exposure to natural levels of parasites during the domestication process, call into question the eco-evolutionary relevance of stress–immune studies in domesticated species (Calisi and Bentley, 2009). To understand the ultimate forces shaping stress–immune interactions, studies in wild organisms will be more informative (Calisi and Bentley, 2009).

Investigations of stress–immune interactions in non-domesticated species are difficult. Most wild species cannot be captured repeatedly, leading many researchers to maintain wild animals in captivity. However, captivity can also be problematic, as some species might alter immune activities or stress responses in light of such a comparatively benign environment whereas others might experience captivity much as imprisonment and undergo physiological disregulation (Mason, 2010). We recently found that the immune system of wild house sparrows [*Passer domesticus* (Linnaeus 1758)] is disregulated in captivity. Leukocyte infiltration of skin (Viswanathan and Dhabhar, 2005) became biased towards granulocytes *versus* lymphocytes over the captivity period (Kuhlman and Martin, 2010), perhaps because of elevations in baseline corticosterone (CORT) in captive compared with free-living individuals. In a more recent study, inflammatory responses to an immunogenic Gram-negative bacterial component [lipopolysaccharide (LPS) from *Escherichia coli*] were amplified in captive house sparrows (Martin et al., 2011): expression of Toll-like receptor 4 and interleukin 1-beta (IL-1β) by circulating leukocytes were elevated in month-long captive *versus* wild birds.

In the present study, we took advantage of the effects of captivity on house sparrow CORT to test the re-allocation hypothesis.

Specifically, we asked whether after 6 weeks in captivity (1) baseline CORT was elevated, and (2) constitutive [*in vitro* bacterial killing ability (Liebl and Martin, 2009b)] and induced (oxidative burst responses) innate immune responses (Sild and Horak, 2010) were reduced more so than the size of the major flight muscle and the ability of birds to perform hovering flight (Veasey et al., 1998). Although the re-allocation hypothesis was proposed to explain (among other things) the effects of glucocorticoids on immune functions over very short periods (e.g. minutes to hours), we expected that the apoptotic and anti-apoptotic effects of glucocorticoids on various cells (Amsterdam et al., 2002; Meagher et al., 1996) might mediate resource re-allocations over longer periods (e.g. days to weeks). We measured baseline CORT because alterations in this hormone might predict changes in performance and/or immune functions (Williams, 2008). We chose these induced and constitutive innate immune functions because they are broadly effective at controlling diverse parasites and they can be measured repeatedly (weekly) from small blood volumes (Millet et al., 2007). We measured vertical flight because it is one of the most energy-demanding movements birds use (Dial et al., 1997). More importantly, it is one of the few performance parameters that can be accurately and repeatedly scored in songbirds [although for this and other metrics (e.g. CORT), some habituation might occur].

MATERIALS AND METHODS

Bird capture, care and morphometrics

Wild adult birds (N=10; three males and seven females) were captured in mist nets from a single location in north St Petersburg, FL, USA, in fall 2011. Fifty microliters of blood were collected within 3 min of capture from the brachial vein after cleaning the area with 100% alcohol and allowing the skin to dry. Within 5 min of collection, blood samples were processed for bacterial killing activity (BKA; see below). Body mass (to 0.1 g), pectoralis width (mm) and furcular fat score (1–8 ordinal scale, 8 maximum) were then recorded for each individual. After capture, birds were held singly in cloth bags (2.5 h maximum) until they were transferred to an animal facility at the University of South Florida. Upon arrival at the facility, birds were housed individually in conventional songbird cages (35×27.5×47.5 cm, width × depth × height) and provided two perches, *ad libitum* access to mixed seeds (Scarlett Natural Finch, Moyer & Sons, Sauderton, PA, USA) and water, and isolated from other disturbance except an ~15 min period daily when their food, water and cage liners were replaced by caretakers. All birds were allowed to see and hear each other throughout the study. Photoperiod was maintained at levels comparable to ambient conditions at the time of capture for the study duration. An additional blood sample (50 µl) and morphometrics were collected weekly for 6 weeks, and on the following day, flight performance tests were conducted (see below). For all captive birds, blood samples were collected within 5 min of entering the housing room. To ensure that all samples were obtained within this period, three to four individuals entered the room simultaneously and bled one to three birds each, always collecting the final sample well within the 5-min window. At the end of the study, birds were released at the site of capture. All procedures meet guidelines for the use of animals in research and were approved by the USF IACUC (#W3202) prior to conducting the work.

Bacterial killing activity

In vitro BKA of blood was assessed following modified laboratory protocols (Liebl and Martin, 2009a). From each blood sample, and within 30 s of sampling, a 1.5 µl aliquot was added to 34.5 µl of CO_2-independent medium in four different microcentrifuge tubes: three to assess anti-*E. coli* (Gram-negative bacteria) activity and the fourth as a blank in spectrophotometric measurements. All samples for BKA were held on ice until they were returned to the laboratory for processing. In the laboratory in a BSL-2 laminar flow hood, 12.5 µl of a 10^5 bacteria ml^{-1} solution [bacteria from lyophilized pellets (Microbiologics, St Cloud, MN, USA) reconstituted in sterile phosphate-buffered saline (PBS)] was added to each diluted blood sample, and 12.5 µl sterile PBS was added to tubes to be used as blanks. Bacteria–blood cocktails were then vortexed vigorously, incubated at 37°C for 1 h and vortexed again. Then, 250 µl of tryptic soy broth was added to all tubes to foster bacterial growth, and the tubes were incubated for 12 h at 37°C. Upon completion of incubation, sample absorbance was quantified using a NanoDrop 1000 spectrophotometer (600 nm filter, ThermoScientific, Wilmington, DE, USA). Each tube was vortexed immediately before absorbance of a 2 µl subsample, from the center of the tube, was measured. Prior to each sample measurement, the spectrophotometer was blanked using the blanks identified above. All samples were referenced against a positive control that was processed and incubated at the same time as the blood samples and consisted of all cocktail components except blood (medium was added to adjust final concentrations). The percentage of bacteria killed was calculated by dividing sample values by the positive control and subtracting this value from 1. In week 2, blood samples became contaminated prior to assay.

Oxidative burst

For oxidative burst, we followed published protocols (Sild and Horak, 2010) using an ABEL Cell Activation kit with Pholasin and Adjuvant-K (Knight Scientific, Plymouth, UK), measuring burst four times for each individual: once at capture and every 2 weeks thereafter. Whole blood samples were held at room temperature until analysis (within 2 h of collection). Samples were assayed individually using a handheld luminometer and a total sample volume of 250 µl (112.5 µl reconstitution and assay buffer, 25 µl 1:100 diluted whole blood, 25 µl Adjuvant-K, 62.5 µl Pholasin). A 30 s baseline luminescence reading was taken, after which 25 µl of *E. coli* 055.B5 LPS was injected directly into the sample. Luminescence readings were then recorded every 0.5 s for 3 min post-injection. For comparisons of burst activity changes over captivity, we calculated two parameters: baseline burst [mean of relative light units (RLU) measurements prior to introduction of LPS into samples)] and peak burst (maximum RLU value post-LPS treatment of blood minus baseline burst, calculated as above).

Corticosterone assay

A commercially available EIA kit (catalog no. 900-097, Assay Designs, Ann Arbor, MI, USA) was used to measure plasma CORT (Breuner et al., 2006). CORT concentrations were measured only four times during the study, once at capture and every 2 weeks thereafter. Briefly, 10% steroid displacement reagent (5 µl) was added to 5 µl of plasma and 5 min later, assay buffer (240 µl) was added to each sample, vortexed and aliquoted in duplicate (100 µl per well) to assay plates (Kuhlman and Martin, 2010). In addition, a standard curve (ranging from 200,000 to 32 pg) was measured in duplicate. Samples and standards were then incubated with conjugated CORT and antibody for 2 h at room temperature while being shaken. Wells were emptied and washed before substrate was added to all wells; plates were incubated 1 h at room temperature without shaking. Stop solution was then added, and each plate was read at 405 nm (corrected at 590 nm to minimize background absorbance).

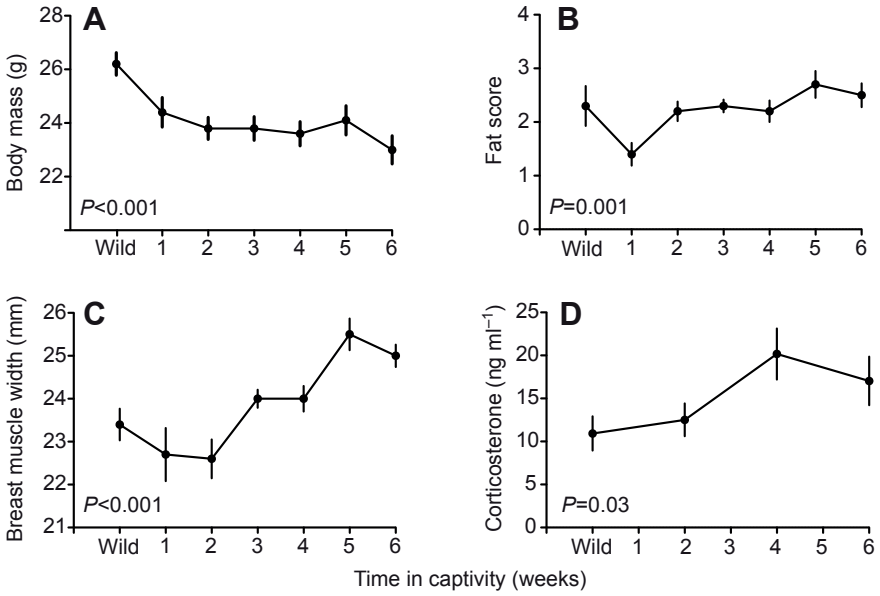

Fig. 1. Captivity duration effects on (A) body mass, (B) fat score, (C) pectoral muscle width and (D) baseline corticosterone in house sparrows. Bars are means ± 1 s.e.m. *P*-values depict effects of time in captivity.

Vertical flight challenge

Vertical flight performance was quantified weekly by assessing the height and duration of hovering flight in a Plexiglas vertical flight chamber (115×21.5×21.5 cm, height × width × depth) housed in a room free of other birds (Blount and Matheson, 2006; Veasey et al., 1998). The first trial was conducted the morning on the day after capture, as (1) birds had just been held in bags for variable periods before cage housing in the aviary and (2) this approach enabled us to sample behavior at the same time of day for all individuals. The chamber included a trapdoor ~10 cm above the floor; below the trapdoor was a white-noise emitter. Both were used to encourage flight once the trial began. To conduct each trial, a video camera was placed in the room prior to trials, equidistant from the flight chamber for all trials. A single bird was then captured from its cage in a separate room, placed in the flight box on the trapdoor, and given ~45 s to accommodate to the conditions, without the experimenter present. To induce vertical flight, the experimenter simultaneously opened the trapdoor and activated the white-noise emitter (both *via* remote control). Flights were then recorded for 45 s, after which the experimenter turned off the white-noise emitter, entered the room and reset the trapdoor. The experimenter then left the room, waited 45 s and repeated the trial. Video recordings of flight behavior were then viewed to quantify four parameters: height and duration of the first vertical flight in response to the dual stimulation of white noise and trapdoor activation. Two birds (one at the time of capture and one 4 weeks post capture) did not perform during trials, but zeroes were included in analyses to be conservative.

Data analysis

BKA values (% positive control killed) were arcsine square-root transformed. Thereafter, no variable was significantly non-normal (one-sample Kolmogorov–Smirnov test), so repeated-measures ANOVA was used for all comparisons. Mauchly's tests were used to test for sphericity, and when the test was violated ($P<0.05$), Greenhouse–Geisser corrections were used to identify significant effects of time on dependent variables. Sex was not evaluated because of the small sample size within each sex. All analyses were performed with SPSS v.18 (IBM, Armonk, NY, USA) at an α-level of ≤ 0.05.

RESULTS

Captivity impacts on morphometrics and corticosterone

Body mass declined over time in captivity ($F_{6,54}=15.3$, $P<0.001$; Fig. 1A), decreasing most in the first week and then remaining stable for the remainder of the study. Fat scores declined in the first week, but tended to recover and remain stable for the remainder of the study ($F_{6,54}=4.4$, $P=0.001$; Fig. 1B). Flight muscle, however, increased in size (~9%) over time in captivity ($F_{6,54}=11.0$, $P<0.001$; Fig. 1C). Baseline CORT also increased over captivity ($F_{3,24}=3.4$, $P=0.03$; Fig. 1D) after one outlier was dropped (baseline CORT at time of capture was >2 s.d. of the mean for other individuals).

Captivity impacts on immune functions

BKA did not change in a simple fashion over captivity ($F_{2.8,25.5}=2.3$, $P=0.10$). BKA tended to decrease in the first weeks of captivity, but recovered by week 5 (Fig. 2A). Baseline (pre-LPS) oxidative burst changed dramatically over time in captivity ($F_{1.5,27}=35.9$, $P<0.001$), increasing rapidly until week 4 and declining thereafter, but not nearly to levels measured at capture (Fig. 2B). Peak burst activity also changed over captivity ($F_{1.7,15.9}=9.9$, $P=0.002$), increasing by 2 weeks of captivity and then returning to near-capture levels by 6 weeks (Fig. 2C).

Captivity effects on flight performance

Hovering duration changed over captivity ($F_{5,45}=7.3$, $P<0.001$), increasing rapidly in the week post-capture and remaining high but decreasing slowly over the remainder of the study (Fig. 3A). Flight height did not change significantly with captivity duration ($F_{2.0,18.4}=2.3$, $P=0.12$), although it too tended to increase the longer birds remained captive (Fig. 3B).

Relationships among traits within individuals

Spearman rank correlation analysis indicated only two significant relationships between (1) means of trait values across all time intervals and/or (2) percent change in trait values. Mean baseline oxidative burst was inversely related to peak oxidative burst ($r=-0.87$, $P=0.001$), and change in fat score was inversely related to change in body mass ($r=-0.65$, $P=0.04$). Thus, at the individual level, there was little evidence that changes in immune traits were directly related to changes in morphological or performance-related traits.

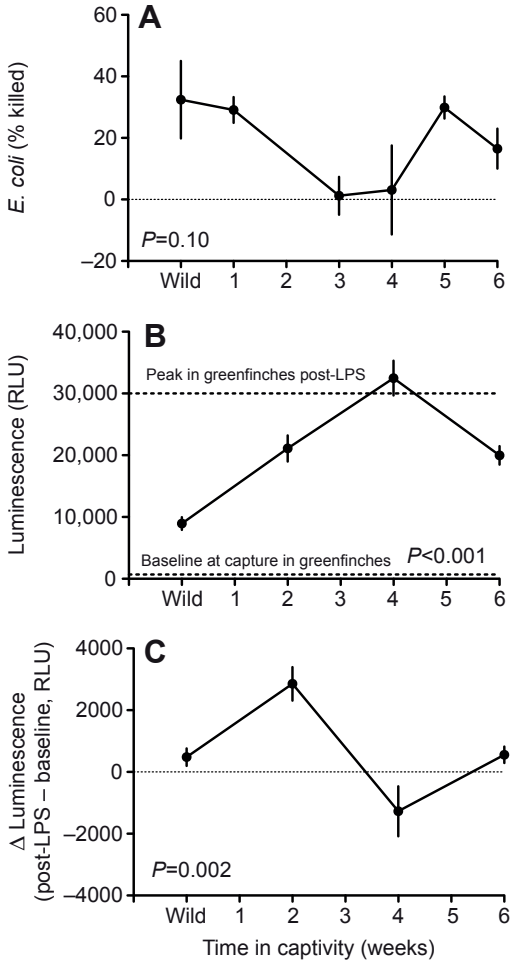

Fig. 2. Captivity duration effects on (A) bacterial killing ability (*Escherichia coli* bacteria) of whole blood, (B) baseline oxidative burst and (C) lipopolysaccharide (LPS)-induced oxidative burst in house sparrows. Bars are means ± 1 s.e.m. *P*-values depict effects of time in captivity.

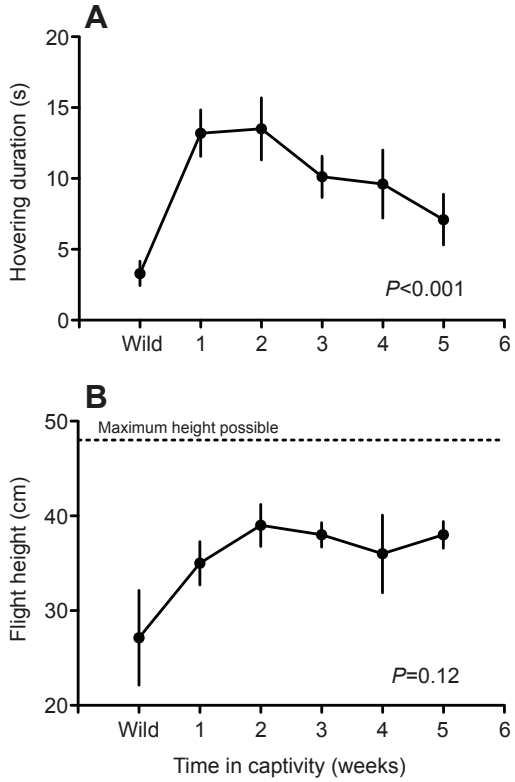

Fig. 3. Captivity duration effects on physical performance in house sparrows: (A) duration of a hovering flight and (B) maximum height of a hovering flight. Bars are means ± 1 s.e.m. *P*-values depict effects of time in captivity.

DISCUSSION

In the present study, body mass declined, fat scores decreased and then recovered, and baseline CORT increased with captivity duration for house sparrows. Immune functions became disregulated (oxidative burst, see below) or declined modestly and then recovered (BKA) over captivity. However, flight muscles grew, and this growth co-occurred with improvements in hovering flight performance. Overall, these effects are consistent with the re-allocation hypothesis: in the presence of elevated glucocorticoids, physical performance (hovering) improved whereas immune function declined. As yet, however, these data provide only indirect, weak support for the re-allocation hypothesis, especially as correlation analyses indicated that changes in endocrine, immune and performance variables were unrelated within individuals. Moreover, our results examine a different time frame than was addressed by the original re-allocation hypothesis, so it would be intriguing to conduct a similar study over even shorter time periods post-stressor. Below, we interpret our results and propose additional, more direct ways to test the re-allocation hypothesis.

Can captivity cause stress in house sparrows?

Captivity effects on baseline CORT vary in house sparrows, sometimes leading to elevations (Kuhlman and Martin, 2010) and other times not (Martin et al., 2011). In the present study, baseline CORT tended to increase with captivity duration. Similar results of captivity have been observed in other wild animals (Romero and Wingfield, 1999), especially soon after capture (Mason, 2010). Basal glucocorticoids are highest in the first few days post-capture in wild kawahai (*Arrapis trutta*) (Davidson et al., 1997), and in chukars (*Alectornis chukar*), individuals lost mass, the ability to release CORT in response to a restraint stressor and corticosterone negative feedback capacity soon after capture, but regained all three abilities within 10 days (Dickens et al., 2009c). Similar patterns have been seen in sympathetic nervous system coordination of heart rate; in captive European starlings (*Sturnus vulgaris*), a series of stressors induced an elevation of baseline heart rate. However, further elevation in heart rate due to an additional stressor was weak for the first several days of captivity (Dickens and Romero, 2009). In light of these effects across various taxa, it seems reasonable to interpret captivity as at least a mild psychological stressor for many species, including house sparrows (Dickens et al., 2009a).

Captivity effects on immune functions

Many species experience immune decrements in captivity (Berzins et al., 2008; Davis and Maerz, 2008; Ewenson et al., 2003; Ewenson et al., 2001), and such results are consistent with the effects of stress hormones on many immune functions (Sorrells and Sapolsky, 2007). Inflammatory cells and processes seem particularly prone to perturbation in captivity (Buehler et al., 2008), although this observation has exceptions (Sepp et al., 2010). House sparrows appear prone to hyper-inflammation over long periods in captivity (Kuhlman and Martin, 2010; Martin et al., 2011). Whereas such

enhancements might be interpreted as protective, these elevations are more likely indicative of immune disregulation with subsequent, strong negative impacts (i.e. immunopathology) on hosts (Graham et al., 2011). In the present study, we examined captivity effects on two innate immune measures: BKA (Millet et al., 2007) and oxidative burst (Sepp et al., 2010; Sild and Horak, 2010). Functionally, the former index is simple to interpret: a higher BKA is likely protective. Over captivity, BKA tended to decrease and then recover to wild-caught values; if captivity is representative of stressors persisting over similar time periods, house sparrows would likely be vulnerable to Gram-negative bacterial infections in the bloodstream for short periods and then recover. The latter index, oxidative burst, is more difficult to interpret functionally, especially given the distinct effects of captivity on baseline and induced (peak) burst responses. Both values represent the activity of heterophils and other granulocytes to release reactive oxygen species (predominantly superoxide). However, for baseline burst, levels represent the activity of cells in the absence of microbial stimuli, whereas for peak burst, levels represent inducibility in response to an immunogenic substance (LPS). We interpret the captivity effects seen here as disregulation because baseline values were several orders of magnitude higher than ever observed in greenfinches (*Carduelis chloris*), the species in which this assay was first validated (lower dashed line in Fig. 2B) (Sild and Horak, 2010) and the only other songbird species to which the assay has been applied. Maintaining large numbers of cells expressing high levels of reactive oxygen species probably causes some oxidative damage to captive birds. Further indicative of disregulation, in greenfinches, LPS treatment of blood greatly elevated burst activity (upper dashed line in Fig. 2B); however, in house sparrows, especially after a few weeks of captivity, LPS treatment could not further elevate burst responses.

Captivity effects on flight muscle and performance

Perhaps the most surprising results of the study were the strong positive effects of captivity duration on hovering ability and pectoralis muscle width. Captivity was expected to have weak but negative effects on these traits because: (1) birds would be able to exercise little in cages [and thus experience atrophy due to disuse (Portugal et al., 2009; Price et al., 2011)], and (2) chronically elevated CORT tends to degrade muscle in domesticated game birds (Dong et al., 2007; Hull et al., 2007) and wild songbirds (Awerman and Romero, 2010; Busch et al., 2008; Gray et al., 1990). As our study was not designed to determine how captivity altered muscle growth and viability in sparrows on the molecular level, we can only speculate as to why house sparrow physical performance improved with captivity duration. One possibility is the re-allocation hypothesis: although captivity altered stress hormone regulation, this disregulation was sufficient to alter only immune functions; muscle size increased and function seemingly improved over the experiment. Indeed, different duration stressors may have distinct effects on different physiological systems in many species. For instance, it is reasonable that wild birds would be under some nutritional stress and that captivity would provide more and more predictable food resources than natural conditions. An abundance of food may promote some systems, but the psychological distress of being held in a cage or handled repeatedly may incite degradation of other cells and tissues. Perhaps in house sparrows (or wild birds generally), lymphoid tissues and cells are more susceptible to CORT or other stress hormones than myocytes. Even though elevated CORT can increase feeding activity (Kitaysky et al., 2001), some physiological systems may be less able than others to balance the enhancing and degrading effects of stressors (Diamond, 1993; Martin et al., 2007).

A second possibility is that house sparrows are physiologically unique, i.e. they do not exhibit a representative response to captivity as other wild animals. They are a close commensal of humans (Anderson, 2006) and one of the world's most broadly distributed species (Schrey et al., 2011). They also exhibit some immune and endocrine novelties seen in few other songbirds (Lee et al., 2005; Martin et al., 2010; Martin et al., 2005). Although unsatisfying at the mechanistic level, these traits, together with the niche that house sparrows occupy, may enable them to grow flight muscles and perform well physically under conditions that would be stressful to other species.

A third possibility is that changes in immune and flight parameters represent distinct circannual rhythms for different tissues, not effects of captivity and/or stress. It was impossible to include a group in our study that did not experience captivity but still served as a reference for trait comparisons. Perhaps the observed changes in flight musculature and hovering ability, as well as oxidative burst, are typical changes that occur at these times of year in this population. Seasonal changes in avian immune functions are well known (Martin et al., 2008). Moreover, in many avian species, muscles can change size on short time scales (Lindström et al., 2000), and size changes can occur without changes in environmental stimuli such as photoperiod (Dietz et al., 1999). Muscle size changes likewise can occur regardless of use/disuse in birds: one group of barnacle geese (*Branta leucopsis*), captive and flightless all their lives, grew and shrank pectoralis muscles as much as 35%, contingent on molt stage (Portugal et al., 2009). Collectively, the effects of stress hormones on the musculature of wild animals warrant greater study, as effects appear contingent on both species and timing (Chin et al., 2009; John-Alder et al., 2009).

A fourth possibility involves the constraints of the different study methods. Some, such as the flight box, may entail habituation such that low values in week 0 followed by an increase and decline thereafter comprise both physical and mental changes. In contrast, changes in immune functions also have inferential limitations. We (and others) assume that quantitative immune variation has functional relevance, but without direct testing, we cannot be sure that these magnitude changes would make individuals more vulnerable to infection or other diseases. In the present study, assays were chosen based on their modest requirements (i.e. blood volume) and lack of the need of training for birds (i.e. flight in a wind tunnel). Whereas other metrics, such as wind tunnel performance, might be less apt to suffer from confounding factors, the training they would require could impose more stress and jeopardize our ability to conduct the study over the necessary time scale (i.e. immediately after capture from the wild).

Correlations among traits

If immune disregulation occurred to free resources for performance and if such effects were mediated by CORT, we might have observed correlations among traits within individuals (Williams, 2008). Although two significant correlations were detected – both (1) peak and baseline oxidative burst and (2) body mass loss and fat mass score were inversely related – neither supported direct effects of changes in one suite of traits with changes in others. This lack of correlation may be due to the time scales over which re-allocations occur, happening faster or slower than our sampling paradigm could detect. Alternatively or additionally, compensation may have occurred in other systems (e.g. neurogenesis, alimentation, etc.). Going forward, it would be rewarding to test the re-allocation

hypothesis directly by using stable isotopes (McCue et al., 2011) or radioisotopes (Zera and Zhao, 2006) of crucial amino acids.

Conclusions

We found indirect support of the re-allocation hypothesis for stress–immune interactions: house sparrow immune functions were disregulated but flight performance and flight muscle size increased in response to captivity. We advocate future research on stress–immune interactions in wild animals, including tests of basic hypotheses as well as practical work to improve animal husbandry and conservation efforts. Such work might elucidate how to help animals cope with translocation (Dickens et al., 2009b), and why some species are more susceptible to infection when in captivity, e.g. captive Pallas' cats (*Otocolobus manul*) are particularly prone to dying from toxoplasmosis (Brown et al., 2005) whereas captive, but not wild, cheetahs (*Acionyx jubatus*) are prone to gastritis from opportunistic *Helicobacter* infections (Terio et al., 1999).

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