

SHORT COMMUNICATION

## Stress in the wild: Chronic predator pressure and acute restraint affect plasma DHEA and corticosterone levels in a songbird

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(Received 10 May 2012; revised 29 July 2012; accepted 30 July 2012)

### Abstract

The effects of chronic stressors on glucocorticoid levels are well described in laboratory rodents, but far less is known about the effects of chronic stressors on wild animals or on dehydroepiandrosterone (DHEA) levels. DHEA can be produced by the adrenal cortex and has prominent antiglucocorticoid properties. Here, we examined wild songbirds to elucidate the relationship between chronic predator pressure and plasma DHEA and corticosterone levels. We measured circulating steroid levels at baseline and after acute restraint in the breeding and nonbreeding seasons. During the breeding season, males in low predator pressure (LPP) environments had higher baseline DHEA levels than males in high predator pressure (HPP) environments. Also, acute restraint decreased DHEA levels in LPP males only but increased corticosterone levels in HPP and LPP males similarly. During the nonbreeding season, DHEA and corticosterone levels were lower than during the breeding season, and acute restraint decreased DHEA levels in both HPP and LPP males. Unlike males, breeding females showed no effect of predator pressure on baseline DHEA or corticosterone levels. These data suggest that naturalistic chronic and acute stressors affect circulating DHEA and corticosterone levels in wild animals and highlight the importance of using multiple endpoints when studying the physiological effects of chronic stress.

**Keywords:** Avian, dehydroepiandrosterone, HPA axis, predation risk, season, sex difference

### Introduction

The effects of chronic stressors on circulating glucocorticoid levels have been well described, however, much less is understood about dehydroepiandrosterone (DHEA) levels. DHEA is an androgen precursor that can be synthesized in the adrenal cortices, gonads, and brain and, in some species, can be regulated by adrenocorticotrophic hormone and acute stressors (Arvat et al. 2000; Torres and Ortega 2003; but see Marinelli et al. 2007). DHEA has antiglucocorticoid and neuroprotective properties (Karishma and Herbert 2002; Maninger et al. 2009; Newman et al. 2010), but few studies have examined the relationship between chronic stress and DHEA levels.

Most studies of stress physiology use inbred laboratory rodents, under highly controlled and

uniform conditions (e.g. 12L:12D photoperiod; constant temperature), and often use stressors that are not typically encountered in the natural environment (e.g. foot shock, cage tilting, strobe light, and white noise; Rosenfeld 2010). We have gained many important insights from these studies, but an important complement to such work is studying the effects of naturalistic chronic and acute stressors on wild animals that are genetically diverse and living in natural conditions.

Here, we examined small (~25 g) wild songbirds, song sparrows (*Melospiza melodia*) that live at either high predator pressure (HPP) or low predator pressure (LPP) sites in southwestern British Columbia, Canada (Zanette et al. 2003; 2006; Clinchy et al. 2004; 2011). At HPP and LPP sites, we measured

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plasma DHEA and corticosterone levels at baseline and after acute restraint (30 min), during the breeding and nonbreeding seasons. Moreover, we examined both males and females. Unlike laboratory rats and mice (but similar to humans), song sparrows have high levels of circulating DHEA (Newman et al. 2008; Newman and Soma 2009). Interestingly, in song sparrows, DHEA is regulated locally in the brain and peripheral tissues by acute stress (Newman and Soma 2011; Pradhan and Soma, 2012), but systemic plasma levels do not increase in response to acute stress (Newman et al. 2008; Soma and Wingfield 2001). Relatively few studies have described the effects of natural chronic stress on glucocorticoid levels in wild animals (Clinchy et al. 2004, 2011; Creel et al. 2009; Sheriff et al. 2011). Here, we examine the effects of chronic stress on DHEA levels in song sparrows. Our data indicate that use of multiple biomarkers, in addition to circulating glucocorticoid levels, will be informative in studies of stress physiology (Travers et al. 2010; Clinchy et al. 2011).

## Materials and methods

### Field protocol

We examined wild adult song sparrows that were part of a long-term study. HPP sites were located near Victoria on Vancouver Island, BC, Canada, and LPP sites were located <2 km offshore on several small coastal islands. These sites have been described in detail (Zanette et al. 2003, 2006). Importantly, the HPP sites induce chronic stress in song sparrows (Clinchy et al. 2004, 2011), and the adverse effects of this chronic predator-induced stress on physiology and fitness have been experimentally verified (Zanette et al. 2006, 2011). Predator pressure is particularly important during the breeding season, when both nests (eggs and nestlings) and adults are at risk. During the breeding season, males and females were captured in mist nets or potter traps on day 6 of the 11-day chick-rearing period (May–July 2005). During the nonbreeding season, we focused on males, and they were captured in mist nets (October 2005). There was no effect of capture method (mist net vs. potter trap) on baseline steroid levels (male DHEA:  $t = 0.81, p = 0.30$ ; female DHEA:  $t = 0.62, p = 0.55$ ; male corticosterone:  $t = 0.14, p = 0.45$ ; female corticosterone:  $t = 0.63, p = 0.54$ ).

### Blood collection

For males, during the breeding and nonbreeding seasons, subjects were captured and a baseline blood sample ( $\sim 150 \mu\text{l}$ ) was collected within 3 min from the brachial vein. To examine the effect of acute restraint, which simulates an acute stressor, such as natural capture by a predator, males were then restrained for

30 min in a cloth bag and another blood sample ( $\sim 150 \mu\text{l}$ ) was collected from the brachial vein (Newman et al. 2008). For females, during the breeding season only, baseline blood samples were collected. Females were not restrained to avoid any effects of acute restraint on female parental behavior. Blood was kept on ice until returned to the laboratory (1–6 h) and centrifuged. Plasma was stored at  $-20^\circ\text{C}$ .

### DHEA and corticosterone analysis

Total (free + bound) DHEA and corticosterone levels were quantified in plasma using specific and sensitive radioimmunoassays (RIA) that have been validated for song sparrow plasma (Newman et al. 2008). Before DHEA RIA, steroids were extracted from plasma using high performance liquid chromatography (HPLC)-grade dichloromethane (Newman et al. 2008). For DHEA, intra-assay variation was 4.8%, and inter-assay variation was 5.0% (low control) and 6.2% (high control). For corticosterone, plasma was assayed directly; intra-assay variation was 2.5%, and inter-assay variation was 4.6% (low control) and 2.9% (high control).

### Statistics

In males, we evaluated the effects of the predator pressure environment, acute restraint, and season on plasma DHEA and corticosterone using a three-factor mixed-design ANOVA, where subject identity was included as a random effect. Significant effects were further investigated using Tukey's honestly significant difference (HSD) *post hoc* test. In females, we evaluated the effect of predator pressure environment on baseline plasma DHEA and corticosterone levels using *t*-tests. Data are presented as mean  $\pm$  SEM, all tests were two tailed, and values of  $p \leq 0.05$  were considered significant.

## Results

### Plasma DHEA and corticosterone levels in males

For plasma DHEA levels in wild male song sparrows, there was a significant three-way interaction between season, predator pressure, and acute restraint ( $F_{1,92} = 8.02, p = 0.006$ ; Figure 1). Baseline DHEA levels were higher during the breeding season than the nonbreeding season (breeding,  $2.17 \pm 0.14 \text{ ng/ml}$ ; nonbreeding,  $1.00 \pm 0.06 \text{ ng/ml}$ ).

In the breeding season, baseline DHEA levels were significantly greater in LPP males than in HPP males (Figure 1A). Furthermore, acute restraint significantly decreased DHEA levels in LPP males only (Figure 1A).

In the nonbreeding season, there was no difference in baseline DHEA levels between HPP and LPP males (Figure 1B). Also, acute restraint significantly

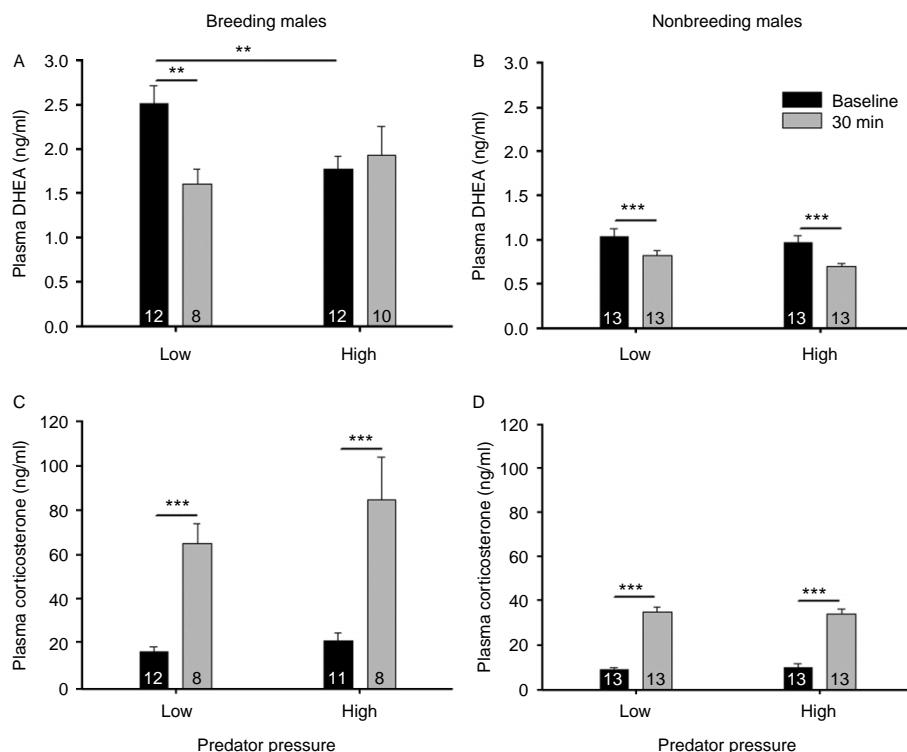


Figure 1. In wild male song sparrows, the effects of chronic LPP or HPP on baseline and stressed (30 min restraint) levels of plasma DHEA (A and B) and corticosterone (C and D) during the breeding season (A and C) and nonbreeding season (B and D). \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$ .

decreased DHEA levels in both HPP and LPP males (Figure 1B).

For plasma corticosterone levels, there was a significant two-way interaction between season and acute restraint ( $F_{1,89} = 11.88, p = 0.0009$ ; Figure 1). Acute restraint increased plasma corticosterone levels in males at HPP and LPP sites (Figure 1C,D); however, the response to acute restraint was nearly two times greater during the breeding season than during the nonbreeding season. Consistent with previous reports on these song sparrows (Clinchy et al. 2004, 2011), plasma corticosterone levels in breeding male song sparrows tended to be lower at LPP sites, although the effect of predator pressure environment was not significant here in our mixed model ( $F_{1,89} = 3.00, p = 0.08$ ).

#### Plasma DHEA and corticosterone levels in females

In contrast to the males, in wild female song sparrows during the breeding season, we found no effect of predator pressure on baseline DHEA levels (Table I). At HPP sites, breeding females had higher baseline

DHEA levels than males ( $t = 2.10, p = 0.04$ ), but there was no sex difference at LPP sites ( $t = 0.84, p = 0.41$ ). Consistent with previous reports on these song sparrows (Clinchy et al. 2011), there was no effect of predator pressure environment on baseline corticosterone levels in females (Table I).

#### Discussion

Wild song sparrows had circulating DHEA and corticosterone levels that differed according to predator pressure and season. During the breeding season, HPP males have lower baseline DHEA levels than LPP males. Acute restraint does not further decrease DHEA levels in HPP males but does decrease DHEA levels in LPP males. During the nonbreeding season, baseline DHEA levels are lower than during the breeding season and do not differ between HPP and LPP males, and acute restraint decreases DHEA levels in males from both environments. Our study demonstrates the effects of a natural chronic stressor on circulating DHEA levels in wild animals.

Table I. Baseline DHEA and corticosterone levels (ng/ml) in wild breeding female song sparrows under LPP or HPP.

Females	Low predator pressure	High predator pressure	<i>t</i> Value	<i>p</i> Value
Plasma DHEA	$3.00 \pm 0.41$ (11)	$2.31 \pm 0.21$ (13)	-1.40	0.18
Plasma corticosterone	$16.31 \pm 2.12$ (13)	$13.81 \pm 1.41$ (13)	-0.98	0.34

Note: Numbers in parentheses indicate sample size and data are means  $\pm$  SEM.

During the breeding season, our data suggest that chronic predator pressure leads to a reduction in baseline DHEA levels. This is consistent with the response to acute restraint, which increased corticosterone in all males, but decreased DHEA only in LPP males. Previous reports show that DHEA has antiglucocorticoid effects, which may be mediated by DHEA metabolism by 3 $\beta$ -HSD to active sex steroids, known to oppose the actions of glucocorticoids (Kalimi et al. 1994; Karishma and Herbert 2002; Newman et al. 2010; Pradhan and Soma, in press). In addition, DHEA decreases expression of 11 $\beta$ -HSD isozyme 1, the enzyme that regenerates active glucocorticoids from inactive metabolites (Apostolova et al. 2005). Furthermore, 7-hydroxy-DHEA derivatives are preferred substrates of 11 $\beta$ -HSD1 (Müller et al. 2006), and thus DHEA metabolites may interfere with local production of active glucocorticoids. Thus, it is possible that chronic predator pressure stimulates DHEA metabolism in the brain and other organs to mitigate the actions of elevated glucocorticoids. The lack of a DHEA response to acute restraint in HPP males may reflect a tonically high level of DHEA metabolism and thus an inability to respond even further when confronted with an acute stressor. Overall, the data indicate that chronic predator pressure affects the balance between DHEA and corticosterone production.

During the breeding season, baseline corticosterone levels showed a trend to be higher in HPP males than in LPP males, however not to the degree previously reported (Clinchy et al. 2004). Our sample size for baseline corticosterone in breeding males ( $n = 23$ ) was half that in the Clinchy et al. (2004) study ( $n = 46$ ), and this provides a parsimonious explanation for why we found a strong trend rather than a significant effect. Such apparent inconsistencies reinforce the idea that baseline glucocorticoid measurements alone may not be sufficient to assess chronic stress. For example, several stress-related psychiatric disorders are associated with decreased cortisol levels (Yehuda and Seckl 2011). And in captive wild-caught songbirds, corticosterone levels are decreased in response to chronic stress (Rich and Romero 2005). Finally, in wild ungulates, chronic stress produces inconsistent changes in fecal glucocorticoid metabolite levels (Creel et al. 2009). These examples highlight the utility of using additional biomarkers, such as DHEA, to examine the physiological effects of chronic stress exposure (Travers et al. 2010; Clinchy et al. 2011).

During the nonbreeding season, a somewhat different pattern emerged. Baseline DHEA and corticosterone levels were lower than during the breeding season, and there was no effect of predator pressure on circulating levels of either steroid. These data may reflect the fact that stressors associated with nest predation are absent during the nonbreeding

season. Importantly, during the nonbreeding season, males at both sites decrease DHEA levels in response to acute stress, suggesting that DHEA metabolism is not tonically high (as might be the case in breeding HPP males).

Baseline DHEA levels were also measured in females during the breeding season. Unlike males, chronic predator pressure did not affect baseline DHEA levels in females. Further experiments are required to determine if acute restraint affects DHEA levels in females. The lack of an effect of chronic predator pressure on baseline corticosterone levels in females is consistent with previous results (Clinchy et al. 2011). Females, again unlike males, decrease plasma corticosteroid-binding globulin levels in response to predator pressure, thereby increasing free corticosterone levels (Clinchy et al. 2011).

To date, there are few studies regarding the relationship between chronic stress and DHEA levels (Jezova and Hlavacova 2008). Our results demonstrate an effect of chronic predator pressure on circulating DHEA levels, suggesting that DHEA plays a role in the stress physiology of vertebrates. This long-term field study also shows that wild song sparrows are useful for investigating the effects of chronic stress on the neuroendocrine system.

## Acknowledgments

We thank Devaleena Pradhan for field assistance; Parks Canada, the Saanich Municipality and private landowners for access to the sites; Dr Joanne Weinberg for technical assistance; and Todd Columbia and Beryl Clinchy for their support.

**Declaration of interest:** This research was supported by fellowships from the Natural Sciences and Engineering Research Council of Canada (NSERC) and Michael Smith Foundation for Health Research (MSFHR) to AEMN; an NSERC Discovery Grant to LZ; and an Operating Grant and New Investigator Award from the Canadian Institutes of Health Research, and a Scholar Award from the MSFHR to KKS. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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