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Metabolic responses to adrenocorticotropic hormone (ACTH) vary with life-history stage in adult male northern elephant seals

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ABSTRACT

Strong individual and life-history variation in serum glucocorticoids has been documented in many wildlife species. Less is known about variation in hypothalamic–pituitary–adrenal (HPA) axis responsiveness and its impact on metabolism. We challenged 18 free-ranging adult male northern elephant seals (NES) with an intramuscular injection of slow-release adrenocorticotropic hormone (ACTH) over 3 sample periods: early in the breeding season, after 70+ days of the breeding fast, and during peak molt. Subjects were blood sampled every 30 min for 2 h post-injection. Breeding animals were recaptured and sampled at 48 h. In response to the ACTH injection, cortisol increased 4–6-fold in all groups, and remained elevated at 48 h in early breeding subjects. ACTH was a strong secretagogue for aldosterone, causing a 3–8-fold increase in concentration. Cortisol and aldosterone responses did not vary between groups but were correlated within individuals. The ACTH challenge produced elevations in plasma glucose during late breeding and molting, suppressed testosterone and thyroid hormone at 48 h in early breeding, and increased plasma non-esterified fatty acids and ketoacids during molting. These data suggest that sensitivity of the HPA axis is maintained but the metabolic impacts of cortisol and feedback inhibition of the axis vary with life history stage. Strong impacts on testosterone and thyroid hormone suggest the importance of maintaining low cortisol levels during the breeding fast. These data suggest that metabolic adaptations to extended fasting in NES include alterations in tissue responses to hormones that mitigate deleterious impacts of acute or moderately sustained stress responses.

1. Introduction

All vertebrates potentially experience stressors, such as a lack of food, limited space, environmental perturbations, and conflicting interactions with conspecifics and other species. While the generalized endocrine response to stress is well studied, the terminology for describing stress responses as distinct from normal homeostatic regulation is more controversial, with several proposed frameworks. Allostasis is the maintenance of stability through changes in homeostatic set points (McEwen and Wingfield, 2003). As the cost (e.g. energetic expenditure, oxidative damage) of allostasis accumulates, the animal enters allostatic overload, which is a state where detrimental effects on the organism body and behavior are evident (Gregory and Wood, 1999; McEwen and Wingfield, 2003; Wikelski and Cooke, 2006). Reactive scope first defines predictive and reactive homeostasis, which allow the animal to respond to circadian, seasonal, and unpredictable variations, respectively (Romero et al., 2009). As reactive homeostasis persists, wear and tear accumulates as a cost of maintaining physiological conditions in the reactive homeostasis range (Romero et al., 2009). This accumulation increases the likelihood of entering homeostatic overload or failure, which potentially compromises short or long-term health (Romero et al., 2009). Despite the differences in terminology, sustained stress can produce deleterious impacts, including suppression of immune and reproductive function (Ben-Eliyahu et al., 1991; Tilbrook et al., 2000). Studies on the impacts of anthropogenic stressors to wildlife have begun to recognize that integration of various stressors, such as pollution, disturbance, alterations in food availability and others may have demographic impacts by increasing the allostatic load on individuals and affecting health and reproduction. This realization has led to the development of the field of conservation physiology (Peery et al., 2004; Regel and Pütz, 1997; Wikelski and Cooke, 2006; Wikelski et al., 2002).

The primary hormonal response to stress is the activation of the hypothalamic–pituitary–adrenal (HPA) axis. In response to stress, the hypothalamus releases corticotrophin-releasing hormone (CRH; Ma et al., 1997), which stimulates the pituitary gland to

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release adrenocorticotropic hormone (ACTH; Antoni et al., 1984). ACTH then acts upon the adrenal cortex, which releases corticosteroids, such as cortisol and aldosterone (Haning and Tait, 1970). These adrenal hormones have wide-ranging physiological effects on metabolism, reproductive hormones, and immune function (Brillon et al., 1995; Connell and Davies, 2005; Khani and Tayek, 2001; Maule et al., 1987; Tilbrook et al., 2000). They are known to interact with and affect the concentrations or reactivity of thyroid hormones (thyroxine, T4; and triiodothyronine, T3), which themselves regulate many metabolic pathways and strongly influence whole-animal metabolism (Danforth and Burger, 1984). Despite how important understanding the integration of endocrine responses to multiple stressors is to ecology and conservation, little is known about how HPA axis function and associated metabolic responses vary with life history stages in most wildlife systems. Understanding natural life-history variation in HPA axis activity and responsiveness is critical to providing a context for interpretation of baseline glucocorticoid levels (Madliger and Love, 2014).

Although there have been numerous studies of stress on terrestrial mammals (Boonstra et al., 2001; Båge et al., 2000), few studies have looked at how the HPA axis integrates stressors in wild marine mammals. Marine mammals are difficult to observe and study due to their pelagic lifestyle. Nevertheless, these animals face a number of anthropogenic stressors, such as pollution, anthropogenic noise, declining prey stocks, and boating/fisheries interactions (Brander, 2007; Meltzer, 1994; Nowacek et al., 2001; Tanabe, 2002). Current information on the impacts of stressors to the HPA axis in marine mammals is sparse and variable across species (St. Aubin and Dierauf, 2001; St. Aubin et al., 2001; St. Aubin and Geraci, 1989). Work with captive or rehabilitated Stellar sea lions and harbor seals has addressed relationships between acute stressors and the function of the HPA axis as reflected in fecal and serum corticosteroids (Gulland et al., 1999; Mashburn and Atkinson, 2004, 2008). Interestingly, while aldosterone, a key hormone in electrolyte balance, is not viewed as a major stress hormone in terrestrial mammals, it has been linked to the stress response in bottlenose dolphins (*Tursiops truncatus*) and harbor seals (*Phoca vitulina*) and has been hypothesized as a key stress hormone in marine mammals (Gulland et al., 1999; Houser et al., 2011; Thomson and Geraci, 1986). Levels of cortisol have been related to fasting durations, lactation, molting and breeding behavior in some phocid species (Boily, 1996; Engelhard et al., 2002; Houser et al., 2007; Lidgard et al., 2008; Ortiz et al., 2001); however, data on how HPA responsiveness varies with fasting and reproductive state in the wild are limited. The combination of food abstinence and reproductive effort is a natural and predictable stressor in pinnipeds, yet little is known about the physiological response to the reproductive fasts as reflected in hormonal stress markers.

Adult male northern elephant seals (NES) provide an ideal system to investigate the alteration in HPA axis function and its metabolic impacts. Male elephant seals have the highest fasting energy expenditure, the longest fasting duration, and exhibit dramatic increases in reproductive effort relative to body size when compared to other phocids (Crocker et al., 2012a). The three month breeding fast is energetically costly and incorporates terrestrial movement and combative male-male competition to establish access to females. During this time, body mass decreases by approximately 40% in dominant males and 30% in subordinate males (Deutsch et al., 1990; Haley et al., 1994). Dominant males experience higher mating success, but at the cost of increased energy expenditure, with successful males having sustained fasting energy expenditures approaching 4 times their standard metabolic rates. (Crocker et al., 2012a). Males return to sea after breeding and then later undergo a second month-long haulout, during which they undergo a catastrophic molt of their pelage (Le Boeuf and Laws, 1994). This also occurs while fasting and presumably incurs additional metabolic costs associated with tissue turnover and pelage synthesis.

Elephant seals lack a cortisol response to handling when chemically sedated, which allows measurement of basal values in natural, field conditions (Champagne et al., 2012a). In marked contrast to conspecific females, cortisol remains stable across breeding in males, although highly variable among individuals (Crocker et al., 2012b). This suggests that potential lipolytic benefits of elevations in cortisol (Crocker et al., 2014) are secondary to other impacts on metabolism, including protein sparing. In contrast, aldosterone concentrations increased across the fast (Ortiz et al., 2006), which may support the hypothesis that aldosterone is an important factor in the stress response (in this case, prolonged nutrient deprivation).

Here we report changes in adrenal, thyroid and sex hormones, as well as key metabolites, in response to an ACTH challenge in breeding and molting adult male northern elephant seals. We measured acute effects for 2 h and then sampled again at 48 h to identify impacts of sustained ACTH stimulation. The ACTH challenge was performed to examine the responsiveness of the HPA axis and its effects on metabolism under the combined natural and predictable stressors of prolonged food and water abstinence, breeding activity and combative intrasexual competition, and the physiological constraints of molting. Our objectives were to determine hormonal makers of the stress response and how body condition and life-history stage relate to variability in the responsiveness of the HPA axis, knowledge which is necessary for understanding the potential impact of additional stressors on the seals, particularly those of anthropogenic origin.

2. Methods

2.1. Study site and study animals

All animal handling procedures were approved by the Sonoma State University Institutional Animal Care and Use Committee and work was conducted under National Marine Fisheries Services marine mammal permit #14636. Fourteen individual adult male northern elephant seals were studied during the breeding and molting season at Año Nuevo State Reserve. The molting period was examined in order to observe alterations in hormones and metabolites outside of the breeding season. Animals were considered adult based on body mass and development of secondary sexual characteristics (Deutsch et al., 1990). If not already present, animals were marked with rear flipper tags (Dalton jumbo Rototags, Oxon, England) and hair dye (Lady Clairol, Stamford, CT, USA).

2.2. Field procedures

Males were sampled at three stages: in early January (Early Breeding), in late February or early March (Late Breeding), and mid-July (Mid Molt). Six males were sampled during each stage. Due to availability at the study site, 4 males were represented in both the early and late breeding samples. Each male was sedated with an intramuscular injection of $\sim 0.3 \text{ mg kg}^{-1}$ of tiletamine HCl and zolazepam HCl (Telazol, Fort Dodge Animal Health, Fort Dodge, IA). Immobilization was maintained with bolus, intravenous injections of ketamine HCl (Ketaset, Fort Dodge Animal Health, Fort Dodge, IA). Blood samples were obtained from the extradural vein via an 18 gauge spinal needle and collected into chilled serum, sodium heparin, and potassium EDTA blood tubes. Samples were placed on ice until returned to the lab. After initial blood samples (pre) were collected, mass was visually estimated