Adrenal sensitivity to stress is maintained despite variation in baseline glucocorticoids in moulting seals

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Stressful disturbances activate the hypothalamic–pituitary–adrenal (HPA) axis and result in the release of glucocorticoid (GC) hormones. This characteristic stress response supports immediate energetic demands and subsequent recovery from disturbance. Increased baseline GC concentrations may indicate chronic stress and can impair HPA axis function during exposure to additional stressors. Levels of GCs, however, vary seasonally and with life-history stage, potentially confounding their interpretation. Our objective was to evaluate HPA axis function across variations in baseline GC levels. Northern elephant seals show substantial baseline variation in GC levels during their annual moulting period. We therefore conducted measurements early, in the middle and at the end of moulting; we simulated an acute stressor by administering adrenocorticotropic hormone and evaluated the changes in circulating hormones and metabolites over the following 2 h. The stress response was characterized by increases in both cortisol and aldosterone (F(7,105) = 153 and 25.3, respectively; P < 0.001). These hormones increased in parallel and the slopes of their relationship varied by study group, suggesting they are regulated in a co-ordinated manner during acute stress in this species. There was no detectable difference in the total release of cortisol or aldosterone among study groups, indicating that the HPA axis remained sensitive to stimulation by adrenocorticotropic hormone despite varying baseline levels of GCs. Acute stress influenced carbohydrate and fat metabolism in all study groups, but protein catabolism was affected to a far lesser degree. These findings suggest that elephant seals, and potentially other pinniped species, are resilient to moderate variations in baseline GC levels and remain capable of mounting a response to additional stressors.

Key words: Stress, marine mammal, hypothalamic–pituitary–adrenal axis, aldosterone

Introduction

Numerous environmental conditions potentially disrupt homeostasis in free-ranging animals. Sufficient disturbance will result in a stress response, characterized by activation of the hypothalamic–pituitary–adrenal (HPA) axis and the release of glucocorticoid (GC) hormones (e.g. cortisol; Fig. 1). Activation of the HPA axis and the release of GCs have wide-ranging consequences and influence several metabolic pathways; for example, at high concentrations GCs increase gluconeogenesis, lipolysis and protein catabolism (Sapolsky et al., 2000). These adjustments support immediate energetic...
demands at the expense of energy reserves. Many stressors result from or are influenced by human activity (Maxwell et al., 2013). Conservation biologists are therefore increasingly using stress assessments to monitor animal health and inform management efforts (Cooke and O’Connor, 2010; Madliger and Love, 2014), but this requires a far more detailed understanding of stress responses in wildlife systems than is currently available (Dantzer et al., 2014).

Baseline levels of GCs provide insight into the stress state of individuals and are frequently measured in free-ranging animals. These levels, however, vary widely among species and with life-history stage (Romero, 2002). The interpretation of these metrics is further complicated because increased baseline GCs may be associated with increased, decreased or unrelated to metrics of fitness, including survival (Busch and Hayward, 2009). This probably results from the concentration-dependent function of GC hormones. At low levels, GCs interact with high-affinity type I receptors and influence metabolic functions; it is not until GC concentrations increase markedly that they bind with low-affinity type II receptors and stimulate a stress response (Romero, 2004). Even GC levels that are chronically increased during predictable life-history events are not necessarily deleterious to the individual (Boonstra, 2013). They may, however, still influence an animal’s capacity to respond to additional stressors. The ability of an individual to mount a response, measured as the magnitude of GC release following a stressor, may provide a better metric of animal fitness (Romero, 2004).

As a result of their amphibious lifestyle, pinnipeds (seals and sea lions) are exposed to a multitude of aquatic and terrestrial stressors during critical life-history stages. For example, many pinnipeds endure prolonged periods of food deprivation simultaneous with energetically costly activities, including breeding, lactation and moulting (Houser et al., 2013). These periods occur at predictable intervals and are associated with hormonal changes that potentially influence the ability to respond to additional stressors (Costa, 1993; Atkinson et al., 2011). For example, circulating cortisol concentrations frequently increase during fasting in pinnipeds (Ortiz et al., 2001; Guinet et al., 2004). Several pinniped populations are in decline (e.g. Steller sea lions, Eumetopias jubatus), while others are stable or increasing (e.g. California sea lions, Zalophus californianus, and northern elephant seals, Mirounga angustirostris; Atkinson et al., 2008). Understanding how normal variation in baseline GC levels influences the capacity of animals to respond to subsequent stressors provides insight into how cumulative stressors may influence the resilience of animals to environmental disturbance and its potential fitness consequences.

Our objective was to evaluate the influence of natural variation in baseline GC concentrations on the HPA axis and its sensitivity to additional stressors. Periods when GC levels are elevated to support life-history functions may limit the ability of animals to respond adequately to additional stressors (Rich and Romero, 2005). For example, GC levels increase during moulting in several pinniped species (Riviere et al., 1977; Ashwell-Erickson et al., 1986; Boily, 1996; Myers et al., 2010; Gobush et al., 2014). Elephant seals are an ideal species in which to investigate the stress response in a free-ranging marine mammal because a considerable amount of previous work has investigated their metabolism and its regulation. Elephant seals are known to have considerable variation in GCs as a function of age, sex and life-history stage (including breeding and moulting; for review see Houser et al., 2013). To investigate the possibility that that increased GC concentrations influence the stress response, we examined the response to a simulated stressor—the administration of exogenous adrenocorticotrophic hormone (exACTH)—across the annual moul of juvenile seals when GC levels are anticipated to vary.

We tested the hypothesis that the magnitude of the stress response would vary during moulting and predicted that increases in baseline GCs would result in a muted response to a simulated stressor. In this species, typical handling procedures using dissociative anaesthetics do not appear to cause a significant stress response, evidenced by stable cortisol concentrations during handling, thus offering a study system amenable to experimental perturbation with minimal handling artifact (Champagne et al., 2012b). To assess the stress response, we quantified several hormones and metabolites. Cortisol and endogenous ACTH were used as metrics of the HPA axis response. Some studies have found that ACTH stimulates aldosterone secretion in marine mammals (St Aubin and Geraci, 1986; Gulland et al., 1999; Ensminger et al., 2014); we therefore also evaluated circulating aldosterone as a potential stress hormone. The thyroid hormone axis may be influenced during stress, notably by promoting the production of reverse triiodothyronine (rT3; Weissman, 1990); thus, rT3 was measured to assess the influence of acute stress on the thyroid axis. The stress response has further downstream consequences on metabolism, so select metabolites were assessed to evaluate metabolic effects. We measured blood urea

![Figure 1: The hypothalamic–pituitary–adrenal axis is activated during stress. Glucocorticoid hormones (e.g. cortisol) are released and influence diverse target tissues. Abbreviations: ACTH, adrenocorticotrophic hormone; CRH, corticotrophin-releasing hormone.](https://academic.oup.com/conphys/article-abstract/3/1/cov004/2571225)
nitrogen (BUN), non-esterified fatty acid (NEFA), glucose and lactate concentrations as metrics of protein catabolism, lipolysis and carbohydrate metabolism, respectively.

Materials and methods

This study was conducted at Año Nuevo state park, San Mateo County, CA, USA. Each spring, juvenile elephant seals haul out on rookeries and undertake a catastrophic molt, completely replacing their pelage. The molting process takes ~1 month, during which elephant seals fast completely from food and water; simultaneously, hormone changes occur, including changes in GCs, to facilitate molting (Champagne et al., 2012a; Houser et al., 2013). Measurements were conducted in 16 yearling northern elephant seals (seven female and nine male) at the beginning (n = 6), middle (n = 3) and end (n = 5) of the spring molting period (early, mid, and late, respectively) in a cross-sectional sampling design. Animal states were determined based on the degree of molten pelage. Based on pilot studies, these stages showed substantial variability in baseline GC concentrations, and the predicted trends were observed in the present study (see Results and Table 1).

Study animals were chemically immobilized as previously described (Kelso et al., 2012). Briefly, sedation was induced with 1 mg/kg tiletamine–zolazepam (Telazol) administered as an intramuscular injection and maintained using periodic intravenous doses of ketamine and diazepam (all drugs from Fort Dodge Laboratories, Fort Dodge, IA, USA). This sedation technique does not elicit a stress response in this species because circulating cortisol concentrations do not increase markedly during sedation (Champagne et al., 2012b). Blood samples were collected via an 18 gauge, 8 cm needle inserted into the extradural vessel. Initial blood samples were collected immediately after study animals were sedated, in order to establish baseline hormonal and metabolite values. An intramuscular dose of 30 U exACTH (Wedgewood Pharmacy, Swedesboro, NJ, USA) was then administered via an 18 gauge 8 cm needle inserted into the lateral musculature (0.22 U/kg, SD 0.007 U/kg). Blood samples were then collected periodically for 2.5 h after exACTH injection (15, 30, 60, 90, 120 and 150 min after administration). At the conclusion of the measurement, seals were weighed using a scale suspended from a tripod (MSI tension dynamometer, Seattle, WA, USA).

The response to exACTH administration was evaluated by quantifying select hormones and metabolites. Hormone responses were measured in serial samples for cortisol, aldosterone and endogenous ACTH, whereas rT₃ was measured in only the initial and final samples (pre and post, respectively). Hormones were assayed using commercially available radioimmunoassay (RIA; cortisol and aldosterone from Siemens, Inc., Washington, DC, USA; and rT₃ from Alpco, Inc., Salem, NH, USA) or enzyme immunoassay (EIA) kits (ACTH from Alpco, Inc.). All kits have been previously validated for northern elephant seals (cortisol, Ortiz et al., 2001; aldosterone, Ortiz et al., 2000; Houser et al., 2001; and ACTH and rT₃, Ensminger et al., 2014). The responses of glucose and lactate were measured in serial samples using a dedicated autoanalyser (YSI, 2300; Yellow Springs Inc., Yellow Springs, OH, USA). Non-esterified fatty acid and BUN concentrations were measured from initial and final samples (pre and post, respectively), using enzymatic colorimetric assays (Wako Diagnostics, Richmond, VA, USA; and Stanbio, Boerne, TX, USA, respectively).

To assess the total response during the experimental period, we calculated the total area under the curve (AUC) over time by summing the areas under the response vs. time polygons between sampling points relative to their initial concentrations (see Fig. 2). Statistical tests were performed in R, version 3.0.2 (R Core Team, 2013). Response variables were logarithmically transformed when necessary to meet distribution and variance assumptions. Differences among groups were assessed using ANOVA or Welch’s one-way test assuming unequal variances, and post hoc pairwise comparisons were conducted using Tukey’s tests. Differences among repeated samples were evaluated using linear mixed models (LMMs), with individual as a random effect and sample time as an ordered fixed effect. Appropriate degrees of freedom within LMMs were estimated using the Kenward–Rogers approximation and random effect and sample time as an ordered fixed effect. Appropriate degrees of freedom within LMMs were estimated using the Kenward–Rogers approximation and P-values calculated in the lmerTest package (Kuznetsova et al., 2014); post hoc comparisons among repeated samples were conducted using Dunnett’s test against the initial (time zero) sample within each group. Goodness of fits were calculated using the

Table 1: Sex, initial mass, hormone and metabolite concentrations for each of the three study groups

| Study group | F/M | Mass (kg) | ACTH (pg/mL) | Cortisol (pg/mL) | Aldosterone (pg/mL) | Glucose (mg/dL) | Lactate (mg/dL) | NEFA (mg/dL) | BUN (mg/dL) | rT₃ (pg/mL) |
|-------------|-----|-----------|--------------|------------------|---------------------|-----------------|---------------|--------------|--------------|-------------|
| Early       | 2/4 | 155 (11)  | 4.08 (1.53)  | 110 (38)         | 233 (77)           | 6.80 (0.69)     | 3.58 (0.77)   | 0.864 (0.168) | 7.43 (0.73)  | 1.47 (0.31)  |
| Mid         | 3/2 | 133 (11)  | 3.83 (0.87)  | 413 (111)        | 1431 (1056)        | 5.52 (1.01)     | 5.17 (0.83)   | 0.906 (0.041) | 9.56 (1.82)  | 3.31 (0.59)  |
| Late        | 2/3 | 121 (9)   | 2.86 (0.83)  | 306 (129)        | 372 (185)          | 6.97 (0.55)     | 3.02 (1.14)   | 1.210 (0.372) | 6.18 (1.81)  | 2.46 (0.17)  |
| P-Value     | NA  | <0.01     | <0.001       | <0.001           | <0.05              | <0.01           | <0.05         | <0.05        | <0.05        |

Results are expressed as mean values (SD). Significant differences were evaluated by ANOVA. P-Values are shown in the bottom row; post hoc pairwise comparisons were performed using Tukey’s HSD test, and differences between study groups are denoted by differing letters within each column. Abbreviations: ACTH, adrenocorticotrophic hormone; BUN, blood urea nitrogen; F, female; M, male; NA, not applicable; NEFA, non-esterified fatty acid; nsd, no significant difference; rT₃, reverse triiodothyronine.
MuMIn package (Kamil, 2014) based on the methods of Nakagawa and Schielzeth (2013) to determine r² metrics within LMMs. Marginal r² (m²) values are reported to describe the variance explained by fixed effects.

Results

Cortisol and aldosterone showed similar overall trends in their baseline concentrations; cortisol was lowest early in moulting ($F_{2,13} = 13.7, P < 0.001$; Table 1) and aldosterone was greatest mid-moult (log₁₀ transformed, $F_{2,13} = 14.7, P < 0.001$; Table 1). There was no detectable difference among initial ACTH concentrations ($P > 0.1$). Glucose and lactate had opposing trends across moulting; glucose concentrations were lowest in the middle of moulting when lactate concentrations were highest ($F_{2,13} = 5.5, 7.5$, respectively; $P < 0.05$; Table 1). There was no detectable difference in NEFA concentrations among study groups ($P > 0.1$; Table 1). Blood urea nitrogen concentrations were lowest at the end of moulting ($F_{2,13} = 6.5, P = 0.01$; Table 1). There was considerable variability in rT₃ concentrations, and each study group was different from the others ($F_{2,13} = 30.2, P < 0.0001$; Table 1).

The timing and magnitude of cortisol, aldosterone and ACTH responses following exACTH administration are shown in Figure 2. Concentrations of both cortisol and aldosterone were significantly elevated following ACTH

Figure 2: The responses of cortisol (A), aldosterone (B) and endogenous adrenocorticotropic hormone (ACTH; C) to the administration of exogenous ACTH (exACTH) for each of the three study states: early, middle and late moulting. Initial values are shown at time zero. Coloured asterisks indicate that the hormone value at that time point and all subsequent time points differs significantly from the initial hormone value (Dunnett’s test, $P < 0.05$); ‘nsd’ indicates no significant difference from time zero for any sample in that group. Error bars represent the SEM at each sampling time. An inset within each panel shows a comparison of the total hormone release over time, as area under the curve (AUC) values, among study groups; no differences in AUC values were detected, and the y-axis units are concentration × minutes (not shown).

Figure 3: Cortisol and aldosterone concentrations were tightly correlated during the stress response (linear mixed model (LMM), $F_{1,113} = 237.5, P < 0.0001$), and this relationship varied by study group (cortisol × state, $F_{2,113} = 17.1, P < 0.0001$); see main text for further description of statistical analysis. Each sample collected in this study for all study animals is represented by filled symbols and colour coded according to moulting state (early, mid or late). Fitted regression lines are derived from the LMM model fits, and marginal r² (m²) values are shown for each group. Open circles are average hormone values at each sampling time (0–150 min) within the study group and are shown for visual clarity only.
administration (cortisol, $F_{7,105} = 153$, $P < 0.001$; and 
log$_{10}$ (aldosterone), $F_{7,105} = 25.3$, $P < 0.001$). In all three 
study groups, cortisol concentration increased within 
15 min and remained elevated for the duration of the exper-
iment (Dunnett’s comparison against the initial sample 
within each group, $P < 0.05$). There was an increase in the 
response time of aldosterone following exACTH adminis-
tration with the progression of moulting; aldosterone was 
significantly increased within 30 min early in moulting, 
45 min in the middle of moulting, and 60 min in the late 
moultng group (Dunnett’s comparison against the initial 
sample within each group, $P < 0.05$). We did not detect a 
difference in the total release of cortisol or aldosterone 
among the study groups, as measured by AUC relative to 
initial concentrations (ANOVA, $P > 0.1$: Fig. 2A and B insets). To assess the relationship between cortisol and aldo-
sterone responses, we ran a linear mixed model including 
moultng state as a factor and individual as a random effect; 
cortisol and aldosterone increased in parallel following exACTH administration ($F_{1,113} = 237.5$, $P < 0.0001$; Fig. 3), 
and the slopes of the relationship varied by study group 
(cortisol × state, $F_{2,113} = 17.1$, $P < 0.0001$).

There was a significant decrease in ACTH following 
exACTH administration ($F_{7,105} = 9.2$, $P < 0.001$). This decline, 
however, did not occur until at least 120 min after exACTH 
administration (Dunnett’s comparison against the initial sam-
ple within each group, $P < 0.05$; Fig. 2C).

Circulating concentrations of glucose and lactate displayed 
opposing responses to exACTH administration; glucose 
increased ($F_{7,105} = 20.6$, $P < 0.001$; Fig. 4A), while lactate 
decreased ($F_{7,105} = 25.4$, $P < 0.001$; Fig. 4B) over time in all 
study groups. There were no detectable differences in the total 
glucose or total lactate responses among study groups as mea-
sured by AUC (ANOVA, $P > 0.1$). To assess the association 
between glucose and lactate, we ran a linear mixed model 
with moultng state as a cofactor and individual as a random effect; 
glucose and lactate were inversely related ($F_{1,113} = 905.1$, 
$P < 0.0001$; Fig. 5), and the slopes of the relationship varied by study group 
(lactate × state, $F_{2,113} = 42$, $P < 0.0001$). Furthermore, to assess the total responses of glucose and lac-
tate, we ran a general linear model between glucose and lactate 
AUC values, with moultng state as a cofactor. The total
responses of glucose and lactate were inversely related \((F_{1,10} = 80.2, P < 0.0001; \text{Fig. } 5)\).

We measured the responses of NEFA, BUN and \(rT_3\) in initial and final samples (time zero ‘pre’ samples and 150 min after exACTH administration ‘post’ samples; Fig. 6). The NEFA levels increased in response to exACTH administration in all three study groups (Student’s paired \(t = 9.0, 4.2\) and 6.0 in early, mid and late groups, respectively; \(P < 0.05\)), and the magnitude of response decreased across moulting (based on the proportional increase in NEFA between pre and post samples; \(F_{2,13} = 6.2, P = 0.013\); Tukey’s \(P < 0.05\)). Blood urea nitrogen showed a more variable response; there was no detectable difference in BUN concentration following exACTH administration early in the moult (\(P > 0.1\)), a significant increase in the middle of the moult (Student’s paired \(t = 4.1, P < 0.05\)) and a small but statistically significant increase at the end of moulting (Student’s paired \(t = 4.2, P < 0.05\)). There was not a strong response of \(rT_3\) to exACTH; there was a small but statistically significant increase early in moulting (Student’s paired \(t = 4.3, P < 0.01\)) and no detectable change in the middle or end of the moult (\(P > 0.1\); Fig. 6C).

To assess the relationship between \(rT_3\) and cortisol, we ran a general linear model with moulting state as a cofactor. There was a close association between initial \(rT_3\) and cortisol concentrations (cortisol, \(F_{1,10} = 210.5, P < 0.0001; \text{Fig. } 7\)), and this relationship varied among study groups (cortisol \(\times\) state, \(F_{2,10} = 10.3, P < 0.01\)). At the end of moulting, \(rT_3\) did not vary with cortisol (\(P > 0.9\)), whereas there were close associations during the early and mid-moulting stages (early, \(r^2 = 0.54, P = 0.058\); mid, \(r^2 = 0.88, P = 0.01\)). This association between initial \(rT_3\) and cortisol concentrations was no longer present 150 min after exACTH administration (\(P > 0.1\); Fig. 7).

**Discussion**

Stimulating the HPA axis with exACTH caused a co-ordinated hormonal and metabolic response in juvenile northern elephant seals. Baseline cortisol and aldosterone concentrations varied 4- to 6-fold during moulting. Despite the fluctuations in the initial corticosteroid concentrations, study animals responded in a similar manner to exACTH administration and reacted with comparable corticosteroid secretion relative to baseline concentrations. Most studies administer 0.5–2.0 U/kg of exACTH (e.g. Wasser et al., 2000; Lien and Huang, 2008). Based on preliminary trials, we used a lower dose of 0.25 U/kg, which resulted in a 3- to 12-fold increase in both cortisol and aldosterone. The response to exACTH administration resulted in significant state-dependent downstream effects on glucose metabolism, lipid availability and protein catabolism, indicated by changes in circulating concentrations of glucose, lactate, NEFA and BUN, respectively.

Baseline concentrations of both cortisol and aldosterone varied substantially and followed similar patterns throughout moulting, with the highest hormone concentrations in the

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**Figure 6:** Circulating concentrations of non-esterified fatty acids (NEFA; A), blood urea nitrogen (BUN; B) and reverse triiodothyronine \((rT_3; C)\) before and 2.5 h after the administration of exogenous ACTH (pre and post, respectively) at the beginning, middle and end of the moulting period (early, mid and late, respectively). Coloured asterisks along the x-axis indicate a significant difference between the pre and post samples (Student’s paired \(t\) test, \(P < 0.05\)); ‘nsd’ indicates no significant difference detected. Letters above the pre groups indicate differences in the initial concentrations among moulting stages (early, mid and late; ANOVA followed by Tukey’s HSD test, \(P < 0.05\)).
middle of the moult (Table 1). The concentration of ACTH, however, remained relatively consistent throughout molting, suggesting an allostatic adjustment altering corticosteroid levels during this life-history stage. Some hormone and metabolite concentrations varied among the study groups following the exACTH administration (e.g. peak cortisol concentrations differed between groups 2.5 h post-administration), but these differences were largely driven by initial conditions. The total response, best measured as AUC values, was not detectably different among study groups for any response variable (e.g. see Fig. 2 in insets for cortisol, aldosterone and ACTH). These data suggest that seals maintained the ability to respond to additional stressors despite changes in baseline stress hormone concentrations. Both cortisol and aldosterone increased immediately following exACTH administration. Despite this increase, elephant seals did not display a significant decrease in ACTH for at least 2 h after exACTH administration (Fig. 2), suggesting delayed negative feedback on the HPA axis.

Cortisol and aldosterone showed similar responses following the administration of exACTH; concentrations increased rapidly for ~60 min and continued to increase for the duration of the 2.5 h sampling period, but at a reduced rate (Fig. 2A and B). The response of cortisol was similar among study groups; average peak cortisol concentrations varied only between 1300 and 1600 nM. There were no detectable differences in total aldosterone release among study groups (inset in Fig. 2B). The greater variability in baseline aldosterone concentrations probably influenced the differences apparent among study groups in the absolute aldosterone concentrations following exACTH administration. Rehabilitated (Gulland et al., 1999) and captive (Keogh, 2011) harbour seals (Phoca vitulina) showed similar patterns following exACTH administration, with sharp increases in aldosterone and cortisol for ~1 h, a plateau, followed by decreased concentrations after ~90 min and 2.5 h, respectively. These studies were conducted in the laboratory rather than field settings and administered an exACTH dose of 0.5 U/kg (Keogh, 2011) and 1.0 U/kg (Gulland et al., 1999), whereas we used a lower mass-specific dose of 0.25 U/kg. Despite this lower dose, cortisol concentrations in elephant seals increased to over 1000 nM in all study groups, nearly twice what was observed in harbour seals. These data suggest that, despite the apparently resilient nature of elephant seals, their HPA axis is quite sensitive to perturbation.

The responses of cortisol and aldosterone were tightly correlated (Fig. 3), further suggesting they are regulated in a co-ordinated manner by ACTH. These hormones share similar synthetic pathways; cortisol is produced by 11β-hydroxylase (coded for by gene CYP11B1 and regulated by ACTH), whereas aldosterone is produced by aldosterone synthase (coded for by a similar gene, CYP11B2, and regulated by the renin–angiotensin–aldosterone system, in humans). An unequal crossover event between these genes can result in a gene variant with a CYP11B1 regulatory element and CYP11B2 catalytic sequence, i.e. regulated by ACTH but coding for aldosterone synthase activity, resulting in glucocorticoid-suppressible hyperaldosteronism (Pascoe et al., 1992; White and Pascoe, 1992). Cortisol and aldosterone seem to be associated more closely in phocid seals than commonly reported in terrestrial mammals. Aldosterone is
typically regulated by the renin–angiotensin–aldosterone system in mammals. This system is activated by reduced renal blood flow and results in production of angiotensin I, which is converted to angiotensin II by angiotensin-converting enzyme (found primarily in the lungs) and subsequently stimulates aldosterone secretion (Porterfield and White, 2007). In a diving mammal, however, renal blood flow varies (Zapol et al., 1979) and the lungs are often not perfused (Ponganis, 2011), potentially limiting angiotensin II production. Thus, the regulation of aldosterone secretion by ACTH may be advantageous in a diving mammal. This potential explanation is purely speculative, but the mechanism of the action of GCs on lipolysis is modulated by dehydrogenases that convert GCs between active (e.g. cortisol) and inactive forms (e.g. cortisone; Peckett et al., 2011). Adipose tissue contains high levels of the activating dehydrogenase that can lead to substantially higher concentrations of GCs at target tissues than those present in the circulation (Masuzaki et al., 2001). Local regulation within adipose tissue may therefore result in high responsiveness to GCs and modulate the response with changing conditions.

We found a close association between cortisol and rT3 in the baseline state but not during acute stress (Fig. 7). Stress typically decreases circulating thyroid hormones, triiodothyronine (T3) and thyroxine (T4), reducing whole-animal energy use, during food limitation, for example (Servatius et al., 2000; Helmreich et al., 2005). The reduction in T3 concentration may be facilitated by increased conversion of T4 to rT3 by an inner-ring deiodinase, primarily D3 (Bianco and Kim, 2006); rT3 binds with T3 receptors but has no biological activity, thus acting as a T3 blocker, further enhancing metabolic suppression. There is some evidence that rT3 concentrations increase during GC treatment in humans (Chopra et al., 1975). The present study suggests that rT3 may track cortisol concentrations over longer time periods (more than several hours). Acute stress events, such as animal handling, may not affect rT3 concentrations. Thus, rT3 may provide a marker of stress in free-ranging animals, because it may be disassociated from proximate handling effects, but further study is required to describe the relationship between stress, GCs and the timing and magnitude of the rT3 response.

In addition to the hormonal changes that occurred following exACTH administration, we evaluated several downstream effects on metabolism (Breuner et al., 2013). Many metabolic features were influenced by the stress response, including lipolysis, protein, and carbohydrate metabolism. We use metabolite values here as indices of whole-animal metabolism; NEFA, BUN, glucose and lactate concentrations as indicators of lipolysis, protein and carbohydrate metabolism, respectively. However, we urge caution when using static metabolite values to infer changes in metabolic pathways; sometimes these are correlated, but often they are not (e.g. see Houser et al., 2007).

Usually, the primary metabolic role of stress-induced GC release is to increase glucose availability in the circulation (Sapolsky et al., 2000). In the present study, however, NEFA was the metabolite most influenced by exACTH administration, increasing by 20–60% compared with initial levels, far greater than the 15–20% increase observed in glucose concentration. These increased NEFA concentrations may result either from increased lipolysis from abundant adipose stores in this species or from reduced fatty acid re-esterification to triglyceride. The increase in NEFA occurred despite already high baseline rates of lipolysis typical of phocid seals (>10 μmol/kg/min; Crocker et al., 2014). The rate of fatty acid re-esterification is also high in elephant seals (Houser et al., 2007), and the increase in circulating NEFA may result from decreased re-esterification induced by GCs rather than increased lipid mobilization (Jeanrenaud, 1967). Despite the lack of any detectable difference among initial NEFA concentrations, the magnitude of response, relative to baseline concentrations, declined with the progression of moult. The action of GCs on lipolysis is modulated by dehydrogenases that convert GCs between active (e.g. cortisol) and inactive forms (e.g. cortisone; Peckett et al., 2011). Adipose tissue contains high levels of the activating dehydrogenase that can lead to substantially higher concentrations of GCs at target tissues than those present in the circulation (Masuzaki et al., 2001). Local regulation within adipose tissue may therefore result in high responsiveness to GCs and modulate the response with changing conditions.
responses in the timing of their change compared with baseline, requiring 30–45, 90 and 120 min in early, mid, and late moulting, respectively (Fig. 4), and their concentrations were inversely correlated during the stress response (Fig. 5).

One typical metabolic consequence of the stress response is that cortisol release stimulates protein catabolism and the release of amino acids into the circulation, providing substrates for gluconeogenesis and increasing glucose availability for immediate energy needs (Sapolsky et al., 2000). This protein catabolism can result in lean tissue degradation during chronic or repeated stress events. Animals adapted to prolonged fasts, such as many marine mammals, including elephant seals, have very low rates of protein degradation (Crocker et al., 1998; Houser and Costa, 2001; Verrier et al., 2009) to maintain organ function during fasts associated with breeding, lactation, development and moulting (Champagne et al., 2012a). Strong increases in glucose demands are also evident during extensive tissue repair and synthesis (Williams and Barbul, 2003). Changing hepatic responsiveness to glucocorticoids across moulting may reflect conflicting metabolic demands for new pelage synthesis and the need to spare mobilized protein. Adaptations to fasting may consequently protect lean tissue from degradation during stress. The substrates required for glucose production may be supplied from other intermediates, such as lactate (Champagne et al., 2006, 2012c; Tavoni et al., 2013). The close association between lactate and glucose (Fig. 5) and the lack of a strong response in BUN concentrations is consistent with this hypothesis (Fig. 6).

The response to exACTH administration was recently reported in adult male northern elephant seals during different life-history stages, namely breeding and mid-moulting (Ensminger et al., 2014). A lower mass-specific dose was used in the much larger adults (0.15 U/kg), but several similarities were observed between their study and the present study. Cortisol increased in both age classes (juveniles and adult males), while ACTH did not show strong suppression, suggesting impaired or delayed negative feedback of the HPA axis. Likewise, aldosterone increased in all study groups (Table 1) and the lack of a strong response in BUN concentrations was consistent with this hypothesis (Fig. 6).

We simulated a stress event by administering exACTH to a model marine mammal species, the northern elephant seal, and evaluated the resulting acute stress response. We found that the HPA axis remained sensitive to stimulation, despite the substantial variation in baseline GC levels that occurred during moulting, suggesting that seals are resilient to the normal variation in GC levels that occur during their normal life histories. Both cortisol and aldosterone increased concurrently during acute stress. Aldosterone is not typically considered part of the stress response in most mammals, but numerous studies have now confirmed its responsiveness to stressors in a variety of marine mammals (Thomson and Geraci, 1986; St Aubin and Dierauf, 2001; Houser et al., 2011). The role played by aldosterone in an adaptive stress response, if any, is currently unknown. Lipid and carbohydrate metabolism were altered during stress, as shown by changes in circulating concentrations of NEFA, glucose and lactate. Circulating BUN concentrations, however, showed far smaller changes, suggesting a reduced influence of stress on protein catabolism in this species. In order to gain a better understanding of the consequences of stress on metabolism and energy use, studies combining stress with additional monitoring techniques (e.g. respirometry or metabolic tracers) will be needed. This work provides further support that baseline circulating GC levels alone are not reliable indicators of stress in free-ranging animals; the natural variation during season and life-history stage must be appreciated before using GC measurements as indices of stress.

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