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# Age and Food Deprivation Affects Expression of the Glucocorticosteroid Stress Response in Magellanic Penguin (*Spheniscus magellanicus*) Chicks

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## ABSTRACT

We examined how the glucocortical stress response in free-living Magellanic penguin (*Spheniscus magellanicus*) chicks changes with age and whether adrenocortical function of chicks within a brood varies in relation to food provisioned by adults. Chicks showed little corticosterone response to capture stress shortly after hatching, an intermediate response around 45-d posthatch, and a robust stress response near fledging. However, in response to an adrenocorticotrophic hormone (ACTH) challenge, hatchlings were capable of secreting corticosterone at adult-like levels. The larger sibling in broods of two showed a similar gradual stress-response development pattern. In contrast, by day 45, when differences in body condition were well established between siblings, the smaller, food-deprived chicks significantly increased baseline levels of corticosterone but showed normal stress-induced levels. Near fledging, baseline levels had returned to normal, but stress-induced levels were lower than expected. Similar to altricial species, normally developing semialtricial Magellanic penguin chicks do not express a robust corticosterone stress response until near fledging. Chronic stressors such as food deprivation cause corticosterone use to be up-regulated earlier than expected. However, in cases of extended chronic stress, down-regulation may ensue, thus avoiding the negative effects of chronically elevated levels of corticosterone.

## Introduction

In response to acute perturbations in the environment, adults in all vertebrate classes possess a hypothalamic-pituitary-adrenal (HPA) stress response that results in an increased secretion of glucocorticosteroids (corticosterone in birds) from the adrenocortical cells (Wingfield and Ramenofsky 1999; Sapolsky et al. 2000). This response acts to reduce the impact of the acute stressor, since glucocorticosteroids facilitate mobilization of energy, allowing escape from or outlasting of the perturbation (Johnson et al. 1992; Wingfield et al. 1995). In cases in which the stressor changes from acute to chronic (i.e., the animal cannot escape from or outlast the perturbation), individuals may experience the negative effects of extended elevation of glucocorticosteroid levels. These negative effects include muscle wasting, impaired immune function, depressed growth, inhibition of reproduction, and, in extreme cases, death (Johnson et al. 1992; Wingfield 1994). Due to these deleterious effects, one might speculate that in cases in which individuals cannot escape perturbations, as in defenseless young, delay in development or suppression of the adrenocortical response to stress would be beneficial (Sims and Holberton 2000).

Among birds, the independence of chicks at hatch varies and is defined as a continuum from altricial to precocial (Starck 1993). Altricial hatchlings are completely dependent on parents for survival: they are featherless, have limited thermoregulatory abilities, are relatively immobile, cannot obtain their own food, and are unable to defend themselves. Precocial hatchlings are fully feathered, have thermoregulatory ability, are fully ambulatory, are capable of finding food, and are able to move away from negative stimuli (Starck 1993). Given this range of capabilities among chicks, it has been shown that variability exists in the level of HPA development at the time of hatch. Precocial chicks typically exhibit a well-developed stress response (Freeman 1982; Wentworth and Hussein 1985), but the HPA stress response of altricial or semialtricial hatchlings is low and shows gradual development over the growth period, culminating in an adult-like response only near the point of independence (Sims and Holberton 2000; Sockman and Schwabl 2001; Love et al. 2003).

Recently, studies have predicted that within altricial or semi-altricial broods, age differences of chicks might result in concomitant unequal development of the HPA stress response: older and thus larger chicks in the brood would have a more

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developed stress response (Heath and Dufty 1998; Sims and Holberton 2000). Although age differences within broods have thus far not been shown to affect HPA development (Heath and Dufty 1998; Sims and Holberton 2000; Sockman and Schwabl 2001), experimental manipulations of both quality and quantity of food given to chicks within broods have resulted in increases in both baseline and stress-induced levels of corticosterone for the food-deprived chicks (e.g., Nunez-de la Mora et al. 1996; Kitaysky et al. 1999, 2001a). Although the actions of corticosterone in response to acute stressors are well known (Wingfield and Ramenofsky 1999), constitutive or baseline levels of corticosterone are also necessary for normal metabolic processes of growth and development (Dallman et al. 1993) and can fluctuate in the course of predictable changes in life-history stages such as migration (Ramenofsky et al. 1995; Holberton et al. 1996). Corticosterone is present in embryonic/prehatch chicks (Sapolsky and Meaney 1986; Tanabe et al. 1986), and constituent levels are likely used in metabolic functions facilitating growth. As such, a complete absence of a corticosterone function in response to more acute stressors is unlikely in posthatch chicks, although it may be underdeveloped or reduced. One example of the function of corticosterone posthatch appears to be its positive correlation to and facilitation of begging, as observed in black-legged kittiwake (*Rissa tridactyla*) chicks (Kitaysky et al. 2001b).

We examined the development of the HPA stress response in free-living chicks of the Magellanic penguin (*Spheniscus magellanicus*). We subjected chicks to a standardized capture-stress protocol (Wingfield et al. 1992) to quantify how chicks respond to an acute stressful stimuli. Magellanic penguin chicks are semialtricial; although still defenseless at hatch, they possess downy feathers, are partially able to thermoregulate, and have some locomotor ability, unlike true altricial species (Starck 1993). Because newly hatched chicks are totally dependent on their parents, we predicted that the development of the stress response in semialtricial Magellanic penguin chicks would be gradual, similar to what is observed in true altricial and other semialtricial species (Sims and Holberton 2000; Sockman and Schwabl 2001; Love et al. 2003). Having quantified how the stress response develops in Magellanic penguin chicks, we next administered exogenous adrenocorticotrophic hormone (ACTH, the anterior pituitary peptide that causes corticosterone secretion by the adrenocortical tissue) to newly hatched chicks to determine whether the adrenal tissue was fully functional at hatching. If adrenal function is underdeveloped at hatching, administration of ACTH should result in no subsequent corticosterone secretion. If, however, corticosterone secretion increases in response to ACTH administration, it suggests that the adrenocortical tissue is fully functional in hatchlings and that any changes in the expression of the stress response as chicks age is occurring above the level of the adrenal gland. Specifically, changes in perception of negative perturbations, at

the level of the brain, may be a significant component of the changes in expression of the stress response as chicks age.

Finally, we used a naturally occurring size/competition asymmetry in the two-chick broods of Magellanic penguins to study the effects of food deprivation on the development of the HPA stress response. Initially, this size asymmetry between nest mates is due to the hatching asynchrony (average, 3–4 d between chicks) in Magellanic penguin broods (Boersma 1991). If the first chick receives a meal before its siblings' hatch, the subsequent size advantage allows the first chick to be favored, and, ultimately, due to food limitation and the competitive size advantage, the smaller chick typically dies (Boersma 1991; Blanco et al. 1996). Rapidity of this mortality depends on factors of adult provisioning and food availability (Boersma and Stokes 1995). The first chick typically maintains any size advantage it obtains early in life; however, the second chick can catch and surpass its older sibling in size in some cases (Boersma 1991). Thus, unlike previous studies examining age alone as a cofactor in development of the stress response in altricial or semialtricial chicks (Sims and Holberton 2000; Love et al. 2003), here, for older chicks, it is body size that is the important factor of competition between siblings, not age alone. Therefore, regardless of hatch order, we predicted that both baseline and stress-induced levels of corticosterone in food-deprived chicks would be greater than that of their well-fed nest mates. We further expected the differences in both baseline and stress-induced corticosterone levels between healthy and undernourished chicks to be most pronounced when the latter was moribund and certain not to survive.

## Material and Methods

### *Study Location and Design*

We conducted fieldwork at the Punta Tombo Provincial Penguin Reserve, Chubut, Argentina (44°02'S, 62°11'W) during the breeding seasons of 1999–2000 and 2001–2002. We divided the chick-rearing period into three stages: hatch (within 5–6 d of hatching), middle-aged (45–60 d estimated posthatch), and near-fledging (90–120 d estimated posthatch). These stages corresponded to November 14–18, January 3–4, and February 6–7 in 1999–2000 and November 13–24, December 26–31, and January 22–26 in 2001–2002. Breeding chronology was earlier in 2001–2002 because of more favorable conditions in that season.

### *Stress Response in Growing Chicks*

To determine how the stress response develops as Magellanic penguin chicks age during each of the three stages, we subjected chicks judged to be healthy and developing normally to the standard capture stress series protocol (Wingfield et al. 1992). We captured and held chicks outside the nest and took sequential blood samples over time to quantify the changes in

corticosterone concentrations in the blood in response to the capture stress. Chicks were held in opaque bags whenever possible, although some of the largest, strongest chicks had to be manually restrained by the researcher. We collected blood samples into heparinized microcapillary tubes after puncture of the interdigital (foot-web) vein. Samples were held on ice in the field until return to camp each afternoon/evening when they were centrifuged to separate plasma. Plasma samples were subsequently frozen at  $-10^{\circ}\text{C}$  until transfer to Seattle for analysis (discussed subsequently). We collected preliminary data in 1999–2000, holding chicks for 30 min and collecting blood samples at under 3 min after visual contact, and at 5, 10, and 30 min postdisturbance. In 2001–2002, we extended the restraint period to 60 min and collected blood samples at under 3 min from visual contact, and at 30 and 60 min postdisturbance. We assumed all samples collected within 3 min of the chick having initially seen the researcher were representative of baseline levels of circulating corticosterone (Wingfield et al. 1982). Nests were chosen such that no chick had observed any other capture disturbance before its sampling.

#### *Adrenal Function at Hatch*

To determine adrenal function of hatchling Magellanic penguin chicks, we injected seven chicks within 1 wk of hatch with 24  $\mu\text{L}$  of exogenous ACTH (Sigma, St. Louis, Mo.; chemical no. A-6303) in saline at a concentration of 0.5 IU/ $\mu\text{L}$ . We removed chicks from their nests and collected a blood sample before injection to determine baseline levels of corticosterone. Chick mass ranged from 154 to 264 g (mean =  $212.5 \pm 15.6$  g;  $N = 7$ ), for a resulting dosage range of 46–78 IU/kg. The concentration of ACTH used for penguins in this study was lower than the 100–200 IU/kg given to passerines to increase corticosterone secretion (Romero et al. 1998a, 1998b). We chose a lower dosage to minimize the possibility of pharmacological overdose likely from direct upward size scaling of passerine concentrations to a larger animal such as the penguin chick (West et al. 1962; Harwood 1963; Schmidt-Nielson 1972). Injections were given intramuscularly in the right thigh and were completed within 5 min of capture for all chicks. After injection, sequential blood samples were taken at 10, 30, and 60 min postcapture. As an injection control, eight other chicks from separate nests of ACTH-injected birds received 25  $\mu\text{L}$  physiological saline (0.9% NaCl).

#### *Effect of Food Deprivation on Corticosterone Response*

For food-deprivation comparisons, at hatching, sibling pairs ( $N = 8$ ) were visually chosen when the smaller chick was no more than 5 d old ( $<175$  g, both chicks well brooded under the adult). We chose middle-aged chicks pairs ( $N = 8$ ) that were in or near a nest with an adult present, with the smaller chick actively begging. For all middle-aged pairs, the larger

chick weighed at least 50% more than the smaller chick. Near fledging, asymmetric chick pairs ( $>50\%$  difference in body mass;  $N = 7$ ) were again chosen, with the larger chick having molted most of its down in preparation for fledging ( $<1/4$  chick down remaining) and the smaller chick now in a moribund state (lethargic, extremely undernourished, and judged by an experienced researcher to be incapable of successfully fledging). During all stages, we assumed chick pairs found together were siblings. Food deprivation comparisons were conducted during the 2001–2002 breeding season.

For hatchlings, we conducted stress series for both siblings on the same day, since typically neither chick had eyes open and the second chick seemed unaware of its sibling's capture. For middle-aged and near-fledging stages, as one chick always responded to the capture of its nest mate, we collected stress series blood samples from only one chick on the first day, randomly assigning which chick was sampled first. We also captured the nonsampled chick on the first day and marked it on the foot with an indelible marker for identification. We conducted the stress series on the marked chick the following day. On the day of their respective stress series, we weighed chicks using a spring balance (nearest 10 g) and measured bill length (BL), bill depth (BD), flipper length (FL), and foot length (FT). We calculated body size index for birds from standardized morphometric measurements as

$$\text{body size index} = 0.938(\text{BL}) + 0.957(\text{BD}) \\ + 0.984(\text{FL}) + 0.966(\text{FT}),$$

where coefficients were component scores from a principal components analysis of standardized morphometric measurements of Magellanic penguin chicks at Punta Tombo (as in Hood et al. 1998).

#### *Radioimmunoassay*

For all studies, we measured corticosterone in plasma samples following a modified direct radioimmunoassay format, without chromatography (Wingfield and Farner 1975), as described by Wingfield et al. (1992). Specifically, plasma samples (10–20  $\mu\text{L}$ ) were equilibrated overnight at  $4^{\circ}\text{C}$  with tritiated corticosterone (20  $\mu\text{L}$  at 2,000 counts per minute [cpm]), brought up to a volume of 200  $\mu\text{L}$  with distilled water. We then extracted samples in 4 mL freshly redistilled dichloromethane, aspirated the organic phase, and dried it at  $40^{\circ}\text{C}$  under nitrogen. Dried samples were reconstituted in 550  $\mu\text{L}$  phosphate-buffered saline and allowed to equilibrate overnight. A 100- $\mu\text{L}$  aliquot was placed into a scintillation vial (with 4.5 mL scintillation fluid) for estimating recoveries. Duplicate 200- $\mu\text{L}$  aliquots were then distributed to assay tubes and equilibrated overnight with 100  $\mu\text{L}$  antiserum and 10,000 cpm tritiated corticosterone. We separated bound from free corticosterone by adding 0.5 mL

dextran-coated charcoal for 12 min at approximately 4°C. Samples were centrifuged for 10 min at 2,000 rpm. We collected supernatants into scintillation vials, added 4.5 mL scintillation fluid, vortexed and counted on a scintillation counter for 10 min or to within 2% variation. All assays contained two water blanks and at least one corticosterone standard (1,000 pg in 50  $\mu$ L assay buffer) for assessment of accuracy and variability. Intra-assay variability was 10%, calculated as the coefficient of variation from an assay of 10 duplicate standards by B.G.W. Samples were run in five assays with an interassay variability of 12.8%, based on the corticosterone standard included in each assay. Extraction efficiency was 87.9%, calculated as the average percent recovery from all assays combined.

#### Data Analysis

We tested for differences in baseline levels of corticosterone between groups (stages, injections, siblings) as well as differences in body mass and body size index using ANOVA. We incorporated Fisher's protected least-squared differences (PLSD) when necessary for post hoc determination of differences between more than two groups. To quantify how the penguin chick's corticosterone levels responded to the stress series protocol, we integrated the corticosterone data using the arithmetic trapezoidal rule (i.e., calculated the area under the curve) from baseline, 10, and 30 min for 1999–2000 data and from baseline, 30-, and 60-min data in 2001–2002. The integrated corticosterone value provided a measure of total corticosterone increase over time, while encompassing the pre-existing levels of circulating corticosterone before disturbance (Breuner et al. 1999; Picard-Hagen et al. 2001). Integrated corticosterone levels were also compared with ANOVA and Fisher's PLSD. For the injection study, we integrated the data using all sampling points (baseline, 10, 30, and 60 min). We log-transformed all corticosterone data before analysis to remove problems of nonnormality and heterogeneity of variances. Results are presented using nontransformed data with bars representing  $\pm 1$  SE and  $\alpha = 0.05$  for statistical comparisons.

## Results

#### Stress Response in Growing Chicks

The corticosterone stress response to capture increased as chicks aged (Fig. 1). Baseline levels of plasma corticosterone differed depending on the age of the chick in 1999–2000 (Fig. 2A;  $F_{2,23} = 9.53$ ,  $P = 0.0010$ ), with baseline levels for young chicks significantly higher than for both older groups (Fig. 2A; Fisher's PLSD,  $P \leq 0.01$  in both cases). However, in 2001–2002, baseline corticosterone for hatchlings was only different from the middle-aged period (Fig. 2B; Fisher's PLSD,  $P = 0.02$ ).

Integrated corticosterone was significantly different between the three age groups in both seasons (Fig. 2C, 1999–2000,  $F_{2,23} = 11.27$ ,  $P = 0.004$ ; Fig. 2D, 2001–2002,  $F_{2,22} = 5.77$ ,

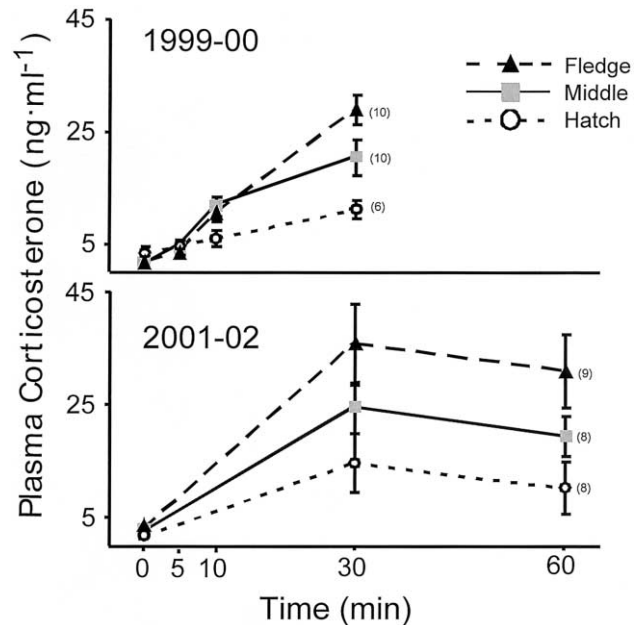


Figure 1. Patterns of corticosterone increase for Magellanic penguin chicks subjected to the capture-stress protocol. Chicks were sampled at three ages—near hatch, middle-aged, and near fledging—during two breeding seasons (1999–2000 and 2001–2002). Sample sizes are included in parentheses with each group.

$P = 0.019$ ). In 1999–2000, integrated corticosterone levels were different among all periods (Fig. 2C; Fisher's PLSD,  $P \leq 0.02$ ). In 2001–2002, integrated corticosterone levels for hatchlings were significantly lower than for both middle-aged chicks and near-fledging chicks (Fig. 2C; Fisher's PLSD,  $P \leq 0.02$  in both cases) but was similar for middle-aged and fledging chicks (Fig. 2D; Fisher's PLSD,  $P = 0.12$ ).

#### Test of Adrenal Function at Hatch

Corticosterone levels increased in response to capture and restraint in both ACTH-injected and saline-injected chicks (Fig. 3). There was no difference in baseline corticosterone for saline-injected and ACTH-injected penguin chicks (Fig. 4A;  $F_{1,13} = 3.16$ ,  $P = 0.10$ ). However, integrated corticosterone levels were different between treatments (Fig. 4B;  $F_{1,13} = 10.16$ ,  $P = 0.007$ ). Furthermore, the integrated corticosterone levels for ACTH-injected hatchlings were not different from integrated corticosterone levels in healthy chicks near fledging (Fig. 4C, up to 30-min integration data only;  $F_{1,14} = 0.92$ ,  $P = 0.35$ ).

#### Effects of Food Deprivation

Near hatching, the large chicks were significantly heavier than small ones (Fig. 5A;  $F_{1,14} = 10.60$ ,  $P = 0.006$ ), but body size index was the same (Fig. 5B;  $F_{1,14} = 2.36$ ,  $P = 0.15$ ). Large

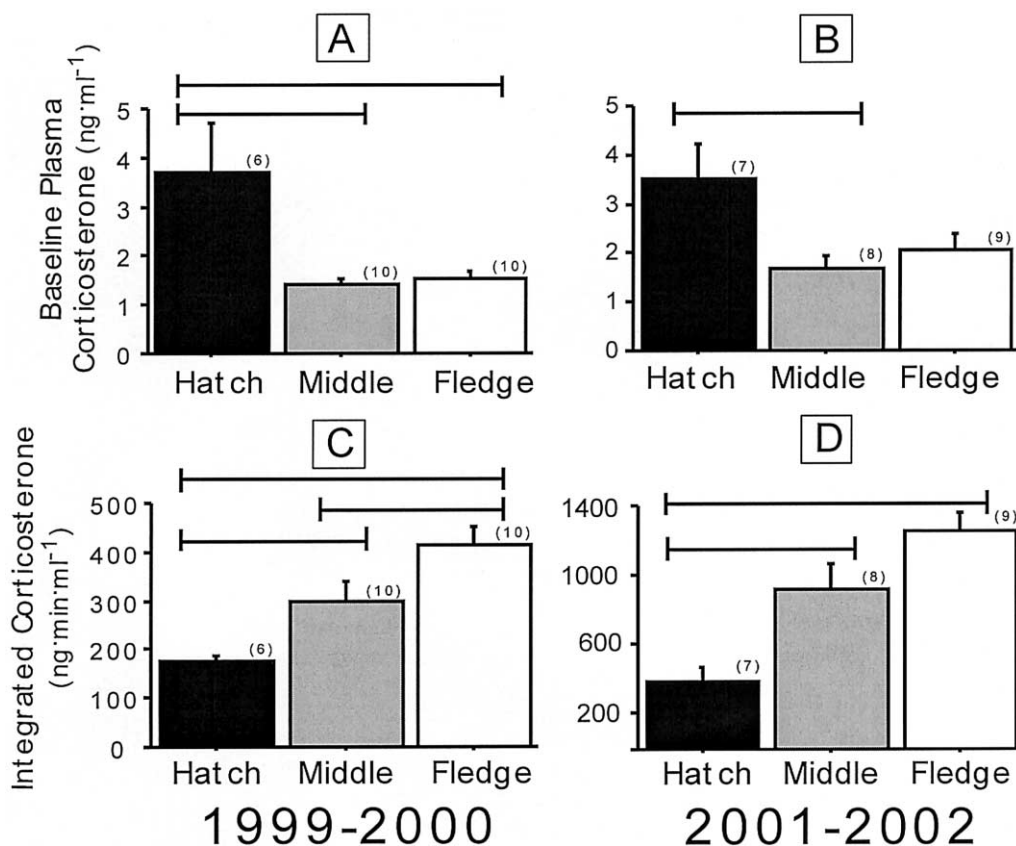


Figure 2. Baseline corticosterone (A and B) and integrated corticosterone (C and D) for Magellanic penguin chicks at three ages (near hatching, middle-aged, and near fledging) in two breeding seasons (1999–2000 and 2001–2002) at Punta Tombo, Argentina. Lines above bars indicate groups that are significantly different from each other at  $\alpha = 0.05$ . Sample sizes for each group are included above each bar.

middle-aged chicks were both significantly heavier (Fig. 5A;  $F_{1,14} = 35.19$ ,  $P < 0.0001$ ) and had a larger body size index (Fig. 5B;  $F_{1,14} = 12.73$ ,  $P = 0.003$ ) than small chicks. This pattern continued for chick pairs sampled near fledging (Fig. 5A, mass:  $F_{1,12} = 53.74$ ,  $P < 0.0001$ ; Fig. 5B, size:  $F_{1,12} = 9.49$ ,  $P < 0.01$ ).

Patterns of corticosterone increases in response to capture and restraint are shown for small and large sibling pairs near hatch, middle-aged, and near fledging during the breeding season (Fig. 6). Near hatching, both baseline and integrated corticosterone were similar for large and small chicks (baseline:  $F_{1,14} = 0.003$ ,  $P = 0.96$ ; integrated:  $F_{1,14} = 0.047$ ,  $P = 0.83$ ; Fig. 7A, 7B, respectively), and there was no difference in baseline corticosterone levels for chicks sampled first or second on the same day (paired  $t = 0.018$ ,  $P = 0.99$ , data not shown). By middle age, baseline corticosterone levels were significantly higher for the small chicks ( $F_{1,14} = 10.41$ ,  $P = 0.006$ ; Fig. 7A), but there was no difference in integrated corticosterone levels between the large and small chicks ( $F_{1,14} = 0.25$ ,  $P = 0.62$ ; Fig. 7B). Near fledging, baseline corticosterone levels were again similar for the large and small chicks ( $F_{1,12} = 0.04$ ,  $P = 0.85$ ;

Fig. 7A), but integrated corticosterone levels were significantly lower for the small chicks ( $F_{1,12} = 6.40$ ,  $P = 0.03$ ; Fig. 7B).

## Discussion

### *Stress Response in Growing Chicks*

As predicted, the HPA stress response in hatching Magellanic penguin chicks was reduced compared with that of older individuals (Fig. 1). The response developed gradually as chicks aged, resulting in an adult-like pattern near fledging (Fig. 1). A similar pattern of development was found in the altricial northern mockingbird, *Mimus polyglottos* (Sims and Holberton 2000), and the semialtricial American kestrel, *Falco sparverius* (Love et al. 2003). The adaptive value of a reduced stress response near hatching may allow altricial and semialtricial young to avoid the negative effects of chronically elevated glucocorticosteroids, including reduced growth rates and muscle catabolism as well as inhibition of thyroid function (Johnson et al. 1992; Wingfield 1994). These negative effects would all be detrimental for defenseless young attempting to gain mass quickly.

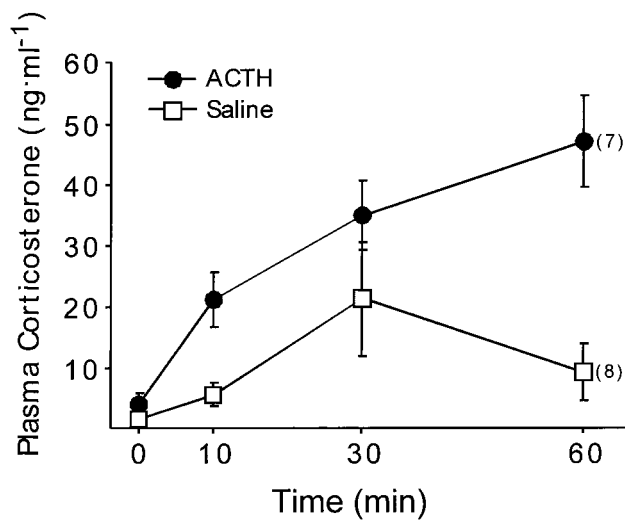


Figure 3. Pattern of corticosterone change over 60 min in Magellanic penguin chicks injected with either adrenocorticotrophic hormone (ACTH) or saline. Sample sizes for each group are included in parentheses.

Thus, a well-developed stress response at hatch is perhaps a characteristic that was selected against as the evolution toward an altricial hatching pattern emerged from the precocial ancestral state.

Although a lack of a well-developed stress response presumably allows defenseless chicks to avoid the effects of chronically high levels of corticosterone, constitutive or baseline levels of corticosterone are necessary for homeostatic processes in all individuals (Dallman et al. 1993). There is evidence that baseline glucocorticoid levels can be elevated in the late stages of embryogenesis (Sapolsky and Meaney 1986; Tanabe et al. 1986), and the lack of a strong stress response immediately after hatch is in fact a hyporesponsive period (e.g., temporarily reduced expression period; Sapolsky and Meaney 1986; Barry et al. 1995a). We have no data to show whether the HPA axis was fully functional before hatch in Magellanic penguin chicks and whether the lack of a stress response shortly after hatch is part of a hyporesponsive period. Regardless, life-history characteristics do appear to play a role, for in precocial species, the period of hyporesponsiveness is either absent or greatly reduced (Freeman and Manning 1984; Sapolsky and Meaney 1986; Holmes et al. 1989). Precocial chicks could likely benefit from the effects of glucocorticosteroids in escape or surviving perturbations, and a well-developed stress response for precocial young is not limited to avian species. Some amphibians, including leopard frogs (*Rana pipiens*) and African clawed frogs (*Xenopus laevis*), have well-developed stress responses throughout all stages of their independent metamorphic development (Glennemeier and Denver 2002).

We found no increases in baseline plasma corticosterone

levels as Magellanic penguin chicks neared fledging (Fig. 2). This was surprising since previous studies tracking baseline corticosterone levels in developing altricial and semialtricial chicks showed increases as fledging approached, with peaks typically being reached in the 1 or 2 d nearest fledging (Heath 1997; Sims and Holberton 2000; Kern et al. 2001). These in-

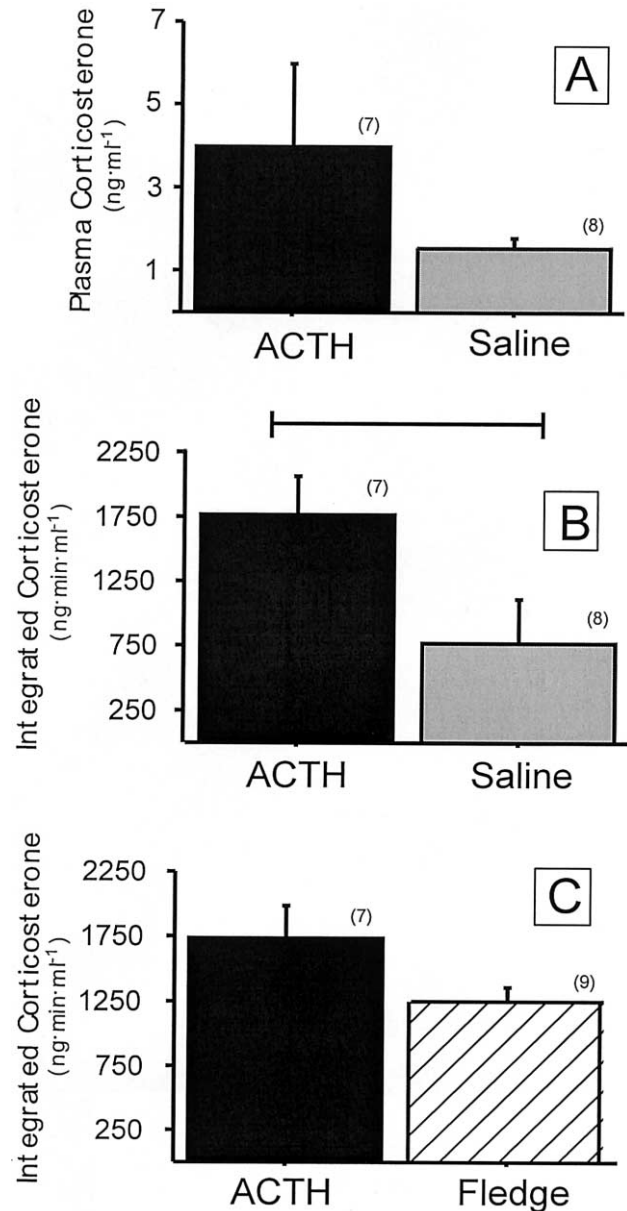


Figure 4. Baseline plasma corticosterone (A) and integrated corticosterone (B) for Magellanic penguin chicks injected with adrenocorticotrophic hormone (ACTH) or saline, as well as levels of integrated corticosterone 45 min postinjection for newly hatched chicks compared with noninjected fledglings (C). Lines above bars indicate groups that are significantly different from each other at  $\alpha = 0.05$ . Sample sizes are included above each bar.

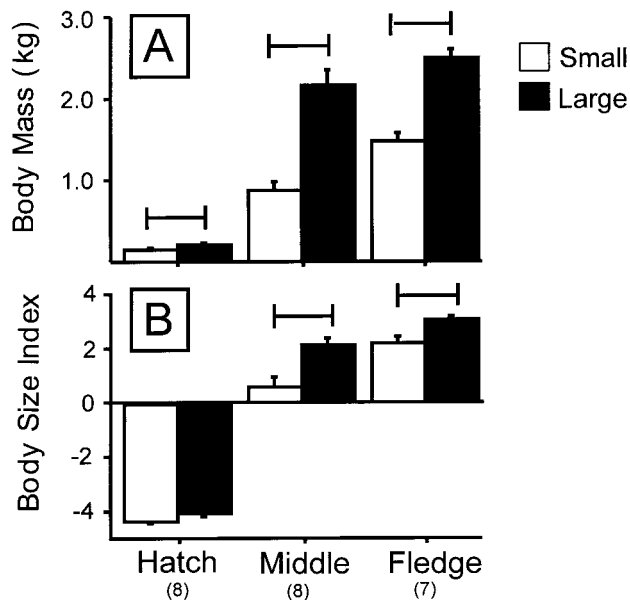


Figure 5. Body mass (A) and body size index (B; calculated from principal components analysis, see text) for large and small chicks in a nest during three periods of the breeding season at Punta Tombo, Argentina. Lines above bars indicate chick groups that are significantly different from each other at  $\alpha = 0.05$ . Number of chick pairs in each period is included in parentheses.

creases have been hypothesized to be beneficial for greater mobilization of energy as chicks prepare for the increased energetic demands of independence. We would expect Magellanic penguin chicks to follow this pattern, since the loss of parental provisioning plus the demands of swimming would increase energetic costs for chicks. We did not know when the chicks that we sampled eventually fledged. Thus, if increases in baseline corticosterone occur only 1 or 2 d before fledging, we may have missed the increase in our chicks if they were sampled more than 1 or 2 d before fledging.

#### Test of Adrenal Function at Hatch

Injection of excess exogenous ACTH into newly hatched chicks resulted in a robust secretion of corticosterone from the adrenocortical tissue compared with saline-injected chicks (Figs. 3, 4B). Corticosterone response in ACTH-injected chicks was similar to levels observed in penguin chicks near fledging (Fig. 4C). Thus, although the ability to elicit a robust secretion of corticosterone in response to stressful stimuli is delayed until near fledging (Fig. 1), it does not appear to be a function of reduced adrenal capability. Instead, some function of changing perception and/or hypothalamic and pituitary components are likely the source of the gradual changes observed in HPA axis maturation with chick development. Indeed, perhaps it is changes in the ability of chicks to perceive what is stressful or

what is not, that is the driving factor in the changes in the expression of the glucocorticosteroid stress response as chicks age. The mature function of adrenal glands in altricial chicks in response to ACTH injection has been documented in chicks of northern mockingbirds (Sims and Holberton 2000). Furthermore, Barry et al. (1995b) showed the same pattern in rainbow trout (*Onchorhynchus mykiss*): ACTH treatment in vitro elicited a robust cortisol (the primary glucocorticosteroid in fish) secretion, even when there was no stress responses elicited in vivo.

#### Effects of Food Deprivation

Lacking a strong secretion of corticosterone in response to acute stressors seems to be an evolutionarily conserved mechanism to avoid chronically elevated corticosterone in young that have

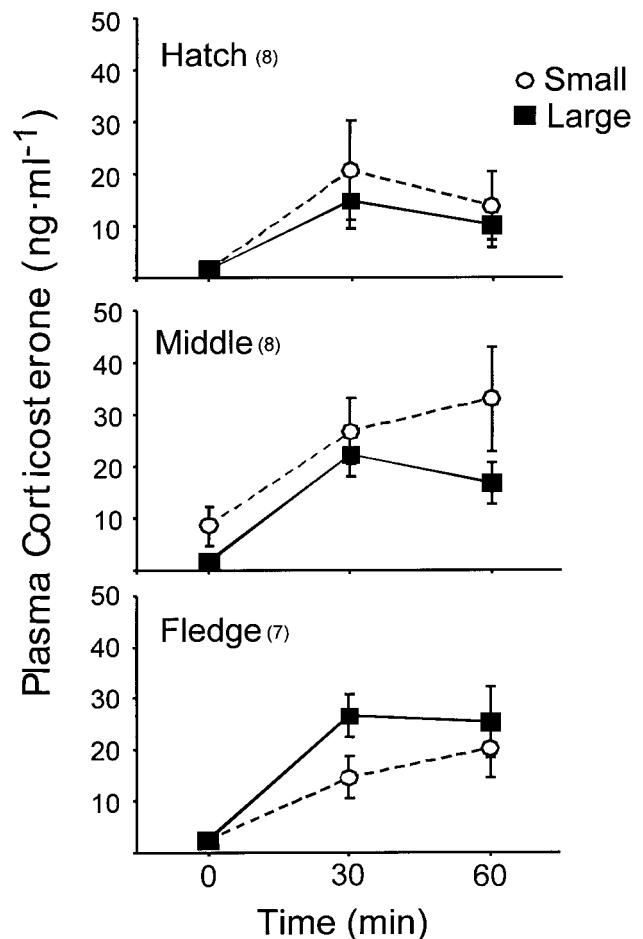


Figure 6. Patterns of corticosterone secretion in response to capture and restraint in small and large sibling Magellanic penguin chicks with size asymmetry due to natural food deprivation during three periods in the breeding season: hatch, middle-aged, and near fledging. Sample sizes (number of pairs in each period) provided in parentheses.



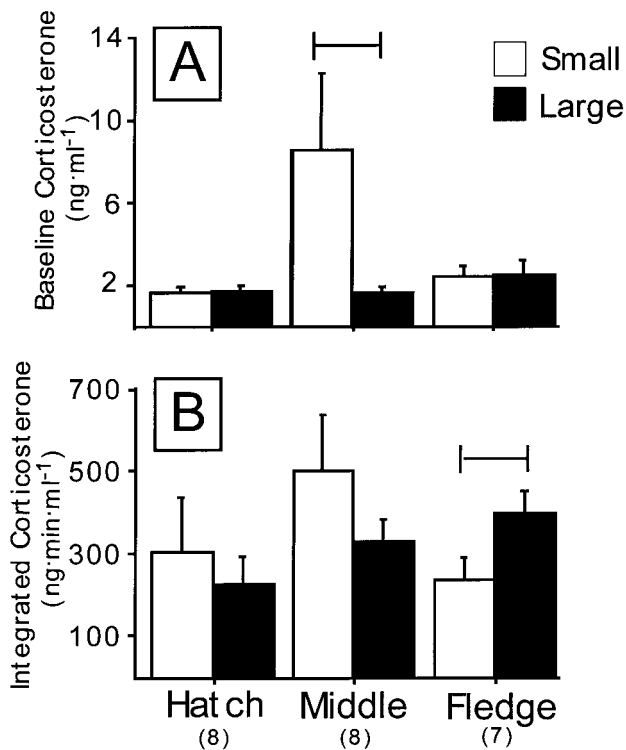


Figure 7. Baseline (A) and integrated corticosterone (B) in small and large Magellanic penguin chicks during three periods of the breeding season. Only baselines for middle-aged chicks and integrated corticosterone for chicks near fledge were significantly different between siblings ( $\alpha = 0.05$ ). Number of pairs in each period are included in parentheses.

no mechanism to escape from perturbations. However, there is growing evidence that corticosterone may serve a function for developing young, specifically in cases where perturbations are long-term (i.e., move from acute to chronic) and thus affect the metabolic processes of energy acquisition and utilization. Experimental manipulation of food intake caused various levels of nutritional stress in the chicks of red-legged kittiwakes, *Rissa brevirostris* (Kitaysky et al. 2001a), black-legged kittiwakes (Kitaysky et al. 1999), and blue-footed boobies, *Sula nebouzii* (Nunez-de la Mora et al. 1996). These studies showed concomitant increases in either baseline or stress-induced levels of circulating corticosterone with food deprivation. Corticosterone also increased in dominant blue-footed booby chicks that previously had a submissive sibling but then were given a more aggressive nest mate (Ramos-Fernandez et al. 2000). Furthermore, corticosterone correlated positively with begging behavior in black-legged kittiwake chicks (Kitaysky et al. 2001b). Thus, despite the gradual appearance of the glucocorticosteroid response to acute stressors in growing chicks (Fig. 1), corticosterone may serve a functional role early in the lives of semi-altricial young in more chronically perturbed situations.

For Magellanic penguin chicks, the normal pattern of corticosterone stress-response development appears to be modified due to the effects of natural food deprivation. This is seen when one compares larger, healthy chicks to smaller, food-deprived individuals across their growth period (Fig. 6). At hatch, while the larger chicks had a mass advantage over their siblings (Fig. 5A), body size index was similar (Fig. 5B), and there were no differences in either baseline (Fig. 7A) or integrated corticosterone levels (Fig. 7B). This was not surprising since hatchlings are incapable of a robust stress response (Fig. 1). Even though the smaller chick's mass decreased below the larger at hatching, the smaller chicks were likely not experiencing a nutritional deficit. Indeed, based on average mass measurements of the smaller chicks ( $149.3 \pm 9.1$  g; Fig. 5A), they had already received at least one meal from their parents, as average hatch mass for Magellanic penguin chicks is approximately 70 g (Boersma et al. 1990).

By middle age, 40–50 d posthatch, both mass and size asymmetries were well established between siblings (Fig. 5). Smaller chicks had significantly higher baseline levels of corticosterone than did the larger nest mate (Fig. 7A), but integrated corticosterone levels were similar (Fig. 7B). The pattern of increased baseline corticosterone for smaller, food-deprived chicks is similar to that observed in laboratory studies of food-deprived black-legged and red-legged kittiwake chicks (Kitaysky et al. 1999, 2001a), and increasing corticosterone levels in response to prolonged food deprivation have been documented in laboratory studies on rats (Tang et al. 1984; García-Belenguer et al. 1993; Kiss et al. 1994). Thus, for Magellanic penguin chicks, increased baseline corticosterone in smaller chicks may be partially due to decreased nutrition condition and the subsequent benefit of increased mobilization of stored energy that is a primary function of corticosterone. However, corticosterone is also known to correlate with increases in begging behavior (Kitaysky et al. 2001b). Although not specifically quantified in our study, middle-aged chicks were chosen for pairs where the smaller chicks were actively begging from adults. Thus, patterns of increased baseline corticosterone in smaller Magellanic penguin chicks may additionally be correlated with their greater amount of begging (Blanco et al. 1996) and the concomitant increased energy demand associated with that activity (Jurisevic et al. 1999; Kilner 2001; Rodríguez-Gironés et al. 2001).

In contrast to the increases in baseline corticosterone levels for small, food-deprived chicks, stress-induced levels of corticosterone were similar for asymmetric middle-aged pairs (Fig. 7B). These results contradict those for food-deprived black- and red-legged kittiwake chicks, which had higher stress responses than did their well-fed siblings (Kitaysky et al. 1999, 2001a). Baseline levels represent a homeostatic maintenance function of corticosterone (Dallman et al. 1993). In contrast, the acute stress-induced levels of corticosterone are assumed to be representative of how an animal perceives and responds to the stressor. In our study, chicks had not experienced capture

before the day they were sampled. Thus, the acute stressor was novel, and both chicks, regardless of their nutritional history and baseline corticosterone levels, had similar stress responses (Fig. 7B). In contrast, kittiwake chicks had experienced multiple handling events (Kitaysky et al. 1999, 2001a). Perhaps the metabolic challenge of food deprivation exacerbated the stress of multiple handlings and thus caused an even greater secretion of corticosterone in response to the acute, but not novel, capture stress in kittiwakes (Kitaysky et al. 1999, 2001a).

In Magellanic penguin chicks, long-term food deprivation—in this case to near starvation—appears to have a very different effect on the corticosterone response compared with less chronic food shortages. Although not specifically followed for confirmation, because of the retarded state of growth in the small, moribund chicks so late in the season, it is highly unlikely that any of them fledged. Indeed, Boersma and Stokes (1995) showed that similarly conditioned chicks always died. Interestingly, these small and large chicks had similar baseline levels of corticosterone (Fig. 7A). Furthermore, integrated corticosterone in small chicks was significantly lower than that in large chicks (Fig. 7B) and not unlike levels observed in hatchlings (Fig. 7B). These findings were opposite to what we predicted for moribund chicks: we thought their baseline and stress-induced corticosterone levels would be elevated compared with those of their healthy siblings.

There is literature on long-term fasting in penguin chicks (Cherel and LeMaho 1985; Cherel et al. 1987; Duchamp et al. 1989, 1991; Pütz and Plötz 1991). However, for the chicks of species thus far examined (emperor penguins, *Aptenodytes forsteri*, and king penguins, *Aptenodytes patagonica*), the mechanisms of their long-term fast is likely very different from our observations of food deprivation in Magellanic penguin chicks. As summarized by Castellini and Rea (1992), food deprivation in species such as king and emperor penguins is due to life-history characteristics that necessitate fasting. In contrast, food deprivation in Magellanic penguin chicks is an unfortunate consequence of parental inability to provision young. Thus, king and emperor penguin chicks are evolutionarily prepared for their period of food deprivation, whereas Magellanic penguin chicks are not prepared to starve. Thus, corticosterone patterns as a response to lack of food between these close phylogenetic species are likely not comparable in this case.

There is limited information about the hormonal consequences of severe starvation. However, the general pattern appears to be an increase in glucocorticoid levels as death approaches. This has been documented in semelparous species of fish (Dickhoff 1989; Kubokawa et al. 1999) and marsupials (Bradley et al. 1976; McDonald et al. 1981) and in humans suffering from anorexia nervosa (Licinio et al. 1996; Muñoz and Argente 2002). However, there do appear to be instances in which long-term but less severe food deprivation causes lower baseline corticosterone levels. These decreases in baseline corticosterone were hypothesized to be due to the habituation

to starvation and thus a mechanism to avoid the negative effects of long-term elevated corticosterone (Rees et al. 1985; Boxwell et al. 1995; Kitaysky et al. 2001a). It is likely that for moribund Magellanic penguin chicks, patterns of low baseline and integrated corticosterone (Fig. 7) are related to the avoidance of long-term elevated corticosterone as well as a physiological shutdown of the endocrine system as these chicks approached death. An eventual decrease in stress response in long-term food deprivation was also observed in captive red-legged kittiwake chicks (Kitaysky et al. 2001a). However, unlike Magellanic penguin chicks, food-deprived kittiwake chicks still had higher stress-induced corticosterone levels than the well-fed control chicks since these chicks were not held to the point of near starvation.

The driving mechanisms for the patterns and modifications in the glucocortical stress response that we observed in Magellanic penguin chicks remains to be resolved. While corticosterone levels appear affected by development and stressors such as food deprivation and starvation, other factors such as the role of corticosterone-binding globulins and rates of clearance (Breuner and Orchinik 2001; Breuner et al. 2003) may also be playing roles in the patterns we have described. However, our results suggest that baseline levels of corticosterone and the glucocorticosteroid stress response are important physiological components in the development of altricial species.

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