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## Dietary restriction causes chronic elevation of corticosterone and enhances stress response in red-legged kittiwake chicks

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**Abstract** Release of corticosterone in hungry kittiwake chicks facilitates begging and allows them to restore depleted energy reserves by increasing parental food provisioning. However, in order to avoid detrimental effects of chronic elevation of corticosterone, chicks might suppress adrenocortical activity in response to prolonged food shortages. In this study we examined temporal dynamics of corticosterone release in red-legged kittiwake (*Rissa brevirostris*) chicks exposed to prolonged restrictions in energy content and/or nutritional quality (low versus high lipid content) of their food. Starting at the age of 15 days, chicks were fed either high- or low-lipid fish at 40%, 65%, and 100% of ad libitum energy intake. Body mass measurements and baseline plasma samples were taken on a weekly basis after beginning of the treatment. After 3 weeks of treatment, chicks were exposed to a standardized acute handling and restraint stress protocol, where in addition to a baseline sample, three plasma samples were taken at intervals up to 50 min. We found that food-restricted chicks had lower body mass, chronically (during 2–3 weeks) elevated baseline and higher acute stress-induced levels of corticosterone compared to chicks fed ad libitum. Low lipid content of food further exacerbated these effects. An increase in baseline levels of corticosterone was observed within a week after energy requirements of food-restricted chicks exceeded their daily energy intake. A tendency for suppression of adrenocortical activity was observed in treatments fed low-lipid diets only at the end of the experiment. We suggest that

nest-bound chicks, if food-stressed, might suffer deleterious effects of chronic elevation of corticosterone.

**Keywords** Corticosterone · Food-stress · Nest-bound chicks · Seabirds

### Introduction

Nest-bound chicks of several species of seabirds respond to food shortages by increasing corticosterone secretion (e.g., blue-footed boobies *Sula nebouxi*, Nunez-de la Mora et al. 1996; black-legged kittiwakes, *Rissa tridactyla*, Kitaysky et al. 1999a). Release of corticosterone (at concentrations similar to baseline levels of food-restricted individuals and/or stress-induced levels of corticosterone in individuals fed ad libitum) in hungry kittiwake chicks allows them to restore depleted energy reserves by facilitating begging and thereby increasing parental food provisioning (Kitaysky et al. 2001). However, chronic elevation of corticosterone at stress-induced levels can be detrimental (Sapolsky et al. 1986; Sapolsky 1992; recent review in Welberg and Seckl 2001). In order to avoid possible detrimental effects of chronic elevation of corticosterone, nest-bound seabird chicks might suppress adrenocortical activity in response to prolonged food shortages.

As a group, seabirds have evolved multiple behavioral and physiological traits allowing them to cope with high variability in food resources (Lack 1968). It might be beneficial for seabird chicks to decrease corticosterone secretion in response to prolonged food shortages. Seabird parents are not always able (or willing) to compensate for a decrease in food availability by increasing their effort in foraging for the young and chick provisioning with food may remain low for extended periods of time (e.g., Kitaysky 1996; Kitaysky et al. 2000). When this is the case, seabird chicks are able to adjust their developmental rate to the rate of energy provisioning by parents and fledge successfully despite severe food shortages (Kitaysky 1999).

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It is not known whether reduced production and/or release of corticosterone is characteristic for slowly developing under-nourished seabird chicks, but a recent study of the domestic turkey (*Meleagris gallopavo*) suggests that a long-term (4 weeks) nutritional stress might cause a diminished adrenocortical activity (Carsia and McIlroy 1998). A suppression of adrenocortical response to an acute stressor was also documented in adult black-legged kittiwakes breeding during long-term food shortages (Kitaysky et al. 1999b). Elevated secretion of corticosterone can change metabolic pathways so that nutritionally stressed individuals rely on catabolism of proteins to fuel their activities (Axelrod and Reisine 1984; Le Ninan et al. 1988; Cherel et al. 1992). As a short-term benefit, a reduced production of corticosterone would allow under-nourished seabird chicks to avoid loss of skeletal muscle proteins. As a long-term benefit, a reduced production of corticosterone would allow slowly developing individuals to elude lasting detrimental effects of chronically increased corticosterone such as a suppressed immune system and neuronal cell death (Sapolsky et al. 1986; Sapolsky 1992; also reviewed in Sapolsky et al. 2000).

The temporal dynamics of corticosterone release in response to prolonged food shortages are not well studied. In the king penguin, *Aptenodytes patagonica*, 3- to 4-month-old chicks are able to tolerate 100 days of natural winter fast without a substantial increase in corticosterone secretion (Le Ninan et al. 1988). However, in seabirds that provision their chicks with food in a continuous manner, elevated levels of corticosterone in chicks were documented within two days of fasting (Nunez-de la Mora et al. 1996), and after 3 weeks of moderate food restriction (Kitaysky et al. 1999a). Although increased corticosterone secretion was shown to be strongly negatively correlated with endogenous fat reserves at the end of 3 weeks food restriction in kittiwake chicks (Kitaysky et al. 1999a), it remains to be shown whether nest-bound seabird chicks experience chronic elevation of corticosterone during prolonged food-shortages.

The functional relationship between diet composition and the physiological condition of young seabirds can be measured using well-characterized responses of adrenocortical hormones to stress (Kitaysky et al. 1999a). Controlled experiments have shown that food-related stress can account for the lower body mass, depleted fat reserves, and elevated baseline levels of corticosterone of birds at fledging when compared to birds raised on ad libitum nutritional regimes (Boag 1987; Kitaysky 1999; Kitaysky et al. 1999a; Romano 2000). If baseline levels of corticosterone appear normal, the pattern and extent of corticosterone secretion following the application of a standardized stressor, such as capture, handling and restraint, indicates increased potential for stress (Wingfield 1994). For example, in black-legged kittiwake chicks, prolonged dietary restrictions enhance adrenocortical response to a standardized stressor even though baseline levels of corticosterone appear normal (Kitaysky et al. 1999a).

In this study we examined the relationship between energy intake, diet quality, baseline levels of corticosterone and standardized acute stress-response (*sensu* Wingfield et al. 1992) in captive red-legged kittiwake (*Rissa brevirostris*) chicks. The major goal of this study was to assess temporal dynamics of corticosterone release in relation to the long-term alterations in quality or energy content of diet and short-term energy demands of growing chicks. For comparison with a natural system, we also measured adrenocortical response of wild red-legged kittiwake chicks exposed to a standardized stressor.

## Materials and methods

Partially incubated eggs of free-living red-legged kittiwakes were collected on St. George Island in the south-eastern Bering Sea and transported to the University of Washington facilities. Eggs were incubated using Lyon incubators at 37.2°C and 56% relative humidity until hatching. Chicks were raised in individual nests at 30°C and 80% relative humidity (newly-hatched to 10 days post-hatch), at 25°C and 70% relative humidity (10–25 days post-hatch), and at outdoor ambient temperatures and humidity until fledging (42–55 days post-hatch). Chicks were hand-fed a mixture of high-lipid forage fish given ad libitum until the experiment. Starting at the age of 15 days post-hatch, either low quality fish, rainbow smelt, *Osmerus mordax* (lipid to protein ratio, LPR = 0.61, hereafter called “low-lipid diet”), or high quality silverside, *Menidia menidia* (LPR = 1.47, hereafter called “high-lipid diet”) were fed to chicks for 21 days (Table 1). The energy content of silverside was calculated from the results of proximate analyses of whole fish (for details of the analysis see Romano 2000, and Kitaysky et al. 1999a). The energy content of rainbow smelt was also calculated from the results of proximate analyses of whole fish (as reported by a commercial provider, Shoreline). Chicks received either 565.1 kJ day<sup>-1</sup> of high-lipid diet (80 g day<sup>-1</sup>, wet fish biomass), 353.2 kJ day<sup>-1</sup> of high-lipid diet (50 g day<sup>-1</sup>), 356.3 kJ day<sup>-1</sup> of low-lipid diet (80 g day<sup>-1</sup>), or 222.7 kJ day<sup>-1</sup> of low-lipid diet (50 g day<sup>-1</sup>). Daily food intake of 7- to 8-week-old captive red-legged kittiwake chicks ( $n = 5$ ) fed high-lipid diet ad libitum was on average  $83.9 \pm 12.54$  g day<sup>-1</sup> (mean  $\pm$  SD; A.S. Kitaysky et al., unpublished observations). Therefore, in this study chick energy intake was at ~40%, 65%, and 100% of the ad libitum ration. To control for possible effects of dietary restrictions other than lipids to protein ratio and daily energy intake, all chicks received an excess of multi-vitamin/mineral supplement (one tablet of USP quality Kirkland Signature on daily basis) in their food. To control for possible effects of captivity on the stress-response of the experimental birds, free-living red-legged kittiwake chicks (similar to the experimental birds' ages) were taken from their nests at St. George Island and exposed to handling stress, and blood samples were taken as described below.

Body mass of post-absorptive chicks was measured every 5 days. To compare chick's energy requirements with its daily energy intake during treatment, we used the equation [ $\log_{10}$  resting metabolic rate (kJ day<sup>-1</sup>) =  $0.87 + 0.70 \log_{10}$  body mass (g, wet)] experimentally derived for growing post-absorptive seabird chicks (Kitaysky 1999). Metabolizable energy of forage fish used in this study was assumed at 87% of total energy intake, as determined earlier for growing black-legged kittiwake chicks (Romano 2000).

A blood sample of undisturbed (collected immediately after taking a chick from the nest) post-absorptive (after overnight fast) chicks was taken on weekly basis starting at the beginning of the experiment. Blood samples were collected by puncturing the alar vein and collecting blood in 100- $\mu$ l heparinized hematocrit tubes. At the end of the experiment, post-absorptive chicks were exposed to a standardized acute handling and restraint stress protocol, where in addition to the first blood sample (baseline) three blood samples (acute stress response) were taken at intervals of 10 min,

**Table 1** Experimental protocols and body mass of red-legged kittiwake chicks (*LPR* lipid to protein ratio). *LPR* ratio for lanternfish (*Stenobrachius* sp.) from Van Pelt et al. (1997)

Diet composition	LPR	Food intake (wet, g d <sup>-1</sup> )	Energy intake (kJ d <sup>-1</sup> )	Body mass at 15 days old		Body mass at 35 days old		<i>n</i>
				Mean	SE	Mean	SE	
Silverside	1.47	80	565.1	181.8	12.4	338.0	3.5	5
Rainbow smelt	0.61	80	356.3	177.2	13.0	237.5	4.9	5
Silverside	1.47	50	353.2	185.3	10.9	262.0	3.7	6
Rainbow smelt	0.61	50	222.7	187.4	9.6	188.9	2.0	5
Lanternfish <sup>a</sup>	2.96	?	?	–	–	339.2 <sup>c</sup>	42.2	5
Lanternfish <sup>b</sup>						373.5 <sup>c</sup>	11.5	2

<sup>a</sup>~30-day-old wild chicks infested with ticks

<sup>b</sup>~30-day-old wild chicks with no ticks

<sup>c</sup>It was not possible to control for the post-absorptive condition of wild chicks and their body mass reflects weight of the bird as well as mass of ingested food and feces

30 min, and 50 min after capture. All captive and wild chicks were bled between 1000 hours and 1400 hours according to the following standardized technique. After each sample, blood flow was stopped by the application of cotton, and birds were placed individually into cloth bags. After blood collection, hematocrit tubes were emptied into 0.5-ml vials, which were stored on ice (in the field) or in a refrigerator at 4°C (in the laboratory). Blood samples were centrifuged and plasma collected within 6 h.

Plasma samples were frozen at -20°C until radioimmunoassay analyses (for details see Wingfield and Farner 1975; Wingfield et al. 1992). The concentrations of corticosterone in plasma samples were measured using 20 µl of plasma. Steroid concentrations were measured in duplicate for each sample after extraction in 4 ml of dichloromethane. Recovery values (ranging from 85% to 97%) following extraction were used to adjust assayed concentrations of steroids. All baseline plasma samples were processed in one assay, and all plasma samples collected during stress-series were processed in the second assay. Intra- and inter-assay coefficients of variation were 5% and 8%, respectively.

All chicks survived the experiment, were fed ad libitum after dietary restriction was lifted, and 2 months after fledging were transferred to Alaska SeaLife Center (Seward, Alaska).

#### Statistical analyses

Body mass and baseline levels of corticosterone prior to the experiment were tested with one-way ANOVA, with treatment as a factor. The effects of the experimental treatments on body mass of chicks at the end of treatment were examined with two-way ANOVA (followed by Tukey Post Hoc test for pairwise comparisons), where daily energy intake and quality of food (*LPR*) were used as factors. The effects of daily energy intake and quality of the food on temporal dynamics of baseline levels of corticosterone were examined with repeated measures two-way ANOVA, where daily energy intake and *LPR* were used as factors and a duration of treatment as a repeated measure. The effects of the experimental treatments on acute stress-induced levels of corticosterone were examined with repeated-measures ANOVA, where experimental treatments were used as factors and samples obtained from the same chick during the acute stress protocol as repeated measures. The effects of chicks' body mass on baseline levels of corticosterone and stress response to handling (expressed as a maximal level of corticosterone achieved during 50 min of restraint) were examined by using regression analyses.

Wild chicks were infested with hard ticks (*Exodes* sp.) making a statistical comparison with not-infested captives invalid. An effect of tick infestation on baseline levels of corticosterone in wild chicks was examined by using linear regression analysis where number of ticks (found feeding on the individual chick) was used as an independent variable.

During statistical analyses, initial data were tested for assumptions required by parametric statistical tests according to Sokal and Rohlf (1981). If these data violated assumptions they

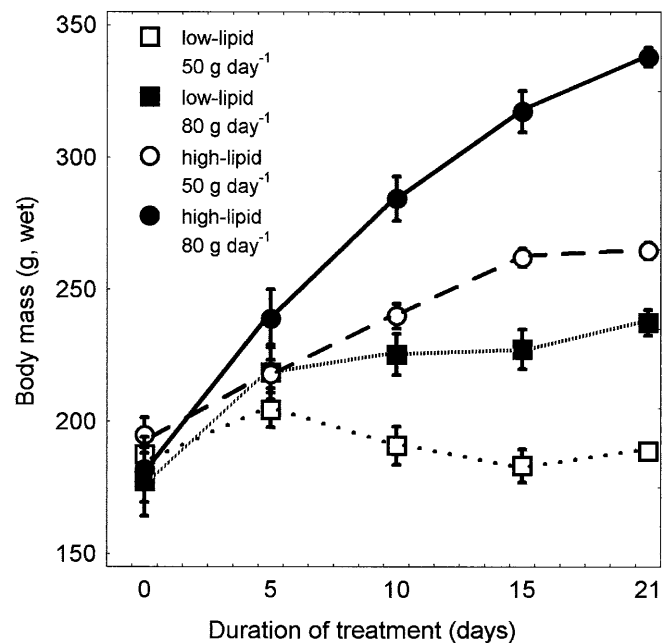
were log<sub>10</sub>-transformed and examined again. All computations were performed by using SYSTAT statistical package (Wilkinson 1992). Statistical significance was assumed at  $P < 0.05$ .

## Results

Body mass and baseline levels of corticosterone were similar among treatments prior to the experiment ( $F_{3, 17} = 0.15$ ,  $P = 0.930$  for body mass, Fig. 1; and  $F_{3, 17} = 1.73$ ,  $P = 0.199$  for baseline corticosterone, Fig. 2, upper panel).

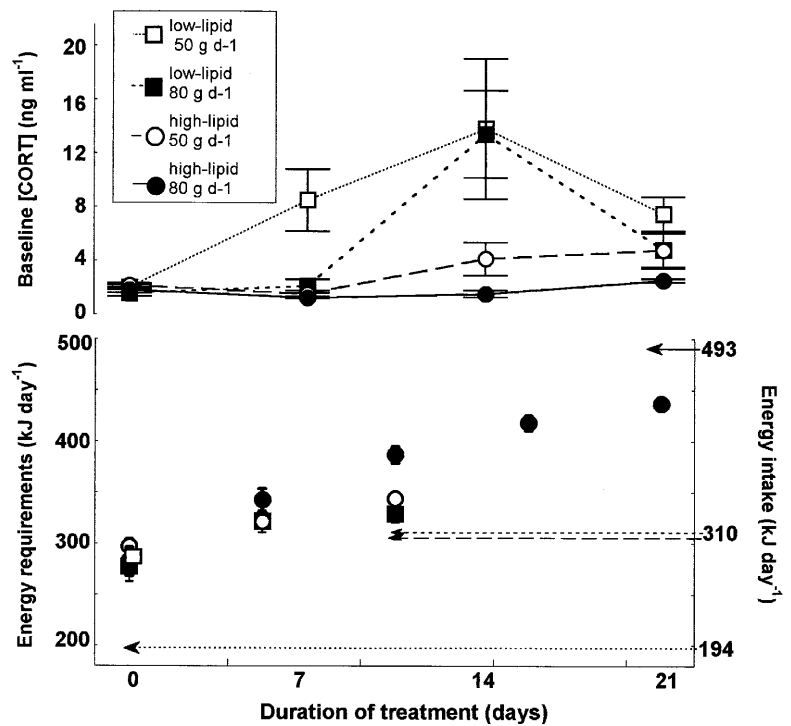
#### Effects of food-stress on growth of body mass

The diets significantly affected body mass of chicks at the end of treatment (daily food intake effect:



**Fig. 1** Growth rate of body mass (means,  $\pm$ SE) of red-legged kittiwake chicks in relation to daily energy intake (40%, 65%, and 100% of the ad libitum), diet composition (lipid to protein ratio, *LPR*: low-lipid = 0.61, high-lipid = 1.47), and duration of treatment

**Fig. 2** *Upper panel:* baseline levels of corticosterone (means,  $\pm$ SE) of red-legged kittiwake chicks in relation to daily energy intake (40%, 65%, and 100% of the ad libitum), diet composition (LPR: low-lipid = 0.61, high-lipid = 1.47), and duration of treatment. *Lower panel:* estimated (see Materials and methods) chicks daily energy requirements (means,  $\pm$ SE; symbols are as in upper panel) versus chicks daily energy intake (indicated with lines for each experimental treatment, as in upper panel)



$F_{1,17} = 282.10$ ,  $P < 0.0001$ ; LPR effect:  $F_{1,17} = 547.70$ ,  $P < 0.0001$ , Table 1). Also, there was a significant effect of interaction between daily food intake and quality (LPR) of food on chick's body mass ( $F_{1,17} = 13.58$ ,  $P < 0.002$ ). In particular, chicks that received  $356.3 \text{ kJ day}^{-1}$  of low-lipid diet were significantly lighter at the end of treatment compared to chicks fed  $353.2 \text{ kJ day}^{-1}$  of high-lipid diet (Tukey post hoc test:  $P = 0.001$ ; Fig. 1).

#### Daily energy requirements versus daily energy intake

Energy requirements of chicks in the different treatments exceeded their energy intake at different times after the beginning of treatment (Fig. 2, lower panel). In particular, metabolizable energy of  $50 \text{ g day}^{-1}$  low-lipid diet was lower than the chick's energy requirements immediately after the start of treatment (Fig. 2, lower panel). Energy requirements of chicks fed either  $80 \text{ g day}^{-1}$  of low-lipid or  $50 \text{ g day}^{-1}$  of high-lipid diets first exceeded metabolizable energy of their food during the 2nd week of treatment (Fig. 2, lower panel). In contrast, metabolizable energy of  $80 \text{ g day}^{-1}$  high-lipid diet exceeded energy requirements of controls during the treatment (Fig. 2, lower panel).

#### Effects of food-stress on temporal dynamics of baseline levels of corticosterone

The experimental treatments had a highly significant effect on baseline levels of corticosterone during treatment (daily food intake effect:  $F_{1,17} = 14.26$ ,  $P = 0.002$ ; LPR effect:  $F_{1,17} = 51.27$ ,  $P < 0.001$ ; daily food in-

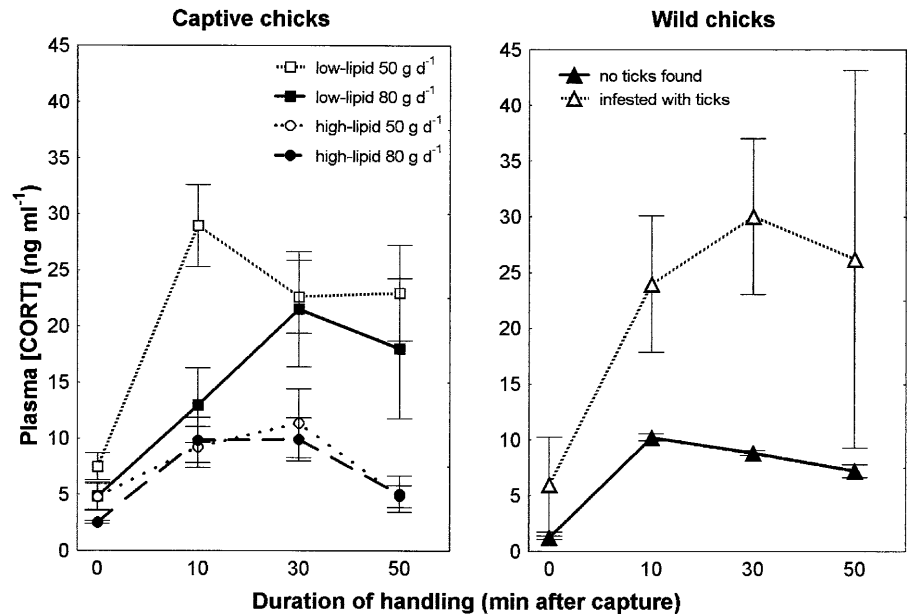
take  $\times$  LPR interaction term:  $F_{1,17} = 0.212$ ,  $P = 0.651$ , Fig. 2, upper panel). Baseline levels of corticosterone were significantly elevated during the experiment in all food-restricted chicks compared to controls (treatment duration effect:  $F_{2,34} = 11.39$ ,  $P < 0.001$ , Fig. 2, upper panel). However, the elevation in baseline levels of corticosterone was significantly higher in chicks that were fed low-lipid diet than in chicks that were fed high-lipid diet (treatment duration  $\times$  LPR interaction term:  $F_{2,34} = 5.04$ ,  $P = 0.012$ , Fig. 2, upper panel). Also, an elevation of baseline levels of corticosterone appeared significantly earlier in chicks that received  $50 \text{ g day}^{-1}$  of low-lipid diet compared to chicks that were fed either  $80 \text{ g day}^{-1}$  of low-lipid or  $50 \text{ g day}^{-1}$  of high-lipid diets (treatment duration  $\times$  LPR  $\times$  daily food intake interaction term:  $F_{2,34} = 3.71$ ,  $P = 0.035$ , Fig. 2, upper panel). In particular, elevation in baseline levels of corticosterone occurred during the 1st week in the most restricted ( $50 \text{ g day}^{-1}$  of low-lipid diet) treatment, and during the 2nd week in the two other food-restricted treatments (Fig. 2, upper panel).

A decrease in baseline levels was observed between day 14 and day 21 of the experiment in the low-lipid treatments but not in the high-lipid treatments (repeated measures ANOVA on baseline levels at days 14 and 21 of treatment; treatment duration  $\times$  LPR interaction term:  $F_{1,18} = 7.7$ ,  $P = 0.012$ , Fig. 2, upper panel).

#### Acute stress-induced levels of corticosterone in captive chicks

All captive chicks responded to an acute standardized stressor with a rapid increase in secretion of corticosterone (Fig. 3, left panel).

**Fig. 3** Adrenal response to a standardized handling and restraint stressor in captive 5-week-old red-legged kittiwake chicks in relation to dietary treatments (*left panel*), and in wild red-legged kittiwake chicks of ages similar to captives (*right panel*)



Captive chicks fed low-lipid diet responded to acute stress significantly faster and achieved significantly higher levels of corticosterone than the chicks fed high-lipid diet (Fig. 3, left panel; LPR effect:  $F_{1, 17}=19.46$ ,  $p<0.001$ ; acute stress effect:  $F_{3, 51}=12.29$ ,  $P<0.001$ ; acute stress $\times$ LPR interaction term:  $F_{3, 51}=3.48$ ,  $P=0.028$ ). The adrenal responses were statistically indistinguishable among the captive chicks that were fed either 50 g day<sup>-1</sup> or 80 g day<sup>-1</sup> of the same diet (Fig. 3, left panel; daily food intake effect:  $F_{1, 17}=2.22$ ,  $P=0.155$ ; daily food intake $\times$ LPR interaction term:  $F_{1, 17}=1.39$ ,  $P=0.255$ ; acute stress $\times$ daily food intake $\times$ LPR interaction term:  $F_{1, 17}=1.76$ ,  $P=0.174$ ).

#### Baseline and acute stress-induced levels of corticosterone in wild chicks

Degree of infestation with hard ticks was significantly positively correlated with baseline levels of corticosterone in wild red-legged kittiwake chicks ( $R^2=0.926$ ,  $F_{1,5}=62.8$ ,  $P=0.001$ , Fig. 4).

All wild chicks responded to a standardized stressor by an increased secretion of corticosterone (Fig. 3, right panel), but the adrenal response of five chicks infested with ticks tended to be higher than that of two chicks with no ticks (Fig. 3, right panel).

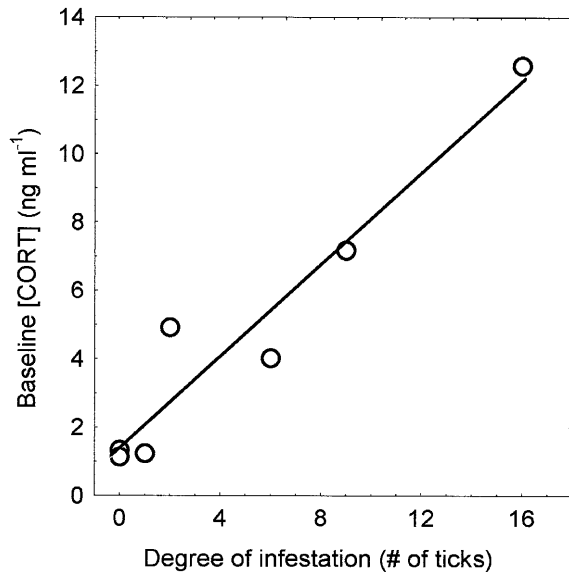
#### Body mass and circulating levels of corticosterone

Among the captive chicks the baseline and maximal acute stress-induced levels of corticosterone were significantly negatively correlated with chick body mass ( $R^2=0.29$ ,  $F_{1, 19}=7.6$ ,  $P=0.013$  and  $R^2=0.25$ ,  $F_{1, 19}=6.46$ ,  $P=0.02$  for the baseline and maximal levels, respectively).

## Discussion

In this study we examined whether long-term experimental variations in energy content and quality of food result in a chronically elevated corticosterone secretion in red-legged kittiwake chicks.

We found that nutritionally stressed chicks had lower body mass and chronically higher baseline levels of corticosterone than chicks fed high-lipid food ad libitum. Baseline levels of corticosterone were elevated for a longer period of time (3 weeks) in chicks that were restricted in both quantity and quality of food, and for shorter period (2 weeks) in chicks that were fed either reduced quantity or quality of food. Timing of an increase in baseline levels of corticosterone in general reflected the time at which energy demands of food-restricted chicks exceeded their energy intake. In particular, metabolizable energy of low-lipid diet given at 50 g day<sup>-1</sup> was lower than the chick's energy requirements immediately at the beginning of treatment (Fig. 2, lower panel), and a significant increase of baseline levels of corticosterone was observed during 1st week of the experiment (Fig. 2, upper panel). Similarly, energy requirements of chicks fed 80 g day<sup>-1</sup> of low-lipid and 50 g day<sup>-1</sup> of high-lipid diets exceeded metabolizable energy of their food during the 2nd week of the experiment and an increase in baseline levels of corticosterone was observed during the 2nd week of treatment (Fig. 2, upper panel). In contrast, metabolizable energy of high-lipid diet given ad libitum exceeded energy requirements of control chicks throughout treatment, which was reflected in consistently low baseline levels of corticosterone of those chicks (Fig. 2, upper panel). We conclude that increased secretion of corticosterone in kittiwake chicks reflects negative energy balance experienced by those chicks. Increased secretion of corticosterone



**Fig. 4** The relationship between infestation with ticks and baseline levels of corticosterone in 5-week-old wild red-legged kittiwake chicks ( $n=7$ )

probably allows under-nourished chicks rely on protein catabolism to fuel their increased activity levels (e.g., begging rates and aggression; Kitaysky et al. 2001; A.S. Kitaysky et al., unpublished observations) during food shortages.

In birds, long-term effects of chronic elevation of corticosterone during early neonatal development are poorly known. However, chronic elevation of glucocorticoids during prenatal development has long-term deleterious effects on the affected mammals (reviewed in Welberg and Seckl 2001). Specifically, chronic elevation of corticosterone (at concentrations similar to stress-induced levels) is known to suppress memory and immune systems, promote wasting of muscle tissue, and cause neuronal cell death (Sapolsky et al. 1986; Sapolsky 1992; also reviewed in Wingfield 1994; Sapolsky et al. 2000). It is not known whether the observed chronic elevation of baseline levels of corticosterone in food-restricted kittiwake chicks was high enough to cause long-term deleterious effects. However, the baseline levels of corticosterone observed in low-lipid diet treatments at the end of the 2nd week of the experiment were similar to maximal levels of corticosterone achieved during acute stress procedure by controls. Thus, the observed chronic elevation of corticosterone was potentially damaging and could cause long-term deleterious effects on food-restricted kittiwake chicks. We recently confirmed this prediction by showing an impaired cognition of young kittiwakes exposed to nutritional stress and/or moderate chronic elevation of corticosterone during early development (A.S. Kitaysky et al., unpublished observations).

At the end of the food-restriction period, baseline levels of corticosterone decreased in chicks fed low-lipid diet. A physiological mechanism(s) that would allow

chronically stressed chicks to reduce baseline levels of corticosterone is not known. It is possible that either clearance rate of plasma corticosterone was higher among chicks on low-lipid diet, or long-term nutritional stress reduced adrenal capacity or adrenal sensitivity (e.g., Rees et al. 1985; Romero et al. 1998). However, although baseline levels of corticosterone decreased between the 2nd and 3rd weeks of the experiment in low-lipid dietary treatments, they were still higher than in controls. Moreover, chicks on low-lipid dietary treatments also had stronger and quicker adrenocortical stress-response to a standardized acute stressor than controls. Therefore, it is unlikely that either adrenal capacity or sensitivity of chronically food restricted red-legged kittiwake chicks were impaired. Regardless of causal factors, a temporal decrease in baseline levels of corticosterone in nest-bound kittiwake chicks exposed to prolonged food shortages might represent a mechanism that allows affected individuals to ameliorate deleterious effects of chronically high concentrations of corticosterone.

Among food-restricted red-legged kittiwake chicks, low-lipid diet enhanced adrenocortical function compared to high-lipid diet. In particular, diets that were isocaloric but different in nutritional quality and fed to chicks at 65% of ad libitum energy intake, resulted in higher baseline and acute stress-induced levels of corticosterone among chicks raised on the low-lipid diet compared to those among chicks raised on the high-lipid diet. Similar results were obtained in an experimental study of black-legged kittiwake chicks (Kitaysky et al. 1999a). Several studies of birds have established that the amount of metabolizable energy is positively correlated with lipids to proteins ratio of the diet (e.g., Buchsbaum et al. 1986; Castro et al. 1989; Romano 2000). Romano (2000) has found that black-legged kittiwake chicks are able to metabolize ~12% more energy from high-lipid compared to low-lipid forage fish and, as a result, they are able to accumulate large fat deposits if fed high-lipid diets. Baseline and acute stress-induced levels of corticosterone reflect total body lipids in kittiwake chicks (Kitaysky et al. 1999a); thus, it is not surprising that in this study we found lower levels of corticosterone in red-legged kittiwake chicks fed high-lipid diet (relatively high body mass) compared to chicks fed low-fat diet (low body mass).

The results of this and earlier studies (Nunez-de la Mora et al. 1996; Kitaysky et al. 1999a) suggest that low endogenous energy reserves in nest-bound semi-precocial seabird chicks cause an elevation in baseline levels of corticosterone and enhance corticosterone secretion following the application of a standardized stressor. This is in contrast to observations of chicks and juveniles of altricial species of birds, where a relationship between body condition and corticosterone secretion was not found (Romero et al. 1998; Schwabl 1999; Sims and Holberton 2000). It has been suggested that development of the hypothalamic-pituitary-adrenal axis and consequently a chick's ability to respond to acute

stressors is associated with chick developmental modes (Schwabl 1999; Sims and Holberton 2000). Specifically, the hypothalamic-pituitary-adrenal axis probably becomes functional later in a chick's life in altricial compared to precocial species of birds. This would explain the discrepancy in the results obtained for different groups of birds.

Among birds that have precocial young, such as the domestic fowl (*Gallus gallus domesticus*) and the mallard (*Anas platyrhynchos*), the baseline plasma concentration of corticosterone in chicks fed ad libitum does not change significantly during the first 4 weeks after hatching (reviewed in Holmes et al. 1990). In mallard chicks fed ad libitum, a restraint for 30 min caused a significant increase in corticosterone concentration and the adrenal stress response was stronger in the chicks during first 2 days post-hatch than between 3 weeks and 4 weeks of post-hatch (Holmes et al. 1990). Similarly, we did not find a significant change in baseline levels of corticosterone among black-legged and red-legged kittiwake chicks fed ad libitum between 2 weeks and 5 weeks of postnatal life (Kitaysky et al. 1999a; this study). Thus, it is likely that the hypothalamic-pituitary-adrenal axis of precocial and semi-precocial chicks is fully developed shortly after hatching. However, studies of adrenocortical stress response of semi-precocial seabird chicks at early ages are needed to confirm this prediction.

Adrenocortical responses of precocial and semi-precocial chicks to nutritional stress and depleted energy reserves are not uniform among different species. Freeman et al. (1981) found that reducing food intake to 75% of ad libitum causes an increase in plasma corticosterone concentration after 1 week of restriction in the precocial chicks of domestic fowl. Increased levels of corticosterone were also recorded in protein-restricted young of the domestic chicken in vivo, and ACTH challenged adrenal steroidogenic cells in vitro (McIlroy et al. 1999; Carsia and Weber 2000). In semi-precocial nest-bound chicks of blue-footed boobies, a short-term food deprivation results in an increase of baseline levels of corticosterone, which was associated with a low body mass (Nunez-de la Mora et al. 1996). In nest-bound chicks of semi-precocial black-legged and red-legged kittiwakes, long-term dietary restriction also causes elevation of baseline levels of corticosterone and enhances adrenocortical response to acute stress (Kitaysky et al. 1999a; this study). In contrast to the results of these studies, Rees et al. (1985) found that adrenocortical responses of immature chicken to an acute stressor and ACTH challenge were reduced by food deprivation. Similarly, increased adrenocortical response to an acute stressor was not associated with dietary restrictions in in vitro study of precocial domestic turkey (*Meleagris gallopavo*, Carsia and McIlroy 1998). Furthermore, in semi-precocial young of American kestrels (*Falco sparverius*), baseline levels of corticosterone were not affected and acute stress-induced levels of corticosterone were reduced by long-term food-restriction (Heath and Dufty

1998). Thus, modulation of adrenocortical activity of chicks in response to food restriction can reflect not only precocial-altricial spectrum of chick development, but other ecological, physiological and behavioral traits of a particular species (e.g., temporal variability in food resources, rate of chick provisioning with food, metabolic responses of chicks to food restrictions, and/or phylogenetic constraints (e.g., Kitaysky 1999). Also, in the above-mentioned experimental studies, the duration of food restriction ranged from 2 days to 4 weeks, and the severity of dietary restriction ranged from complete food deprivation to alterations of quality of diet fed ad libitum. As the results of this current study show, the temporal dynamics of adrenocortical response to nutritional stress are complex, which might account for the discrepancy in the results obtained by different studies of related/same species of birds.

We are convinced that the results found in this study were not merely a consequence of captive conditions, but in fact reflect responses we could expect to see in free-living red-legged kittiwakes. Other studies of adrenal response in young birds to a standardized acute stressor did not indicate that those birds habituated to frequent handling (Freeman et al. 1981; Dufty and Belthoff 1997; Heath and Dufty 1998; Kitaysky et al. 1999a). Baseline and acute-stress induced levels of corticosterone recorded for captive chicks in this study were within the range observed in free-living red-legged kittiwake chicks. Furthermore, baseline and acute stress-induced levels of corticosterone in free-living (not tick infested) and captive chicks fed ad libitum were similar, whereas baseline and acute stress-induced levels of corticosterone were similar between nutritionally stressed captive chicks and free-living chicks infested with ticks. It is possible that tick infestation causes severe metabolic challenges to young red-legged kittiwakes, as has been reported for chicks of other species of birds (reviewed in Duffy 1983), which could result in enhanced adrenocortical function of tick-infested wild chicks similar to under-nourished captive chicks. Finally, captive chicks in all treatments were subject to similar controlled conditions. The only differences between them were the energy content and nutritional quality of their food. Thus, it is reasonable to conclude that the results of this study demonstrate differences in the functional responses of red-legged kittiwake chicks to variations in energetic content and nutritional quality of their food.

To the best of our knowledge, this paper represents the first experimental demonstration of the temporal dynamics of adrenocortical response of nest-bound semi-precocial chicks in relation to long-term dietary restrictions. The results of this study suggest that for a realistic assessment of adrenocortical response of chicks to nutritional stress it is crucial to understand the temporal dynamics of corticosterone release in relation to diet composition and a chick's energy requirements. Non-manipulative observations and a single assessment of adrenocortical activity at the end of long-term food-restriction experiments might lead to equivocal results.

Specifically, our results indicate that there are three phases of adrenocortical response of semi-precocial nest-bound kittiwake chicks to long-term food shortages, which are different from previously described phases of fasting in young and adult birds (e.g., Cherel et al. 1988; Le Ninan et al. 1988). Phase I – initial elevation of baseline levels of corticosterone, occurs shortly after chicks started experiencing negative energy balance and is associated with a decrease in growth of body mass; phase II – chronically elevated baseline levels of corticosterone, no change or slight decrease in body mass; phase III – a suppression of corticosterone secretion, no change in body mass. An extent and duration of each phase probably depends on a chick's ability to accumulate fat reserves prior to a food shortage, how severe dietary restrictions are, and developmental and physiological responses to food shortages specific for a particular group/species of birds.

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