What Factors Drive Prolactin and Corticosterone Responses to Stress in a Long-Lived Bird Species (Snow Petrel *Pagodroma nivea*)?

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ABSTRACT

Life-history theory predicts that individuals should adapt their parental investment to the costs and benefits of the current reproductive effort. This could be achieved by modulating the hormonal stress response, which may shift energy investment away from reproduction and redirect it toward survival. In birds, this stress response consists of a release of corticosterone that may be accompanied by a decrease in circulating prolactin, a hormone involved in the regulation of parental care. We lack data on the modulation of the prolactin stress response. In this study, we tested the hypothesis that individuals should modulate their prolactin stress response according to the fitness value of the current reproductive effort relative to the fitness value of future reproduction. Specifically, we examined the influence of breeding status (failed breeders vs. incubating birds) and body condition on prolactin and corticosterone stress responses in a long-lived species, the snow petrel *Pagodroma nivea*. When facing stressors, incubating birds had higher prolactin levels than failed breeders. However, we found no effect of body condition on the prolactin stress response. The corticosterone stress response was modulated according to body condition but was not affected by breeding status. We also performed an experiment using injections of adrenocorticotropic hormone (ACTH) and found that the modulation of the corticosterone stress response was probably associated with a reduction in ACTH release by the pituitary and a decrease in

adrenal sensitivity to ACTH. In addition, we examined whether prolactin and corticosterone secretion were functionally linked. We found that these two hormonal stress responses were not correlated. Moreover, injection of ACTH did not affect prolactin levels, demonstrating that short-term variations in prolactin levels are not governed directly or indirectly by ACTH release. Thus, we suggest that the corticosterone and prolactin responses to short-term stressors are independent and may therefore mediate some specific components of parental investment in breeding birds. With mounting evidence, we suggest that examining both corticosterone and prolactin stress responses could be relevant to parental investment in vertebrates.

Introduction

According to life-history theory, individuals should adjust their parental investment to reflect the fitness costs and benefits of the current reproductive effort (Stearns 1992). Under adverse environmental conditions, the demands of competing life-history traits cannot be maintained at the maximal level, and the energy allocated to reproduction can be increased only at the expense of that allocated to survival and future breeding opportunities ("the cost of reproduction"; Williams 1966). In this context, the suite of stress-avoidance mechanisms could be the proximate factors that regulate parental decisions and parental investment (Zera and Harshman 2001). For example, endocrine mechanisms mediate many interactions between environmental stressors, physiological state, and behavior (Wingfield 1994; Ricklefs and Wikelski 2002).

The regulation of corticosterone secretion by the hypothalamo-pituitary-adrenal (HPA) axis has been extensively studied because of its role in the stress response (Ricklefs and Wikelski 2002; Wingfield and Sapolsky 2003). Indeed, plasma levels of corticosterone usually increase in response to potential stressors, and this rise triggers behavioral and physiological changes that promote survival by helping individuals to escape life-threatening situations (the "emergency life-history stage" sensu Wingfield et al. 1998). For instance, elevations of corticosterone can inhibit reproductive function and redirect energy allocation from reproduction to self-maintenance in breeding vertebrates (Silverin 1986; Wingfield and Sapolsky 2003). Because corticosterone secretion is involved in the regulation of the trade-off between reproduction and survival, ecophysiologists have suggested that the sensitivity of the HPA axis to stress could be a reliable measurement of parental investment in vertebrates (Wingfield et al. 1995; Ricklefs and Wikelski 2002; Holberton

and Wingfield 2003; Wilson and Holberton 2004). Indeed, a number of studies have effectively demonstrated that corticosterone secretion in response to a standardized stress protocol is modulated according to environmental conditions, physiological conditions, and life-history stages (Wingfield et al. 1995; O'Reilly and Wingfield 2001; Meddle et al. 2003; Moore and Jessop 2003; Wingfield and Sapolsky 2003; Lendvai et al. 2007).

Recently, two studies have suggested that the hormone prolactin might also mediate the life-history trade-off between reproduction and survival in free-living birds (Chastel et al. 2005; Angelier et al. 2007a). Prolactin is a pleiotropic hormone that is known to have more than 300 separate biological activities in vertebrates (Freeman et al. 2000). Specifically, this hormone is involved in parental behavior (Buntin 1996), and several studies have demonstrated that prolactin enhances the expression of parental behaviors in breeders (Buntin 1996; Wang and Buntin 1999) and nonbreeders (Vleck et al. 1991; Schoech et al. 1996; Khan et al. 2001; Angelier et al. 2006). Moreover, there is now strong evidence that circulating prolactin levels can decrease in response to short-term stressful events in some bird species—that is, the prolactin stress response (Sharp et al. 1989; Chastel et al. 2005; Angelier et al. 2007a; Verreault et al. 2008; but see Maney et al. 1999)—and this decrease could ultimately trigger nest desertion if prolactin levels remain low during a prolonged period (Cherel et al. 1994; Chastel and Lormée 2002; Groscolas et al. 2008). Recently, it has been demonstrated that breeding individuals can modulate their prolactin response to stress with respect to the fitness value of the current reproductive effort (the "brood value hypothesis" sensu Lendvai et al. 2007; Angelier et al. 2007a). Thus, an attenuation of the prolactin response to stress could maintain parental behavior despite stressful conditions and therefore maximize the current reproductive investment (Chastel et al. 2005; Angelier et al. 2007a).

Although the prolactin response to stress shows potential for mediating the life-history trade-off between reproduction and survival, only two studies have focused on the prolactin response to stress in relation to parental investment in birds (Chastel et al. 2005; Angelier et al. 2007a). We lack data on the influence of environmental conditions, physiological state, and life-history stages on the prolactin stress response in birds. Understanding how the prolactin stress response is modulated according to these factors could help to determine its functional significance in mediating parental investment and life-history strategies in birds. In this study, we examined the influence of (1) breeding status (breeders vs. failed breeders) and (2) body condition on both prolactin and corticosterone responses to a standardized stress protocol in a long-lived species, the snow petrel *Pagodroma nivea*.

First, we examined the differences in prolactin and corticosterone stress responses between incubating birds and failed breeders. According to the "parental-care hypothesis" (Wingfield et al. 1995), the attenuation of the stress response may be linked to parental care, with a lower stress response expected to be observed in individuals that provide high-quality parental care. Such an attenuation of the stress response would be the

mechanism by which individuals could modulate their parental investment (sensu Clutton-Brock 1991; corticosterone: Wingfield and Sapolsky 2003; prolactin: Chastel et al. 2005). According to this hypothesis, we predict that the stress response of breeding birds should be reduced in comparison with that of failed breeders because breeders invest energy in parental care whereas failed breeders do not. Thus, we predict that the magnitude of the decrease in prolactin levels and the magnitude of the increase in corticosterone levels in response to stress should be lower in incubating birds than in failed breeders (the parental-care hypothesis; Wingfield et al. 1995; O'Reilly and Wingfield 2001; Chastel et al. 2005).

Second, we tested whether hormonal stress responses were correlated with an individual's body condition. Because snow petrels are long-lived birds (Chastel et al. 1993; Jenouvrier et al. 2005), they should not jeopardize their survival and future breeding opportunities for current ones (Drent and Daan 1980). Therefore, birds in poor body condition should reduce their parental investment and redirect energy allocation processes from reproduction to self-maintenance when facing stressful events. We therefore predict that the magnitude of the decrease in prolactin levels and that of the increase in corticosterone levels in response to stress should be higher in breeding individuals of poor body condition (the "body condition hypothesis"; Lynn et al. 2003; but see Holberton and Able 2000; Long and Holberton 2004).

Third, we examined the proximate causes of the modulation of the corticosterone stress response in snow petrels. Because pituitary adrenocorticotropic hormone (ACTH) controls adrenal corticosterone release, an attenuation of the corticosterone stress response could result from changes in the adrenal's sensitivity to ACTH (Romero and Wingfield 1998; Romero et al. 1998b, 1998c; Sims and Holberton 2000; Meddle et al. 2003; Romero 2006). In this study, we tested whether the adrenal's sensitivity to ACTH varied with body condition in brooding snow petrels.

Finally, we studied the functional link between prolactin and corticosterone secretion. We examined (1) whether baseline levels of corticosterone and prolactin were correlated and (2) whether these two hormonal stress responses were correlated. In laboratory rodents, prolactin secretion is governed by many neurohormonal pathways, which can also be associated with stress physiology (reviewed in Freeman et al. 2000). Moreover, previous studies have suggested that corticosterone and prolactin levels are linked by closely related interactions that may influence parental behaviors, feeding, and energy regulation in birds (Koch et al. 2002, 2004; Criscuolo et al. 2006). However, it remains unclear how stress can result in reduced circulating prolactin levels in breeding birds. In this study, we tested whether prolactin secretion might be affected by the HPA axis and particularly by the physiological and hormonal consequences of the release of pituitary ACTH. This hormone could potentially affect pituitary prolactin secretion because corticosterone and prolactin secretions have been found to be interconnected in birds (Koch et al. 2002, 2004; Chastel et al. 2005; Criscuolo et al. 2006; Angelier et al. 2007b).

Material and Methods

Study Site and Species

Snow petrels were studied on Ile des Pétrels, Pointe Géologie Archipelago, Terre Adélie (66°40′S, 140°01′E), Antarctica. Snow petrels are long-lived birds with high survival probability and low fecundity (Chastel et al. 1993). Sexual maturity is acquired at 10 yr of age, on average, and birds breed annually, although each year, a small proportion of birds forgo breeding (Jenouvrier et al. 2005). During the incubating and brooding periods, both parents alternate incubating and brooding the single egg/chick. Once the chick becomes thermally emancipated, both parents forage at sea to feed their chick and restore their own body reserves. In this species, males and females provide similar amounts of parental care (Warham 1990).

Blood Sample, Body Condition, and Breeding Success

The field study was carried out during the austral summer 2004/2005 (December 16–30, 2004). During the last part of the incubation period, 64 adults were captured while on the nest (48 incubating birds and 16 failed breeders). Each bird was sampled only once. In order to test the influence of breeding status on hormonal stress response, we captured adults incubating their egg at the nest (called, hereafter, incubating birds; $n = 48$) and adults that had already lost their egg at the time of capture (called, hereafter, failed breeders; $n = 16$). Time of egg loss was not known for these birds. All birds were bled according to the standardized technique described by Wingfield (1994). Immediately after capture, an initial blood sample was collected from the alar vein with a 1-mL heparinized syringe and a 25-gauge needle. A second sample was taken 30 min after the first. Petrels were kept in individual opaque cloth bags between the initial and second blood samples. In the field, blood samples were put into ice until they were centrifuged. All the birds were sampled during a moderate time window (between 1000 and 1800 hours) to avoid any potential effect of diurnal cycles on prolactin and corticosterone levels. Indeed, baseline and stress-induced prolactin and corticosterone levels were not correlated with the time of day of sampling ($P > 0.05$ in all cases). All birds were weighed to the nearest 2 g with a Pesola scale, and their skull length (head + bill) was measured to the nearest 0.5 mm. During handling of incubating birds, their eggs were covered with cotton and kept warm. At the end of the sampling process, petrels were immediately returned to the nest.

Adrenal Response to ACTH

To test the sensitivity of both prolactin and corticosterone secretion to ACTH, another field study was carried out during the austral summer 2005/2006 (from January 27 to February 5, 2006). During the brooding period, 18 birds were captured on the nest and administered either ACTH (ACTH group) or Ringer's solution (control group). Within 5 min of handling, 8 snow petrels were given an intraperitoneal injection of ACTH (A-6303, Sigma: 133 μg of porcine ACTH and 200 μL of phys-

iological saline), and 10 snow petrels were given an intraperitoneal injection of Ringer's solution (200 μL of physiological saline). This method of injection of ACTH has previously been validated by an experimental study (Wilson and Holberton 2001). Moreover, this method has also been validated for injection of other hormones through osmotic pumps (Horton et al. 2007). In many studies, exogenous ACTH has been delivered by intrajugular injection. In our study, we used this alternative method (intraperitoneal injection) in order to avoid the potential drawbacks associated with intrajugular injection (see Wilson and Holberton 2001). All birds were then kept in individual opaque cloth bags for 30 min (from the time of injection to the time of the blood sample), and a blood sample was then collected to measure corticosterone and prolactin. All birds were weighed to the nearest 2 g with a Pesola scale, and their skull length (head + bill) was measured to the nearest 0.5 mm.

Molecular Sexing and Hormone Assays

Blood samples were centrifuged, and plasma was decanted and stored at $-20°$C until assayed. After centrifugation, red cells were kept frozen as well for molecular sexing. The sex was determined by polymerase chain reaction amplification of part of two CHD genes present on the sex chromosomes at the Centre d'Etudes Biologiques de Chizé (CEBC), as detailed in Weimerskirch et al. (2005). Plasma concentrations of corticosterone were determined first by radioimmunoassay at the CEBC, as previously described (Lormée et al. 2003). Because blood samples were collected within 3 min of capture, they were considered to reflect baseline levels of corticosterone and prolactin (Chastel et al. 2005; Romero and Reed 2005). Plasma concentrations of prolactin were determined with the remaining plasma by a heterologous radioimmunoassay at the CEBC, as detailed in Cherel et al. (1994). The prolactin assay has previously been validated in snow petrels (Angelier et al. 2007a). For two petrels sampled in 2004/2005, we did not have enough plasma to assay either baseline or stress-induced prolactin levels, and we therefore excluded them from the analyses of prolactin levels ($n = 62$). All samples were run in one assay for both hormones (intra-assay variation; corticosterone: 7.8%, prolactin: 8.3%).

Statistics

All analyses were performed with SAS statistical software (SAS Institute, ver. 9.1). Only one partner from each nest was studied, so the data obtained from male and female petrels were considered independent samples. For all variables tested, normality assumptions were checked by plotting the residuals against the predicted value and by running the Shapiro-Wilk test for normality. There were no indications that normality assumptions were violated. We used repeated-measures models (repeated-measures general linear models [GLMs]) to determine whether (1) the standardized stress protocol elevated corticosterone levels and reduced prolactin levels; (2) the corticosterone and

prolactin stress responses differed between sexes or by breeding status; and (3) these hormonal stress responses were correlated with body condition in males and in females. Then we used some specific contrasts of these repeated-measures GLMs to test specific hypotheses regarding baseline and stress-induced hormonal levels ("contrast" statement of SAS). Thus, we examined whether baseline prolactin or corticosterone levels and stress-induced prolactin or corticosterone levels varied with sex (females vs. males) and breeding status (incubating birds vs. failed breeders). Because contrast statements do not allow examination of the influence of a continuous variable (body condition) on a dependent variable (hormonal levels), we then used classic GLMs to examine whether baseline and stress-induced hormone levels were correlated with body condition.

Because male and female snow petrels differ greatly in size, shape, and fat content (Barbraud and Jouventin 1998), body condition was calculated separately for each sex by use of the residuals from a reduced major-axis regression of body mass against skull length (Green 2001). Because body condition was calculated separately by sex, we could not analyze female and male data together to test an effect of body condition on hormone levels and hormonal stress responses. Therefore, we tested whether the corticosterone and prolactin stress responses were correlated with body condition in males and then in females. We also examined whether baseline prolactin levels and stress-induced prolactin levels were correlated with baseline corticosterone levels and stress-induced corticosterone levels, respectively, by using GLMs with sex and breeding status as factors and corticosterone levels (baseline or stress induced) as a covariate.

Finally, we investigated the influence of the ACTH treatment and body condition on stress-induced prolactin and corticosterone levels in brooding birds. Because body condition was calculated separately by sex, we first analyzed the influence of ACTH and body condition on stress-induced hormone levels in males and then in females. We used GLMs with ACTH treatment as a factor, body condition as a covariate, and ACTH treatment × body condition as an interaction. In this case, we did not use repeated-measures models because we were able to measure only stress-induced hormone levels (after 30 min).

Results

Breeding Status, Sex, and Hormonal Levels

Snow petrels responded to the capture/restraint protocol with an increase in circulating corticosterone levels and a concomitant decrease in circulating prolactin levels (repeated-measures GLM; corticosterone: $F_{1,60} = 328.81$, $P < 0.001$; prolactin: $F_{1,58} = 28.78$, $P < 0.001$; Fig. 1). However, these changes in corticosterone and prolactin levels were not affected by sex (repeated-measures GLM; corticosterone: $F_{1,60} = 0.08$, $P = 0.781$; prolactin: $F_{1,58} = 0.04$, $P = 0.840$), breeding status (repeated-measures GLM; corticosterone: $F_{1,60} = 0.05$, $P = 0.826$; prolactin: $F_{1,58} = 0.94$, $P = 0.337$), or their interaction (repeated-measures GLM; corticosterone: $F_{1,60} = 1.24$, $P = 0.269$; prolactin: $F_{1,58} = 0.13$, $P = 0.722$).
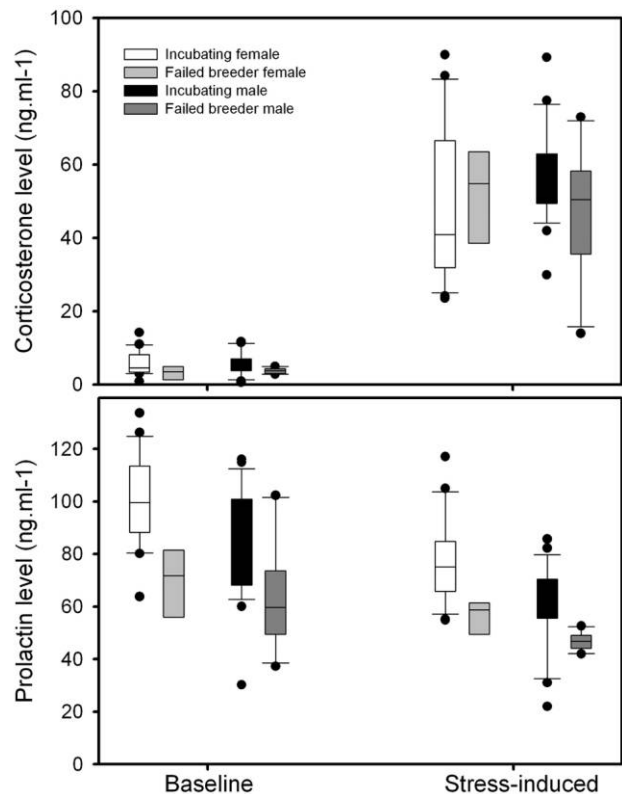

Figure 1. Prolactin and corticosterone response to a standardized stress protocol (capture/handling) of failed breeders and incubating birds in snow petrels. Box-and-whisker plots represent the data: the top and bottom of the boxes represent the top and bottom quartiles, respectively; the line across the box represents the mean. The ends of the whiskers represent the fifth and ninety-fifth percentiles, and the circles represent outliers.

Baseline prolactin levels were significantly higher in females than in males (repeated-measures GLM, contrast: $F_{1,58} = 4.53$, $P = 0.038$; Fig. 1) and significantly lower in failed breeders than in incubating birds (repeated-measures GLM, contrast: $F_{1,58} = 25.19$, $P < 0.001$; Fig. 1). Baseline corticosterone levels did not differ between sexes (repeated-measures GLM, contrast: $F_{1,60} < 0.01$, $P = 0.975$; Fig. 1) or by breeding status (repeated-measures GLM, contrast: $F_{1,60} = 0.38$, $P = 0.539$; Fig. 1). In addition to highly significant effects of sex and breeding status, baseline prolactin levels were negatively correlated with baseline corticosterone levels (GLM: $F_{1,58} = 9.27$, $P = 0.003$; Fig. 2).

Stress-induced prolactin levels were significantly higher in females than in males (repeated-measures GLM, contrast: $F_{1,58} = 5.84$, $P = 0.019$; Fig. 1) and significantly lower in failed breeders than in incubating birds (repeated-measures GLM, contrast: $F_{1,58} = 13.32$, $P < 0.001$; Fig. 1). Stress-induced corticosterone levels did not differ between sexes (repeated-measures GLM, contrast: $F_{1,60} = 0.18$, $P = 0.671$; Fig. 1) and were similar between failed breeders and incubating birds (repeated-measures GLM, contrast: $F_{1,60} = 0.87$, $P = 0.356$; Fig. 1). Sex and breeding status had highly significant effects on stress-induced prolactin levels, but stress-induced prolactin levels were
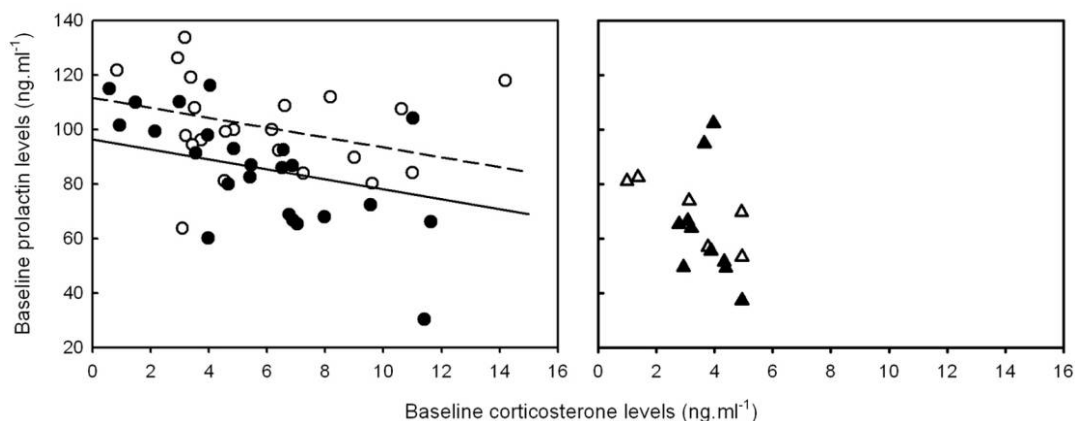
Figure 2. Relationships between baseline prolactin levels and baseline corticosterone levels in incubating snow petrels (*circles*) and failed breeders (*triangles*). Open symbols denote females, and filled symbols denote males. Baseline prolactin levels were significantly and negatively correlated with baseline corticosterone levels in incubating birds (*solid line*, males; *dashed line*, females; slope [mean $\pm$ SE] $= -1.82 \pm 0.79$) but not in failed breeders.

not correlated with stress-induced corticosterone levels (GLM: $F_{1,58} = 0.29$, $P = 0.595$).

Body Condition and Hormonal Levels

Both female and male snow petrels responded to the capture/restraint protocol with an increase in circulating corticosterone levels and a concomitant decrease in circulating prolactin levels (repeated-measures GLM: $P < 0.001$ for both sexes and both hormones). These changes in prolactin levels were not correlated with body condition (repeated-measures GLM: $P > 0.500$ for both sexes). However, the increase in corticosterone levels tended to be lower when females were in good condition (repeated-measures GLM: $F_{1,27} = 3.35$, $P = 0.078$), but this was not the case in males (repeated-measures GLM: $F_{1,33} = 0.02$, $P = 0.883$). Baseline prolactin levels were not correlated with body condition in either sex (GLM; females: $F_{1,25} = 0.15$, $P = 0.706$; males: $F_{1,31} = 0.25$, $P = 0.621$; Fig. 3). Baseline corticosterone levels were negatively correlated with body condition in incubating and failed-breeder females (GLM: $F_{1,26} = 10.03$, $P = 0.004$; Fig. 3) but not in males (GLM: $F_{1,32} = 0.94$, $P = 0.340$; Fig. 3). Moreover, baseline corticosterone levels varied between incubating females and failed-breeder females when the effect of body condition was taken into account (GLM: $F_{1,26} = 9.89$, $P = 0.004$; Fig. 3). Stress-induced prolactin levels were not correlated with body condition in either males (GLM: $F_{1,31} = 1.12$, $P = 0.298$; Fig. 4) or females (GLM: $F_{1,25} = 0.43$, $P = 0.519$; Fig. 4). Stress-induced corticosterone levels were negatively correlated with body condition in females (GLM: $F_{1,26} = 4.84$, $P = 0.037$; Fig. 4) but not in males (GLM: $F_{1,32} = 0.03$, $P = 0.864$; Fig. 4).

Sensitivity of Prolactin and Corticosterone Secretion to ACTH

From the ACTH experiment in 2006, we found no effect of ACTH treatment, body condition, or their interaction on stress-induced prolactin levels in brooding males and brooding females (Table 1; Fig. 5). Furthermore, we found that ACTH-treated brooding females had higher stress-induced corticosterone levels than control ones (Table 1; Fig. 5). Moreover, there was an additive effect of body condition (Table 1): females in poor body condition had higher stress-induced corticosterone levels (Fig. 5). There was no significant effect of the interaction ACTH treatment × body condition on stress-induced corticosterone levels in females ($P > 0.20$). Regarding males, we found no effect of ACTH treatment, body condition, or their interaction on stress-induced corticosterone levels (Table 1; Fig. 5).

Discussion

In this study, we found that corticosterone and prolactin levels increased and decreased, respectively, in response to an acute stress. Although this stress-induced effect has been widely reported for corticosterone, the stress-induced decrease in prolactin levels has been reported in only a few species (Sharp et al. 1989; Maney et al. 1999; Chastel et al. 2005; Angelier et al. 2007a; Verreault et al. 2008). At the physiological level, this decrease in prolactin levels could be due to either a decline in pituitary production or an increase in metabolism and prolactin clearance rate (via kidneys). Moreover, this decline might also result from our bleeding protocol: the standardized capture/restraint stress protocol requires repeated blood sampling and thus loss of blood volume. Sampled individuals may have restored their blood volume by using interstitial fluid from surrounding tissues, and this may have resulted in hemodilution (Dufty 2008). However, the decrease in prolactin levels in our study cannot result only from such a hemodilution because we reported a ~20% decrease in prolactin levels, whereas only a very small amount of blood was collected (maximum: 400 μL).
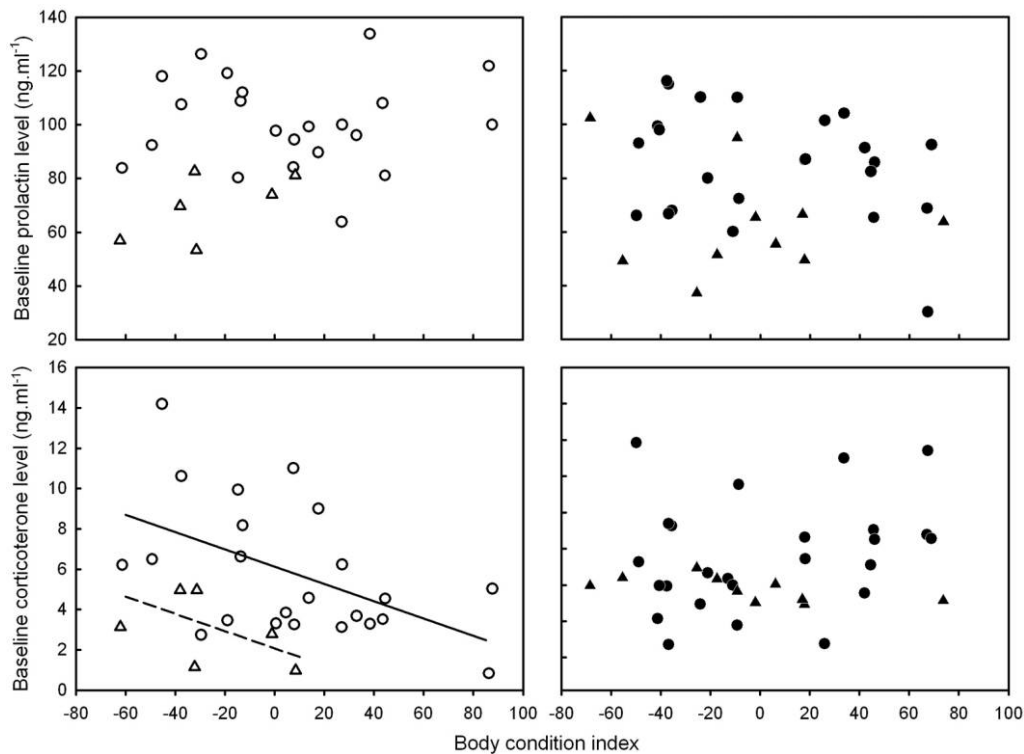
Figure 3. Relationships between body condition and baseline prolactin and corticosterone levels in incubating snow petrels (*circles*) and failed breeders (*triangles*). Open symbols denote females, and filled symbols denote males. Baseline corticosterone levels were negatively correlated with body condition in incubating females (*solid line*) and failed-breeder females (*dashed line*).

Parental-Care Hypothesis

As predicted and previously reported in another seabird species (Chastel et al. 2005), we found that incubating snow petrels had higher prolactin levels than failed breeders when facing stressors. Higher stress-induced prolactin levels in incubating petrels did not result from a lower rate of decrease in prolactin levels but rather from higher baseline prolactin levels. Indeed, we did not find any difference in the prolactin stress response between failed breeders and incubating petrels in our study. However, we must keep in mind that hormones must reach some specific values (above or below a threshold) to interact with a receptor and to have stress-induced physiological or behavioral effects (see Romero 2004; Landys et al. 2006). Therefore, the biological response to stress of an individual is likely to depend on the stress-induced hormone levels that are reached rather than on the hormonal stress response itself (i.e., the difference between stress-induced and baseline levels). Therefore, the difference in stress-induced prolactin levels between failed breeders and incubating birds may have a biological and, probably, an ultimate significance. At the proximate level, it is likely that lower baseline prolactin levels in failed breeders resulted from the loss of the egg: stimuli from the egg or the chick triggers prolactin secretion in birds (El Halawani et al. 1980; Hall 1987; Book 1991; Chastel and Lormée 2002; Massaro et al. 2007). Moreover, circulating prolactin levels of failed breeders did not seem to return to the very low levels

observed in nonbreeders of passerine species (Deviche and Sharp 2001; Badyaev and Vleck 2007; Holberton et al. 2008). This suggests that quite a high prolactin secretion could be maintained in snow petrels without any parental stimuli (as observed in other seabird species; Lormée et al. 1999; Vleck et al. 2000). Moreover, prolactin levels may also remain elevated because birds are approaching the annual prolactin peak associated with the onset of photorefractoriness that occurs during breeding (reviewed in Dawson et al. 2001; Sockman et al. 2006).

Elevated prolactin levels are functionally involved in the will to provide parental care (incubating, brooding, and provisioning behaviors; Buntin 1996; Wang and Buntin 1999; Freeman et al. 2000; Duckworth et al. 2003), to stay on the nest despite stressors (Angelier et al. 2007a), and to come back to the nest after temporary nest desertion (Angelier et al. 2009). Therefore, at the ultimate level, the maintenance of elevated stress-induced prolactin levels in incubating snow petrels could be highly adaptive because it reduces the occurrence and the duration of temporary egg neglect and therefore the deleterious effect of stressful events on the egg's survival (Chaurand and Weimerskirch 1994; Olson et al. 2006). However, we must also note that reducing the expression of parental cares and thus prolactin levels in response to stress might be necessary to avoid very high costs in terms of survival; this could explain why no bird reduced prolactin at all. Regarding failed breeders, snow petrels
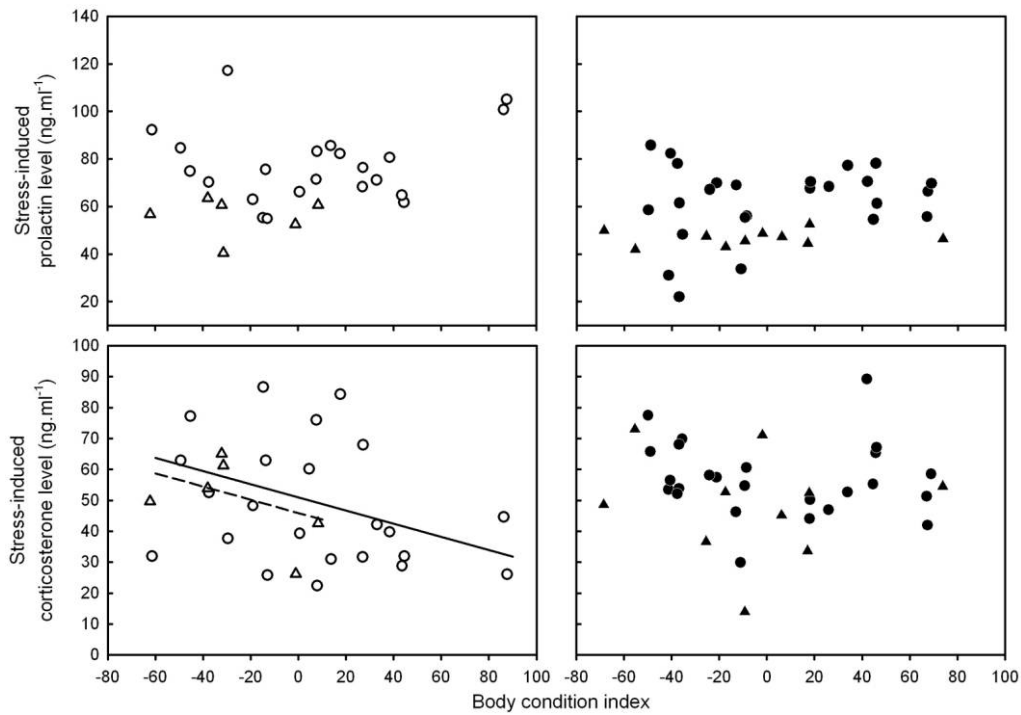
Figure 4. Relationships between body condition and stress-induced prolactin and corticosterone levels in incubating snow petrels (*circles*) and failed breeders (*triangles*). Open symbols denote females, and filled symbols denote males. Stress-induced prolactin levels were not correlated with body condition in either of the sexes or either breeding status. Stress-induced corticosterone levels were negatively correlated with body condition in incubating (*solid line*) and failed-breeder (*dashed line*) females but not in males.

attend their own nest for several weeks after the breeding failure has occurred, and it is possible that such behavior requires the maintenance of moderately elevated prolactin levels. At the ultimate level, such behavior might play a role in the ability of birds to retain a nest or a mate from one breeding season to another, as suggested in other seabird species (Cadiou et al. 1994).

On the other hand, and contrary to our prediction, rates of corticosterone increase and stress-induced corticosterone levels were similar in incubating birds and failed breeders, demonstrating that the corticosterone stress response is not modulated according to breeding status in snow petrels during the incubation period (failed breeders vs. incubating birds). Alternatively, these birds may not have physiologically returned to the "nonbreeding state" at the time of sampling, explaining why their corticosterone stress response was similar to that of incubating birds. Indeed, we do not know exactly when these failed breeders lost their eggs, which could have been just before being sampled. However, this is very unlikely because (1) we found an effect of breeding failure on another endocrine mechanism (prolactin levels), and (2) this similarity in the corticosterone stress response between breeding birds and failed breeders has already been reported in other bird species (Wingfield et al. 1999; Chastel et al. 2005). In vertebrates, elevated stress-induced corticosterone levels are involved in the activation of an "emergency life-history stage," which aims to promote survival at the expense of other life-history related activ-

ities, such as breeding (Wingfield et al. 1998). Therefore, our results suggest that incubating birds and failed breeders have the same capacity to respond to a potential stressor: they are similarly motivated to shift from their current life-history stage to such an emergency life-history stage. Thus, they may be similarly prone to nest desertion in response to prolonged stress, especially if nest desertion is under the influence of corticosterone secretion (Silverin 1986). However, these corticosterone-induced effects might be attenuated by the maintenance of elevated stress-induced prolactin levels. For instance, incubating birds could be less prone to desert the nest in response to short-term stressors than failed breeders because of higher stress-induced prolactin levels (Angelier et al. 2007a). In addition, we note that baseline corticosterone levels of incubating females were slightly higher than those of failed breeder females. However, this difference is quite low (only a few ng mL^{-1}), and it probably results from higher energetic demands in breeding birds (Landys et al. 2006).

In short, elevated stress-induced corticosterone levels probably enhance the will to shift from the current life-history stage to an emergency life-history stage (Wingfield et al. 1998), and elevated stress-induced prolactin levels probably reduce (1) the will to leave the egg, the chick, or the nest unprotected (Angelier et al. 2007a) and (2) the duration of temporary nest desertion if escape behavior occurs (Angelier et al. 2009). Such a combination of hormonal stress responses in breeding birds could be adaptive because it may adjust parental decisions to the

Table 1: Model selection and general linear model to test the influence of body condition and injection of adrenocorticotropic hormone (ACTH) on stress-induced prolactin and corticosterone levels in brooding snow petrels

Dependent and Independent Variable	Male			Female		
	df	F	P	df	F	P
Stress-induced prolactin level:						
Body condition	1, 5	.09	.776	1, 7	.86	.385
ACTH treatment	1, 5	.01	.916	1, 7	.39	.553
Stress-induced corticosterone level:						
Body condition	1, 5	.11	.756	**1, 7**	**8.11**	**.025**
ACTH treatment	1, 5	<.01	.935	**1, 7**	**5.88**	**.046**

Note. Boldface indicates significant factors. Interaction terms are not reported in the table because they were eliminated from all the models through a step-down approach ($P > 0.15$).

situation encountered and to the fitness value of the current reproductive event. Moreover, elevated stress-induced corticosterone levels and low stress-induced prolactin levels in failed breeders probably maximize a bird's survival by enhancing escape behavior and by delaying return to the breeding site, where the stressor still potentially occurs.

Body Condition Hypothesis

Because snow petrels in poor body condition should reduce their parental investment in order to maximize their survival, we predicted that birds in poor body condition should be more sensitive to stress during the breeding period (the body condition hypothesis; Lynn et al. 2003; but see Holberton and Able 2000 and Long and Holberton 2004 regarding preparation for migration). Contrary to this prediction, we found no effect of body condition on the prolactin stress response in male or female snow petrels. On the other hand, and in agreement with this prediction, decreased prolactin secretion in response to the energetic constraints of fasting has previously been shown (Cherel et al. 1994; Angelier et al. 2007b), and two studies have reported that baseline prolactin levels and body condition can be correlated in breeding birds (Weimerskirch and Cherel 1998; O'Dwyer et al. 2006). The relationship between prolactin and body condition appears to be complex and nonlinear because prolactin secretion decreases only when individuals have reached a certain low threshold in body condition (Cherel et al. 1994). Similarly, the sensitivity of prolactin secretion to stress might be increased only when parents reach a certain low threshold in body condition. In our study, breeding snow petrels might not have reached this low threshold; the result, therefore, is the absence of a relationship between baseline prolactin levels, the prolactin stress response, and body condition.

We did find, however, that stress-induced corticosterone levels of females in poor body condition tended to be higher than those of females in good body condition, in accord with our prediction. Unlike that of females, the corticosterone stress response of male snow petrels was unaffected by body condition. In snow petrels, females are smaller and desert the nest more often than males (Barbraud and Jouventin 1998; Barbraud and

Chastel 1999), suggesting, therefore, that they are more energetically constrained than males. Elevated baseline corticosterone levels are commonly associated with low energy availability (Landys et al. 2006), which may explain the elevated baseline corticosterone levels found in females in poor body condition. Regarding male snow petrels, body reserves may not have reached the threshold below which parental survival is threatened, which might explain why neither baseline corticosterone levels nor corticosterone stress response were correlated with body condition (Cherel et al. 1988; Romero and Wikelski 2001). Moreover, other factors could have affected hormone levels in our study (e.g., the effect of age on hormone levels: Heidinger et al. 2006; Angelier et al. 2007a, 2007b; changes in hormone levels across an incubation shift: Hector and Goldsmith 1985; Hector and Harvey 1986), and we have to note that these factors might have masked some relationships between condition, breeding status, and hormone levels.

As previously reported in several seabird species, prolactin levels were higher in females than in males (Lormée et al. 2000). This pattern is still not well understood because males and females provide similar amounts of parental care in the great majority of seabirds (Vleck 1998).

Proximate Causes of Corticosterone Release Modulation

When body condition was considered, mean corticosterone levels of females challenged by an ACTH injection were higher than those of controls, demonstrating that the adrenal glands can secrete more corticosterone if more ACTH is available to these glands. This result demonstrates that modulations of the corticosterone stress response in female petrels (see Adams et al. 2005 for an example) probably result from variations in the secretion of ACTH by the pituitary (at least during the brooding stage, because we injected only brooding birds with ACTH). Of course, it suggests that variations in the secretion of ACTH-releasing factors may also be involved in the modulation of the corticosterone stress response (corticotropin-releasing factor, arginine vasotocin, and mesotocin; Romero et al. 1998a, 1998b). Moreover, we found no effect of the ACTH treatment × condition interaction on stress-induced corticosterone levels, demonstrat-
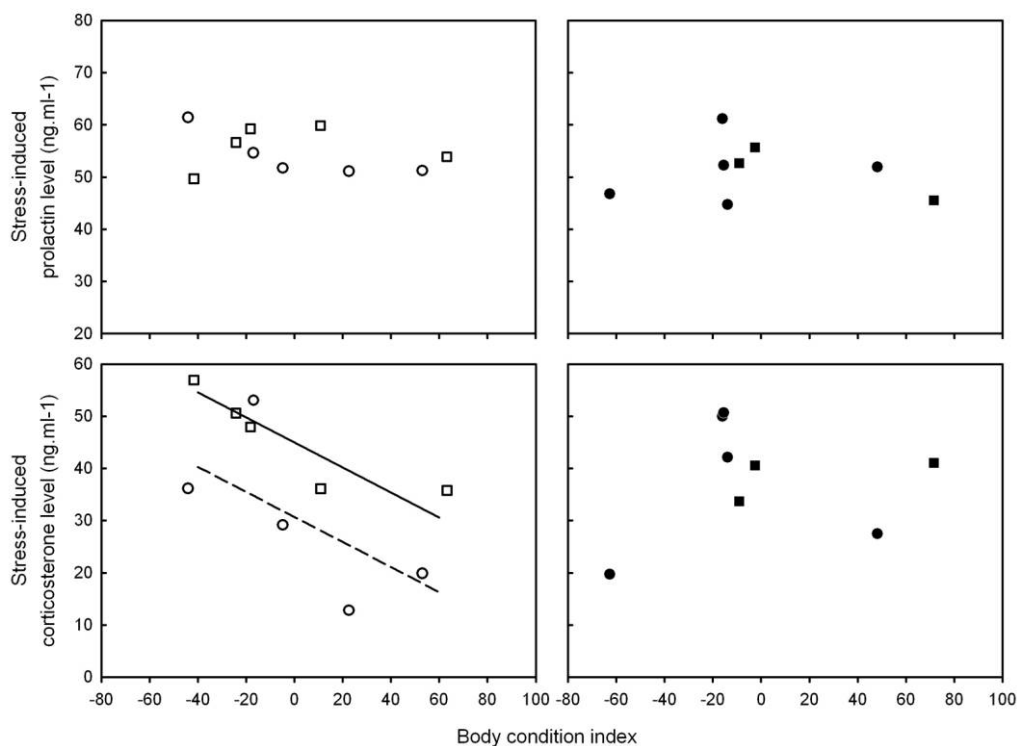
Figure 5. Relationships between body condition and stress-induced prolactin/corticosterone levels in adrenocorticotropic hormone (ACTH)–treated (*squares*) and control (*circles*) snow petrels during the brooding period. Stress-induced corticosterone levels were negatively correlated with body condition in ACTH-treated females (*solid line*) and control ones (*dashed line*). Open symbols denote females, and filled symbols denote males.

ing that adrenal sensitivity to ACTH did not vary with body condition. Therefore, the modulation of corticosterone stress response with body condition in females probably resulted from variations in the release of ACTH by the pituitary. Supporting this interpretation, changes in the release of ACTH have already been shown to regulate corticosterone stress response in birds (Romero et al. 1998a, 1998b, 1998c; Romero and Wingfield 1998; Meddle et al. 2003; Romero 2006). For instance, Romero (2006) found that an experimental increase in circulating ACTH levels stimulates corticosterone secretion of house sparrows (*Passer domesticus*) during several life-history stages (molting, wintering, and breeding).

In males, we found that injection of ACTH did not stimulate corticosterone secretion whatever their body condition, suggesting, therefore, that males might be insensitive to an increased release of ACTH by the pituitary. Such an insensitivity has already been reported in several bird species during various life-history stages (Romero et al. 1998b, 1998c; Romero and Wingfield 1998; Romero 2006) and suggests that adrenal glands might already be saturated by the endogenous ACTH. However, our sample size is quite small, and future studies are definitely needed to confirm this interpretation.

Functional Link between Corticosterone and Prolactin

In birds, there is growing evidence that plasma levels of corticosterone and prolactin are linked by closely related inter-

actions that can influence parental behavior, energy regulation, and feeding activity (Koch et al. 2002, 2004; Criscuolo et al. 2006). However, the functional link between corticosterone and prolactin is poorly known, especially in free-living vertebrates. In this study, we found a negative relationship between the baseline corticosterone and prolactin levels of snow petrels. This result suggests that long-term variations in corticosterone levels may be involved in the regulation of prolactin secretion (Freeman et al. 2000). This interpretation is supported by several laboratory experiments conducted with captive rodents (Freeman et al. 2000). For instance, blockade of corticosterone receptors by administration of mifepristone enhances prolactin secretion (van Der Schoot et al. 1990). Moreover, chronic administration of ACTH and maintenance of elevated corticosterone over a long period results in decreased prolactin secretion (Kanyicska et al. 1983; Fekete et al. 1984; Yelvington et al. 1984).

Although some studies have shown that both corticosterone and prolactin secretions are affected by short-term stressful events (Chastel et al. 2005; Angelier et al. 2007a), no study has found a relationship between the corticosterone and prolactin stress responses in breeding birds. In snow petrels, we found no relationship between stress-induced corticosterone and prolactin levels, and we suggest with mounting evidence that the sensitivity of corticosterone and prolactin secretion to temporary, stressful events is not governed by the same physio-

logical mechanisms. Moreover, we found no effect of ACTH injection on stress-induced prolactin levels, demonstrating that the short-term decrease in prolactin levels in response to stress was not regulated by the release of ACTH by the pituitary. In humans, a temporary experimental increase in ACTH levels has been reported to induce a reduction in prolactin secretion. This effect appears to be mediated through a stimulatory effect of ACTH on corticosterone secretion, which reduces prolactin secretion (Bratusch-Marrain et al. 1982; Hubina et al. 2002). Because our experimental injection of ACTH has been effective in increasing corticosterone levels (at least in females), our results suggest that a corticosterone-mediated reduction in prolactin levels might occur only when elevated corticosterone levels are maintained during a prolonged period (more than 30 min) in breeding snow petrels. In our study, short-term variations in prolactin secretion are more likely regulated primarily by other biological substances quickly secreted in response to stress, such as dopamine, serotonin, histamine, oxytocin, vasopressin, and vasoactive intestinal peptide (El Halawani et al. 1991; Sharp et al. 1998; Maney et al. 1999; Vleck and Patrick 1999; Freeman et al. 2000). To sum up, these results support the idea that the corticosterone and prolactin responses to short-term stressors could be independent and might, therefore, mediate some specific components of parental investment in breeding birds.

Conclusion

Few data exist on the effect of breeding status and body condition on both prolactin and corticosterone stress responses (Chastel et al. 2005). In this study, we showed that hormonal stress responses could be modulated according to these factors in a seabird species. These results suggest that the resistance to stress in breeding birds can be mediated by several hormonal mechanisms, such as corticosterone and prolactin stress responses. Each of these responses probably governs specific behavioral and physiological mechanisms (Buntin 1996; Landys et al. 2006), and consequently, studying both of these hormonal stress responses may allow one to better evaluate the influence of ecological factors on parental investment. To better understand the functional link between parental investment and the prolactin stress response, further studies should examine this stress response in more bird species and in relation to different life-history strategies.

Acknowledgments

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