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Exogenous corticosterone and nest abandonment: A study in a long-lived bird, the Adélie penguin

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ABSTRACT

Breeding individuals enter an emergency life-history stage when their body reserves reach a minimum threshold. Consequently, they redirect current activity toward survival, leading to egg abandonment in birds. Corticosterone (CORT) is known to promote this stage. How and to what extent CORT triggers egg abandonment when breeding is associated with prolonged fasting, however, requires further investigation. We manipulated free-living male Adélie penguins with CORT-pellets before their laying period. We then examined their behavioral response with respect to nest abandonment in parallel with their prolactin levels (regulating parental care), and the subsequent effects of treatment on breeding success in relieved birds. Exogenous CORT triggered nest abandonment in 60% of the treated penguins ~14 days after treatment and induced a concomitant decline in prolactin levels. Interestingly, prolactin levels in treated penguins that did not abandon their nest were higher at the point of implantation and also after being relieved by females, when compared with abandoning penguins. Among successful birds, the treatment did not affect the number of chicks, nor the brood mass.

Our results show the involvement of CORT in the decision-making process regarding egg abandonment in Adélie penguins when incubation is associated with a natural long fast. However, we suggest that CORT alone is not sufficient to trigger nest abandonment but that 1) prolactin levels need to reach a low threshold value, and 2) a rise in proteolysis (i.e. utilization of protein as main energy substrate) seems also to be required.

Introduction

During the demanding breeding period, life-history theory predicts that individuals should favor their own survival over that of their offspring, when breeding becomes too costly in terms of body maintenance (Stearns, 1992). Once the parents' energetic state reaches a low threshold, they enter an emergency life-history stage triggering physiological and behavioral changes that enhance survival (Wingfield et al., 1998). One example of such behavior is egg abandonment in birds.

Several studies examining the mechanisms that mediate life-history trade-offs in vertebrates, have emphasized the importance of glucocorticoids. Corticosterone (CORT), the primary glucocorticoid hormone in birds, has been linked to the promotion of an emergency life-history stage (Wingfield et al., 1998) and has also been associated with the reduction or suppression of parental care (Wingfield, 2003; Wingfield and Sapolsky, 2003). For instance, exogenous CORT increases foraging activity at the expense of chick brooding/guarding in black-legged kittiwakes *Rissa tridactyla* (Kitaysky et al., 2001) and elevated CORT levels have been linked to egg abandonment in incubating fasting penguins (Groscolas et al., 2008; Spée et al., 2010). However, Criscuolo and colleagues (Criscuolo et al., 2005) showed that an experimentally-induced rise in CORT concentration alone was not sufficient to trigger nest desertion in incubating female common eider ducks *Somateria mollissima*. The authors suggested that the lack of effect could be due to 1) a too short duration of treatment since CORT concentration returned to its baseline level within four days of treatment, 2) a too high dose and/or 3) another mechanism involving other endocrine factors.

Recent studies strongly suggest that CORT could affect other endocrine factors that promote the expression of parental care (Angelier et al., 2009; Criscuolo et al., 2005). In this context, the pituitary hormone prolactin is of particular interest, since it is involved in the initiation and the maintenance of avian incubation behavior (Buntin, 1996). The relationship between CORT and prolactin is nevertheless complex, since there is evidence that these hormones can act additively or synergistically in some avian species to stimulate parental provisioning of young (ring doves *Streptopelia risoria*, Koch et al., 2004; mourning doves *Zenaida macroura*, Miller et al., 2009). Moreover, studies support the idea that the

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effects of these two hormones on parental behavior are antagonistic (reviewed in Angelier and Chastel, 2009). Correlative studies revealed that an increase in CORT levels resulting from acute stress or prolonged energy constraints is associated with a decrease in prolactin levels (Angelier et al., 2007b; Chastel et al., 2005; Groscolas et al., 2008). In agreement with these observations, experimental studies have shown that exogenous administration of CORT leads to a reduction in plasma prolactin concentrations (Angelier et al., 2009; Criscuolo et al., 2005). These studies suggest that the secretion of CORT and prolactin might be functionally linked. We therefore suggest that increased CORT levels, induced by prolonged energy constraints like fasting, could act through prolactin concentrations and thereby stimulate egg abandonment. The finding that Adélie penguins with high CORT concentrations and low prolactin levels desert their nest, whereas birds with both high CORT and prolactin levels do not (Spée et al., 2010), strongly supports this hypothesis.

Long-lived seabirds are good models to study the underlying endocrine mechanisms that mediate the decision to promote self-maintenance at the expense of current reproduction. In seabirds and especially in penguins, reproduction is associated with long fasting periods (up to four months in the emperor penguin *Aptenodytes forsteri*; Groscolas and Robin, 2001), since foraging and breeding areas are generally far apart. In Adélie penguins *Pygoscelis adeliae*, males and females both migrate to the breeding colony, where females fast until the clutch is complete. As they return to sea to feed, males usually take on the first incubation shift (Ainley, 2002). Thereafter both parents alternate in caring for the egg(s) and chick(s). However, birds are likely to abandon incubation, once energy reserves are nearing a critical point of exhaustion. In this case, they enter a proteolytic stage, the so-called phase III of fasting (PIII). At this stage, hormonal and metabolic shifts characteristic of a switch from lipid to protein utilization (as revealed by increased uric acid levels) occur (Cherel et al., 1988; Robin et al., 1998; Spée et al., 2010). In addition to these physiological adjustments, marked behavioral changes have been reported for birds entering PIII, such as an increase in locomotor activity (Robin et al., 1998; Spée et al., 2011) and nest abandonment (Groscolas et al., 2008; Spée et al., 2010). Given their long life span, the optimal strategy for an incubating penguin entering PIII is to abandon the current reproductive effort in favor of its own survival, thereby ensuring future reproductive attempts.

In this study, we firstly examined whether the maintenance of high CORT levels can induce abandonment of reproduction in free-living Adélie penguins. We experimentally increased CORT levels to mirror prolonged energy constraints and the activation of an emergency life-history stage and examined to what extent this affected the completion of incubation (whether penguins abandoned their nest in response to the treatment or not). Secondly, knowing that prolactin plays a key role in the control of parental behavior in birds and that this hormone might also be linked with CORT, we examined whether CORT administration affected prolactin levels. Finally, because CORT generally increases foraging effort (Kitaysky et al., 2001; Angelier et al., 2007a) and subsequent reproductive success (Criscuolo et al., 2005), we also monitored the effects of the treatment on foraging trip duration in treated males and their partners and the number of surviving chicks.

Materials and methods

Field procedure

The study was conducted in Dumont d'Urville Station (66°40′S; 140°01′E), Adélie land, Antarctica, during the 2007–2008 austral summer and was approved by the ethics committee of the French Polar Institute (IPEV) and the Terres Australes et Antarctiques Françaises (TAAF).

Free-living male Adélie penguins were captured on two occasions (Fig. 1). Penguins were captured for the first time between the periods of pair formation and egg-laying. A blood sample was collected from the wing vein within 5 min of capture as Vleck and colleagues (Vleck et al., 2000b) showed that handling durations of less than 5 min had no effect on CORT levels in Adélie penguins. Samples were transferred into tubes pre-treated with heparin and centrifuged (5000 rpm for 10 min at 4 °C). Plasma was then collected and kept frozen in aliquots at −20 °C until laboratory analysis. Each penguin was then implanted with CORT pellets, placed subcutaneously in the nape of the neck. It has been shown that the action of CORT on locomotor activity

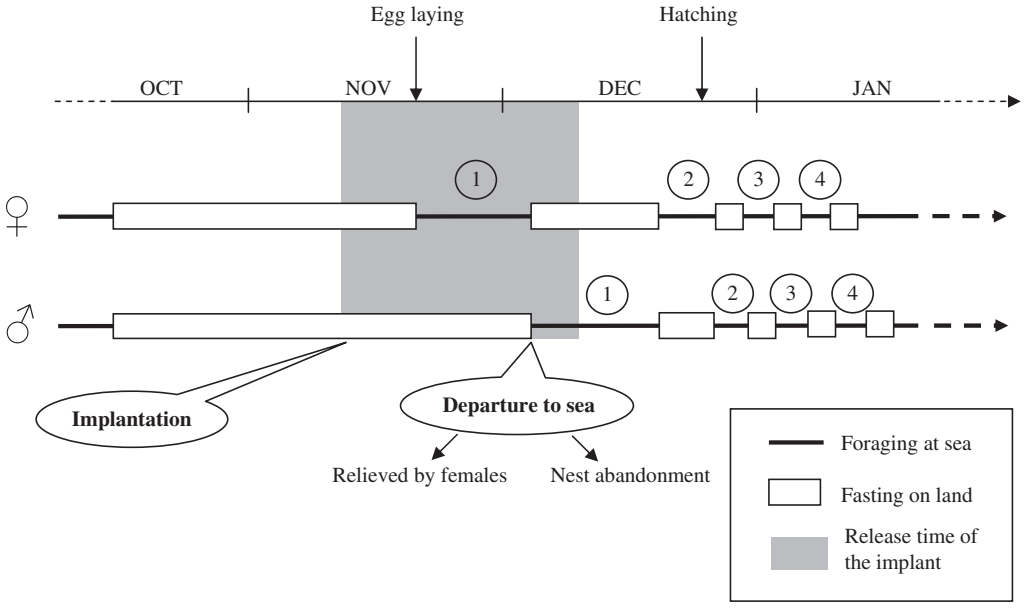

Fig. 1. Study protocol. Male Adélie penguins were weighed and blood samples were taken at the time of implantation and when they departed to sea (when their partner had returned or when they abandoned their nest). Numbers indicate successive foraging trips of males and their female partners. The gray area represents the period during which CORT was released from the implant. See Materials and methods section for details.

depends on its concentration (Breuner and Wingfield, 2000; Spée et al., 2011). This is the reason why birds were implanted with 10 (C10; n = 15) or 100 (C100; n = 15) mg of CORT in the present study. Control penguins (n = 29) underwent the same protocol but without pellet insertion. CORT pellets (21 days release, G-111) were obtained from Innovative Research of America (Sarasota, FL, USA). These two doses, defined as low and high dose of CORT, were chosen according to data obtained on previous work run on captive Adélie penguins (Spée et al., 2011). Indeed, penguins treated with the highest dose (C100) showed metabolic, hormonal and behavioral (increased locomotor activity) changes characterizing control birds in PIII of fasting. All birds were weighed with an electronic balance (Ohaus, ± 2 g) and marked with a Nyanzol-D mark, painted on the breast feathers. Sex was determined by a combination of parameters including cloacal inspection before egg laying, incubation routine (males usually take on the first incubation shift; Ainley, 2002), and measure of plasma lipemia (i.e. the amount of lipids present in the bloodstream). Indeed, near the time of laying, females have to produce a clutch of eggs that requires the deposition of large amounts of yolk lipids. Accordingly, they exhibit higher plasma lipemia before egg-laying than males (Beaulieu et al., 2010a; Kern et al., 2005). This latter method has already been used for sex determination in Adélie penguins by Vleck and colleagues (Vleck et al., 2000a).

Males were recaptured at the end of the first incubation shift, when they left the colony to refeed at sea, either because (1) their partner had returned to relieve them or (2) because they abandoned their nest. Among the control birds, two males abandoned the nest while 27 were relieved by females. In C10-penguins only one bird abandoned the nest, while 14 were relieved by females. In C100-birds nine abandoned the nest and six were relieved by females. During this second capture, birds were weighed and another blood sample was taken.

Nests were observed once every 2–3 h to determine (1) which birds were present on the nest and potential nest abandonments, (2) the date of egg laying, (3) the number of eggs per pair, and (4) the date of hatching. However, in cases in which males exhibited characteristics associated with imminent abandonment (e.g. low body mass [~3.5 kg; (Cockrem et al., 2006; Spée et al., 2010)] approaching the threshold into PIII of fasting and below which they are prone to abandon their nest, lessened attentiveness to egg(s) (during incubation), nests were check hourly. We also monitored foraging trip durations (during incubation and the chick-rearing stage, until the seventh foraging trip in succession) in males and their partners. The number of chicks was determined 1 week after hatching and during the crèche stage, when they reached their peak body mass (~42–45 days after hatching; Ainley, 2002). At this latter stage, chicks were weighed with an electronic balance (Ohaus, ± 2 g). Reproductive success was estimated from the number of chicks surviving to this latter stage, since after this point chick mortality is usually very rare (Beaulieu et al., 2010c; Beaulieu et al., 2009; Clarke et al., 2002).

Laboratory analyses

Plasma CORT concentrations were determined by immunoassay according to guidelines provided by the manufacturer (AssayPro, AssayMax Corticosterone ELISA Kit, EC3001-1). Plasma concentrations of prolactin were determined by a heterologous radioimmunoassay (RIA) at the Centre d'Etude Biologiques de Chizé (CEBC; France). Pooled plasma samples of Adélie penguins produced a dose response curve that paralleled chicken prolactin standard curves (bAFP 4444BQ, source: Dr. Parlow, N.H.P.P. Harbor-UCLA Medical Center, Torrance). Intra and inter assay variations for prolactin were 6% and 9%, respectively, while they were 5% and 7%, respectively for CORT.

Plasma levels of uric acid were measured on undiluted plasma (25 µl) using enzymatic colorimetric tests (Sigma Diagnostics, St Louis, MO, USA).

Data analyses and statistics

To estimate the time-course over which CORT levels changed following C100-implantation, we separate birds sampled at the time of departure from sea into groups using the Sturges' rule. We obtained 5 classes with CORT values (ng/ml) ranging as follow: 5.78 ± 0.68 at day 0 (n = 15); 16.52 ± 6.93 at day 9 (n = 2); 20.85 ± 0.89 at days 13–14 (n = 2); 37.08 ± 9.84 at days 15–16 (n = 4); 8.09 ± 2.62 at days 17–19 (n = 4) and 8.82 ± 2.21 at days 20–22 (n = 2); this increase following CORT treatment being in agreement with previous data using similar pellets in eider ducks (Bourgeon and Raclot, 2006). It is of note that there is likely no confounding effect between sampling time and nesting status, since birds were relieved by females between 15 and 22 days after the time of implantation while penguins abandoned their nest between 9 and 19 days after the treatment. We found an effect of the day of sampling on CORT levels (Kruskal–Wallis: $H = 15.85$, $p < 0.001$). CORT levels were significantly increased at days 9–16 compared to day 0 ($p < 0.001$). Then, CORT levels dropped at days 17–22 to a level similar to that obtained at day 0 ($p = 0.78$). Moreover, circulating levels of CORT measured in C100-birds were within the range of CORT obtained in Adélie penguins in response to capture and handling (up to 78.7 ng/ml, Cockrem et al., 2009), thus being physiologically relevant.

We used a generalized linear mixed model (GzLM) with a binomial distribution family to examine the effect of treatment on the percentage of nest abandonment. Because the resulting groups were unbalanced, we did not use a general linear model to analyze the effect of "treatment" (control, C10, C100) and "incubation success" (relieved by females or nest abandonment) on most of the variables investigated (date of implantation and departure to sea; body mass and body condition index at these stages; fasting duration between the time of implantation and departure to sea; daily body mass loss; dates of egg-laying and hatching; number of egg(s), chick(s), and brood mass). In fact, due to the small number of abandoning birds within the control and C10-groups (n = 2 and n = 1, respectively) and the large number of abandoning C100-penguins (n = 9), the statistical results would automatically be biased. Instead, we considered control, C10, and C100-penguins relieved by females and we did not conduct statistical analyses on penguins that abandoned their nest. General linear models (GLM) were used to compare body mass, fasting duration, incubation duration, and daily body mass loss between groups. A generalized linear mixed model (GzLM) with a gamma distribution was used to compare the brood mass because data were not distributed normally but were skewed to the right. GzLM's with a Poisson distribution were used for count data (date of implantation, departure to sea, egg-laying and hatching, number of eggs and chicks).

Concerning hormonal changes in response to treatment, we did not include control birds in the statistical analyses because of the small sample size of abandoning penguins (n = 2). We used a general linear mixed model (GLMM) for CORT analysis and a generalized estimating equation (GEE) with a gamma distribution for prolactin analysis since prolactin data were not distributed normally. We included "incubation success" (relieved by females or nest abandonment), "sampling period" (the day of implantation and the day of departure to sea) and their interaction as fixed factors, with "sampling period" being a repeated measure.

We compared uric acid levels between the time of departure to sea and the point of implantation in each group of C100-treated penguins with Wilcoxon tests.

As the duration of the first foraging trip, which takes place during the incubation stage, and the duration of subsequent trips differ in Adélie penguins, comparisons between groups (control and C100) were carried out separately. To compare the duration of the first foraging trip between control and C100 penguins in males and their partners, we used a Student's *t*-test or a Mann–Whitney test, when

normality was not met (for females). A GEE with a gamma distribution was used to compare the duration of subsequent foraging trips in males and their partners. We included individuals as a random factor and "treatment" (control and C100), "trip number" (from the second to the seventh foraging trip in succession) and their interactions as fixed factors, with "trip number" being a repeated measure.

For multiple comparisons we used Bonferroni post hoc tests. Analyses were performed using SPSS (Vers. 16.02; SPSS Inc., Chicago, Ill., USA). Results are expressed as means ± SE and differences were considered as statistically significant when $p<0.05$.

Results

Nest abandonment

We found a strong effect of treatment on nest abandonment in free-living male Adélie penguins (6.9%, 6.7%, and 60% in control, C10, and C100-penguins, respectively; Wald $\chi^2=14.4$, df = 2, $p<0.001$). C100-birds were seven times more likely to abandon their nest than control ($p<0.001$) and C10-penguins ($p=0.001$).

Profile of abandoning birds (control, C10, and C100-penguins)

We did not conduct statistical analyses on penguins that abandoned their nest due to the small number of abandoning birds within the control and C10-groups ($n=2$ and $n=1$, respectively). However, respective values can be seen in Table 1 and Fig. 2.

C100-penguins that abandoned their nest appear to have spent less time ashore between the time of implantation and their departure to sea, and seem to have left the colony to refeed at sea with a higher body mass than that of control and C10-birds.

Profile of birds relieved by females (control, C10, and C100-penguins)

At the time of implantation (implantation date was similar between groups, Wald $\chi^2=1.29$, df = 2, $p=0.53$; Table 1), control, C10, and C100-birds that were relieved by females had similar body masses ($F_{2,44}=2.81$, $p=0.07$; Table 1). Fasting duration for males between the time of implantation and their departure to sea was also similar between groups ($F_{1,9}=0.46$, $p=0.63$; Fig. 2a). In contrast, daily body mass loss was significantly different between groups ($F_{2,35}=11.5$, $p<0.001$; Fig. 2c). C100-penguins had a daily body mass loss that was 15% and 20% greater than that of C10 ($p=0.007$) and control birds ($p<0.001$), respectively. The date of departure from sea differed between control, C10, and C100-penguins that were relieved by females (Wald $\chi^2=8.58$, df = 2, $p=0.01$; Table 1) but body masses were similar at this stage ($F_{2,35}=1.71$, $p=0.20$, Fig. 2b).

We found no significant effect of treatment on laying date (Wald $\chi^2=0.16$, df = 2, $p=0.92$; Table 1) and the number of eggs (Wald $\chi^2=0.19$, df = 2, $p=0.91$; Table 1) in birds relieved by females. In contrast, the treatment significantly affected hatching date (Wald $\chi^2=39.7$, df = 2, $p<0.001$; Table 1). Eggs of control birds hatched ~1 and 2 days earlier than that of C10 and C100-penguins, respectively ($p<0.001$ for both). However, when considering the duration of the incubation period, we found no effect of the treatment ($F_{2,37}=1.97$, $p=0.15$; Table 1). The treatment did not affect the number of chicks, neither 1 week after hatching (Wald $\chi^2=0.42$, df = 2, $p=0.81$; Table 1), nor when chicks reached their peak body mass (Wald $\chi^2=0.25$, df = 2, $p=0.88$; Table 1). Moreover, the brood mass at this latter stage was not affected by the treatment (Wald $\chi^2=0.57$, df = 2, $p=0.75$; Table 1).

Uric acid levels in C100-birds

At the time of departure to sea, uric acid levels significantly increased in C100-birds that abandoned their nest compared to the point of implantation (from 0.19 ± 0.01 mmol/l to 0.35 ± 0.04 mmol/l; $Z=-2.31$, $p=0.02$). This trend was not significant in C100-penguins that were relieved by females (0.20 ± 0.02 mmol/l vs 0.30 ± 0.05 mmol/l; $Z=-1.76$, $p=0.08$).

CORT and prolactin levels in control penguins

We did not conduct statistical analyses on control penguins due to the small number of abandoning birds ($n=2$). However, respective values can be seen in Fig. 3.

In abandoning penguins, CORT levels tend to increase while prolactin concentrations tend to decline. In contrast, hormone profiles of birds that were relieved by females did not appear to show any changes.

CORT and prolactin levels in both abandoning and relieved C100-penguins

CORT levels changed significantly in all treated-penguins during the study period (Wald $\chi^2=9.54$, df = 1, $p=0.002$; Fig. 3a). At the time of implantation (initial levels), circulating levels of CORT were 70% lower than when they left the colony to refeed at sea (final levels). However, the incubation success did not influence plasma levels of CORT (Wald $\chi^2=0.67$, df = 1, $p=0.41$) and were not influenced by the interaction between the incubation success and the sampling period (Wald $\chi^2=0.43$, df = 1, $p=0.51$).

In contrast, there was a significant effect of the incubation success ($F_{1,12}=6.94$, $p=0.02$; Fig. 3b) and the sampling period ($F_{1,12}=47.47$, $p<0.001$) on prolactin concentrations. In other word, C100-penguins that were relieved by females (C100 Re) had higher initial and final

Table 1
Profile of control and CORT treated-penguins (C10 and C100) according to their incubation success.

Completion of the 1st incubation shift	Relieved by females			Nest abandonment		
Treatment	Control (n = 27)	C10 (n = 14)	C100 (n = 6)	Control (n = 2)	C10 (n = 1)	C100 (n = 9)
Date of implantation	16/11 ± 0.64[a]	16/11 ± 1.06[a]	17/11 ± 0.98[a]	16/11 for both	16/11	16/11 ± 0.80
Body mass at implantation (kg)	4.75 ± 0.06[a]	5.10 ± 0.15[a]	4.94 ± 0.21[a]	4.69 and 4.49	4.89	5.18 ± 0.14
Departure date	6/12 ± 0.62[a, b]	7/12 ± 0.15[a]	5/12 ± 0.47[b]	13/12 and 6/12	9/12	30/11 ± 1.02
Laying date	20/11 ± 0.47[a]	20/11 ± 0.62 [a]	20/11 ± 0.95[a]	18/11 and 21/11	24/11	19/11 ± 0.62
No. of eggs per pair	1.78 ± 0.08[a]	1.93 ± 0.07[a]	2.0 ± 0.0[a]	2 for both	1	1.78 ± 0.15
Hatching date	24/12 ± 0.48[a]	25/12 ± 0.62[b]	26/12 ± 0.68[b]	–	–	–
Incubation duration (days)	34.54 ± 0.49[a]	35.45 ± 0.58[a]	36.60 ± 0.98[a]	–	–	–
No. of chicks per pair*	1.23 ± 0.13 [a]	1.36 ± 0.20[a]	1.60 ± 0.24[a]	–	–	–
No. of chicks per pair $	1.13 ± 0.11[a]	1.31 ± 0.20[a]	1.40 ± 0.24[a]	–	–	–
Brood mass $	4.29 ± 0.26[a]	5.73 ± 0.58 [a]	5.26 ± 0.93[a]	–	–	–

C10 and C100: male Adélie penguins implanted with 10 and 100 mg of CORT, respectively. Results are expressed as means ± SE. Among birds that were relieved by females, values with different superscript letters are significantly different from each other. Statistical analyses were not conducted in abandoning birds because of their small number within the control and C10-groups. * one week after the hatching date; $ when chicks reached their peak body mass (42 to 45 days after hatching; Ainley, 2002).

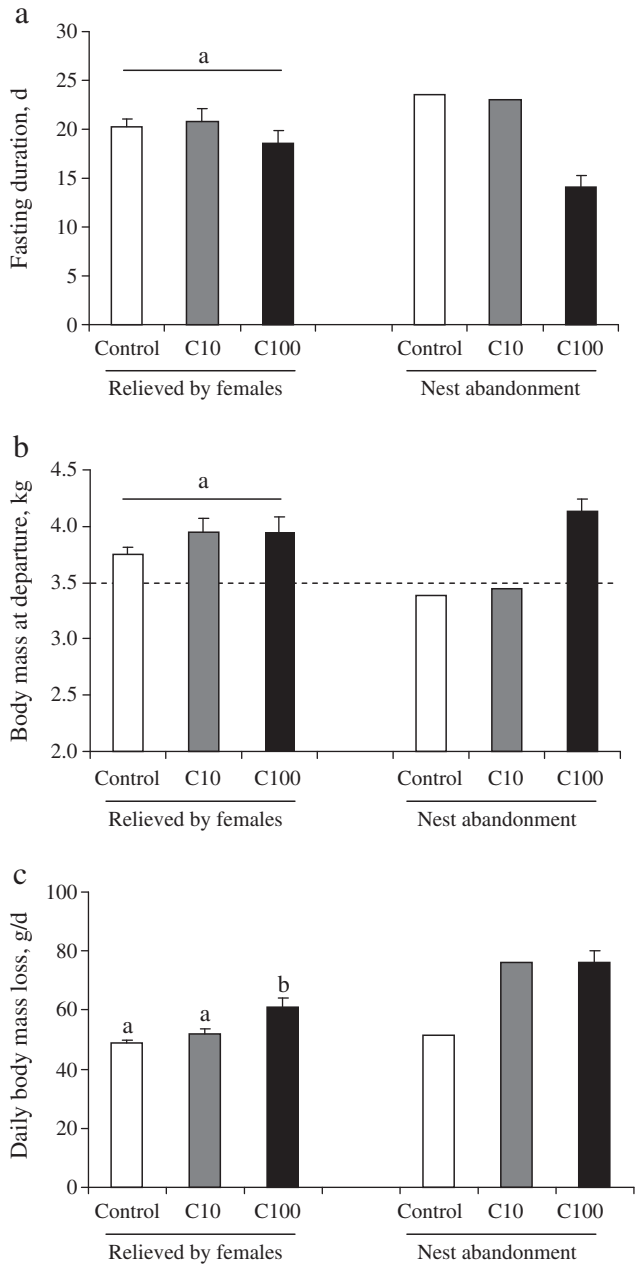

Fig. 2. Effect of CORT implants on fasting duration (a), body mass at the time of departure to sea (b), and daily body mass loss (c) in free-living male Adélie penguins, according to incubation success (relieved by females or nest abandonment). C10 and C100 represent penguins implanted with 10 and 100 mg of CORT, respectively. The dotted line (b) represents the body mass threshold below which control male Adélie penguins enter phase III of fasting and spontaneously abandon their nest (Cockrem et al., 2006; Spée et al., 2010). Results are means ± S.E. For all graphs, among birds relieved by female bars with different superscript letters are significantly different from each other. Statistical analyses were not conducted in control and C10 abandoning birds because of their small sample size.

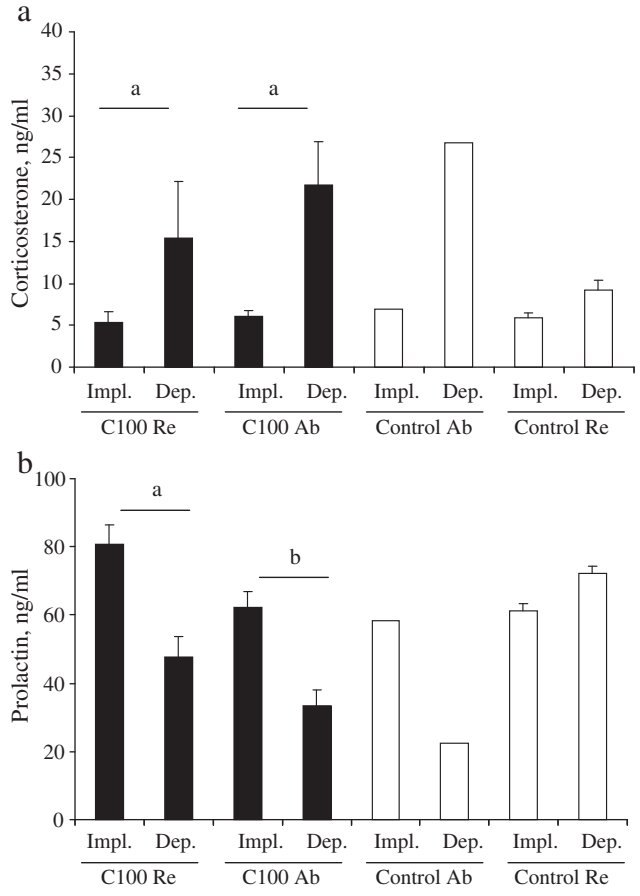

Fig. 3. Effect of CORT implants on corticosterone (a) and prolactin (b) levels in free-living male Adélie penguins. Impl., the time of implantation; Dep., the time of departure to sea (after relieved by females or after nest abandonment); C100 Re, birds implanted with 100 mg of CORT that were relieved by females; C100 Ab, birds implanted with 100 mg of CORT that abandoned their nest; Control Ab, control penguins that abandoned their nest; Control Re, control penguins that were relieved by females. Results are means ± S.E. Among birds relieved by females different superscript letters indicate significant difference between groups. Control birds were not included in the statistical analyses because of the small sample size of abandoning penguins.

prolactin levels than abandoning C100-penguins (C100 Ab). Moreover, prolactin decline between the initial and final sampling stage was similar in both treated-groups, as indicated by a lack of interaction between the incubation success and the sampling period with respect to the effect on plasma prolactin levels ($F_{1, 12} = 0.20$, $p = 0.66$).

Foraging trip duration in control and C100-penguins that were relieved by partners

Duration of the first foraging trip in males, which takes place during the incubation stage, was affected by the treatment ($t = 2.32$, $df = 30$, $p = 0.03$; Fig. 4a). C100-penguins spent 13% less time at sea, when compared with control birds. Subsequent foraging trip duration was also affected by the treatment (Wald $\chi^2 = 29.7$, $df = 1$, $p < 0.001$), trip number (Wald $\chi^2 = 295$, $df = 5$, $p < 0.001$), and their interaction (Wald $\chi^2 = 249$, $df = 5$, $p < 0.001$; Fig. 4a). C100-penguins spent 75% more time at sea during their second foraging trip, when compared with control birds ($p < 0.001$). However, during subsequent foraging trips there were no significant differences between groups.

Treatment of male penguins had no effect on foraging trip duration of their female partners, neither during incubation ($U = 50$, $p = 0.36$; Fig. 4b), nor during the chick-rearing period (Wald $\chi^2 = 1.71$, $df = 1$, $p = 0.19$; Fig. 4b), indicating that females did not compensate.

Discussion

In our study, exogenous CORT (high dose, C100) induced an important proportion of egg abandonment in free-living Adélie penguins, which was paralleled by a decline in prolactin levels. We suggest that CORT alone is not sufficient to trigger nest abandonment and that 1) prolactin levels may need to reach a low threshold value and 2) an increase in proteolysis should occur to stimulate nest desertion.

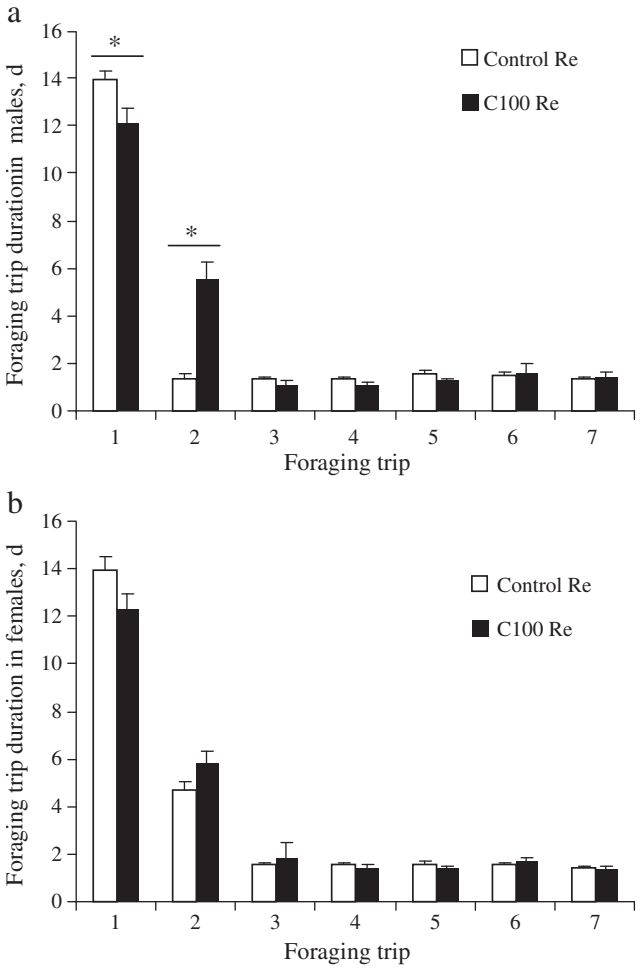

Fig. 4. Effect of CORT implants on foraging trip duration (in days) in free-living male Adélie penguins relieved by females (a) and in their female partners (b). C100 represents males implanted with 100 mg of CORT. Results are means ± S.E. Asterisks indicate significant differences between groups for a given foraging trip.

CORT treatment and egg abandonment

Our experimentally-induced rise in CORT levels induced a significant rate of nest abandonment in the group treated with the high dose of CORT (C100). Nest abandonment was 60%, while it was around 7% in control and C10-penguins. In contrast to C100-penguins, birds in these two other groups had body masses at the time of abandonment that were lower than the critical body mass threshold which typically characterizes the entrance into PIII in male Adélie penguins (Cockrem et al., 2006; Spée et al., 2011). Moreover, the time that the C10-penguin spent fasting before deserting their nest was similar to that of abandoning control birds. Thus, the most likely explanation for the only abandoned nest in C10-penguins that we observed is reach of a low threshold in body reserves rather than a direct response to low dose of CORT.

In species where incubation is shared by both parents, the time one parent spent foraging may constrain the time the partner spent fasting (Tveraa et al., 1997). In this direction, the reach of a critical body mass leading to nest abandonment can result from delayed relief by partners (Davis, 1982; Watanuki, 1993). The male's ability to sustain a long-term fast is also affected by their body mass at the beginning of the fast. For instance, penguins arriving in the colony with a low body mass due to adverse environmental conditions reached the critical levels for nest abandonment at an earlier date than usual (Cockrem et al., 2006). In the present study, whether abandoning males had late-returning females or whether they had low body mass at the beginning of the incubation shift is difficult to assess because of the small number of deserting birds.

We found that C100-penguins abandoned their nest ~14 days (range: from 9 to 19 days) after the start of the treatment, while control penguins deserted ~23 days after manipulation. The fact that the effect of CORT takes a long time to develop suggests that the effect of CORT on the triggering of nest abandonment is indirect and probably depends on the sensitivity of individuals to CORT (see below). The increase in body mass loss observed in C100-birds could have contributed to their shorter stay on the nest compared to control penguins. This could partly reflect a shift in the substrate used to support cellular metabolism (i.e. a switch from lipid utilization to protein catabolism) and notably the relative contribution of lipid and protein to energy expenditure. Indeed, the increase in body mass loss observed in late fasting birds was paralleled to the use of protein as main energy substrate (Cherel et al., 1988; Robin et al., 1998). We found here an increase in plasma uric acid levels (reflecting protein catabolism) in C100-penguins that abandoned their nest. This metabolic change may thus have contributed to prompt penguins to go to sea to refeed.

The progressive effect of CORT on prolactin levels (Angelier et al., 2009) might also explain the lag time before nest desertion. In fact, contrary to the response of CORT and prolactin to acute stress, there is evidence that responses of these two hormones to long-term stressors such as prolonged energy constraints or experimental administration of CORT are linked (Criscuolo et al., 2005; Angelier et al., 2009). However, the inhibitory action of CORT on plasma prolactin seems to be complex, since prolactin levels decrease only slowly and progressively in response to an increase in CORT levels (Angelier et al., 2009). Further experimental studies are needed to determine how CORT and prolactin are functionally linked during fasting, the factors involved in these changes, and how these two hormones interact to affect parental attentiveness.

Why some treated birds did not abandon their nest

Egg abandonment occurs when CORT secretion is strongly stimulated (Groscolas et al., 2008; Spée et al., 2010). We found that all C100-treated birds had similar CORT levels while their behavioral response differed. There are many downstream factors of CORT secretion, which can affect the behavioral and physiological outcome of a CORT increase. For instance, the binding of CORT to corticosteroid binding globulin (CBG) may regulate the action of CORT by altering its amount reaching target tissues (Breuner and Orchinik, 2002). According to the free hormone hypothesis, the unbound hormone is the biologically active fraction, able to enter cells and activate receptors. In this framework, the primary role of CBG would be to regulate the bioavailability and clearance rate of CORT (Ekins, 1990). It has been reported that free baseline CORT levels of nest-abandoning female European starlings *Sturnus vulgaris* are significantly higher than those of non-abandoning birds (Love et al., 2004). In this context, one could hypothesize that C100-penguins that did not abandon their nest have a higher CBG capacity, which would make them less sensitive to exogenous CORT. Furthermore, a number of studies in birds highlighted a deactivation of the hypothalamic–pituitary–adrenal axis after CORT administration, which leads to a down-regulation of the endogenous CORT secretion (Busch et al., 2008; Müller et al., 2009). However, because the exogenous dose of CORT we implanted was very high in comparison to endogenous baseline levels of CORT in penguins, such down-regulation might not explain the selective behavioral response we observed.

C100-birds that abandoned their nest had significantly higher uric acid levels at departure to sea than at the point of implantation, while this was not the case for C100-penguins that were relieved by their partner. This difference was not due to sampling date since among relieved and abandoning birds, samples can have been collected at the

same period. Also, uric acid concentrations do not peak then decline but rather continue to increase as body reserves deteriorates (Spée et al., 2011). Moreover, we have to be cautious about the non-significant results for relieved birds, regarding the low sample size ($n = 6$) and the p value ($p = 0.08$). Thus, we are only able to suggest that proteolysis could be an important factor in triggering refeeding behavior, as already proposed (Criscuolo et al., 2005). Moreover, proteolytic systems are coordinately up regulated in the skeletal muscles of rodents during late fasting when plasma levels of CORT are high (Bertile et al., 2003). This supports the view that effect of CORT on the triggering of nest abandonment is partly related to its catabolic peripheral action on protein metabolism.

Interestingly, the other main difference between C100-birds relieved by their partner (C100 Re) and abandoning C100-penguins (C100 Ab) was their prolactin profile. The magnitude of the decrease in prolactin levels between the time of implantation and departure to sea was similar in both groups (41% and 46% for C100-birds relieved by females and C100-penguins that abandoned their nest, respectively), but they had different initial (at the time of implantation) and final prolactin concentrations (when relieved by their partner or when they abandoned their nest). Prolactin levels were significantly higher in C100-birds relieved by their partner when compared with abandoning C100-penguins. If we consider the rate of decrease in prolactin levels between the two groups (expressed as the percentage of prolactin decline per day), we found that prolactin levels decreased more rapidly in abandoning birds, when compared with C100-birds relieved by their partners (3.5% and 1.8% per day, respectively; Fig. 5). Angelier and Chastel (2009) proposed that the rate of decrease in prolactin levels in response to a standardized stress protocol (capture followed by 30 min restraint) is directly related to the motivation of the individual to maintain parental care. The same hypothesis could apply to the present results.

The differences in incubation behavior among C100-treated birds emphasize the need of prolactin levels to reach a minimum threshold value before nest desertion is triggered. The profile of C10-birds support our hypothesis of a threshold level of prolactin under which nest desertion is triggered since 1) C10-penguins that did not abandoned their nest (relieved by females) had still high prolactin levels (as control birds relieved by females), and 2) the C10-bird that abandoned its nest showed a marked decline in prolactin levels: from 63.8 to 25.1 ng/ml. There is growing evidence in the literature that the relationship between parental behavior and prolactin levels is non-linear and depends on a threshold value of prolactin levels (Angelier and Chastel, 2009). It has been proposed that after reaching a minimum threshold level of prolactin, post-hatching parental care can no longer be provided or maintained (Boos et al., 2007; Criscuolo et al., 2002). One would also expect that the decline in prolactin levels could be a consequence of the nest abandonment. Indeed, whether the decline in prolactin levels precedes the decision to desert the nest or merely follows remains unclear. However, a study conducted in meerkats *Suricata suricatta* supports the former course of events as high prolactin levels preceded the decision to engage in parental care (Carlson et al., 2006). Whether meerkats and penguins are distant in a taxonomic point of view, this study offers insight into the time-course of events linking hormones and behavior. In this direction, Angelier and colleagues (2009) showed in black-legged kittiwakes that an experimental increase in CORT levels was accompanied by a decrease in prolactin level and subsequently by a reduction of nest attendance. To clearly disentangle the two hypothesis (either prolactin is a cause or a consequence of nest abandonment), it would be interesting to determine whether high levels of CORT would cause nest abandonment in birds whose levels of prolactin are maintained by exogenous hormones administration.

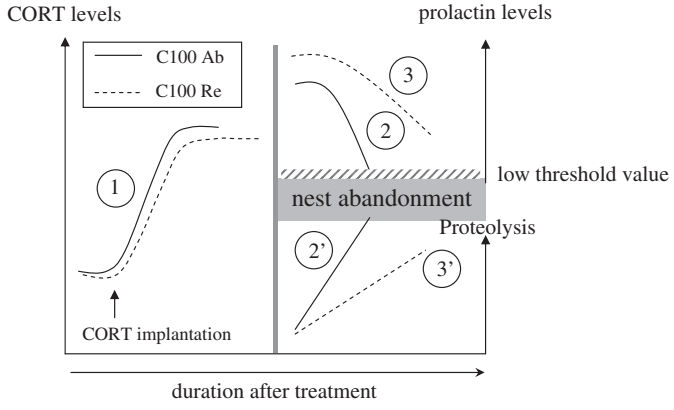

Fig. 5. Schematic representation of how exogenous CORT acts on prolactin levels and proteolysis, leading to nest abandonment (or not) in free-living male Adélie penguins. (1) The implantation of CORT induced a similar rise in CORT levels in both groups (C100 Ab, birds implanted with 100 mg of CORT that deserted their nest ~14 days after the start of the treatment, solid line; and C100 Re, birds implanted with 100 mg of CORT that were relieved by females ~19 days after the start of the treatment; dotted line). (2) Initial prolactin levels were lower in treated birds that abandoned their nest when compared with birds relieved by females. The rate of decline in prolactin levels in response to the CORT treatment was also greater in the former group, so that their prolactin levels reached a low threshold value, triggering egg abandonment. (3) By contrast, prolactin levels in treated birds relieved by females declined at a slower rate and did not reach the low threshold value until males were successfully relieved by females. Moreover, C100-birds that abandoned their nest (2′) had significantly higher uric acid levels at departure to sea than at the point of implantation, while this was not the case for C100-penguins that were relieved by their partner (3′). An increase in proteolysis could thus be an important factor in triggering refeeding behavior.

Why did initial prolactin levels differ between birds?

In penguins, prolactin levels increase gradually throughout courtship and peak during the middle of the incubation phase (Vleck et al., 1999). We could simply assume that C100-penguins that were relieved by females had higher prolactin levels at the time of implantation because they were sampled at an earlier point of their breeding cycle. However, this was not the case as all birds were sampled at the same point in time and their laying dates were similar, indicating that reproductive cycles were synchronized.

In addition, there is evidence that breeding experience and age can affect baseline prolactin levels. Most often, inexperienced parents (Angelier et al., 2007b; Angelier et al., 2006; Christensen and Vleck, 2008) or young birds (Angelier et al., 2007b; Deviche et al., 2000; Preault et al., 2005) have lower prolactin levels than experienced or older breeders. Future studies carried out with birds either of known age or for which information about the biological age is measurable (by determining telomere length for example; Monaghan and Haussmann, 2006) will allow to test the hypothesis that C100-penguins relieved by females were older breeders, while C100-birds that abandoned their nest belonged to inexperienced parents.

Effect of treatment on foraging trip duration and reproductive success

C100-penguins that were relieved by females spent less time at sea during their first foraging trip when compared with control birds. They also had high CORT levels when they left the colony to undertake their first foraging trip, at which point they were still within the delivery period of the implant. It has been reported earlier that Adélie penguins with high CORT levels before a foraging trip spend less time at sea and engage in a greater foraging effort, while foraging success is lower (Angelier et al., 2008). We can thus assume that treated birds were not as efficient as usual during their first foraging trip and needed to replenish their body reserves when undertaking their second foraging trip. Accordingly, the second foraging trip in these birds was longer than in control birds. Foraging trip duration of the partner of CORT treated males was not different from that of partners of control birds. In accordance with our result, Beaulieu and colleagues found that females of handicapped male Adélie penguins

(i.e. equipped with a large dummy device which increases the cost of foraging leading penguins to spend more time to forage at sea than control birds) did not change their foraging trip duration (Beaulieu et al., 2010b). However, whether CORT affects foraging performance (e.g. diving behavior or spatial distribution) or whether it influences parental provisioning rates requires further examination. Another explanation for the shorter first foraging trip may involve the lower prolactin levels of departing C100-penguins, since prolactin has been shown to be involved in stimulating feeding and parental provisioning of young in some avian species (Buntin et al., 1999; Koch et al., 2004).

We found that reproductive success (as determined by the number of chicks that survived until they reached peak body mass) and brood mass were similar in control and C100-birds relieved by their partners. Accordingly, this suggests that their adjustment in foraging behavior was sufficient to ensure self-maintenance and survival of their chicks.

Conclusions and perspectives

This study illustrates the involvement of CORT in the decision-making process regarding egg abandonment in penguins, when incubation is associated with a natural long fast. However, we suggest that CORT alone is not sufficient to trigger nest abandonment but that 1) prolactin levels need to reach a low threshold value and 2) a rise in proteolysis (i.e. utilization of protein as main energy substrate) seems also to be required (Fig. 5). Interestingly, C100-penguins that were relieved by females had higher initial and final prolactin levels than abandoning C100-birds. This suggests that high prolactin levels prior to the incubation fast prompt penguins to maintain parental care in stressful situations, such as induced by prolonged energy constraints. We suggest that penguins with higher initial prolactin levels could be "better parents" (see Preault et al., 2005). The finding that reproductive success in these penguins was similar to that of control birds (similar number of chicks that survived until they reached their peak body mass) is consistent with this suggestion but this remains to be tested experimentally in further studies.

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