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Exogenous corticosterone mimics a late fasting stage in captive Adélie penguins (*Pygoscelis adeliae*)

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Spée M, Marchal L, Thierry A, Chastel O, Enstipp M, Le Maho Y, Beaulieu M, Raclot T. Exogenous corticosterone mimics a late fasting stage in captive Adélie penguins (*Pygoscelis adeliae*). *Am J Physiol Regul Integr Comp Physiol* 300: R1241–R1249, 2011. First published February 23, 2011; doi:10.1152/ajpregu.00762.2010.— Fasting is part of penguin's breeding constraints. During prolonged fasting, three metabolic phases occur successively. Below a threshold in body reserves, birds enter phase III (PIII), which is characterized by hormonal and metabolic shifts. These changes are concomitant with egg abandonment in the wild and increased locomotor activity in captivity. Because corticosterone (CORT) enhances foraging activity, we investigated the variations of endogenous CORT, and the effects of exogenous CORT on the behavioral, hormonal, and metabolic responses of failed breeder Adélie penguins. Untreated and treated captive male birds were regularly weighed and sampled for blood while fasting, and locomotor activity was recorded daily. Treated birds were implanted with various doses of CORT during phase II. Untreated penguins entering PIII had increased CORT (3.5-fold) and uric acid (4-fold; reflecting protein catabolism) levels, concomitantly with a rise in locomotor activity (2-fold), while prolactin (involved in parental care in birds) levels declined by 33%. In CORT-treated birds, an inverted-U relationship was obtained between CORT levels and locomotor activity. The greatest increase in locomotor activity was observed in birds implanted with a high dose of CORT (C100), locomotor activity showing a 2.5-fold increase, 4 days after implantation to a level similar to that of birds in PIII. Moreover, uric acid levels increased three-fold in C100-birds, while prolactin levels declined by 30%. The experimentally induced rise in CORT levels mimicked metabolic, hormonal, and behavioral changes, characterizing late fasting, thus supporting a role for this hormone in the enhanced drive for refeeding occurring in long-term fasting birds.

locomotor activity; long-lived seabird; long-term fasting; glucocorticoid

DURING THEIR LIFE CYCLE, SEVERAL animal species alternate periods of fasting and feeding. For instance, long fasting periods are common in seabird species, especially penguins, since they feed exclusively at sea, while they have to spend time ashore to breed and molt (36). This natural situation of spontaneous prolonged fasting can lead to energy reserve exhaustion and, consequently, to the abandonment of current reproduction.

Three distinct metabolic phases have been described during prolonged fasting in birds as well as in rodents, based on patterns of daily body mass loss and substrates used to support cellular metabolism (3, 11, 12, 16, 29). Phase I (PI) is a brief period of adaptation in which catabolism of fat stores increases, while phase II (PII) is a long period of economy characterized by a constant rate of body mass loss and by protein sparing. The latter is indicated by low levels of uric acid, the production of which is the result of protein catabolism (12, 29). At this stage of fasting, plasma levels of β-hydroxybutyrate (βOHB) are elevated, indicating that lipids are the main energy substrate (12, 29). If a minimum body reserve threshold is reached, animals enter phase III (PIII). At this stage, hormonal and metabolic shifts occur, which are characteristic for a switch from lipid to protein utilization. In addition to these physiological adjustments, marked behavioral changes have been reported for birds entering PIII. For example, incubating king penguins *Aptenodytes patagonicus* (17) and Adélie penguins (31) were observed to abandon their egg(s), while nonbreeding fasting emperor penguins *Aptenodytes forsteri* increased locomotor activity (29). These behavioral changes, which are the visible manifestations of endogenous metabolic and endocrine shifts, have been interpreted to reflect an increase in the drive to refeed (17, 19, 24).

There are different ideas about how such increased drive for refeeding is mechanistically triggered. At the central levels, the expression of several hypothalamic neuropeptides that are involved in the control of feeding behavior has been reported for rodents during periods of prolonged fasting (5). It was found that the hypothalamic response to long-term fasting (in PIII) is mediated by the orexigenic system, rather than by the anorexigenic system. The regulation of neuropeptide Y (NPY) and agouti-related peptide (AGRP) expression seems to be primarily involved in the response to prolonged fasting and could mediate the late enhanced drive for refeeding. Indeed, it has been shown that the hypothalamic mRNA expression of both NPY and AGRP sharply increased in rats in PIII compared with PII (5). This supports the view that the central effects of long-term fasting are mediated mainly by neuropeptides synthesized in the arcuate nucleus of the hypothalamus (5). Nevertheless, the nature and exact mechanisms of this enhanced drive for refeeding, which is associated with the late fasting stage, remain partly unknown at the peripheral level. Hormones are known to play an important role in the control of feeding behavior, and, in penguins, they might act as mediators between metabolic shifts and behavioral changes (19). It can, therefore, be validly proposed that this enhanced drive for refeeding is triggered by the hormonal adjustments that characterize the entrance into PIII.

In birds, parental behavior is controlled by two hormones with opposite effects: *1*) prolactin, the major hormone that stimulates incubation and parental care (8, 39), and *2*) cortico-

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sterone (CORT), the main avian glucocorticoid, which plays a role in a wide variety of physiological and behavioral processes (25). A rise in plasma levels of CORT was linked to the entrance into PIII in king and emperor penguins (13, 29) and occurs concomitantly with an increase in locomotor activity (29).

Foraging activity was shown to increase in response to CORT treatment in several species of mammals (9) and birds (2, 7, 23). Experimental studies suggest that CORT is involved in the initiation of food intake during short fasting periods. For example, CORT was shown to stimulate locomotor activity and food searching in white-crowned sparrows, *Zonotrichia leucophrys* (2), while it enhances foraging in breeding black-legged kittiwakes, *Rissa tridactyla,* at the mid-chick-rearing stage (23). Hence, it is tempting to propose that CORT initiates foraging behavior during late fasting, once critical reserve exhaustion is reached. It would thereby redirect bird behavior from a costly activity (e.g., reproduction) to a behavior promoting survival (e.g., departure to refeed) (38). However, implantation of exogenous CORT in female common eiders, *Somateria mollissima,* did not lead to nest abandonment (15). This absence of behavioral changes could be due to *1*) a short treatment duration, since CORT concentrations returned to basal levels within 4 days of implantation and/or *2*) a too high dose of CORT, which was possibly not biologically relevant.

In the present study, we investigated the role of CORT in the induction of locomotor activity reflecting an increase in the drive to refeed and the associated metabolic and hormonal changes that occur during late fasting in Adélie penguins. We mainly focused on male penguins because they undertake the longest fasting period during courtship/incubation [up to 50 days (35)] and, thus, might be prone to reserve exhaustion that can lead to nest desertion. First, we validated the fasting phases of captive birds, by examining the relationship between uric acid levels and body mass of untreated birds and by defining the body mass threshold, below which they enter PIII. Plasma levels of metabolites are known to be reliable indicators of the nutritional state and fasting phases in birds (13, 20, 29). We used a correlative approach to examine the natural time-course changes of hormones (plasma levels of CORT and prolactin), metabolites (plasma levels of uric acid, βOHB, nonesterified fatty acid and glucose), and behavior (locomotor activity) in fasting captive birds. Second, we investigated whether exogenous CORT might induce *1*) a switch in the substrate used to support cellular metabolism (as revealed by plasma metabolites), *2*) a decrease in prolactin levels, and *3*) an increase in locomotor activity. Since the effect of CORT on locomotor activity depends on its concentration (7), we used various doses of CORT.

MATERIALS AND METHODS

Study Area and Birds

The study was conducted in Dumont d'Urville Station (66°40'S, 140°01′E), Adélie Land, Antarctica, during three austral summers (2004–2005, 2006–2007, and 2007–2008). The captive birds in the current study were failed breeders, i.e., they started a reproductive cycle but discontinued incubation prematurely. This was unrelated to parental body condition but explained by extrinsic factors, such as bad weather and flooding. The protocol performed on Adélie penguins received the approval of the Ethics Committee of the French Polar Institute Paul Emile Victor, and authorizations were given by the Terres Australes et Antarctiques Françaises.

Study 1: correlative approach. During the austral summer of 2004–2005, a total of 12 male and 5 female Adélie penguins were captured over the course of the breeding season. They were kept in a pen (5.2 m × 2.4 m), where they fasted until their body mass fell below the minimum body mass threshold that marks the entrance into PIII in male Adélie penguins, i.e., ~ 3.5 kg (14). The number of birds present at any time in the pen never exceeded eight individuals. After 1 or 2 days of captivity, penguins were equipped with two pedometers each (Dista F100, Decathlon, France) to record their locomotor activity. Pedometers were coated with mastic to waterproof them and attached to the feathers at hip level (one to the left and one to the right) using cyanoacrylate glue (Loctite). Preliminary analysis showed that the two pedometers deployed with each bird gave comparable readings. This enabled us to use recordings from one instrument only, if the other was lost. Locomotor activity was measured daily. Birds were weighed every other day using an electronic balance (Ohaus; ± 2 g), and blood samples were taken frequently during the fasting period (between 3 and 10 times, depending on the initial body mass and daily body mass loss of individuals). Blood samples (~3 ml) were collected from the alar vein within 5 min of capture, a time recommended by Vleck et al. (34), to assess baseline CORT levels in Adélie penguins. No more than 2 or 3 birds were sampled per day to avoid biases in CORT levels. Samples were transferred in tubes pretreated with heparin or EDTA and centrifuged (5,000 rpm for 10 min at 4°C). Plasma was then collected and kept frozen in aliquots at −20°C until analyzed.

Study 2: experimental approach. During the austral summers of 2006–2007 and 2007–2008, a total of 28 males was captured and kept in a pen as described above. After a few days in captivity, they were implanted with CORT pellets of either 10 (n = 5; C10), 50 (n = 4; C50), 100 (n = 8; C100), or 200 mg (n = 4; C200). Birds were quickly anesthetized with isoflurane in pure O_2 administered via a hood placed over the head. Then, birds were implanted with CORT pellets of different doses. CORT pellets (21-day release, G-111) were obtained from Innovative Research of America (Sarasota, FL) and implanted in the nape of the neck. For this, a small patch of skin was disinfected with alcohol and betadine (iodine solution), and a small incision, equal to the size of the pellet, was made. The implant was inserted subcutaneously, and the incision was closed with one or two stitches, cleaned with betadine, and, finally, sprayed with aluminum powder. Control penguins (n = 7) underwent the same protocol, but either no pellet was inserted or a placebo pellet was used. The overall procedure took less than 5 min. CORT-implanted birds with different doses were held together with the total number of birds never exceeding eight individuals. All birds were weighed every other day, and their locomotor activity was recorded daily as described above. Blood samples (~3 ml) were taken as follows: treated penguins were sampled at the time of implantation (*day 0*; before implantation), 3 days after implantation (*day 3*), and on the last day that they spent inside the pen (*days 7–11*, depending on the initial body mass and daily body mass loss of individuals). Control penguins were sampled at *day 0* and when they entered PIII.

All penguins from *studies 1* and *2* were released on the edge of the breeding colony (and thus close to the sea, which is free of sea ice at this period of the year) at the end of the experiments.

Plasma Analysis: Metabolites and Hormones

Concentrations of uric acid, βOHB, nonesterified fatty acid (NEFA), and glucose were measured by the enzymatic colorimetric method using commercial kits (uric acid: Sigma Diagnostics, St. Louis, MO; βOHB, NEFA, glucose: Randox Laboratories, Crumlin, UK). The determination was performed on undiluted plasma (uric acid: 25 μl; βOHB: 20 μl; NEFA: 12.5 μl; glucose: 10 μl).

CORT concentrations were determined by a quantitative competitive sandwich enzyme immunoassay technique, according to guide-

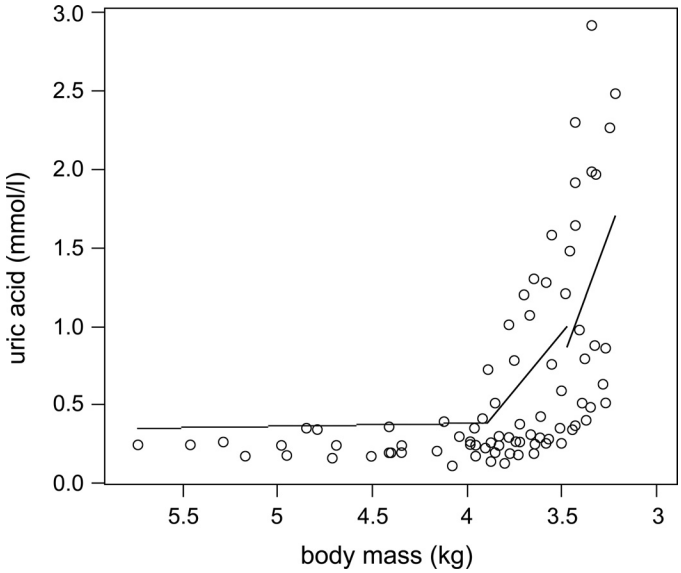

Fig. 1. Plasma levels of uric acid in relation to body mass changes in captive male Adélie penguins (untreated birds; $n = 12$). The lines within the figure were determined using one dynamic segmented regression analysis for all untreated male birds. Two breakpoints were detected. This allowed us to distinguish three stages during the fasting period and also to identify the corresponding body mass.

lines provided by the manufacturer (AssayMax corticosterone ELISA kit, EC3001-1; AssayPro, St. Charles, MO). Plasma concentrations of prolactin were determined by a heterologous radioimmunoassay at the Centre d'Etude Biologiques de Chizé (Villiers en Bois, France). Pooled plasma samples of Adélie penguins produced a dose-response curve that paralleled chicken prolactin standard curves (bAFP 4444BQ, source: Dr. Parlow, National Hormone and Peptide Program Harbor-UCLA Medical Center, Torrance, CA). Intra-assay and inter-assay coefficients of variation for CORT were 5% and 7%, respectively. The corresponding values for prolactin were 6% and 9%, respectively.

Determination of Fasting Phases and Statistical Analysis

Plasma levels of uric acid are a reliable indicator of fasting phases in birds (13, 20, 29). The entrance into late fasting (PIII) results from reaching a threshold of low body reserves and is not controlled by fasting duration (29). In the current study, we used one dynamic segmented regression analysis [segmented package from R; (27)] for all untreated male birds to identify breakpoints in the relationship between uric acid levels and body mass (*study 1*). This analysis is a regression model in which the relationships between body mass and uric acid levels are piecewise linear, namely represented by straight lines connected at values called breakpoints. Using this method, we were able to distinguish three stages during the fasting period (PII; PII-PIII, which corresponds to a transition from PII to PIII; and PIII) and also to identify the corresponding body mass (Fig. 1). These three stages were taken as reference points for the subsequent analysis. Because captive penguins are failed breeders, they were already in a fasting state when they were captured and kept in the pen. This is the reason why the *phase I* of fasting cannot be described in these birds. We also performed a dynamic regression analysis for untreated female birds (*study 1*). However, because of the small sample size, we were able to identify only two fasting stages (PII and PIII).

Overall, our data were analyzed by the construction of general linear mixed models [GLMM; (21)], allowing for the control of pseudoreplication by the inclusion of a random factor (i.e., individuals identity). Comparisons of body mass and plasma parameters in relation to fasting stages in untreated males and females (*study 1*) were analyzed with a GLMM. We included the sampling stage (birds were sampled for blood between 3 and 10 times, depending on individuals) as repeated factor, and fasting stages (PII, PII-PIII, PIII for males and PII, PIII for females) as a fixed factor. Normality of residuals was assessed using a Shapiro-Wilk test. When normality was not met and the distribution of data was skewed to the right, a generalized estimating equation (GEE) with a gamma distribution was used. The relationships between locomotor activity and plasma levels of CORT and prolactin were tested using Spearman's rank correlation.

In treated birds (*study 2*), the effects of CORT implants on body mass, plasma metabolites, and locomotor activity were analyzed using GLMM or GEE, when normality was not met. We included "treatment," "sampling stage," and their interaction as a fixed factor, with "sampling stage" being a repeated measure. The year during which the experiment was conducted was also added as a fixed factor. The percentage decrease in prolactin levels in response to prolonged fasting in control birds (between PII and PIII) and in CORT-implanted birds (between the time of implantation and *days 7–11*) was compared using a one-way ANOVA. In C100 penguins, the effect of CORT implants on prolactin levels was analyzed using a GLMM with "treatment," "day relative to implantation" (repeated measure), and their interaction as a fixed factor. The relationship between locomotor activity and prolactin in C100 birds was tested using Spearman's rank correlation.

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

RESULTS

Study 1: Correlative Approach

Validation of fasting phases in untreated male Adélie penguins: body mass, body mass loss, plasma parameters, and locomotor activity. Twelve untreated male Adélie penguins, weighing 4.65 ± 0.16 kg, were captured and kept in a pen. They entered into PII-PIII and PIII at a mean body mass of 3.90 ± 0.16 kg and 3.47 ± 0.15 kg, respectively (dynamic regression of analysis). During the various fasting stages, their mean body mass was as follows: 4.16 ± 0.12 kg in PII, 3.77 ± 0.11 kg during the PII-PIII transition, and 3.39 ± 0.11 kg in PIII. Penguins spent on average 10.75 ± 2.25 days in PII, 4.25 ± 0.30 days in PII-PIII, and 2.92 ± 0.26 days in PIII. It is important to note that the duration of phase II reported here represents only one part of the whole PII period, knowing that penguins were already in this stage of fasting when they were captured (see above). The daily body mass loss depended on the nutritional state (Wald $\chi^2 = 57.1$, df = 2, $P < 0.001$; Table 1), with penguins in PII-PIII and PIII losing 25% and 71% more body

Table 1. *Profile of untreated captive male Adélie penguins according to fasting stage*

Nutritional state	PII	PII–PIII	PIII
Body mass loss, $g \cdot kg^{-1} \cdot day^{-1}$	17.45 ± 0.52[a]	21.88 ± 0.77[b]	29.88 ± 1.56[c]
bOHB, mmol/l	1.29 ± 0.14[a]	1.10 ± 0.13[a,b]	0.85 ± 0.12[b]
NEFA, mmol/l	0.84 ± 0.09[a]	0.82 ± 0.08[a]	0.75 ± 0.06[a]
Glucose, mmol/l	12.24 ± 0.61[a]	12.53 ± 0.55[a]	13.10 ± 0.45[a]

Results are expressed as means ± SE ($n = 10–12$). PII, phase II of fasting; PII-PIII, transition from phase II to phase III of fasting; PIII, phase III of fasting; βOHB, β-hydroxybutyrate; NEFA, nonesterified fatty acids. Within each line, values that do not share the same superscript letter are significantly different ($P < 0.05$).

mass per kilogram per day, respectively, than penguins in PII ($P < 0.001$ for both). Moreover, nutritional state influenced plasma levels of βOHB ($F_{2,24} = 6.60$, $P = 0.005$; Table 1). Concentrations of βOHB were 34% lower during PIII, when compared with PII ($P = 0.01$). In contrast, plasma levels of NEFA ($F_{2,28} = 4.26$, $P = 0.27$; Table 1) and glucose ($F_{2,28} = 0.50$, $P = 0.61$; Table 1) did not differ significantly between the different nutritional states of male penguins.

In untreated males, the nutritional state affected CORT levels ($F_{2,15} = 8.63$, $P = 0.003$; Fig. 2*A*), prolactin concentrations (Wald $\chi^2 = 8.63$, df = 2, $P = 0.01$; Fig. 2*B*), and locomotor activity (Wald $\chi^2 = 36.4$, df = 2, $P < 0.001$; Fig. 2*C*). CORT concentrations and locomotor activity in PIII were 253% and 91% higher, respectively, than in PII ($P < 0.01$ for both). In contrast, prolactin concentrations were 33% lower in PIII, when compared with PII ($P = 0.03$). Locomotor activity was positively correlated with plasma CORT levels ($r_s = 0.69$, $P < 0.001$; Fig. 2*D*) and negatively correlated with prolactin concentrations ($r_s = -0.48$, $P = 0.01$; Fig. 2*E*).

Validation of fasting phases in untreated female Adélie penguins: body mass, plasma parameters, and locomotor activity. Untreated female Adélie penguins entered into PIII at a mean body mass of 3.24 ± 0.07 kg (dynamic regression of analysis). Their mean body mass was 3.80 ± 0.03 kg in PII and 3.11 ± 0.03 kg in PIII. The nutritional state of females affected plasma levels of CORT ($F_{1,3} = 19.7$, $P = 0.02$), βOHB ($F_{1,5} = 8.00$, $P = 0.03$), and prolactin (Wald $\chi^2 = 66.3$, df = 1, $P < 0.001$; Table 2). CORT levels were 130% higher in PIII than in PII, while plasma levels of βOHB and prolactin were 23% and 53% lower, respectively, in PIII when compared with PII. NEFA ($F_{1,6} = 2.39$, $P = 0.17$; Table 2) and glucose levels ($F_{1,8} = 0.004$, $P = 0.95$; Table 2) were not influenced by the nutritional state of birds. Locomotor activity was also affected by the fasting stage of

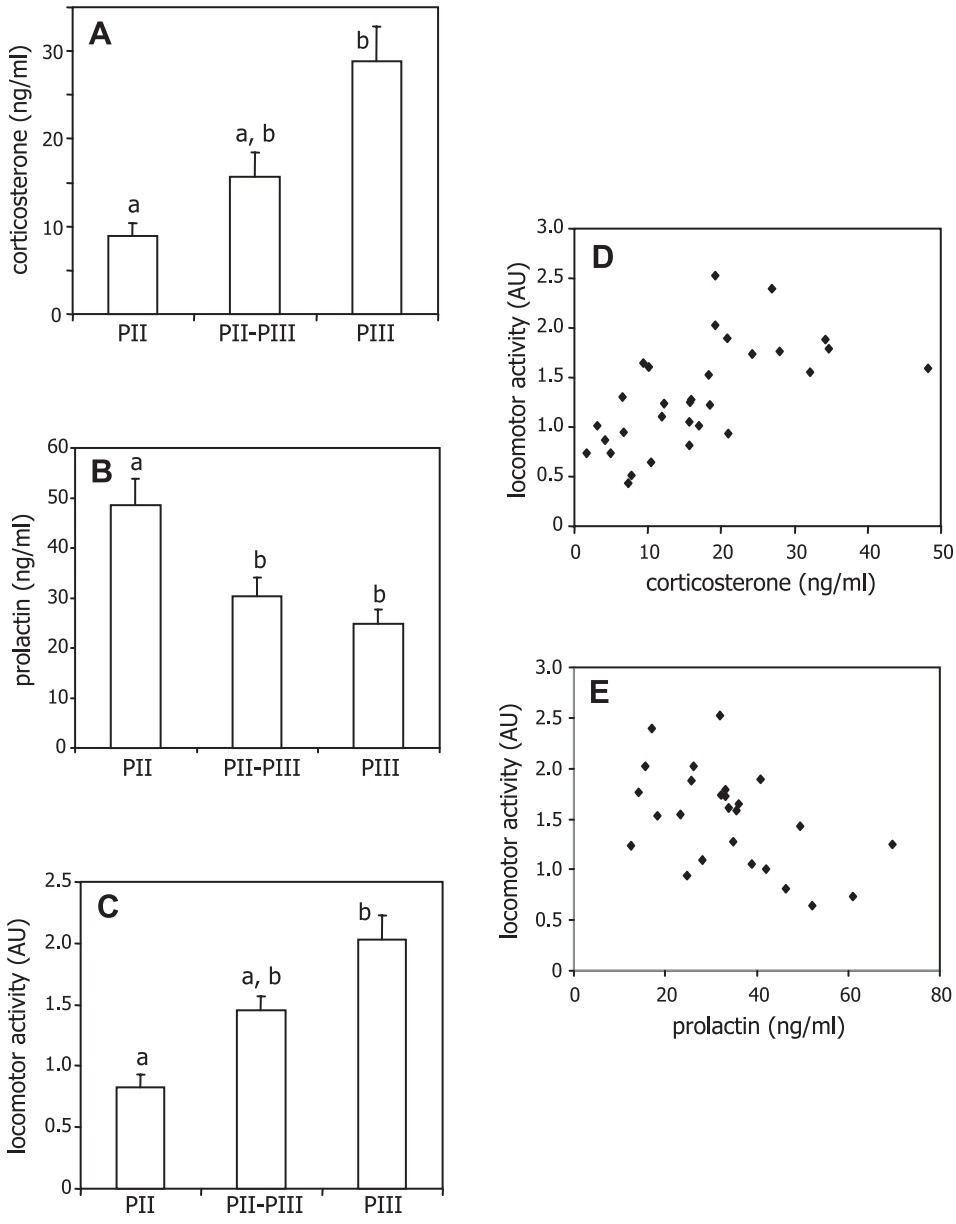

Fig. 2. Plasma levels of corticosterone (CORT; *A*; $n = 12$), prolactin (*B*; $n = 8$), and locomotor activity (*C*; $n = 12$) in relation to fasting stage in captive male Adélie penguins (untreated birds). Values are expressed as means ± SE. Bars not sharing the same superscript letter are significantly different ($P < 0.05$). Relationship between plasma levels of CORT and locomotor activity (*D*; $n = 12$) and between prolactin levels and locomotor activity (*E*; $n = 8$) in male captive Adélie penguins (untreated birds).

Table 2. *Profile of untreated captive female Adélie penguins according to fasting stage*

Nutritional state	PII	PIII
βOHB, mmol/l	1.11 ± 0.20[a]	0.86 ± 0.17[b]
NEFA, mmol/l	0.92 ± 0.08[a]	0.81 ± 0.09[a]
Glucose, mmol/l	12.36 ± 0.43[a]	12.75 ± 0.48[a]
CORT, ng/ml	11.74 ± 3.17[a]	27.01 ± 3.33[b]
Prolactin, ng/ml	56.49 ± 0.99[a]	26.53 ± 1.55[b]
Locomotor activity, AU	1.21 ± 0.03[a]	2.14 ± 0.14[b]

Results are expressed as means ± SE (n = 4–5). CORT, corticosterone; AU, arbitrary units. Within each line, values that do not share the same superscript letter are significantly different ($P < 0.05$).

females (Wald $\chi^2 = 21.0$, df = 1, $P < 0.001$; Table 2) and was 77% higher during PIII than during PII.

Study 2: Experimental Approach

Effects of CORT implants on CORT levels and locomotor activity. As expected, plasma levels of CORT were selectively affected by the CORT treatment (Wald $\chi^2 = 62.2$, df = 4, $P < 0.001$) and by the interaction treatment × day relative to implantation (Wald $\chi^2 = 137.2$, df = 7, $P < 0.001$; Fig. 3*A*). At the time of implantation (*day 0*), CORT concentrations in C10, C50, C100, and C200 penguins were similar to control birds in PII (all $P > 0.05$). In all CORT-implanted penguins, with the exception of C10 penguins ($P = 0.78$), plasma levels of CORT increased significantly between *day 0* and *day 3* (Fig. 3*A*). This increase was 2.9-fold for C50 penguins, 3.3-fold for C100 penguins, and 5.4-fold for C200 penguins (all $P < 0.001$). On *day 3*, CORT levels of C50 and C100 penguins were not different from those of control birds in PIII (P=1.00 and P=0.07, respectively). However, CORT levels of C200 birds on *day 3* were significantly higher than those of control penguins in PIII ($P < 0.001$). At the third sampling stage (*days 7–11*, depending on individuals), CORT levels in C50 and C100 penguins were not different from those on *day 3* ($P > 0.99$) and were similar to those of control birds in PIII ($P > 0.99$; Fig. 3*A*).

Locomotor activity was selectively affected by CORT treatment (Wald $\chi^2 = 21.0$, df = 4, $P < 0.001$; Fig. 3*B′*). In C10, C50, and C200 penguins, locomotor activity was similar to that of control birds in PII ($P > 0.99$ for all). In C100 birds, however, locomotor activity was significantly higher than that of control birds in PII ($P = 0.002$) and, in fact, similar to that of control birds in PIII ($P > 0.99$). Moreover, we found that locomotor activity was affected by the interaction

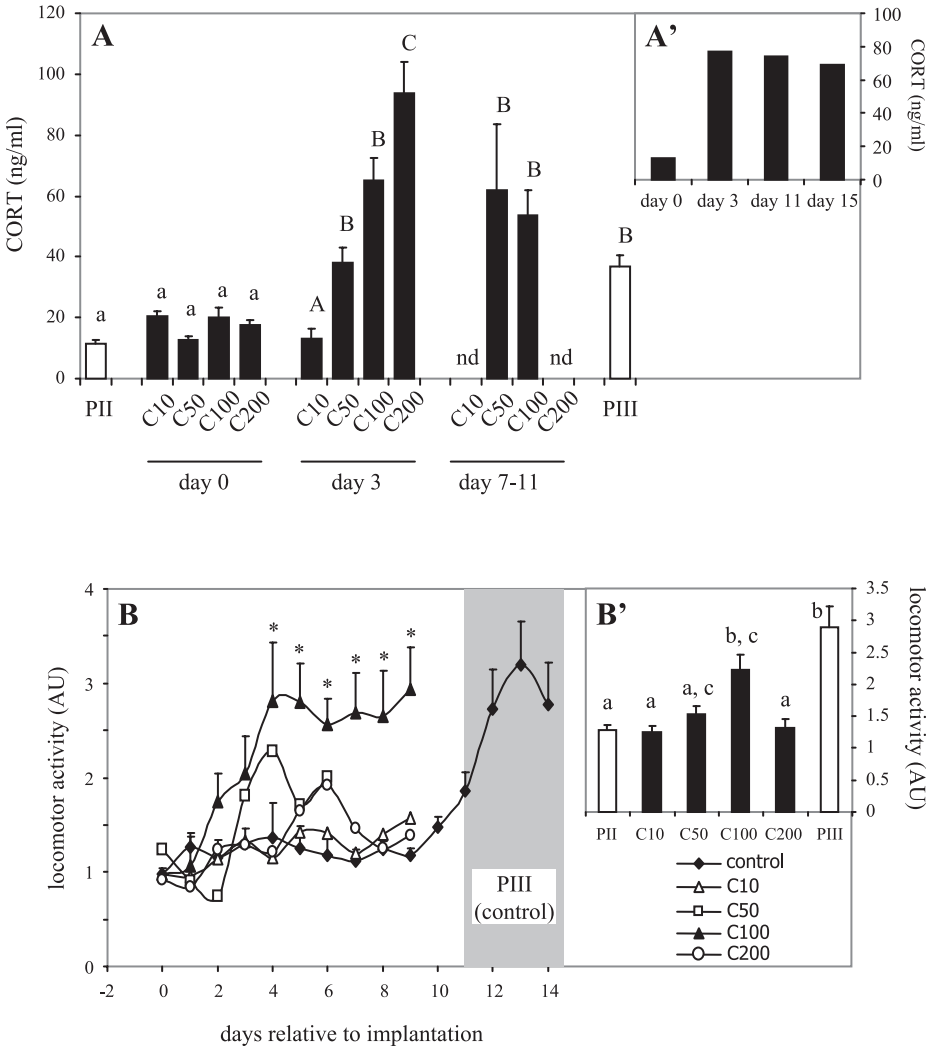

Fig. 3. *A*: plasma levels of CORT in control captive male Adélie penguins in phase II (PII) and phase III (PIII) of fasting (open bars, n = 7) and in CORT-implanted birds (solid bars, n = 4–8), in relation to sampling stage. CORT concentrations of CORT-implanted birds at *day 0* were compared with those of control birds in PII (see superscript letter "a"), while CORT levels at *day 3* and *days 7–11* were compared with those of control birds in PIII (see superscript letters "A,B,C"). nd, not determined. *Inset A′*: characteristic profile of CORT levels in one C100-treated bird weighing 5.8 kg and kept 17 days in the pen. *B*: changes in locomotor activity relative to fasting phase in control captive male Adélie penguins (n = 7) and relative to implantation day in CORT-treated penguins (n = 4–8). *$P < 0.05$ in C100 vs. control. *Inset B′*: effects of treatment on locomotor activity (mean value of locomotor activity over the entire treatment duration). Penguins were implanted with 10 (C10), 50 (C50), 100 (C100), and 200 (C200) mg of CORT. Values are expressed as means ± SE. Bars not sharing the same superscript letter are significantly different ($P < 0.05$).

treatment × day relative to implantation (Wald $\chi^2 = 6.836\times10^{12}$, df = 24, $P < 0.001$; Fig. 3*B*). In C100 penguins, locomotor activity reached a plateau at *day 4*, after which it remained stable. During this time, it was ~2.5 times higher than that of control penguins in PII ($P < 0.005$ for each given day) and was similar to that of control birds in PIII ($P > 0.05$).

Effects of CORT implants on body mass and plasma metabolites. Body mass was affected by the treatment (Wald $\chi^2 = 69.7$, df = 4, $P < 0.001$), the sampling stage (*day 0* and *day 3* for CORT-implanted birds, PII and PIII for control birds; Wald $\chi^2 = 201$, df = 1, $P < 0.001$), and their interaction (Wald $\chi^2 = 51.4$, df = 4, $P < 0.001$; Table 3). At the time of implantation, the body mass of control birds in PII was similar to that of C10 ($P > 0.99$), C50 ($P > 0.99$), and C200-birds ($P = 0.32$). However, body mass of C100 penguins was significantly higher at this point ($P < 0.001$). At the subsequent sampling stage, all CORT-implanted birds had a significantly higher body mass than control birds in PIII ($P < 0.001$ for each comparison). Similarly, uric acid levels were affected by the treatment (Wald $\chi^2 = 54.4$, df = 4, $P < 0.001$), the sampling stage (Wald $\chi^2 = 70.3$, df = 1, $P < 0.001$) and their interaction (Wald $\chi^2 = 61.0$, df = 4, $P < 0.001$; Table 3). At the time of implantation, uric acid levels of all CORT-implanted birds (C10, C50, C100, and C200 penguins) were similar to those of control birds in PII ($P < 0.05$ for each comparison). Three days after implantation, plasma uric acid levels remained unchanged in C10 penguins ($P > 0.99$). However, in all other CORT-implanted penguins, uric acid concentrations were significantly increased at this point, when compared with *day 0* (C50: 2.2-fold, $P = 0.01$; C100: 2.8-fold, $P < 0.001$; C200: 2.4-fold, $P < 0.001$). At the subsequent sampling stage (i.e., *day 3*), only C100 birds had uric acid levels similar to those of control birds in PIII ($P > 0.99$). Furthermore, the treatment ($F_{4,41} = 2.79$, $P = 0.04$) and the sampling stage ($F_{1,41} = 16.0$, $P < 0.001$) also affected βOHB levels, but their interaction was not significant ($F_{4,41} = 0.64$, $P = 0.63$; Table 3). βOHB concentrations between C10 and C100 penguins differed significantly ($P = 0.04$). Plasma levels of NEFA were affected by the treatment ($F_{4,21} = 6.03$, $P = 0.002$), so that C10 penguins had higher NEFA concentrations than control, C50, and C100 penguins. In addition, glucose levels were also affected by the treatment ($F_{4,41} = 6.32$, $P < 0.001$), so that glucose concentrations were higher in C100 and C200 penguins, when compared with control and C50 birds. However, NEFA and glucose levels were not affected by the sampling stage ($F_{1,41} = 3.11$, $P = 0.08$ and $F_{1,20} = 0.67$, $P = 0.42$ for NEFA and glucose, respectively) and by the interaction between treatment × sampling stage ($F_{4,20} = 0.35$, $P = 0.84$ and $F_{4,41} = 1.42$, $P = 0.24$ for NEFA and glucose, respectively; Table 3).

Effects of CORT implants on prolactin levels. Prolactin levels in C100 penguins were significantly affected by sampling stage ($F_{2,16} = 15.18$, $P < 0.001$; Fig. 4*A*). After 8–11 days of treatment, prolactin concentrations in these birds were significantly lower than on *day 0* ($P < 0.001$). However, while prolactin levels declined in all birds (between PII and PIII in control birds and between the time of implantation and *days 7–11* in CORT-implanted birds), the overall decline (expressed in percentage) was not significant ($F_{4,22} = 1.81$, $P = 0.16$; Fig. 4*B*). We found a significant negative relationship between prolactin levels and locomotor activity in C100 penguins ($r_s = -0.52$, $P = 0.03$; Fig. 4*C*), but this was not the case for C10, C50, and C200 birds (not shown).

DISCUSSION

The present study shows that an experimentally induced rise in CORT levels of Adélie penguins, which resulted in high but physiologically relevant circulating levels of CORT, mimicked metabolic, hormonal, and behavioral changes characteristic for the phase of late fasting.

Untreated Male and Female Birds: "Validation" of the Adélie Penguin Model

Minimum body mass threshold. We found that untreated male Adélie penguins in Dumont d'Urville entered PIII of fasting at a body mass of ~ 3.5 kg (Fig. 1). This value was obtained from males with different initial body masses (indicating that fasting duration differed) in studies conducted during several consecutive years between 2002 and 2009 (not shown). This indicates that such a minimum threshold might be relatively fixed for a given species, while differences between the sexes have to be accounted. Our results lend support to the critical body mass value reported by Cockrem et al. (14) for male Adélie penguins on Ross Island. Given our results, this critical body mass value in male Adélie penguins is consistent

Table 3. *Body mass and plasma levels of metabolites in control and CORT-implanted captive male Adélie penguins in relation to treatment and sampling stage (day relative to implantation in treated birds and fasting phase in control birds)*

	Day Relative to Implantation	C10 (n = 5)	C50 (n = 4)	C100 (n = 8)	C200 (n = 4)	Fasting Phase (Control Birds)	Control (n = 7)
Body mass, kg	0	4.31 ± 0.22^{a,b}	4.37 ± 0.23^{a,b}	4.89 ± 0.16^{a}	4.46 ± 0.09^{a,b}	PII	4.13 ± 0.03^{b}
	3	4.10 ± 0.29^{a}	4.07 ± 0.20^{a}	4.51 ± 0.17^{a}	4.11 ± 0.12^{a}	PIII	3.39 ± 0.05^{b}
Uric acid, mmol/l	0	0.19 ± 0.03^{a}	0.17 ± 0.01^{a}	0.25 ± 0.03^{a}	0.19 ± 0.03^{a}	PII	0.17 ± 0.01^{a}
	3	0.26 ± 0.01^{a}	0.36 ± 0.06^{a,b}	0.70 ± 0.06^{c}	0.46 ± 0.05^{b}	PIII	0.88 ± 0.21^{b,c}
βOHB, mmol/l	0	1.83 ± 0.42	1.74 ± 0.31	1.07 ± 0.12	1.44 ± 0.08	PII	1.79 ± 0.27
	3	1.44 ± 0.41	0.98 ± 0.13	0.72 ± 0.12	0.87 ± 0.15	PIII	0.88 ± 0.21
NEFA, mmol/l	0	1.42 ± 0.27	0.88 ± 0.11	0.68 ± 0.08	0.99 ± 0.06	PII	0.84 ± 0.14
	3	1.41 ± 0.39	0.68 ± 0.11	0.60 ± 0.07	0.99 ± 0.13	PIII	0.71 ± 0.12
Glucose, mmol/l	0	14.34 ± 0.37	13.27 ± 1.89	14.98 ± 0.27	18.22 ± 0.72	PII	13.05 ± 0.78
	3	14.84 ± 1.38	10.75 ± 0.29	15.23 ± 0.23	14.32 ± 1.81	PIII	12.63 ± 0.54

Results are expressed as means ± SE. Penguins were implanted with 10 (C10), 50 (C50), 100 (C100), and 200 (C200) mg of CORT, respectively. Superscript letters were not added when the interaction between the treatment and the sampling stage was not significant. Within each line, values that do not share the same superscript letter are significantly different ($P < 0.05$). See the RESULTS section for further details.

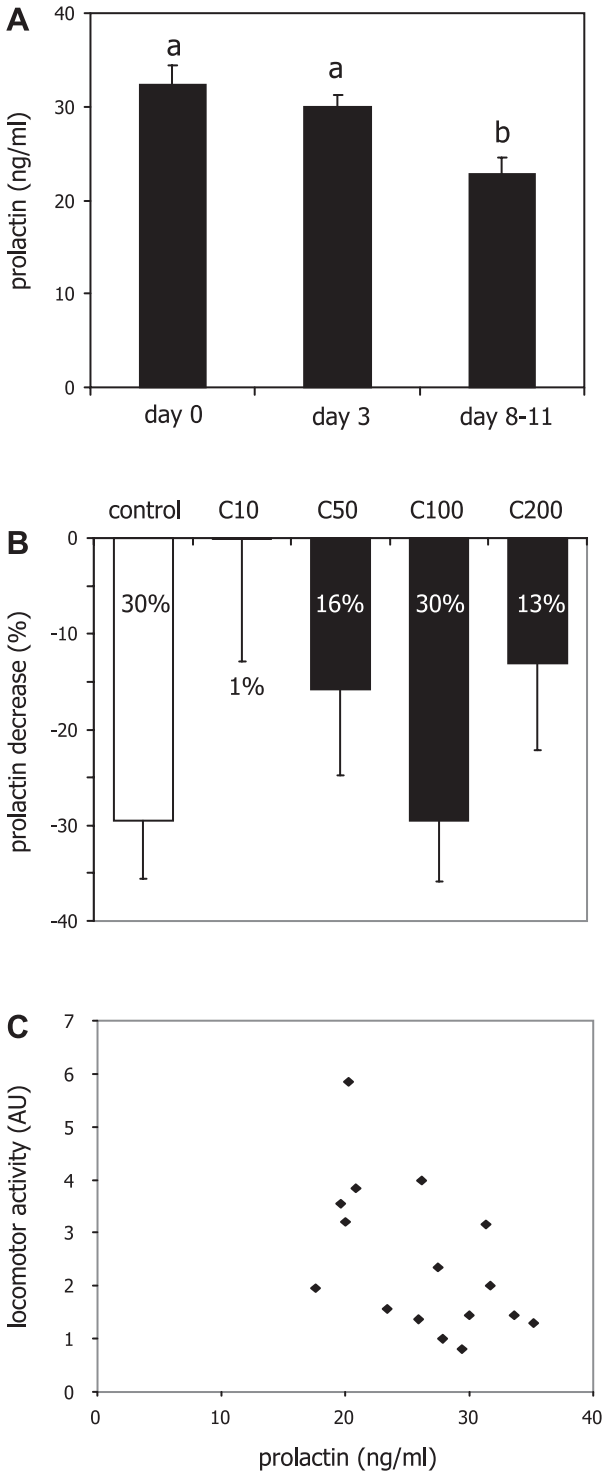

Fig. 4. *A*: effect of C100-CORT implants on prolactin levels in captive male Adélie penguins (n = 8). Values are expressed as means ± SE. Bars not sharing the same superscript letter are significantly different ($P < 0.05$). *B*: decrease in prolactin levels (%) in response to prolonged fasting in control birds (between PII and PIII) and in CORT-implanted birds (between the time of implantation and *days 7–11*). Penguins were implanted with 10 (C10), 50 (C50), 100 (C100), and 200 (C200) mg of CORT. *C*: relationship between plasma levels of prolactin and locomotor activity in C100-implanted penguins.

across studies and the same for both study sites. When below a body mass threshold of ~ 3.2 kg, female Adélie penguins entered PIII and displayed metabolic, hormonal, and behavioral changes, similar to that of males (Table 2). Hence, females also have the capacity to trigger the enhanced drive for refeeding, promoting their survival in case of critical reserve exhaustion. Mean body mass of male and female Adélie penguins when arriving in the colony was closed to 5.3–6.0 kg and 4.7–5.2 kg, respectively (32). With critical body masses ~ 3.5 kg and ~3.2 kg for males and females, respectively, these birds should be able to support comparable exhaustion of energy reserves (~34–42% and ~32–38% of body mass loss for males and females, respectively).

CORT, prolactin, and locomotor activity. As expected, untreated Adélie penguins in PIII had high levels of CORT (Fig. 2*A*). This is in agreement with studies conducted in king penguins (13) and emperor penguins (29). Moreover, the fact that PIII is reached when CORT secretion is strongly stimulated supports the idea that this hormone reflects food stress in birds (22). CORT has numerous biological effects, notably by regulating carbohydrate, lipid, and protein metabolism and is thus expected to play a major role during periods of nutritional limitation (30). Prolactin has the opposite effect of CORT in the control of parental behavior, stimulating incubation in birds (8). In the present study, captive birds were failed breeders; i.e., they started a reproductive cycle but prematurely discontinued incubation, regardless of parental body condition. In previous studies, prolactin levels remained nearly unchanged after nest failure in Adélie penguins (33) and in emperor penguins (26). However, in our study, prolactin levels at the point of capture were low in some birds, indicating that they might have lost their eggs several days ago (33). As was previously reported for emaciated king penguins (10, 18), we also found that prolactin concentration sharply decreased in PIII (Fig. 2*B*), suggesting that this hormone can be modulated by marked energy constraints and/or stressful situations. Entrance into PIII of fasting is also associated with behavioral changes and Adélie penguins in our study showed an increased locomotor activity at this stage (Fig. 2*C*). In untreated penguins, locomotor activity was positively related with CORT levels (Fig. 2*D*), while there was a negative relationship between locomotor activity and prolactin concentrations (Fig. 2*E*). These results support the idea of a potential involvement of both CORT and prolactin in the induction of the increased drive for refeeding that occurs in birds during late fasting. Altogether, our results from untreated birds illustrate that Adélie penguins entering into PIII show metabolic, hormonal, and behavioral changes that are similar to those of other penguin species (13, 29). This emphasizes the usefulness of the Adélie penguin model for further investigation.

CORT-Implanted Penguins: Experimental Study

Effects of CORT implants on CORT levels and locomotor activity. As expected, CORT levels increased with the implanted dosage (Fig. 3*A*), attesting that our experimental approach was operative. We found that exogenous CORT did not exert a proportional dose-response effect on locomotor activity, but the response rather had the shape of an inverted-U curve (mean value of locomotor activity over the entire treatment duration; Fig. 3*B*). While high levels of CORT (C100)

increased locomotor activity of birds, low (C10), intermediate (C50), and very high (C200) CORT levels had little (C50) or no effect (C10 and C200; Fig. 3*B*). An inverted-U relationship between CORT levels and locomotor activity was also reported for Gambel's white-crowned sparrows (*Zonotrichia leucophrys gambelii*). In these birds, only intermediate levels of CORT activated behavior, while high levels (but still within the range of CORT levels measured in free-living sparrows in response to capture and handling) had no effect (7). In the present study, circulating levels of CORT measured in C100 birds (63 ng/ml at *day 3*; 52 ng/ml at *days 8–11*) fell within the range of CORT levels obtained from untreated male Adélie penguins in PIII (from 23 to 67 ng/ml) and were, therefore, physiologically relevant.

Lack of a behavioral shift in penguins treated with the highest CORT dosage. Circulating CORT levels measured in C200 birds reached 94 ng/ml at *day 3* of treatment and decreased slightly thereafter (Fig. 3*A*). Such extremely high CORT concentrations have been measured in Adélie penguins before. Cockrem et al. (14) reported CORT levels for Adélie penguins that left the colony to refeed at sea that ranged between 10.7 and 110.4 ng/ml, after birds had been captured and handled for ~30 min. However, it is possible that the highest CORT dose used in our study (C200) was outside the range of physiological relevance for birds at this fasting stage. Accordingly, this dosage might have been unable to mimic the transition between PII and PIII, leading to the lack of a behavioral response. Furthermore, it should be beneficial to an animal to initiate behavioral responses in a way that is appropriate for the severity of the stressor. Hence, very high circulating levels of CORT could reflect severe environmental perturbations that may be incompatible with increased activity and food-searching behavior, as suggested by Breuner and Wingfield (7). On the other hand, there are many factors downstream of CORT secretion that can affect the behavioral and physiological outcome of a CORT increase. For instance, the binding of CORT to corticosteroid binding globulin (CBG) may regulate its action by altering the amount of CORT reaching target tissues (6). It is possible that the lack of a response in C200 penguins was related to a strong buffering action induced by an increase in CBG capacity. However, in raptor nestlings, the implantation of CORT pellets induced an increase in CBG capacity that resulted only in an attenuated increase of free CORT levels, attesting that total CORT levels were buffered only to a small degree (28). Future studies examining the time course of CBG capacity and free CORT levels in response to a stressor of different severity (or to several doses of CORT), should provide further insight into the mechanisms that regulate CORT actions in fasting seabirds.

Effects of CORT implants on plasma metabolites. In C100 penguins, we observed a shift from lipid to protein utilization, as reflected by the increase in uric acid levels and the decrease in βOHB concentrations (Table 3). This observation is in agreement with the role of CORT in the fasting-induced rise in protein utilization that was reported in rats (9). Interestingly, the highest dose of CORT in our study (C200), which did not affect locomotor activity, also provoked a less pronounced effect on uric acid levels, when compared with C100 penguins (Table 3). Similar to the dose-dependent effect of CORT on locomotor activity, it seems that the action of CORT on protein breakdown also depends on its concentration. Criscuolo et al. (15) found that protein catabolism after CORT implantation of incubating female common eider ducks was not as high as that of control females in PIII (0.21 mmol/l vs. 0.75 mmol/l). Because none of the treated females abandoned their nest, the authors suggested that an increase in proteolysis could be an important factor in triggering refeeding behavior. In this context, it was recently shown that proteolytic systems in the skeletal muscles of rodents are only slightly and selectively induced during PII of fasting, while they are strongly upregulated, in a coordinated fashion, during late fasting (4). Hence, one could hypothesize that the effect of CORT on the escape behavior may depend on a synergistic action, with its catabolic peripheral action taking place in the muscle.

Effects of C100 implants on prolactin levels. Some studies have emphasized that the secretion of CORT and prolactin might be mechanistically linked (1, 15). In our study, the C100 pellet was clearly the appropriate dose that mimicked metabolic and behavioral changes, characteristic of PIII in Adélie penguins. Therefore, in our analysis, we focused on C100 penguins to examine how exogenous CORT affected prolactin levels. We found a strong decrease in prolactin concentration 8–11 days after treatment, at which point it was 30% lower than on *day 0* (Fig. 4*B*). This decline in prolactin levels could be indirect and due to the arrival at a minimum body mass (10, 18). However, 8–11 days after treatment, the average body mass of C100 penguins was 3.74 ± 0.13 kg, and, therefore, above the critical body mass. This suggests that CORT levels affect circulating prolactin concentrations before birds reach the critical body mass threshold. We found a negative relationship between prolactin levels and locomotor activity in C100 penguins (Fig. 4*C*). Hence, it could be that the effect of CORT implants on behavioral changes of penguins may be reinforced through an effect on prolactin levels. Support for this idea comes from a study in black-legged kittiwakes, where a short-term increase in CORT levels was accompanied by a 30% decrease in prolactin and a subsequent reduction in nest attendance (1).

Perspectives and Significance

Overall, our study emphasizes the role of CORT in the rise in locomotor activity and the associated metabolic and hormonal changes occurring in phase III. This supports the view that this hormone plays a key role in the enhanced drive for refeeding that occurs in fasting birds below its body mass threshold; such a role remains to be validated in free-living penguins.

Interestingly, we observed that the time course of the response following CORT treatment in our study on Adélie penguins (2–4 days) is markedly different from that observed in Gambel's white-crowned sparrows (15 min) by Breuner and Wingfield (7). This could be related to the life-history strategy of penguins at the breeding stage. In contrast to white-crowned sparrows, penguins spontaneously fast for a long period during incubation, and they are prepared to cope with this nutritional constraint (13, 29, 35). This energy-demanding period would thus be predictable and, therefore, not considered as stressful (37). This also led us to suggest that in penguins, CORT could exert its action through indirect effects on specific targets, which remain to be identified. One behavioral consequence of PIII is nest abandonment in free-ranging birds. To examine the

role of hormones in the orchestration of nest abandonment, it would be of great interest to investigate the respective role of CORT and prolactin in nest desertion in breeding birds using experimental approaches.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES

1. **Angelier F, Clément-Chastel C, Welcker J, Gabrielsen GW, Chastel O.** How does corticosterone affect parental behaviour and reproductive success? A study of prolactin in black-legged kittiwakes. *Funct Ecol* 23: 784–793, 2009.
2. **Astheimer LB, Buttemer WA, Wingfield JC.** Interactions of corticosterone with feeding, activity and metabolism in passerine birds. *Ornis Scand* 23: 355–365, 1992.
3. **Belkhou R, Cherel Y, Heitz A, Robin JP, Le Maho Y.** Energy contribution of proteins and lipids during prolonged fasting in the rat. *Nutr Res* 11: 365–374, 1991.
4. **Bertile F, Le Maho Y, Raclot T.** Coordinate upregulation of proteolytic-related genes in rat muscle during late fasting. *Biochem Biophys Res Commun* 311: 929–934, 2003.
5. **Bertile F, Oudart H, Criscuolo F, Le Maho Y, Raclot T.** Hypothalamic gene expression in long-term fasted rats: relationship with body fat. *Biochem Biophys Res Commun* 303: 1106–1113, 2003.
6. **Breuner CW, Orchinik M.** Plasma binding proteins as mediators of corticosteroid action in vertebrates. *J Endocrinol* 175: 99–112, 2002.
7. **Breuner CW, Wingfield JC.** Rapid behavioral response to corticosterone varies with photoperiod and dose. *Horm Behav* 37: 23–30, 2000.
8. **Buntin JD.** Neural and hormonal control of parental behavior in birds In: *Advances in the Study of Behavior*, edited by Rosenblatt JS and Snowdon CT. New York: Academic, 1996, p. 161–213.
9. **Challet E, Le Maho Y, Robin JP, Malan A, Cherel Y.** Involvement of corticosterone in the fasting-induced rise in protein-utilization and locomotor activity. *Pharmacol Biochem Behav* 50: 405–412, 1995.
10. **Cherel Y, Mauget R, Lacroix A, Gilles J.** Seasonal anf fasting-related changes in circulating gonadal steroids and prolactin in king penguins, *Aptenodytes patagonicus*. *Physiol Zool* 67: 1154–1173, 1994.
11. **Cherel Y, Robin JP, Heitz A, Calgari C, Le Maho Y.** Relationship between lipid availability and protein utilization during prolonged fasting. *J Comp Physiol* 162: 305–313, 1992.
12. **Cherel Y, Robin JP, Le Maho Y.** Physiology and biochemistry of long-term fasting in birds. *Can J Zool Rev Can Zool* 66: 159–166, 1988.
13. **Cherel Y, Robin JP, Walch O, Karmann H, Netchitailo P, Le Maho Y.** Fasting in king penguin. I. Hormonal and metabolic changes during breefing. *Am J Physiol Regul Integr Comp Physiol* 254: R170–R177, 1988.
14. **Cockrem JF, Potter MA, Candy EJ.** Corticosterone in relation to body mass in Adelie penguins (*Pygoscelis adeliae*) affected by unusual sea ice conditions at Ross Island, Antarctica. *Gen Comp Endocrinol* 149: 244–252, 2006.
15. **Criscuolo F, Chastel O, Bertile F, Gabrielsen GW, Le Maho Y, Raclot T.** Corticosterone alone does not trigger a short term behavioral shift in incubating females common eiders *Somateria mollissima*, but does modify long-term reproductive success. *J Avian Biol* 36: 306–312, 2005.
16. **Goodman MN, Larsen PR, Kaplan MM, Aoki TT, Young VR, Ruderman NB.** Starvation in rats. II. Effect of age and obesity on protein sparing and fuel meatbolism. *Am J Physiol Endocrinol Metab* 239: E277–E286, 1980.
17. **Groscolas R, Decrock F, Thil MA, Fayolle C, Boissery C, Robin JP.** Refeeding signal in fasting-incubating king penguins: changes in behavior and egg temperature. *Am J Physiol Regul Integr Comp Physiol* 279: R2104–R2112, 2000.
18. **Groscolas R, Lacroix A, Robin JP.** Spontaneous egg or chick abandonment in energy-depleted king penguins: A role for corticosterone and prolactin? *Horm Behav* 53: 51–60, 2008.
19. **Groscolas R, Robin JP.** Long-term fasting and re-feeding in penguins. *Comp Biochem Phys A* 128: 645–655, 2001.
20. **Jenni-Eiermann S, Jenni L.** What can plasma metabolites tell us about the metabolism, physiological state and condition of individual birds? An overview. *Biol Cons Fauna* 102: 312–319, 1998.
21. **Kachman SD.** An introduction to generalized linear mixed models. *Proceedings: Implementation Strategies for National Beef Catyle Evaluation*, Athens, GA: USDA NCR, 2000, p. 59–73.
22. **Kitaysky AS, Piatt JF, Wingfield JC.** Stress hormones link food availability and population processes in seabirds. *Mar Ecol Prog Ser* 352: 245–258, 2007.
23. **Kitaysky AS, Wingfield JC, Piatt JF.** Corticosterone facilitates begging and affects resource allocation in the black-legged kittiwake. *Behav Ecol* 12: 619–625, 2001.
24. **Koubi H, Robin JP, Dewasme G, Le Maho Y, Frutoso J, Minaire Y.** Fasting-induced rise in locomotor activity in rats coincides with increased protein utilization. *Physiol Behav* 50: 337–343, 1991.
25. **Landys MM, Ramenofsky M, Wingfield JC.** Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. *Gen Comp Endocrinol* 148: 132–149, 2006.
26. **Lormée H, Jouventin P, Chastel O, Mauget R.** Endocrine correlates of parental care in an Antarctic winter breeding seabird, the emperor penguin, *Aptenodytes forsteri*. *Horm Behav* 35: 9–17, 1999.
27. **Muggeo VMR.** Segmented: an R package to fit regression models with broken-line relationships. *R News* 8/1: 20–25, 2008.
28. **Müller C, Almasi B, Roulin A, Breuner CW, Jenni-Eiermann S, Jenni L.** Effects of corticosterone pellets on baseline and stress-induced corticosterone and corticosteroid-binding-globulin. *Gen Comp Endocrinol* 160: 59–66, 2009.
29. **Robin JP, Boucontet L, Chillet P, Groscolas R.** Behavioral changes in fasting emperor penguins: evidence for a "refeeding signal" linked to a metabolic shift. *Am J Physiol Regul Integr Comp Physiol* 274: R746–R753, 1998.
30. **Sapolsky RM, Romero LM, Munck AU.** How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Rev* 21: 55–89, 2000.
31. **Spée M, Beaulieu M, Dervaux A, Chastel O, Le Maho Y, Raclot T.** Should I stay or should I go? Hormonal control of nest abandonment in a long-lived bird, the Adélie penguin. *Horm Behav* 58: 762–768, 2010.
32. **Vleck CM, Bucher TL, Reed JM, Kristmundsdottir AY.** Changes in reproductive hormones and body mass through the reproductive cycle in the Adélie penguin (*Pygoscelis adeliae*), with associated data on courting-only individuals. *Proceedings of the 22nd International Ornithological Congress,* edited by Adams N and Slotow R. Durban, South Africa: University of Natal, 1999, p. 1210–1223.
33. **Vleck CM, Ross LL, Vleck D, Bucher TL.** Prolactin and parental behavior in Adélie penguins: effects of absence from nest, incubation length, and nest failure. *Horm Behav* 38: 149–158, 2000.
34. **Vleck CM, Vertalino N, Vleck D, Bucher TL.** Stress, corticosterone, and heterophil to lymphocyte ratios in free-living Adelie penguins. *Condor* 102: 392–400, 2000.
35. **Vleck CM, Vleck D.** Physiological condition and reproductive consequences in Ade'lie penguins. *Integr Comp Biol* 42: 76–83, 2002.
36. **Williams TD.** *The Penguins (Bird Families of the World).* New York: Oxford University Press, 1995.
37. **Wingfield JC.** Control of behavioural strategies for capricious environments. *Anim Behav* 66: 807–815, 2003.
38. **Wingfield JC, Maney DL, Breuner CW, Jacobs JD, Lynn S, Ramenofsky M, Richardson RD.** Ecological bases of hormone-behavior interactions: The "emergency life history stage". *Am Zool* 38: 191–206, 1998.
39. **Youngren OM, Elhalawani ME, Silsby JL, Phillips RE.** Intracranial prolactin perfusion induces incubation behavior in turkey hens. *Biol Reprod* 44: 425–431, 1991.