

Factors Affecting Plasma Concentrations of Prolactin in the Common Eider *Somateria mollissima*

François Criscuolo, Olivier Chastel,* Geir. Wing Gabrielsen,† André Lacroix,* and Yvon Le Maho

Centre d'Ecologie et Physiologie Energétiques, CNRS, 23 rue Becquerel, F-67087 Strasbourg Cedex 2, France;

*Centre d'Etudes Biologiques de Chizé, CNRS, F-79360 Villiers-En-Bois, France; and †Norwegian Polar Institute, The Polar Environmental Center, Hjalmar Johansensgate 14, N-9296 Tromsø, Norway

Accepted November 30, 2001

In the common eider only the females incubate while they fast for 25 days. Thus, since they rely entirely on their body reserves for successful incubation, they can be defined as capital incubators. To assess the potential effects of their initial body mass, the incubation duration, and depletion in body reserves on prolactinemia, blood samples of eiders were analyzed during the breeding cycle and an experimental manipulation of the duration of incubation. Levels of circulating prolactin increased at the onset of incubation and then reached a high and stable level during incubation before increasing sharply before hatching. The prolactin level decreased significantly upon hatching. Captive females deprived from their eggs exhibited a rapid decrease in prolactinemia, suggesting that egg stimuli are necessary to prolactin secretion. Aunts, i.e., helper females caring for conspecific young, presented prolactin levels higher than nonbreeding captive females but not significantly different from those of females at hatching. Plasma prolactin at hatch was directly related to body mass loss. Birds with shortened incubation have higher body masses and showed higher levels of prolactinemia at hatching than the control group, in accordance with the idea that circulant prolactin at hatching is linked to body condition. Females which underwent an extended incubation (and started to eat again) displayed a low body mass and a high prolactinemia. These data therefore suggest that refeeding, albeit increasing the risk of predation, enhances prolactin secretion and allows the bird to

continue incubation despite that it has reached a poor body condition. © 2002 Elsevier Science (USA)

INTRODUCTION

Prolactin is involved in the initiation and maintenance of avian incubation behavior (Hall, 1991; Buntin, 1996; Sharp *et al.*, 1998). In most birds, tactile and visual stimuli from nest, eggs, or incubating mate stimulate prolactin secretion (Buntin, 1996). Removal of these stimuli results in a decrease in plasma prolactin and the termination of parental behavior (Buntin, 1986; Hall and Goldsmith, 1983; Ramsey *et al.*, 1985). However, studies conducted on different avian species demonstrate that prolactin secretion can be maintained regardless of direct stimulations from the nest or eggs. Albatrosses (*Diomedea* sp.) coming back from feeding excursions still present high prolactin levels despite their long absences from the nest (Hector and Goldsmith, 1985). Prolactin remains unaffected by nest failure in emperor (*Aptenodytes forsteri*) and king penguins (*A. patagonicus*; Lormée *et al.*, 1999; Jouventin and Mauget, 1996) as well as in females Adélie penguin (*Pygoscelis adeliae*, Vleck *et al.*, 2000), suggesting that prolactin secretion could be endogenously timed in some pelagic seabirds (Hector and Goldsmith, 1985; Garcia *et al.*, 1996; Lormée *et al.*, 1999).

In birds, incubation behavior competes with foraging, thus inducing restricted access to food. Anorexia has evolved as a means to ensure greater nest attendance (Mrosovsky and Sherry, 1980), to enhance the chances of hatching success. Accordingly, the amount of adult body reserves available to incubate is a key factor in determining incubation constancy (Aldrich and Raveling, 1983; Chastel *et al.*, 1995; Chaurand and Weimerskirch, 1994). For the king penguin, the circulating prolactin level seems to be decreased only when the bird reaches a critical threshold in its body reserves (when body lipids neared exhaustion in the phase III of fasting; Cherel *et al.*, 1994). This suggests a potential control of prolactin secretion (thus of parental behavior) by adult body condition in capital incubators, i.e., birds which does not feed during incubation.

The common eider *Somateria mollissima* presents a particular breeding strategy since only the female incubates for 24-26 days (Korschgen, 1977). Therefore, like the male Emperor penguin *A. forsteri* (Le Maho, 1977), the female eider can be defined as a true capital incubator (Drent and Daan, 1980) since it relies entirely on their body nutrient reserves during incubation. Moreover, the common eider presents a post-hatch alloparental behavior, nonbreeding or failed breeders (aunts) tending for conspecific youngs in large crèches (see Öst, 2000). However, this parental behavior depends of body fuels reserves since only birds in good body condition take care of the ducklings (Bustnes and Erikstad, 1991).

The first aim of this study was to describe the variations of circulant prolactin during the breeding cycle, from prelaying to hatching, to define whether prolactin in eiders follows a comparable changes to those previously observed in other ducks (Hall and Goldsmith, 1983). The relationship between natural clutch size and prolactin was examined to test whether parental investment in incubation is done in relation to the reproductive value of the clutch (i.e., clutch size; Erikstad and Tveraa, 1995).

In a second step, we tried to establish the hormonal link between body condition and parental care. We first checked whether prolactin can mediate posthatch parental care (levels of helper females) and is endogenously controlled (i.e. by adult body reserves). It should be expected that females in good condition also have high prolactin levels, which promote brood-

ing of youngs. The breeding season is reduced to the short arctic summer for the female eider nesting in Svalbard, and a previous work pointed out the fact that the incubation period is shortened by 1 or 2 days in this population (Parker and Holm, 1990). Moreover, we showed that female eiders in Svalbard only have a limited safety margin in their body reserves and change their incubation behavior (i.e., by taking longer and more frequent recesses) when facing an extended incubation period, even of only 1 day (Crisuolo *et al.*, 2001). Accordingly, we wondered if prolactin levels can be potentially modified during this extended incubation, thus being involved in these behavioral modifications. To do so, incubation length was experimentally shortened or prolonged by swapping eggs between nest with different laying dates. Female eiders facing an artificially extended incubation start to feed again (Crisuolo *et al.*, 2001). To test whether the nutritional status of the bird can influence its prolactin concentration, we determine the plasma concentrations of triacylglycerols (feeding indice) and of β -hydroxybutyrate (fasting indice, Le Maho *et al.*, 1981) of prolonged incubating females.

METHODS

The study was conducted in Kongsfjorden, on the western coast of the Svalbard Archipelago (78°55 N), on female eiders nesting close to the research station of Ny-Ålesund (June and July 1998 and 1999) and on Prins Heinrich Island (1999 and 2000). In 1998 and 1999, egg laying started in mid-June, and breeding conditions were comparable since in both years the disappearance of the snow and the spring break-up of the sea-ice inside the fjord were delayed. In 2000, the season started earlier (in early June) because the sea-ice already disappeared at the end of May.

Blood Sampling

A total of 177 females and 12 males were sampled during the 3 years of the study. Before they were released, their body mass was measured with a portable electronic balance (± 2 g) and clutch size was recorded. Blood (2.5 ml) was collected from the brachial vein with a 5-ml nonheparinized syringe and a

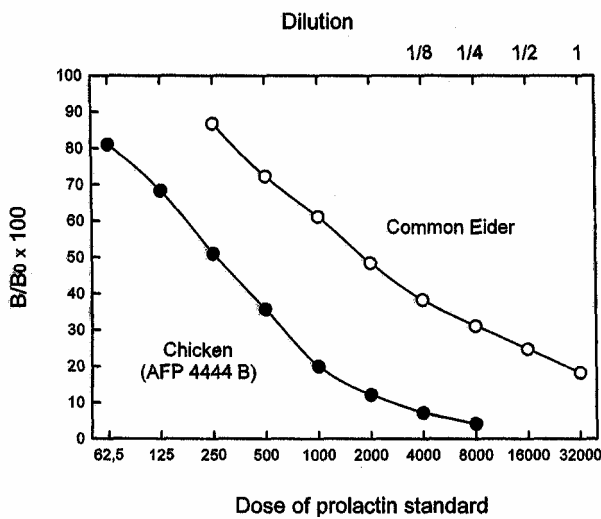


FIG. 1. Dose-response curves of chicken and females common eider prolactin. Prolactin standards are expressed in pg/tube.

21-gauge hypodermic needle. It was transferred to 5-ml tubes containing anticoagulant agent and kept on ice during the transport to the laboratory. The samples were then centrifuged at 5000 rpm at 2°, and 100 µl of plasma was aliquoted into several 0.5-ml microtubes. The plasma was stored in a -20°C freezer until analysis.

Prolactin and Metabolites Assays

The plasma concentrations of prolactin were determined by radioimmunoassays at the CEBC (Mauget *et al.* 1995; Lormée *et al.*, 1999, 2000). Pooled plasma samples produced dose-response curves that paralleled the chicken prolactin standard curves (source, Dr. Parlow, NHPP Harbor-UCLA Medical Center, Los Angeles, CA, Fig. 1). The intraassay coefficient of variation was 3.3% (*n* = 4 duplicates). Three assays were performed, with a variation of 14.7%. Prolactin levels of females was significantly higher in 1999 compared to 1998 and 2000 (ANOVA, *n* = 116, Tukey test, *P* < 0.0005). As prolactin levels measured in 1998 and 2000 did not differ, the data were pooled for the analysis of the effect of body mass and relative mass loss on prolactin level at hatching.

Plasma triacylglycerols and β-hydroxybutyrate levels of prolonged incubating females were measured enzymatically using commercial kits (Boehringer, Mannheim, Germany).

Prolactin Changes during Breeding

In 1999, a total of 59 females were sampled throughout the breeding cycle which was divided into three reproductive stages: (i) the prelaying stage, females being caught on the beach using a net; (ii) the incubation period (from clutch completion to day 24), the birds then being caught on the nest using a bamboo pole with a nylon snare; and (iii) the hatching period when females were caught still on the nest, but with at least one hatched egg. During this period, the non-breeding females or females which had failed in reproduction sitting close to a nest containing newly hatching ducklings (hereafter called aunts, see Schmutz *et al.*, 1982) were caught with a net. Male eiders were caught with a net as well during the prelaying and early incubation stages, before they left the breeding grounds to molt. By determining the prolactin levels of aunts and of males which do not participate either to incubation nor to brooding, we intended to underscore the potential relationship between prolactin and posthatch parental care.

Body Mass and Prolactin Levels

Because female eiders lose 30-40% of their initial body mass during incubation (Korschgen, 1977; Parker and Holm, 1990; Gabrielsen *et al.*, 1991), we studied the relationships between initial (days 1-5) and final (days 21-24) body masses, between the proportion of mass loss during incubation and prolactin levels in the 3 years of the study. In 1998 and 2000, we measured the proportion of mass loss by dividing daily mass loss (g/day), by initial body mass (g). The lower this rate of body mass loss, the bigger the initial body lipid reserves (Cherel and Groscolas, 1998).

Effect of Clutch Size on Prolactin Levels

Since investment in incubation varies with the clutch size in eider, the relationship between the number of eggs and the plasma concentration of prolactin was studied on free incubating females, during early, mid, and late incubation, both in 1998 and 2000 (*n* = 47) and in 1999 (*n* = 41).

Effect of Eggs and Nest on Prolactin Levels

Prolactin changes of females deprived from their nests were studied to estimate the importance of eggs and nests stimuli in the control of prolactin secretion. In 2000, five females were therefore caught on the nest after at least 1 week of incubation and held in captivity for on average 3 weeks to measure changes in plasma levels of prolactin in birds without eggs to incubate. Blood samples were taken at capture and then once every 5 days. The experimentation was stopped when fasting females entered into the first day of phase III (see Le Maho *et al.*, 1981), and then the last blood sample was taken. After the experiment, the birds were refed and released. Phases of fasting are characterized by the daily change in body mass per unit body mass (dm/mdt or $(mass_{final} - mass_{initial})/days$ of fasting/massinitial; Le Maho *et al.*, 1981). Phase II corresponds to a long period of economy, during which the rate of body mass loss and the body protein utilization are low, most of the energy being derived from lipids. Phase III is critical because both rates of body mass loss and protein utilization are increasing while body lipids neared depletion (Cherel *et al.*, 1988).

Manipulation of Incubation Duration and Hatching Date

Incubation duration was artificially shortened or prolonged in free incubating females in 2000 ($n = 35$). By modifying the hatching date, we investigated whether the prolactin changes at hatching are dependent on the body condition or not. Hatching dates were changed by manipulating the length of the incubation period during the breeding season. We exchanged eggs from nests with different laying dates to create: (i) a shortened incubation group—females that had a reduced incubation period with ducklings hatching after only 18.3 ± 0.4 days of incubation (6.6 days before the normal hatching date, $n = 10$); (ii) a prolonged incubation group—females that had a prolonged incubation period with ducklings not yet hatched after 27.6 ± 0.6 days of incubation (2.7 days after the normal hatching date, $n = 9$) (in this group, plasma concentrations of triacylglycerols and β -hydroxybutyrate were determined to explore the relation between the prolactin levels and the nutrient status of

the females; (iii) a prolonged hatching group—prolonged females that had ducklings after 28.8 ± 1.0 days of incubation (3.9 days after the normal hatching date, $n = 5$); and (iv) two control groups—females sampled at the hatching stage, after a natural incubation period of 24.9 ± 0.3 days of incubation ($n = 11$), and females sampled during natural incubation (after 13.7 ± 2.2 days, $n = 10$). The discrepancy in the number of incubation days in the shortened and prolonged groups is due to the difficulty in catching birds several times and to the high rate of egg predation in unattended nests during extended incubation (Crisuolo *et al.*, 2001). Eggs were exchanged during the first week of incubation and blood sampling were taken at the dates given for all groups.

Statistical Analysis

Changes in plasma prolactin during the breeding season and the hatching date experimentation were compared using a one-way ANOVA (a level of significance is 0.05), followed by post-ANOVA Tukey tests. For captive females, an ANOVA for repeated measurements was used to compare initial and phase II prolactin levels. Correlations between prolactin level, body condition, and clutch size were made using Pearson correlation coefficients. Values are expressed as means \pm SE.

RESULTS

Prolactin Changes during Breeding

Plasma concentrations of prolactin varied significantly over the breeding cycle (ANOVA, $n = 59$, $P < 0.05$, Fig. 2). Prolactin titers increased from the prelaying stage to the beginning of incubation (Tukey test, $P < 0.002$). Thereafter, prolactin values remained un-changed (from days 0-3 to days 11-20). Before hatching (days 21-24), prolactin concentrations increased significantly and dropped at hatching (Tukey test, $P < 0.002$). Prolactin concentration of aunts was lower than those of incubating females (Tukey test, $P < 0.002$) and similar to those of prelaying and hatching females (Tukey test, $P > 0.002$). Furthermore, aunts had prolactin levels significantly higher than captive females

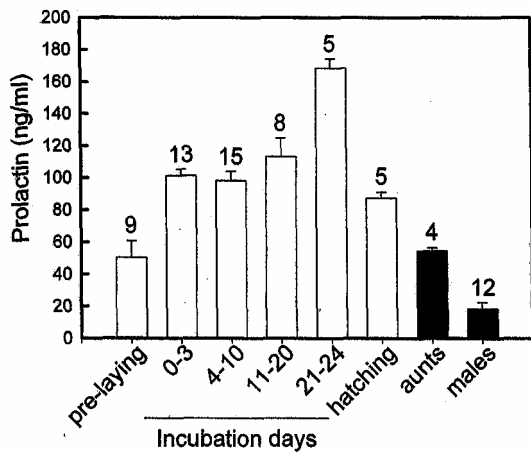


FIG. 2. Average plasma concentrations of prolactin in female common eiders during the reproductive cycle. Sample sizes are indicated above the vertical bars. Are also indicated the average prolactin levels for aunts (helper females) and male eiders.

in phase II (normal energetic situation; 54.35 ± 2.03 vs 31.41 ± 3.86 ng/ml, Student's *t* test, $P < 0.05$).

Prolactin concentration of males was significantly lower than the levels of hatching females and aunts (Tukey test, $P < 0.002$), but comparable to the levels of prelaying females (Tukey test, $P > 0.002$).

Body Mass and Prolactin Levels

There was no significant relationship between initial body mass (days 0-3) and initial plasma concentrations of prolactin in 1999 ($r = -0.406$, $N = 13$, $P = 0.169$) and in 1998 and 2000 ($r = 0.246$, $N = 15$, $P = 0.377$). The plasma concentrations of prolactin measured at the end of incubation (days 21-24) were un-related to final body mass in 1999 ($r = 0.419$, $N = 5$, $P = 0.483$) and in 1998 and 2000 ($r = 0.436$, $N = 8$, $P = 0.280$). However, the final prolactinemia of birds sampled at hatching was negatively correlated to the relative daily mass loss in 1998 and 2000 ($r = -0.625$, $N = 11$, $P = 0.04$, Fig. 3).

Clutch Size and Prolactin Levels

We did not detect any significant relationship between natural clutch size (two to six eggs) and plasma prolactin concentrations neither in 1998 and 2000 during early (days 0-3, $r = -0.454$, $N = 14$, $P = 0.103$), mid (days 3-20, $r = 0.174$, $N = 26$, $P = 0.394$), and late

incubation (days 21-24, $r = 0.346$, $N = 7$, $P = 0.447$), nor in 1999 during early (days 0-3, $r = -0.023$, $N = 13$, $P = 0.941$), mid (days 4-20, $r = -0.012$, $N = 23$, $P = 0.957$), and late incubation (days 21-24, $r = 0.451$, $N = 5$, $P = 0.446$).

Effect of Eggs and Nest on Prolactin Levels

The prolactin concentrations of incubating females that were removed from their nest and held in captivity dropped from 68.28 ± 1.33 ng/ml (level at capture) to 35.36 ± 4.93 ng/ml after only 3 days of captivity (ANOVA for repeated measures, $N = 5$, $P < 0.05$, Fig. 4).

Manipulation of Incubation Duration and Hatching Date

At hatching, prolactin levels and body masses differed significantly between the shortened, prolonged, and control groups (ANOVA, $P < 0.05$). Indeed, the prolactin concentration of the shortened group was significantly higher than the level of the control group at hatching (Tukey test, $P < 0.02$). Likewise, the shortened group exhibited a higher body mass at hatching (Tukey test, $P < 0.02$, Fig. 5). Females of the prolonged group showed a higher plasma concentration of prolactin than the control hatching group (Tukey test, $P < 0.02$), but had a comparable body mass at hatching (Tukey test, $P > 0.02$, Fig. 5).

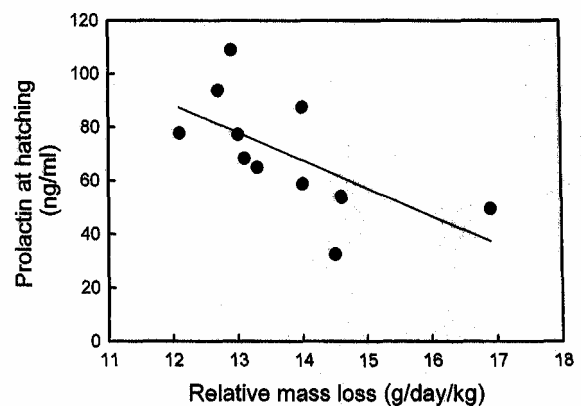


FIG. 3. Prolactin levels at hatching of female common eiders sampled in 1998-2000, in relation to the relative body mass lost (daily mass loss/initial body mass) during incubation.

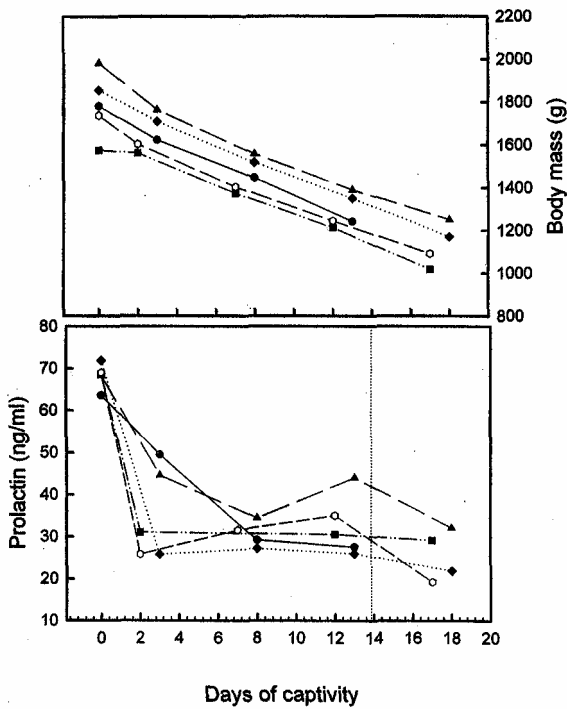


FIG. 4. Changes in body mass and prolactin concentrations of five captive female common eiders deprived from their eggs after 1 week of incubation. Prolactin values shown on this figure were measured during phase II of fasting (of variable duration depending of the initial adiposity of the bird). The dotted line give the average expected hatching date of the five females. Each symbol refers to an individual female.

Despite a lower body mass (Student's *t* test, $P < 0.05$, Fig. 6), females sampled after 2.7 days of prolonged incubation presented a prolactin level not sig-

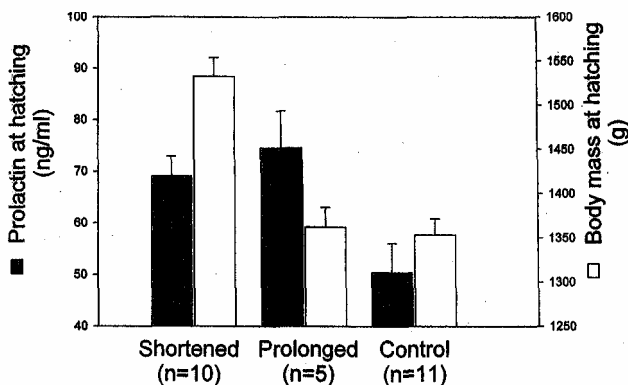


FIG. 5. Average prolactin concentrations and body masses of female common eiders, after 18.3 ± 0.4 (shortened), 28.8 ± 1.0 (pro-longed), and 24.9 ± 0.3 (control) days of incubation.

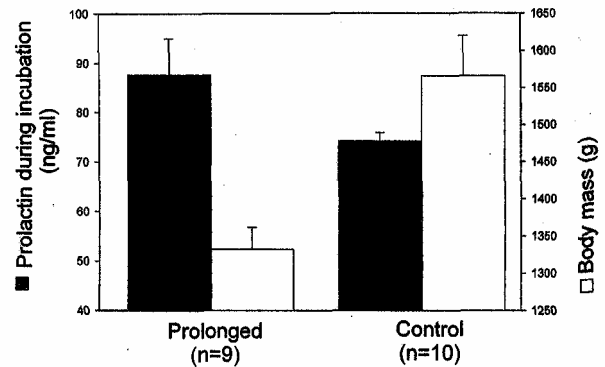


FIG. 6. Average prolactin concentrations and body masses of female common eiders during incubation, after 27.6 ± 0.6 (delayed) and 13.7 ± 2.2 (control) days of incubation.

nificantly different from a control group of females incubating since only 13.7 ± 2.2 days (Student's *t* test, $P = 0.10$). Moreover, among these females which started to feed again, those presenting the lower β -hydroxybutyrate/triacylglycerols ratio also showed higher prolactin levels ($r = -0.895$, $N = 9$, $P < 0.05$, Fig. 7).

DISCUSSION

Prolactin during the Breeding Cycle

Thus, in eider ducks, prolactin concentration rises at the beginning of incubation and remains consistently

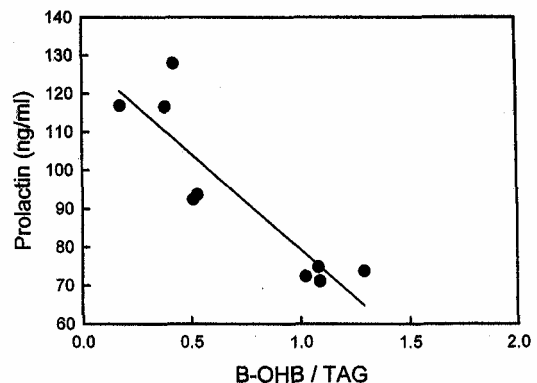


FIG. 7. Prolactin levels of incubating female eiders sampled after 27.6 ± 0.6 (delayed) days of incubation, in relation to their nutritional status estimated by the ratio β -hydroxybutyrate (B-OHB, indice of fasting)/triacylglycerols (TAG, indice of feeding).

high until hatching. Then it decreases significantly (Fig. 2). Those captive females which were interrupted in incubation do not maintain an elevated circulating prolactin comparable to that of incubating females. Hall (1987) also described the collapse of prolactin secretion after the complete removal of the clutch in females mallard *Anas platyrhynchos*. Meijer (1995) demonstrated that hens of red junglefowl (*Gallus gallus spadiceus*) stop incubation when they can neither touch nor see their eggs. However, one can wonder if the prolactin decrease in captive eiders is not the consequence of the stress induced by the captivity? We need further information to answer this question, but Jouvantin and Mauget (1996) did not detect any decrease in prolactin levels of king penguins maintained in captivity for 48 h. In a previous experiment conducted on female eider, we did not detect any drop in prolactin during a 30-min acute stress response to capture and handling (unpublished data). In conclusion, our data emphasize the importance of the eggs and/or nest stimuli (Hall and Goldsmith, 1983; Ramsey *et al.*, 1985), which therefore induce, at least partly, the first rise and the upholding of prolactin in eiders.

The profile of plasma prolactin concentration establishes that prolactin increases in eiders during days 21-24, when the eggs are close to hatching (Fig. 2). Prolactin secretion can be modified by numerous stimuli affecting the brood patch (Hall, 1987). Thus, the activity of the duckling at the end of its development (tapping and vocalizations) could induce a rise of prolactin secretion, thus stimulating nest attentiveness as observed in geese (Thompson and Raveling, 1987; Reed *et al.*, 1995). Measurement of prolactin concentrations of a bird belonging to the experimentally prolonged group support this idea since when incubation was delayed by 10 days (hatching at day 34), it did not show the prehatching rise in prolactin at day 24. However, since this rise in prolactinemia was not previously described in other species, its role needs to be precisely determined in a further study.

Clutch Size and Prolactin

In our study the number of eggs a female eider is incubating had no effect on her prolactin concentrations. This result is in accordance with the findings that pied flycatchers *Ficedula hypoleuca* and mallards *A. platyrhynchos* incubating clutches of different size

show no difference in prolactin stimulation (Hall, 1987; Hall and Goldsmith 1983; Silverin and Goldsmith, 1983). This suggests that the number of eggs does not exert a quantitative effect on prolactin (Silverin and Goldsmith, 1983). In contrast, male Wilson's phalarope *Phalaropus tricolor* exhibits lowered prolactin levels when its clutch size is experimentally reduced or enlarged (Delehanty *et al.*, 1997). For the phalarope which have a determinate clutch of four eggs, any modification of the egg number induces the reduction of the reproductive value of the current breeding attempt (Delehanty and Oring, 1993). Still, an experimental manipulation of clutch size is needed in common eider to verify the influence of clutch size on prolactin secretion, the optimal clutch size in eiders being a compromise between the allocation of body reserves to eggs, incubation, and care of chicks (Erikstad and Tveraa, 1995).

Mass Loss, Prolactin Levels at Hatching, and Posthatch Parental Care

Breeding can be a costly activity for capital incubators (which depend entirely on their body reserves) by reducing the chance of adult survival if body reserves are extensively depleted at the end of incubation (Korschgen, 1977). In this case, clutch abandonment may have an adaptive value if the cost of incubation is too high, and if brood abandonment could lead to higher chances of adult survival, and allows future reproductive attempts (Stearns, 1992; Erikstad *et al.*, 1998). For these species, fasting for several weeks does not represent an energetic stress until the birds reach a critical threshold in their endogenous body fuels. As was shown in the incubating male king penguin, which because it feeds far out at sea cannot forage without abandoning its egg, prolactin levels are only decreased by an extensive body reserve depletion, i.e., when entering the critical fasting phase (namely, phase III, Cherel *et al.*, 1994). Likewise, we did not find a direct relationship between body mass and prolactin levels in incubating female eiders, because the birds seldom reach a phase III during normal incubation (Crisuolo *et al.*, 2001). Such a result is consistent with the incubation strategy of capital incubators, which because they depend almost exclusively on their endogenous nutrient reserves are able to lose a large amount of body mass without threatening their reproduction or

survival (Cherel *et al.*, 1988; Moreno, 1989). On the other hand, a deteriorated adult body condition at hatching could have an impact on the posthatch parental behavior. An interesting point of eider reproduction strategy is the posthatch brood amalgamation, which consists of several females tending for ducklings (Öst, 2000). Several studies focused on the reasons leading to brood abandonment in eiders (Bustnes and Erikstad, 1991; see Öst, 2000). They accord to present the *energetic salvage strategy hypothesis* as the most probable. According to this hypothesis, females may become brood abandoners if reaching a poor body condition at the end of incubation, thereby increasing their lifetime fitness (they cannot forage and take care of the young in the same time because feeding and breeding areas are different; Prestrud and Mehlum, 1991) but also attempting to salvage some reproductive success when unable to provide themselves parental care (Öst, 2000). Our data give some support to this hypothesis, since we found that prolactin levels at hatching were negatively correlated with the proportion of body mass lost during incubation. Females that lost a higher proportion of body mass at the end of incubation also exhibit lower prolactin levels and should present a higher rate of brood abandonment (see Bustnes and Erikstad, 1991; Johnsen *et al.*, 1994). However, this correlation does not demonstrate a causal relationship between the body mass loss and the prolactin levels. The variations in prolactinemia could also be due to its implication in the regulation of lipid metabolism in birds (Garrison and Scow, 1975; Hall *et al.*, 1986).

Contrary to what is observed in mallards *A. platyrhynchos* (Hall and Goldsmith, 1983) and turkey hens (*Meleagris gallopavo*; Opel and Proudman, 1989), circulating prolactin in eiders does not decrease to basal levels at hatching (similar to the level of nonincubating captive females in fasting phase II), but is maintained high despite the presence of ducklings. The prolactin level of aunts is also comparable to the level of hatching females. This suggests a possible relationship between posthatching parental care and circulating prolactin at the end of incubation. Therefore, females in good body condition at hatching should present high levels of prolactin and take care of their chicks. Likewise, females without their own ducklings (failed breeders) but in adequate body condition should be more sensitive to duckling stimuli (visual

and tactile). Schmutz *et al.* (1982) found that females which have lost more body mass (breeders which failed when close to hatching) were never observed to behave as aunts afterward, in contrast to females which failed early in the reproductive season and thus have restored their body mass at the time of brooding. However, to confirm the hypothesis of the role of prolactin in the promotion of posthatching parental care in eiders, serial bleeding during the days following the hatching stage will give valuable information. Indeed, prolactin levels continue to decrease until day 4 after hatching in precocial birds (Oring *et al.*, 1988).

Previous studies have shown that brooding of chicks and alloparental behavior can be regulated by a similar endocrine mechanism as incubation (Oring *et al.*, 1988; Vleck *et al.*, 1991; Schoech *et al.*, 1996). In barheaded geese (*A. indicus*), despite the decline in prolactin at hatching, females brooding goslings have higher prolactin levels than those that have failed in reproduction (Dittami, 1981). Gorman (1973) found a comparable result, since prolactin levels in pituitaries of female eiders present in crèche were lower than those of incubating birds, but higher than basal levels of nonbreeding females. In contrast, Schmutz *et al.* (1982) recorded low prolactin levels of eider aunts in Hudson Bay. They suggested that aunts are both failed breeders and nonbreeders that are selecting nesting sites for future years which are temporarily attracted to broods. Despite a lack of information about aunt behavior in Svalbard, we think that this discrepancy in the prolactin levels of aunts may be population dependent, as seems to be the case for crèche formation between eiders living in northern Finland (energetic salvage strategy hypothesis, Öst, 2000) and in the St. Lawrence River Estuary (*accidental hypothesis*, Munro and Bédard, 1977). However, prolactin is probably not the single hormone involved in the control of the parental behavior in eiders. Indeed, corticosterone is known to redirect behavioral and metabolic processes from high energy demanding to emergency activities that potentiate adult bird survival (Silverin, 1986). Thus, determining the concentrations of corticosterone in late incubating females, and its potential relationship with body condition, could help to determine the endogenous mechanisms leading to parental care decisions.

Body Mass, Refeeding, and Prolactin Levels

Prolactin levels in eiders do not simply reflect the presence of eggs, but are under the control of body condition as well. This idea is supported by the hatching date experiment. By shortening the incubation duration (duckling hatching earlier than in the 25-day incubation group), we found that the circulating prolactin is maintained above the level of the control group (Fig. 5). That the shortened group also had a significantly higher body mass than the control group fits well with a relationship between an adequate body condition at hatching and a high final prolactin level (thus promoting postincubating parental care). Moreover, male phalaropes with a shortened incubation period presented an earlier decrease of prolactin than the control group (Oring *et al.*, 1988). The fact that female eiders do not show the same decrease despite a shortened incubation accentuates the idea of a threshold level of prolactin concentration, at least just after hatching, to promote posthatch parental behavior in eiders.

Prolactin levels of the prolonged groups (before and after hatching) are maintained even after 3.9 days of prolonged incubation. This result suggests that an endogenous timing mechanism is not the primary factor regulating the prolactin decrease in eiders, in contrast to albatrosses (Hector and Goldsmith, 1985). Female eider is known to incubate nonviable eggs for 19 days longer than the normal incubation time (Crisuolo *et al.*, 2001). These observations and our results indicate that eider prolactin concentration appears more sensible to environmental stimuli (i.e., presence of eggs). A similar observation was recently made on Adelie penguins by Vleck *et al.* (2000), as there was no modification of prolactin plasma levels after increasing or decreasing the incubation period by about 10 days. These authors suggested that such a phenomenon could be adaptive in species with a naturally variable incubation period (see also Oring *et al.*, 1988). Nevertheless, the natural duration of incubation period does not seem to be subject to variation in eiders (24.9 days in this study; 24.5 days in Parker and Holm, 1990). We rather suggest that the maintenance of prolactin is due to the fact that prolonged birds start to feed again. Indeed, females facing an extended incubation period take longer and more frequent recesses devoted to dive cycles (Crisuolo *et al.*, 2001), in such a way that they hold their body mass at the same level

as the normal hatching birds. Although circulating prolactin should decrease when body condition deteriorates (i.e., during an artificially prolonged incubation), the prolonged females did not show a drop of prolactinemia while still incubating or at hatching. This suggests that refeeding becomes the predominant regulating factor of prolactin secretion or uncouples the influence of the presence of eggs and of body condition on prolactin (Fig. 7). Prolactin is modified in fowls by short fasting (decrease) and refeeding (increase, see Hall *et al.*, 1986). Nevertheless, this behavioral change has a cost in terms of reproduction success. Refeeding for incubating eiders necessitates a dive cycle which can last more than 1 h (F. Criscuolo *et al.*, unpublished data), thus enhancing the chances of predation of the unattended nest (Mehlum, 1991). For a long-lived bird such as the common eider, a tradeoff between current reproductive success and future reproduction is essential. Since the feeding areas for adults and ducklings are often separate, the decision to ensure posthatching parental care is indeed of primary importance for adult survival. We suggest that prolactin in female eiders is partly under the influence of adult body condition and energetic flux which allow the bird to behave or not as a brooding female. A direct link between investment in current reproduction and the amount of body reserves available is still unestablished in birds, but the leptin, implicated in reproduction in mammals, could be a good candidate (Bruneau *et al.*, 1999).

ACKNOWLEDGMENTS

Financial support for this work was provided by the Institut Français pour la Recherche et Technologie Polaires, the Norwegian Polar Institute and the European Community (LSF funding to Ny-Ålesund station). The authors thank the staff of the Norwegian Polar Research Station in Ny-Ålesund and of the CEBC in Chizé and of CEPE in Strasbourg for their help and kindness; H. Lundvigsen, W. Moskal, T. Zorn, J. Durant, and C. Trouvé for their technical assistance during field experiment and prolactin assays; and F. Bertile, D. Gremillet, and S. Massemin for their advises on an earlier draft.

REFERENCES

- Aldrich, T. W., and Raveling, D. G. (1983), Effects of experience and body weight on incubation behavior of Canada geese. *Auk* **100**, 670-679.

- Bruneau, G., Vaisse, C., Caraty, A., and Monget, P. (1999). La leptine: Une clé pour la reproduction. *Med. Sci.* **15**, 191-196.
- Buntin, J. D. (1986). Role of prolactin in avian incubation behaviour and care of young: Is there a causal relationship? *Ann. N. Y. Acad. Sci.* **474**, 252-267.
- Buntin, J. D. (1996). Neural and hormonal control of parental behaviour in birds. *Adv. Study Behav.* **25**, 161-213.
- Bustnes, J. O., and Erikstad, K. E. (1991). Parental care in the common eider (*Somateria mollissima*): Factors affecting abandonment and adoption of young. *Can. J. Zool.* **69**, 1538-1545.
- Chastel, O., Weimerskirch, H., and Jouventin, P. (1995). Influence of body condition on reproductive decision and reproductive success in the Blue Petrel. *Auk* **112**(4), 964-972.
- Chaurand, T., and Weimerskirch, H. (1994). Incubation routine, body mass regulation and egg neglect in the Blue Petrel *Halobaena caerulea*. *Ibis* **136**(3), 285-290.
- Cherel, Y., Robin, J.-P., Walch, O., Karmann, H., Netchitailo, P., and Le Maho, Y. (1988). Fasting in king penguins. I. Hormonal and metabolic changes during breeding. *Am. J. Physiol.* **254**, R170-R177.
- Cherel, Y., Mauget, R., Lacroix, A., and Gilles, J. (1994). Seasonal and fasting-related changes in circulating gonadal steroids and prolactin in King penguins, *Aptenodytes patagonicus*. *Physiol. Zool.* **67**, 1154-1173.
- Cherel, Y., and Groscolas, R. (1998). Relationships between nutrient storage and nutrient utilization in long-term fasting birds and mammals. In "Proceedings of the 22nd International Congress of Ornithology, Durban, South Africa."
- Criscuolo, P., Gabrielsen, G. W., Gendner, J.-P., and Le Maho, Y. (2001). Incubation behaviour and body mass regulation in female Common Eider (*Somateria mollissima*). *J. Avian Biol.*, in press.
- Delahanty, D. J., and Oring, L. W. (1993). Effect of clutch size on incubation persistence in male Wilson's phalaropes (*Phalaropus tricolor*). *Auk* **110**, 521-528.
- Delahanty, D. J., Oring, L. W., Fivizzani, A. J., and El Halawani, M. E. (1997). Circulating prolactin of incubating male Wilson's phalaropes corresponds to clutch size and environmental stress. *Condor* **99**, 397-405.
- Dittami, J. P. (1981). Seasonal changes in the behavior and plasma titers of various hormones in barheaded geese, *Anser indicus*. *Z. Tierpsychol.* **55**, 289-324.
- Drent, R. H., and Daan, S. (1980). The prudent parent: Energetic adjustments in avian breeding. *Ardea* **68**, 225-252.
- Erikstad, K. E., Fauchald, P., Tveraa, T., and Steen, H. (1998). On the cost of reproduction in long-lived birds: The influence of environmental variability. *Ecology* **79**, 1781-1788.
- Erikstad, K. E., and Tveraa, T. (1995). Does the cost of incubation set limits to clutch size in the Common Eider *Somateria mollissima*? *Oecologia* **103**, 270-274.
- Gabrielsen, G. W., Melhum, P., Karlsen, H. E., Andresen, Ø., and Parker, H. (1991). Energy cost during incubation and thermoregulation in the female Common Eider *Somateria mollissima*. *Norsk Polarinstitutt Skrifter* **195**, 51-62.
- Garcia, V., Jouventin, P., and Mauget, R. (1996). Parental care and the prolactin secretion pattern in the king penguin: An endogenously timed mechanism? *Horm. Behav.* **30**, 259-265.
- Garrison, M. M., and Scow, R. O. (1975). Effect of prolactin on lipoprotein lipase in crop sac and adipose tissue of pigeons. *Am. J. Physiol.* **228**, 1542-1544.
- Gorman, M. L. (1973). Pituitary prolactin levels in the Common Eider *Somateria mollissima*. *Ornis. Scand.* **4**, 123-125.
- Hall, M. R., and Goldsmith, A. R. (1983). Factors affecting prolactin secretion during breeding and incubation in the domestic duck (*Anas platyrhynchos*). *Gen. Comp. Endocrinol.* **49**, 270-276.
- Hall, M. R., Harvey, S., and Chadwick, A. (1986). Control of prolactin secretion in birds: a review. *Gen. Comp. Endocrinol.* **62**, 171-184.
- Hall, M. R. (1987). External stimuli affecting incubation behavior and prolactin secretion in the duck (*Anas platyrhynchos*). *Horm. Behav.* **21**, 269-287.
- Hall, M. R. (1991). Endocrinological and behavioral changes associated with the onset of incubation in the duck. *Physiol. Behav.* **50**, 311-316.
- Hector, J. A. L., and Goldsmith, A. R. (1985). The role of prolactin during incubation: comparative studies of three Diomedea albatrosses. *Gen. Comp. Endocrinol.* **60**, 236-243.
- Johnsen, I., Erikstad, K. E., and Saether, B. E. (1994). Regulation of parental investment in a long-lived seabird, the puffin *Fratercula arctica*. *Oikos* **71**, 273-278.
- Jouventin, P., and Mauget, R. (1996). The endocrine basis of the reproductive cycle in the king penguin (*Aptenodytes patagonicus*). *J. Zool. Lond.* **238**, 665-678.
- Korschgen, C. E. (1977). Breeding stress of female Eiders in Maine. *J. Wild. Manage.* **41**(3), 360-373.
- Le Maho, Y. (1977). The Emperor penguin: A strategy to live and breed in the cold. *Am. Sci.* **65**, 680-683.
- Le Maho, Y., Vu Van Kha, H., Koubi, H., Dewasme G., Girard, J., Ferré, P., and Cagnard, M. (1981). Body composition, energy expenditure, and plasma metabolites in long-term fasting geese. *Am. J. Physiol.* **241**, E342-E354.
- Lormée, H., Jouventin, P., Chastel, O., and Mauget, R. (1999). Endocrine correlates of parental care in an antarctic winter breeding seabird, the Emperor penguin, *Aptenodytes forsteri*. *Horm. Behav.* **35**, 9-17.
- Lormée, H., Jouventin, P., Lacroix, A., Lallemand, J., and Chastel, O. (2000). Reproductive endocrinology of tropical seabirds: Sex-specific patterns in LH, steroids, and prolactin secretion in relation to parental care. *Gen. Comp. Endocrinol.* **117**, 413-426.
- Mauget, R., Garcia, V., and Jouventin, P. (1995). Endocrine basis of the reproductive pattern of the Gentoo penguin (*Pygoscelis papua*): Winter, breeding and extended laying period in northern populations. *Gen. Comp. Endocrinol.* **98**, 177-184.
- Meijer, T. (1995). Importance of tactile and visual stimuli of eggs and nest for termination of egg laying of Red Junglefowl. *Auk* **112**, 483-488.
- Mehlum, F. (1991). Egg predation in a breeding colony of the Common Eider *Somateria mollissima* in Kongsfjorden, Svalbard. *Norsk Polarinstitutt Skrifter* **195**, 37-45.
- Moreno, J. (1989). Strategies of mass change in breeding birds. *Biol. J. Linn. Soc.* **37**, 297-310.
- Mrosovsky, N., and Sherry, D. F. (1980). Animal anoxerias. *Science* **207**, 837-842.

- Munro, J., and Bédard, J. (1977). Crèche formation in the Common Eider. *Auk* **94**, 759-771.
- Opel, H., and Proudman, J. A. (1989). Plasma prolactin levels in incubating Turkey hens during pipping of the eggs and after introduction of poults into the nest. *Biol. Reprod.* **40**, 981-987.
- Oring, L. W., Fivizzani, A. J., Colwell, M. A., and El Halawani, M. E. (1988). Hormonal changes associated with natural and manipulated incubation in the sex-role reversed Wilson's phalarope. *Gen. Comp. Endocrinol.* **72**, 247-256.
- Öst, M. (2000). "Feeding Constraints and Parental Care in Female Eiders," thesis.
- Parker, H., and Holm, H. (1990). Patterns of nutrient and energy expenditure in female Common Eiders nesting in the arctic. *Auk* **107**, 660-668.
- Prestrud, P., and Mehlum, F. (1991). Population size and summer distribution of the Common Eider *Somateria mollissima* in Svalbard, 1981-1985. *Norsk Polarinstitutt Skrifter* **195**, 9-20.
- Ramsey, S. M., Goldsmith, A. R., and Silver, R. (1985). Stimulus requirements for prolactin and LH secretion in incubating ring doves. *Gen. Comp. Endocrinol.* **59**, 246-256.
- Reed, A., Hughes, R. J., and Gauthier, G. (1995). Incubation behavior and body mass of female greater snow geese. *Condor* **97**, 993-1001.
- Schoech, S. J., Mumme, R. L., and Wingfield, J. C. (1996). Prolactin and helping behaviour in the cooperative breeding Florida scrub-jay, *Aphelocoma c. coerulescens*. *Anim. Behav.* **52**, 445-456.
- Sharp, P. J., Dawson, A., and Lea, R. W. (1998). Control of luteinizing hormone and prolactin secretion in birds. *Comp. Biochem. Physiol. C* **119**, 275-282.
- Schmutz, J. K., Robertson, R. J., and Cooke, F. (1982). Female sociability in the common eider during brood rearing. *Can. J. Zool.* **60**, 3326-3331.
- Silverin, B., and Goldsmith, A. (1983). Reproductive endocrinology of free living Pied flycatchers (*Ficedula hypoleuca*): Prolactin and FSH secretion in relation to incubation and clutch size. *J. Zool. Lond.* **200**, 119-130.
- Silverin, B., and Goldsmith, A. (1984). The effects of modifying incubation on prolactin secretion in free-living pied flycatchers. *Gen. Comp. Endocrinol.* **55**, 239-244.
- Silverin, B. (1986). Corticosterone-binding proteins and behavioral effects of high plasma levels of corticosterone during the breeding period in the pied flycatcher. *Gen. Comp. Endocrinol.* **64**, 67-74.
- Silverin, B., and Goldsmith, A. (1990). Plasma prolactin concentrations in breeding pied flycatchers (*Ficedula hypoleuca*) with an experimentally prolonged brooding period. *Horm. Behav.* **24**, 104-113.
- Stearns, S. C. (1992). "The Evolution of Life History." Academic Press, London.
- Thompson, S. C., and Raveling, D. G. (1987). Incubation behavior of Emperor geese compared with other geese: Interactions of predation, body size and energetics. *Auk* **104**, 707-716.
- Vleck, C. M., Mays, N. A., Dawson, J. W., and Goldsmith, A. R. (1991). Hormonal correlates of parental and helping behavior in cooperatively breeding Harris' hawks (*Parabuteo unicinctus*). *Auk* **108**, 638-648.
- Vleck, C. M., Ross, L. L., Vleck, D., and Bucher, T. L. (2000). Prolactin and parental behavior in Adélie penguins: Effects of absence from nest, incubation length, and nest failure. *Horm. Behav.* **38**, 149-158.