31 Relationship between nutritional status and prolactin levels in the Common Eider, a capital incubator

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ABSTRACT

In Common Eider, only females incubate while fasting for 25 days. When their body condition is deteriorated at hatching, females often abandon their ducklings. To therefore investigate how body condition may mediate parental care in eiders, we studied the effect of a change in the duration of incubation on their plasma prolactin, i.e. the main parental hormone in birds. Birds with shortened incubation have higher body masses and showed higher levels of plasma prolactin levels at hatching than the control group, suggesting that circulant prolactin at hatching is linked to body condition. The females that underwent an extended incubation started to feed again and displayed a "normal" body mass but unexpectedly a very high plasma prolactin concentration.

Key Words: *Somateria mollissima,* prolactin, Svalbard, incubation, feeding patterns

INTRODUCTION

Prolactin is involved in the initiation and maintenance of incubation behaviour in birds (Hall 1991, Buntin 1996, Sharp *et al.* 1998), in addition to its large variety of actions (i.e. in lipid metabolism, Hall *et al.* 1986). Eggs act as tactile and visual stimuli in most birds, since their removal results in a decrease in plasma prolactin and the termination of parental behaviour (Buntin 1986, Hall & Goldsmith 1983, Ramsey *et al.* 1985). However, prolactin secretion can be modulated by several factors such as food restriction (Hall *et al.* 1986) or be endogenously timed as it has been showed in pelagic birds, prolactin levels being maintained regardless of direct stimulations from the nest or eggs (Hector & Goldsmith 1985, Lormée *et al. 1*999, Garcia *et al.* 1996, Jouventin & Mauget 1996, Vleck *et al.* 2000). Incubation behaviour competes with foraging, thus inducing restricted access to food. Anorexia has evolved as a means to ensure greater nest attendance (Mrosovsky & Sherry 1980), to enhance the chances of hatching success. However, the amount of adult body reserves available to incubate is a key factor in determining incubation constancy (Aldrich & Raveling 1983, Chastel *et al.* 1995, Chaurand & Weimerskirch 1994, Cherel *et al.* 1994). This suggests a potential control of prolactin secretion (thus of parental behaviour) by adult body condition in capital incubators, i.e. birds which does not feed during incubation.

The female Common Eider *Somateria mollissima* can indeed be defined as a true capital incubator (Drent & Daan 1980) since it relies entirely on its body nutrient reserves during 24-26 days of incubation (Korschgen 1977). Moreover, the female behaves as a brood-tender or not, depending on its remaining body reserves at hatching (Bustnes & Erikstad 1991). Knowing that plasma prolactin concentration is high in female eiders taking care of the brood (Criscuolo *et al,* in press) as observed in other species (Ball 1991), we checked whether prolactin is endogenously controlled (i.e. by adult body reserves). To modify the female body condition at hatching, the incubation length was experimentally shortened or prolonged by swapping eggs between nest with different laying dates. Prolonged incubating females were found to start feed again, since we observed successive dive cycles during recesses at sea (Criscuolo *et al.* in press). Thus, for these females, to test whether the nutritional status of the bird can influence its prolactin concentration, we determined the plasma concentrations of triacylglycerols as a sign of feeding and of β-hydroxybutyrate as a sign of fasting (Le Maho *et al.* 1981).

METHODS

The study was conducted in Kongsfjorden, on the western coast of the Svalbard Archipelago (78°55' N), on female eiders nesting on Prins Heinrich Island (1998 and 2000) from early June to mid-July.

Blood sampling

A total of 35 females were used in this study. Before they were released, their body mass was measured with a portable electronic balance (± 2 g) and clutch size recorded. Blood (2.5 ml) was collected from the brachial vein with a 5 ml non-heparinized syringe and a 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°C, and 100 ml 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 C.E.B.C. (Mauget *et al.* 1995, Lormée *et al.* 1999, Lormée *et al.* 2000). Pooled plasma samples produced dose-response curves that paralleled the chicken prolactin standard curves (source: Dr. Parlow, N.H.P.P. Harbor-UCLA Medical Center, U.S.A.). The intra-assay coefficient of variation was 3.3 % (n=4 duplicates). Three assays were performed, with a variation of 14.7%. 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, Ger-many).

Body mass and prolactin levels

In the three years of the study, because female eiders lose 30-40 % of their initial body mass during incubation (Korschgen 1977, Parker & 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 1998 and 2000, we measured the proportion of mass loss by dividing daily mass loss (g / day), by initial body mass (g). The rate of body mass loss is inversely related to the initial body lipid reserves (Cherel & Groscolas 1998).

Manipulation of Incubation duration and hatching date

Incubation duration was artificially shortened or prolonged in free incubating females in 2000 (n=35). We indeed exchanged eggs from nests with different hatching 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 iiii) two control groups: females sampled at the hatching stage, after a natural incubation period of 24.9 ± 0.3 days of incubation (n=ll), 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 to catch birds several times and to the high rate of egg predation in unattended nests during extended incubation (Criscuolo *et al.,* in press). Eggs were exchanged during the first week of incubation and blood samples were taken at the dates given for all groups.

Statistical analysis

Changes in plasma prolactin during the experimentation were compared using a one-way ANOVA (alpha level of significance is 0.05), followed by post-ANOVA Tukey tests. Correlations between prolactin level, body condition and metabolite concentrations were made using Pearson correlation coefficients. Values are expressed as means ± standard error (SE).

RESULTS

Body mass and prolactin levels

There was no significant relationship between initial body mass (days 0-3) and initial plasma concentrations of prolactin *(r* = 0.246, *N*= 15, *P* = 0.377). The plasma concentrations of prolactin measured at the end of incubation (days 21 -24) were unrelated to final body mass *(r* = 0.436, *N* = 8, *P* = 0.280). However, the final plasma prolactin levels of birds sampled at hatching was negatively correlated to the relative daily mass loss *(r* = -0.625, *N = 11, P* = 0.04, Fig. 1).

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. 2). Females of the
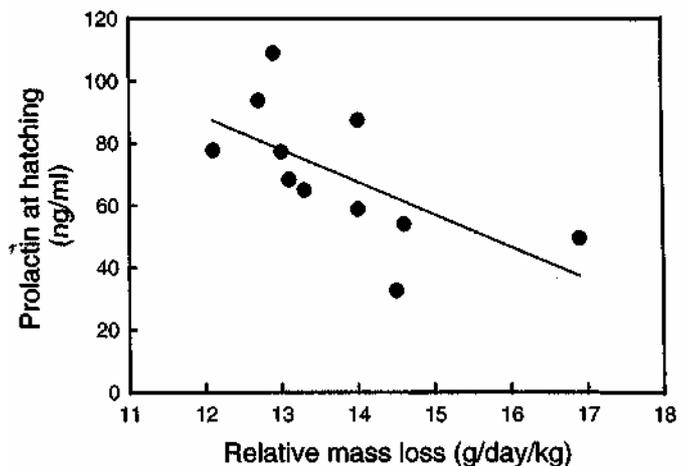

Fig. 1. Prolactin levels at hatching of female common eiders, in relation to the relative body mass lost (daily mass loss/initial body mass) during incubation.
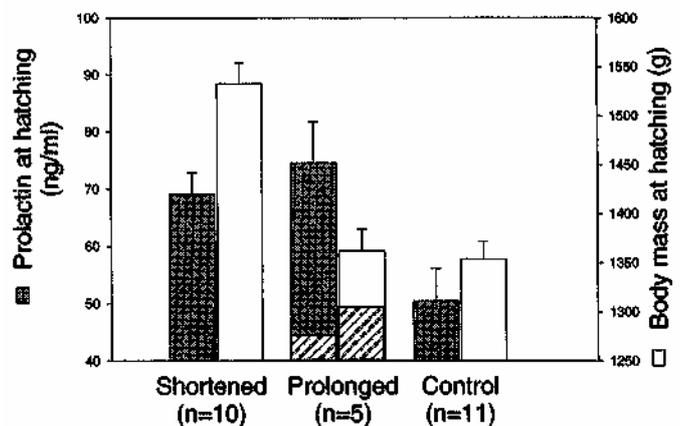

Fig. 2. Average prolactin concentrations and body masses of female common eiders at hatching, after 18.3 ± 0.4 (shortened), 24.9 ± 0.3 (control) and 28.8 ± 1.0 (prolonged) days of incubation. (▨) represents the expected levels in body mass and prolactin levels of females that have faced a prolonged incubation period.
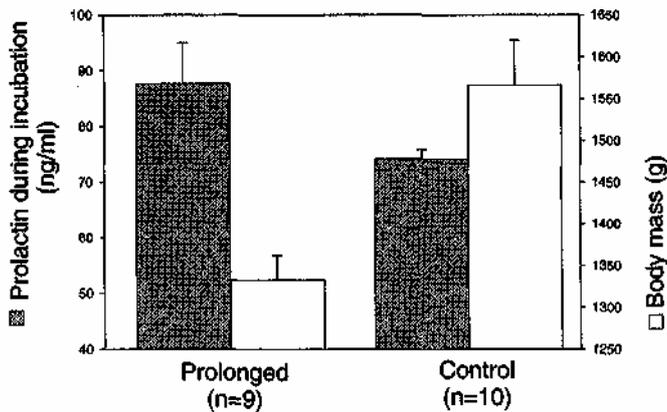
Fig. 3. Average prolactin concentrations and body masses of female common eiders during incubation, after 27.6 ± 0.6 (delayed) and 13.7 ± 2.2 (control) days of incubation.
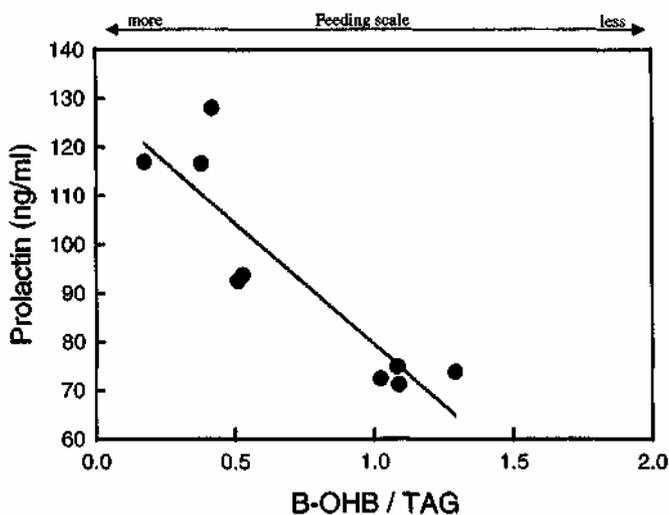

Fig. 4. Prolactin levels of incubating female eiders (n = 9) sampled after 27.6 ± 0.6 (delayed) days of incubation, in relation to their nutritional status estimated by the ratio ß-hydroxybutyrate (B-OHB, indice of fasting) / triacylglycerols (TAG, indice of feeding).

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. 2).

Despite a lower body mass (Student's t-test, $P < 0.05$, Fig. 3), females sampled after 2.7 days of prolonged incubation presented a prolactin level not significantly 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 (that has been feeding) also showed higher prolactin levels $(r = -0.895, N = 9, P < 0.05$, Fig. 4).

DISCUSSION

Mass loss, prolactin levels at hatching and post-hatch parental care
We did not find a direct relationship between body mass and prolactin levels in incubating female eiders. Such a result is consistent with the incubation strategy of capital incubators. Indeed, because those species depend almost exclusively on their endogenous nutrient reserves, they 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 post-hatch parental behaviour. Several studies tried to determine the reasons leading to brood abandonment in eiders (Bustnes & 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 youngs in the same time because feeding and breeding areas are different; Prestrud & 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 exhibited lower prolactin levels and should present a higher rate of brood abandonment (see Bustnes & Erikstad 1991, Johnsen *et al.* 1994). However, this correlation do not demonstrate a causal relationship between the body mass loss and the prolactin levels. The variations in plasma prolactin levels could also be due to its implication in the regulation of lipid metabolism in birds (Garrison and Scow 1975, Hall *et al.* 1986).

Previous studies have shown that brooding of chicks and alloparental behaviour can be regulated by a similar endocrine mechanism as incubation (i.e. by prolactin, Oring *et al.* 1988, Vleck *et al.* 1991, Schoech *et al.* 1996). In barheaded geese *(Anser 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 non-breeding females. However, prolactin is probably not the single hormone involved in the control of the parental behaviour in eiders. Indeed, corticosterone is known to redirect behavioural 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 at hatching was maintained above the level of the control group (Fig. 2). 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 post-incubating parental care).

Prolactin levels of the prolonged groups (before and after hatching) were maintained even after 3.9 days of prolonged incubation. This suggests that an endogenous timing mechanism is not the primary factor regulating the prolactin decrease in eiders, in contrast to albatrosses (Heector & Goldsmith 1985). Female eider is known to incubate nonviable eggs for 19 days longer than the normal incubation time (Criscuolo *et al.,* in press). 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 & Holm 1990). We rather suggest that the high prolactin levels are the consequence that prolonged birds start to feed again (Criscuolo *et al.* in press), in such a way that they hold their body mass at the same level as the normal hatching birds. Although circulating prolactin would have decreased when body condition deteriorates (i.e. during an artificially prolonged incubation), the prolonged females did not show a drop of plasma prolactin levels 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. 4). Prolactin is modified in fowls by short fasting (decrease) and refeeding (increase, see Hall *et al.* 1986). Nevertheless, this behavioural change has a cost in term of reproduction success. Refeeding for incubating eiders necessites a dive cycle which can last more than one hour (Criscuolo *et al.* in press), thus enhancing the chances of predation of the unattended nest (Mehlum 1991).

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