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► **To cite this version:**

S. Bourgeon, T. Raclot. Corticosterone selectively decreases humoral immunity in female eiders during incubation.. *Journal of Experimental Biology*, 2006, 209 (24), pp.4957-4965. 10.1242/jeb.02610 . hal-00129345

HAL Id: hal-00129345

<https://hal.science/hal-00129345>

Submitted on 8 Feb 2007

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1 **Corticosterone selectively decreases humoral immunity in female eiders**
2 **during incubation**

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17 Running title: Corticosterone and immunity in birds

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1 SUMMARY

2 Immunity is hypothesized to share limited resources with other physiological functions and
3 this may partly account for the fitness costs of reproduction. Previous studies have shown that
4 the acquired immunity of female common eider ducks (*Somateria mollissima*) is suppressed
5 during their incubation **during which they entirely** fast. Corticosterone was proposed to be an
6 underlying physiological mechanism for such immunosuppression. Therefore, the current
7 study aimed to assess the effects of exogenous corticosterone on acquired immunity in captive
8 eiders. To this end, females were implanted with corticosterone pellets at different stages of
9 their incubation fast. We measured **total immunoglobulin levels**, T-cell-mediated immune
10 response, body mass and corticosterone levels in these females and compared them with those
11 of control females prior to and after manipulation (i.e. corticosterone pellet implantation). To
12 mimic corticosterone effects on body mass, we experimentally extended fasting duration in a
13 group of females termed 'late fasters'. Implanted females had corticosterone levels 6 times
14 higher and **lost 35 % more mass** than control females. Corticosterone levels in 'late fasters'
15 were similar to those in control females but body mass was 8 % lower in the former. The
16 decrease in the **immunoglobulin levels** of **corticosterone** implanted females was twice as high
17 as in control females, while the T-cell-mediated immune response was not significantly
18 affected by the treatment. We found a decrease in the T-cell-mediated immune response only
19 in 'late fasters' (by 60 %), while the **immunoglobulin level** was not lower in this group than in
20 corticosterone implanted or control females. Our study shows that in incubating eiders,
21 exogenous corticosterone decreased only **humoral immunity**. We suggest that the
22 immunosuppressive effect of corticosterone could be mediated through its effects on body
23 reserves. Further experiments are required to determine the relationship between body
24 condition and immune system in fasting birds.

25 **Key-words:** Birds; Body reserves; Fasting; Glucocorticoids; Immunosuppression

1 INTRODUCTION

2

3 Life-history theory assumes that a major trade-off occurs between reproduction and survival,
4 so that the costs associated with a given reproductive effort might have a deleterious impact
5 on adult survival and future reproduction (Williams, 1966; Stearns, 1992). This concept is
6 based on physiological trade-offs between resource-demanding activities within an individual.
7 Recently, the costs of immune defenses have been emphasized (Gustafsson et al., 1994;
8 Sheldon and Verhulst, 1996; Råberg et al., 1998). Namely, parental effort may induce
9 immunosuppression in birds (Moreno et al. 1999). Acquired immunity can be classified into
10 humoral immunity (mediated by B-lymphocytes) and cell-mediated immunity (mediated by
11 T-lymphocytes) (Roitt et al., 1998). Deerenberg et al. (1997) showed in captive zebra finches
12 (*Taeniopygia guttata*) that their humoral immunity was progressively reduced when the
13 reproductive effort is increased. Similarly, when raising experimentally enlarged broods,
14 female pied flycatchers (*Ficedula hypoleuca*) exhibited reduced T-cell-mediated responses
15 (Moreno et al., 1999). Previous studies have shown that the acquired immunity of female
16 common eiders (*Somateria mollissima*) is suppressed during the incubation fast (Hanssen et
17 al., 2004; Bourgeon et al., 2006), while its experimental activation has strong negative effects
18 on the fitness of female eiders (Hanssen et al., 2004). Whatever the ultimate factors
19 explaining such an immunosuppression in breeding eider ducks, the underlying physiological
20 mechanisms still remain unknown. Among potential proximate factors underlying
21 immunosuppression during reproduction, a link between immunocompetence and hormonal
22 changes has been proposed (see Deerenberg et al., 1997).

23 Glucocorticoids are an essential component of the endogenous immunoregulatory
24 network, while also being associated with stress. Hence, these hormones establish a close
25 endocrine link between immunocompetence and stress (Apanius, 1998). Råberg et al. (1998)

1 hypothesized that corticosterone, secreted during stressful activities, reduces the acquired
2 immune function. However, while the T-cell-mediated immune response was suppressed by
3 **experimentally** elevated corticosterone levels in non-breeding New-Jersey house sparrows
4 (*Passer domesticus*; Martin II et al., 2005), it did not significantly covary with **natural**
5 corticosterone concentrations in breeding barn swallows (*Hirundo rustica*; Saino et al., 2002).
6 Similarly, Bourgeon et al. (2006) found that in female common eiders both components of the
7 acquired immunity decreased independently of plasma corticosterone levels, which itself did
8 not vary significantly over the incubation period. **However, in several bird species, organisms**
9 **are metabolically prepared for a long-term fast** (Le Maho et al., 1981; Cherel et al., 1988;
10 **Lindgård et al., 1992**). Indeed, fasting is first characterized by glycogen reserves exhaustion
11 (phase I) before a long period of protein sparing and preferential mobilization of fat stores
12 (phase II), which is followed by a period of increased net protein catabolism (phase III).
13 **While phases I/II are characterized by the maintenance of low plasma levels of corticosterone,**
14 **phase III of fasting is associated with an increase in plasma corticosterone levels. Such an**
15 **elevation could be responsible for the increase in protein catabolism (proteolysis), because**
16 **corticosterone is known to mobilize peripheral calorie stores for glucose production and**
17 **energy utilization (Robin et al., 1998). Depending on conditions during the breeding season,**
18 **incubating female eiders can enter phase III of fasting (see Le Maho et al., 1981; 1983).**

19 Our main objective in this study was to examine the effects of increased plasma
20 corticosterone levels on both components of the acquired immunity in wild female common
21 eiders at different stages of their incubation fast. To this end, female eiders, nesting in the
22 high arctic, were implanted with corticosterone pellets both at the beginning and at the end of
23 incubation. Subsequently, **female total immunoglobulin levels**, T-cell-mediated immune
24 response, body mass, and plasma corticosterone were measured and compared with those in
25 control birds prior to and after manipulation. To mimic corticosterone effects on body mass,

1 fasting duration in a group of females (termed 'late fasters') was experimentally extended.
2 Body mass loss in these females was therefore increased, while corticosterone levels
3 remained unaltered (birds remained in phase II and did not reach phase III). This allowed us
4 to discriminate the effects of increased body mass loss on the acquired immunity from those
5 of elevated corticosterone levels. We predicted that an increased plasma corticosterone level,
6 which increases proteolysis during the female fast, should have immunosuppressive effects.
7 Implanted females should therefore show T-cell-mediated immune responses and/or an
8 immunoglobulin level lower than in control females but similar to that of 'late fasters'.
9

10 MATERIALS AND METHODS

11

12 The study was conducted in a common eider colony on Prins Heinrich Island, Kongsfjorden,
13 Svalbard Archipelago (78°55' N, 20°07' E) between June and July 2005. The breeding colony
14 in the study site contained about 105 nests. Females in the study area laid between one and
15 five eggs, but a clutch size of three to four eggs was most common (20 % and 61 %, respectively,
16 N=105). Eider ducklings are precocial and are cared for by the female only.
17 Incubation lasts between 24 and 26 days (Korschgen, 1977). All birds started their incubation
18 between June 07 and June 15, the main laying period for the colony. Ducks that laid their
19 eggs after this period were not considered in this study. Ambient temperatures in June and
20 July ranged from 2 to 10°C.

21

22 Sampling protocol

23 Nests were checked at least every second day throughout the study period. This was done to
24 determine initial clutch size but also to investigate the rate of egg predation and nest
25 desertion. A clutch of eggs was considered complete when no additional egg was laid during a

1 two-day period (Yoccoz et al., 2002). Females which suffered partial egg predation were
2 excluded from this study. Female eiders were caught on their nests using a bamboo pole with
3 a nylon snare. Blood was collected from the brachial vein within three minutes of capture. It
4 was stored in tubes containing EDTA (an anticoagulant agent) and kept on ice until being
5 centrifuged in the laboratory (at 10,000 rpm, for five minutes, at 4°C). Plasma samples were
6 stored at -20°C and subsequently used to measure immunoglobulin and corticosterone levels.
7 After blood sampling, body size was recorded (wing and tarsus lengths) and birds were
8 weighed with a portable electronic balance (± 2 g).

9

10 Experimental groups and corticosterone implantation

11 A total of 36 females with mean clutch sizes of three to four eggs (3.69 ± 0.08 eggs, N=36)
12 (mean \pm SE) was used in this study. Freely incubating females were classified into two
13 experimental groups: corticosterone (N=18) and control females (N=18). Because previous
14 results have shown that both components of the acquired immune system are decreased
15 during incubation in eider ducks (Bourgeon et al., 2006), females from both groups were
16 caught during the first part of incubation (10.64 ± 0.62 days, N=22) (mean \pm SE) (11 females
17 from each group) and near the end of incubation (20.07 ± 0.24 days, N=14) (7 females from
18 each group). After capture, a blood sample was taken, and body size and mass were recorded.
19 Half of the females were then implanted with corticosterone pellets (see below), while the
20 others underwent the same procedure without actual implantation. Birds were held in cages
21 for the following 5 days at ambient temperatures, with snow given as fresh water *ad libitum*.
22 Four days after the treatment (18.31 ± 0.87 days into their incubation, N=36) (mean \pm SE),
23 another blood sample was taken, a PHA skin test conducted (see below), and body mass
24 determined. Birds were released 24 hours later, after the PHA skin test had been read.
25 Additionally, in a group termed 'late fasters', which consisted of 7 captive females with mean

1 clutch sizes from two to five eggs (3.43 ± 0.53 eggs, $N=7$) (mean \pm SE), fasting duration was
2 experimentally extended. Birds remained in phase II of fasting during experimentation and
3 never entered into phase III. This protocol allowed to discriminate the effects of increased
4 body mass loss on the acquired immunity from those of elevated corticosterone levels. To this
5 end, these females had been incubating eggs for at least 23 days (24.86 ± 0.40 days, $N=7$)
6 (mean \pm SE), i.e. eggs were close to hatching. We took a blood sample and recorded body size
7 and mass. Females were then held in captivity for five days (29.86 ± 0.40 days into their
8 incubation, $N=7$) (mean \pm SE), at which point a further blood sample was taken, a PHA skin
9 test performed (see below), and body mass recorded. Females were released 24 hours after
10 this, when the PHA skin test had been read.

11 Corticosterone pellets (100 mg, 21 day release, G-111) were obtained from Innovative
12 Research of America (Sarasota, Florida, USA). The implants slowly release the hormone
13 which enters the bloodstream. In preliminary trials we found that this dose was sufficient to
14 induce marked increases in the level of plasma corticosterone and accelerate body mass loss
15 as early as two days after the start of treatment (S. Bourgeon et al., unpublished data). For the
16 implantation of the corticosterone pellets we followed the recommendations of the
17 manufacturer. Briefly, a small patch of skin at the back side of the birds' neck was shaved and
18 disinfected using alcohol and betadine (iodine solution). A small incision equal to the size of
19 the pellet was made and the implant was inserted underneath the skin. The skin was closed
20 with a single stitch, using surgical thread. The wound was cleaned with betadine and sprayed
21 with an aluminium powder. The surgical procedure required less than 10 minutes. Control
22 animals underwent the identical procedure, without actual insertion of a pellet.

23
24
25

1 T-cell-mediated immune response: PHA skin test

2 For this test we challenged one wing-web with the mitogenic phytohemagglutinin (PHA),
3 while the other wing-web (control) was injected with phosphate buffered saline (PBS).
4 Briefly, 100 μl of 5 $\text{mg}\cdot\text{ml}^{-1}$ PHA (Sigma L 8754) in PBS were injected intradermally into the
5 right wing-web, while the left wing-web was injected with an equal volume of PBS. This
6 procedure was shown to induce little physiological stress in birds (Merino et al., 1999).
7 Injection sites on the wing-web were measured with a micrometer calliper (three readings)
8 just before and 24 h after injection with PHA or PBS. The T-cell-mediated immune response
9 was taken as the difference between the two wing-web swellings.

10

11 Immunoglobulin levels: ELISA test

12 A sensitive ELISA method was used to determine the amount of serum immunoglobulins in
13 eider duck blood. This method using commercial anti-chicken antibodies has so far been
14 validated in six wild avian species (Martinez et al., 2003). Despite the fact that Anseriforms
15 have an additional immunoglobulin isotype (IgY), which is not found in other birds (Parham,
16 1995), we assumed linear cross-reactivity. Accordingly, the values obtained were used as
17 **relative immunoglobulin levels.**

18 To determine the linear range of the sigmoid curve, ELISA plates were coated with serial
19 dilutions of serum (100 μl) in carbonate–bicarbonate buffer (0.1 M, pH = 9.6) and incubated
20 overnight at 4°C. We selected the data obtained from trials using the serum dilution nearest to
21 the centre of its linear range. ELISA plates were then coated with 100 μl of diluted serum
22 samples from female eiders (two samples per female diluted to 1/32000 in carbonate-
23 bicarbonate buffer) and incubated for 1 h at 37°C. After a second incubation overnight at 4°C,
24 the plates were washed with a solution (200 μl) of phosphate buffer saline and Tween (PBS-
25 Tween), and a diluent (100 μl), containing 5% powdered milk in PBS was added. Following

1 incubation for 1 h at 37°C, the plates were washed with PBS-Tween buffer. 100 µl of anti-
2 chicken conjugate (Sigma A 9046) was added at 1:250 and the plates were incubated for 2 h
3 at 37°C. After three washes, the plates were filled with 100 µl of a solution consisting of 2,2'-
4 azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid (ABTS) and concentrated hydrogen
5 peroxide diluted to 1:1000. Following incubation for 1 h at 37°C, absorbance was measured at
6 405 nm using a plate spectrophotometer (Awareness Technology, Inc., Palm City, FL 34991,
7 USA).

8 9 Assessment of the corticosterone levels

10 Corticosterone concentrations were determined by radioimmunoassay (RIA) in our laboratory
11 using an ¹²⁵I RIA double antibody kit from ICN Biomedicals (Costa Mesa, CA, USA). The
12 corticosterone RIA had an intra-assay variability of 7.1 % (N=10 duplicates) and an inter-
13 assay variability of 6.5 % (N=15 duplicates).

14 15 Statistical analysis

16 Statistical analysis was conducted with SPSS 12.0.1 (SPSS Inc., Chicago, IL, USA). Values
17 are means ± standard error (SE). Corticosterone levels were not normally distributed
18 (Kolmogorov-Smirnov test, P<0.05). Hence, data were log transformed to meet parametric
19 assumptions, before parametric tests were used. Repeated measures two-way ANOVA was
20 used to test for the effects of the treatment and incubation stage on corticosterone levels, body
21 mass and **immunoglobulin level**. Two-way ANOVA was used to test for the effects of the
22 treatment and incubation stage on the T-cell-mediated immune response. Linear regression
23 analysis was used to assess the relationships between all parameters measured.

24

25

1 RESULTS

2

3 Tables 1 and 2 provide biological, hormonal and immune profiles for the female eider ducks
4 (before and after implantation) sampled during the first part and near the end of their
5 incubation period, respectively. Initial clutch size, tarsus and wing lengths, days of
6 incubation, body mass, immunoglobulin level and corticosterone levels were not significantly
7 different between the experimental and control group before manipulation (i.e. corticosterone
8 pellet implantation) at both incubation stages.

9

10 Effects of corticosterone implants on body mass:

11 As expected, implants induced a significant increase in corticosterone levels (repeated
12 measures two-way ANOVA: effects of repetition: $F_{1,32}=65.95$, $P<0.0001$; effects of treatment:
13 $F_{1,32}=54.16$, $P<0.0001$; effects of incubation stage: $F_{1,32}=0.06$, $P=0.81$; interaction: $F_{1,32}=0.73$,
14 $P=0.40$). Four days after implantation, corticosterone levels in implanted birds were 6 times
15 higher than in control females, independent of incubation stage. Moreover, corticosterone
16 levels in 'late fasters' were far below the levels of implanted females. In fact, they were not
17 significantly different from the levels in control females sampled at the end of their incubation
18 period (T-test: $t=-0.03$; $N=25$; $P=0.97$). Body mass in corticosterone implanted females was
19 significantly decreased by 18 %, while it only decreased by 12 % in control females (repeated
20 measures two-way ANOVA: effects of repetition: $F_{1,32}=1191.35$, $P<0.0001$; effects of
21 treatment: $F_{1,32}=30.46$, $P<0.0001$; effects of incubation stage: $F_{1,32}=0.10$, $P=0.33$; interaction:
22 $F_{1,32}=0.78$, $P=0.38$). Four days after implantation, implanted females were significantly lighter
23 than control females (by 8 %), independent of the incubation stage. In fact, body mass loss
24 per day in implanted females was 35 % higher than in control females, regardless of the
25 incubation stage (two-way ANOVA: effects of treatment: $F_{1,32}=25.89$, $P<0.0001$; effects of

1 incubation stage: $F_{1,32}=1.29$, $P=0.26$; interaction: $F_{1,32}=0.01$, $P=0.92$). Corticosterone levels in
 2 birds during early incubation were negatively related to body mass only after the implantation
 3 (Table 3), when high corticosterone levels were associated with low body mass values.
 4 However, there was no significant relationship between these parameters in birds near the end
 5 of their incubation period, neither before nor after implantation (Table 4). Finally, body mass
 6 of 'late fasters' was not significantly different from that of implanted females but was
 7 significantly lower than in control females sampled at the end of their incubation (8 %; Table
 8 2) (repeated measures one-way ANOVA: effects of repetition: $F_{1,18}=707.63$, $P<0.0001$;
 9 effects of **treatment**: $F_{2,18}=10.89$, $P=0.001$).

10

11 Effects of corticosterone on the T-cell-mediated immune response and **immunoglobulin level**:

12 Corticosterone implants had no significant effect on the T-cell-mediated immune response
 13 (two-way ANOVA: effects of **treatment**: $F_{1,32}=2.49$, $P=0.12$; effects of incubation stage:
 14 $F_{1,32}=2.94$, $P=0.10$; interaction: $F_{1,32}=0.003$, $P=0.95$; Fig. 1). Responses in implanted females
 15 were similar to that of control females, independent of the incubation stage. However, the
 16 immune response in 'late fasters' was significantly reduced when compared with implanted
 17 and control females at the end of their incubation (53 % and 63 %, respectively) (one-way
 18 ANOVA: effects of **treatment**: $F_{2,18}=9.39$, $P=0.002$; Fig. 1). There was no relationship
 19 between the T-cell-mediated immune response and plasma corticosterone levels at any stage
 20 (Tables 3 and 4). However, there was a positive significant relationship between the T-cell-
 21 mediated immune response and body mass but only during early incubation (Tables 3 and 4),
 22 so that the immune response was stronger in heavier females.

23 After four days, the **immunoglobulin level** in implanted females was significantly
 24 decreased by 45 and 33 %, when sampled during early and late incubation, respectively. The
 25 corresponding decline in **immunoglobulin level** of control females was only 25 % and 25 %

1 (repeated measures two-way ANOVA: effects of repetition: $F_{1,32}=105.10$, $P<0.0001$; effects
2 of **treatment**: $F_{1,32}=7.34$, $P=0.01$; effects of incubation stage: $F_{1,32}=7.14$, $P=0.01$; interaction:
3 $F_{1,32}=2.77$, $P=0.11$; Fig. 2), indicating that corticosterone pellet implantation had a negative
4 effect on the female **immunoglobulin level**. This effect was stronger when implantation
5 occurred during early incubation rather than during late incubation. The **immunoglobulin**
6 **level** in 'late fasters' was not significantly different from that of implanted or control females
7 sampled near the end of their incubation (repeated measures one-way ANOVA: effects of
8 repetition: $F_{1,18}=78.15$, $P<0.0001$; effects of **treatment**: $F_{2,18}=0.74$, $P=0.49$; Fig. 2). We found
9 no relationship between the immunoglobulin and plasma corticosterone levels, neither before
10 nor after the treatment during early incubation. The same held true for the period after
11 implantation, when birds were near the end of incubation. By contrast, both parameters were
12 negatively related before implantation of ducks that were near the end of the incubation
13 period. High corticosterone levels were then associated with a low **immunoglobulin level**
14 (Table 4; Fig. 3). Furthermore, there was no relationship between the **immunoglobulin level**
15 and body mass, neither before nor after the treatment at any stage of incubation (Tables 3 and
16 4). Finally, we did not find a relationship between both components of the acquired immunity
17 after hormone implantation at any stage of the eider duck incubation period (Tables 3 and 4).
18 Hence, the **immunoglobulin level** was independent of the T-cell-mediated immune response.

19

20 **DISCUSSION**

21

22 It has previously been shown that the acquired immunity is significantly decreased during the
23 incubation fast of female common eiders (Hanssen et al., 2004; Bourgeon et al., 2006).
24 **Among other potential scenarios**, reduced immunocompetence during reproduction has been
25 attributed to hormonal regulation, in particular through the action of glucocorticoids

1 (Deerenberg et al., 1997; Råberg et al., 1998). Hence, the main objective of the current study
2 was to investigate the potential physiological mechanisms underlying such
3 immunosuppression. We therefore assessed the effects of exogenous corticosterone on both
4 components of the acquired immune system in female eiders during different stages of their
5 incubation fast. In addition, a group of females whose fasting duration was experimentally
6 extended was used to discriminate the effects of increased body mass loss on the acquired
7 immunity from those of elevated corticosterone levels.

8 Experimentally increased plasma corticosterone levels only affected one of the two
9 components of the acquired immunity. The **immunoglobulin level** in implanted females was
10 significantly decreased when compared with that of control females. This response was
11 strongest when birds were implanted at the beginning of their incubation fast. However, the
12 T-cell-mediated immune response was not significantly affected by the treatment.
13 Paradoxically, there was no significant relationship between plasma corticosterone and
14 **immunoglobulin levels** after the implantation at any incubation stage. By contrast, before the
15 treatment in ducks that were near the end of their incubation, high corticosterone levels
16 seemed to be associated with a low **immunoglobulin level**.

17 **Whatever the effects of exogenous corticosterone** and similar to an earlier
18 investigation (Bourgeon et al., 2006), we did not find a significant relationship between both
19 components of the acquired immunity. This lends support to the view that variations in one
20 component of the acquired immunity are not necessarily a reliable indicator of changes in the
21 other (Norris and Evans, 2000). In fact, in the present study, the **immunoglobulin level**
22 seemed to be more sensitive to corticosterone treatment than the T-cell-mediated immune
23 response. In control females, the **immunoglobulin level** significantly decreased throughout
24 incubation, while the T-cell-mediated immune response did not vary significantly. This would
25 seem to contrast with the finding of a previous investigation (Bourgeon et al., 2006)

1 supporting that both components significantly decrease throughout the incubation fast of eider
2 ducks. This apparent discrepancy could hold in the fact that smaller sample sizes have been
3 used, what is reinforced by high variances observed in this immune response. Moreover, we
4 can not exclude the possibility that effects of corticosterone on the T-cell-mediated immune
5 response might require more time (see Dhabhar and Mc Ewen, 1997) and/or higher
6 corticosterone concentrations. In the current study, a significant decrease in the T-cell-
7 mediated immune response was only observed in 'late fasters', while their immunoglobulin
8 level was not lower than that of corticosterone implanted or control females. Implanted
9 females significantly lost more weight than control females, which is consistent with the
10 observation by Cherel et al. (1988) that high levels of corticosterone increase proteolysis in
11 fasting birds. In a preliminary study on captive female eiders, high doses of exogenous
12 corticosterone, administered for a few days, induced a rise in plasma levels of uric acid,
13 indicating protein breakdown (Criscuolo et al., 2005). Corticosterone levels of 'late fasters' in
14 the current study were similar to that of control females but body mass was 8 % lower in the
15 former. Hence, the T-cell-mediated immune response appears to be more sensitive to body
16 mass loss than to elevated levels of corticosterone, supporting the view of an indirect effect of
17 corticosterone on this immune parameter. Accordingly, in the present study we found a
18 positive relationship between body mass and T-cell-mediated immune response, where the
19 response was stronger in females with a greater body mass. By contrast, the immunoglobulin
20 level appears to be more sensitive to high corticosterone levels than to body mass loss.
21 Nevertheless, corticosterone levels and body mass were negatively related in the current
22 study, so that high levels of corticosterone were associated with low body mass. Our results
23 agree with previous findings from breeding black-legged kittiwakes (*Rissa tridactyla*), where
24 high corticosterone levels were associated with a marked decline in body condition (Kitaysky
25 et al., 1999). In this context, it would be interesting for future studies 1) to experimentally

1 extend the fasting duration of eiders until they reach phase III of fasting or 2) to find free-
2 ranging birds which spontaneously shift from lipid to protein utilization, so that the effects of
3 both elevated corticosterone levels and decreased body mass on the acquired immunity can be
4 examined.

5 The present study showed that an increasing body mass loss, caused either by
6 corticosterone administration, or by an experimental extension of fasting duration, **negatively**
7 **affected the birds' immunoglobulin level and their T-cell-mediated immune response,**
8 **respectively.** This result lends support to the resource-limitation hypothesis which predicts
9 that investment in costly behaviours, such as reproduction, reduces the amount of resources
10 available to other systems, such as the immune system (**Sheldon and Verhulst, 1996**; Råberg
11 et al., 1998). However, evidence for an energetically costly immune response is still equivocal
12 (Råberg et al., 1998; Eraud et al., 2005; Verhulst et al., 2005). **Whatever the ultimate factors**
13 **explaining immunosuppression during reproduction,** corticosterone was proposed to regulate
14 immunosuppression in incubating birds (Deerenberg et al., 1997; Råberg et al., 1998; Saino et
15 al., 2003). However, during fasting corticosterone should be maintained at low levels to avoid
16 metabolism disorders, such as protein catabolism (Cherel et al., 1988). In the current study we
17 did not find marked variations in corticosterone levels during the incubation period of control
18 and 'late fasting' females. However, 'late fasters' had a lower T-cell-mediated immune
19 responses than corticosterone implanted or control females. This would support the view that
20 fasting duration and/or body composition (see below) might be relevant parameters for
21 immunosuppression.

22 Other aspects of immunosuppression, namely humoral immunity, could be mediated
23 by factors related to fuel utilization or body mass loss. Such a relationship between energy
24 storage/mobilization and immunocompetence might plausibly be mediated through nutritional
25 and/or endocrine factors (Apanius, 1998). Exogenous administration of corticosterone is

1 likely to increase proteolysis. Consequently, lean body mass will be decreased, while the
2 energy stored as lipids within the body will be spared. Hence, for the same final body mass,
3 adiposity of 'late fasters' should be lower than for corticosterone implanted females as
4 reported to be the case in dark-eyed juncos (*Junco hyemalis*) treated with corticosterone (Gray
5 et al., 1990). Currently, adipose tissue is perceived as an active participant in the regulation of
6 essential and prominent body processes such as immune homeostasis (Matarese and La Cava,
7 2004). This raises the question of how body reserves might control the immune system
8 (Demas and Sakaria, 2005). Some adipose humoral signals, such as leptin, are generated in
9 proportion to fat stores and act on feedback control systems to influence numerous biological
10 processes (Löhmus and Sundtröm, 2004; Matarese et al., 2005). In fact, leptin is secreted
11 primarily by adipose tissue and has been shown to enhance a variety of immunological
12 parameters in mammals (Lord et al., 1998; Faggioni et al., 2001; Demas et al., 2003) and
13 birds (Löhmus et al., 2004). Consequently, leptin levels might be lower in 'late fasters' when
14 compared with corticosterone implanted eiders. To gain further insight into the role that leptin
15 plays for the immune system of fasting birds, plasma measurements of leptin and
16 manipulation of its circulating concentrations would be useful.

17 In conclusion, exogenous corticosterone decreased only one component of the
18 acquired immune system in incubating female eider ducks. While the treatment significantly
19 decreased their immunoglobulin level, their T-cell-mediated immune response was not
20 affected. Implanted females lost significantly more weight than control birds. Females, whose
21 fasting duration was experimentally extended, increasing body mass loss, displayed lower T-
22 cell-mediated immune responses than implanted females, while their corticosterone levels
23 remained at baseline values. Consequently, the immunosuppressive effect of corticosterone
24 appears to be mediated by its effect on body reserves, which have been shown to play an
25 important role in the regulation of the immune system. For example, leptin, which conveys

1 information on energy availability, could be involved in the observed immunosuppression.
2 Further experiments are required to determine the relationship between body condition and
3 immune function in incubating female eiders. Our results raise the question of the
4 physiological mechanisms which can explain the effects of corticosterone on the immune
5 response. Furthermore, could it be that depending on its concentration, this hormone is able to
6 trigger different responses, as has already been reported in the context of foraging behaviour
7 (Wingfield et al., 1998).

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1 **ACKNOWLEDGMENTS**

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3 Financial support for this study was provided by the Institut polaire français Paul Emile
4 Victor (IPEV) and through a fellowship from the French MENRT to S. Bourgeon. The study
5 was approved by the Norwegian Animal Research Authority and the Ethic Committee of the
6 Institut Polaire Français Paul-Emile Victor. Permission to work on Common Eiders was also
7 granted by the Governor of Svalbard.

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1 **Table 1.** Profiles for both experimental groups of captive female eiders (corticosterone and
 2 control group) which were sampled during their early phase of incubation. ‘Time of sampling’
 3 indicates incubation stage. Values are means \pm SE. Lower case a and b indicate a significant
 4 difference between groups (T-tests).

Before implantation	Group 1: Corticosterone females (N=11)	Group 2: Control females (N=11)
Initial clutch size (eggs)	3.82 \pm 0.12 ^a	3.54 \pm 0.16 ^a
Tarsus length (cm)	6.15 \pm 0.05 ^a	6.21 \pm 0.04 ^a
Wing length (cm)	29.14 \pm 0.15 ^a	29.45 \pm 0.17 ^a
Time of sampling (days)	10.27 \pm 0.76 ^a	11.00 \pm 1.00 ^a
Body mass at sampling (g)	1687 \pm 39 ^a	1753 \pm 34 ^a
Immunoglobulin level (absorbance units)	1.04 \pm 0.06 ^a	0.94 \pm 0.07 ^a
Corticosterone (ng.ml ⁻¹)	14.34 \pm 2.17 ^a	14.11 \pm 1.63 ^a
After implantation	Group 1: Corticosterone females (N=11)	Group 2: Control females (N=11)
Time of sampling (days)	14.27 \pm 0.76 ^a	15.00 \pm 1.00 ^a
Body mass at sampling (g)	1430 \pm 33 ^a	1575 \pm 32 ^b
Total body mass loss (g)	257.09 \pm 13.12 ^a	178.55 \pm 8.42 ^b
Immunoglobulin level (absorbance units)	0.57 \pm 0.05 ^a	0.70 \pm 0.05 ^a
T-cell-mediated immune response (mm)	0.72 \pm 0.14 ^a	0.97 \pm 0.18 ^a
Corticosterone (ng.ml ⁻¹)	109.90 \pm 8.16 ^a	14.70 \pm 1.85 ^b

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1 **Table 2.** Profiles for the three experimental groups of captive female eiders (corticosterone,
 2 control, and ‘late fasters’), which were sampled near the end of their incubation. ‘Time of
 3 sampling’ indicates incubation stage. Values are means \pm SE. Lower case a, b and c indicate a
 4 significant difference between groups (one-way ANOVA).

Before implantation	Group 1: Corticosterone females (N=7)	Group 2: Control females (N=7)	Group 3: Late fasting females (N=7)
Initial clutch size (eggs)	3.71 \pm 0.18 ^a	3.71 \pm 0.18 ^a	3.43 \pm 0.53 ^a
Tarsus length (cm)	6.16 \pm 0.05 ^a	6.07 \pm 0.04 ^a	6.01 \pm 0.06 ^a
Wing length (cm)	29.19 \pm 0.24 ^a	29.14 \pm 0.20 ^a	29.29 \pm 0.18 ^a
Time of sampling (days)	20.29 \pm 0.29 ^a	19.86 \pm 0.40 ^a	24.86 \pm 0.40 ^b
Body mass at sampling (g)	1549 \pm 27 ^a	1571 \pm 37 ^a	1441 \pm 36 ^b
Immunoglobulin level (absorbance units)	0.70 \pm 0.06 ^a	0.70 \pm 0.04 ^a	0.82 \pm 0.07 ^a
Corticosterone (ng.ml ⁻¹)	18.36 \pm 3.09 ^a	18.35 \pm 4.45 ^a	13.26 \pm 1.99 ^a
After implantation	Group 1: Corticosterone females (N=7)	Group 2: Control females (N=7)	Group 3: Late fasting females (N=7)
Time of sampling (days)	24.29 \pm 0.29 ^a	23.86 \pm 0.40 ^a	29.86 \pm 0.40 ^b
Body mass at sampling (g)	1315 \pm 23 ^{a,b}	1394 \pm 36 ^a	1285 \pm 27 ^b
Total body mass loss (g)	234.00 \pm 15.93 ^a	177.14 \pm 9.54 ^b	155.43 \pm 10.45 ^b
Immunoglobulin level (absorbance units)	0.47 \pm 0.03 ^a	0.52 \pm 0.04 ^a	0.57 \pm 0.05 ^a
T-cell-mediated immune response (mm)	1.00 \pm 0.13 ^a	1.27 \pm 0.18 ^a	0.47 \pm 0.08 ^b
Corticosterone (ng.ml ⁻¹)	111.10 \pm 18.06 ^a	22.33 \pm 5.29 ^b	16.72 \pm 2.21 ^b

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1 **Table 3.** Results for linear regressions between immune parameters, body mass, and
 2 corticosterone level in captive female eiders, sampled during the first part of incubation. Signs
 3 given into brackets indicate positive or negative relationships.

Before implantation	Body mass (g)	Immunoglobulin level (absorbance units)	Corticosterone (ng.ml ⁻¹)	
Body mass (g)	-	F _{1,21} =1.19, P=0.29 (+)	F _{1,21} =0.07, P=0.80 (+)	
Immunoglobulin level (absorbance units)		-	F _{1,21} =3.99, P=0.06 (+)	
Corticosterone (ng.ml ⁻¹)			-	

After implantation	Body mass (g)	Immunoglobulin level (absorbance units)	T-cell-mediated immune response (mm)	Corticosterone (ng.ml ⁻¹)
Body mass (g)	-	F _{1,21} =2.37, P=0.14 (+)	F _{1,21} =5.86, P=0.02 (+)	F _{1,21} =6.80, P=0.02 (-)
Immunoglobulin level (absorbance units)		-	F _{1,21} =3.58, P=0.07 (+)	F _{1,21} =2.68, P=0.12 (-)
T-cell-mediated immune response (mm)			-	F _{1,21} =1.13, P=0.30 (-)
Corticosterone (ng.ml ⁻¹)				-

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1 **Table 4.** Results for linear regressions between immune parameters, body mass, and
 2 corticosterone level in captive female eiders, sampled near the end of incubation. Signs given
 3 into brackets indicate positive or negative relationships.

Before implantation	Body mass (g)	Immunoglobulin level (absorbance units)	Corticosterone (ng.ml ⁻¹)	
Body mass (g)	-	F _{1,20} =3.00, P=0.10 (-)	F _{1,20} =0.26, P=0.61 (-)	
Immunoglobulin level (absorbance units)		-	F _{1,20} =7.70, P=0.01 (-)	
Corticosterone (ng.ml ⁻¹)			-	

After implantation	Body mass (g)	Immunoglobulin level (absorbance units)	T-cell-mediated immune response (mm)	Corticosterone (ng.ml ⁻¹)
Body mass (g)	-	F _{1,20} =4.16, P=0.05 (-)	F _{1,20} =0.17, P=0.68 (+)	F _{1,20} =0.43, P=0.52 (-)
Immunoglobulin level (absorbance units)		-	F _{1,20} =1.16, P=0.29 (-)	F _{1,20} =2.76, P=0.11 (-)
T-cell-mediated immune response (mm)			-	F _{1,20} =1.04, P=0.32 (+)
Corticosterone (ng.ml ⁻¹)				-

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1 **FIGURE LEGENDS**

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3 **Fig 1.** Effects of treatment on wing-web swelling in female eiders: corticosterone implanted
4 (hatched bars), sham implanted (plain bars), and 'late fasting' females (cross hatched bars).
5 Values are means \pm SE. Lower case a and b indicate a significant difference between groups
6 (LSD post-hoc tests).

7

8 **Fig 2.** Effects of treatment on immunoglobulin level before (hatched bars) and after (plain
9 bars) manipulation in female eiders. Values are means \pm SE. Lower case a, b, c and d indicate
10 a significant difference between groups (LSD post-hoc tests).

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12 **Fig 3.** Relationship between corticosterone level and immunoglobulin level before
13 implantation in female eiders sampled near the end of incubation.

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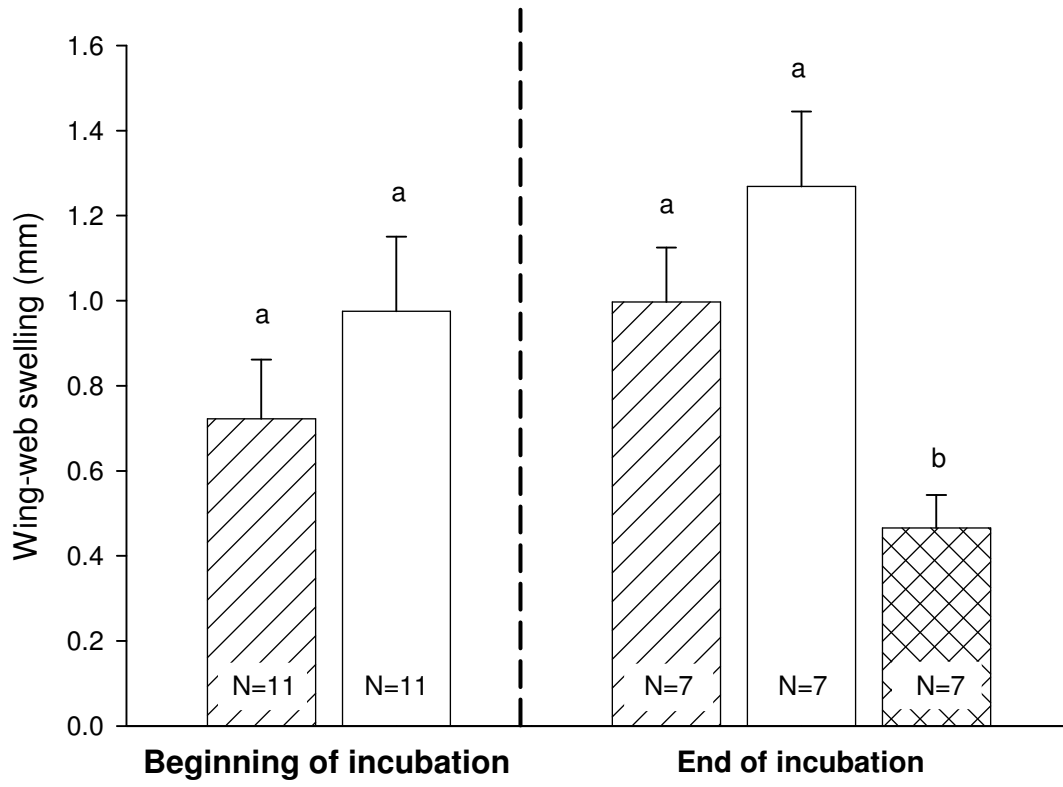
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1 **FIGURE 1.**

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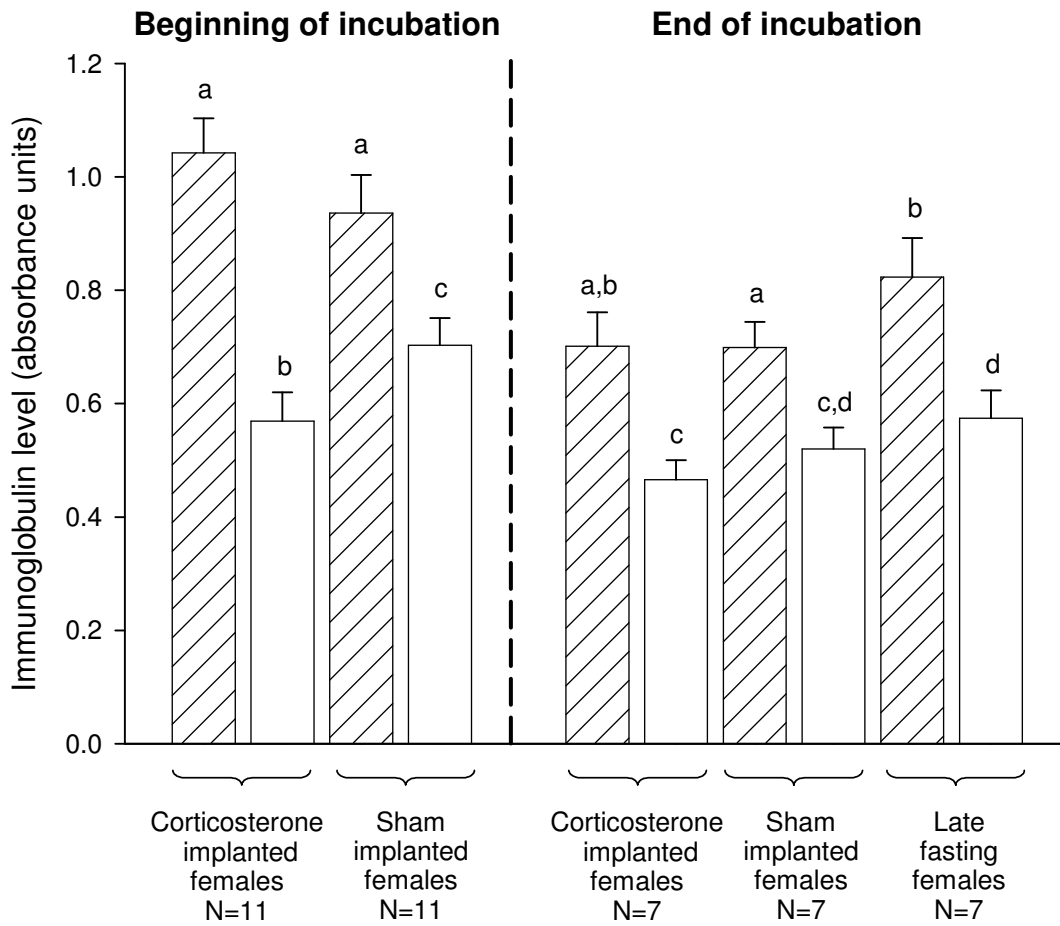
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1 **FIGURE 2.**

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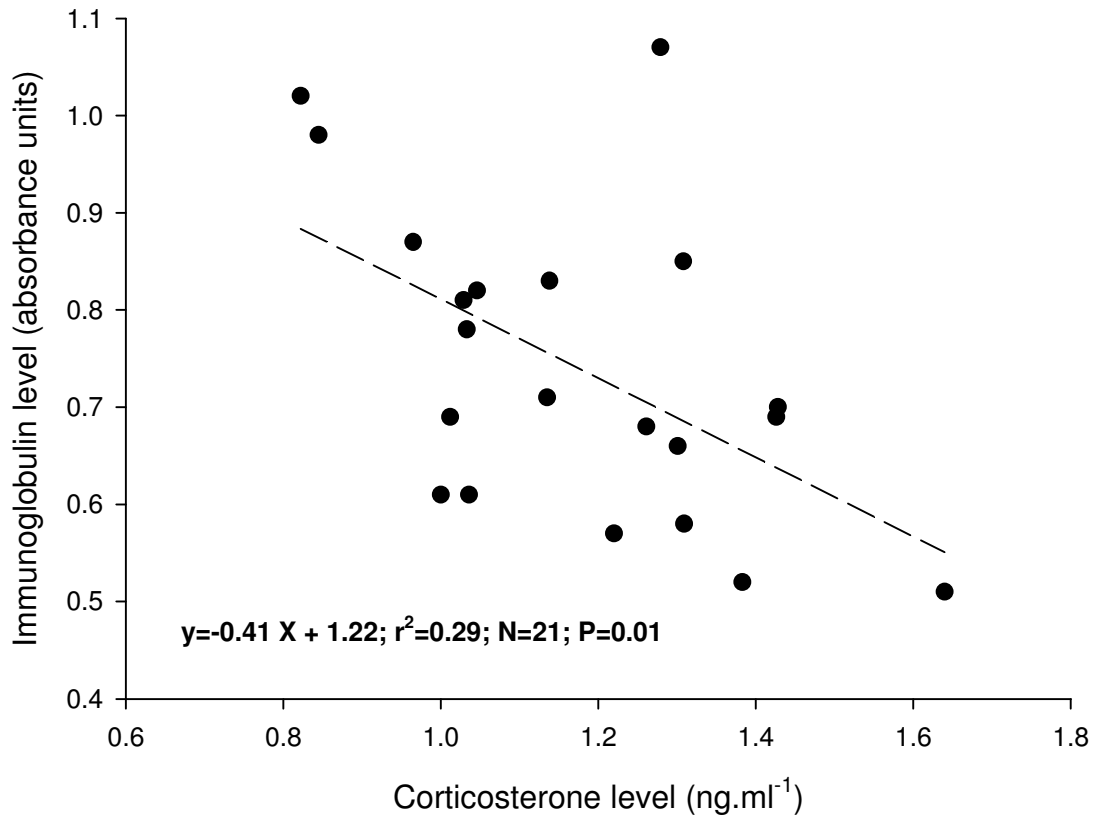
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1 **FIGURE 3.**

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