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## Triiodothyronine suppresses humoral immunity but not T-cell-mediated immune response in incubating female eiders (*Somateria mollissima*).

S. Bourgeon, T. Raclot

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1 **Triiodothyronine suppresses humoral immunity but not T-cell-mediated**  
2 **immune response in incubating female eiders (*Somateria mollissima*).**

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5 Sophie Bourgeon and Thierry Raclot

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9 *Institut Pluridisciplinaire Hubert Curien (IPHC), Département Ecologie, Physiologie et*  
10 *Ethologie (DEPE), UMR 7178 CNRS-ULP,*

11 *23 rue Becquerel, F-67087 Strasbourg Cedex 2, France*

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17 Running title: Triiodothyronine and acquired immunity in eiders

18

19 Address for correspondence:

20 Sophie Bourgeon

21 IPHC, Département Ecologie, Physiologie et Ethologie, UMR 7178 CNRS-ULP,

22 23 rue Becquerel, F-67087 Strasbourg Cedex 2, France

23 Tel: (33) 3.88.10.69.15

24 Fax: (33) 3.88.10.69.06

25 e-mail: [sophie.bourgeon@c-strasbourg.fr](mailto:sophie.bourgeon@c-strasbourg.fr)

1 **ABSTRACT**

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3 Immunity is believed to share limited resources with other physiological functions and this  
4 may partly account for the fitness costs of reproduction. Previous studies have shown that the  
5 acquired immunity of female common eiders (*Somateria mollissima*) is suppressed during the  
6 incubation fast. To save energy, triiodothyronine (T<sub>3</sub>) is adaptively decreased during fasting in  
7 most bird species, despite T<sub>3</sub> levels are maintained throughout incubation in female eiders.  
8 However, the relationship between thyroid hormones and the immune system is not fully  
9 understood. The current study aimed to determine the endocrine mechanisms that underlie  
10 immunosuppression in incubating female eiders. To this end we assessed the effects of  
11 exogenous T<sub>3</sub> on both components of the acquired immune system in 42 free-ranging  
12 incubating birds. Half of the females were implanted with T<sub>3</sub> pellets, while the other half  
13 sham implanted served as control. We measured variations in the immunoglobulin index, the  
14 T-cell-mediated immune response, body mass, and plasma parameters in both groups before  
15 and after manipulation. T<sub>3</sub> levels in implanted females were 4 times higher and mass loss was  
16 40 % greater than in control females. Implanted females also showed an 18 % decrease in the  
17 immunoglobulin index, while the T-cell-mediated immune response was not significantly  
18 affected by the treatment. Finally, the treatment did not induce any significant changes in  
19 corticosterone levels. Our study shows that exogenous T<sub>3</sub> decreased only one component of  
20 the acquired immune system. We suggest that the immunosuppressive effect of T<sub>3</sub> could be  
21 mediated by its effects on body fat reserves. Further experiments are required to determine 1)  
22 the relationship between adiposity and immune function, 2) the adaptive significance of  
23 immunosuppression during incubation in eiders.

24

25 **Key-words:** Acquired immunity; Birds; Body fat reserves; Fasting; Thyroid hormones

# 1 INTRODUCTION

2

3 Life-history theory predicts a trade-off between an organism's current reproductive effort and  
4 its future survival and reproductive success, where an increased reproductive effort could be  
5 deleterious to adult survival (Williams, 1966; Stearns, 1992). One potential mechanism  
6 whereby current reproductive effort may incur long-term reproductive costs is through a  
7 resource shift away from the immune system (Gustafsson et al., 1994; Sheldon and Verhulst,  
8 1996; Råberg et al., 1998). Acquired immunity consists of two components (Roitt et al.,  
9 1998): humoral immunity (mediated by B-lymphocytes) and cell-mediated immunity  
10 (mediated by T-lymphocytes). Previous studies have shown that the acquired immunity of  
11 female common eiders (*Somateria mollissima*) is suppressed during the incubation fast  
12 (Hanssen et al., 2004; Bourgeon et al., 2006a) while its experimental activation has strong  
13 negative effects on the fitness of female eiders (Hanssen et al., 2004; Hanssen et al., 2005;  
14 Hanssen, 2006). However, Bourgeon et al. (2006a) found that both components of the  
15 acquired immunity decrease independently of each other. Whatever the adaptive significance  
16 of such an immunosuppression in breeding eider ducks may be, the underlying physiological  
17 mechanisms are also largely unknown. Deerenberg et al. (1997) suggested that a reduced  
18 immunocompetence during reproduction could be related to hormonal changes.

19 Thermoregulation and energy metabolism are partially regulated by triiodothyronine  
20 ( $T_3$ ) in mammals and birds (McNabb, 1995). While female eider ducks fast throughout  
21 incubation (Korschgen, 1977; Gabrielsen et al., 1991; Criscuolo et al., 2000), they also have  
22 to maintain a high body temperature, required for egg incubation (Criscuolo et al., 2003).  
23 Criscuolo et al. (2003) showed that  $T_3$  levels in female eiders were maintained throughout  
24 incubation. Circulating  $T_3$ , thermogenesis, and basal metabolic rate (BMR) were found to be  
25 positively correlated in precocial species during fasting (Sechman et al., 1989; Gabarrou et

1 al., 1997). Furthermore, daily energy expenditure and the T-cell-mediated immune response  
2 were negatively correlated in female pied flycatchers (*Ficedula hypoleuca*; Moreno et al.,  
3 2001). However, a depressed level of thyroid hormones led to a suppressed cytotoxic T-cell  
4 activity in mallard ducks (*Anas platyrhynchos*) (Fowles et al., 1997), while it increased the *in*  
5 *vitro* T-cell proliferative response to mitogenic stimulation in chickens (Williamson et al.,  
6 1990). Hence, the relationship between thyroid hormones and the immune system is far from  
7 understood (Smits et al., 2002) and remains to be clarified.

8         The main objective of our study was to examine the effects of increased plasma T<sub>3</sub>  
9 levels on both components of the acquired immunity in free-ranging female common eiders  
10 during their incubation phase. To this end, incubating females were implanted with T<sub>3</sub> pellets  
11 and compared to control birds. We subsequently measured variations of the female  
12 immunoglobulin index, the T-cell-mediated immune response, body mass, and plasma  
13 parameters and compared these with measurements from control birds prior to and after  
14 manipulation (i.e. implantation of T<sub>3</sub> pellets or sham implantation). We predicted that  
15 increased plasma T<sub>3</sub> levels, which will increase the energy expenditure of incubating female  
16 eiders, should have immunosuppressive effects. Implanted females should therefore show  
17 lower T-cell-mediated immune responses and/or a reduced immunoglobulin index than  
18 control birds.

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## 1 MATERIALS AND METHODS

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3 The study was conducted in a common eider duck colony on Storholmen Island,  
4 Kongsfjorden, Svalbard Archipelago (78°55' N, 20°07' E) between June and July 2005. This  
5 breeding colony contained about 900 nests. Females laid between 1 and 6 eggs, but a clutch  
6 size of 4 to 5 eggs was most common (49.35 % and 24.62 %, respectively, N=845). Eider  
7 ducklings are precocial and are cared for by the female only. Incubation lasts between 24 and  
8 26 days (Korschgen, 1977). All birds started their incubation between June 7 and June 13, the  
9 main laying period for the colony. Ducks that laid their eggs after this period were not  
10 considered in this study. Ambient temperatures in June and July ranged from 2 to 10°C.

11

### 12 Sampling protocol

13 Nests were checked at least every two days throughout the study period. This was done to  
14 determine initial clutch size but also to investigate the rate of egg predation and nest  
15 desertion. A clutch of eggs was considered complete when no additional egg was laid during a  
16 two-day period (Erikstad and Tveraa, 1995). Female eiders were caught on their nests using a  
17 bamboo pole with a nylon snare. Blood was collected from the brachial vein within three  
18 minutes of capture, stored in tubes containing EDTA (an anticoagulant agent) and kept on ice  
19 until further processing. In the laboratory blood was centrifuged at 10,000 rpm for five  
20 minutes at 4°C. Plasma samples and blood cells were separately stored at -20°C and plasma  
21 was subsequently used to measure immunoglobulin, T<sub>3</sub>, and corticosterone levels. After blood  
22 sampling, body size was recorded (wing and tarsus lengths) and birds were weighed with a  
23 portable electronic balance ( $\pm 2$  g).

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## 1 Experimental groups and T<sub>3</sub> implantation

2 A total of 42 females with a mean clutch size of 4 to 5 eggs ( $4.17 \pm 0.38$  eggs; mean  $\pm$  SD)  
3 were used in this study and captured on three occasions. To prevent nest desertion, we only  
4 caught birds that were already incubating for at least five days. Since both components of the  
5 acquired immunity decrease during whole incubation (Bourgeon et al., 2006a), we limited the  
6 present study to a relatively short time period, so that natural immunological changes  
7 occurring as the fasting is proceeding would not have confounding effects. Females were split  
8 into two experimental groups: birds with implanted T<sub>3</sub> pellets (N=21) and control females  
9 (N=21). Females from both groups were caught after about 10 days of incubation ( $9.95 \pm 0.33$   
10 days, N=42; mean  $\pm$  SE). When taking the first blood sample, we also recorded body size and  
11 body mass. Before release, half of the females were implanted, while the others underwent the  
12 same procedure without actual implantation. All females were recaptured 6 to 8 days later  
13 ( $16.57 \pm 0.42$  days, N=42; mean  $\pm$  SE), when a second blood sample was taken and the PHA  
14 skin test was conducted (see below). Birds were again weighed before their release. Finally,  
15 all birds were captured 24 hours later to read the PHA skin test. Only nests which did not  
16 suffer any predation were included in our study.

17 T<sub>3</sub> pellets (100 mg, 21 day release, T-261) were purchased from Innovative Research of  
18 America (Sarasota, Florida, USA). In preliminary trials, this dose was sufficient to induce a  
19 marked increase in plasma T<sub>3</sub> levels, while also causing an increased loss in body mass  
20 (Bourgeon et al., unpublished observation). T<sub>3</sub> pellets were implanted subcutaneously at the  
21 back side of the birds' neck. For this, we shaved the skin of the concerned area and  
22 disinfected using alcohol and betadine (iodine solution). A small incision, equal to the size of  
23 the pellet was made and the implant was positioned underneath the skin. The skin was closed  
24 with a single stitch, using surgical thread. The wound was cleaned with betadine and sprayed  
25 with an aluminium powder.

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

2 To evaluate the T-cell-mediated immune response, 100 µl of 5 mg.ml<sup>-1</sup> PHA (Sigma L 8754)  
3 in phosphate-buffered saline (PBS) were injected intradermally in the right wing-web. It has  
4 been shown previously that birds suffer little physiological stress from PHA injection (Merino  
5 et al., 1999). The left wing-web was injected with an equal volume of PBS as a control. The  
6 thickness of each wing-web was measured with a micrometer calliper (three readings), just  
7 prior to and 24 h after injection. The cell-mediated immune response was calculated as the  
8 difference in wing-web swelling between the mitogen-injected and control site. Since the  
9 PHA skin test could only be conducted once with each animal (a second injection would lead  
10 to a secondary immune response), we injected all birds 6 to 8 days after manipulation.

11

12 Immunoglobulin levels: ELISA test

13 The amount of serum immunoglobulins in avian blood has been assessed using a sensitive  
14 ELISA method. Commercial antichickens conjugate antibodies were used as reported by  
15 Martínez et al. (2003). This method has been validated in six wild avian species. Although the  
16 method has not been validated for Anseriforms, we assumed a linear cross-reactivity, despite  
17 the fact that Anseriforms have an additional immunoglobulin isotype (IgY), which is not  
18 found in other birds (Parham, 1995). We used the measurements obtained from these tests as  
19 an immunoglobulin index.

20 The serum dilution in eiders was determined by coating ELISA plates with serial dilutions of  
21 serum (100 µl) in carbonate-bicarbonate buffer (0.1M, pH=9.6) to investigate the linear range  
22 of the sigmoid curve. Data obtained from trials using the serum dilution nearest to the centre  
23 of its linear range were selected. To be coated, ninety-six-well ELISA plates were filled with  
24 100 µl of diluted serum samples from female common eiders (two samples per female, diluted  
25 to 1/32000 in carbonate-bicarbonate buffer). The plates were first incubated for 1 h at 37°C



1 and then incubated overnight at 4°C. After washing the plates once with 200 µl of a solution  
2 of phosphate buffer saline and Tween (PBS-Tween), 100 µl of a solution containing 5%  
3 powdered milk in PBS was added. After a second incubation (1 h at 37°C) and a wash with  
4 PBS-Tween buffer, 100 µl of antichicken antibodies, diluted 1:250 (Sigma A 9046), were  
5 added and the plates were incubated for 2 h at 37°C. After three washes, the plates were  
6 finally filled with 100 µl of a solution (peroxide diluted in ABTS (2,2'-azino-bis-(3-  
7 ethylbenzthiazoline-6-sulphonic acid)) 1:1000). Following incubation (1 h at 37°C), the plates  
8 were read using a 405 nm wavelength filter (Awareness Technology, Inc., Palm City, FL,  
9 USA).

10

#### 11 Assessment of the T<sub>3</sub> and corticosterone levels

12 T<sub>3</sub> and corticosterone concentrations were determined in plasma by radioimmunoassay (RIA)  
13 in our laboratory using a <sup>125</sup>I RIA double antibody kit from ICN Biomedicals (Costa Mesa,  
14 CA, USA). The RIA for T<sub>3</sub> and corticosterone had an intra-assay variability of 5.1 % (N=20  
15 duplicates) and 7.1 % (N=10 duplicates), respectively. Inter-assay variability was 7.0 %  
16 (N=59 duplicates) for T<sub>3</sub> and 6.5 % (N=15 duplicates) for corticosterone.

17

#### 18 Statistical analyses

19 Statistical analysis was conducted with SPSS 12.0.1 (SPSS Inc., Chicago, IL, USA). Values  
20 are means ± standard error (SE), unless otherwise indicated. Since all data were normally  
21 distributed (Kolgomorov-Smirnov test, P>0.05), we used parametric tests. Repeated measure  
22 ANOVA was used to test for treatment effects on T<sub>3</sub> levels, body mass, corticosterone levels,  
23 and the immunoglobulin index. One-way ANOVA was used to test for the effects of the  
24 treatment on the T-cell-mediated immune response. Linear regressions were used to assess the  
25 relationships between all measured parameters.

## 1 **RESULTS**

2

3 Table 1 provides details about the female eiders used in this study. There was no significant  
4 difference between the two groups for any of the parameters measured (see Table 1) before  
5 manipulation. We also ensured that females from both groups were at a comparable  
6 incubation stage, when they were caught to undergo the PHA skin test (Table 1).

7

### 8 Effects of T<sub>3</sub> on body mass and corticosterone levels:

9 As expected, implants induced a significant increase in T<sub>3</sub> levels (repeated measures  
10 ANOVA: effects of repetition:  $F_{1,40}=186.38$ ,  $P<0.0001$ ; effects of group:  $F_{1,40}=124.29$ ,  
11  $P<0.0001$ ; interaction:  $F_{1,40}=150.03$ ,  $P<0.0001$ ). After 6 days, T<sub>3</sub> levels were 4 times higher in  
12 implanted females than in control birds (Table 1). Body mass decreased significantly in T<sub>3</sub>  
13 implanted females (15 %), while this decrease was less pronounced in control females (9 %;  
14 repeated measures ANOVA: effects of repetition:  $F_{1,40}=524.05$ ,  $P<0.0001$ ; effects of group:  
15  $F_{1,40}=0.05$ ,  $P=0.82$ ; interaction:  $F_{1,40}=23.70$ ,  $P<0.0001$ ). Consequently, body mass of T<sub>3</sub>  
16 females after 6 days was significantly lower than that of control females. In fact, body mass  
17 loss per day was 40 % greater in T<sub>3</sub> females than in control females (Table 1). However,  
18 linear regression analysis showed no significant relationship between T<sub>3</sub> levels and body  
19 mass, neither before nor after the treatment (Table 2). Corticosterone levels were not  
20 significantly affected by T<sub>3</sub> implants (repeated measures ANOVA: effects of repetition:  
21  $F_{1,40}=27.12$ ,  $P<0.0001$ ; effects of group:  $F_{1,40}=0.01$ ,  $P=0.93$ ; interaction:  $F_{1,40}=0.003$ ,  $P=0.96$ ).  
22 While plasma corticosterone levels after 6 days were increased by 60 %, this increase was  
23 similar for both groups (Table 1). Again, we did not find a significant relationship between T<sub>3</sub>  
24 levels and plasma corticosterone, neither before nor after the treatment (Table 2). Similarly,

1 the relationship between corticosterone levels and body mass was not significant, neither  
2 before nor after the treatment (Table 2).

3

4 Effects of T<sub>3</sub> on immunoglobulin index and T-cell-mediated immune response:

5 Implanted females showed a similar T-cell-mediated immune response than control females  
6 (Table 1), indicating that T<sub>3</sub> implants had no significant effect on this parameter. Similarly,  
7 the relationship between T<sub>3</sub> levels after implantation and the T-cell-mediated immune  
8 response was not significant (Table 2). By contrast, the immunoglobulin index was  
9 significantly decreased in implanted females (18 %), while it actually increased by about 5 %  
10 in control females (repeated measures ANOVA: effects of repetition:  $F_{1,40}=2.15$ ,  $P=0.15$ ;  
11 effects of group:  $F_{1,40}=0.05$ ,  $P=0.83$ ; interaction:  $F_{1,40}=8.17$ ,  $P=0.007$ ; Fig. 1). Surprisingly,  
12 there was a significant positive relationship between T<sub>3</sub> levels and the immunoglobulin index  
13 only before the treatment (Table 2). This would indicate that high T<sub>3</sub> levels were associated  
14 with a high immunoglobulin index before the treatment but not thereafter (Fig. 2).

15 There was no significant relationship between the two components of the acquired immunity  
16 after hormone implantation (Table 2). Hence, the immunoglobulin index was independent of  
17 the T-cell-mediated immune response. Also, there was no significant relationship between the  
18 immunoglobulin index and plasma corticosterone levels or body mass, neither before nor after  
19 the treatment (Table 2). Furthermore, the T-cell-mediated immune response was not  
20 significantly related to neither plasma corticosterone levels, nor body mass (Table 2).

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## 1 **DISCUSSION**

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3 Previous studies have shown that the acquired immunity is significantly decreased during the  
4 incubation fast of female common eiders (Hanssen et al., 2004; Bourgeon et al., 2006a). To  
5 save energy,  $T_3$  is adaptively decreased during fasting in most bird species (Harvey and  
6 Klandorf, 1983). This raises the question of the relationship between thyroid hormones and  
7 the immune system. Hence, the main objective of the current study was to investigate the  
8 endocrine mechanisms underlying the immunosuppression reported during fasting in  
9 incubating birds. To this end we assessed the effects of exogenous  $T_3$  on both components of  
10 the acquired immune system in free-ranging female eider ducks.

11 Experimentally increased plasma  $T_3$  levels affected only one of the two components of  
12 the acquired immunity. While the immunoglobulin index was significantly decreased in  
13 implanted females, when compared with control females, the T-cell-mediated immune  
14 response was not significantly affected by the treatment. Interestingly, while exogenous  $T_3$   
15 decreased the immunoglobulin index, high  $T_3$  levels before the treatment were associated with  
16 a high immunoglobulin index. Similar to previous results (Bourgeon et al., 2006a), we did not  
17 find a significant relationship between both components of the acquired immunity. This  
18 supports the view that variations in one component are not necessarily a reliable indicator of  
19 changes in the other, as suggested by Norris and Evans (2000). In the present study, the  
20 immunoglobulin index was more sensitive to  $T_3$  treatment than was the T-cell-mediated  
21 immune response. This is in accordance with previous results (Bourgeon et al., 2006a), which  
22 showed that, for the same time period, the immunoglobulin index decreases two times faster  
23 than the T-cell-mediated immune response in incubating eiders. We can therefore not exclude  
24 the possibility that effects of  $T_3$  on the T-cell-mediated immune response might require more  
25 time and/or higher  $T_3$  concentrations.

1           The treatment did not induce any significant changes in corticosterone levels. After 6  
2 days, corticosterone levels in T<sub>3</sub> implanted females were similar to that of control females.  
3 Glucocorticoids, which are secreted during stressful activities, are an essential component of  
4 the endogenous immunoregulatory network (Apanius, 1998). Furthermore, Råberg et al.  
5 (1998) suggested that corticosterone reduces the adaptive immune function. However, since  
6 corticosterone levels in our study were not affected by the treatment, the observed decrease in  
7 the immunoglobulin index might be mediated by T<sub>3</sub>, independently of corticosterone. Indeed,  
8 an experimental study performed on the same species showed that exogenous corticosterone  
9 significantly decreased immunoglobulin index (Bourgeon and Raclot, 2006). Moreover, in the  
10 current study, we did not find a significant relationship between corticosterone levels and the  
11 immunoglobulin index or the T-cell-mediated immune response, neither before, nor after the  
12 treatment. This is in agreement with results from an earlier study (Bourgeon et al., 2006a),  
13 which found that plasma corticosterone levels did not vary throughout the incubation period  
14 of female eiders.

15           T<sub>3</sub> partially regulates thermoregulation and energy metabolism in mammals and birds  
16 (McNabb, 1995). Accordingly, T<sub>3</sub> implanted females in our study lost significantly more  
17 weight than did control females. Norris and Evans (2000) proposed that immunocompetence  
18 may be fixed by the BMR, which is determined by energy reserves. Such a view on the  
19 relationship between energy metabolism and immunocompetence is supported by the  
20 existence of nutritional and endocrine factors that regulate both of these processes (Apanius,  
21 1998). Since eider ducks do not feed during incubation, limited resources might be allocated  
22 for the reconstitution of tissues affected by gluconeogenesis, and, therefore be unavailable for  
23 the maintenance of immunity (Saino et al., 2002). Moreover, adipose tissue is no longer  
24 regarded only as a fat store but also as an important endocrine organ, responsible for the  
25 synthesis and secretion of several hormones and proteins (Ahima and Flier, 2000). It has

1 recently been described as an active participant in the regulation of essential and prominent  
2 body processes, such as immune homeostasis (Matarese and La Cava, 2004). Some adipose  
3 humoral signals are generated in proportion to fat stores and act on feedback control systems  
4 to influence food intake and energy expenditure (Matarese and La Cava, 2004). This latter  
5 fact raises the question of the hormonal control of the immune system by body reserves  
6 (Demas and Sakaria, 2005). The peptide hormone leptin is secreted primarily by adipose  
7 tissue and has been shown to enhance a variety of immunological parameters in mammals  
8 (Lord et al., 1998; Faggioni et al., 2001) and birds (Löhmus et al., 2004). Since circulating  
9 levels of leptin are generally proportional to the amount of body fat (Löhmus and Sundström,  
10 2004; Matarese et al., 2005), decreases in body fat stores may affect immunity via changes in  
11 endocrine signalling (Demas and Sakaria, 2005). Exogenous administration of  $T_3$  is likely to  
12 increase BMR, further depleting body energy stores. Consequently, leptin levels might be  
13 lowered in  $T_3$  implanted birds, when compared with the controls. While the effects induced by  
14 leptin might favour survival under hostile conditions, concomitant starvation leads to  
15 immunosuppression and impaired fertility, because energy-consuming processes are switched  
16 off by leptin (Matarese and La Cava, 2004). The immunosuppressive effect of exogenous  $T_3$   
17 could therefore be mediated by leptin. For an examination of the role of this hormone in the  
18 immune function of fasting eiders, direct manipulation of leptin concentrations would be  
19 helpful (Löhmus et al., 2004). In addition, since we found that an experimentally induced  
20 increase in the utilization of body reserves led to immunosuppression, it seems likely that  
21 immunocompetence is related to the amount of body fat available. Initial body condition and  
22 the subsequent utilization of endogenous reserves might therefore determine the observed  
23 variations in immune functioning throughout incubation. Females with a good initial body  
24 condition would be less prone to suffer immunosuppression during incubation than females  
25 with a bad initial body condition.

1           While the main goal of our study was to investigate the physiological mechanisms  
2 underlying immunosuppression in breeding eiders, it might also provide evidence regarding  
3 its adaptive significance. Two hypotheses, which are not mutually exclusive, have been  
4 proposed to explain the immunosuppression observed during breeding. The  
5 immunopathology-avoidance hypothesis states that during heavy physical workloads, such as  
6 encountered during reproduction, the risk of an autoimmune response increases (Råberg et al.,  
7 1998). To decrease the risk of auto immunopathology, immunocompetence is down regulated.  
8 Råberg et al. (1998) suggested that such a down regulation would be mediated by  
9 corticosterone. However, in the present study we did not find a significant relationship  
10 between corticosterone levels and the T-cell-mediated immune response or the  
11 immunoglobulin index. Nevertheless, our present results do not allow us to exclude this  
12 hypothesis. Notably, we did not measure the heat shock protein (HSP) levels which are more  
13 appropriate for detecting chronic or long-term exposure to stressors (Martinez-Padilla et al.,  
14 2004 ; Tomás et al., 2004). HSPs have been shown to significantly increase over incubation in  
15 breeding eiders, further partially supporting the immunopathology-avoidance hypothesis in  
16 this species (Bourgeon et al., 2006b). The second hypothesis, termed the resource-limitation  
17 hypothesis, predicts that the investment in costly behaviours, such as reproduction, will  
18 reduce the amount of resources available to other systems, such as the immune system  
19 (Råberg et al., 1998). For this hypothesis to be valid, an energetic or nutritional cost  
20 associated with the maintenance and activation of the immune system is required (Råberg et  
21 al., 1998). While evidence for an energetically costly immune response is still equivocal  
22 (Råberg et al., 1998; Eraud et al., 2005; Verhulst et al., 2005), the present study shows that an  
23 increase in energy expenditure, caused by T<sub>3</sub> administration, had a negative effect on the  
24 females' immunoglobulin index. This would lend support to the latter hypothesis, illustrating  
25 the trade-off between one component of immunity and other resource demanding activities.

1           In conclusion, exogenous T<sub>3</sub> decreased only one component of the acquired immune  
2 function in incubating female eiders. While their immunoglobulin index was significantly  
3 decreased after T<sub>3</sub> administration, their T-cell-mediated immune response was not affected.  
4 Since T<sub>3</sub> implants did not induce changes in corticosterone concentrations, this would suggest  
5 that glucocorticoids were not involved in the observed decrease in the immunoglobulin index.  
6 Weight loss was significantly greater in T<sub>3</sub> implanted females than in control females. The  
7 immunosuppressive effect of T<sub>3</sub> might therefore be mediated by its effects on energy  
8 expenditure and/or body fat reserves. In fact, leptin, which conveys information on energy  
9 availability, could be responsible for the observed immunosuppression in our study. Further  
10 experiments are required to shed more light onto the relationship between leptin, body  
11 condition and the immune system in incubating female eider ducks. Finally, despite the fact  
12 that short-term energetic costs have not been observed in female eiders injected with three  
13 different non-pathogenic antigens while their survival was compromised (Hanssen et al.,  
14 2004), the adaptive significance of immunosuppression during incubation in eider ducks still  
15 remains to be documented.

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2

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1 **REFERENCES**

2

3 Ahima, R.S., Flier, J.S., 2000. Adipose tissue as an endocrine organ. Trends Endocrin. Met.  
4 11, 327-332.

5 Apanius, V., 1998. Stress and Immune Defense. Adv. Stud. Behav. 27, 133-153.

6 Bourgeon, S., Criscuolo, F., Le Maho, Y., Raclot, T., 2006a. Phytohemagglutinin response  
7 and immunoglobulin index decrease during incubation fasting in female Common Eiders.  
8 Physiol. Biochem. Zool. 79 (4), 793-800.

9 Bourgeon, S., Martinez, J., Criscuolo, F., Le Maho, Y., Raclot, T., 2006b. Fasting-induced  
10 changes of immunological and stress indicators in breeding female eiders. Gen. Comp.  
11 Endocrinol. 147, 336-342.

12 Bourgeon, S., Raclot, T., 2006. Corticosterone selectively decreases humoral immunity in  
13 female eiders during incubation. J. Exp. Biol. 209, 4957-4965.

14 Criscuolo, F., Gauthier-Clerc, M., Gabrielsen, G.W., Le Maho, Y., 2000. Recess behaviour of  
15 the incubating Common Eider *Somateria mollissima*. Polar Biol. 23, 571-574.

16 Criscuolo, F., Raclot, T., Le Maho, Y., Gabrielsen, G.W., 2003. Do T<sub>3</sub> levels in incubating  
17 Eiders reflect the cost of incubation among clutch sizes? Physiol. Biochem. Zool. 76 (2), 196-  
18 203.

19 Deerenberg, C., Arpanius, V., Daan, S., Bos, N., 1997. Reproductive effort decreases  
20 antibody responsiveness. Proc. R. Soc. B 264, 1021-1029.

21 Demas, G.E., Sakaria, S., 2005. Leptin regulates energetic tradeoffs between body fat and  
22 humoral immunity. Proc. R. Soc. B 272, 1845-1850.

23 Eraud, C., Duriez, O., Chastel, O., Faivre, B., 2005. The energetic cost of humoral immunity  
24 in the Collared Dove, *Streptopelia decaocto*: is the magnitude sufficient to force energy-based  
25 trade-offs? Funct. Ecol. 19, 110-118.

1 Erikstad, K.E., Tveraa, T., 1995. Does the cost of incubation set limits to clutch size in  
2 common eiders *Somateria mollissima*. *Oecologia* 103, 270-274.

3 Faggioni, R., Feingold, K.R., Grunfeld, C., 2001. Leptin regulation of the immune response  
4 and the immuno-deficiency of malnutrition. *FASEB J.* 15, 2565-2571.

5 Fowles, J.R., Fairbrother, A., Kervliet, N.I., 1997. Effects of induced hypo- and  
6 hyperthyroidism on immune function and plasma biochemistry in mallards (*Anas*  
7 *platyrhynchos*). *Comp. Biochem. Physiol.* 118 C (2), 213-220.

8 Gabarrou, J.F., Duchamp, C., Williams, J., Geraert, P.A., 1997. A role for thyroid hormones  
9 in the regulation of diet-induced thermogenesis in birds. *Brit. J. Nutr.* 78, 963-973.

10 Gabrielsen, G.W., Mehlum, F., Karlsen, H.E., Andresen, Ø., Parker, H., 1991. Energy cost  
11 during incubation and thermoregulation in the female Common Eider *Somateria mollissima*.  
12 *Norsk Polarinstitutt Skrifer* 195, 51-62.

13 Gustafsson, L., Nordling, D., Andersson, M.S., Sheldon, B.C., Qvarnström, A., 1994.  
14 Infectious diseases, reproductive effort and the cost of reproduction in birds. *Philos. T. Roy.*  
15 *Soc. B* 346, 323-331.

16 Hanssen, S.A., 2006. Costs of an immune challenge and terminal investment in a long-lived  
17 bird. *Ecology* 87 (10), 2440-2446.

18 Hanssen, S.A., Hasselquist, D., Folstad, I., Erikstad, K.E., 2005. Cost of reproduction in a  
19 long-lived bird: incubation effort reduces immune function and future reproduction. *Proc. R.*  
20 *Soc. B* 272, 1039-1046.

21 Hanssen, S.A., Hasselquist, D., Folstad, I., Erikstad, K.E., 2004. Costs of immunity: immune  
22 responsiveness reduces survival in a vertebrate. *Proc. R. Soc. B* 271, 925-930.

23 Harvey, S., Klandorf, H., 1983. Reduced adrenocortical function and increased thyroid  
24 function in fasted and refed chickens. *J. Endocrinol.* 98, 129-135.

1 Korschgen, C.E., 1977. Breeding stress of female Eiders in Maine. *J. Wildlife Manage.* 41  
2 (3), 360-373.

3 Lõhmus, M., Olin, M., Sundström, L.F., Troedsson, M.H.T., Molitor, T.W., El Halawani, M.,  
4 2004. Leptin increases T-cell immune response in birds. *Gen. Comp. Endocrinol.* 139, 245-  
5 250.

6 Lõhmus, M., Sundström, L.F., 2004. Leptin and social environment influence the risk-taking  
7 and feeding behaviour of Asian blue quail. *Anim. Behav.* 68, 607-612.

8 Lord, G.M., Matarese, G., Howard, J.K., Baker, R.J., Bloom, S.R., Lechler, R.I., 1998. Leptin  
9 modulates the T-cell immune response and reverses starvation-induced immunosuppression.  
10 *Nature* 394, 897-901.

11 Martínez, J., Tomás, G., Merino, S., Arriero, E., Moreno, J., 2003. Detection of serum  
12 immunoglobulins in wild birds by direct ELISA: a methodological study to validate the  
13 technique in different species using antichickens antibodies. *Funct. Ecol.* 17, 700-706.

14 Martinez-Padilla, J., Martínez, J., Davila, J.A., Merino, S., Moreno, J., Millán, J., 2004.  
15 Within-brood size differences, sex and parasites determine blood stress protein levels in  
16 Eurasian kestrel nestlings. *Funct. Ecol.* 18, 426-434.

17 Matarese, G., La Cava, A., 2004. The intricate interface between immune system and  
18 metabolism. *Trends Immunol.* 5 (4), 193-200.

19 Matarese, G., Moschos, S., Mantzoros, C.S., 2005. Leptin in immunology. *J. Immunol.* 173,  
20 3137-3142.

21 McNabb, F.M.A., 1995. Thyroid hormones, their activation, degradation and effects on  
22 metabolism. *J. Nutr.* 125, S1773-S1776.

23 Merino, S., Martínez, J., Møller, A.P., Sanabria, L., De Lope, F., Perez, J., Rodriguez-  
24 Caabeiro, F., 1999. Phytohaemagglutinin injection assay and physiological stress in nestling  
25 house martins. *Anim. Behav.* 58, 219-222.

1 Moreno, J., Sanz, J.J., Merino, S., Arriero, E., 2001. Daily energy expenditure and cell-  
2 mediated immunity in pied flycatchers while feeding nestlings: interaction with moult.  
3 *Oecologia* 129 (4), 492-497.

4 Norris, K., Evans, M.R., 2000. Ecological immunology: life history trade-offs and immune  
5 defense in birds. *Behav. Ecol.* 11, 19-26.

6 Parham, P., 1995. The duck's dilemma. *Nature* 374, 16-17.

7 Råberg, L., Grahn, M., Hasselquist, D., Svensson, E., 1998. On the adaptive significance of  
8 stress-induced immunosuppression. *Proc. R. Soc. B* 265, 1637-1641.

9 Roitt, I.M., Brostoff, J., Male, D.K., 1998. *Immunology*. London, Mosby.

10 Saino, N., Incagli, M., Martinelli, R., Møller, A.P., 2002. Immune response of male barn  
11 swallows in relation to parental effort, corticosterone plasma levels, and sexual  
12 ornamentation. *Behav. Ecol.* 13, 169-174.

13 Sechman, A., Niezgodá, J., Soboconski, R., 1989. The relationship between basal metabolic  
14 rate (BMR) and concentrations of plasma thyroid hormones in fasting cockerels. *Folia Biol.-*  
15 *Prague* 37, 83-90.

16 Sheldon, B.C., Verhulst, S., 1996. Ecological immunology: costly parasite defenses and trade-  
17 offs in evolutionary ecology. *Trends Ecol. Evol.* 11 (8), 317-321.

18 Smits, J.E., Fernie, K.J., Bortolotti, G.R., Marchant, T.A., 2002. Thyroid hormone  
19 suppression and cell-mediated immunomodulation in American kestrels (*Falco sparverius*)  
20 exposed to PCBs. *Arch. Environ. Con. Tox.* 43, 338-344.

21 Stearns, S.C., 1992. *The evolution of life histories*. Oxford University Press, Oxford.

22 Tomás, G., Martínez, J., Merino, S., 2004. Collection and analysis of blood samples to detect  
23 stress proteins in wild birds. *J. Field Ornithol.* 75, 281-287.

24 Verhulst, S., Riedstra, B., Wiersma, P., 2005. Brood size and immunity costs in zebra finches  
25 *Taeniopygia guttata*. *J. Avian Biol.* 36, 22-30.

1 Williams, G.C., 1966. Natural selection, the costs of reproduction, and a refinement of Lack's  
2 principle. Am. Nat. 100, 687-690.

3 Williamson, R.A., Davison, T.F., Payne, L.N., 1990. Effects of thyroid hormones on humoral  
4 and cell-mediated immune responses in the domestic fowl (*Gallus domesticus*). Dev. Comp.  
5 Immunol. 14, 305-318.

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1 **TABLES**

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3 **Table 1.** Profiles for both experimental groups of free-ranging incubating female eiders,  
 4 before and after implantation of T<sub>3</sub> pellets. Control animals were sham implanted. Values are  
 5 means ± SE.

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Before implantation	Group 1: T <sub>3</sub> females (N=21)	Group 2: Control females (N=21)	T-test	P
<b>Initial clutch size (eggs)</b>	4.19 ± 0.09	4.14 ± 0.08	0.40	0.69
<b>Tarsus length (cm)</b>	6.15 ± 0.03	6.08 ± 0.04	1.57	0.12
<b>Wing length (cm)</b>	29.30 ± 0.11	29.00 ± 0.16	1.56	0.12
<b>Incubation stage at sampling (days)</b>	10.09 ± 0.49	9.81 ± 0.45	0.43	0.67
<b>Body mass at sampling (g)</b>	1836 ± 31	1784 ± 28	1.25	0.13
<b>Immunoglobulin index (absorbance units)</b>	0.85 ± 0.04	0.78 ± 0.04	1.51	0.25
<b>Triiodothyronine (pg.ml<sup>-1</sup>)</b>	12.26 ± 0.74	10.14 ± 0.89	1.84	0.07
<b>Corticosterone (ng.ml<sup>-1</sup>)</b>	12.17 ± 1.30	11.94 ± 1.01	0.14	0.89

After implantation	Group 1: T <sub>3</sub> females (N=21)	Group 2: Control females (N=21)	T-test	P
<b>Incubation stage at sampling (days)</b>	17.14 ± 0.60	16.00 ± 0.56	1.38	0.17
<b>Body mass at sampling (g)</b>	1590 ± 29	1624 ± 27	-0.87	0.39
<b>Body mass loss per day (g)</b>	35.62 ± 1.72	25.62 ± 1.44	4.46	<0.0001
<b>Immunoglobulin index (absorbance units)</b>	0.72 ± 0.04	0.82 ± 0.04	-1.63	0.11
<b>T-cell-mediated immune response (mm)</b>	0.98 ± 0.11	0.92 ± 0.11	0.42	0.68
<b>Triiodothyronine (pg.ml<sup>-1</sup>)</b>	44.76 ± 2.25	11.90 ± 1.28	12.68	<0.0001
<b>Corticosterone (ng.ml<sup>-1</sup>)</b>	19.45 ± 1.84	19.38 ± 1.82	0.03	0.98

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1 **Table 2.** Results for linear regressions between immune parameters, body mass, T<sub>3</sub>, and  
 2 corticosterone levels in free-ranging incubating female eiders.

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Before implantation	Body mass (g)	Immunoglobulin index (absorbance units)	Triiodothyronine (pg.ml <sup>-1</sup> )	Corticosterone (ng.ml <sup>-1</sup> )
Body mass (g)	-	F <sub>1,41</sub> =2.16, P=0.15	F <sub>1,41</sub> =2.90, P=0.10	F <sub>1,41</sub> =0.69, P=0.41
Immunoglobulin index (absorbance units)		-	F <sub>1,41</sub> =9.91, P=0.003	F <sub>1,41</sub> =0.52, P=0.47
Triiodothyronine (pg.ml <sup>-1</sup> )			-	F <sub>1,41</sub> =0.65, P=0.43



After implantation	Body mass (g)	Immunoglobulin index (absorbance units)	T-cell-mediated immune response (mm)	Triiodothyronine (pg.ml <sup>-1</sup> )	Corticosterone (ng.ml <sup>-1</sup> )
Body mass (g)	-	F <sub>1,41</sub> =0.01, P=0.91	F <sub>1,41</sub> =0.20, P=0.66	F <sub>1,41</sub> =0.35, P=0.56	F <sub>1,41</sub> =0.05, P=0.82
Immunoglobulin index (absorbance units)		-	F <sub>1,41</sub> =0.35, P=0.56	F <sub>1,41</sub> =1.03, P=0.31	F <sub>1,41</sub> =1.11, P=0.30
T-cell-mediated immune response (mm)			-	F <sub>1,41</sub> =0.38, P=0.54	F <sub>1,41</sub> =0.72, P=0.40
Triiodothyronine (pg.ml <sup>-1</sup> )				-	F <sub>1,41</sub> =0.01, P=0.92

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1 **FIGURE LEGENDS**

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3 **Figure 1.** Effects of T<sub>3</sub> administration on the immunoglobulin index in free-ranging  
4 incubating female eiders. Shown is the immunoglobulin index of T<sub>3</sub> implanted (  ) and  
5 control females (  ) before and after manipulation. Values are mean ± SE. T<sub>3</sub>,  
6 triiodothyronine. Lower case a and b indicate a significant difference between groups (T-  
7 tests).

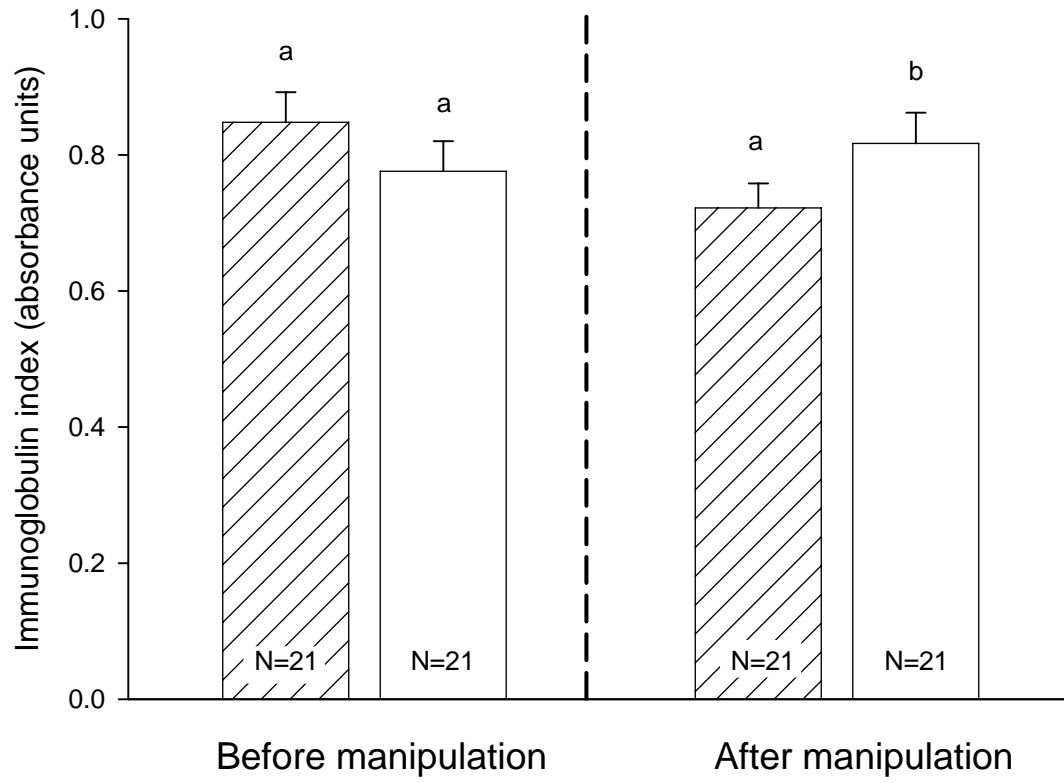
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9 **Figure 2.** Relationship between T<sub>3</sub> level and the immunoglobulin index in free-ranging  
10 incubating female eiders before implantation. T<sub>3</sub>, triiodothyronine.

1 **FIGURE 1.**

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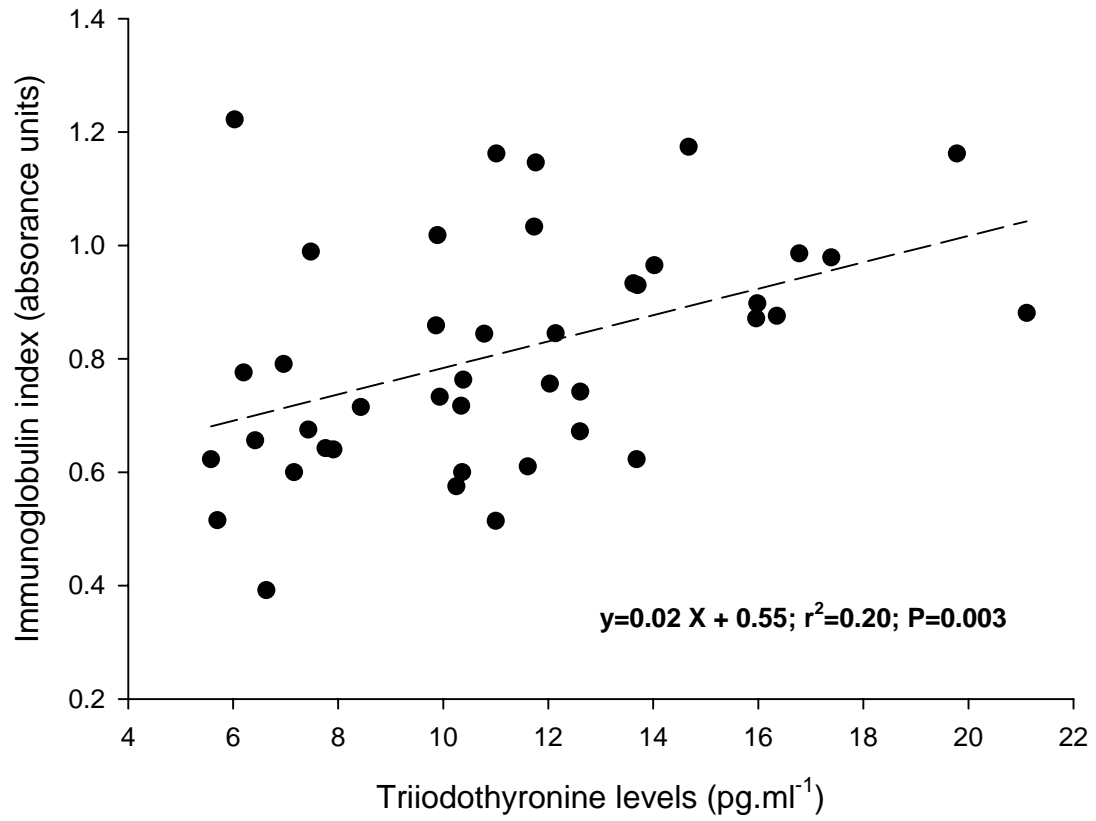
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1 **FIGURE 2.**

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