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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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17 Running title: Triiodothyronine and acquired immunity in eiders

18

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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 (T₃) is adaptively decreased during fasting in
7 most bird species, despite T₃ 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 T₃ on both components of the acquired immune system in 42 free-ranging
12 incubating birds. Half of the females were implanted with T₃ 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. T₃ 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 T₃ decreased only one component of
20 the acquired immune system. We suggest that the immunosuppressive effect of T₃ 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 T₃
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 T₃ 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 T₃ pellets or sham implantation). We predicted that
15 increased plasma T₃ 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, T₃, 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 (± 2 g).

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25

1 Experimental groups and T₃ implantation

2 A total of 42 females with a mean clutch size of 4 to 5 eggs (4.17 ± 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 T₃ pellets (N=21) and control females
9 (N=21). Females from both groups were caught after about 10 days of incubation (9.95 ± 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 ± 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 T₃ 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 T₃ levels, while also causing an increased loss in body mass
20 (Bourgeon et al., unpublished observation). T₃ 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⁻¹ 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 T₃ and corticosterone levels

12 T₃ and corticosterone concentrations were determined in plasma by radioimmunoassay (RIA)
13 in our laboratory using a ¹²⁵I RIA double antibody kit from ICN Biomedicals (Costa Mesa,
14 CA, USA). The RIA for T₃ 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 T₃ 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 T₃ 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 T₃ on body mass and corticosterone levels:

9 As expected, implants induced a significant increase in T₃ 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, T₃ levels were 4 times higher in
12 implanted females than in control birds (Table 1). Body mass decreased significantly in T₃
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 T₃
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 T₃ females than in control females (Table 1). However,
18 linear regression analysis showed no significant relationship between T₃ levels and body
19 mass, neither before nor after the treatment (Table 2). Corticosterone levels were not
20 significantly affected by T₃ 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 T₃
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 T₃ 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 T₃ implants had no significant effect on this parameter. Similarly,
7 the relationship between T₃ 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 T₃ levels and the immunoglobulin index
13 only before the treatment (Table 2). This would indicate that high T₃ 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 T₃ 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 T₃, 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 T₃ partially regulates thermoregulation and energy metabolism in mammals and birds
16 (McNabb, 1995). Accordingly, T₃ 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 (Löhms et al., 2004). Since circulating
9 levels of leptin are generally proportional to the amount of body fat (Löhms 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 (Löhms 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 T₃ 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 T₃ decreased only one component of the acquired immune
2 function in incubating female eiders. While their immunoglobulin index was significantly
3 decreased after T₃ administration, their T-cell-mediated immune response was not affected.
4 Since T₃ 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 T₃ implanted females than in control females. The
7 immunosuppressive effect of T₃ 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 **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 T₃ pellets. Control animals were sham implanted. Values are
 5 means ± SE.

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

After implantation	Group 1: T ₃ females (N=21)	Group 2: Control females (N=21)	T-test	P
Incubation stage at sampling (days)	17.14 ± 0.60	16.00 ± 0.56	1.38	0.17
Body mass at sampling (g)	1590 ± 29	1624 ± 27	-0.87	0.39
Body mass loss per day (g)	35.62 ± 1.72	25.62 ± 1.44	4.46	<0.0001
Immunoglobulin index (absorbance units)	0.72 ± 0.04	0.82 ± 0.04	-1.63	0.11
T-cell-mediated immune response (mm)	0.98 ± 0.11	0.92 ± 0.11	0.42	0.68
Triiodothyronine (pg.ml⁻¹)	44.76 ± 2.25	11.90 ± 1.28	12.68	<0.0001
Corticosterone (ng.ml⁻¹)	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, T₃, 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 ⁻¹)	Corticosterone (ng.ml ⁻¹)
Body mass (g)	-	F _{1,41} =2.16, P=0.15	F _{1,41} =2.90, P=0.10	F _{1,41} =0.69, P=0.41
Immunoglobulin index (absorbance units)		-	F _{1,41} =9.91, P=0.003	F _{1,41} =0.52, P=0.47
Triiodothyronine (pg.ml ⁻¹)			-	F _{1,41} =0.65, P=0.43

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

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1 **FIGURE LEGENDS**

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3 **Figure 1.** Effects of T₃ administration on the immunoglobulin index in free-ranging
4 incubating female eiders. Shown is the immunoglobulin index of T₃ implanted () and
5 control females () before and after manipulation. Values are mean ± SE. T₃,
6 triiodothyronine. Lower case a and b indicate a significant difference between groups (T-
7 tests).

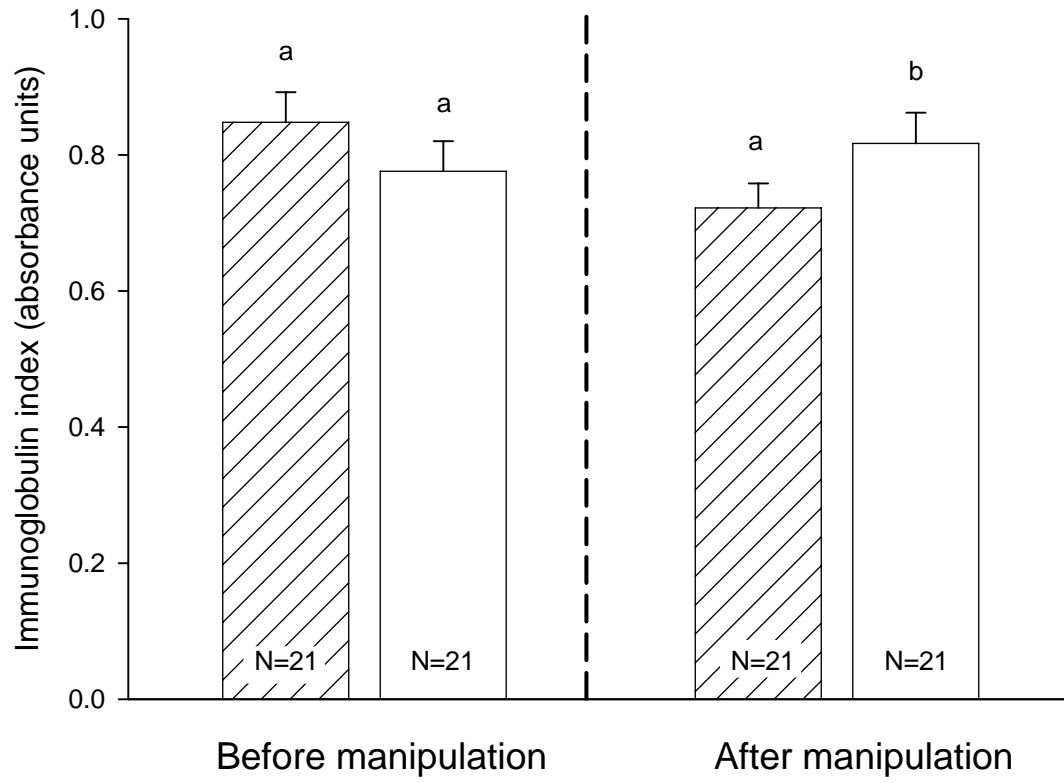
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9 **Figure 2.** Relationship between T₃ level and the immunoglobulin index in free-ranging
10 incubating female eiders before implantation. T₃, triiodothyronine.

1 **FIGURE 1.**

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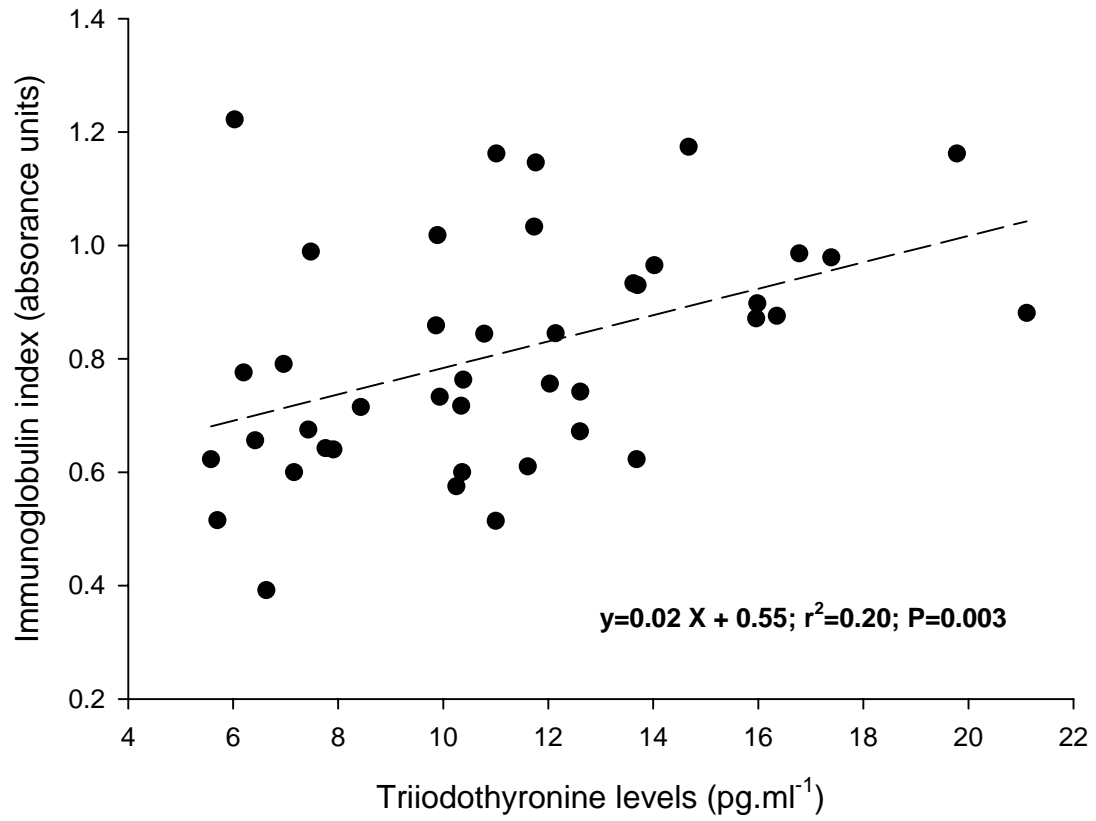
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1 **FIGURE 2.**

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