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The effects of testosterone on immune function in quail selected for divergent plasma corticosterone response

Mark L. Roberts[1,*], Katherine L. Buchanan[2], Matthew R. Evans[3], Raul H. Marin[4] and Daniel G. Satterlee[5]

[1]Division of Biology, Imperial College London, Silwood Park Campus, Buckhurst Road, Ascot, Berkshire SL5 7PY, UK, [2]School of Life and Environmental Sciences, Faculty of Science and Technology, Deakin University, Pigdons Road, Geelong VIC 3127, Australia, [3]Centre for Ecology and Conservation, School of Biosciences, University of Exeter, Cornwall Campus, Penryn, Cornwall TR10 9EZ, UK, [4]Cátedra de Química Biológica-ICTA, Facultad de Ciencias Exactas Físicas y Naturales, Universidad Nacional de Córdoba, Córdoba, Argentina and [5]School of Animal Sciences, Louisiana State University Agricultural Center, Baton Rouge, LA 70803, USA

*Author for correspondence (m.l.roberts@imperial.ac.uk)

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SUMMARY

The immunocompetence handicap hypothesis (ICHH) suggests that the male sex hormone testosterone has a dual effect; it controls the development and expression of male sexually selected signals, and it suppresses the immune system. Therefore only high quality males are able to fully express secondary sexual traits because only they can tolerate the immunosuppressive qualities of testosterone. A modified version of the ICHH suggests that testosterone causes immunosuppression indirectly by increasing the stress hormone corticosterone (CORT). Lines of Japanese quail (*Coturnix japonica*) selected for divergent responses in levels of plasma CORT were used to test these hypotheses. Within each CORT response line (as well as in a control stock) we manipulated levels of testosterone in castrated quail by treatment with zero (sham), low or high testosterone implants, before testing the birds' humoral immunity and phytohaemagglutinin (PHA)-induced immune response, as well as body condition. The PHA-induced response was not significantly affected by CORT selected line, testosterone treatment or their interaction. There was, however, a significant effect of CORT line on humoral immunity in that the control birds exhibited the greatest antibody production, but there was no significant effect of testosterone manipulation on humoral immunity. The males in the sham implant treatment group had significantly greater mass than the males in the high testosterone group, suggesting a negative effect of high testosterone on general body condition. We discuss these results in the context of current hypotheses in the field of sexual selection.

Key words: testosterone, corticosterone, immunity, stress, Japanese quail, PHA, SRBC, selection lines, body mass.

INTRODUCTION

The immunocompetence handicap hypothesis (ICHH) (Folstad and Karter, 1992) attempts to explain the evolution of male sexual signalling and female preferences that involves an interaction between the hormonal control of male sexual advertisement and the immune system. It incorporates Zahavi's (Zahavi, 1975) handicap hypothesis, which states that extravagant male sexual traits have evolved because they offer females an honest sign of quality. Only phenotypically and/or genetically relatively higher quality males can afford to fully express such traits, since the traits are costly; for example, the trait may make the possessor more conspicuous to predators. The ICHH is also an extension of Hamilton and Zuk's (Hamilton and Zuk, 1982) parasite theory, which suggests that male sexual traits give females an honest indication of a male's capacity to withstand the physiological costs of parasites and pathogens. The mechanism proposed by the ICHH suggests that testosterone (T) serves a dual role in mediating both sexual signal expression and immunosuppression (Folstad and Karter, 1992). High levels of T result not only in full signal expression but also in a concomitant reduction in immunocompetence. Therefore only high quality males can afford to both fully express sexual traits and be able to resist or tolerate parasite/pathogen attack.

One of the main assumptions of the ICHH is that elevated T has a deleterious effect on the immune system (Folstad and Karter, 1992). There is good evidence of the immunosuppressive nature of T in laboratory mammals (Grossman, 1985), and several manipulative avian studies have found a deleterious effect of elevated T on humoral immunity (Duffy et al., 2000; Peters, 2000; Casto et al., 2001; Buchanan et al., 2003; Owen-Ashley et al., 2004; Deviche and Cortez, 2005), as well as on cell-mediated immunity (Duffy et al., 2000; Casto et al., 2001; Owen-Ashley et al., 2004; Deviche and Cortez, 2005; Boughton et al., 2007; Fargallo et al., 2007). However, other studies have found no such effect on either humoral (Hasselquist et al., 1999; Roberts et al., 2007a) or cell-mediated immunity (Buchanan et al., 2003; Greenman et al., 2005; Roberts et al., 2007a). In addition, in a recent meta-analysis Roberts and colleagues (Roberts et al., 2004) found that when controlling for multiple studies on the same species, the only convincing empirical evidence in vertebrates supporting the ICHH comes from the measurement of ectoparasite loads on T-manipulated individuals, particularly in reptile species.

In a study on the house sparrow (*Passer domesticus*), Evans and colleagues (Evans et al., 2000) found that T implantation impaired antibody production. However, after statistically controlling for the effect of the glucocorticoid steroid hormone corticosterone (CORT, which increased with T), T was found to enhance immunocompetence. Therefore it is possible that in studies that have found an apparently deleterious effect of T on immunity it was

CORT that was the immunosuppressive agent. This stress-mediated version of the ICHH [first suggested by Møller (Møller, 1995)] may be a better model than the original ICHH in explaining an immunosuppressive effect of T, if high T levels result in high CORT levels and an associated reduction in immune function; T in this scenario is therefore indirectly immunosuppressive through its positive effects on CORT production.

CORT is secreted in response to stress due to the stimulation of the hypothalamus–pituitary–adrenal axis, and is involved in the mobilization of energy stores (glucose), the shutdown of digestive processes and increasing the peripheral blood supply (Silverin, 1998; Buchanan, 2000). CORT also has behavioural effects such as reducing reproductive activity, increasing dispersal behaviour and increasing foraging activity (Wingfield et al., 1997; Breuner et al., 1998; Silverin, 1998). T implantation increases plasma CORT in several avian species (Schoech et al., 1999; Duffy et al., 2000; Evans et al., 2000; Poiani et al., 2000; Owen-Ashley et al., 2004), and there is good evidence to suggest that CORT can be immunosuppressive when chronically elevated (e.g. Harvey et al., 1984; Wingfield et al., 1997; Råberg et al., 1998; Buchanan, 2000; Sapolsky et al., 2000).

Both T and CORT have been found to affect body condition (measured as the ratio of body mass to structural size); experimentally elevated T has been found to decrease body condition (Ros, 1999; Wikelski et al., 1999; Clotfelter et al., 2004; Mougeot et al., 2004; Roberts et al., 2007a), but the opposite has also been found (Briganti et al., 1999; Chastel et al., 2005). Increased CORT levels have also been found to be negatively related to body condition (Schwabl, 1995; Hood et al., 1998; Kitaysky et al., 2001; Sockman and Schwabl, 2001; Perfito et al., 2002; Breuner and Hahn, 2003; Pereyra and Wingfield, 2003; Love et al., 2005; Perez-Rodriguez et al., 2006; Roberts et al., 2007a). However, some studies have found no relationship between either CORT (Lormee et al., 2003; Lynn et al., 2003) or T (Buttemer and Astheimer, 2000; Alonso-Alvarez et al., 2002) and body condition. Indeed, some studies have found a positive association between CORT levels and body mass (Landys-Cianelli et al., 2002). Again, because the hormones may covary, it is unclear what (if any) effect either hormone may have on general body condition. Relating either CORT or T negatively to condition could suggest an indirect cost of maintaining high levels of these hormones. It is also possible that chronic effects of either hormone on body condition will eventually have indirect, negative effects on immunocompetence (Acquarone et al., 2002; Cucco et al., 2002; Fargallo et al., 2002; Lifjeld et al., 2002).

In order to assess the effects of testosterone and corticosterone simultaneously on avian immunocompetence and mass we sought to manipulate the hormones independently. Populations of Japanese quail (*Coturnix japonica*) were selected for low or high levels of adrenocortical responsiveness (plasma CORT response to brief mechanical restraint), with an additional randomly bred stock that served as a control (see Satterlee and Johnson, 1988). It is important to note that we have no evidence to suggest that the birds differed in basal corticosterone levels, so any effect of corticosterone was mediated through either changes in peak corticosterone levels or other physiological processes linked to the selection for differing corticosterone response to stress. However, there is evidence to suggest that peak and basal corticosterone levels covary (Cockrem and Silverin, 2002; Romero and Wingfield, 1998; Schoech et al., 1999), suggesting that pleiotropic effects might be responsible and therefore directional manipulation of one might affect the other. As the birds experienced stress due to interactions with other birds and general husbandry disturbance we expected corticosterone levels to rise above basal levels during the course of the experiment. We predicted that under the ICHH birds within the high T treatment group should demonstrate the lowest immune response to experimental challenges, and males with the lowest T should have the highest immune response. In contrast if CORT is immunosuppressive and there is no effect of T (thus supporting the stress-mediated ICHH), birds in the high CORT line should show the lowest immune response. If the costs associated with either hormone are energetic or metabolic rather than immunological, then this should be apparent as differences in body mass between the treatment groups.

We previously conducted a similar experiment on domesticated zebra finches (*Taeniopygia guttata*), selected in duplicate lines (2× high CORT, 2× low CORT and 2× control) for divergent levels of peak CORT over four generations. Levels of heritability of response were 20% in the high CORT lines but were lower in the low CORT lines, and there was a significant difference in peak CORT between the lines (Evans et al., 2006). In 2004, 4 years after the initiation of selection, testosterone levels were manipulated (Roberts et al., 2007a), but no significant differences in immune response were found between the selection lines or testosterone treatment groups. The present study aimed to test the same hypothesis in a different species – the Japanese quail – to ascertain whether the results obtained from the zebra finch experiment were species specific or whether we would find a lack of evidence for either T or CORT being immunosuppressive in other species selected for different levels of peak CORT.

MATERIALS AND METHODS

Selection programme and housing

Male Japanese quail (*Coturnix japonica*, Temminck and Schlegel 1849) were unselected (controls) or selected over several generations for divergent plasma CORT responses to brief mechanical immobilization. After 12 generations of initial pedigree selection (Satterlee and Johnson, 1988), the low (LS) and high (HS) stress lines differed significantly in plasma CORT response to restraint, having mean (±s.e.) CORT levels of 4.5 (±0.53) and 12.8 (±0.82) ng ml^{-1}, respectively (Satterlee and Johnson, 1988). More recent genetic history (from G_{13} to G_{34}; three generations beyond the one studied herein – G_{31}) verifies that, despite periodic relaxation of selection, maintenance of divergent adrenocortical responsiveness of a similar magnitude (2- to 4-fold CORT differences between the lines at 30 and 15 min after 5 min of handling) to the genetic selection stressor has been consistently maintained (Satterlee et al., 2000; Jones et al., 2002; Satterlee et al., 2002; Marin and Satterlee, 2004; Satterlee et al., 2006; Satterlee et al., 2007). The control line was also maintained by the University of Louisiana, as part of the experimental assessment of the effects of selection for varying corticosterone response to stress, and had the same history of husbandry regime.

The birds were kept in a room with a 14 h:10 h L:D photoperiod. In order to limit testes growth (to simplify later castration), after sexing birds by plumage coloration at 26 days of age, 135 male quail (45 males from each line and 45 controls) were placed on a short photoperiod (6 h of light daily). The birds were randomly housed in 45 cages, each cage containing a LS, a HS and a control male. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Louisiana.

Implantation procedure

All 135 males on the short photoperiod regime were castrated under general anaesthetic beginning at 9 weeks of age to ensure removal

of the effects of endogenous T production. A gaseous anaesthetic (Sevofluorane) was used, delivered at a mean concentration of 2% in air and at 1 l min^{-1}. Each bird was anaesthetized for 5 min before surgery; this period was always sufficient to ensure the animal was fully unconscious. An incision was made in the dorsal body wall between the last two ribs, and the testes removed using curved forceps. The body wall was then closed with surgical suture. Immediately after the castration, a Silastic implant containing no (zero), a low or a high amount of T (see below) was inserted into an incision made in the skin of the neck. This incision was also closed by surgical suture. The birds were fully conscious and alert within a few minutes of cessation of the anaesthetic.

The birds from each selected line (LS and HS) and the controls were randomly and equally allocated to one of three treatment groups: empty implant, low T implant and high T implant. This resulted in there being 15 males in each of nine CORT (LS, HS or control stock)×T-implant groups. The birds in the treatment groups were given one of the following: (1) an empty Silastic implant (10 mm in length; Dow Corning tubing, number 602-252; Midland, MI, USA; inner diameter 1.57 mm, outer diameter 2.41 mm), the sham implanted group; (2) one 10 mm long T implant packed with crystalline T (Sigma T-1500; St Louis, MO, USA), the low T group; or (3) two implants of T (each 10 mm long), the high T group. These implant dimensions have been used previously in Japanese quail to produce natural physiological levels of T in gonadectomized males (Castagna et al., 1999). The efficacy of the implants was confirmed by measurement of cloacal gland foaming, which is known to be T dependent (Siopes and Wilson, 1975; Seiwert and Adkins-Regan, 1998). The degree of foam production was measured between 22 and 31 days after castration and implantation of testosterone. It was counted in individuals on an arbitrary scale (integers from 0, or no foam, to 4, greater amount of foam production) (Satterlee et al., 2002).

PHA-induced immune response

The males' immunity was assessed by PHA injection in the same chronological order as their castration, on average 9.5 days (range: 8–11 days) after castration. This is an immune challenge that has been widely used in avian behavioural ecology (Duffy et al., 2000; Casto et al., 2001; Buchanan et al., 2003; Roberts et al., 2007a; Roberts et al., 2007b) and stimulates the proliferation of multiple immune cells and involves both the innate and adaptive elements of the immune system (Martin et al., 2006). Each male was injected with the mitogen phytohaemagglutinin (PHA-P, Sigma) intradermally into the left wing web (Lochmiller et al., 1993). Each male received 100 µl of a suspension of 0.32 mg PHA-P in 0.1 ml phosphate buffered saline (1× PBS) (Lochmiller et al., 1993). A spessimeter (Alpa s.r.l. Milan, Italy) was used to measure the wing web before injection (as a control measurement), and at 24 h after injection to obtain the wing web swelling in response to the mitogen, because little further swelling occurs after this time point (Martin et al., 2006). All the males were then weighed to the nearest 1 g using a Pesola spring balance, and their right tarsus lengths were measured to the nearest 0.01 mm with digital callipers.

Humoral immune response

Challenge by sheep red blood cell (SRBC) injection is also a frequently employed non-pathogenic test of the immune system in birds. It provokes an antibody response, thereby testing the humoral component of immune defence (Evans et al., 2000; Peters, 2000; Ardia et al., 2003; Buchanan et al., 2003; Hanssen et al., 2004). Two days after the completion of the PHA challenges, the experimental males were blood sampled. Approximately 150 µl of blood was collected into heparinized capillary tubes and centrifuged at 11,000 ***g*** for 15 min. The plasma was removed and heat treated at 56°C for 30 min in a water bath. The samples were then stored at –20°C and subsequently tested for cross-reaction to SRBC before exposure to the antigen. This was carried out as a control assay to ensure that anti-SRBC haemagglutinins were not present in the blood before immunization. Two days later the primary humoral immune response of the males was tested. Each male was immunized with 300 µl of a 10% solution of thrice-washed SRBC by intraperitoneal injection. Similar amounts of SRBC have been used with this species previously (Shimizu et al., 2004). Ten days later blood samples were taken. Twelve days after the initial injection the males were inoculated again with SRBC to test the secondary antibody response. Ten days after the secondary injection the birds were again blood sampled in the manner described above. Thus, in total three blood samples were taken from each manipulated male. The time frame of blood sampling was similar to that of previous avian experiments that employed SRBC challenge (Snoeijs et al., 2007). The mean period between the castration–T implant treatments and the primary antibody assay was 26 days; the mean period between these treatments and the secondary antibody assay was 38 days. The antibody response was assayed using a standard haemagglutination technique (Hay and Westwood, 2002). Briefly, the plasma was serially diluted across a V-form microtitre plate. A sample of 2% SRBC was added to all of the wells of the tray and the tray was then incubated at 37°C for 1 h in a water bath. Haemagglutination is evident when the antibodies in the plasma form a thin film of blood cells that covers the surface of the well. The most dilute titre of plasma exhibiting agglutination was recorded.

Statistical analyses

One bird died at an early stage of the experiment, leaving 134 individuals that were included in the subsequent analyses (see figure legends for sample sizes). The degree of cloacal gland foaming was compared between treatment groups by the use of ANOVA. The degree of swelling exhibited by the wing web injected with PHA was used as the dependent variable in a ReML (restricted maximum likelihood model). T treatment group, CORT line (and their interaction), body mass, tarsus length, and tier (level of cage from floor) nested within housing unit (battery) were the fixed effects, while cage number and family were included as random effects. The residuals of the model conformed to a normal distribution and were homoscedastic. A minimal model was obtained from the maximal model by stepwise deletion of non-significant terms ($P>0.05$).

In a separate analysis, antibody response (measured as the proportion of wells out of 12 that exhibited agglutination) was used as the dependent variable and the fixed and random effects were the same as used in the PHA model. The residuals of the model conformed to a normal distribution and were homoscedastic, so again a ReML model was used for this analysis. A minimal model was obtained from the maximal model by stepwise deletion of non-significant terms ($P>0.05$).

Finally, because either hormone could mediate its effect through changes in body mass, and important body size effects were detected in our previous study on zebra finches (Roberts et al., 2007b), body mass was used as a dependent variable with all other variables mentioned included as explanatory variables. The residuals derived from body mass regressed against tarsus length are not thought to be an appropriate measure of condition (Darlington and Smulders, 2001; Green, 2001), therefore mass was used as a measure

of condition with tarsus length included as a covariate. This approach has been recommended as the most appropriate method of statistically controlling for known sources of variation in dependent variables (Darlington and Smulders, 2001; Garcia-Bertou, 2001; Green, 2001). The residuals of the model conformed to a normal distribution and were homoscedastic, so a ReML model was used for this analysis. All analyses were carried out using Genstat (Genstat 6th Edition, VSN International, Hemel Hempstead, Herts, UK) and S-Plus 2000 (Insightful Corporation, Seattle, WA, USA).

RESULTS

Testosterone manipulation

ANOVA showed that the T treatment groups differed significantly in cloacal gland foaming ($F_{2,126}$=238.41, P<0.001; mean±s.e.: empty implant group 0.04±0.04; low T implant group 2.26±0.14; high T implant group 3.00±0.08). ANOVA also showed a significant main effect of CORT line on cloacal foaming ($F_{2,126}$=6.16, P<0.01). *Post-hoc* tests showed that the LS group had a significantly higher mean foam production (1.93±0.21) than their HS counterpart (1.48±0.21) with the control group showing an intermediate value (1.88±0.23). No significant interaction was observed between line and testosterone treatment ($F_{4,126}$=1.89, P>0.05).

PHA-induced immune response

There was no significant effect of CORT line on the size of wing web swelling at 24 h after PHA injection (Wald statistic=5.2, d.f.=2, P=0.074), but there was a trend for the unselected control stock to exhibit the greatest swelling (mean±s.e.: high line 0.692±0.05; low line 0.656±0.06; control stock 0.809±0.06). There was no significant difference between T groups in PHA response (Wald statistic=1.1, d.f.=2, P=0.587). The housing location had a significant effect on the wing web swelling (unit×tier interaction: Wald statistic=11.72, d.f.=5, P=0.039). There was no significant CORT×T interaction (P>0.05).

Secondary antibody response

Only unit (housing battery; Wald statistic=5.5, d.f.=1, P=0.019) and CORT line (Wald statistic=7.2, d.f.=2, P=0.028) (Fig. 1) remained significant after stepwise deletion from the maximal model. On inspection of the adjusted means, the control stock had a higher secondary response than either the LS or HS lines (Fig. 1). *Post-hoc* tests showed that the difference was significant between the control stock and the low CORT line. No differences between the LS and HS lines were observed. T treatment had no effect on antibody response (Wald statistic=0.01, d.f.=2, P=0.997) and neither did a CORT×T group interaction (P>0.05).

Body condition

The explanatory variables that had a significant effect on body condition, measured as body mass, were a unit×tier (housing) interaction (Wald statistic=19.1, d.f.=3, P<0.001), tarsus length (a positive association; Wald statistic=61.3, d.f.=1, P<0.001) and T treatment group. Empty implant-treated males had a significantly greater body mass than high T implant males (Wald statistic=10.1, d.f.=1, P=0.006), with low T implant males being of intermediate mass (Fig. 2). CORT line had no effect on body mass (Wald statistic=1.37, d.f.=2, P=0.505).

DISCUSSION

There is no evidence from this study to provide support for the ICHH or its stress-mediated modification. No significant effects of CORT (line influences) or T (implant influences) on immune response were detected. In particular, the PHA-induced immune response was not significantly affected by either T treatment group or CORT line. These results are contrary to those of several other studies that found a negative effect of T implantation on the cell-mediated immune response (Duffy et al., 2000; Casto et al., 2001; Owen-Ashley et al., 2004), but are in agreement with others that found no effect of CORT manipulation (Martin et al., 2005; Roberts et al., 2007a) or T manipulation (Buchanan et al., 2003; Roberts et al., 2007a) on the cell-mediated response. One possible reason for this is that our immune tests were not appropriate for testing general immunocompetence levels in this species. These tests are certainly crude tests of aspects of immune function, which may not accurately assess the ability to counter real pathogens (Viney et al., 2005). Another possibility is that the experimental conditions were not appropriate for detecting a meaningful change in immune function, if such an effect could only be detected, for example, when resources are in short supply. Although this is possible, there are many manipulative studies which have demonstrated effects of changes to the endocrine system on immunocompetence, using similar immune tests and without dietary restrictions (e.g. Duffy et al., 2000; Peters, 2000; Casto et al., 2001).

Immunity did not differ significantly between the CORT selected lines; a possible explanation for this is that basal corticosterone levels are more important than peak levels in controlling immunocompetence. In a similarly designed experiment on zebra finches selected for divergent levels of peak CORT and with T

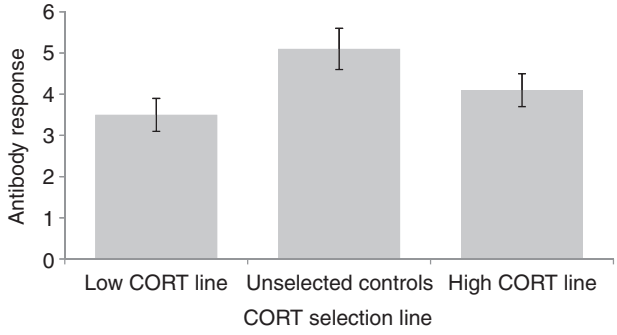

Fig. 1. Mean ± s.e. secondary humoral response (number of agglutinated wells) of Japanese quail to sheep red blood cell injection in unselected controls, low corticosterone (CORT) line (LS) and high corticosterone line (HS) (LS line N=45; HS line N=44; controls N=45; see text for details).

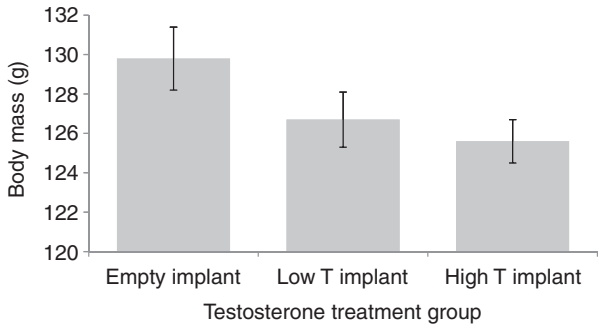

Fig. 2. Mean ± s.e. body mass of Japanese quail in different testosterone (T) treatment groups (Empty implant group N=44; Low T implant group N=45; High T implant group N=45; see text for details).

manipulated, Roberts and colleagues (Roberts et al., 2007a) also found no differences in immune response between birds selected for different levels of CORT, or between different T treatment groups; however, actual hormone titres did significantly interact positively with a measure of humoral immunity. In the present experiment we did not obtain individual CORT and T titres and so cannot test whether they were related to immune response. The possibility exists that actual CORT and/or T titres may have shown significant relationships with immunocompetence, either alone or in interaction with each other or treatment groups, rather than the treatment groups alone. The control birds showed a greater secondary antibody response than either the LS or HS lines, which showed similar responses to each other, and there was a trend for the control birds to exhibit a greater swelling than the selected birds; however, this was not significant. Body condition, measured as body mass, was significantly higher in the empty implant-treated males than in the high T implant males, suggesting a potential cost of elevated T.

Intermediate levels of plasma CORT response to stress, such as would be expected in the controls, may be the optimal solution in terms of mounting an immune response. It is possible that if stressor-induced CORT levels are too low, then the hormone may not be able to optimally fulfil its functions in adaptive instigation of the 'flight or fight' syndrome and attendant physiological and behavioural responses to stressful stimuli (Buchanan, 2000). Indeed, some studies have found an immuno-enhancing effect of high peak CORT levels on the cell-mediated immune response (Dhabhar and McEwen, 1999; Dhabhar, 2000; Dhabhar, 2002; Roberts et al., 2007b). Conversely, if stressor-induced CORT levels are too high, then the negative aspects of hyper-CORT secretion and activity may manifest themselves, possibly by producing immunosuppression and a reduction in reproductive activity (Wingfield et al., 1997). Alternatively, the fact that the control males exhibited greater immune responses than the two stress line groups may suggest that the controls had a more robust immune response than the other groups for reasons other than actual CORT level effects, such as greater genetic heterozygosity, given that there is considerable evidence to suggest that low genetic diversity is linked to low immune function (Whiteman et al., 2006; Hale and Briskie, 2007; Reid et al., 2007; Ross-Gillespie et al., 2007). The selected lines were likely to be more inbred than the control stock (mating of half-siblings was unavoidable for selection purposes), although we have no data on heterozygosity levels within the lines to confirm this.

Regardless of line, the empty implant-treated males had significantly greater body mass than the high T implant males. Several studies have found a negative effect of T on body condition or body mass in birds (Ros, 1999; Wikelski et al., 1999; Clotfelter et al., 2004; Mougeot et al., 2004), and this result to some extent agrees with our (Roberts et al., 2007a) finding that male zebra finches in a high T treatment group suffered greater mass loss than those in other treatment groups, but in that case only with increasing CORT titres. A possible mechanism for this negative effect of T on mass is that an increase in metabolic rate or activity occurs in response to elevated levels of plasma T (Buchanan et al., 2001). However, previous avian studies that have investigated the effects of T on body mass and metabolic rate or energy expenditure have reported mixed results (Wikelski et al., 1999; Buttemer and Astheimer, 2000; Lynn et al., 2000; Buchanan et al., 2001). We did not measure energy expenditure or metabolic rate in this study, but T manipulation certainly had a significantly negative effect on male body mass. This result does not lend support to the hypothesis that T is immuno-enhancing due to increased body condition (Evans et al., 2000). However, this hypothesis assumes that high T males have greater access to dietary resources through dominance of subordinates resulting in increased body mass. This in turn allows more resources to be available to the dominant or high T individual for immune defence. Males within each T group were housed together; therefore, no difference should have existed in T levels between males in the same cages. In addition, food was not a limiting factor as the birds were fed *ad libitum*. The high T implant males behaved more aggressively to each other than males in the other groups (M.L.R., unpublished observations), and frequently pecked each other or attempted to mount and copulate. This may well have reduced the overall condition of the birds in the high T groups. On inspection of Fig. 2 it can be seen that there was little difference in body mass between the low T group and the high T group, suggesting that the lower level of T in the empty implant-treated males was responsible for the differences in body mass.

Considering that T implantation had a negative effect on body mass, and body mass in previous studies has been positively related to immunocompetence (Acquarone et al., 2002; Cucco et al., 2002; Fargallo et al., 2002; Lifjeld et al., 2002), it seems somewhat surprising that T did not have a negative effect on immune response. The reasons for this are unclear; however, it is possible that individuals trade-off condition (as fat reserves) for immunocompetence (McNamara and Buchanan, 2005); the result is no perceived cost of T to immunity but a corresponding cost to condition, as found in our study. Future studies that manipulate T levels should control the amount of food provided to the experimental individuals, or better still should measure the amount eaten per day by individuals in different T treatment groups. In conclusion, the results of the present study are in general consistent with the results obtained previously (Roberts et al., 2007a). No line or treatment effects of CORT or T, respectively, were found on immunocompetence, and the supposition that either hormone is immunosuppressive in all circumstances is therefore not validated.

LIST OF ABBREVIATIONS

CORT	corticosterone
HS	high stress
ICHH	immunocompetence handicap hypothesis
LS	low stress
PHA	phytohaemagglutinin
SRBC	sheep red blood cells
T	testosterone

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