# Testosterone manipulation postcastration does not alter cloacal gland growth differences in male quail selected for divergent plasma corticosterone stress response[1]

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**ABSTRACT** Japanese quail selected for reduced (low-stress, LS) rather than exaggerated (high-stress, HS) plasma corticosterone response to brief restraint have consistently shown greater cloacal gland (CG) development, an androgen-dependent trait. In this study, the effects of testosterone implants on levels of plasma testosterone and CG development in castrated LS and HS quail were determined. Stress-line males were castrated and randomly allocated to 1 of 3 testosterone treatments: the empty testosterone (ET), low testosterone (LT), or high testosterone (HT) implant group. Cloacal gland volume was determined at 4 weekly intervals that represented ranges of 1 to 9 d, 8 to 17 d, 15 to 24 d, and 22 to 31 d after castration and testosterone implantation. Levels of plasma testosterone were also assessed at the end of the study. Development of the CG was affected by quail line (LS > HS), testosterone treatment (HT > LT > ET), and time of measurement (1 to 9 d < 8 to 17 d < 15 to 24 d = 22 to 31 d after castration and testosterone implantation). A significant interaction between testosterone treatment and time of measurement on CG volume was also detected (with CG volume generally increasing with time in LT- and HT-treated quail, but not in ET-treated quail). However, even though HT implant treatments induced higher CG development than did LT treatments beyond the first interval of CG volume measurement, and despite the finding of greater CG volumes in LS than HS quail during the last 2 measurement intervals within each of the LT and HT groups, no interaction was observed between testosterone implant dosages and quail stress line on CG volume. Thus, by the end of the study, regardless of testosterone dose, CG volume was consistently greater in LS quail than in their HS counterparts. In addition, although, as expected, the testosterone implant treatment significantly altered levels of plasma testosterone (HT > LT > ET), neither quail line nor its interaction with testosterone treatment affected plasma testosterone. The present findings suggest that the often-observed depressed CG development in the HS line may be independent of testosterone effects.

**Key words:** cloacal gland, corticosterone, testosterone, Japanese quail

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## INTRODUCTION

In avian species, the administration of corticosterone (Deviche et al., 1982; Joseph and Ramachandran, 1993) or adrenocorticotropin (Edens, 1987) and the activation of the hypothalamic-pituitary-adrenal (**HPA**) axis by various nonspecific systemic stressors (Deviche, 1983; Edens, 1987) have been associated with depression in the hypothalamic-pituitary-testicular (**HPT**) axis, which, in turn, presents a plausible explanation for the observation of stress-induced inhibition of male reproductive functions. In rodents, glucocorticoid action is also known to inhibit the expression of testosterone-biosynthetic enzymes and thereby reduce the steroidogenic capacities of Leydig cells (Payne and Sha,


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1991; Orr et al., 1994). Glucocorticoids may also control mitosis and induction of apoptosis (Schwartzman and Cidlowski, 1993; King and Cidlowski, 1998) in a variety of target cells.

It has been postulated that corticosterone may inhibit gonadotropin-releasing hormone and therefore the secretion of follicle-stimulating hormone, luteinizing hormone (**LH**), or both, reducing the positive effects of these gonadotropins on the testes and the LH-induced secretion of testosterone (Satterlee et al., 2000; Satterlee and Marin, 2006). Several studies have also shown that glucocorticoids may induce repression of proteins needed for formation of the hormone receptors, intracellular signaling molecules, and steroidogenic enzymes that are components of stress-mediated suppression of reproductive cell types (Welsh et al., 1999; Sapolsky et al., 2000).

The development of the cloacal gland (**CG**) in Japanese quail (*Coturnix coturnix japonica*) is an androgen-dependent phenomenon and is considered a reliable indicator of testicular development and sexual activity (Coil and Wetherbee, 1959; McFarland et al., 1968; Sachs, 1969; Siopes and Wilson, 1975; Oishi and Konishi, 1983; Delville et al., 1984). Thus, CG hypertrophy is an excellent nondestructive indicator of male quail gonadal development. Male Japanese quail selected for reduced (low-stress, **LS**) rather than exaggerated (high-stress, **HS**) plasma corticosterone response to brief mechanical restraint (Satterlee and Johnson, 1988) show CG of larger size and higher foam production (Satterlee et al., 2002). Cloacal gland development and foam production also occur sooner in LS than in HS quail, line differences that persist well into adulthood (Marin and Satterlee, 2004). This comparative advantage in CG development of LS quail has been observed even when animals were held on short (nonphotostimulatory) day lengths (Satterlee and Marin, 2004; Satterlee et al., 2007). In all these reports, it was proposed that the genetically controlled line differences in adrenocortical responsiveness (HS > LS) may have resulted in circulating corticosterone conditions during growth and development that interacted divergently with the HPT axis, such that greater releases of testosterone were an underlying feature causing the accelerated puberty and enhanced reproductive fitness observed in LS quail. Indeed, we have also found that, in comparison with their HS counterparts, adult male LS quail possess higher testicular weights and greater copulatory efficiency, as estimated by the number of times that copulation sequences are completed once they have been initiated (Marin and Satterlee, 2003, 2004; Satterlee and Marin, 2004). Such findings have led us to propose that steady-state or reproductive event-driven gonadotropin-releasing hormone-induced pituitary LH and follicle-stimulating hormone release are compromised in HS quail by day-to-day stress-induced exaggerated blood corticosterone responses that are expected to occur routinely in the HS line. If so, a further expected consequence would be a reduction in gonadotropin-induced testicular growth and circulating levels of testosterone. Reduction in testosterone would, in turn, lessen the prime stimulus for CG growth and function (at least in terms of initial foam production). However, no clear differences in plasma testosterone were found between LS and HS quail when a single blood sample was taken at the end of the day-length manipulation study cited above (Satterlee et al., 2006). On the other hand, testicular endocrine activity is typically known to increase immediately before and during the breeding season (Bartke et al., 1987), so failure to reveal differences between the stress lines in plasma testosterone concentrations in the single snapshot blood sampling study of Satterlee et al. (2006) may have been due to the use of a sampling procedure that did not encompass a period when the testes and CG were experiencing rapid growth.

Considering the factors mentioned above, the present study was conducted to determine whether the previously demonstrated quail stress line differences in CG development (LS > HS) are related to different levels of circulating testosterone. To control for levels of testosterone, males were first castrated (to remove the effects of endogenous testosterone production), and then birds from each stress line were randomly allocated into testosterone treatment groups that were designed to represent increasing amounts of testosterone replacement therapy—empty testosterone (**ET**), low testosterone (**LT**), and high testosterone (**HT**) implant groups. In this way, blood levels of testosterone and corticosterone could be manipulated so that CG development could be compared between the stress lines and testosterone treatments independent of gonadal contributions of testosterone. It is important to note that although birds in the stress lines do not differ in the basal corticosterone levels, because of the apparent nonspecific nature of divergent stress responsiveness in the stress lines (Satterlee and Johnson, 1985; Jones et al., 1992a,b, 1994, 1999, 2000; Jones and Satterlee, 1996; Satterlee et al., 2000; Satterlee and Marin, 2006; Davis et al., 2008; Kembro et al., 2008; Guzman et al., 2009), we would expect differential adrenocortical responses consistent with the genetic background of each line to have occurred often during the course of the study. For example, divergent stress line plasma corticosterone responses (HS > LS) likely occurred 1) at times of routine maintenance chores (e.g., during disturbances associated with daily manual feed replenishment, removal, and scraping of droppings pans); 2) during bird capture, crating, transport, and handling for the purposes of hatching, leg and wing banding, and housing; 3) during the complex and stressful treatment procedures associated with testes removal and testosterone implantation (e.g., anesthesia and surgeries); 4) during immune testing procedures; and 5) as a result of the weekly CG measurements made after castration and testosterone treatment.

## MATERIALS AND METHODS

### Animals and Housing

Male Japanese quail (*C. coturnix japonica*) from 2 lines selected for either an LS or HS plasma corticosterone response to brief mechanical immobilization (Satterlee and Johnson, 1988) were studied. The same birds used herein were also used in a study reported by Roberts et al. (2009), wherein testosterone manipulation on castrate LS vs. HS humoral immunity and phytohemagglutinin-induced immune responses were determined. The more recent genetic history (from $G_{13}$ to $G_{34}$; 3 generations beyond the one studied herein, $G_{31}$) verifies that, despite periodic relaxation of selection, divergent adrenocortical responsiveness of a similar magnitude (2-fold corticosterone differences between the lines, HS > LS) to the selection stressor has been consistently maintained (Satterlee et al., 2000, 2002, 2006, 2007; Jones et al., 2002; Marin and Satterlee, 2004). Moreover, an examination of corticosterone differences in the yolks of eggs collected from LS and HS hens at $G_{32}$ (the generation that immediately followed the one presently studied) showed that line differences (HS > LS) persist (Hayward et al., 2005).

Details of the rearing and brooding procedures used in the present study can be found elsewhere (Jones and Satterlee, 1996; Satterlee and Marin, 2004). Because Japanese quail are extremely photosensitive (Robinson and Follett, 1982; Mills et al., 1997), after sex identification by plumage coloration (28 d of age), the present quail were kept under a short photoperiod regimen (6 h of light daily) to limit testis growth and to simplify later castration (see below). Forty-five males of each selected line, representing 3 to 4 breeder males from each of the 12 families within a line, were randomly selected for study. Males were housed in a 4-tier pedigree cage unit (Alternative Cage Designs, Alternative Design Manufacturing and Supply Inc., Siloam Springs, AR). Cages measured 51 × 15 × 27 cm (length × width × height). In each cage, 2 experimental quail (1 LS + 1 HS) were housed along with a third, unselected filler male (hatched and managed under identical conditions described for the selected line males; see above). The filler male used by Roberts et al. (2009), and therefore in the present study, also provided for the same stocking density as was used in the study by Satterlee and Marin (2004) of photoperiod-induced changes in CG physiology.

### Castration and Implantation Procedures

Between 9 and 10 wk of age, all males (see below) were castrated under general anesthesia to ensure removal of the effects of endogenous testosterone production, as described by Roberts et al. (2009). A gaseous anesthetic (Sevoflourane, Baxter Healthcare Corp. Deerfield, IL) was used. It was delivered at a mean concentration of 2% in air and at 1 L/min. Each bird was anesthetized for 5 min before surgery; this period was always sufficient to ensure the bird was fully unconscious. An incision was made in the dorsal body wall between the last 2 ribs, and the testes were removed using a curved forceps. The body wall was then closed with surgical sutures. Immediately after castration, a Silastic tube implant(s) containing various amounts of testosterone (see below) was inserted into an incision made in the skin of the neck. This incision was also closed by surgical suture. All birds were fully conscious and alert within a few minutes of cessation of the anesthetic.

Birds from each selected line (45 LS + 45 HS) were randomly and equally allocated to 1 of 3 testosterone treatments: the ET, LT, or HT implant group. This resulted in there being 15 males in each line × testosterone treatment group, for a total of 90 birds used in the study. For the testosterone implant treatment, birds were given either 1) an empty Silastic tube implant (10 mm in length; Dow Corning tubing, number 602-252, Dow Corning Corp., Midland, MI; inner diameter: 1.57 mm, outer diameter: 2.41 mm), designated the sham-implanted ET group; 2) one 10-mm-long Silastic tube packed with crystalline testosterone (Sigma T-1500), designated the LT implant group; or 3) 2 Silastic tubes (each 10 mm long) filled with testosterone, designated the HT implant group. These size implants have been used previously in Japanese quail to produce natural physiological levels of testosterone in gonadectomized males (Castagna et al., 1999). To reach the total number of 90 birds used in the study, the castrations and subsequent testosterone implant procedures took 9 consecutive days to complete. Therefore, to maintain the best possible balance between the numbers of birds within each stress line × testosterone implant treatment group undergoing surgical procedures on a daily basis, a minimum of 1 male, and occasionally 2 males, from each line × implant group were operated on daily, following a set cage order (each cage containing an LS and HS male) that alternated sequentially between the 3 testosterone implant treatments (ET, LT, and HT).

### CG Volume Measurements

Cloacal gland volume (**CVOL**) was determined weekly over a 4-wk period in all males, beginning at 11 wk of age. Because of the complexities and constraints of the time needed to complete all the surgical procedures described above, these study intervals correlated to time period ranges of 1 to 9 d, 8 to 17 d, 15 to 24 d, and 22 to 31 d after castration and implantation. Cloacal gland size measurements, length (mm) and width (mm), were made using a digital caliper, and CVOL was calculated from these measurements according to the formula proposed by Chaturvedi et al. (1993; $4/3 \times 3.5414 \times a \times b^2$, where $a = 0.5 \times$ long axis and $b = 0.5 \times$ short axis). To confirm the efficacy of castrations and testosterone

implantations on CG physiology further, foam gland expression was also measured at the end of the study (at the 22- to 31-d interval after castration and implantation). The results of CG foam measurements were reported by Roberts et al. (2009) and therefore will not be repeated herein. To avoid potential effects of different operators, the same experimenter made all CG measurements. Body weight measurements were also made during testosterone treatments, and the results showed no differences between LS and HS quail (Roberts et al., 2009). Because no differences in BW were found between LS and HS quail in that study and because the additional CG measurements (foam production) made in the same study were done at the end of the present experiment, these variables could not have affected the present CVOL and plasma testosterone outcomes. In addition, Satterlee et al. (2006) proposed that HPA and HPT axis alterations are likely independent of differences in BW because these are consistently found to be similar between the lines at various adult ages. However, we submit that the immune response treatments of phytohemagglutinin-A and sheep red blood cell challenges made by Roberts et al. (2009) during an earlier time period that was coincident between the 2 studies constituted potential advantageous treatment instances wherein yet further divergent adrenocortical stress line responses might have occurred.

### Testosterone Measurements

Blood sampling was conducted for the testosterone assay at the end of the study (4 wk after the castration and implantation treatments). Blood (100 μL) was drawn from the brachial vein. Samples were subsequently centrifuged for 15 min at 886 × *g*, and plasmas were drawn off and stored at −20°C until assay. Testosterone concentrations were measured in plasma samples by direct RIA using antitestosterone antiserum (code 8680-6004, Biogenesis, Poole, UK) and [$^{125}$I]testosterone label (code 07-189126, ICN, Boldon, UK; Parkinson and Follett, 1995). The assay was run with 50% binding at 0.53 ng of testosterone/mL, and a detection limit of 0.015 ng of testosterone/mL. The interassay CV was 7.1% and the intraassay CV was 4.0%.

### Statistical Analyses

The CVOL data were subjected to a repeated measures ANOVA that examined the main effects of the between-subject variables, testosterone treatment (ET, LT, and HT) and quail stress line (LS and HS); the within-subject variable, time of sampling (1 to 9 d, 8 to 17 d, 15 to 24 d, and 22 to 31 d after castration and testosterone implantations); and their interactions. Levels of plasma testosterone data were subjected to a 2-way ANOVA that used a 2 × 3 factorial arrangement of treatments (the 2 quail stress lines and the 3 testosterone implant treatments). Post hoc Fisher least significant difference tests were used to partition the main effect means associated with testosterone implant treatment and time of sampling as well as all interactive effect means. Analysis of variance assumptions were verified in all cases, and significant effect levels were set at a *P*-value of 0.05 or less.

## RESULTS

Cloacal volume was significantly affected by quail line (LS > HS; $F_{1,84} = 5.96$, $P < 0.02$), testosterone treatment ($F_{2,84} = 204.44$, $P < 0.001$; ET < LT < HT, $P < 0.05$ by Fisher's least significant difference), and time of sampling after castration and testosterone implantation ($F_{3,252} = 65.60$, $P < 0.001$; 1 to 9 d < 8 to 17 d < 15 to 24 d = 22 to 31 d, $P < 0.05$ by Fisher's least significant difference). A significant interaction between testosterone treatment and time of sampling ($F_{6,252} = 17.85$; $P < 0.001$) on CVOL was also detected (data not shown; but, as expected, CVOL generally increased with time after castration and implantation in the LT and HT groups, but not in the ET group). No interaction between quail stress line and testosterone treatment on CVOL was detected ($F_{2,84} = 1.77$; $P > 0.17$).

Figure 1 depicts the changes in mean CVOL responses of LS and HS male quail across the 4 weekly intervals of CVOL measurement after castration and testosterone treatment. Fisher's least significant difference post hoc tests showed significant differences ($P < 0.05$) in mean CVOL as follows: the LS and HS groups that were given no testosterone replacement therapy postcastration, and the ET control groups), which showed similarly depressed and no further CVOL development postcastration. However, both LS and HS quail, when treated with testosterone, similarly increased their CVOL as soon as 1 to 9 d after castration and testosterone treatment. By 8 to 17 d after castration and testosterone implantation, further increases in CVOL were observed that were dose dependent (HT > LT), but not line dependent. By 15 to 24 d after castration and testosterone implantation and thereafter, both line and testosterone dose were effective in partitioning CVOL mean responses according to the following order: LS-HT > HS-HT > LS-LT > HS-LT. The 2 highest mean CVOL values (that were also similar to one another) were found in the LS quail group treated with the highest dose of testosterone and observed at the last 2 time intervals (15 to 24 d and 22 to 31 d after castration and testosterone implantation, respectively).

The CG foam measurements reported by Roberts et al. (2009) at 22 to 31 d after castration and testosterone implantation essentially mimicked the present CVOL findings observed herein at the same time interval. That is, no foam production was evident in ET-treated birds, whereas increasing amounts of CG foam were seen in LT- and HT-treated quail, respectively. Low-stress quail treated with testosterone implants postcastration also showed greater CG foam production than did similarly testosterone-treated HS-quail.

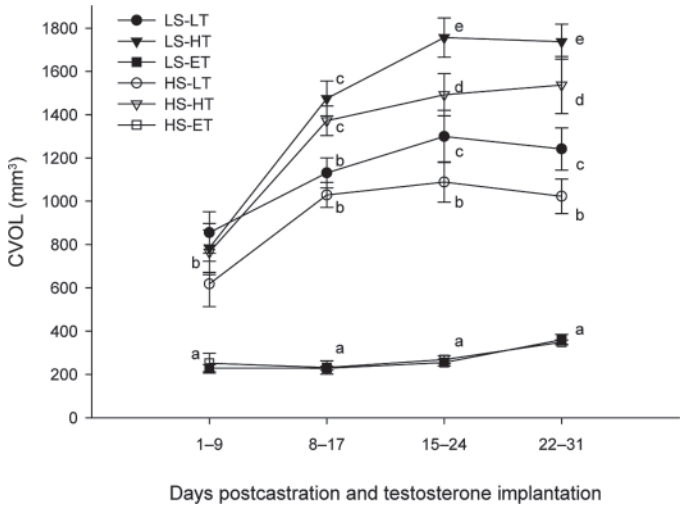


**Figure 1.** Mean (±SE; vertical bars) cloacal gland volume (CVOL) in quail lines selected for reduced (LS, low-stress) or exaggerated (HS, high-stress) plasma corticosterone response to brief mechanical restraint that were castrated and treated with empty testosterone (ET), low testosterone (LT), or high testosterone (HT) implants. Means with no common letter (a–e) differ significantly ($P < 0.05$).

Figure 2 depicts mean levels of plasma testosterone in the LS and HS male quail treated with an ET, LT, or HT implant postcastration. Neither quail line ($F_{1,77} = 1.39$; $P = 0.24$) nor its interaction with testosterone treatment ($F_{2,77} = 1.01$; $P = 0.37$) affected levels of plasma testosterone at the end of the study. However, as expected, a significant effect of testosterone treatment dose ($F_{2,77} = 8.00$; $P < 0.001$) on plasma testosterone levels was evident. Fisher least significant difference post hoc tests showed that, regardless of the quail line, HT-implanted males possessed higher ($P < 0.05$) mean ± SE levels of plasma testosterone than did males of the LT- and ET-implanted groups (12.70 ± 1.48, 8.99 ± 1.26, and 5.67 ± 0.61 ng/mL, respectively).

## DISCUSSION

This study evaluated the changes in mean CVOL responses of LS and HS male quail after castration and testosterone replacement therapy treatment. As expected, no CG development was observed in control LS and HS quail (males that were not implanted with testosterone after castration), whereas CG development was observed when birds were implanted with testosterone. Previous reports have shown a positive correlation between CG size and blood testosterone levels (Mills et al., 1997; Mohan et al., 2002). Not surprisingly, then, the highest mean CVOL values were found in those quail groups that were treated with the highest dose of testosterone, especially after 8 to 17 d of administration, the same treatment groups that showed the greatest levels of plasma testosterone at the end of the study. In addition, in agreement with the quail studies of Ottinger et al. (1997) and Biswas et al. (2007), our CVOL results show that the maximum effects of testosterone implants on CG development occurred between 2 and 3 wk after testosterone implantation, effects that were dose dependent. Thus, once again, the present results confirm that testosterone replacement postcastration is necessary for CG growth in Japanese quail and that CG development is dependent on the testosterone dose administered after castration. Our CVOL and plasma testosterone findings also lend further support to the well-documented contention that CG development is an androgen-dependent phenomenon (Coil and Wetherbee, 1959; McFarland et al., 1968; Sachs, 1969; Siopes and Wilson, 1975; Oishi and Konishi, 1983; Delville et al., 1984). Interestingly, even though the present HT implant treatments induced greater CG development than did the LT implant treatments, no significant interaction was observed between the effects of testosterone dose implanted and quail stress line. That is, LS and HS castrated quail that were implanted with either an LT or HT dose eventually showed (i.e., by the last 2 sampling intervals) significant differences in their CG development consistent with what we have reported previously (LS > HS; Satterlee et al., 2002, 2006, 2007; Marin and Satterlee, 2004; Satterlee and Marin, 2004).

As mentioned in the materials and methods section, CG foam measurements made at the end of the study, as reported by Roberts et al. (2009), essentially mimicked the present CVOL findings made at the same time interval. Thus, collectively, the CVOL and foam findings attest to the effectiveness of the castration surgeries and the ability of testosterone replacement therapy to reestablish CG growth and foam production in castrated quail.

We have discussed elsewhere (Satterlee et al., 2000, 2002, 2006; Marin and Satterlee, 2004; Satterlee and Marin, 2004) the evidence that the administration of corticosterone or adrenocorticotropin, as well as activation of the HPA axis by various nonspecific systemic

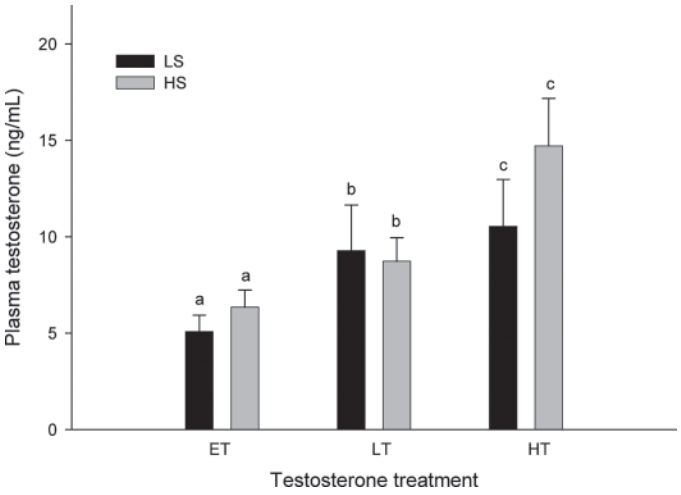


**Figure 2.** Mean (±SE; vertical bars) plasma testosterone (ng/mL) in quail lines selected for reduced (LS, low-stress) or exaggerated (HS, high-stress) plasma corticosterone response to brief mechanical restraint that were castrated and treated with empty testosterone (ET), low testosterone (LT), or high testosterone (HT) implants. Means with no common letter (a–c) differ significantly ($P < 0.05$).

stressors, is associated with depression in the HPT axis in birds. The mechanism of action proposed may also include (as mentioned in the introduction section) the induction or repression of proteins needed for the formation of testosterone hormone receptors, intracellular signaling molecules, and steroidogenic enzymes (Welsh et al., 1999; Sapolsky et al., 2000). This led us to our former hypothesis that one or more of these potential relationships may explain why stress-induced inhibition of avian male reproductive functions is often reported (Deviche et al., 1982; Deviche, 1983; Edens, 1987; Joseph and Ramachandran, 1993). Furthermore, CG development appears to respond not only to direct plasma testosterone intervention, but also to the nonreduced metabolites of testosterone. For example, Massa et al. (1980) and Delville et al. (1984) have identified several testosterone metabolites that stimulated CG growth in castrated birds. Therefore, the CG growth response in castrated birds to either plasma testosterone or its metabolites suggests that both testosterone and testosterone-metabolite receptors exist in the CG. However, based on our present results (that show differential LS and HS CG growth), it seems that yet other unidentified factors besides testosterone and testosterone metabolites (the latter of which were not measured herein) can also affect CG development. In the present study, we indirectly evaluated an aspect of the reproductive performance of castrated LS and HS quail by measuring their CG development in response to 2 regimens of testosterone replacement therapy (a theoretically low and high steady-state dose of testosterone delivered by differential testosterone implantation). Although plasma testosterone was once again measured only at the end of the study, no differences were found in circulating levels of this androgen between LS and HS quail. It should be further noted that within each dose of testosterone administered (either LT or HT), similar plasma testosterone levels were consistently detected between quail of the LS and HS stress lines. Nevertheless, higher CG development was still observed in LS quail compared with HS quail by the end of the study. Therefore, although the present results continue to support our premise that a strong negative relationship exists between activation of the HPA axis and CG development (i.e., herein, HS quail once again showed a reduced CVOL in comparison with LS quail), because this relationship appears to be independent of testosterone intervention, an alternative mechanism to explain this negative relationship is needed. One possibility may lie in the existence of an extragonadal effect of corticosterone on the CG itself, wherein corticosterone may induce alterations such as downregulation of the testosterone, testosterone-metabolite receptors, or both (see above). However, the stress-mediated suppression on CVOL (through a corticosterone effect on testosterone or testosterone metabolite CG receptors) was not completely suppressive because CVOL was not reduced to zero in size in the testosterone-treated groups. This proposed effect is evidenced by the fact that, in the HS line (the line genetically predisposed toward exaggerated plasma corticosterone responsiveness), CG growth did not reach the same magnitude as that achieved by the LS quail after testosterone replacement therapy. Thus, the present findings suggest the existence of a partial stress-mediated suppression of the testosterone effects on CG development.

Presently, a somewhat surprising finding was the much higher than expected levels of plasma testosterone at the end of the study in ET-treated quail of both stress lines. Because CVOL in quail of both lines was considerably dampened in comparison with the LS and HS quail that received testosterone replacement therapy, and because CG foam production was essentially zero at this time for ET-treated birds [see Roberts et al., 2009; CG foam = 0.04 ± 0.04 on a 0 (no foam) to 4 (maximum foam) scale] at this time, testaments to the effectiveness of the castrations, plasma testosterone levels would logically be expected to be almost nil as well. However, plasma testosterone levels of 5.0 and 6.3 ng/mL were found in LS-ET and HS-ET quail, respectively. Interestingly, high levels of testosterone secretion were also reported in intact and castrated male quail in another study that used short days (photic castration), suggesting additional testosterone secretion from extragonadal sources (Boswell et al., 1995). The mechanism of partial suppression of testosterone effects on CG development mentioned above may also help explain the low CG size observed in the ET-treated birds in spite of the unexpected testosterone levels detected in them.

It is also important to recall that, in previous studies with noncastrated birds, LS male quail not only showed larger CG sizes and accelerated CG development when compared with HS quail, but LS birds also possessed greater testicular weights (Marin and Satterlee, 2004; Satterlee and Marin, 2004) and enhanced reproductive behavior, as evidenced by a lower latency to the first grab, a higher number of cloacal contacts, and an improved copulatory efficiency (Marin and Satterlee, 2003). Thus, the stress-induced inhibitions of male reproductive functions mentioned above appear to be influencing the HPT axis at various levels.

When somewhat similar photoperiod constraints as were used in the present study were applied to intact stress line quail (i.e., rearing under continuous short day lengths of 6 h beginning at hatch; Satterlee et al., 2007), whereas LS males exhibited greater CG development, a CVOL mean of only 1,500 $mm^3$ was achieved at 150 d of the experiment. In contrast, under the experimental conditions of the present study, although testosterone was administered to castrated birds at levels that have been demonstrated to restore physiological plasma testosterone values reflective of sexually active individuals (Balthazart et al., 1984; Castagna et al., 1999), mean CVOL in the LS-HT males was found, as soon as 15 to 24 d postcastration, to be almost 17% higher (1,754 $mm^3$) than the average CVOL reported by Satterlee et al. (2007). Therefore, the administration of a high, but

theoretically physiological, dose of exogenous testosterone was able to produce a more rapid and greater CG development in light-deprived castrated LS males than that seen under nonstimulatory photoperiodic conditions using intact subjects.

In summary, regardless of the endocrine mechanisms that underlie the presently observed line differences in CVOL, the continued finding of greater CG development in males of the LS line represents an intuitively desirable trait associated with selection for reduced adrenocortical responsiveness. Avian geneticists should consider incorporating such selection into their breeding programs.

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