

Testosterone and Avian Life Histories: The Effect of Experimentally Elevated Testosterone on Corticosterone and Body Mass in Dark-Eyed Juncos

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To assess whether alterations in the normal pattern of testosterone (T) secretion might be beneficial or detrimental, we studied a breeding population of dark-eyed juncos in which we elevated T experimentally and measured its effect on potential correlates of fitness. We treated both free-living and captive males with implants that were either empty (C-males, controls) or packed with T (T-males, experimentals). Timing of implant varied and was designed to mimic natural peak breeding levels except that peaks were either prolonged or premature. We bled the birds at recapture and analyzed their plasma, and that of their female mates, for T and corticosterone (B). We also measured body mass and fat score in free-living T- and C-males. In the field, T-implants elevated T and kept it elevated for at least a month. Experimental males also had higher B than controls. In captives, the effect of the implants on plasma T was detectable within 24 hr. B in captive T-males was again higher than in captive C-males. In females, neither T nor B differed between mates of T- and C-males. T-males implanted in early spring lost more mass between implant and recapture in late spring than did controls and also had lower fat scores when recaptured. When implants were inserted in summer, treatment did not influence mass. Elevated T in early spring apparently hastened the transition from the winter to the breeding mode of fat storage. We suggest that prolonged elevation of testosterone might be selected against because of the association between T and B. Premature elevation of T might be costly because of the resultant loss of mass and fat reserves, which could lead to mortality when spring snowstorms prevent access to food. © 1991 Academic Press, Inc.

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Testosterone (T) influences many aspects of physiology and behavior in birds. Physiological effects include metabolic rate (Hännöler and Prinzinger, 1979; Feuerbacher and Prinzinger, 1981), lipid storage (Wingfield, 1984), and timing of molt (Runfeldt and Wingfield, 1985; Schleussner, Dittami, and Gwinner, 1985; Nolan, Ketterson, Ziegenfus, Cullen, and Chandler, submitted for publication). Behaviors affected include parental behavior (Silverin, 1980; Hegner and Wingfield, 1987; Oring, Fivizzani, and El Halawani, 1989), vocal behavior (Wada, 1981, 1982, 1986; Harding, Walters, Collado, and Sheridan, 1988; Gyger, Karakashian, Dufty, and Marler, 1988), aggressive behavior (Wingfield, Ball, Dufty, Hegner, and Ramenofsky, 1987; Wingfield, Hegner, Dufty, and Ball, 1990), and locomotor activity (Wada, 1982, 1986).

Recent studies of wild birds indicate that these T-induced changes in physiology and behavior may influence fitness in ways that are sometimes beneficial and sometimes detrimental (Wingfield *et al.*, 1990). Experimental elevation of plasma T can increase territory size (Watson and Parr, 1981; Wingfield, 1984; but see Beletsky, Orians, and Wingfield, 1990) and lead to the acquisition of additional mates in normally monogamous birds (Watson and Parr, 1981; Wingfield, 1984). It can also depress offspring growth and survival (Silverin, 1980; Hegner and Wingfield, 1987; but see Ketterson, Nolan, Wolf, and Ziegenfus, submitted for publication) and lower adult survival (Dufty, 1989; but see Ketterson *et al.*, submitted for publication, a). Finally, T can suppress molt and can delay the termination of breeding by males (Runfeldt and Wingfield, 1985; Nolan *et al.*, submitted for publication).

The typical temporal pattern of plasma T during the breeding season in monogamous, temperate-zone songbirds exhibits one or two peaks in early spring followed by a return to basal levels (see Wingfield *et al.*, 1990, for review). Deviations from this pattern, in magnitude of the peaks or timing of their onset or decline, might be expected to have consequences for fitness. Our objectives were to determine what some of these consequences might be by treating free-living and captive male dark-eyed juncos (*Junco hyemalis*) with implants packed with testosterone (experimental or T-males) or left empty (control or C-males). In some years implants were introduced in spring, before the natural period of peak testosterone secretion, thereby accelerating the onset of the peak. In other years we treated males after breeding had begun and prolonged the peak's duration.

More specifically, among males our objectives were to document the magnitude and time course of the effects of implants on plasma T and to discover any correlated effects on physiology including any changes in plasma levels of corticosterone (B) and in body mass.

With respect to females, our objective was to document hormone levels in the mates of T-males and C-males. We hypothesized on the basis of

an earlier experiment that females mated to T-males might have higher testosterone than control females. In that experiment, when we removed male members of pairs and replacement males appeared and courted the females, T was elevated not only in the replacement males but also in the courted females (Ketterson, Nolan, Wolf, Dufty, Ball, and Wingfield, unpublished data). Similarly, we predicted that mates of T-males might also have higher B. This prediction was based on the observation that when juncos are feeding nestlings, females deprived of male assistance increase their own feeding rates to compensate (Wolf, Ketterson, and Nolan 1990), which is accompanied by a rise in B (Ketterson *et al.*, unpublished data). Because T-males feed their young at lower rates than C-males (Ketterson *et al.*, submitted for publication, a), we expected B to be elevated in the mates of T-males.

METHODS

Species and Study Area

We studied a sedentary subspecies of dark-eyed juncos (*Junco hyemalis carolinensis*), near Pembroke, Virginia, at the University of Virginia's Mountain Lake Biological Station, during the summers of 1986–1989. Our study area was at the higher elevations of the Appalachian Mountains (see Wolf, 1987, for further description). Juncos are apparently monogamous passerines in which females build the nest and incubate, but both sexes feed and defend nestlings and fledglings.

Treatment and Blood Sampling

In 1986–1988 we captured and implanted males whose mates were incubating eggs or tending newly hatched young (May 20–June 23). Males were anesthetized and given 20-mm implants of Silastic tubing (0.58 mm, i.d.; 0.77 mm, o.d., Dow Corning), either packed with testosterone (Sigma Chemical) or left empty, and sealed with Silastic glue (Dow Corning). Implants were placed along the flank beneath the left wing. We removed implants from all males that we recaptured after July 15.

Males and their mates that we recaptured between May 29 and July 15 were bled. Time between implant and blood sampling ranged from 6 to 60 days, but birds were all at the same stage of reproduction, tending nestlings. In order to minimize the effects that our capture efforts might have on the birds' natural hormonal states, we captured most of them either in nets that intercepted them as they flew to the nest or in Potter traps baited with seed. In the few cases (two T-males, three C-males) in which we played tape-recorded calls of the young to facilitate capture, capture was completed in less than 2 min. All bleeding was completed within 10 min of the moment of initial disturbance, i.e., capture or playback calls, and in all comparisons involving B, we corrected statistically for the effect of bleeding time.

To determine how rapidly the implants affected plasma T we treated 18 male captives (6 controls, 12 experimentals) that we caught between June 5 and July 13, 1988 outside our study area. One day after implant we took blood samples from two T-males and one C-male and repeated this procedure on different males 2 days after implant, 3 days after implant, and so on for 6 days, until all the birds had been sampled once. We held these birds individually in large outdoor aviary cages ($1.7 \times 2.4 \times 2.4$ m), in which they were able to see and hear one another but not to interact in other ways. For bleeding, we caught them within 90 sec of our arrival at the aviary and completed bleeding within 10 min of arrival ($\bar{x} = 7.1$ min, SE = 0.35, $N = 18$).

Hormone Assays

Samples were spun in a clinical centrifuge and the plasma stored at -20°C . The 1986 and 1987 samples were analyzed in two assays performed at Rockefeller University by Dufty and Ball and the 1988 samples were analyzed in a single assay at Indiana University by Johnsen. Procedures were as described by Wingfield and Farner (1975), Wingfield, Newmann, Hunt, and Farner (1982a), and Ball and Wingfield (1987).

Briefly, plasma samples were equilibrated overnight with 2000 cpm of labeled T and B, extracted with 4.5 ml dichloromethane, and separated on celite/glycol columns using 20 and 50% ethyl acetate in isooctane, respectively, after DHT was removed using 10% ethyl acetate. There were no consistent interassay effects, and water blanks and standard concentrations were of similar high quality in both sets of assays. The expected value for the T standards was 0.250 ng, and the observed average was 0.252 ng ($N = 8$, SE = 7.42, c.v. = 8.34%). For B the expected value for the standards was 1.000 ng, and observed values averaged 1.020 ng ($N = 6$, SE = 24.5, c.v. = 5.63%). Recoveries for T averaged 0.54 ($N = 102$, SE = 0.01), 0.57 ($N = 108$, SE = 0.01), and 0.66 ($N = 75$, SE = 0.01); B recoveries averaged 0.72 (SE = 0.01), 0.70 (SE = 0.01), and 0.71 (SE = 0.01). We omitted samples from analyses (a) if recoveries were lower than 50%, or (b) in the case of T, if volumes of the plasma samples were lower than 50 μl .

Body Mass and Fat Scores

Before the 1989 breeding season began, we netted and trapped juncos during March and early April, implanted them as described above, and weighed them (Pesola spring balance, nearest 0.1g). We recaptured them later and weighed them again, and in many cases measured visible fat at recapture (scale 0–5, as described by Nolan and Ketterson, 1983). Because juncos tend to fatten in response to recent air temperatures (Nolan and Ketterson, 1983), it was important to confine attention to birds caught more or less at the same time. Consequently, we compared all individuals

recaptured during the last half of April, when weather can still be cold and snowy at high altitudes, computing change in mass between implanting and recapture. All birds had been implanted for at least a week and all were at about the same stage of reproduction (egg laying had not begun).

During the breeding seasons of 1987 and 1988 (May–August), we weighed males just before the young were ready to leave the nest (age 10 days, hatching day = 0). All had been implanted for at least 5 days. We did not record fat because fat scores are almost invariably 0 during summer (personal observation).

RESULTS

Hormone Concentrations

Testosterone in free-living males. We compared 14 T-males recaptured between 6 and 51 days after implant with 13 C-males recaptured 12 to 47 days after implant. T-implants led to sustained elevation of T for at least 40 days (Spearman's r relating T to days since implant, $N = 14$, $r_s = 0.304$, n.s.), and T was also unrelated to time since implant in C-males ($N = 13$, $r_s = -0.249$, n.s.).

When we combined samples from males without regard to time since implant, T averaged 6.42 ng/ml (SE = 0.94) in T-males, which differed significantly from the levels in C-males, 2.10 ng/ml (SE = 0.50, ANOVA, $P = .001$, Fig. 1).

Corticosterone in free-living males. A rank-order correlation between B and time since implant in T-males was positive and approached significance ($N = 16$, $r_s = 0.499$, $P < .10$). In C-males, B showed no significant relation to time since implant ($N = 7$, $r_s = 0.402$, n.s.).

When treatments were compared without respect to time since implant, corticosterone was twice as high in T-males ($\bar{x} = 23.3$ ng/ml, SE = 2.90) as in C-males ($\bar{x} = 10.4$ ng/ml, SE = 1.61, ANOVA, $P = .013$, Fig. 2). Time elapsed between initial disturbance at capture (e.g., hitting the net, entering the trap) and completion of bleeding (bleeding time) was entered into a second ANOVA as a covariate. Bleeding time was significant ($P = .020$), but, when its effect was corrected for, B-levels in T- and C-males still differed significantly ($P = .017$).

We asked whether levels of T and B were related, computing a rank-order correlation coefficient between T and B for T-males alone ($N = 14$, $r_s = 0.222$, $P > .5$), C-males alone ($N = 7$, $r_s = 0.286$, $P > 0.5$), and T- and C-males combined ($N = 21$, $r_s = 0.516$, $P < .02$). Results indicate that B covaried with T, even among controls, but the correlation was significant only when treatment groups were combined.

Testosterone in captive males. The full response to T-implants was apparent after 24 hr and levels did not appear to vary with time in the following 6 days. For Days 1 through 6 after implant, the daily values

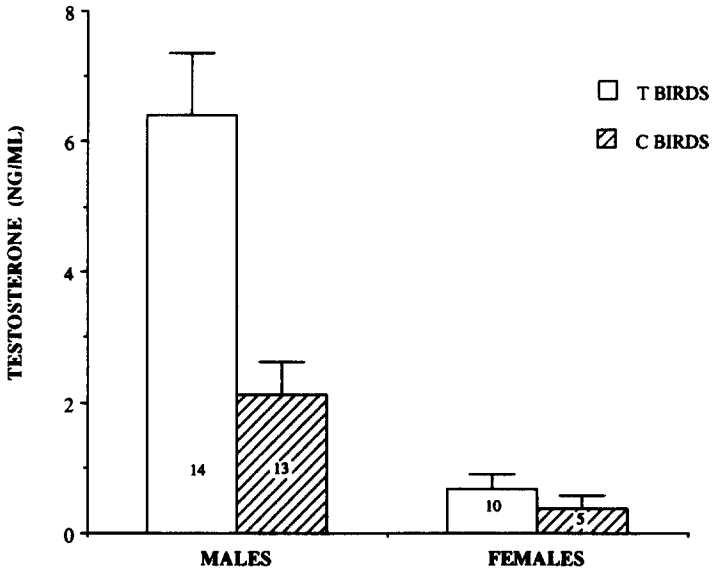


FIG. 1. Testosterone concentration (ng/ml, $\bar{x} \pm 1$ SE) of free-living male and female dark-eyed juncos classified according to treatment of the male (T, T-implanted males; C, males with empty implants). Sample sizes are displayed in histogram bars. All juncos had nestlings when sampled.

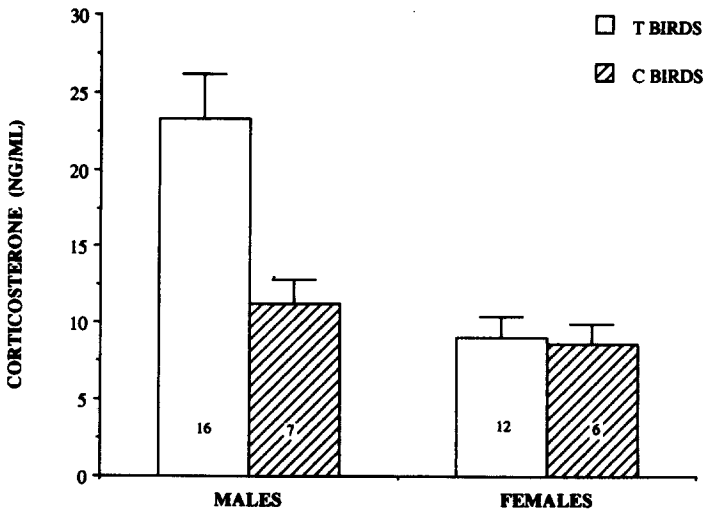


FIG. 2. Corticosterone concentration (ng/ml, $\bar{x} \pm 1$ SE) of free-living male and female dark-eyed juncos classified according to treatment of the male (T, T-implanted males, C, males with empty implants). Sample sizes are displayed in histogram bars. All juncos had nestlings when sampled.

(average of 2/day for T-males, 1/day for C-males), all in ng/ml, were 16.4 vs. 0.1, 10.2 vs. 1.3, 8.4 vs. 7.1, 10.3 vs. 0.6, 8.0 vs. 0.1, and 9.4 vs. 1.4 for T- and C-males, respectively. When data were combined across days, the average plasma concentration for T-males was 10.48 ng/ml ($N = 12$, $SE = 1.46$), which was considerably higher than the value for C-males, 1.8 ng/ml ($N = 6$, $SE = 1.10$; ANOVA, $P < .001$).

Corticosterone in captive males. Corticosterone was also elevated in aviary-held males that received T-implants. For Days 1 through 6 after implant, the daily values (average of 2/day for T-males, 1/day for C-males), all in ng/ml, were 25.1 vs. 35.4, 72.7 vs. 3.1, 25.8 vs. 12.4, 23.5 vs. 10.2, 14.1 vs. 19.1, and 27.9 vs. 19.4 for T-males and C-males, respectively. When data were combined across days, B in T-males averaged 25.5 ng/ml ($N = 12$, $SE = 3.25$), which was greater than the 16.6 ng/ml observed in C-males ($N = 6$, $SE = 4.50$). Whether the difference was significant depended upon the test employed (ANOVA, $P < .07$, Mann-Whitney U, $P < .05$).

Hormone concentrations in females. T was lower in females than in males of both treatment groups, and did not differ according to treatment of their mate (females of T-males, $N = 10$, $\bar{x} = 0.68$ ng/ml, $SE = 0.21$; females of C-males, $N = 5$, $\bar{x} = 0.37$ ng/ml, $SE = 0.19$; ANOVA, $P = .360$, Fig. 1). The treatment group to which a male belonged also had no effect on his mate's B. In female mates of T-males, B averaged 9.1 ng/ml ($N = 12$, $SE = 1.36$), while in mates of C-males it averaged 8.6 ng/ml ($N = 6$, $SE = 1.33$; ANOVA, $P = .542$, Fig. 2).

Body Mass and Fat Scores in Free-Living Birds

Body mass and fat scores in spring. In 1989, when males were implanted before breeding, T influenced body mass. The treatment groups did not differ in mass prior to treatment (T-males, first capture $\bar{x} = 23.0 \pm 0.212$ g; 0.212 g; C-males, $\bar{x} = 22.9 \pm 0.29$ g, t test, $P > .8$), but at recapture during the last half of April, C-males were heavier ($N = 32$, $\bar{x} = 22.4$ g, $SE = 0.19$) than T-males ($N = 32$, $\bar{x} = 21.6$ g, $SE = 0.17$, ANOVA, $P < .05$, Fig. 3). Similarly, loss of mass between initial capture and recapture was greater among T-males than controls (1.3 g vs. 0.51 g, t test, $P < .05$), and the rate of loss (loss divided by the interval in days between capture and recapture) was also greater in T-males than in controls (ANOVA, $P < .03$).

We compared fat scores in the second half of April to determine whether the differences in mass should be attributed to differences in stored energy. Scores were recorded for 14 C-males, and scores for 6 were greater than 0. Of 16 T-males, the score of only 1 was greater than 0 (Fisher's Exact = 0.025).

Body mass during the breeding season. During 1987 and 1988, body mass of T-males averaged 20.9 g ($N = 18$, $SE = 0.21$) at the time young

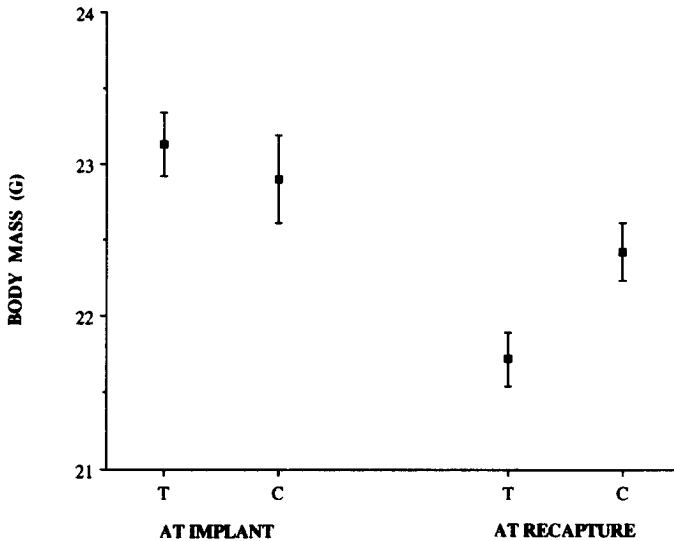


FIG. 3. Body mass ($\bar{x} \pm 1$ SE) of free-living dark-eyed juncos at the time they were implanted in March–April and upon recapture in the last half of April. T and C refer to juncos with testosterone-filled ($N = 32$) and empty implants ($N = 32$), respectively.

left the nest, while C-males averaged 21.0 g ($N = 13$, $SE = 0.25$; ANOVA, $F = 0.15$, $P = .70$) at the same stage. Thus among males implanted during the breeding season, mass did not differ according to treatment.

DISCUSSION

Testosterone in Males

As expected, plasma levels of T rose significantly after treatment with T. Data from the field indicate that T remained elevated for at least 40 days, and data from captives indicate that the implants took effect within 24 hr of treatment. As to the magnitude of the effect in the field, T-implants produced an average plasma concentration of 6.4 ng/ml, as compared to 2.1 ng/ml for controls. The value for controls is identical to that seen in an earlier study of unimplanted (nonexperimental) males, also 2.1 ng/ml (Ketterson *et al.*, unpublished data). Both the C-males in this study and the unimplanted males in the earlier study were tending nestlings when they were sampled, a stage of reproduction when T is normally low (Wingfield and Moore, 1988; Wingfield *et al.*, 1990). These low midseason values contrast with data we obtained from control males that we bled in 1989 in early spring, just prior to the onset of reproduction. T averaged between 5.5 and 9.8 ng/ml (depending upon whether they were first-year males (5.5) or adults (9.8), unpublished data), so we conclude that the testosterone implants in the present study induced levels

close to the physiological maximum and maintained these levels for most or possibly all of the breeding season.

Corticosterone in Males

Unlike the findings of Wingfield (1984) and Hegner and Wingfield (1987) for T-implanted song sparrows (*Melospiza melodia*) and house sparrows (*Passer domesticus*), respectively, treatment of male juncos with T, both free-living and captive, was associated with a rise in B. This suggests that treatment with T may alter physiology or behavior in juncos in ways that cause stress, leading to increased secretion of B (Siegel, 1980), which in turn might impose a cost in the form of immunosuppression or alteration of metabolic pathways (e.g., Gross, Siegal, and DuBose, 1980; Sturkie, 1986; Chrousos, Loriaux, and Gold, 1988; Folstad, Nilssen, Halvorsen, and Andersen, 1989). Alternatively, treatment with T might alter the nature of the response to stress of capture.

However, another explanation for higher plasma B, as opposed to increased secretion, is a T-induced increase in corticosteroid binding proteins (CBP) (see Silverin, 1986; Daniel and Assenmacher, 1974; and Wingfield and Farner, 1980). CBP binds B in a form that is not biologically active and protects it from degradation in the circulation, thereby increasing its half life. Our assay does not distinguish between bound (inactive) and free (active) corticosterone.

In some birds, androgens are believed to increase the concentration of CBP, and thus apparent secretion (Silverin, 1986; Daniel and Assenmacher, 1974; Wingfield and Farner, 1980), but in other birds (Peczely, 1979) and in a number of mammals (Bradley, 1987; Gala and Westphal, 1965; Kley, Hermann, Morgner, and Kruskemper, 1973) androgens decrease CBP. Further study will be required to determine whether the observed increase in B in T-males reflects greater secretion.

Assuming for now that the difference does represent a difference in secretion, we know of only one mechanism that has been proposed to explain how high T might act directly to increase secretion of B. If T were to act as a glucocorticoid antagonist, it might compete with B for binding sites in the pituitary and interfere with the normal negative feedback of B upon its own secretion (Buttery and Vernon, 1983; Wilson, 1988). More typically we have come to expect an inverse relationship between B and T, in which high corticosterone can lead to suppression of the hypophyseal-gonadal axis, and thus of T (Moore, Thompson, and Marler, 1991).

Indirectly, T might influence the bird's general level of activity (Harvey, Phillips, Rees, and Hall, 1984) or the frequency or intensity of its aggressive encounters with conspecifics (e.g., Sapolsky, 1987), possibly leading to increased secretion of B. This relationship between T and B obviously deserves further study.

Hormone Concentrations in Females

We had expected that females mated to T-males might have higher levels of T than those of females mated to controls under certain natural circumstances (see introduction). T increases in female pied flycatchers (*Ficedula hypoleuca*) when it is high in their mates (Silverin and Wingfield, 1982), and T is higher in female house sparrows mated to T-implanted males (Hegner and Wingfield, 1987). However, we did not observe this effect.

We had also anticipated that B might be higher in females mated to T-males because female juncos that increase their rate of delivery of food to nestlings sometimes have higher B (Ketterson *et al.*, unpublished data; see introduction). Also, B is known to vary positively with brood size in female pied flycatchers (Silverin, 1982). However, we found no difference in B between female mates of T- and C-males.

Body Mass and Fat Scores

Our finding of lower body mass and lower subcutaneous fat scores in males implanted with T in early spring is similar to a finding by Marler and Moore (1988a), who report that male mountain spiny lizards treated with T lost more mass than controls (see also Dark, Whaling, and Zucker, 1987). Although the differences in mass between our treatment groups were not large, they were statistically significant and they were accompanied by a difference in fat score. Obviously, small differences in means can only be statistically significant when within-group variation is small. Juncos regulate mass closely in response to recent weather conditions (Nolan and Ketterson, 1983). Small differences in stored energy can be very important when juncos have no access to food and must use this stored energy while fasting (Ketterson and Nolan, 1976, 1983).

It is widely known that body mass and, more particularly, fat levels of seasonally breeding species are lowest in summer when the gonads are enlarged, so we might expect some relationship between gonadal steroids and fat stores. Furthermore, an increase in gonadal steroids is frequently associated with a decrease in the ratio of fat to muscle (see Wade and Gray, 1979, for a review of the mammalian literature). The decrease in mass and reduction of fat could follow from any of several mechanisms: reduced food consumption, increased activity, increased standard metabolic rate, decreased fat synthesis, or increase in the cost of thermoregulation. With the exception of food consumption, testosterone has been reported to effect all these changes (e.g., Wada, 1981, 1982; Hännöler and Prinzing, 1979; Feuerbacher and Prinzing, 1981; Thapliyal, Lai, Pati, and Gupta, 1983).

We emphasize that T predicted mass in male juncos only when implants

were inserted before breeding had begun (see also Wingfield, 1984). The most likely explanation for the seasonal difference in effect would seem to be that T ordinarily plays a role in the transition from a winter to a summer mode of regulation of body mass and fat stores by male juncos. By artificially elevating T in early spring we may have accelerated this transition, but, once it is made, subsequent administration of T evidently produces no effect on mass or fat.

An alternative possibility is that the rise in corticosterone induced by treatment with T caused the loss of mass. When stressors increase B, the typical effect is catabolism of muscle as animals convert protein to glucose (Sturkie, 1986). The result is loss of mass. We made no direct effort to estimate muscle mass, but in order to assign fat scores it is necessary to examine the abdominal region and the furcula, and this examination exposes the pectoral muscle to view. In juncos that have been fasting during winter, loss of muscle and the consequent prominence of the sternum are quite noticeable during inspection for fat score. Therefore we think we would have noticed a loss of muscle, and, because we did not, we think it is unlikely that T-males had degraded flight muscle. Furthermore, treatment with B in some species has been associated both with increased food intake (Nagra, Breitenbach, and Meyer, 1963) and increased fat deposition (Wingfield and Silverin, 1986; Gray, Yarien, and Ramenofsky, 1990), neither of which is consistent with the loss in mass and fat that we found in T-implanted juncos.

CONCLUSION

The data in this paper suggest that costs could be associated with deviations from the typical pattern of testosterone secretion in juncos. An increase in T early in the year (which reduces energy reserves) could impair survival after exposure to spring snow storms. Such storms are not uncommon in the higher mountains of Virginia (e.g., on May 7, 1989, 5.1 cm of snow fell, and male juncos left their territories and ranged widely in search of food). Individuals that accelerated this spring increase in T could be eliminated by such weather.

Maintaining T at high levels throughout the summer could also be disadvantageous, if the associated increase in corticosterone were to suppress acquired immunity (Folstad *et al.*, 1989; Gross *et al.*, 1980). However, we observed no differences in summer mortality between the treatment groups (Ketterson *et al.*, submitted for publication). Persistence of high T into autumn would very likely suppress molt. Whether these potential costs could be offset by benefits in the form of increased ability to attract mates or ability to obtain extra-pair fertilizations is the subject of our current research.

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