*Department of Biology and Center for the Integrative Study of Animal Behavior,
Indiana University, Bloomington*

The Effects of Experimentally Elevated Testosterone and Food Deprivation on Food Consumption and Prey Size Preferences in Male Dark-Eyed Juncos (*Junco hyemalis*, Emberizidae: Passeriformes)

Ethan D. Clotfelter, Val Nolan Jr & Ellen D. Ketterson

Abstract

Numerous studies have shown that the experimental elevation of circulating levels of testosterone reduces parental behaviour in male birds, particularly the provisioning of young. The mechanisms responsible for this change in behaviour are not fully understood. In this study, we examine the effects of elevated testosterone on food consumption and prey selection, both of which have potential consequences for nestling provisioning behaviour. We manipulated testosterone and performed two experiments on a captive, non-breeding population of male dark-eyed juncos (*Junco hyemalis*) on long day-lengths. In the first experiment, we subjected juncos to 3 h of food deprivation and compared food consumption and prey size selection by males with elevated testosterone (testosterone males) to that of control males. Testosterone males consumed more food than control males and showed a preference for larger prey. In a second experiment in which small prey were more abundant than large prey, food consumption and prey size preferences did not differ between testosterone and control males. We also manipulated the duration of food deprivation in the second experiment. Males of both treatments consumed more small prey under conditions of mild (1 h) or moderate (5 h) food deprivation and consumed more large prey under conditions of intermediate (3 h) food deprivation. We discuss our results and the effects that testosterone has on self-maintenance behaviour and male parental effort.

Corresponding author: Ethan Clotfelter, Department of Biology, Providence College, 549 River Avenue, Providence, RI 02918, USA. E-mail: eclotfel@providence.edu

Introduction

Androgens such as testosterone affect many behavioural and physiological traits in vertebrates (Wade 1976; Balthazart 1983; Marler & Moore 1988; Wingfield et al. 1990). One area that has received considerable attention is the negative impact that testosterone has on parental effort, particularly in birds (Silverin 1980; Hegner & Wingfield 1987; Oring et al. 1989; Ketterson et al. 1992; Ketterson & Nolan 1999). This relationship is robust across a range of avian species, but the mechanisms responsible are not fully understood.

Numerous studies in birds have examined the relationship between testosterone and male parental effort by measuring the expression of alternative behaviours. Elevated testosterone reduces parental effort in many species by increasing the time and energy that males allocate to mating effort. Males implanted with testosterone perform more courtship behaviour, defend larger territories, and defend those territories more aggressively than control males (Wingfield 1984; Chandler et al. 1994; Alatalo et al. 1996). The trade-off between parental effort and alternative behaviours, such as self-maintenance behaviour, has received little attention. No study has examined the ways in which testosterone affects parental effort by increasing food consumption or decreasing foraging efficiency. The lack of data in this area is surprising, given the evidence that elevated testosterone causes hyperphagia (Wade & Gray 1979; Deviche 1992, 1995; Wikelski et al. 1999; Lynn et al. 2000) and may affect foraging performance (Andrew & Rogers 1972; Archer 1974; Rogers & Andrew 1989). It is also surprising considering that foraging for self-maintenance employs the same behavioural repertoire that is used in foraging to provision offspring.

In the current study, we examine the way in which elevated testosterone affects food consumption and prey selection in male birds that typically contribute a significant proportion of parental effort. Our model system is the dark-eyed junco (*Junco hyemalis*), a species that has been the subject of extensive behavioural and physiological research in our laboratory (see Ketterson & Nolan 1999 for summary). We conducted two experiments on a captive, non-breeding group of males that were photo-stimulated into breeding condition by exposure to long day-lengths. In order to isolate the effect of testosterone on self-feeding behaviour from its effect on parental provisioning, we observed the effect of experimentally elevated testosterone on food consumption and prey size preference. The results will help elucidate the relationship between elevated testosterone and male parental effort in birds by providing a better understanding of the effects of testosterone on self-maintenance.

Methods

Study Species

The experiments described below were conducted on 28 adult male dark-eyed juncos (*Junco hyemalis carolinensis*) caught at the Mountain Lake Biological

Station in Giles County, Virginia, USA (37°22′ N, 80°32′ W) between 1997 and 1999 (see Chandler et al. 1994 for a description of the study site). Dark-eyed juncos (Order Passeriformes, Family Emberizidae) are socially monogamous birds, and both parents feed the young a diet that consists primarily of arthropods (Nolan et al. in press). We transported juncos to the Kent Farm Bird Observatory in Bloomington, IN (39°10′ N, 86°31′ W), during the year in which they were caught.

Housing Rooms

The birds were housed in mixed-sex groups and were used in mate-choice studies in the spring and summer of 1999 (Parker-Renga et al., unpubl. data). In Nov. 1999, we placed 28 birds in two 27-m^3 rooms connected by an open window. Each room had cedar chips on the floor and contained numerous branches and perching sites. We provided the birds with the following ad libitum: mixed seed (millet, cracked corn, and sunflower seeds), a dietary supplement (ground mixture of commercial dog food, carrots, and hardboiled eggs), dishes of sand, and vitamin-enriched water. During 1 wk per month, they were given water treated with sulfamethazine sodium to prevent coccidiosis. Between 1 and 15 Dec. 1999, we gradually increased the photoperiod in these rooms from 9 : 15 h light : dark (approximate ambient photoperiod) to 15 : 9 h light : dark (approximate maximum summer photoperiod).

Implants

On 14 Dec. 1999, half of the males were implanted subcutaneously with two implants made from silastic tubing and sealed with silastic glue (Dow Corning; i.d. = 1.47 mm, o.d. = 1.95 mm, length = 12 mm). Half the males received implants packed with 10 mm of crystalline testosterone (Sigma Chemical) and half received empty, control implants. The treatment of the first male was determined by a coin toss, and thereafter the treatment alternated between testosterone and control. Previous research has shown that circulating levels of testosterone are approximately three times greater in testosterone males than in control males (e.g. 6.42 ± 0.94 ng/ml and 2.10 ± 0.50 ng/ml, respectively; Ketterson et al. 1991). Testosterone levels of testosterone males are not pharmacological, but rather are typical of maximum levels observed during the early breeding season (Ketterson & Nolan 1992). Implants and implanting techniques were identical to those used previously by our research group (Ketterson et al. 1991, 1992; Schoech et al. 1998).

For each bird, we measured body mass, body fat, and body condition. Mass was measured to the nearest 0.1 g with a Pesola balance. The body fat index is a measure of the visible fat reserves in the furcular fossa and abdominal regions, on a scale of 0–5 following Helms & Drury (1960). The body condition index is a measure of the fullness of the pectoralis muscle on a scale of 1–3 following Gosler (1991). Body fat and condition indices were measured by a single observer (E.D.C.) to eliminate problems of inter-observer reliability. These indices were measured

prior to implantation (14 Dec. 1999) and again during the first experiment (13–26 Jan. 2000). The observer recorded body fat and condition before checking the hormone implants to reduce the possibility of unconscious bias.

After implanting the birds, we separated the housing rooms by closing the connecting window. We assigned the juncos to one of two rooms, with seven testosterone males and seven control males in each (i.e. 14 juncos to each of the two rooms). Room assignments were made randomly within a treatment. We eliminated several males from one or both experiments because they had lost their implants or had suffered from harassment by other males.

Testing Rooms

For each trial in both experiments, we removed the focal juncos from the housing rooms and transported them to testing rooms. One testosterone male and one control male were tested simultaneously in separate, identical testing rooms. This paired design removed such confounding effects as time of day and number of day since implantation. We randomly assigned the first testosterone male and first control male to one of the two testing rooms; thereafter testosterone males and control males were alternated in each room.

Testing rooms were identical to housing rooms in light, dimensions, and availability of perches. There were no cedar chips on the floor to ensure accurate counting of mealworms after each trial. Water, but no food, was available to the birds. Testing rooms were swept out after each trial. A junco in a testing room was visually isolated, but could hear the bird in the adjoining testing room and those in the housing rooms.

Experiment 1

We conducted expt 1 between 13 and 26 Jan. 2000. After the birds had an opportunity to feed in the morning (06:00–08:00 h), we transferred the focal males to the testing rooms for 3 h of food deprivation and acclimation. We then introduced two glass dishes (10 cm diameter), each containing 10 large and 10 small mealworms (*Tenebrio molitor*), whose masses were recorded before each trial. Large mealworms were 5–6 times heavier than small mealworms (large: 0.352 ± 0.079 g (SE), n = 20; small: 0.061 ± 0.014 g, n = 20).

We covered the mealworms with a 3–4-cm layer of cedar chips identical to those used on the floor of the housing rooms. We covered the mealworms to ensure that males were required to actively search for food. The birds were allowed 60 min to feed on the mealworms in the dishes. Following each trial, we recorded the final mass of the remaining large and small mealworms in each room.

Experiment 2

This experiment was conducted between Feb. 1 and Feb. 4 2000 and differed in three respects from the first. First, we put the mealworms in

27×42 cm trays filled with cedar chips to increase the difficulty of the foraging task. Secondly, we changed the number of mealworms and the ratio of size classes. In each tray, we placed five large mealworms and 30 small mealworms (approximately equal biomass of each prey type). Thirdly, we varied the duration of food deprivation among three treatments: mild (1 h), intermediate (3 h), or moderate (5 h). Three sets, each consisting of a testosterone male and a control male, were subjected to each of the three food deprivation treatments (i.e. 18 birds total). Juncos in the 3-h and 5-h deprivation treatments were kept in closed buckets for 2 h and 4 h, respectively, then allowed 1 h each to acclimatize to the testing room. Juncos in the 1-h deprivation treatment were moved directly to the testing room. The juncos ceased all alarm calling and began singing 5–10 min after entering the testing room, suggesting that 60 min is an adequate acclimation period. As in expt 1, the birds were allowed to forage for 60 min and the masses of remaining large and small mealworms were recorded after each trial.

Statistical Analyses

We analysed our data using SYSTAT 6.0 (Wilkinson 1996). All statistical tests were two-tailed. Results presented include means $\pm$ SE, and differences were considered significant at $p < 0.05$. In each experiment, we performed multiple linear regressions to determine the proportion of the variance in food consumption explained by the following independent variables: room in which the focal bird was housed, date of the trial, time elapsed since implantation, and hour of day of the trial. We also included as variables body mass, body fat, and body condition of the subject bird at the time of trial. None of these variables had a significant effect on food consumption or prey size preferences in either experiment, and were not considered in subsequent analyses.

Results

General

Males in both treatments decreased in body mass, body fat, and body condition from the time we implanted them (14 Dec.) to the time of their participation in the first experiment (13–26 Jan.). Paired t-tests revealed that these decreases were highly significant (body mass: $t = 13.10$, $df = 26$, $p < 0.001$, Fig. 1a; body fat: $t = 5.97$, $df = 26$, $p < 0.001$, Fig. 1b; body condition: $t = 7.90$, $df = 26$, $p < 0.001$, Fig. 1c). Hormone treatment had no effect on change in body mass (ANCOVA, $F_{1,52} = 2.46$, $p = 0.12$), but testosterone males showed significantly greater decreases in body fat ($F_{1,52} = 6.75$, $p = 0.012$) and body condition ($F_{1,52} = 4.14$, $p = 0.047$) than control males.
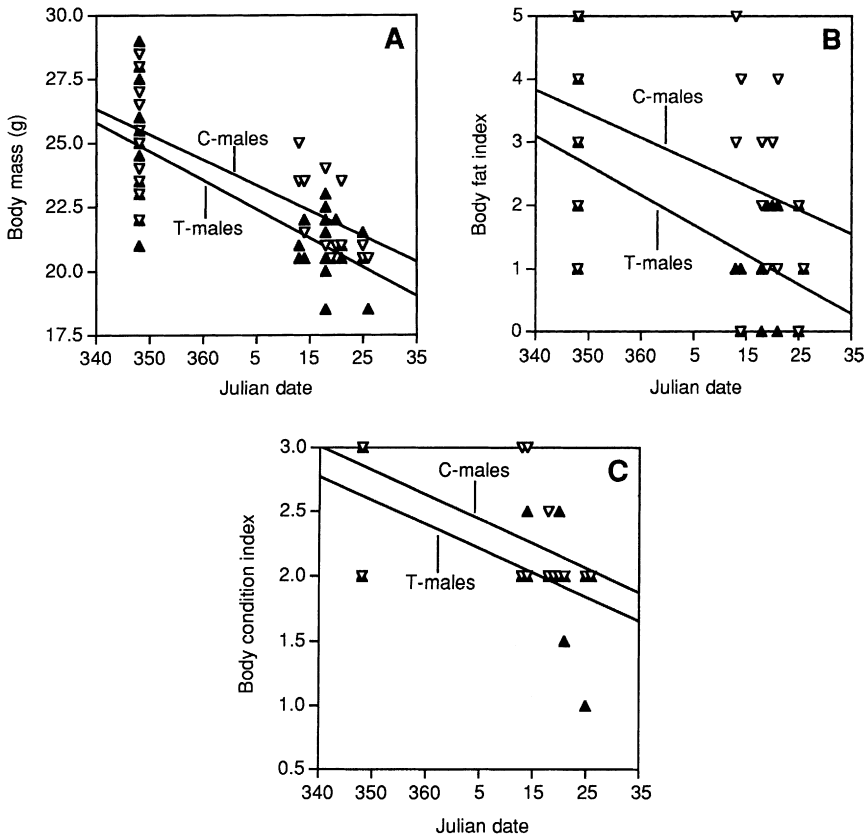
Fig. 1: Changes in body mass (A), body fat (B) and body condition (C) between implantation (14 Dec. 1999 = Julian date 348) and the start of experiments (13–26 Jan. 2000 = Julian date 13–26). Solid triangles represent males with testosterone (T) implants and open triangles represent males with control (C) implants. Lines are the results of linear regressions on Julian date

Experiment 1

When offered equal numbers of large and small mealworms mixed together, the total mass of small mealworms consumed by testosterone males and control males was identical (testosterone males: 0.21 ± 0.058 g (SE); control males: 0.21 ± 0.059 g; t = 0.097, df = 24, ns; Fig. 2). Testosterone males consumed significantly more large mealworms, however, than did control males (testosterone males: 0.74 ± 0.13 g; control males: 0.35 ± 0.095 g; t = −2.45, df = 24, p = 0.022; Fig. 2). As a consequence, the total mass consumed by testosterone males was greater than that of control males (testosterone males: 0.94 ± 0.14 g; control males: 0.57 ± 0.094 g; t = −2.26, df = 24, p = 0.033).
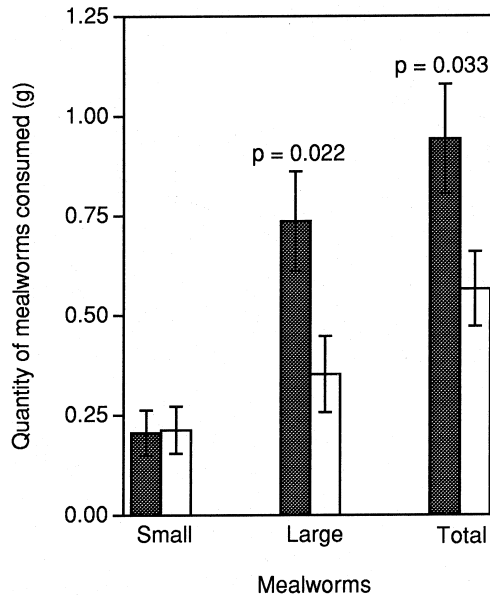
Fig. 2: Differences in consumption of small, large and total mealworms by male treatment in expt 1. Solid bars represent mealworm consumption by testosterone males and open bars represent control males. p-Values were derived from paired t-tests (n = 13 pairs of testosterone males and control males)

Experiment 2

When we provided male juncos with more small mealworms than large mealworms, hormonal treatment had no effect on prey size preference. Testosterone males and control males ate similar quantities both of small mealworms (testosterone males: 0.53 ± 0.085 g; control males: 0.53 ± 0.074 g; $t = -0.066$, $df = 16$, ns) and of large mealworms (testosterone males: 0.32 ± 0.10 g; control males: 0.23 ± 0.082 g; $t = -0.63$, $df = 16$, ns).

The duration of food deprivation had a moderate effect on the consumption of small prey (ANOVA, $F_{2,12} = 3.64$, $p = 0.058$), but hormonal treatment did not (ANOVA, $F_{1,12} = 0.006$, ns; Fig. 3). Both testosterone males and control males consumed more small mealworms after mild food deprivation (1 h) than after intermediate or moderate deprivation (3 h or 5 h). Conversely, males of both treatments consumed significantly more large mealworms after intermediate deprivation than after mild or moderate deprivation (male treatment: ANOVA, $F_{1,12} = 0.88$, ns; food deprivation: ANOVA, $F_{2,12} = 10.44$, $p = 0.002$; Fig. 3). Interaction terms for both ANOVAs were non-significant.

Discussion

Body mass, body fat, and body condition decreased significantly when we induced breeding condition by photo-stimulation. Final masses and body fat
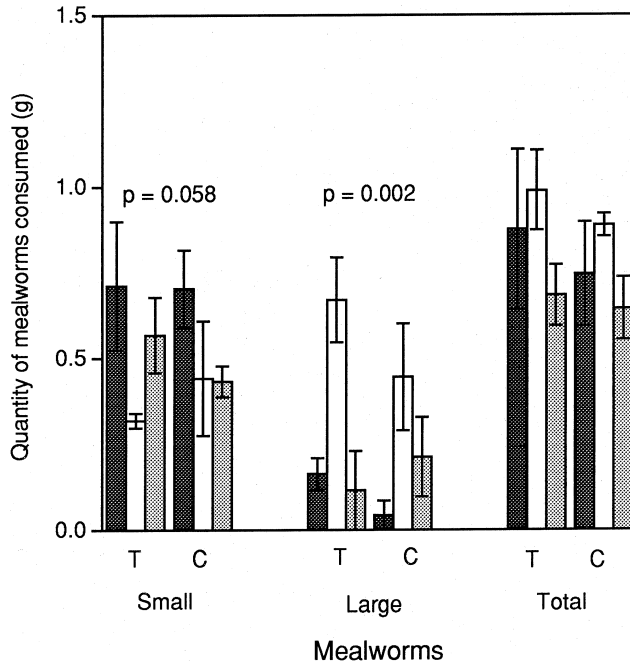
Fig. 3: Differences in consumption of small and large mealworms in expt 2 by male treatment (testosterone vs. control) and duration of food deprivation. Dark gray bars represent mealworm consumption after 1 h of food deprivation; open and light gray bars represent birds subjected to 3 h and 5 h of food deprivation, respectively. p-Values were derived from an ANOVA with male treatment and duration of food deprivation as factors

scores were similar to those reported from free-living populations under natural photoperiods (Ketterson et al. 1991; Deviche 1995). Juncos treated with testosterone lost slightly more body mass than control males in the period between implantation and our experiments. Body fat and body condition significantly decreased during the same period. These results are consistent with those from other studies, including several on dark-eyed juncos (Ketterson et al. 1991; Deviche 1995; Wikelski et al. 1999; Lynn et al. 2000).

Implantation with testosterone resulted in hyperphagia in expt 1. Hyperphagia has been reported as a consequence of elevated testosterone in numerous studies, including several on dark-eyed juncos (Wade 1976; Wade & Gray 1979; DeViche 1992, 1995; Wikelski et al. 1999; Lynn et al. 2000). One of the many physiological consequences of high testosterone in male juncos is an elevation of corticosterone (B) levels (Ketterson et al. 1991; Klukowski et al. 1997; Schoech et al. 1999). Elevated B converts protein to glucose (Sturkie 1986), which may explain the reduction in body condition which we observed in testosterone males. In other words, hyperphagia in testosterone males may be a consequence of B-induced muscle catabolism. We did not measure corticosterone levels in the current study.

The biomass of large prey exceeded that of small prey in expt 1, meaning that juncos had a greater chance of finding a large mealworm if they foraged randomly. Under these conditions, testosterone males consumed significantly more large mealworms than control males. Paszkowski & Moermond (1984) studied foraging by ovenbirds (*Seiurus aurocapillus*) on large and small mealworms and found that handling time increased 10-fold with only a 2- or 3-fold increase in prey mass. Preliminary results from a biochemical study of *Tenebrio molitor* by a commercial vendor suggest that the nutritional content of large and small mealworms is proportional to worm mass (Grubco Incorporated). This suggests that taking large prey may not be cost-effective for dark-eyed juncos, but the question remains to be tested. If free-living males with naturally or experimentally elevated testosterone show a bias for large prey similar to the one we observed in our captive population, they may have to increase their foraging time to achieve the same total energy intake as males without a prey-size bias.

In expt 2 we increased the abundance of small prey, which resulted in similar prey consumption by testosterone males and control males. This suggests that hyperphagia in testosterone males is reduced when the relative abundance of the preferred prey type (large mealworms) is reduced. This result illustrates the need for further study of the effects of testosterone on foraging behaviour and food intake. Experiment 2 also showed that males of both hormone treatments consumed more large mealworms under intermediate levels of food deprivation than under mild or severe deprivation. The difference was most pronounced in testosterone males (Fig. 2), but the effect of hormone treatment was not statistically significant. If variation in prey size preference exists among males, some of it may be due to variation in corticosterone levels. As mentioned above, male juncos with elevated testosterone have chronically higher corticosterone levels than control males. Food deprivation, on the other hand, can cause short-term increases in corticosterone (Astheimer et al. 1992). The ways in which testosterone, corticosterone, and hunger level interact to affect foraging behaviour (e.g. prey size preferences) are unclear and merit further study (Gray et al. 1990).

Taken together, our experiments showed that elevated testosterone results in hyperphagia and selection of large prey items by male dark-eyed juncos. Both of these effects have potential consequences for parental behaviour. If hyperphagia results in increased food consumption by testosterone males, it may contribute to the reduced provisioning rates we have observed in testosterone males (Ketterson et al. 1992; Schoech et al. 1998). If testosterone males are biased toward selecting larger prey, this could have consequences for nestling survival. Large prey items are less easily apportioned to multiple offspring (Mock 1984), and even for an individual nestling, such prey may be inappropriate food until the nestling reaches adult size. Our experiments demonstrate that self-maintenance behaviour such as food consumption may play a larger role than previously considered in determining the balance between mating effort and parental effort by male birds. Future studies should examine the trade-off between parental behaviour and self-maintenance behaviour in free-living populations.

Acknowledgements

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