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Testosterone increases activity but not daily energy expenditure in captive male dark-eyed juncos, *Junco hyemalis*

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Plasma testosterone (T) levels in male dark-eyed juncos peak early in the breeding season, then decline. If T enhances opportunities for reproductive success, as suggested by previous experiments, why does elevated T not occur naturally? To address this question, we prolonged the early peak level throughout the breeding season and explored potential energetic costs of maintaining elevated T. We measured daily energy expenditure (DEE) of treated males (T-males) and controls (C-males) using doubly labelled water (DLW). We also conducted behaviour scans of T- and C-males housed in outdoor aviaries. DEE was not higher in T-males than in C-males. However, T-males did increase locomotion and foraging and decrease rest and self-maintenance. These results suggest that elevated T may increase the contribution of some components of DEE and lower the contribution of others. Furthermore, the T-induced decrease in allocation of time to rest and maintenance may represent a long-term cost that has led to selection against the maintenance of elevated T beyond the natural early spring peak.

Male dark-eyed juncos *Junco hyemalis*, like males of most monogamous bird species, show a peak in plasma testosterone (T) levels in early spring, after which levels decline (Ketterson & Nolan 1992). In contrast, males of most polygynous bird species maintain T at high levels much beyond early spring (Wingfield 1984; Beletsky et al. 1995). Male juncos that are caught in spring, implanted with T, and released to live free on their territories maintain plasma T at levels comparable to the spring peak throughout the breeding season. These T-males engage in behaviour atypical of that of unmanipulated males and control males (C-males), including (1) occupying larger home ranges at some stages of breeding (Chandler et al. 1994); (2) increasing the frequency of song, which in control males functions in pair formation (Ketterson et al. 1992; Chandler et al. 1994; Titus 1998); and (3) reducing paternal care of nestlings (Ketterson et al. 1992). As a result of these behavioural changes, T-males probably are more likely than C-males to encounter and attract extrapair mates. Female juncos prefer T-males to C-males in mate-choice trials (Enstrom et al. 1997), and free-living T-males are more likely than C-males to produce young by extrapair fertilization (Raouf et al. 1997). This gain in number of young sired appears to be offset by the greater mortality of nestlings in the nests of T-males' social mates, probably because of reduced paternal care (Ketterson & Nolan 1992; Raouf et al. 1997). As a result, the production of nestlings of T- and C-males appears equal. One might therefore expect to find some naturally occurring phenotypes comparable to the T-males produced experimentally. The apparent absence of such males leads us to expect that some cost(s) of prolonged T constrains male juncos to the observed natural early spring peak.

There are a variety of pathways by which T could influence an organism's fitness. For example, it might decrease immunocompetence (Hillgarth et al. 1996; Deerenberg et al. 1997), or reduce longevity by adversely affecting energy expenditure (Marler et al. 1995; Daan et al. 1996). We focused on energy relationships and asked whether one possible selective factor limiting juncos to their early season peak could be the energetic cost of maintaining elevated T for an entire breeding season. Increased T might affect energy expenditure in two ways: it might influence resting metabolic rate, or it might increase other aspects of daily energy expenditure (DEE, which incorporates resting metabolism, but also includes thermoregulatory costs and activity costs).

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To determine whether elevated T increases energy expenditure, we injected T- and C-males housed in outdoor aviaries with doubly labelled water (DLW), measured DEE of the treatment groups, and compared their activity levels.

METHODS

Animals

Forty male dark-eyed juncos were caught in 1995 in mist nets and potter traps (late April, $N=5$; 19 June–22 July, $N=35$) near the University of Virginia's Mountain Lake Biological Station, Giles County, Virginia (37°22'N, 80°31'W). The five males captured in late April were allowed to acclimate to captivity for 8 weeks before experiments began; the remaining 35 males were used in experiments that began on the day of capture. Treatments using the males caught in April allowed us to ask whether there were effects that recent introduction to captivity may have had on the behaviour and DEE of the other males. Following capture, we weighed birds to the nearest 0.1 g (Pesola spring scale, accuracy ± 0.3%) and determined age (yearling or older) based on eye colour, body size and plumage characteristics (Ketterson & Nolan 1992). We conducted four replicate trials, each for 1 week; eight males were used in each trial (but see below for the exceptional treatment of trial 2).

On day 1 of each trial (the day of capture for 35 of the males), we implanted birds with either testosterone-filled or empty implants, as described below. On days 3 and 4, we observed behaviour. On days 5 and 6, we carried out DLW procedures, except that a temporary equipment failure caused us to delay DLW measures until day 17 for birds in trial 2.

Implants

On day 1, we assigned males to dyads whose members we matched for age and similarity of mass, presence or absence of avian foot pox and amount of visible subcutaneous fat. Dyad members were randomly designated as T-male and C-male, then anaesthetized using metofane (Schering-Plough Animal Health, Union, Missouri, U.S.A.), and we implanted two 10-mm segments of silastic tubing (Dow Corning, Midland, Michigan, U.S.A.; i.d.=1.47 mm, o.d.=1.96 mm) subcutaneously along the flank beneath the left wing. T-males received implants packed with crystalline testosterone (T, Sigma Chemical, St Louis, Missouri, U.S.A.), and C-males received empty implants; implants were sealed with silastic glue (Dow Corning). Because the double-sample DLW method required that two blood samples be collected from birds over a 24-h period (see below), we did not collect additional blood samples from males to assay for plasma T levels. In previous studies, assays of plasma collected from caged T-males during the first week after implantation revealed that levels of circulating T were within the normal physiological range typical of the early season peak (Ketterson et al. 1991). Additionally, several studies have successfully increased plasma T levels in dark-eyed juncos using implants identical to those used in this study (Ketterson & Nolan 1992; Chandler et al. 1997; Enstrom et al. 1997; Raouf et al. 1997).

Housing

Following implantation, a dyad was placed in one of four outdoor aviaries and allowed 24–48 h for acclimation to captivity and for stabilization of T levels in T-implanted males (Ketterson et al. 1991; unpublished data). Aviaries were aligned in a row, separated from one another by approximately 1 m. Each aviary was divided down the middle so that it consisted of two 63-m^3 compartments. The aviaries' outer walls were screen wire, which was covered with opaque cloth; clear plastic sheeting covered the tops, and natural tree cover provided approximately equal shade among compartments. Each compartment contained similarly placed perches. Members of a dyad were randomly assigned to a compartment. This assignment of a T-male/C-male dyad to each aviary controlled for any possible cage-related differences in microclimate. Dyad members could not see one another or the males in adjoining aviaries. We supplied seed and water ad libitum, and five mealworms daily to each bird.

Behavioural Observations

On days 3 and 4, each dyad was observed between 0600 and 0830 hours (morning observation period) and again between 1530 and 1800 hours (evening observation period). A single observer was hidden behind a blind 10 m from the aviaries. We determined the order of observation randomly and recorded behaviour every 15 s for 15 min. The observer was unaware of the treatment group. We grouped behaviour into six categories: (1) foraging: searching for food while standing in the food dish, or eating, or drinking; (2) vocalizing: singing long-range song (advertising song; see Titus 1998); (3) alert perching: perching while looking about the cage; (4) sleeping: closed eyes; (5) preening: preening or bill-wiping; and (6) moving: locomotor activity not included in categories 1–5. For each observation period, we calculated mean proportions of time spent in these behaviours, except for vocalizing. The proportional data were arcsine transformed to stabilize variances. To assure that the various proportions did not sum to one (and thus that categories would be independent of one another), we excluded one category of behaviour from statistical analysis: alert perching. We analysed the data by four two-way analyses of variance (ANOVAs) with time and treatment as factors; sequential Bonferonni adjustment of critical values was performed for multiple comparisons (Sokal & Rohlf 1995). We calculated the mean number of long-range songs heard, combining the morning and evening observation periods for each treatment group. To determine differences in number of vocalizations between treatment groups, we performed a Scheirer–Ray–Hare

extension of the Kruskal–Wallis test for a two-way non-parametric ANOVA (Sokal & Rohlf 1995). Statistical results are reported as means ± SE.

Doubly Labelled Water Procedures

DLW procedures to determine DEE began on day 5 for all weeks except week 2; as stated, birds used in week 2 were injected on day 17. Between 0830 and 1100 hours, we took each bird to a nearby laboratory, weighed it to the nearest 0.1 g, and assigned a fat score from 0 to 5 based on visible subcutaneous fat stores (0=no visible fat, 5=bulging fat bodies) (Wingfield & Farner 1978; Nolan & Ketterson 1983). Birds were then given an intramuscular injection of 50 µl of DLW ($^3HH^{18}O$, containing 97 atom % ^{18}O and ca. 0.6 Mbq ^3H). After injection, birds were held in opaque bags (which minimized activity and energy expenditure) for 1 h to allow the DLW to equilibrate with systemic water levels. To determine baseline isotope levels, we then collected 40–80 µl of blood (alar vein, 26 gauge needle) in heparinized microhaematocrit capillary tubes, which we flame-sealed, labelled and stored at 4°C (Webster & Weathers 1989). We then returned birds to their aviary cages. We placed Hobo® temperature-data loggers (accuracy: 0.7°C at 25°C) in four of the eight cages to collect ambient temperature data at 4-min intervals for 24 h. All cages were approximately equally shaded during the day, and placement of the temperature-data loggers was similar in each cage. To determine the loss rate of the isotopes over a 24-h period, we again caught each bird exactly 24 h after DLW injection, collected 40–80 µl of blood, and flame-sealed and stored the capillary tubes at 4°C.

We continued to hold birds in captivity until their faeces were radiation-free, at which point we removed implants and released individuals at their capture sites.

Calculation of DEE

Due to loss of some samples in shipping, we determined DEE of 24 males (11 T-males, 13 C-males). We determined DEE with either the double-sample (N=17) or the single-sample (N=7) DLW technique (Webster & Weathers 1989). Blood samples were shipped to the University of California, Davis where they were micro-distilled to obtain pure water, which was flame-sealed in glass tubes (Wood et al. 1975). Samples were assayed for ^3H activity (Searle model Mark III liquid scintillation counter, toluene-Triton X 100-PPO scintillation cocktail) and for ^{18}O content by cyclotron-generated proton activation of ^{18}O to ^{18}F with subsequent counting of the positron-emitting ^{18}F in a Packard Gamma-Rotomatic counting system (Wood et al. 1975). The natural (background) abundance of ^{18}O in blood obtained from three uninjected juncos averaged 0.2016%. We calculated CO^2 production and water efflux from equations (1) and (2), respectively, of Webster & Weathers (1989).

As stated, the birds' diet was restricted to seed and mealworms. Assuming mealworms consist of 67% water and have a dry-matter energy content of 17.1 kJ/g (Weathers & Sullivan 1989), we calculated that males obtained 5.7 kJ/g of mealworm. Because each male was given only five mealworms per day, we assumed that most of the energy was obtained from seeds (commercial mixture with an approximate energy equivalent of 24.6 kJ/litre CO_2; Weathers & Nagy 1984).

Five males lost body mass during the 24-h DLW experiment, so we adjusted their energy equivalents for catabolism of body tissue. Klaassen & Biebach (1994) estimated the composition of tissue lost by fasting garden warblers, *Sylvia borin*, to be 73% fat, 8% dry protein and 19% water. We assumed energy densities of 39.6 kJ/g for fat and 18.3 kJ/g for dry protein catabolized to uric acid (King 1957). We calculated CO_2 production resulting from catabolism of body tissue as 1.10 litres of CO_2 per gram mass lost and assigned this CO_2 an energy equivalent of 27.7 kJ/litre.

Although the birds in Klaassen & Biebach's (1994) study were migrating and our juncos were not, studies show that fasting nonmigrants obtain 7–15% of their energy from protein and that, on average, fasting birds obtain 15% of their energy from protein, 5% from carbohydrate and 80% from fat (King 1957). The values from Klaassen & Biebach (1994) are (on a dry-matter basis) 10% protein and 90% fat, which fall well within the previous ranges. Additionally, the energy equivalents of protein and fat are very similar, 26.9 kJ/litre and 27.8 kJ/litre, respectively (a 3% difference). Thus, regardless of the assumptions made about the ratio of protein to fat in the mass of the tissue that was lost, our calculations of DEE and our conclusions would be virtually unchanged.

Sixteen males gained mass during the experiment. For these we assumed that the retained tissue had the same composition as the tissue catabolized by fasting birds and that each gram increase resulted in the production of 0.53 litres of CO_2 with an energy equivalent of 10 kJ/litre (Weathers & Sullivan 1989). We analysed the resulting data with a two-way ANOVA; factors were (1) treatment and (2) week in which DLW measures were made (i.e. trial number). Statistical results are reported as means ± SE.

RESULTS

Daily Energy Expenditure (DEE)

We determined DEE of 24 birds (11 T-males, 13 C-males). We found no significant difference in DEE between treatment groups (C-males: 59.89 ± 4.52 kJ/day, T-males: 63.401 ± 5.07 kJ/day; Fig. 1; ANOVA: $F_{1,16}$= 0.371, P=0.55, data adjusted for changes in body mass). There was no effect of time (i.e. trial number) ($F_{3,16}$=2.17, P=0.132), nor was there an interaction of time and treatment ($F_{3,16}$=0.221, P=0.880). Similarly, when DEE values were not adjusted for changes in body mass, the CO_2 production of C-males and T-males were statistically equivalent (C-males=5.74 ± 1.22 ml CO_2/gh, T-males= 5.97 ± 1.25 ml CO_2/gh; t test: t_{22}=0.44, P=0.67). During the 4 weeks of the study, differences in mean ambient temperature between aviary compartments were within 1°C over the 24-h periods in which DEE was measured.

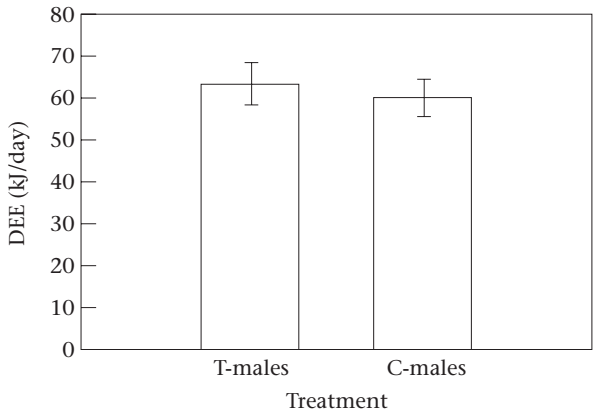

Figure 1. Results of doubly labelled water measurements of daily energy expenditure (DEE) in male juncos implanted with testosterone (T-males) or empty implants (C-males). DEE values are means±SE.

Similarly, within weeks, differences between compartments in maximum ambient temperatures were within 5°C, and differences in minimum ambient temperatures, within 0.5°C. Mean, maximum and minimum daily ambient temperature values varied significantly across the 4 weeks of the study (Friedman repeated measures ANOVA by ranks: $P<0.001$, $P=0.033$ and $P<0.001$, respectively); however, a linear regression of DEE against daily mean temperature revealed no significant effect of temperature on DEE (DEE=138.01*mean temperature, $R^2=0.142$, $F_1=3.642$, $P=0.0695$).

Both within and across treatment groups, there was no effect of age (yearling or older) on DEE (two-way ANOVA: $F_{1,20}=2.757$, $P=0.112$). Of the 24 males for which DEE was determined, 19 were captured immediately before they were used in this study, and five were allowed to acclimate to captivity for 8 weeks before use in this study (see Methods). The males that were used in the experiments beginning on the day of capture did not differ in DEE from the five males that had been in captivity since April, regardless of treatment (two-way ANOVA: $F_{1,20}=0.285$, $P=0.599$). A t test revealed no difference in DEE between the birds in trial 2 that were implanted 17 days prior to DLW measures and all other birds implanted 5 days prior ($t_{22}=-0.642$, $P=0.528$).

Body Mass

Prior to treatment, males assigned to treatment groups did not differ in body mass (C-males: 20.6 ± 0.21 g; T-males: 20.5 ± 0.32 g; t test: $t_{34}=-0.181$, $P=0.317$). Similarly, there was no significant difference in the average amount of body mass lost per day during the entire experiment (T-males lost 0.15 ± 0.06 g/day, C-males lost 0.083 ± 0.034 g/day; t test: $t_{34}=-1.030$, $P=0.310$). We also measured changes in body mass during the DLW portion of the experiment. During the DLW measurements, T-males gained 0.10 ± 0.56 g (range −1.20–0.80 g), whereas C-males gained 0.42 ± 0.46 g (range −0.50–1.30 g). These values did not differ significantly (t test: $t_{22}=1.06$, $P=0.30$). Initial body mass of the two groups was also similar at the beginning of DLW measurements: T-males weighed 19.5 ± 1.3 g and C-males weighed 20.0 ± 0.8 g (t test: $t_{22}=1.09$, $P=0.29$). Both T- and C-males carried little visible fat, and thus average fat scores did not differ between treatments at any point in the study.

Behaviour

Behavioural observations were conducted for 19 T-males and 19 C-males. T-males spent more time foraging (ANOVA followed by sequential Bonferronni test: $F_{1,1}=7.85$, $P=0.0065$, critical value=0.0127) and moving ($F_{1,1}=6.65$, $P=0.0119$, critical value=0.017) than C-males, and less time sleeping ($F_{1,1}=6.38$, $P=0.014$, critical value=0.025) and preening ($F_{1,1}=4.43$, I=0.039, critical value=0.05) (Fig. 2).

Regardless of treatment, males spent more time sleeping and less time moving during the evening than the morning observation period (sleeping: $F_{1,1}=17.93$, $P<0.001$, critical value=0.025; moving: $F_{1,1}=56.41$, $P<0.001$, critical value=0.017), but time of day had no effect on foraging ($F_{1,1}=2.10$, $P=0.15$, critical value=0.0127) or preening ($F_{1,1}=2.31$, $P=0.13$, critical value=0.05). There were no significant differences between treatment groups in mean number of long-range songs (Kruskal–Wallis test, Scheirer–Ray–Hare extension: $H_{1,1}=0.074$, $P=0.79$) and no effect of time of day on mean number of long-range songs ($H_{1,1}=0.0002$, $P=0.99$).

DISCUSSION

DEE as measured by the DLW method includes the costs of thermoregulation, resting metabolism and activity. Earlier studies have shown that administration of exogenous T to male birds increases the frequency of energetically demanding activities such as locomotion, vocalization and aggression (Wada 1981, 1986; Massa & Bottoni 1987; Ketterson et al. 1992), suggesting that T may enhance DEE. However, in our study, although administration of T increased locomotor activity, it did not affect DEE or body mass. These results suggest that if T treatment raised activity costs, it also directly or indirectly produced a compensatory change in some other component of DEE. Unfortunately, the DLW method alone does not permit determination of components of DEE. Nevertheless, we can ask whether compensation more likely involved substitution (e.g. heat generated by activity might have substituted for the cost of thermoregulation) or energetic trade-offs (e.g. cost of greater activity during the day may have been compensated for by lower resting metabolism at night).

Focusing first on thermoregulation, we do not think that thermoregulatory needs differed between treatment groups. The design of the experiments allowed for comparison of DEE for dyads placed in adjacent aviaries and measured on the same days, which should have controlled for any small cage-related differences in air temperature. The cages were similarly shaded, so we do not think that energy expended for thermoregulation differed

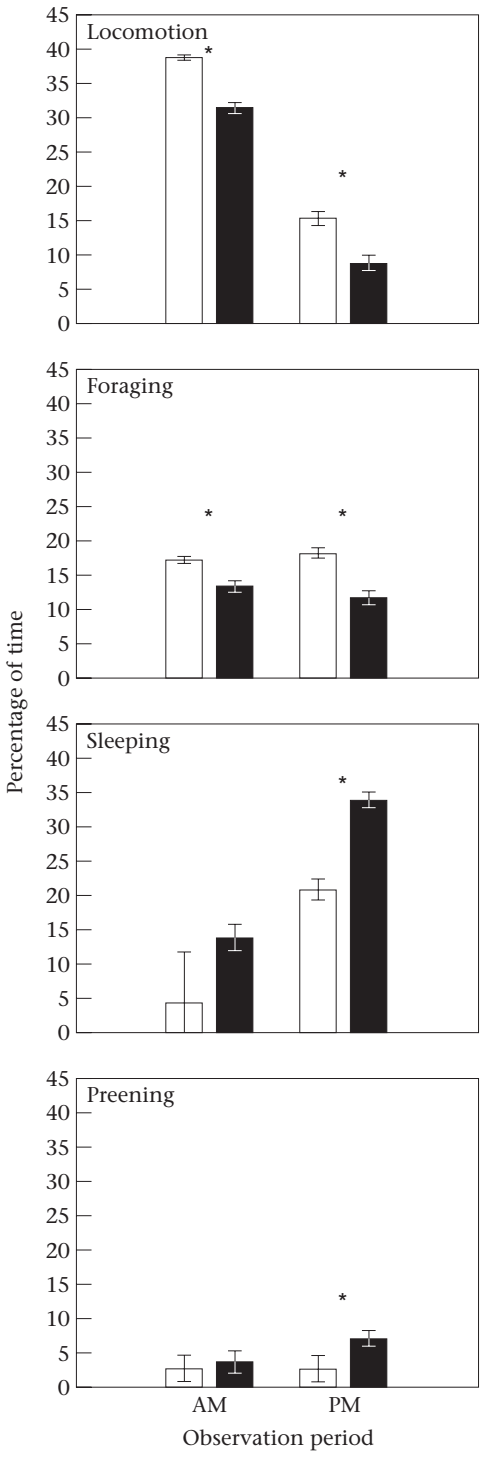

Figure 2. Results of behaviour scans of captive male juncos implanted with testosterone implants (□: T-males) or empty implants (■: C-males). AM: 0600–0830 hours; PM: 1530–1800 hours. Treatment-related differences in mean percentage of time males spent engaged in each activity are indicated by asterisks (*P<0.05). Values are means±SE.

between treatment groups due to differences in time spent exposed to direct sunlight within aviaries. Even if sun were a concern, high temperatures result in a much smaller increase in metabolic rate than low temperatures (Weathers 1981). Thus, as air temperature is an adequate index of thermal environment, the lack of a correlation between DEE and air temperature is sufficient to exclude temperature effects in this study. The possibility remains that heat generated by activity may have been used by T-males to meet a portion of their daytime thermoregulatory costs (Ketterson & King 1977).

If thermoregulatory needs of T- and C-males did not differ and heat generated by activity did not play a role in DEE, then another possible explanation for the lack of difference in DEE between T- and C-males is that low-intensity activities such as foraging and preening may have little impact on total DEE. In small birds, energy devoted to resting metabolism and thermoregulation can comprise 70% of DEE, leaving only 30% for physical activity (Walsberg 1983; Weathers & Sullivan 1989). If this is true of captive juncos, even significant differences in time spent foraging and moving may not have generated treatment-related differences in DEE. The following calculations illustrate the relationship between activity costs and DEE for our juncos. For C-males, DEE averaged 59.9 kJ/day, of which 70% (41.9 kJ/day) may have been devoted to resting metabolism and thermoregulation. Assuming resting and thermoregulatory costs were the same in T- and C-males, T-males would have devoted 21.5 kJ/day to activity (63.4–41.9 kJ/day), or 19% more energy than C-males. Although the behaviour of T-males thus likely imposed higher energy costs for activity than the behaviour of C-males, the difference becomes statistically lost in the context of total DEE. This emphasizes the importance of partitioning energy costs between maintenance and activity categories (Weathers et al. 1984; Weathers & Sullivan 1989); something we were unable to do in the present study. Whether increased allocation of energy to activity may entail fitness costs in T-males despite DEE being unchanged remains to be studied.

However, if we relax the above assumption that resting costs were the same in T- and C-males, there are numerous possible explanations for why DEE of T-males might equal that of C-males despite T-males' higher level of activity. One possibility that deserves further investigation is that resting metabolic rate might be lower in T- than C-males.

Deerenberg et al. (1998) found that experimentally increasing perch-hopping levels in captive zebra finches, *Taeniopygia guttata*, induced a decrease in nocturnal resting metabolic rate. DEE (measured by the DLW method), however, did not differ between birds with increased activity levels and birds with normal activity levels, apparently because the birds compensated for increased activity metabolism by reducing their energy expenditure at night, resulting in no net difference in total energy expended over a 24-h period. Similarly, in a study of captive male Gambel's white-crowned sparrows, *Zonotrichia leucophrys gambelii*, Wikelski et al. (1999) found that T-implantation increased activity levels and decreased diurnal and nocturnal resting metabolic rate. Although Wikelski et al.'s (1999) study did not include a measure of DEE, these results suggest that the T-induced rise in activity levels may often be

accompanied by a reduction in energy expended for resting metabolism.

On the other hand, T treatment did not alter resting metabolic rate in male mountain spiny lizards, *Sceloporous jarrovi*, although field metabolic rate (analogous to DEE) was higher in T-males than in controls apparently owing to an increase in territorial defence (Marler et al. 1995). Unlike birds, lizards devote little energy to thermoregulation, and their DEE consists largely of activity and resting metabolism. Thus, with no compensatory decline in resting metabolic rate in these lizards, increased activity metabolism resulted in an elevation of DEE.

Finally, Deviche (1992) found no effect of T treatment on resting metabolism of captive male dark-eyed juncos measured during the active period of the day. Deerenberg et al. (1998) and Wikelski et al. (1999) suggest that birds with high activity levels during the day may compensate for the increased daytime energy expenditure with a reduction in resting metabolism at night (and possibly during the inactive hours of the day). If so, we would not expect a difference in the daytime resting metabolic rates of T- and C-males, which is consistent with Deviche's results.

Behaviour

T-males increased the time allocated to locomotor activity and to foraging, but spent less time sleeping and preening. Given that DEE did not differ between T- and C-males, and that T-males spent more time foraging than C-males, it is surprising that T-males did not gain significantly more mass (and have a higher fat score) than controls. It is possible that food intake was not proportional to time spent foraging for T-males. However, Deviche (1992) also found that T-implanted male juncos increased food consumption without altering body mass or fat index. One explanation for this result is that T may have depressed foraging efficiency (see Nagy 1987; Bednekoff & Houston 1994), which might have caused our T-males to devote more time than C-males to foraging without increasing their net energy intake. It is also possible that T depressed energy assimilation efficiency in the gut. In mammals, resting metabolic rate is associated with differences in the ability of the digestive tract to assimilate nutrients and energy substrates. This is reflected primarily in differences in the area of absorptive mucosal surface of the small intestine (Karasov & Diamond 1985; Konarzewski & Diamond 1994). If T indeed had an effect to reduce resting metabolic rate in our birds (as in white-crowned sparrows; Wikelski et al. 1999), it is possible that this might result in a reduction in intestinal area and, consequently, a reduced absorption efficiency in the gut. Clearly, further research is necessary to support these ideas.

Regardless, if free-ranging T-males devote more time to foraging, as did our captive T-males, they might face disadvantages, even if they managed to maintain normal mass. Increased foraging might make them more vulnerable to predators (Bednekoff & Houston 1994; but see also Marler & Moore 1991), and a reduction in time devoted to rest and self-maintenance could be disadvantageous. In our study, T-males spent less time sleeping and preening than did C-males. Reduced preening may increase the probability of parasite infection, suggesting additional research on the relative parasite loads of T- and C-males.

In summary, we found that although high T increased foraging behaviour and locomotor activity in captive male juncos, it did not increase DEE. Thus, our findings do not support the hypothesis that maintaining prolonged high T levels is disadvantageous in this species because it leads to elevated DEE. Our data are consistent with the idea that T may directly or indirectly affect resting metabolic rate and/or nighttime energy expenditure (Deerenberg et al. 1998; Wikelski et al. 1999). Additionally, we found that T-treated birds reduced time allocated to rest and self-maintenance. The influence of T on foraging and maintenance behaviours, in concert with a possible reduction in resting metabolic rate, may represent an important cost to male juncos whose plasma T is tonically elevated throughout a breeding season.

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