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Testosterone effects on avian basal metabolic rate and aerobic performance: Facts and artefacts

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Keywords

Testosterone, effects, avian, basal, metabolic, rate, aerobic, performance, Facts, artefacts

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Testosterone effects on avian basal metabolic rate and aerobic performance: Facts and artefacts[☆]

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Abstract

We examined the effects of cage size and testosterone (T) levels on basal and peak metabolic rates (BMR and PMR, respectively) and on pectoral and leg muscle masses of male house sparrows (*Passer domesticus*). Birds were housed either in small birdcages or in flight aviaries for at least 2 weeks prior to the initial metabolic evaluations. They were then implanted with either empty or T-filled silastic capsules and remeasured 5–6 weeks later. Birds treated with single T implants achieved breeding levels (4–6 ng/mL) and one group given double implants reached 10 ng/mL. There was no effect of T on BMR or PMR in any group studied, but there was an effect of caging. Caged birds showed significant reductions in PMR over the course of captivity, whereas PMR in aviary-housed birds were indistinguishable from their free-living counterparts. Testosterone treatment significantly increased leg muscle mass in caged birds, but had no effect on muscle mass in aviary-housed sparrows. We conclude that testosterone has no direct effect on sparrow metabolic rate or muscle mass, but may interact with cage conditions to produce indirect changes to these variables.

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Keywords: Basal metabolic rate; Cage effects; Muscle mass; Oxygen consumption rate; House sparrows; Testosterone; Peak metabolic rate; Aerobic scope

1. Introduction

Testosterone (T) serves as an activating signal that prepares male vertebrates for reproduction. In many species, this steroid hormone also serves to stimulate the expression of male secondary sexual characteristics, but the pattern and amplitude of T secretion varies markedly according to the breeding system (Wingfield et al., 1990). Males of polygynous species (most mammals; some birds) have significantly higher and more protracted secretion of T throughout the breeding season than males showing biparental care (most birds; some mammals; Wingfield et al., 1990). Such pronounced differences in T secretion among breeding males are believed to result from inherent trade-offs between the benefits of having high T during mate acquisition and a range of costs associated with main-

taining elevated T, which have been shown experimentally to reduce male survivorship (Dufty, 1989). One factor responsible for decreased survivorship is T-induced reduction in immune function, an effect demonstrated in both birds and mammals (see reviews by Grossman, 1984; Schuurs and Verheul, 1990; Olsen and Kovacs, 1996; Besedevsky and del Ray, 1996).

Although the functional bases of T-affected immunosuppression remain unresolved, some have suggested that the energy and nutrients demanded by breeding activities limit the resources that can be allocated to immune functions (Gustafsson et al., 1994; Sheldon and Verhulst, 1996; Svensson et al., 1998). Because T levels are elevated in males during breeding, it is difficult to gauge the extent to which immunosuppression is due to direct effects of T on components of the immune system, versus indirect effects through T-induced increases in energy-demanding activities.

One intriguing consequence of T-induced immunosuppression is that it might result in greater allocation of resources for aerobic performance in immune-challenged breeding males. Such outcomes would permit males to remain vigorous during mate

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attraction and mate guarding, periods associated with greatly elevated T levels (Hegner and Wingfield, 1986). This could be demonstrated experimentally by testing whether T-treated birds display higher peak metabolic rates when immune challenged compared to control birds. Before engaging in such a study, however, it is important to evaluate the effect of T on factors associated with peak metabolic rate in birds and how these might be affected by caging conditions. We therefore have undertaken preliminary studies to examine the influence of cage size and T treatment on basal and peak metabolic rates and locomotor muscle size in male house sparrows (*Passer domesticus*).

2. Material and methods

2.1. Experimental animals

All house sparrows used in these studies were captured from free-living populations residing in the Illawarra region of New South Wales, Australia. Birds were captured outside the breeding season and held in either small cages indoors (2 birds per cage; 40 × 60 × 40 cm; room maintained at 22 °C) or in flight aviaries (up to 12 birds per aviary; 4.5 × 3.6 × 2.5 m) exposed to ambient conditions. All birds were provided commercial finch seed mix, shell grit and water *ad libitum*.

2.2. Experimental protocols

2.2.1. Study I (Cage)

Twelve adult males were captured in early autumn and placed in small, indoor cages and were given 2 weeks of adjustment before measuring basal and peak metabolic rates (BMR and PMR, respectively). Birds at this time of year have regressed gonads with minimal testosterone secretion. Seven birds were then implanted subcutaneously with a testosterone-filled silastic tube (see below for details) and the other 5 birds (controls) received an empty implant. Peak metabolic rate was remeasured in these birds 3 and 6 weeks later, and BMR was re-evaluated 6 weeks after receiving implants. Birds were returned to holding cages for a 2-h recovery and rehydration period after PMR measurements, removed to obtain a blood sample, and then killed by simultaneous cervical dislocation and thoracic compression to determine pectoral and leg muscle wet and dry masses (see Chappell et al., 1999 for details).

2.2.2. Study II (Aviary I)

Thirty-three adult males were captured in early autumn and housed in outdoor flight cages and given either 2 testosterone-filled implants (10 TT birds), one empty and one T-filled silastic implant (13 T birds), or a pair of empty silastic implants (10 controls). Birds were captured in flight cages 4 weeks later and placed in small holding cages for a 2-h recovery and rehydration period. They were then removed to obtain a blood sample, and then killed to determine pectoral and leg muscle masses.

2.2.3. Study III (Aviary II)

Eleven adult males were captured in early winter and housed in outdoor flight cages and had BMR and PMR measured after

2 weeks of adjusting to captivity. They then received either an empty silastic implant (6 controls) or a single implant filled with testosterone (5 T birds). PMR and BMR were remeasured 5 weeks later and a blood sample collected to determine T levels.

2.3. Testosterone implant procedure

Birds were implanted subcutaneously with either empty silastic capsules (control birds) or one (T birds) or two silastic tubes filled with crystalline testosterone (TT birds; Sigma Chemical Co.; T-1500). The capsules were prepared from 6 mm lengths of medical-grade silastic tubing (1.47 mm inner diameter; 1.96 mm outer diameter; Dow Corning), which were sealed at each end with silastic glue (Dow Corning). Birds were given anaesthetic (methoxyflurane) and had small incisions made to permit subcutaneous insertion of implants on the sides of their thorax. The resultant wounds were sealed with medical-grade cyanoacrylate adhesive (Vetbond).

2.4. Hormone sampling and analyses

Blood samples were collected in heparinised microhaematocrit tubes from an alar vein following venipuncture with a 26-g needle. Up to 200 µl of blood was collected at this time when evaluating plasma T levels. Blood samples were placed in an ice-filled container (ca. 1 °C) for no more than 1 h before being centrifuged at 6000 rpm for 5 min in a Hettich EBA 12 with an haematocrit rotor. Harvested plasma was frozen at –20 °C until being used for hormonal analysis. Plasma T content was evaluated using a double-antibody radioimmunoassay kit (Pantex, Santa Monica, California, USA; Cat. No. 135) with an intra-assay coefficient of variation of 10% at the lower end of the standard curve (0.4 ng/mL).

2.5. Basal metabolic rate measurements

Measurements were initiated at least 6-h after the birds had last fed, in the dark, during the birds' rest-phase. We measured BMR at temperatures between 29 and 31 °C, a range we found to be thermoneutral for this species (Chappell et al., 1999). Birds were placed in 2-L chambers fitted with a perch and having an airflow maintained at 500 mL/min by mass flow controllers (Tylan Model FC-280S). Oxygen consumption rates were evaluated by comparing oxygen content of inlet and outlet air for each chamber using a Sable Systems FC-1 oxygen analyser in combination with an electronic stream selector (Sable Systems Respirometer Multiplexer V 2.0). Voltage output from the oxygen analyser was recorded at 5-s intervals and each bird was sampled for 11 min/h. All reported values of oxygen consumption are corrected to standard temperature and pressure (STP) conditions and have been calculated using Eqn. 2 of Hill (1972). Basal rates of oxygen consumption (BMR) for each bird represent the mean of the 2 lowest 5-min averages of oxygen uptake recorded during two separate sampling periods during the 12-h measurement period (see Buttemer et al., 1991 for details).

2.6. Peak metabolic rate measurements

We measured oxygen consumption rates ($\dot{V}O_2$) during intense exercise within an enclosed motorised wheel to gauge exercise-induced metabolic rate maxima, which we designate as peak metabolic rate (PMR). The system is nearly identical to that described by Chappell et al. (1999). Briefly, the wheel (31 cm diameter, 12 cm width, 8 L volume) consists of transparent plastic sides with a carpet-lined rim on its interior. Airflow is provided to the wheel at a rate of 5 L min⁻¹ STP using a mass-flow controller (Hastings). Air leaving the chamber was dried and rendered CO₂-free using Drierite and soda lime, respectively, before being analysed for O₂ content (Sable Systems FC-1). Output from the oxygen analyser and flow meter were recorded on a Macintosh computer fitted with an A-to-D converter and custom-designed software (WartHog Systems).

For each exercise bout, a bird was placed in the stationary, towel-covered wheel and allowed 2–3 min to settle before exercise was initiated. The towel was then removed and the wheel rotated at a speed of ca. 0.3 m s⁻¹ initially, but gradually advanced to match the speed of each bird's takeoff and landing. Ping-pong balls placed inside the wheel provided added incentive for flight. Birds tended to flap vigorously during the first 2–4 min of exercise, but showed signs of less flapping and more running as exercise progressed, at which time the wheel was stopped. The maximum $\dot{V}O_2$ was computed from the highest instantaneous $\dot{V}O_2$ measured during a 1-min interval of exercise (Chappell et al., 1999).

2.7. Muscle masses

Birds were weighed (accuracy of ± 0.05 g), after which they were killed instantly by simultaneous cervical dislocation and thoracic compression. During the dissection, immediately following, we removed the entire right breast muscle complex (*musculus supracoracoideus* and *musculus pectoralis*) from the skeleton. We also removed the entire right leg and detached all muscles and tendons proximal to the tibiotarsus-tarsometatarsus joint. We were careful to dissect each bird identically. Muscles were trimmed of fat and connective tissue, washed in physiological saline, blotted dry, and weighed (± 0.1 mg). They were then dried to constant mass at 60 °C and re-weighed. Since both muscles were obtained from the right side only, total muscle mass was calculated by doubling this value.

2.8. Data analysis

Evaluations of T effects on BMR and PMR were examined using paired-sample Student's *t*-tests of values measured for each bird before and after implant insertion. T levels of T-treated and control captive birds were compared using ANOVA with intergroup differences identified using Fisher's Protected Least Significant Difference (PLSD). Comparison of intergroup post-implant BMR and aerobic scope values were performed using ANOVA. Because locomotor muscles are influenced by the mass they move, identification of treatment effects requires elimination of mass-related influences. This was done by first

regressing dry muscle mass against fresh body mass and then examining the residuals of the various treatments by ANOVA. Examination of temporal effects on PMR of caged sparrows was determined using a two-way ANOVA for treatment and repeated measures (time after capture). Values stated below represent means ± 1 standard error.

3. Results

3.1. Testosterone levels and morphological comparisons

Birds implanted with T-filled implants had significantly higher T levels than those with empty implants (Table 1; $P < 0.01$ for all comparisons). Body mass varied significantly among the study groups ($F_{7,60} = 2.908$; $P = 0.01$), but this was due to birds in Study III being significantly heavier than all other birds ($P < 0.03$). Thus, although there were substantial differences in T levels, body mass did not differ between birds within a given study cohort.

Despite similarity in overall body mass among treatments, closer examination of body constituents revealed substantial variation in their relative size (Figs. 1 and 2). Leg muscle dry mass correlated significantly with fresh body mass ($r = 0.72$; 55 df; $P = 0.0001$) and analysis of residuals revealed that T-treated males held in small cages had significantly larger leg muscles than all other treatment groups studied ($P < 0.05$; Fig. 1), with the exception of aviary birds given double T implants ($P = 0.08$). None of the other treatment groups differed significantly in relative leg muscle mass. Pectoral dry muscle mass also correlated significantly with fresh body mass ($r = 0.70$; 55 df; $P = 0.0001$). Although T-treated birds held in small cages tended to have relatively larger pectoral muscles (Fig. 2), there was no effect of T-treatment on relative pectoral size in captive birds ($P > 0.05$ for all comparisons). By contrast, free-living birds had significantly smaller pectoral muscle mass for their size than all captive birds, with the exception of control birds held in small cages ($P = 0.12$; Fig. 2).

3.2. Metabolic rate measurements

With the exception of control birds held in aviaries, the pre-implant values of BMR tended to be higher in captive birds than

Table 1
Body mass and plasma testosterone concentration of birds used in this study

Group	Body mass, g	Plasma testosterone, ng mL ⁻¹
Cage controls	22.8 $\pm$ 0.4	0.13 $\pm$ 0.04
Cage T-treated	23.5 $\pm$ 0.5	3.93 $\pm$ 0.41
Aviary I controls	24.2 $\pm$ 0.6	0.21 $\pm$ 0.09
Aviary I T-treated	24.0 $\pm$ 0.5	6.1 $\pm$ 0.7
Aviary I TT-treated	24.1 $\pm$ 0.7	10.5 $\pm$ 1.5
Aviary II controls	25.8 $\pm$ 0.5	0.18 $\pm$ 0.05
Aviary II T-treated	26.3 $\pm$ 0.5	3.9 $\pm$ 0.4

Control birds are implanted with empty silastic capsules, T-treated birds are implanted with a single T-filled capsule, and TT-treated birds are implanted with 2 T-filled capsules. Cage refers to a small standard bird cage, whereas aviary represents a flight cage.

Values below represent group means $\pm$ one standard error.

Fig. 1. Average leg muscle mass as a percent of total body mass in male house sparrows held either in small cages (Cage) or in flight aviaries (Aviary). Captive birds were given either an empty implant (C), a single (T), or double implants (TT) of testosterone-filled silastic capsules. Average leg muscle mass of free-living (FL) males (Chappell et al., 1999) is shown for comparison. Error bars represent one standard error. See text for statistical comparisons.

measurements made 5–6 weeks after receiving implants (Fig. 3). These differences, however, only reached statistical significance in the T-treated birds held in small cages ($t=3.841$; $P=0.01$; 6 *df*). Regardless of cage type, post-implant BMR values were statistically indistinguishable between all treatment groups ($F_{3,19}=1.582$; $P=0.227$).

Peak metabolic rate declined significantly over the course of captivity for sparrows held in small cages (Fig. 4; $F_{2,20}=4.718$, $P=0.02$), but there was no effect of treatment on this decline ($F_{1,10}=1.908$, $P=0.197$). By contrast, PMR was unchanged over the course of captivity in both aviary-housed control birds ($t=1.331$, $P=.24$, 5 *df*) and T-treated birds ($t=0.557$, $P=0.607$,

Fig. 2. Average pectoral muscle mass as a percent of total body mass in male house sparrows held in cages and receiving treatments as in Fig. 1. Average pectoral muscle mass of free-living (FL) males (Chappell et al., 1999) is shown for comparison. Error bars represent one standard error. See text for statistical comparisons.

Fig. 3. Average basal metabolic rate (BMR) of male house sparrows held either in small cages (Cage) or in flight aviaries (Aviary). Birds receiving testosterone-filled silastic capsules are identified by the suffix T, whereas those given empty silastic capsules are denoted by the suffix C. Open columns signify pre-implant measurements, filled columns represent post-implant measurements, and error bars represent one standard error. See text for statistical comparisons.

4 *df*). The PMR differences between captive birds was significantly affected by caging ($F_{1,19}=13.12$, $P=0.002$), but not by T treatment ($F_{1,19}=2.16$, $P=0.16$), and there was no significant interaction between these factors ($P=0.59$).

The ratio between PMR and BMR is termed aerobic scope and represents the relative expandability of metabolism towards aerobic performance. The aerobic scope differed significantly among captive and free-living house sparrows (Fig. 5; $F_{4,29}=3.14$, $P=0.029$). These differences are a consequence of caged control birds having a significantly lower aerobic scope than all aviary-housed birds ($P<0.05$ for all

Fig. 4. Average peak metabolic rates of sparrows measured over an 11-week period of being held in small cages. Birds were first measured 2 weeks after being placed in cages and again at 3 and 6 weeks after receiving either empty (filled circles) or testosterone-filled silastic capsules (open circles). Error bars represent one standard error.

Fig. 5. The relation between aerobic scope (peak metabolic rate/BMR) and cage type (small cage=C; flight cage=aviary) in male house sparrows. Those receiving empty or testosterone-filled silastic capsules are denoted by the suffixes C and T, respectively. Values shown represent means and associated standard errors. Average aerobic scope measured in free-living (FL) males (Chappell et al., 1999) is shown for comparison.

comparisons). The aerobic scope of T-treated cage birds was statistically indistinguishable from all other groups.

4. Discussion

A fundamental requirement for relevant assessment of reproductive hormone effects on a given species is to experimentally attain circulating levels that are representative of breeding animals. The levels of T resulting from our use of T-filled implants were all within the physiological levels and durations reported for male house sparrows during the early breeding season (Hegner and Wingfield, 1986). Our studies are thus likely to reveal T-affected metabolic and morphological variations that free-living males might experience when going from non-breeding to breeding stages.

4.1. Morphological considerations

One of main outcomes of this study was to show that housing in small cages as opposed to flight aviaries can bias the results of experiments attempting to measure T-effects on muscle size in birds. Had we restricted our study to birds confined to small cages, we would have concluded that elevated T results in hypertrophied leg muscles in house sparrows. Because this response was not noted in T-treated birds housed in flight aviaries, it appears to be an interactive effect of cage and treatment. Small cages restrict the types of movement that birds undertake to combinations of very short flapping flight and hopping between perches. It is possible that T-treated birds hopped more frequently than control birds in the small cages, a situation noted in T-treated white-crowned sparrows (*Zonotrichia leucophrys gambelii*; Wikelski et al., 1999). Such an effect would likely result in exercise-induced hypertrophy of leg musculature in more active birds.

The absence of testosterone effects on relative size of pectoral musculature in all captive house sparrows further suggests that T does not directly lead to muscular hypertrophy in this species. This is unlike the situation found among some smaller mammalian species, where lean muscle mass was significantly correlated with testes size (Schulte-Hostedde et al., 2003). Perhaps aerodynamic requirements place greater constraint on size dimorphism in birds, particularly in species showing biparental care such as the house sparrow. The lack of testosterone-induced muscular hypertrophy in our captive males is consistent with studies showing no gender-related differences in pectoral and leg muscle masses in adult house sparrows during the breeding season (Chappell et al., 1999). The relatively smaller pectoral muscles of free-living compared to caged sparrows in our study may be due to lower daily frequencies of short-distance and hovering flight compared to caged birds. It is well known that takeoffs and hovering are the most expensive phases of bird flight (Pennycuik, 1968) and, accordingly, may give rise to increased muscle size through power-training effects.

4.2. Basal metabolic rate

We can state unequivocally that T had no effect on BMR in the birds we studied. The strongest evidence for this comes from the lack of difference in BMR measured before and after the same individuals received T implants. This result accords with findings of some other studies (Rautenberg, 1952/1953; Hänssler and Prinzinger, 1979; Buttemer and Astheimer, 2000), but differs from those demonstrating reduced BMR (Wikelski et al., 1999) or increased BMR (Buchanan et al., 2001) in T-treated versus control birds. It is important to recognise, however, that an unambiguous interpretation of T effects on the metabolic rate of birds requires two things: T levels must be maintained at physiologically relevant levels and the conditions of metabolic measurement must permit basal metabolic rates (BMR) to be obtained.

BMR is the minimum rate of aerobic metabolism that an endothermic animal can achieve while maintaining its resting body temperature. This requires that the animal be in a thermoneutral environment, under postabsorptive conditions, asleep during the rest-phase of its daily cycle, and free of any disturbance. Such conditions are easily defined, but may be difficult to achieve, particularly for species that are sensitive to disturbances. Metabolic rates are very labile and can be more than 50% higher in an animal that is motionless, but alert, compared to when it is asleep (Buttemer et al., 1986). For animals that settle quickly in respirometry chambers, metabolic measurements may be at basal levels for very long periods, whereas for those that take a long time to settle and remain asleep for only brief periods, BMR may only occur on a transient basis, if at all. Consequently, BMR may not be measurable in birds that remain restless during their period of measurement, whether from endogenous or external influences.

The studies of Rautenberg (1952/1953) and Hänssler and Prinzinger (1979) are among the first to address T effects on avian BMR. They both found castrated male birds to have lower

BMR than intact males, but importantly, T treatment failed to increase the BMR of these castrates. Unfortunately, because plasma T levels were not measured in their research, we do not know if physiological levels of T were obtained. By contrast, more recent research using T-filled silastic tubes have successfully achieved physiological levels of T in their study animals (Wikelski et al., 1999; Buttemer and Astheimer, 2000; Buchanan et al., 2001), but with very different effects on BMR. Wikelski et al. (1999) report that T-treated white-crowned sparrows have a lower BMR than control males, Buttemer and Astheimer (2000) found T treatment to have no effect on BMR of white-plumed honeyeaters (*Lichenostomus penicillatus*), while Buchanan et al. (2001) found BMR to vary directly with level of T in castrated house sparrows. Because of the profound evolutionary implications that T-induced variation in BMR has for breeding males, a closer examination as to why these results might differ is warranted.

A comparison of BMR for species given physiological levels of T reveals that some studies reported values for control or pre-implanted birds that greatly exceeded the basal metabolic rates predicted for a passerine bird of their size (Fig. 6). The metabolic rates reported by Wikelski et al. (1999) for white-crowned sparrows exposed to long-day photoperiods before receiving T-filled implants are nearly 60% above both predicted (Aschoff and Pohl, 1970) and measured basal metabolic rates for this species (King, 1964; Buttemer et al., 1991). This is most likely due to nocturnal restlessness and perhaps pre-migratory movements, *zugunruhe*, commonly seen in migratory species

exposed to long-day conditions. Such nocturnal activities make it nearly impossible to identify periods of basal metabolism, which is probably why Wikelski et al. (1999) termed their results resting rather than basal metabolic rates. Similarly, the house sparrow metabolic results reported by Buchanan et al. (2001) also suggest that their measurement conditions were not conducive for BMR determination. The intact house sparrows of their study, for example, have BMR values over 50% higher than predicted (Fig. 6). Although their T-treated birds do show a progressive rise in BMR with increased levels of T (Fig. 6), the exceptionally high BMR values shown by intact birds with much lower T levels cast doubt on their conclusion that T produces a rise in basal metabolic rate. An alternate interpretation of their results is that their birds did not fully settle in the limited time allocated to these measurements (3 h), and that those with higher testosterone levels settled least. By contrast, longer-term metabolic measurements of both white-plumed honeyeaters (Buttemer and Astheimer, 2000) and house sparrows of the present study had pre-treatment BMR values close to those predicted for passerines (Fig. 6) and, most importantly, there was no effect of T on BMR in either species. Furthermore, the slightly lower than predicted BMR we measured in house sparrows agrees with values measured by Hudson and Kinsey (1966) for North American sparrows collected from a similar latitude.

Although some have criticised BMR as being metabolically unrepresentative of what animals normally experience, it is a physiological baseline that defines the minimum cost of living for an endothermic animal (Hulbert and Else, 2004). Careful measurement of BMR is needed to distinguish direct from indirect effects of T on metabolic rate. That is, if an animal's BMR rose significantly following T treatment, then one could conclude that T had a direct effect on that individual's metabolic processes. If, however, an individual's BMR was not affected by T, but its overall overnight energy expenditure was elevated, then T would be eliciting an indirect effect on metabolism, perhaps by making the animal more wary or by increasing its activity levels. These distinctions between direct and indirect effects have very important consequences for free-living animals. Direct effects of T on metabolic rate would result in persistently elevated rates of energy expenditure, but indirect effects are context-dependent and thus capable of being ameliorated by behaviour. It is with this distinction that we conclude that T has no direct effect on avian metabolic rate.

4.3. T effects on peak metabolic rate

A key objective of our studies was to examine the effects of T and cage conditions on house sparrow aerobic metabolic performance before commencing experiments on the interaction between T and immune challenge on peak metabolic rate (PMR). Because these experiments will require multiple measurements of each individual's PMR, it is critical that these aerobic measurements are repeatable. The repeatability of PMR has been confirmed in red jungle fowl (Chappell et al., 1996) and also in aviary-housed house sparrows (Warne, 2000). Such repeatability, however, depends importantly on caging

Fig. 6. Nocturnal metabolic rates of hormonally manipulated male birds, as percent of predicted values, in relation to plasma testosterone levels. Predicted values use the allometric equations of Aschoff and Pohl (1970) for passerine birds along with the body mass reported for each treatment group, except for the house sparrows of Buchanan et al. (2001) where a 25 g body mass was assumed. Species shown are white-crowned sparrows (*Zonotrichia leucophrys*; Wikelski et al., 1999) intact birds (filled circles) and castrates (open circles); house sparrows (*Passer domesticus*) studied by Buchanan et al., 2001 (open squares=castrates; filled square=intact birds) or present study (filled triangles=aviary-house birds; open triangles=caged birds); white-plumed honeyeaters (*Lichenostomus penicillatus*; Buttemer and Astheimer, 2000) (open diamonds=captive birds; filled diamond=free-living males). Variance has been omitted for clarity.

conditions. House sparrows in small cages showed progressive declines in PMR, despite T-treated birds having locomotor muscles as large or larger than aviary-housed or free-living birds. This demonstrates the importance of flight history on attaining peak aerobic output in these animals. After cage effects are accounted for, there was no effect of T treatment on PMR in sparrows. This suggests that free-living birds will gain no direct aerobic benefit from elevated T levels while breeding. The aerobic scope values we recorded for aviary-housed sparrows were indistinguishable from those measured on recently captured free-living birds (Chappell et al., 1999), and nearly double the peak values reported previously for birds under exposure to extreme cold (Hinds et al., 1993). We are thus confident birds held in flight cages can maintain their aerobic capacity to levels commensurate with their free-living counterparts.

5. Concluding remarks

Physiologists often attempt to understand the underlying bases of biological processes and must, by necessity, control as many variables as possible to target the ones of interest. This sometimes involves capturing free-living animals and holding them under controlled conditions while in captivity. The present study demonstrates how such situations may give rise to erroneous conclusions. If, for example, one wished to examine whether T caused muscular hypertrophy in birds, one would conclude this to be the case if birds were held in small cages, but would conclude the opposite if examining aviary-housed or free-living birds. Artefacts of caging would also emerge in studies of aerobic capacity. In a similar vane, unless conditions of measurement promote full rest by the subject being evaluated, metabolic readings may vary for reasons not accounted for by the researcher. Thus, the variable effects of T reported for different species may be as much a consequence of variations between researchers in experimental design and execution as in actual differences between species. It is important to remember that even the best considered designs are fraught with potential for artefactual outcomes.

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