Journal of Fish Biology (2004) **65,** 895–905
doi:10.1111/j.1095-8649.2004.00484.x, available online at http://www.blackwell-synergy.com

Androgen levels and energy metabolism in *Oreochromis mossambicus*

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(Received 13 August 2003, Accepted 28 May 2004)

Two studies were conducted to test the relationship between androgens and routine metabolism in the Mozambique tilapia *Oreochromis mossambicus*. In the first study, endogenous levels of plasma levels of androgens and oxygen consumption rate were measured. In accordance with expectations routine metabolism corrected for metabolic body mass, was positively correlated with the behaviourally active metabolite of testosterone, 11-ketotestosterone, but not with testosterone itself. In the second study levels of 11-ketotestosterone were experimentally elevated, which increased the lowest values of (corrected) routine metabolism, indicating a positive relationship with standard metabolism. These results show the importance of measuring reproductive hormones, and are supportive of the hypothesis that elevated levels of androgens are a costly trait.

Key words: 11-ketotestosterone; metabolic rate; *Oreochromis mossambicus*; testosterone; tilapia.

INTRODUCTION

In many vertebrates circulating levels of sex-steroids such as androgens are elevated during periods of inter-sexual competition and androgen treatment facilitates courtship and aggressive behaviours (Nelson, 1999; Oliveira *et al.*, 2001). Changes in androgen levels during the reproductive season have been shown to correlate with changes in energy allocation (Leonard *et al.*, 2002). Experimental treatment with androgens has been shown to increase growth rate in fishes (Ron *et al.*, 1995), which is an important trait in fishes since larger males are more successful in establishing and defending high quality territories or spawning areas (Côté & Hunte, 1989; Magnhagen & Kvarnemo, 1989). Moreover, androgen treatment has been shown to increase the mass of sonic muscles, which are sexually dimorphic and play a role in spawning behaviour

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(Connaughton & Taylor, 1995). Androgens may thus be viewed as competition hormones that respond to the social environment and prepare the individual for the high levels of competition during the reproductive season (Oliveira *et al.*, 2001).

In support of such a function of androgens in competition in fishes, a positive correlation has been found between androgen levels and the acquisition of dominance (Oliveira *et al.*, 1996), and between androgen dependent traits (*i.e.* aggression) and dominance (Metcalfe *et al.*, 1995; Cutts *et al.*, 1998). Interestingly, in several fishes standard metabolism (r_s), *i.e.* the metabolism measured during periods of inactivity, has been shown to positively correlate with dominance (Clarke, 1992; Metcalfe *et al.*, 1995; Yamamoto *et al.*, 1998; Cutts *et al.*, 2001; McCarthy, 2001). A relationship between androgens and r_s would imply energetic costs of elevated levels of these hormones independent of their direct effects on behaviour. Such costs have been predicted to explain individual variation in androgen levels, but so far experiments in birds analysing these costs have given both positive and negative results (Wikelski *et al.*, 1999; Buchanan *et al.*, 2001).

The goal of the present study was to test the relationship between androgens: the behaviourally active 11-ketotestosterone (11-KT), and its precursor, testosterone (T), and metabolic rate (r) in the Mozambique tilapia *Oreochromis mossambicus* Peters. This species was chosen because of its aggression in territorial interactions (Baerends & Baerends van Roon, 1950), while dominance and high levels of aggression correlate with high levels of 11-KT in *O. mossambicus* (Oliveira *et al.*, 1996; Oliveira & Almada, 1998). Individuals of this species adapt readily to tests in metabolic chambers (Job, 1969; Kutty, 1972), and their low levels of activity make them suitable for measurements of r_s (Ginneken *et al.*, 1997).

MATERIALS AND METHODS

ANIMALS

Male Mozambique tilapia were 2 to 4 years old, and kept in 750–800 l aquaria at the animal housing facilities of the Instituto Superior de Psicologia Aplicada, Lisbon, Portugal. Aquaria had on average three males and three females. Males formed typical dominance hierarchies in the aquaria. Dominant males were easy to recognize because they showed a contrasting black and white colouration whereas subdominants had pale colouration (Baerends & Baerends van Roon, 1950). Furthermore, only the dominant males defended a territory where other males were chased off. To standardize the experiments, variation in social status was controlled for by selecting only males that were holding dominant positions for the studies. Water in the aquaria was filtered (bottom filter with sand), continuously aerated and kept at a temperature of 26° C (range ± 1° C). The photoperiod regime was 13L : 11D, which corresponded to the photoperiod during the reproductive period in the fish's natural home range (Trewavas, 1983). The switch between the light and dark period was rapid.

EXPERIMENTAL PROTOCOL

Study 1

In order to standardize all measurements, males ($n = 19$, mean ± s.d. body mass, M, 56·9 ± 14·7 g and standard length, L_S, 12·4 ± 1·2 cm) were caught from the stock fish and

individually housed in 121 aquaria (experimental day 1), visually isolated from other males. Fish were kept in isolation during 7 days in which they were fed proportionally to their body mass (9 g $kg^{-0{\cdot}8}$ day^{-1} of pellet food) during the first 6 days. This was followed by 1 day fasting to avoid the interference of heat increment of feeding on oxygen consumption measurements. At experimental day 8, M and L_S were measured, and the fish were placed individually in chambers for oxygen consumption measurements. At experimental day 10, within 1 min after capture, a blood sample was taken and M was measured. All fish were returned to their original groups. Food pellets were made at the Department of Aquaculture Systems and Animal Nutrition in the Tropics and Subtropics, University of Hohenheim, Germany, and contained 42·0% crude protein, 9·9% crude lipid, 11·3% crude ash and 1 g of food pellets contained 20·3 kJ gross energy.

Study 2

A similar protocol was followed as for study 1 with the following adjustments: 1) at experimental day 1, 14 males (M, $55{\cdot}7 \pm 14{\cdot}9$ g and L_S, $12{\cdot}3 \pm 1{\cdot}2$ cm) were implanted with a silastic tube containing 11-KT and castor oil, and 13 males (M, $56{\cdot}0 \pm 16{\cdot}5$ g and L_S, $12{\cdot}6 \pm 1{\cdot}1$ cm) were given a silastic tube containing castor oil only before placing them in isolation; 2) activity measurements were carried out at experimental day 10; 3) blood samples were drawn and M was measured at experimental day 12, after which they were returned to their original groups.

INDIRECT CALORIMETRIC SYSTEM AND OXYGEN REGISTRATION

To study energy metabolism, an open flow-through respirometry system (Cech, 1990) was used, designed to record oxygen concentrations sampled from eight different respiration chambers at constant intervals. Respiration chambers were made from acrylic plastic (Perspex) (1·2 cm thick, dimensions $15{\cdot}4 \times 15{\cdot}4 \times 26{\cdot}2$ cm). An identical chamber containing no fish was used as a control to correct for possible consumption of oxygen by algae and bacteria. From a header tank with a constant water level, water was provided under constant pressure (gravity) to each chamber. Water flow to the chambers was restricted (Kristall microtip, Carl Roth GmbH, Germany) to *c.* 120 ml min^{-1}, and turnover time for the chambers was *c.* 51 min. Flow rates, measured by hand, remained constant for weeks. Flow rates were chosen so that the difference in oxygen concentration between the outflow of the control chamber and the chambers containing fish were generally in the range of 0·5–1·0 mg O_2 l^{-1} The water entering each chambers was also passed through an electronic flowmeter (Durchfluss-Impulsgeber POM Opto, Conrad Electronics, Germany), whose signal was converted and logged to a computer to control for blockages.

Water was returned to the header tank where it was continuously aerated and filtered over charcoal (Eheim, Germany). Water was gradually refreshed (turnover time *c.* 1 week) and air-conditioning of the room kept water at a temperature of 26° C (range ± 1° C).

Automated continuous flow sampling (eight electronically operated solenoid valves) was applied to obtain oxygen consumption measurements per chamber at 150 s intervals (three values per hour per chamber). To wash out the water sampled from the previous chamber, no oxygen measurements were taken during the first 147 s of sampling. During the last 3 s, three measurements were taken from the signal of the sensor (CellOx® fitted with stirrer type R2 300 in a through flow cell type D201 and attached to a dissolved oxygen meter type Oxi 197; WTW GmbH, Germany) averaged, and logged to a computer for further analyses.

CALCULATION OF OXYGEN CONSUMPTION RATE

To allow the fish to adapt to the respiration chambers, all calculations of oxygen consumption rate were based on values recorded on the second day of oxygen measurements, beginning at experimental day 8 of the studies at the start of the light phase at

0800 hours until the end of the dark phase. Metabolic rates were calculated using the method of Niimi (1978) which corrects for a time-lag due to washout delays caused by the volume of water in the respiration chamber (Steffensen, 1989; Cech, 1990). The commonly used allometric factor of 0·8 was used to correct oxygen consumption rates (r) for M [$rM^{-0·8}$ (mmol O_2 h^{-1} $kg^{-0·8}$)] (Job, 1969; Bishop, 1999; Speakman *et al*., 2002). None of the observed relationships between oxygen consumption rate and M differed significantly from the expected one (one sample t-tests, all NS).

All oxygen measurements were carried out on post-absorptive individuals (experimental day 8) and refer to routine metabolic rates (r_s) since no continuous activity measurements were carried out (Cech, 1990). *Oreochromis mossambicus* males spend most time motionless on the bottom of the chamber, and sometimes metabolic rates reach very low levels. Ginneken *et al*. (1997) argued that such low peaks in metabolism would not reliably represent r_s of *O. mossambicus* since such levels were not sustained (Fry, 1971). Therefore running averages were calculated of two consecutive measurements of oxygen consumption rate for each subject and all values in which two values had >10% difference were discarded. From these values the minimum value was selected to estimate r_s as resting routine metabolic rate (r_{sr}). The maximum routine metabolic rate (r_{sm}) was also selected. This value is lower than the 'activity metabolic rate' because males were not stressed to show maximum swimming activity for which a swim respirometer is required (Cech, 1990).

HORMONAL TREATMENT AND ANALYSES OF STEROIDS

Silastic tubes (internal diameter 1·6 mm, outer diameter 2·4 mm) were filled with 20 µl of 5 µg 11-KT per µl castor oil or with 20 µl castor oil only. Fish were anaesthetized in MS-222 (dilution 1 : 10 000) and a small incision was made in the abdomen through which the silastic implants were inserted. After insertion the cut was closed with glue. This procedure lasted *c*. 2 min per fish and all fish recovered quickly after placing them in aerated water. None of the fish died during the study. After treatment, the fish were individually housed for 1 week in 12 l aquaria, visually isolated from other males.

A 0·3 ml blood sample was drawn from the caudal vein with a 1 ml syringe (taking *c*. 1 min per male). Blood plasma was stored at −20° C until steroid extraction. Free, sulphate and glucuronide steroid fractions were extracted from 50 µl plasma using the methodology described in Scott & Vermeirssen (1994). Steroid residues were re-suspended in 1 ml assay buffer and stored again at −20° C until assayed for T and 11-KT. The radioimmunoassays and cross reactions for T and 11-KT are described in Scott *et al*. (1984) and Kime & Manning (1982). Intra-assay and interassay precision (CV) were 7·5 and 12·4% for T and 8·2 and 11·6% for 11-KT.

ACTIVITY MEASUREMENTS

In study 2, at the start of the experimental day 10, a transparent sheet was placed on top of each respiration chamber. These sheets had one horizontal line over the length and two lines over the width, to partition the chamber in six equal parts. Half hour focal observations were carried out in 2·5 h intervals, two times during the morning and two times during the afternoon. As a measurement of activity the number of times the head of the focal individual passed one of the lines was recorded. Observations were carried out from behind a blind.

STATISTICAL TREATMENT

In study 2, two data points were discarded from the analyses. In both cases the oxygen consumption measurements were invalid due to blockage of the flow regulators. Data were tested for normality by means of Kolgomorov–Smirnov and none of the distributions significantly deviated from the normal distribution (all $P > 0·10$), and therefore parametric statistics were applied (Pearson correlations and t-tests). All statistics were

calculated using the SPSS 11.0 package (SPSS Inc., Chicago, U.S.A). Two-sided P values were used. α was set to 0·05. Data are given as means ± S.E.

RESULTS

STUDY 1

Temporal patterns in oxygen consumption rate

During the day r_s slowly decreased and the lowest value ($1{\cdot}74 \pm 0{\cdot}09$ mmol $O_2\,h^{-1}\,kg^{-0{\cdot}8}$) was reached during the night-time [0420 hours; Fig. 1(a)], and r_{sr} amounted to $1{\cdot}46 \pm 0{\cdot}07$ mmol $O_2\,h^{-1}\,kg^{-0{\cdot}8}$. In 37% of the males r_{sr} occurred during the daytime. Routine metabolism reached highest levels in the morning just after switching on the light [0820 hours, $3{\cdot}48 \pm 0{\cdot}37$ mmol $O_2\,h^{-1}\,kg^{-0{\cdot}8}$; Fig. 1(a)]. The r_{sm} was $3{\cdot}94 \pm 0{\cdot}30$ mmol $O_2\,h^{-1}\,kg^{-0{\cdot}8}$ and occurred in all fish during the light period (74% of the males reached r_{sm} between 0800 and 1000 hours).

Androgen levels and oxygen consumption rate

Plasma levels of 11-KT were positively correlated with r_{sr} and r_{sm} (Fig. 2; partial correlations corrected for M: r_{sr}, $r = 0{\cdot}46$, $P = 0{\cdot}05$; r_{sm}, $r = 0{\cdot}63$, $P < 0{\cdot}01$). Plasma levels of T did not correlate significantly with metabolic rate (partial correlations corrected for M: $r < 0{\cdot}29$, all NS).

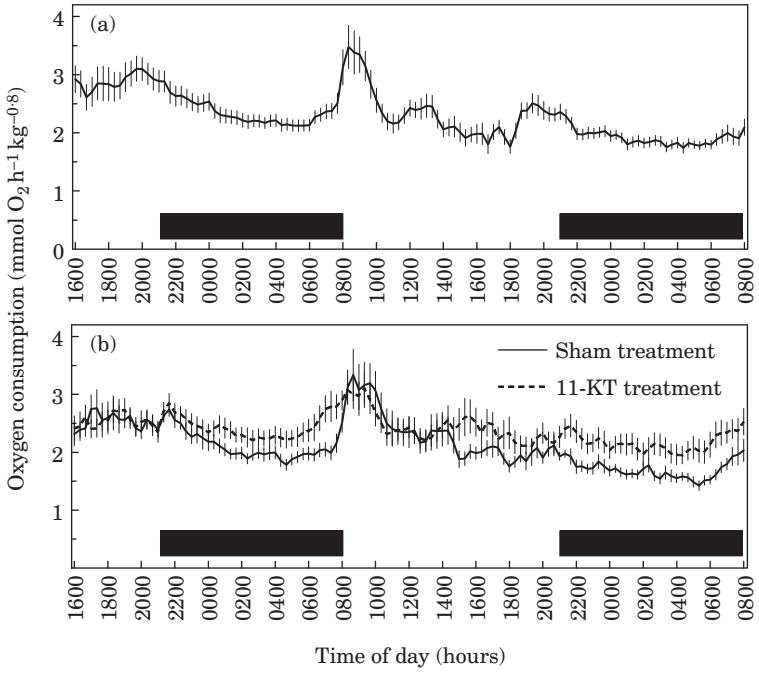

FIG. 1. Time course of oxygen consumption rate (mean ± S.E.) during the first 2 days of study (a) 1 and (b) 2. All metabolic rates are corrected for differences in metabolic body mass. ■, the dark period.

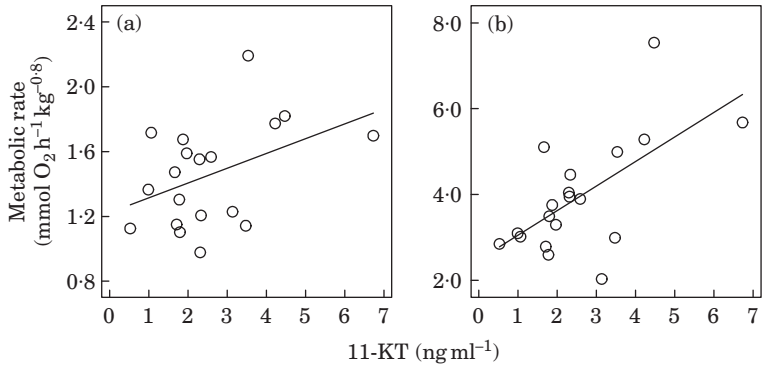

FIG. 2. The relationship between (a) resting (r_{sr}) and (b) maximum (r_{sm}) routine metabolic rate and plasma levels of 11-ketotestosterone. All metabolic rates are corrected for differences in metabolic body mass.

STUDY 2

Androgen levels and general activity

Treatment with 11-KT caused a significant increase in plasma levels of 11-KT (control males, $1{\cdot}34 \pm 1{\cdot}31$ ng ml^{-1}; 11-KT treated males, $2{\cdot}45 \pm 0{\cdot}83$ ng ml^{-1}; two sample *t*-test, d.f. $= 23$, $P = 0{\cdot}02$). Levels of 11-KT were within the range of endogenous levels that were measured in study 1 (range 0·5 to 6·7 ng ml^{-1}).

The mean level of activity during social isolation in sham treated males was 23 ± 31 events per 30 min and in 11-KT treated males, 28 ± 50 events per 30 min. This difference was not statistically significant [Mann–Whitney *U*-test, $P > 0{\cdot}05$.

Effects of 11-KT treatment on routine metabolism

Routine metabolism followed the same diurnal pattern as was found in study 1 (Fig. 1). The 11-KT treated males showed a comparable peak as sham treated males in r_s at the start of the day [Fig. 1(b), control: 0840 hours, $3{\cdot}48 \pm 0{\cdot}37$ mmol O_2 h^{-1} $kg^{-0·8}$; 11-KT: 0920 hours, $3{\cdot}11 \pm 0{\cdot}26$ mmol O_2 h^{-1} $kg^{-0·8}$], but it decreased less during the day and stayed relatively high during the night in 11-KT treated males compared to control males [Fig. 1(b); control: 0520 hours, $1{\cdot}42 \pm 0{\cdot}09$ mmol O_2 h^{-1} $kg^{-0·8}$; 11-KT: 0420 hours, $1{\cdot}95 \pm 0{\cdot}15$ mmol O_2 h^{-1} $kg^{-0·8}$]. Oxygen consumption rate started to increase 2 h before the lights were switched on.

As expected from study 1, r_{sr} was significantly higher in 11-KT treated individuals than in controls (Fig. 3; two-sample *t*-test, d.f. $= 23$, $P = 0{\cdot}02$), however, r_{sm} was not significantly different between the groups (Fig. 3; two-sample *t*-test, d.f. $= 23$, $P > 0{\cdot}05$).

DISCUSSION

Automated continuous measurement of oxygen consumption rate was used to study the relationship between energy metabolism and androgen hormones in male *O. mossambicus*. In the first study, endogenous blood plasma levels of 11-KT positively correlated with resting, and maximum routine metabolism.

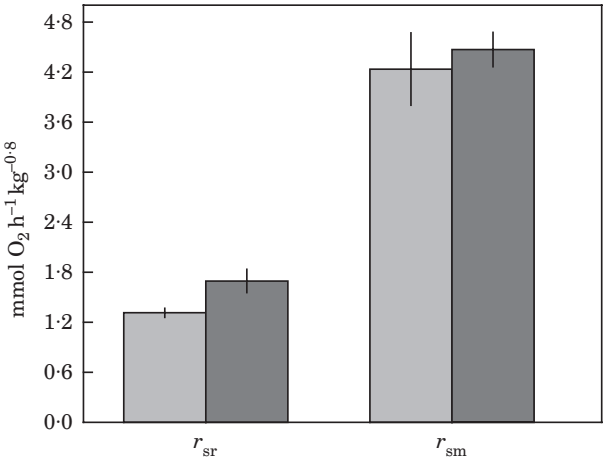

FIG. 3. The effect of 8 days experimental treatment on resting and maximum routine metabolic rate (r_{sr} and r_{sm}). The 11-KT treated group (■) was given a silastic implant filled with 11-KT and castor oil. The sham group (▒) was given a silastic implant filled with castor oil only.

In a second study, in which these relationships were tested by manipulating levels of 11-KT by applying implants, a positive effect of androgens on r_{sm}, was confirmed, but no effect of the hormone was found on r_{sm}. The studies might have been less suited for testing effects of androgens on r_{sm}, since the respiration chambers did not allow the highest metabolism of the males to be measured in a standardized way during maximum swimming performance. Therefore the highest routine metabolism during spontaneous activity in the chambers was selected. Androgens are expected to mediate changes in behavioural activity only in a social context (Nelson, 1999), and individual variation in spontaneous activity was found not to be related to the treatment with androgens.

Lowest but stable values of r_s were calculated from measurements in post-absorptive animals, preventing any effect of digesting food on the metabolic rates (Fry, 1971). The average level of r_{sr} in control males in the study was $1{\cdot}4 \pm 0{\cdot}1$ mmol $O_2\,h^{-1}\,kg^{-0{\cdot}8}$. Ginneken *et al.* (1996) found in *O. mossambicus* under comparable conditions to the present study (second day in respirometer) but at lower temperature (20° C *v.* 26° C in the present study), a value of $2{\cdot}8 \pm 0{\cdot}4$ mmol $O_2\,h^{-1}\,kg^{-1}$, in fish of unspecified sex of $25{\cdot}3 \pm 1{\cdot}3$ g, which would give a value of $1{\cdot}3 \pm 0{\cdot}2$ mmol $O_2\,h^{-1}\,kg^{-0{\cdot}8}$. Caulton (1978) studied r_s of *O. mossambicus* at a range of temperatures. When data were selected of comparable body mass (30–100 g) and temperature (25° C) as in the present study, an average r_s of $1{\cdot}5 \pm 0{\cdot}1$ mmol $O_2\,h^{-1}\,kg^{-0{\cdot}8}$ ($n = 6$; sex unspecified) was found. Both values from the literature overlap with the values for r_{sr} found in the present study. Also, r_{sr} levels are within the range of routine metabolism found by Becker & Fishelson (1986) in two related species of the genus *Oreochromis* [26° C, *O. aureus* (Steindachner) and *O. niloticus* (L.): 1·4 mmol $O_2\,h^{-1}\,kg^{-0{\cdot}8}$]. Maximum r_s levels (*c.* 4 mmol $O_2\,h^{-1}\,kg^{-0{\cdot}8}$) were in the range of those obtained from *O. mossambicus* that are swimming in a routine state of activity at a swimming

speed of *c.* two body lengths s^{-1} (*i.e.* 3·7 mmol $O_2\,h^{-1}\,kg^{-0·8}$; Job, 1969; Kutty, 1972). This level of activity is low in comparison to activity during agonistic interactions.

On average oxygen consumption rate was lowest at the end of the night period. This suggests that measuring oxygen consumption rate during the night period would be sufficient for obtaining r_{sr}. *Oreochromis mossambicus*, however, showed a very low oxygen consumption rate during the day (almost one-third of the animals had the lowest values during the daytime). As stressed before by Cech (1990), these data show the importance of performing continuous measurements of oxygen consumption rate during the night and day to assess the lowest values of metabolism. Furthermore, the data suggest that variation in endogenous levels of androgens might cause differences of *c.* 40% between the highest and lowest levels of r_{sr} measured. This might have consequences for estimating levels of r_s.

Reproductively active dominant males were selected for the study and therefore the selected animals probably had a high endogenous production of androgen. In study 2, levels of 11-KT were significantly higher in androgen treated animals than in sham-treated animals. Nevertheless, the average hormone levels were not higher than those of the untreated animals in study 1. This indicates that the increases in hormone levels due to the hormonal treatment were occurring within the physiological range. It furthermore suggests that the surgery carried out on all animals in the second study slightly suppressed the endogenous hormone production. Production of androgens may further have been suppressed due to the social isolation during the study. Levels of T observed in another study on *O. mossambicus* were about six times higher (6–13 ng ml^{-1}) than those in this study (Rocha & Reis-Henriques, 1996).

Resting routine metabolism correlated positively with plasma levels of 11-KT but not with T. In teleosts, a strong relationship has been found between social stimulation, aggressive behaviour and 11-KT, whereas such a strong relationship has not been found for T (Borg, 1994; Oliveira *et al.*, 2002). This suggests that in fishes 11-KT is the main androgen causing both peripheral effects on energy metabolism and central effects on aggressive behaviour.

The positive effect of 11-KT treatment on r_{sr} confirms the expectation that androgen levels enhance metabolic rates during rest. Metabolism measured during inactivity is usually equalled with basal metabolism, which is the minimum metabolism required for sustaining the critical physiological body functions (Fry, 1971; Cech, 1990). Metabolic rates measured during resting, however, may include physiology not related to maintenance, such as heat increment of feeding (specific dynamic action) and anabolic processes (*i.e.* growth). Androgens are known for their effects on anabolic processes (Tsai & Sapolsky, 1996), and this peripheral effect of androgens might support the central effects of androgens on aggressive behaviour and thereby the success in aggressive competition (Wingfield *et al.*, 1990; Oliveira *et al.*, 2002). In favour of such a relationship, a positive relationship has been found between r_s and dominance for teleosts (Clarke, 1992; Metcalfe *et al.*, 1995; Yamamoto *et al.*, 1998; Cutts *et al.*, 2001; McCarthy, 2001) and birds (Røskaft *et al.*, 1986; Högstad, 1987; Bryant & Newton, 1996), and between dominance, aggression and androgens (Oliveira, 1998).

The present results indicate a positive relationship between routine metabolism and the behaviourally active androgen hormone 11-KT, but not its precursor T. This supports the hypothesis that elevated levels of androgens are a costly trait (Wikelski *et al*., 1999; Buchanan *et al*., 2001). The functional significance of an androgen dependent increase in metabolism during inactivity should still be clarified. One possibility, congruent with the positive relationship between dominance and standard metabolism referred to above, is that it 'gears up' the animal in order to respond more rapidly to social challenges (Tsai & Sapolsky, 1996).

We are very grateful to E. Couto for running the radioimmunoassay and to P. Lawrence who constructed the respiratory system. RFO kindly provided the facilities for a sabbatical leave of KB. The research and the sabbatical leave were funded by a VW-Foundation grant, which also provided an assistant fellowship for AFHR to work within the scope of this grant. This study is part of a research grant funded to RFO by the Portuguese Foundation for Science and Technology – FCT (POCTI/BSE/38484/01). RFO's research is also supported by the Plurianual Program of FCT (R&D Unit 331/94). Procedures involving animals are in compliance with the 'Principles of animal care', publication No. 86-23, revised 1985 of the National Institute of Health, U.S.A., and with the current laws of Portugal.

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