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Costly steroids: egg testosterone modulates nestling metabolic rate in the zebra finch

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The transfer of non-genetic resources from mother to the offspring often has considerable consequences for offspring performance. In birds, maternally derived hormones are known to influence a variety of morphological, physiological and behavioural traits in the chick. So far, the range of these hormonal effects involves benefits in terms of enhanced growth and competitive ability as well as costs in terms of immunosuppression. However, since yolk hormones can enhance growth and begging activity, high levels of these hormones may also involve energetic costs. Here, we show experimentally that elevated levels of prenatal testosterone increase resting metabolic rate in nestling zebra finches (*Taeniopygia guttata*). Surprisingly, however, elevation of prenatal testosterone did not result in higher growth rates and, thus, differences in resting metabolism do not seem to be linked to nestling growth. We conclude that apart from immunosuppressive effects, high levels of egg steroids may also entail costs in terms of increased energy expenditure.

Keywords: maternal hormones; maternal effects; testosterone; metabolism; *Taeniopygia guttata*

1. INTRODUCTION

Maternal hormones can have a considerable impact on offspring phenotype in many vertebrate species (e.g. Clark & Galef 1995; McCormick 1999; Uller & Olsson 2003; Groothuis *et al.* 2005*a*). In oviparous species, hormones transferred from the mother to the egg vary remarkably within as well as among clutches (e.g. Conley *et al.* 1997; Janzen *et al.* 1998; McCormick 1999; Groothuis *et al.* 2005*a*). Recent studies on birds suggest that this variation may be maintained owing to a trade-off between beneficial and costly effects of egg hormones on offspring performance. Egg hormones promote pre- and postnatal growth (e.g. Groothuis *et al.* 2005*a*; Gil *et al.* 2006; but see Sockman & Schwabl 2000) as well as competitive ability of the chicks (e.g. Groothuis *et al.* 2005*a*; von Engelhardt *et al.* 2006), but they also negatively affect immune function of the offspring (Andersson *et al.* 2004; Navara *et al.* 2005; Groothuis *et al.* 2005*b*; Müller *et al.* 2005; but see Tschirren *et al.* 2005; Navara *et al.* 2006). However, since egg hormones increase growth and activity levels, they may not only entail costs in terms of immunosuppression, but also in terms of increased energy expenditure. It has previously been suggested that such costs would be manifested in higher metabolic rates in chicks hatching from eggs with high hormone levels (Eising *et al.* 2003; Gil 2003). The only study so far addressing this cost experimentally has not found any effects of egg hormones on chick metabolism (Eising *et al.* 2003). Neither resting metabolic rate nor daily energy expenditure was increased in precocial black-headed gull (*Larus ridibundus*) chicks that were exposed to elevated prenatal androgen levels. In the present study, we report data from an altricial passerine, the zebra finch (*Taeniopygia guttata*), showing a positive relationship between elevated prenatal testosterone levels and nestling resting metabolic rate.

2. MATERIAL AND METHODS

(a) *General*

For the experiment, we bred zebra finches in a captive population held in indoor facilities at Lund University, Sweden. Male and female zebra finches were paired randomly and housed in individual breeding cages (80×40×80 cm) with food (commercial seed mixture), water and cuttle bone provided ad libitum. A nest box and nesting material (coconut fibres and cotton wool) were supplied for breeding. Birds were maintained under constant temperature (20±2°C) and photoperiod (14 L : 10 D). Birds subjected to egg treatment and respirometry measurements (see below) were the offspring of 30 zebra finch pairs, which each contributed one clutch to the experiment.

(b) *Testosterone injections*

Eggs were collected on the day of laying, replaced with artificial ones and kept in an incubator at constant temperature (37°C) until day 4. This allowed us to monitor embryo survival and to discard infertile eggs. We used the same injection protocol as previously described in von Engelhardt *et al.* (2006), thereby manipulating the levels of egg testosterone (T) within the physiological range of the species. On day 3 of incubation, eggs were injected with either 500 pg T in 5 µl of sterile sesame oil (T-eggs) or 5 µl sterile sesame oil only (control eggs). On day 4, eggs were placed into foster nests. Whole clutches were randomly assigned to either T or control treatment and cross-fostered to randomly assigned nests. There was no significant difference in hatching success ($F_{1,29}=0.09$, $p=0.77$) or brood size ($F_{1,29}=0.90$, $p=0.35$) between the egg treatment groups.

(c) *Nestling growth and sexing*

As a measure of nestling growth, we used the increase in nestling body mass between hatching, day 10 and day 20 of the nestling period. Body mass was measured to the nearest 0.01 g. The sex of most nestlings was determined at the age of 40–50 days when they started to moult into sex-specific plumage. However, seven nestlings used in this experiment died before the age of 40 days. Molecular sex determination was used to determine the sex of those nestlings (Bradbury & Blakey 1998).

(d) *Resting metabolic rate*

Resting metabolic rate (RMR) was measured at 25°C in a four-channel open- circuit respirometer (see Nilsson & Råberg 2001 for technical details) when the nestlings were 14–15 days old.

(e) *Statistical analysis*

Statistical analyses were conducted with SAS v. 9.1 for Windows. When analysing the differences in RMR, we used linear mixed models (Proc Mixed; Littell *et al.* 2004) with egg treatment, sex and age as fixed factors, nest and respirometer channel as random factors and body mass as a covariate. To test for differences in growth rate, we used mixed model repeated measures ANOVA (Littell *et al.* 2004) with nest as random factor, egg treatment as fixed factor and individual identity as the subject. Random effects were estimated with the likelihood ratio test (Littell *et al.* 2004). Non-significant factors, covariates and their interactions ($p>0.1$) were sequentially removed from the models. The Sattherthwaite approximation was used to calculate the denominator degrees of freedom (Littell *et al.* 2004). All tests were two-tailed.

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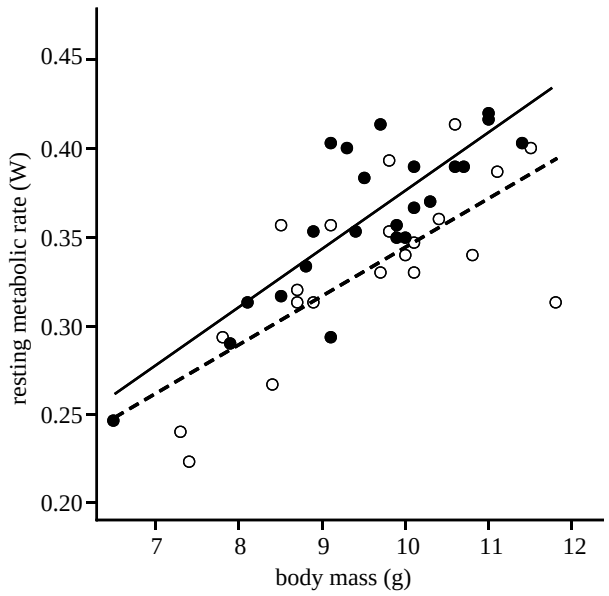

Figure 1. Relationship between body mass and RMR for offspring hatched from eggs with elevated testosterone levels (filled circles, solid line; $n=23$) and control offspring (open circles, broken line; $n=22$).

3. RESULTS

Offspring hatched from eggs with elevated T-levels had significantly higher RMR at 14–15 days of age than offspring hatched from control eggs (figure 1; table 1). The effect of egg treatment was independent of the sex or the exact age of the nestling. RMR was strongly influenced by individual body mass, but the effect of egg treatment on RMR appears not to be due to treatment-specific differences in growth rate. Growth rate from hatching to the age of 20 days did not differ between the two treatment groups (change in body mass over time by treatment interaction, effect of egg treatment, both $p>0.28$). No effect of sex on growth rate was found (change in body mass over time by sex interaction, effect of sex, both $p>0.35$). Moreover, body mass during RMR measurements (day 14 or 15) was not affected by egg treatment ($F_{1,38.8}=0.46$, $p=0.50$) or sex ($F_{1,27.5}=0.22$, $p=0.64$), and there was no significant interaction between sex and treatment ($F_{1,39.4}=0.91$, $p=0.35$).

Table 1. Linear mixed model analysis of RMR with egg treatment, age (it was not significant and hence removed from the model) and sex as fixed factors, nest and respirometer channel as random factors and body mass as a covariate. Non-significant interactions were sequentially removed from the model. For fixed effects, the test statistic is F; for random effects, it is χ^2.

factors	d.f.	F-/χ^2-value	p-value
egg treatment	1,26.5	6.84	0.015
body mass	1,30.3	88.75	<0.0001
sex	1,28.0	3.71	0.06
sex×body mass	1,30.5	3.55	0.07
nest		7.0	0.008
channel		3.7	0.054

4. DISCUSSION

RMR of 14–15 days old zebra finch chicks hatched from T-eggs was about 8% higher than that of chicks hatched from control eggs (figure 1). Thus, in contrast to the black-headed gull (Eising *et al.* 2003), high androgen levels seem to be associated with higher energy expenditure in the zebra finch.

The mechanism through which yolk testosterone can influence RMR in zebra finch chicks is not clear. Egg testosterone may affect RMR indirectly, through influences on nestling development rate. In contrast to previous studies on zebra finches (von Engelhardt *et al.* 2006), we found no effect of egg treatment on body mass or growth rate. It is therefore unlikely that differences in RMR are directly linked to nestling growth.

von Engelhardt *et al.* (2006) found that elevation of T in zebra finch eggs increased begging rate, at least in female offspring. Increased requirements for energy expenditure often translate into higher RMR (Nilsson 2002) and, thus, higher RMR in T-chicks may have resulted from higher levels of (begging) activity. Unfortunately, we have no data available on the begging activity of our chicks and, thus, we do not know whether T-chicks begged indeed longer or more intensely than controls.

Testosterone may also affect RMR more directly, inducing physiological changes in the nestlings. Such changes may include short- and/or long-term organizational effects of testosterone on the size or processing rate of internal organs potentially affecting RMR. Alternatively, egg hormone treatment may induce permanent differences in the ability to secrete hormones. We do not know whether egg treatment caused differences in circulating hormone levels in the nestlings, but higher blood levels of testosterone or related steroids may result in higher metabolic rates as has been found in adult birds and fishes (Buchanan *et al.* 2001; Ros *et al.* 2004). Moreover, testosterone is also known to increase anabolic processes at the cellular level (Tsai & Sapolsky 1996).

Our study clearly demonstrates that metabolic costs must be taken into account when referring to the trade-off between costs and benefits of maternal egg androgen levels. However, to be able to pinpoint the mechanisms and to explain variation between species in the effect of egg hormones on RMR, more studies are badly needed. Higher metabolic rates may not only be costly in terms of higher energy expenditure. The increase in metabolism in T-nestlings may be coupled with an overproduction of free oxygen radicals (Perez-Campo *et al.* 1998) and, thus, an increased risk of oxidative stress. Moreover, further studies are also needed to examine whether egg treatment may also involve long-term effects on offspring metabolic rate.

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