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. 2005a). 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. 2005a). 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. 2005a; 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. 2005b; 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 nestling 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 submitted 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 (F1.29 = 0.09, p = 0.77) or brood size (F1.29 = 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 Satterthwaite approximation was used to calculate the denominator degrees of freedom (Littell et al. 2004). All tests were two-tailed.
of T in zebra finch eggs increased begging rate, at
ling growth. It is therefore unlikely
offspring hatched from control eggs (figure 1). Thus, in
from T-eggs was about 8% higher than that of chicks
RMR of 14–15 days old zebra finch chicks hatched
4. DISCUSSION
Offspring hatched from eggs with elevated T-levels
or more intensely than controls.
Testosterone may also affect RMR more directly,
changes may include short- and/or long-term organiz-
nestlings, but higher blood levels of testosterone or
metabolic rate. 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 energy expenditure. The increase in metab-
olism in T-nestlings may be coupled with an over-
production of free oxygen radicals ( Perez-Campo
et al. 2001; Ros et al. 2004). Moreover, testosterone is
also known to increase anabolic processes at the
cellular level ( Tsai & Sapolsky 1996 ).

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Swedish Research Council. The experiment was approved
by the Malmo ¨/Lund ethical committee for animal research.

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 χ².

<table>
<thead>
<tr>
<th>factors</th>
<th>d.f.</th>
<th>F-χ²-value</th>
<th>p-value</th>
</tr>
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<td>egg treatment</td>
<td>1,26.5</td>
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<td>body mass</td>
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<td>&lt;0.0001</td>
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<td>sex</td>
<td>1,28.0</td>
<td>3.71</td>
<td>0.06</td>
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<tr>
<td>sex×body mass</td>
<td>1,30.5</td>
<td>3.55</td>
<td>0.07</td>
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<td>nest</td>
<td>7.0</td>
<td>0.008</td>
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<tr>
<td>channel</td>
<td>3.7</td>
<td>0.054</td>
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</table>

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

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).
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The effect of egg treatment was independent of the sex or the exact age of the nesting. 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). More-
over, body mass during RMR measurements (day 14
or 15) was not affected by egg treatment (F1,38.8 =
0.46, p = 0.50) or sex (F1,27.5 = 0.22, p = 0.64), and
there was no significant interaction between sex and
treatment (F1,30.4 = 0.91, p = 0.35).

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 nesting 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 nest-
ling growth.

von Engelhardt et al. (2006) found that elevation
of T in zebra finch eggs increased begging rate, at

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).


