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Sex-specific effects of prenatal testosterone on nestling plasma antioxidant capacity in the zebra finch

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SUMMARY

Trans-generational transfer of non-genetic, maternal resources such as hormones can have a substantial influence on offspring phenotype in many vertebrate species. In birds, maternal androgens enhance both growth and competitive behaviour, but also suppress the immune system. It has been hypothesised that high levels of egg androgens could also influence the prooxidant–antioxidant balance through their positive effect on growth and metabolism. We tested this hypothesis in the zebra finch (*Taeniopygia guttata*). Eggs were injected with testosterone dissolved in sesame oil or sesame oil only (control). We subsequently assessed the effect of the egg hormone manipulation on nestling growth and nestling plasma total antioxidant capacity (TAC). Growth rates of zebra finch nestlings were not significantly affected by egg hormone treatment. However, male offspring hatched from eggs with experimentally elevated testosterone had reduced plasma TAC at 10 days of age compared with male offspring hatching from control eggs. At the age of 34 days, males had similar plasma TAC irrespective of egg treatment. No effects of egg testosterone manipulation on nestling plasma TAC were found in females. Our results demonstrate that embryonic exposure to elevated levels of testosterone modulates chick antioxidant status, but this seems to be independent of chick growth. Sex-specific effects of prenatal testosterone on plasma TAC of zebra finch nestlings may have important consequences for sex allocation.

Key words: maternal effects, antioxidants, hormones, early development.

INTRODUCTION

Understanding the mechanisms that contribute to variation in offspring phenotype is crucial for the understanding of life history strategies and evolutionary processes (Stearns, 1992). Variation in offspring phenotype can be attributed to differences in genetic as well as non-genetic influences. One important non-genetic factor affecting the formation of the offspring phenotype is trans-generational transfer of maternal resources. Maternal resources such as nutrients, antibodies or hormones, can have a substantial impact on morphological, physiological and behavioural traits of the offspring (Mousseau and Fox, 1998). Maternally derived hormones in particular, have been identified as important mediators of offspring growth and behaviour in many vertebrate species (e.g. Schwabl, 1993; Clark and Galef, 1995; McCormick, 1999; Rhen and Crews, 2002).

Avian mothers convey significant quantities of androgens to their eggs, which are known to positively influence embryonic development, post-natal growth and competitive behaviour of the offspring (for a review, see Groothuis et al., 2005a). However, there is large inter-female variation in the amount of androgens transferred to the eggs. Recent studies suggest that this variation may be maintained because the positive effects on growth and behaviour are counterbalanced by negative effects on the immune function of the offspring (e.g. Groothuis et al., 2005b). Both the T-cell mediated and the humoral immune system of young birds seem to be negatively influenced by high yolk androgen levels (Andersson et al., 2004; Navara et al., 2005; Groothuis et al., 2005a; Groothuis et al., 2005b) [but see also Tschirren et al. and Navara et al. (Tschirren et al., 2005; Navara et al., 2006)]. It has been argued that there may be a trade-off between growth and immune function because both are energetically costly (Müller et al., 2005).

In addition to the negative effects on immune function, it has been hypothesized that high amounts of maternally derived egg steroids also may involve costs in terms of increased susceptibility to oxidative stress as a consequence of accelerated growth (Royle et al., 2001; Groothuis et al., 2006; Martin and Schwabl, 2008). This hypothesis assumes that enhanced offspring development rate mediated by egg steroids is associated with increased cell metabolism and a concomitant increase in the production of reactive oxygen species (ROS) (see Martin and Schwabl, 2008). ROS, which are produced during normal metabolic processes by the mitochondria, can have severe cytotoxic effects as they can oxidize macromolecules such as proteins or DNA (e.g. Finkel and Holbrook, 2000; Fang et al., 2002).

Enzymatic antioxidants such as superoxide dismutase as well as dietary antioxidants such as the vitamins E and C scavenge ROS and protect the organism from oxidative damage (Finkel and Holbrook, 2000; Barja, 2004). However, when there is an overproduction of ROS in relation to the antioxidant defences available this can result in oxidative stress (Finkel and Holbrook, 2000). In birds, only one study has directly assessed the effect of accelerated growth on the prooxidant–antioxidant system. Alonso-Alvarez et al. (Alonso-Alvarez et al., 2007a) found that high nestling growth rate in the zebra finch (*Taeniopygia guttata* Vieillot 1817) was associated with increased susceptibility of red blood cells to oxidative damage. This is consistent with the above hypothesis that an increase in growth rate mediated by egg androgens could potentially lead to changes in the prooxidant–antioxidant balance.

A recent study on zebra finches has further shown a positive effect of experimentally elevated yolk testosterone levels on nestling resting metabolic rate (Tobler et al., 2007). Assuming a positive

link between oxygen consumption and ROS production, such as has been found in humans (Loft et al., 1994), this would also support the idea that zebra finch young hatching from eggs with elevated testosterone might be more susceptible to oxidative stress. Some caution must be used, however, when relating metabolic activity directly to ROS production. Uncoupling proteins located in the mitochondrial membrane help reduce ROS production by altering membrane proton gradients (e.g. Brand, 2000; Speakman et al., 2004; Balaban et al., 2005), which means that the relationship between metabolism and ROS production is not a simple linear one. Nevertheless, it is suggestive that high levels of egg androgens may influence the prooxidant–antioxidant balance through their effect on growth and/or metabolism.

Although logically appealing, the hypothesis that high levels of egg androgens may influence the prooxidant–antioxidant balance has never been tested. In this study, we therefore experimentally manipulated testosterone levels in zebra finch (*Taeniopygia guttata*) eggs to investigate the effect of prenatal testosterone on the chicks' antioxidant defences, i.e. plasma total antioxidant capacity (TAC). The zebra finch is well suited to investigate this aspect given the previous work on this species (see above). Moreover, it has been shown that experimental elevation of yolk testosterone results in enhanced growth and begging of female offspring (von Engelhardt et al., 2006) (but see Tobler et al., 2007). If, as has been hypothesized, elevated levels of yolk testosterone would represent a cost in terms of increased susceptibility to oxidative stress, compensatory responses of the prooxidant–antioxidant system might be expected. Recent studies suggest that animals increase plasma antioxidant levels in response to increased ROS production to improve protection against oxidative damage (Barja 2004; Legatt et al., 2007; Cohen et al., 2008a; Cohen et al., 2008b). Therefore, we hypothesized that chicks hatching from eggs with elevated testosterone levels may have higher plasma levels of antioxidants compared with chicks hatching from control eggs.

MATERIALS AND METHODS

Study species and husbandry

We used a captive population of zebra finches 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 nest material (coconut fibres and cotton wool) were supplied for breeding. Birds were maintained under constant temperature (20±2°C) and photoperiod (14 h:10 h L:D). Birds subjected to egg treatment and blood-sampling were the offspring of 35 zebra finch pairs, which each contributed one clutch to the experiment.

Testosterone injections

During egg-laying, nests were checked every morning and freshly laid eggs were replaced with artificial ones. Collected eggs were kept in an incubator at constant temperature (37°C) until day 4. This allowed us to monitor embryo survival and to discard infertile eggs. On day 3 of incubation, eggs were injected with either 500 pg testosterone in 5 µl of sterile sesame oil (T eggs) or 5 µl sterile sesame oil only (control eggs) [see von Engelhardt et al. (von Engelhardt et al., 2006) for a detailed technical description of the injection method]. In zebra finches, females transfer more testosterone to their clutch when they are mated to an attractive male (Gil et al., 1999; von Engelhardt, 2004) and there is a concomitant increase in all eggs. The injection dose of testosterone used in our study corresponds approximately to the difference in testosterone + dihydrotestosterone measured in yolks of eggs from females paired with attractive males *versus* unattractive males and, thus, mimics a natural scenario (see also von Engelhardt et al., 2006). The hole in the egg shell was sealed with a tiny drop of superglue (Super Attak; Loctite Sweden AB, Göteborg, Sweden). All eggs within a clutch received the same treatment and clutches were randomly assigned to treatment groups. After injection, eggs were immediately placed back into the incubator. On day 4, eggs with live embryos were placed in foster nests. Whole clutches were randomly assigned to foster parents. The number of eggs that failed to hatch because of reasons other than infertility was similar in both treatment groups (19 control and 16 T eggs). Moreover, the frequency of hatching failures not due to infertility was not significantly different between the treatment groups (χ^2=0.43, d.f.=1, P=0.51). There was also no significant difference in the number of hatched chicks between the treatment groups [$F_{1,33}$=0.74, P=0.40; T broods: 2.5±0.2 chicks (mean ± 1 s.e.m.), control broods: 2.3±0.2 chicks].

Nestling growth

Eggs within a clutch were marked individually on the day of laying. On the day of hatching, we marked offspring dorsally with nontoxic color pens and if possible identified from which egg a chick hatched. In cases where two or three chicks hatched synchronously and we could not determine the exact egg numbers, we use averages between the possible egg numbers (e.g. 1.5 for a chick from either first or second egg). At the age of 8–11 days chicks were banded with aluminium rings. Nestling body mass was measured to the nearest 0.01 g on days 1 (hatching day), 10 and 34 post hatch. Day 10 represents a period of intensive growth and at this stage most nestlings have obtained more than 50% of their final body mass. At day 34 nestlings have almost reached their final body mass and start to become independent from the parents. For the periods 1–10 and 11–34 days of age we computed the growth rate as the increment in body mass (body mass 2 minus body mass 1).

Assessment of nestling antioxidant capacity

We collected a blood sample (~40 µl) from the brachial vein at day 10 and again from the jugular vein (~90 µl) at day 34 within 3 min of removal of the bird from the cage. All blood samples were centrifuged at 1000 ***g*** and plasma was stored at –50°C until further analysis. Plasma TAC of zebra finch chicks was measured using a commercial test kit (Abel®-41M2) purchased from Knight Scientific Limited (Plymouth, UK; http://www.knightscientific.com). Abel®-41M2 is a chemiluminescent test that allows the assessment of the TAC of a test sample (e.g. plasma) to prevent oxidation by peroxynitrite, which has high oxidant potential and occurs naturally in inflammatory cells, such as neutrophils and macrophages.

The basic principle of the test is as follows. The test uses the photoprotein Pholasin®, which emits light in the presence of free radicals, other reactive oxygen species and peroxidase enzymes. Antioxidants in the sample under test compete with Pholasin® for the reactive oxygen species peroxynitrite ($ONOO^-$) which is produced in the assay by the reaction between superoxide and nitric oxide, released simultaneously and continually from a 2.5 mmol l^{-1} solution of 3-morpholino-sydnonimine HCl ($C_6H_{10}N_4O_2$ HCl; SIN-1). In the absence of other antioxidants, Pholasin® emits light with gradually increasing intensity, reaching a peak after a few minutes. If there are antioxidants in the sample, they will compete with the Pholasin® for the peroxynitrite and this will delay the time at which the peak luminescence occurs. The more peak luminescence is delayed, the higher is the TAC of the test sample.

Samples were run in duplicates on 96-well microplates provided with the test kit. 5 µl samples (zebra finch plasma) were dissolved in 95 µl assay buffer per well, then, 50 µl Pholasin® was pipetted into each well. The plates were then transferred to a BMG-labtechnologies Lumistar Galaxy microplate luminometer and incubated for 5 min at 30°C. After the incubation period, 50 µl of 2 mg ml^{-1} SIN-1 solution was injected into each well using an automatic dispenser. The plates were then read in the luminometer at 38-s intervals for 69.7 min (110 cycles) at 30°C. Each plate contained two blanks (Pholasin® and SIN-1 only) and five duplicates of a diluted standard using vitamin E analogue (VEA) 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid. The time until peak luminescence (i.e. TAC) for the test sample is then expressed in VEA equivalent units µmol l^{-1}, which can be directly derived from the linear regression formula of the standard curve making it possible to compare samples between plates. The two samples from the same individual were run on the same plate. The mean of the duplicate values expressed in standard VEA equivalent units µmol l^{-1} was used in the statistical analyses. Intra- and inter-assay variation was 2% and 13%, respectively.

Data analysis and sample sizes

Data were analysed using mixed models in SAS System for Windows 9.1 (PROC MIXED) (Litell et al., 2004). Nest was included in all models as a random factor to control for non-independence of nestlings sharing the same genetic parents as well as the same rearing environment. The original models for growth rate analyses contained the following fixed effects: egg treatment, nestling sex and mean brood size and all two-way interactions. Mean brood size was calculated as the mean number of nestlings between days 1 and 10, and between days 11 and 34. The original models for analyses with plasma TAC further included both the growth rate during the 1–10 days of age period as well as growth rate during the 11–34 days of age period. The effect of egg number and respective interactions with egg treatment, nestling sex and brood size were originally included in all models to test for effects of laying order. However, the effect of egg number was not significant in any of the models (P>0.21 in all cases) and it was therefore excluded from all models. Initial full models were reduced in a stepwise backward procedure removing non-significant terms (P>0.1) subsequently from the models. Random factors were estimated with the likelihood ratio test as described by Littell et al. (Littell et al., 2004). The Satterthwaite approximation was used to calculate the denominator degrees of freedom in all models (Littell et al., 2004). Residuals were tested for normality and homoscedasticity. VEA units were log-transformed to normalise the data. The significance level was set at P<0.05.

The data set includes measures from 30 T (15 males and 15 females) and 36 control (21 males and 15 females) offspring. However, sample sizes varied between analyses because we could not obtain the correct hatchling masses of nine chicks (3 T males, 3 T females and 3 control males) owing to simultaneous hatching or hatching during late afternoon/evening. We further failed to measure body mass of five 10-day old chicks (1 T male, 2 control males, 2 control females). Three chicks (1 T male and 2 control females) were not blood sampled on day 10 because they were considered too small.

RESULTS

Nestling growth

Hatchling mass of zebra finch chicks did not differ between egg treatment groups [egg treatment: $F_{1,46.2}$=0.44, P=0.51; sex: $F_{1,40.9}$=0.96, P=0.33; egg treatment by sex interaction: $F_{1,46.8}$=2.71, P=0.11; LSM (± s.e.m.) 0.86±0.03 g and 0.84±0.03 g for the egg treatment and the control group]. Growth rate between 1 and 10 days was not significantly affected by egg treatment, sex or brood size (F<1.55, d.f.≥1,26, P>0.21 in all cases; 6.80±0.30 g and 6.67±0.26 g for the egg treatment and control group respectively; 6.50±0.25 g and 6.96±0.28 g for males and females). Growth rate between 10 and 34 days of age was negatively influenced by brood size (parameter estimate ± s.e.m.: –0.72±0.29; $F_{1,33.3}$=6.04, P=0.019), but was unaffected by egg treatment and sex (F<2.75, d.f.≥1,32, P>0.10 for both factors and their interaction; 4.89±0.32 g and 4.46±0.30 g for the egg treatment and control group, respectively; 4.87±0.25 g and 4.47±0.26 g for males and females). Early growth during the first 10 days of age was negatively correlated with growth during days 10–34 (–0.48±0.09, $F_{1,37.7}$=26.5, P<0.001). This means that nestlings with a high growth rate during the first 10 days of their life had slower growth afterwards, whereas nestlings with low growth rate during the first 10 days showed accelerated growth later. Body mass at the age of 34 days did not differ between egg treatment groups or sexes (F<2.80, d.f.≥1,34, P>0.10 in both cases; 12.61±0.27 g and 11.99±0.25 g for T and control offspring; 12.37±0.23 g and 12.22±0.24 g for males and females).

Nestling antioxidant capacity

The effect of egg treatment on plasma TAC of 10-day-old nestlings depended on nestling sex as shown by the significant interaction (Fig. 1, Table 1). Male, 10-day-old nestlings hatched from testosterone-treated eggs had lower plasma TAC than nestlings hatched from control eggs ($F_{1,19.9}$=4.54, P=0.046), whereas this effect was not apparent in females ($F_{1,15.1}$=0.90, P=0.36) (Fig. 1). Also, in T offspring, plasma TAC on day 10 was significantly higher in females than in males ($F_{1,14.7}$=6.03, P=0.027), whereas no such difference was found for control offspring ($F_{1,28.9}$=1.93, P=0.18; Fig. 1). Growth rate for the 1–10 day period was not related to plasma TAC at day 10 ($F_{1,45.3}$=0.29, P=0.60). Plasma TAC of 34-day-old nestlings was positively, albeit weakly, correlated to the growth rate during the 10–34-day period (Table 1). None of the other variables included in the model (sex, egg treatment, brood size and growth rate during the 1–10-day period) explained a significant part of the

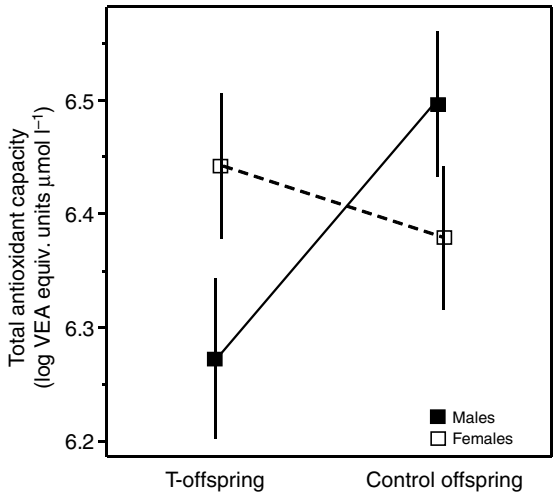

Fig. 1. Significant interaction of the egg hormone manipulation and sex on nestling plasma total antioxidant capacity 10 days after hatching (see also Table 1). Values are means ±1 s.e.m. for T-offspring (males, N=14; females, N=15) and control offspring (males, N=21; females, N=13).

Table 1. Summary of the fixed effects influencing nestling plasma total antioxidant capacity at the age of 10 and 34 days

Parameters	Estimate ± s.e.m.	d.f.	F/χ^2	P
TAC day 10				
Egg treatment	0.07±0.1	1,29.4	1.44	0.24
Sex	0.14±0.09	1,49.3	0.01	0.93
Egg treatment × sex	–0.28±0.13	1,49.6	5.23	0.026
Brood size	0.10±0.04	1,28.6	3.40	0.076
Nest	–	1	5.5	0.010
TAC day 34				
Growth rate	0.06±0.02	1,45.9	7.36	0.009
Nest	–	1	2.3	0.044

TAC, total antioxidant capacity.
Nest was included as a random factor in the models. Non-significant effects (P>0.1) were backward eliminated from the model and are thus not included in the table. For fixed effects, the test statistic is F; for random effects, it is χ^2 (see Materials and methods).

variation in nestling plasma TAC at day 34 (F<2.02, d.f.≥1,28, P>0.16 in all cases; VEA units 840.5±54.1 and 825.0±46.0 for T and control males, 786.2±53.1 and 758±51.8 for T and control females, respectively).

DISCUSSION

We found that experimental elevation of egg testosterone levels negatively affected plasma total antioxidant capacity of zebra finch nestlings. However, the effect of the experimental manipulation was sex and age specific. Elevated levels of yolk testosterone reduced plasma TAC in male but not female nestlings at the age of 10 days. At the age of 34 days, i.e. the stage when the offspring become independent from the parents, males and females had similar blood levels of antioxidants, irrespective of egg treatment.

Effects of egg treatment

Differences in antioxidant status between the treatment groups at day 10 do not appear to be linked to nestling growth. Zebra finch nestlings from both treatment groups had similar growth rates. This contrasts with an earlier study on zebra finches in which females profited from elevation of egg testosterone (with the same dose injected) in terms of enhanced growth and begging (von Engelhardt et al., 2006). Differences in the effect of egg testosterone manipulation on growth rates may be due to population differences in maternal egg androgens, as zebra finch females from our population appear to transfer somewhat higher amounts of testosterone to their eggs compared with the population used by von Engelhardt et al. (von Engelhardt et al., 2006) (M.S., unpublished data).

The sex-specific effect of egg treatment on nestling antioxidant capacity at day 10 seems not to be due to differential effects of elevated yolk testosterone on nestling metabolism. The positive effect of elevated yolk testosterone on nestling metabolism recently reported for zebra finches (Tobler et al., 2007) was independent of nestling sex. Thus, if plasma antioxidant levels were directly influenced by metabolic activity, both males and females would have been expected to show changes in plasma antioxidant levels. However, one problematic aspect when relating metabolic activity and ROS production is that this relationship appears not to be a simple (linear) one. Uncoupling proteins located in the mitochondrial membrane can lower ROS production by changing membrane proton gradients (Brand, 2000; Echtay et al., 2002; Speakman et al., 2004; Balaban et al., 2005), which results in reduced ATP production and higher energy requirements (e.g. Speakman et al., 2004; da Silva et al., 2008). Given this potential trade-off between ROS production and ATP production efficiency it will depend on the current energy requirements and the shape of the trade-off whether and how much animals will rely on antioxidant defense systems for ROS protection. Consequently, higher metabolic activity may not necessarily result in changes of plasma antioxidant levels. Notably, a recent comparative study on birds investigating the association between life-history variables and plasma antioxidants showed a relatively weak association between basal metabolic rate and plasma antioxidants (Cohen et al., 2008b).

A major component of circulating plasma TAC is uric acid, the main product of nitrogen catabolism in birds. Levels of circulating uric acid can vary depending on the amount of food ingested (e.g. Kolmstetter and Ramsay, 2000) and, thus, it could be argued that lower plasma TAC in male nestlings may result from lower food intake of these males. However, if this was the case, we would expect it to be translated into different growth rates which we did not observe.

It is probable that egg testosterone manipulation influenced the prooxidant–antioxidant balance more directly through other mechanisms than growth and metabolism. Egg treatment may have induced permanent physiological changes in male zebra finch chicks, which in turn negatively affected their antioxidant defences at the age of 10 days. Such changes may, for example, include differences in the ability to secret hormones. If egg hormone treatment were to result in consistently higher nestling blood levels of testosterone or related steroids this would probably affect plasma total antioxidant capacity. It has been shown that high levels of circulating testosterone impair antioxidant enzymes in rat and rabbit testicular tissues (Chainy et al., 1997; Aydilek et al., 2004) [but see Peltola et al. (Peltola et al., 1996)]. Moreover, high testosterone blood levels have recently been shown also to negatively influence red blood cell resistance to a free radical attack in adult zebra finch males (Alonso-Alvarez et al., 2007b). However, high levels of blood testosterone are also known to positively affect the levels of dietary antioxidants, i.e. carotenoids (Blas et al., 2006), possibly through direct effects of testosterone on lipoproteins or digestive enzymes, which regulate carotenoid availability (e.g. Woo et al., 1993; McGraw et al., 2006). Unfortunately, we did not measure circulating testosterone levels in the blood. It must also be noted that the link between yolk androgens and circulating, endogenous androgens in nestlings is not well established. Exposure to high levels of prenatal testosterone may also involve changes in availability and affinity of steroid hormone receptors on proteins regulating the prooxidant–antioxidant system and, thus, may not necessarily involve elevated levels of circulating testosterone.

How should the difference in plasma TAC of male nestlings at the age of 10 days be interpreted? It has been hypothesized that elevated levels of yolk testosterone could represent a cost for the offspring in terms of increased susceptibility to oxidative stress (see Introduction). Recent studies suggest that animals increase antioxidant levels as a compensatory response to increased ROS production (Barja 2004; Legatt et al., 2007; Cohen et al., 2008a; Cohen et al., 2008b). Therefore, if T offspring were exposed to higher levels of ROS, a concomitant increase in plasma antioxidant levels would be expected. However, we found that plasma TAC in male T nestlings was lower than in control male nestlings, which does not match with this expectation. We can only speculate about the potential causes and consequences of the difference in plasma TAC between testosterone and control male nestlings. One

possibility is that lowered antioxidant status in T males reflects depletion of antioxidant defences as a consequence of increased ROS production (see Alonso-Alvarez et al., 2007a). Alternatively, lowered antioxidant levels could also be a response to lowered ROS production, for example as a result of enhanced bioavailability of dietary carotenoids (see above). Thus, it is premature to conclude that reduced antioxidant capacity during the early nestling phase represents a viability cost to male offspring. Further studies are needed to establish the fitness consequences resulting from the differences in antioxidant status.

Differences between the sexes

The finding of a sex-specific effect of egg treatment on antioxidant status in 10-day-old zebra finch nestlings is not surprising given that previous work has demonstrated that prenatal androgens affect growth and begging sex specifically in this species (von Engelhardt et al., 2006) [but see Tobler et al. (Tobler et al., 2007)]. Moreover, other studies have shown male and female zebra finch chicks have different nutritional requirements during early development (Martins 2004; Arnold et al., 2007) and cope differently with sibling competition (De Kogel and Prijs, 1996; Bradbury and Blakey, 1998; Kilner, 1998). Thus, the sex-specific differences in plasma TAC due to egg testosterone treatment probably have different consequences in male and female offspring. Variation in egg testosterone levels may therefore be an important factor for sex allocation in this species (see von Engelhardt et al., 2006).

Interestingly, female zebra finches have been shown to transfer higher amounts of androgens to their eggs when paired with a more attractive male (Gil et al., 1999; von Engelhardt, 2004). Moreover, yolk antioxidants are also known to vary with male attractiveness. When paired to an attractive male, females transfer less antioxidants in first-laid eggs than females paired with an unattractive male, but more in later-laid eggs (Williamson et al., 2006). More attractive males may be more likely to sire offspring of high genetic quality or provide more benefits in terms of territory quality or parental care and, thus, father offspring of higher viability. Hence, it has been hypothesised that female zebra finches may fine tune development of individual offspring and sex ratio depending on the offspring's survival prospects by adjusting the relative amount of yolk androgens to the amount of yolk antioxidants (e.g. Royle et al., 2001; Groothuis et al., 2006; Williamson et al., 2006). Given the results from our study it is probable that maternal antioxidants play an important role in the modulation of egg steroid effects.

In conclusion, we provide evidence that prenatal exposure to high levels of testosterone influences the antioxidant defence system of the offspring, at least during the early developmental period. The finding of a sex-specific effect may have implications for avian sex allocation.

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