

## BRIEF COMMUNICATION

# High Yolk Testosterone Transfer Is Associated with an Increased Female Metabolic Rate

Barbara Tschirren<sup>1,\*</sup>  
 Ann-Kathrin Ziegler<sup>1</sup>  
 Cindy I. Canale<sup>1</sup>  
 Monika Okuliarová<sup>2</sup>  
 Michal Zeman<sup>2</sup>  
 Mathieu Giraudeau<sup>1,3</sup>

<sup>1</sup>Department of Evolutionary Biology and Environmental Studies, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland; <sup>2</sup>Department of Animal Physiology and Ethology, Faculty of Natural Sciences, Comenius University, 84215 Bratislava, Slovak Republic; <sup>3</sup>Centre for Ecology and Conservation, University of Exeter, Penryn, TR10 9FE Cornwall, United Kingdom

Accepted 5/21/2016; Electronically Published 7/1/2016

## ABSTRACT

Yolk androgens of maternal origin are important mediators of prenatal maternal effects. Although in many species short-term benefits of exposure to high yolk androgen concentrations for the offspring have been observed, females differ substantially in the amount of androgens they transfer to their eggs. It suggests that costs for the offspring or the mother constrain the evolution of maternal hormone transfer. However, to date, the nature of these costs remains poorly understood. Unlike most previous work that focused on potential costs for the offspring, we here investigated whether high yolk testosterone transfer is associated with metabolic costs (i.e., a higher metabolic rate) for the mother. We show that Japanese quail (*Coturnix japonica*) females that deposit higher testosterone concentrations into their eggs have a higher resting metabolic rate. Because a higher metabolic rate is often associated with a shorter life span, this relationship may explain the negative association between yolk testosterone transfer and female longevity observed in the wild. Our results suggest that metabolic costs for the mother can balance the short-term benefits of yolk testosterone exposure for the offspring, thereby contributing to the maintenance of variation in maternal yolk hormone transfer in natural populations.

**Keywords:** maternal effects, reproductive physiology, metabolic rate, trade-off, maintenance of variation, metabolic costs, yolk hormone transfer.

## Introduction

Maternally transferred yolk androgens are important mediators of prenatal maternal effects in birds and other oviparous species (Schwabl 1993). Many experimental studies have shown that exposure to high androgen concentrations before birth can have beneficial short-term effects on offspring, including an enhanced begging capacity or boosted growth (reviewed in Groothuis et al. 2005b; Gil 2008). This has raised the question of why not all females deposit high androgen concentrations into their eggs and how the large between-female variation in yolk androgen deposition is maintained in natural populations.

In an attempt to answer this question, researchers have mostly focused on potential costs of prenatal exposure to high androgen concentrations for the offspring, including immunosuppression (Groothuis et al. 2005a; Tschirren et al. 2005), metabolic costs (Eising et al. 2003; Tobler et al. 2007; Nilsson et al. 2011), or sexual antagonism (Ruuskanen et al. 2012; Tschirren 2015). The results of these studies are mixed. Costs associated with yolk androgen transfer for the mother, on the other hand, have received less attention (but see Pilz et al. 2003; Gil et al. 2006), even though the recent finding that female collared flycatchers (*Ficedula albicollis*) that deposit high testosterone concentrations into their eggs live shorter (Tschirren et al. 2014) indicates that direct costs for the mother may be substantial.

A negative association between resting metabolic rate (RMR) and life span is the cornerstone of the rate of living hypothesis (Pearl 1928), and evidence for such a relationship has been found among, as well as within, species (Ruggiero et al. 2008; but see, e.g., Duarte and Speakman 2014). If testosterone transfer to the eggs is associated with metabolic costs for the mother, this could provide a proximate explanation for why females that deposit high testosterone concentrations into their eggs live shorter. Here, we tested whether high maternal testosterone transfer is associated with an increased female metabolic rate in a precocial bird, the Japanese quail (*Coturnix japonica*), to explore whether metabolic costs for the mother may constrain the evolution of maternal yolk hormone deposition.

\*Corresponding author; e-mail: barbara.tschirren@ieu.uzh.ch.

## Material and Methods

### Study Population

The study was conducted in a population of Japanese quail (*Coturnix japonica*) maintained at the University of Zurich, Switzerland. Males and females were housed in separate outdoor aviaries (7 m × 5.5 m each). For reproduction, male-female pairs ( $N = 40$  pairs; age: 154–184 d) were transferred to cages (122 cm × 50 cm × 50 cm) within our breeding facility. Cages contained ad lib. food, water, grit, a source of calcium, a shelter, and a sand bath. The bottom of the cages was lined with sawdust. The breeding facility was kept on a 16L:8D cycle at  $20^\circ \pm 3^\circ\text{C}$  (see Pick et al. 2016 for details).

### Yolk Testosterone Analysis

We collected the fifth egg of each female on the day it was laid (natural clutch size: 7–14 eggs; Hoffmann 1988), separated yolk and albumen, weighed ( $\pm 0.01$  g) and homogenized the yolk, and froze it at  $-20^\circ\text{C}$  ( $N = 40$ ). Yolk testosterone (yolk T) extraction and radioimmunoassay were performed following previously published protocols (Okuliarová et al. 2011). In short, 100–110 mg of yolk was spiked with approximately 2,500 dpm of [ $^3\text{H}$ ]-testosterone (PerkinElmer) and extracted twice with a mixture of diethyl and petroleum ether. Yolk testosterone concentrations (pg/mg yolk) were quantified in 10  $\mu\text{L}$  aliquots using [1,2,6,7- $^3\text{H}$ ]-testosterone (PerkinElmer; specific activity 63.47 Ci/mmol) and a specific antibody generated in rabbits against testosterone-3-(carboxy-methyl) oxime bovine serum albumin conjugate (Zeman et al. 1986). The sensitivity of the assay was  $1.62 \pm 0.17$  pg per tube. The mean recovery rate  $\pm$  SD was  $79.3\% \pm 6.4\%$ . The samples were analyzed in two assays. The intra- and interassay coefficients of variation were 4.7% and 6.5%, respectively. Measured yolk testosterone concentrations were log transformed before analysis.

### Metabolic Rate

RMR of females ( $N = 40$ ) was measured over six consecutive nights (seven birds per night) in sealed plastic metabolic chambers (3.9 L, 234 mm × 165 mm × 165 mm; Lock & Lock, Hanacobi) during the birds' rest phase (6 p.m.–8:30 a.m.) in a dark room at  $25^\circ\text{--}28^\circ\text{C}$ , which is within the species' thermoneutral zone (Ben-Hamo et al. 2010). Food was withdrawn from the cages for 2 h before measurements started, in order to ensure a postabsorptive state. We measured the fractional content of oxygen and carbon dioxide in the air using an eight-channel open-flow respirometry system (Sable Systems, Las Vegas). Before each trial, the  $\text{CO}_2$  analyzer was zeroed using  $\text{CO}_2$ -free air (dry nitrogen, 99.99%  $\text{N}_2$ , PanGas) and spanned using a 1.002% mol  $\text{CO}_2$  mixture (balance  $\text{N}_2$ , PanGas). The  $\text{O}_2$  analyzer was spanned to 20.95% by flushing dry air through the system. During a trial, external air was pumped into the chambers (seven containing a bird and one empty control chamber) at a flow rate of 1,650–1,700 mL/min (dual-channel bench field

pump, 3.5 lpm maximal flow per channel; PP-2-1, Flow Bar mass flow meter FB-8-1, Sable Systems). All gas flow connections passed through ultra-low-permeability Tygon tube (i.d. 8 mm).

Each recording sequence lasted 45 min and consisted of one round of measurements of  $\text{O}_2$ ,  $\text{CO}_2$ , flow rate, and temperature for each of the seven chambers containing a bird. The empty control chamber was measured at the beginning and end of each sequence. Excurrent air from the chambers was pushed through a RM-8-2 respirometry multiplexer (Sable Systems) programmed to serially divert individual gas streams every 5 min. A subsample of each gas stream (250 mL/min) was pulled through a dessicant column (magnesium perchlorate, Sigma-Aldrich) before being analyzed every second over a 5-min period by a fuel cell  $\text{O}_2$  analyzer and a dual-wavelength infrared bench  $\text{CO}_2$  analyzer (Foxbox, Sable Systems). To avoid taking measurements during the drift period (i.e., warming up) of the Foxbox, we turned it on 4 h before the first recording started. Because of residual air in the system, we excluded the first 100 s of each 5-min measurement. In total, we obtained 18–20 200-s measurement sequences per bird during the course of the night.

Oxygen consumption rates ( $\dot{V}\text{O}_2$ , mL/min) were determined by comparing the oxygen content of the metabolic chamber containing birds ( $\text{Fe}$ ) and the empty control chamber ( $\text{Fi}$ , baseline). Baseline  $\text{O}_2$  and  $\text{CO}_2$  were determined by regressing all control chamber readings against time for each 45-min sequence.  $\dot{V}\text{O}_2$  was calculated using the following equation, which corrects for flow rate (FR) and  $\text{CO}_2$  concentration:  $\dot{V}\text{O}_2 = \text{FR} \times [(\text{FiO}_2 - \text{FeO}_2) - \text{FeO}_2 \times (\text{FeCO}_2 - \text{FiCO}_2)] / (1 - \text{FeO}_2)$ . We determined the mean of 60 consecutive seconds of lowest  $\dot{V}\text{O}_2$  within a range of variation of  $<0.015$  mL/min (i.e., threefold the standard deviation of the control chamber). RMR (W or J/s) was estimated from  $\dot{V}\text{O}_2$  and the respiratory exchange ratio (RER;  $\dot{V}\text{CO}_2/\dot{V}\text{O}_2$ ) using the thermal equivalence data in Withers (1992). The average RER was 0.70, indicating a predominance of lipid metabolism. The birds were weighed ( $\pm 0.1$  g) after the RMR measurement. Metabolic rate was measured after egg collection (see above). All females were in breeding condition (i.e., egg laying) and in the same stage of the breeding cycle when RMR was measured. All experiments conform to the relevant regulatory standards and were conducted under licenses provided by the Veterinary Office of the Canton of Zurich, Switzerland (195/2010, 14/2014, 156).

### Statistical Analysis

We tested for an association between yolk T concentration and female RMR using a linear mixed model that included female RMR and body mass as fixed effects. In addition, we ran the same model but with the residuals of a linear regression of RMR on body mass (residual RMR) as fixed effect. Because some of the females used in this study were sisters, we included family ID ( $N = 29$  families) as a random effect in the models. Residuals of the models were normally distributed.  $P$  values were obtained by comparing two nested models, with and without the variable of interest, using likelihood ratio

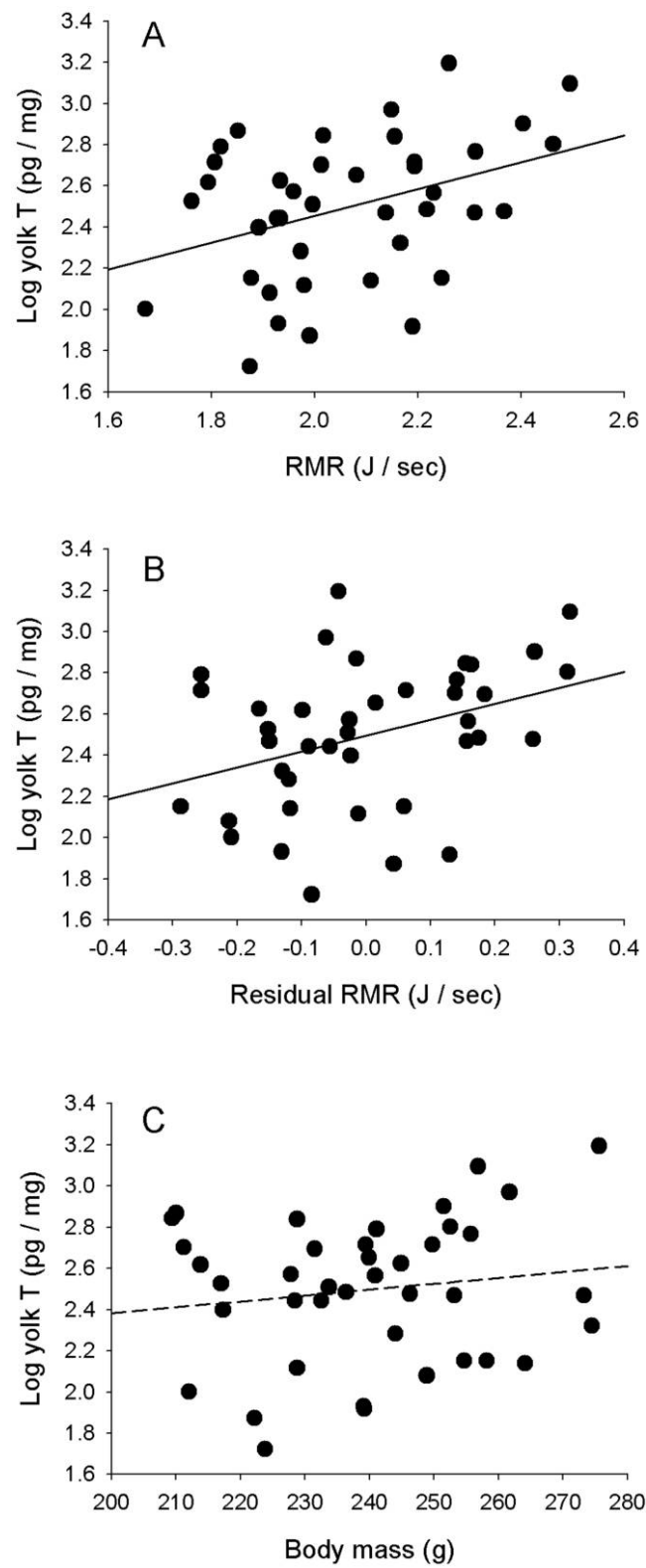


Figure 1. Relationship between testosterone concentrations in the eggs and a female's resting metabolic rate (RMR; A), the residuals of a regression of female RMR on body mass (residual RMR; B), and a female's body mass (C;  $N = 40$  females).

tests. All analyses were performed in R (R Development Core Team 2011).

## Results

We observed a significant positive association between the amount of testosterone a female transfers to her eggs and her RMR ( $\chi^2 = 5.496$ ,  $P = 0.019$ ; fig. 1A). Similarly, there was a positive association between yolk T and a female's residual RMR ( $\chi^2 = 5.243$ ,  $P = 0.022$ ; fig. 1B). Although there was a strong positive relationship between RMR and body mass ( $\chi^2 = 18.688$ ,  $P < 0.001$ ), no significant association between a female's body mass and the T concentration in her eggs was observed ( $\chi^2 = 0.423$ ,  $P = 0.516$ ; fig. 1C). The results were similar when analyzing yolk T content instead of yolk T concentration (yolk T content – mother RMR:  $\chi^2 = 6.781$ ,  $P = 0.009$ ; yolk T content – residual mother RMR:  $\chi^2 = 6.566$ ,  $P = 0.010$ ).

## Discussion

Our study shows that the transfer of high T concentrations to the eggs is associated with an increased female metabolic rate. If the increased RMR associated with high yolk T transfer affects a female's daily energy budget, which is likely (Buchanan et al. 2001), the resulting allocation trade-off may play a key role in balancing costs and benefits of differential maternal egg provisioning and contribute to the maintenance of variation in maternal yolk hormone transfer among females.

Currently, we can only speculate about the proximate mechanisms that underlie the observed relationship between yolk T concentrations and female metabolic rate. Components involved in the endocrine control of reproductive physiology could either directly or indirectly explain the association. For example, the production of testosterone in the theca and granulosa cells of the follicular wall (Hackl et al. 2003) or the transfer of testosterone from the site of production to the developing yolk might be energetically demanding. Alternatively, the observed association could be indirect, mediated, for example, by circulating T levels in the mother. Indeed, experimental studies have shown that high plasma T levels can lead to an increased metabolic rate in both birds (Buchanan et al. 2001) and men (Welle et al. 1992). However, this scenario is unlikely given that in quail maternal plasma T levels and T concentrations in the eggs appear to be unrelated (Hackl et al. 2003; Okuliarová et al. 2011). Finally, behavioral differences among females may indirectly affect both yolk testosterone transfer and metabolism (van Oers et al. 2011; Bouwhuis et al. 2014).

Irrespective of the exact physiological or behavioral mechanisms that link testosterone transfer to the egg and female metabolic rate, costs associated with a high metabolic rate (e.g., Ruggero et al. 2008; Dowling and Simmons 2009) may (at least partly) explain why females that transfer high yolk T concentrations to their eggs have a shorter life span (Tschirren et al. 2014). Ultimately, our study highlights that to understand the evolution of maternal effects as well as their mediators, it is crucial to consider the costs and benefits for all players involved.

## Acknowledgments

We thank the quail team for help with animal husbandry and data collection and three reviewers for comments on the manuscript. The study was supported by the Swiss National Science Foundation (PP00P3\_128386 and PP00P3\_157455) and the Fonds zur Förderung des akademischen Nachwuchses.

## Literature Cited

- Ben-Hamo M., B. Pinshow, M.D. McCue, S.R. McWilliams, and U. Bauchinger. 2010. Fasting triggers hypothermia, and ambient temperature modulates its depth in Japanese quail *Coturnix japonica*. *Comp Biochem Physiol A* 156: 84–91.
- Bouwhuis S., J.L. Quinn, B.C. Sheldon, and S. Verhulst. 2014. Personality and basal metabolic rate in a wild bird population. *Oikos* 123:56–62.
- Buchanan K.L., M.R. Evans, A.R. Goldsmith, D.M. Bryant, and L.V. Rowe. 2001. Testosterone influences basal metabolic rate in male house sparrows: a new cost of dominance signalling? *Proc R Soc B* 268:1337–1344.
- Dowling D.K. and L.W. Simmons. 2009. Reactive oxygen species as universal constraints in life-history evolution. *Proc R Soc B* 276:1737–1745.
- Duarte L.C. and J.R. Speakman. 2014. Low resting metabolic rate is associated with greater lifespan because of a confounding effect of body fatness. *Age* 36. doi:10.1007/s11357-11014-19731-11353.
- Eising C.M., G.H. Visser, W. Müller, and T.G.G. Groothuis. 2003. Steroids for free? no metabolic costs of elevated maternal androgen levels in the black-headed gull. *J Exp Biol* 206:3211–3218.
- Gil D. 2008. Hormones in avian eggs: physiology, ecology and behavior. *Adv Study Behav* 38:337–398.
- Gil D., A. Marzal, F. de Lope, M. Puerta, and A.P. Møller. 2006. Female house martins (*Delichon urbica*) reduce egg androgen deposition in response to a challenge of their immune system. *Behav Ecol Sociobiol* 60:96–100.
- Groothuis T.G.G., C.M. Eising, C. Dijkstra, and W. Müller. 2005a. Balancing between costs and benefits of maternal hormone deposition in avian eggs. *Biol Lett* 1:78–81.
- Groothuis T.G.G., W. Müller, N. von Engelhardt, C. Carere, and C. Eising. 2005b. Maternal hormones as a tool to adjust offspring phenotype in avian species. *Neurosci Biobehav Rev* 29:329–352.
- Hackl R., V. Bromundt, J. Daisley, K. Kotrschal, and E. Möstl. 2003. Distribution and origin of steroid hormones in the yolk of Japanese quail eggs (*Coturnix coturnix japonica*). *J Comp Physiol B* 173:327–331.
- Hoffmann E. 1988. *Coturnix* quail. Hsien, Taipei.
- Nilsson J.F., M. Tobler, J.A. Nilsson, and M.I. Sandell. 2011. Long-lasting consequences of elevated yolk testosterone for metabolism in the zebra finch. *Physiol Biochem Zool* 84: 287–291.

- Okuliarová M., T.G.G. Groothuis, P. Skrobánek, and M. Zeman. 2011. Experimental evidence for genetic heritability of maternal hormone transfer to offspring. *Am Nat* 177: 824–834.
- Pearl R. 1928. *The rate of living*. University of London Press, London.
- Pick J.L., P. Hutter, and B. Tschirren. 2016. In search of genetic constraints limiting the evolution of egg size: direct and correlated responses to artificial selection on a prenatal maternal effector. *Heredity* 116:542–549. doi:10.1038/hdy.2016.16.
- Pilz K.M., H.G. Smith, M.I. Sandell, and H. Schwabl. 2003. Interfemale variation in egg yolk androgen allocation in the European starling: do high-quality females invest more? *Anim Behav* 65:841–850.
- R Development Core Team. 2011. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. <http://www.R-project.org>.
- Ruggiero C., E.J. Metter, V. Melenovsky, A. Cherubini, S.S. Najjar, A. Ble, U. Senin, D.L. Longo, and L. Ferrucci. 2008. High basal metabolic rate is a risk factor for mortality: the Baltimore Longitudinal Study of Aging. *J Gerontol A* 63: 698–706.
- Ruuskanen S., B. Doligez, N. Pitala, L. Gustafsson, and T. Laaksonen. 2012. Long-term fitness consequences of high yolk androgen levels: sons pay the costs. *Funct Ecol* 26:884–894.
- Schwabl H. 1993. Yolk is a source of maternal testosterone for developing birds. *Proc Natl Acad Sci USA* 90:11446–11450.
- Tobler M., J.A. Nilsson, and J.F. Nilsson. 2007. Costly steroids: egg testosterone modulates nestling metabolic rate in the zebra finch. *Biol Lett* 3:408–410.
- Tschirren B. 2015. Differential effects of maternal yolk androgens on male and female offspring: a role for sex-specific selection? *PLoS ONE* 10:e0133673. doi:10.1371/journal.pone.0133673.
- Tschirren B., E. Postma, L. Gustafsson, T.G.G. Groothuis, and B. Doligez. 2014. Natural selection acts in opposite ways on correlated hormonal mediators of prenatal maternal effects in a wild bird population. *Ecol Lett* 17:1310–1315.
- Tschirren B., V. Saladin, P.S. Fitze, H. Schwabl, and H. Richner. 2005. Maternal yolk testosterone does not modulate parasite susceptibility or immune function in great tit nestlings. *J Anim Ecol* 74:675–682.
- van Oers K., K.L. Buchanan, T.E. Thomas, and P.J. Drent. 2011. Correlated response to selection of testosterone levels and immunocompetence in lines selected for avian personality. *Anim Behav* 81:1055–1061.
- Welle S., R. Jozefowicz, G. Forbes, and R.C. Griggs. 1992. Effect of testosterone on metabolic rate and body composition in normal men and men with muscular dystrophy. *J Clin Endocrinol Metab* 74:332–335.
- Withers P.C. 1992. *Comparative animal physiology*. Saunders College, Fort Worth, TX.
- Zeman M., J. Kosutzky, and E. Bobakova. 1986. Testosterone concentration in the seminal plasma of cocks. *Br Poult Sci* 27:261–266.