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

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Brief Communication

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High yolk testosterone transfer is associated with an increased female metabolic rate

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Running title: Metabolic costs of yolk testosterone transfer

What Is Already Known: Yolk hormones of maternal origin are important mediators or prenatal maternal effects in oviparous species. Within populations, there is large variation

in yolk hormone transfer observed among females, but the mechanisms that contribute to the maintenance of this variation are poorly understood.

What This Study Adds: Unlike most previous work that has focused on potential costs for the offspring, we here show that yolk hormone transfer is associated with metabolic costs for the mother. These costs for the mother can balance beneficial effects on offspring and thereby contribute to the maintenance of variation in maternal yolk hormone transfer in animal populations.

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 if 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 (RMR). Because a higher metabolic rate is often associated with a shorter lifespan, 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 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 lifespan 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 if high maternal testosterone transfer is associated with an increased female metabolic rate in a precocial bird, the Japanese quail (*Coturnix japonica*) to explore if metabolic costs for the mother may constrain the evolution of maternal yolk hormone deposition.

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 x 5.5 m each). For reproduction, male-female pairs (N = 40 pairs; age: 154-184 days) were transferred to cages (122 x 50 x 50 cm) within our breeding facility. Cages contained *ad libitum* 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 16 : 8 h light : dark cycle at 20 ± 3°C (see Pick et al. 2016 for details).

Yolk testosterone analysis

We collected the 5th egg of each female on the day it was laid (natural clutch size: 7-14 eggs (Hoffmann 1988)), separated yolk and albumen , weighed (± 0.01g) and homogenised the yolk, and froze it at –20°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 were spiked with approximately 2500 dpm of [^{3}H]-testosterone (PerkinElmer, USA) and extracted twice with a mixture of diethyl and petroleum ether. Yolk testosterone concentrations (pg / mg yolk) were quantified in 10µl aliquots using [1,2,6,7-^{3}H]-testosterone (PerkinElmer, USA, 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 ± 0.17 pg per tube. The mean recovery rate ± SD was 79.3 ± 6.4%. The samples were analyzed in two assays. The intra- and inter-assay coefficients of variation were 4.7% and 6.5%, respectively. Measured yolk testosterone concentrations were log transformed before analysis.

Metabolic rate

Resting 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 x 165 x 165mm, Lock & Lock, Hanacobi Co. Ltd., Korea) during the birds' rest phase (6pm – 8:30am) in a dark room at 25-28°C, which is within the species' thermoneutral zone (Ben-Hamo et al. 2010). Food was withdrawn from the cages for two hours before measurements started to ensure a post-absorptive 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, USA). Before each trial, the CO_2 analyzer was zeroed using CO_2-free air (dry nitrogen, 99.99% N_2, PanGas, Switzerland) and spanned using a 1.002 % mol CO_2 mixture (balance N_2, PanGas, Switzerland). The O_2 analyzer was spanned to 20.95% by flushing dry air through the system. During a

122 trial, external air was pumped into the chambers (seven containing a bird and one empty
123 control chamber) at a flow rate of 1650-1700 ml / min (Dual channel bench field pump,
124 3.5lpm maximal flow per channel, PP-2-1; Flow Bar Mass Flow Meter FB-8-1, Sable
125 System, USA). All gas flow connections passed through ultra-low permeability Tygon
126 tube (internal diameter: 8 mm).
127 Each recording sequence lasted 45 minutes and consisted of one round of measurements
128 of O_2, CO_2, flow rate and temperature for each of the seven chambers containing a bird.
129 The empty control chamber was measured at the beginning and the end of each sequence.
130 Excurrent air from the chambers was pushed through a RM-8-2 respirometry multiplexer
131 (Sable Systems International, Las Vegas, USA) programmed to serially divert individual
132 gas streams every five minutes. A subsample of each gas stream (250 ml / min) was
133 pulled through a dessicant column (magnesium perchlorate, Sigma-Aldrich, USA) before
134 being analyzed every second over a five minute period by a fuel cell O_2 analyzer and a
135 dual wavelength infrared bench CO_2 analyzer (Foxbox, Sable System, USA). To avoid
136 taking measurements during the drift period (i.e. warming up) of the Foxbox, it was
137 turned on 4 hours before the first recording started. Because of residual air in the system,
138 we excluded the first 100 seconds of each 5 min measurement. In total, we obtained 18-
139 20 200 sec measurement sequences per bird during the course of the night.
140 Oxygen consumption rates (VO_2, ml / min) were determined by comparing the oxygen
141 content of the metabolic chamber containing birds (Fe) and the empty control chamber
142 (Fi, baseline). Baseline O_2 and CO_2 were determined by regressing all control chamber
143 readings against time for each 45 minute sequence. VO_2 was calculated using the
144 following equation, which corrects for flow rate (FR) and CO_2 concentration: $VO_2 = FR$

145 * ((FiO$_2$ - FeO$_2$) - FeO$_2$ * (FeCO$_2$ - FiCO$_2$)) / (1 - FeO$_2$). We determined the mean of 60
146 consecutive seconds of lowest VO$_2$ within a range of variation of < 0.015 ml / min (i.e.,
147 three-fold the standard deviation of the control chamber). RMR (W or J / sec) was
148 estimated from VO2 and the respiratory exchange ratio (RER; VCO2 / VO2) using the
149 thermal equivalence data in Withers (1992). The average RER was 0.70, indicating a
150 predominance of lipid metabolism. The birds were weighed (± 0.1g) after the RMR
151 measurement. Metabolic rate was measured after egg collection (see above). All females
152 were in breeding condition (i.e. egg laying) and in the same stage of the breeding cycle
153 when RMR was measured.
154 All experiments conform to the relevant regulatory standards and were conducted
155 under licenses provided by the Veterinary Office of the Canton of Zurich, Switzerland
156 (195/2010; 14/2014; 156).
157
158 *Statistical analysis*
159 We tested for an association between yolk T concentration and female RMR using a
160 linear mixed model that included female RMR and body mass as fixed effects. In addition,
161 we ran the same model but with the residuals of a linear regression of RMR on body mass
162 (residual RMR) as fixed effect. Because some of the females used in this study were
163 sisters, we included Family ID (N = 29 families) as a random effect in the models.
164 Residuals of the models were normally distributed. *P* values were obtained by comparing
165 two nested models, with and without the variable of interest, using likelihood ratio tests.
166 All analyses were performed in R (R Development Core Team 2011).
167

168 **Results**

169 We observed a significant positive association between the amount of testosterone a
170 female transfers to her eggs and her RMR (χ^2 = 5.496, P = 0.019; Fig. 1A). Similarly,
171 there was a positive association between yolk T and a female's residual RMR (χ^2 = 5.243,
172 P = 0.022; Fig. 1B). Although there was a strong positive relationship between RMR and
173 body mass (χ^2 = 18.688, P < 0.001), no significant association between a female's body
174 mass and the T concentration in her eggs was observed (χ^2 = 0.423, P = 0.516; Fig. 1C).
175 The results were similar when analyzing yolk T content instead of yolk T concentration
176 (yolk T content – mother RMR: χ^2 = 6.781, P = 0.009; yolk T content – residual mother
177 RMR: χ^2 = 6.566, P = 0.010).
178
179 **Discussion**

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

186 Currently, we can only speculate about the proximate mechanisms that underlie
187 the observed relationship between yolk T concentrations and female metabolic rate.
188 Components involved in the endocrine control of reproductive physiology could either
189 directly or indirectly explain the association. For example, the production of testosterone
190 in the theca and granulosa cells of the follicular wall (Hackl et al. 2003) or the transfer of

191 testosterone from the site of production to the developing yolk might be energetically
192 demanding. Alternatively, the observed association could be indirect, mediated, for
193 example, by circulating T levels in the mother. Indeed, experimental studies have shown
194 that high plasma T levels can lead to an increased metabolic rate in both birds (Buchanan
195 et al. 2001) and men (Welle et al. 1992). However, this scenario is unlikely given that in
196 quail maternal plasma T levels and T concentrations in the eggs appear to be unrelated
197 (Hackl et al. 2003; Okuliarová et al. 2011). Finally, behavioral differences among
198 females may indirectly affect both yolk testosterone transfer and metabolism (van Oers et
199 al. 2011; Bouwhuis et al. 2014).
200 Irrespective of the exact physiological or behavioural mechanisms that link testosterone
201 transfer to the egg and female metabolic rate, costs associated with a high metabolic rate
202 (e.g. Ruggiero et al. 2008; Dowling and Simmons 2009), may (at least partly) explain
203 why females that transfer high yolk T concentrations to their eggs have a shorter lifespan
204 (Tschirren et al. 2014). Ultimately, our study highlights that to understand the evolution
205 of maternal effects as well as their mediators, it is crucial to consider the costs and
206 benefits for all players involved.
207

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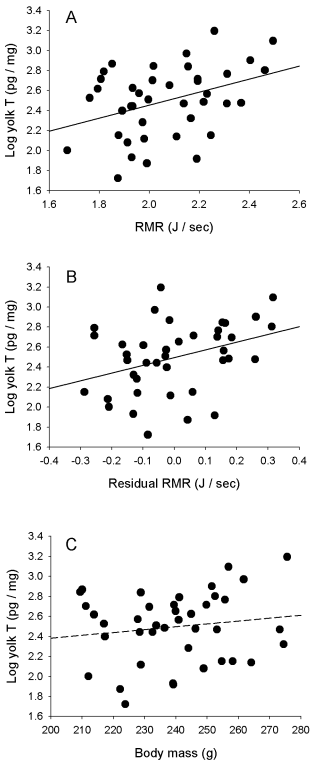
289 **Figure Legend**

290

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



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