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

Tschirren, Barbara ; Ziegler, Ann-Kathrin ; Canale, Cindy I ; Okuliarová, Monika ; Zeman, Michal ; Giraudeau, Mathieu

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.

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

4

5 Barbara Tschirren[a*], Ann-Kathrin Ziegler[a], Cindy I. Canale[a], Monika Okuliarová[b],

6 Michal Zeman[b] & Mathieu Giraudeau[ac]

7 [a]Department of Evolutionary Biology and Environmental Studies, University of Zurich,

8 Winterthurerstrasse 190, 8057 Zurich, Switzerland

9 [b]Department of Animal Physiology and Ethology, Faculty of Natural Sciences, Comenius

10 University, Bratislava, Slovak Republic

11 [c] Centre for Ecology and Conservation, University of Exeter, Penryn, TR10 9FE

12 Cornwall, UK

13

14 *Correspondence: Barbara Tschirren, Department of Evolutionary Biology and

15 Environmental Studies, University of Zurich, Winterthurerstrasse 190, 8057 Zurich,

16 Switzerland; email: barbara.tschirren@ieu.uzh.ch, phone: +41 44 635 47 77, Fax: +41 44

17 635 47 80

18

19 Running title: Metabolic costs of yolk testosterone transfer

20

21

22 **What Is Already Known:** Yolk hormones of maternal origin are important mediators or

23 prenatal maternal effects in oviparous species. Within populations, there is large variation

24   in yolk hormone transfer observed among females, but the mechanisms that contribute to

25   the maintenance of this variation are poorly understood.

26

27   **What This Study Adds:** Unlike most previous work that has focused on potential costs

28   for the offspring, we here show that yolk hormone transfer is associated with metabolic

29   costs for the mother. These costs for the mother can balance beneficial effects on

30   offspring and thereby contribute to the maintenance of variation in maternal yolk

31   hormone transfer in animal populations.

32

33 **Abstract**

34 Yolk androgens of maternal origin are important mediators of prenatal maternal effects.

35 Although in many species short-term benefits of exposure to high yolk androgen

36 concentrations for the offspring have been observed, females differ substantially in the

37 amount of androgens they transfer to their eggs. It suggests that costs for the offspring or

38 the mother constrain the evolution of maternal hormone transfer. However, to date the

39 nature of these costs remains poorly understood. Unlike most previous work that focused

40 on potential costs for the offspring, we here investigated if high yolk testosterone transfer

41 is associated with metabolic costs (i.e. a higher metabolic rate) for the mother. We show

42 that Japanese quail (*Coturnix japonica*) females that deposit higher testosterone

43 concentrations into their eggs have a higher resting metabolic rate (RMR). Because a

44 higher metabolic rate is often associated with a shorter lifespan, this relationship may

45 explain the negative association between yolk testosterone transfer and female longevity

46 observed in the wild. Our results suggest that metabolic costs for the mother can balance

47 the short-term benefits of yolk testosterone exposure for the offspring, thereby

48 contributing to the maintenance of variation in maternal yolk hormone transfer in natural

49 populations.

50

51 **Keywords:** maternal effects; reproductive physiology; metabolic rate; trade-off;

52 maintenance of variation; metabolic costs; yolk hormone transfer

53 **Introduction**

54 Maternally transferred yolk androgens are important mediators of prenatal maternal

55 effects in birds and other oviparous species (Schwabl 1993). Many experimental studies

56 have shown that exposure to high androgen concentrations before birth can have

57 beneficial short-term effects on offspring, including an enhanced begging capacity or

58 boosted growth (reviewed in Groothuis et al. 2005b; Gil 2008). This has raised the

59 question why not all females deposit high androgen concentrations into their eggs, and

60 how the large between-female variation in yolk androgen deposition is maintained in

61 natural populations.

62   In an attempt to answer this question, researchers have mostly focused on

63 potential costs of prenatal exposure to high androgen concentrations for the offspring,

64 including immunosuppression (Groothuis et al. 2005a; Tschirren et al. 2005), metabolic

65 costs (Eising et al. 2003; Tobler et al. 2007; Nilsson et al. 2011) or sexual antagonism

66 (Ruuskanen et al. 2012; Tschirren 2015). The results of these studies are mixed. Costs

67 associated with yolk androgen transfer for the mother, on the other hand, have received

68 less attention (but see Pilz et al. 2003; Gil et al. 2006), even though the recent finding that

69 female collared flycatchers (*Ficedula albicollis*) that deposit high testosterone

70 concentrations into their eggs live shorter (Tschirren et al. 2014) indicates that direct

71 costs for the mother may be substantial.

72 A negative association between resting metabolic rate (RMR) and lifespan is the

73 cornerstone of the rate of living hypothesis (Pearl 1928), and evidence for such a

74 relationship has been found among, as well as within species (Ruggiero et al. 2008; but

75 see e.g. Duarte and Speakman 2014). If testosterone transfer to the eggs is associated with

76 metabolic costs for the mother, this could provide a proximate explanation for why

77 females that deposit high testosterone concentrations into their eggs live shorter. Here,

78 we tested if high maternal testosterone transfer is associated with an increased female

79 metabolic rate in a precocial bird, the Japanese quail (*Coturnix japonica*) to explore if

80 metabolic costs for the mother may constrain the evolution of maternal yolk hormone

81 deposition.

82

83 **Material and Methods**

84 *Study population*

85 The study was conducted in a population of Japanese quail (*Coturnix japonica*)

86 maintained at the University of Zurich, Switzerland. Males and females were housed in

87 separate outdoor aviaries (7 x 5.5 m each). For reproduction, male-female pairs (N = 40

88 pairs; age: 154-184 days) were transferred to cages (122 x 50 x 50 cm) within our

89 breeding facility. Cages contained *ad libitum* food, water, grit, a source of calcium, a

90 shelter and a sand bath. The bottom of the cages was lined with sawdust. The breeding

91 facility was kept on a 16 : 8 h light : dark cycle at $20 \pm 3°C$ (see Pick et al. 2016 for

92 details).

93

94 *Yolk testosterone analysis*

95 We collected the 5th egg of each female on the day it was laid (natural clutch size: 7-14

96 eggs (Hoffmann 1988)), separated yolk and albumen , weighed ($\pm 0.01g$) and

97 homogenised the yolk, and froze it at $-20°C$ (N = 40). Yolk testosterone (yolk T)

98 extraction and radioimmunoassay were performed following previously published

99   protocols (Okuliarová et al. 2011). In short, 100-110 mg of yolk were spiked with

100   approximately 2500 dpm of [^{3}H]-testosterone (PerkinElmer, USA) and extracted twice

101   with a mixture of diethyl and petroleum ether. Yolk testosterone concentrations (pg / mg

102   yolk) were quantified in 10μl aliquots using [1,2,6,7-^{3}H]-testosterone (PerkinElmer,

103   USA, specific activity 63.47 Ci / mmoL) and a specific antibody generated in rabbits

104   against testosterone-3-(carboxy-methyl) oxime bovine serum albumin conjugate (Zeman

105   et al. 1986). The sensitivity of the assay was 1.62 ± 0.17 pg per tube. The mean recovery

106   rate $\pm$ SD was $79.3 \pm 6.4\%$. The samples were analyzed in two assays. The intra- and

107   inter-assay coefficients of variation were 4.7% and 6.5%, respectively. Measured yolk

108   testosterone concentrations were log transformed before analysis.

109

110   *Metabolic rate*

111   Resting metabolic rate (RMR) of females (N = 40) was measured over six consecutive

112   nights (seven birds per night) in sealed plastic metabolic chambers (3.9 l, 234 x 165 x

113   165mm, Lock & Lock, Hanacobi Co. Ltd., Korea) during the birds' rest phase (6pm –

114   8:30am) in a dark room at 25-28°C, which is within the species' thermoneutral zone

115   (Ben-Hamo et al. 2010). Food was withdrawn from the cages for two hours before

116   measurements started to ensure a post-absorptive state. We measured the fractional

117   content of oxygen and carbon dioxide in the air using an eight-channel open flow

118   respirometry system (Sable Systems, Las Vegas, USA). Before each trial, the CO_2

119   analyzer was zeroed using CO_2-free air (dry nitrogen, 99.99% N_2, PanGas, Switzerland)

120   and spanned using a 1.002 % mol CO_2 mixture (balance N_2, PanGas, Switzerland). The

121   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 ($\chi^2 = 5.496$, $P = 0.019$; Fig. 1A). Similarly,

171   there was a positive association between yolk T and a female's residual RMR ($\chi^2 = 5.243$,

172     $P = 0.022$; Fig. 1B). Although there was a strong positive relationship between RMR and

173   body mass ($\chi^2 = 18.688$, $P < 0.001$), no significant association between a female's body

174   mass and the T concentration in her eggs was observed ($\chi^2 = 0.423$, $P = 0.516$; Fig. 1C).

175   The results were similar when analyzing yolk T content instead of yolk T concentration

176   (yolk T content – mother RMR: $\chi^2 = 6.781$, $P = 0.009$; yolk T content – residual mother

177   RMR: $\chi^2 = 6.566$, $P = 0.010$).

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

213   **Literature Cited**

214   Ben-Hamo M., B. Pinshow, M.D. McCue, S.R. McWilliams, and U. Bauchinger. 2010.

215   Fasting triggers hypothermia, and ambient temperature modulates its depth in

216   Japanese quail *Coturnix japonica*. Comp Biochem Physiol A 156:84-91.

217   Bouwhuis S., J.L. Quinn, B.C. Sheldon, and S. Verhulst. 2014. Personality and basal

218   metabolic rate in a wild bird population. Oikos 123:56-62.

219   Buchanan K.L., M.R. Evans, A.R. Goldsmith, D.M. Bryant, and L.V. Rowe. 2001.

220   Testosterone influences basal metabolic rate in male house sparrows: a new cost

221   of dominance signalling? Proc R Soc B 268:1337-1344.

222   Dowling D.K. and L.W. Simmons. 2009. Reactive oxygen species as universal

223   constraints in life-history evolution. Proc R Soc B 276:1737-1745.

224   Duarte L.C. and J.R. Speakman. 2014. Low resting metabolic rate is associated with

225   greater lifespan because of a confounding effect of body fatness. Age

226   36:doi:10.1007/s11357-11014-19731-11353.

227   Eising C.M., G.H. Visser, W. Müller, and T.G.G. Groothuis. 2003. Steroids for free? No

228   metabolic costs of elevated maternal androgen levels in the black-headed gull. J

229   Exp Biol 206:3211-3218.

230   Gil D. 2008. Hormones in avian eggs: Physiology, ecology and behavior. Adv Study

231   Behav 38:337-398.

232   Gil D., A. Marzal, F. de Lope, M. Puerta, and A.P. Møller. 2006. Female house martins

233   (*Delichon urbica*) reduce egg androgen deposition in response to a challenge of

234   their immune system. Behav Ecol Sociobiol 60:96-100.

235   Groothuis T.G.G., C.M. Eising, C. Dijkstra, and W. Müller. 2005a. Balancing between

236   costs and benefits of maternal hormone deposition in avian eggs. Biol Lett 1:78-

237   81.

238   Groothuis T.G.G., W. Müller, N. von Engelhardt, C. Carere, and C. Eising. 2005b.

239   Maternal hormones as a tool to adjust offspring phenotype in avian species.

240   Neurosci Biobehav Rev 29:329-352.

241   Hackl R., V. Bromundt, J. Daisley, K. Kotrschal, and E. Möstl. 2003. Distribution and

242   origin of steroid hormones in the yolk of Japanese quail eggs (*Coturnix coturnix*

243   *japonica*). J Comp Physiol B 173:327-331.

244   Hoffmann E. 1988. Coturnix quail. Yi Hisien Publishing Co., Taipei.

245   Nilsson J.F., M. Tobler, J.A. Nilsson, and M.I. Sandell. 2011. Long-lasting consequences

246   of elevated yolk testosterone for metabolism in the Zebra finch. Physiol Biochem

247   Zool 84:287-291.

248   Okuliarová M., T.G.G. Groothuis, P. Skrobanek, and M. Zeman. 2011. Experimental

249   evidence for genetic heritability of maternal hormone transfer to offspring. Am

250   Nat 177:824-834.

251   Pearl R. 1928. The rate of living. University of London Press, London.

252   Pick J.L., P. Hutter, and B. Tschirren. 2016. In search of genetic constraints limiting the

253   evolution of egg size: direct and correlated responses to artificial selection on a

254   prenatal maternal effector. Heredity. doi: 10.1038/hdy.2016.16.

255   Pilz K.M., H.G. Smith, M.I. Sandell, and H. Schwabl. 2003. Interfemale variation in egg

256   yolk androgen allocation in the European starling: do high-quality females invest

257   more? Anim Behav 65:841-850.

258   R Development Core Team. 2011. R: A language and environment for statistical

259   computing.http://www.R-project.org.

260   Ruggiero C., E.J. Metter, V. Melenovsky, A. Cherubini, S.S. Najjar, A. Ble, U. Senin,

261   D.L. Longo, and L. Ferrucci. 2008. High basal metabolic rate is a risk factor for

262   mortality: The Baltimore Longitudinal Study of Aging. J. Gerontol. A 63:698-706.

263   Ruuskanen S., B. Doligez, N. Pitala, L. Gustafsson, and T. Laaksonen. 2012. Long-term

264   fitness consequences of high yolk androgen levels: sons pay the costs. Funct Ecol

265   26:884-894.

266   Schwabl H. 1993. Yolk is a source of maternal testosterone for developing birds. Proc

267   Natl Acad Sci U S A 90:11446-11450.

268   Tobler M., J.A. Nilsson, and J.F. Nilsson. 2007. Costly steroids: egg testosterone

269   modulates nestling metabolic rate in the zebra finch. Biol Lett 3:408-410.

270   Tschirren B. 2015. Differential effects of maternal yolk androgens on male and female

271   offspring: a role for sex-specific selection? PLoS ONE 10:e0133673.

272   doi:0133610.0131371/journal.pone.0133673.

273   Tschirren B., E. Postma, L. Gustafsson, T.G.G. Groothuis, and B. Doligez. 2014. Natural

274   selection acts in opposite ways on correlated hormonal mediators of prenatal

275   maternal effects in a wild bird population. Ecol Lett 17:1310-1315.

276   Tschirren B., V. Saladin, P.S. Fitze, H. Schwabl, and H. Richner. 2005. Maternal yolk

277   testosterone does not modulate parasite susceptibility or immune function in great

278   tit nestlings. J Anim Ecol 74:675-682.

279   van Oers K., K.L. Buchanan, T.E. Thomas, and P.J. Drent. 2011. Correlated response to

280   selection of testosterone levels and immunocompetence in lines selected for avian

281   personality. Anim Behav 81:1055-1061.

282   Welle S., R. Jozefowicz, G. Forbes, and R.C. Griggs. 1992. Effect of testosterone on

283   metabolic rate and body composition in normal men and men with muscular

284   dystrophy. J Clin Endocrinol Metab 74:332-335.

285   Withers P.C. 1992. Comparative animal physiology. Saunders College Pub., Fort Worth.
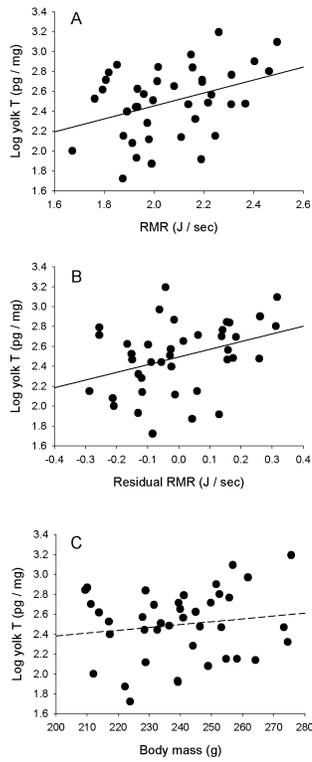
286   Zeman M., J. Kosutzky, and E. Bobakova. 1986. Testosterone concentration in the

287   seminal plasma of cocks. Br Poult Sci 27:261-266.

288

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).

294