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The metabolic cost of avian egg formation: possible impact of yolk precursor production?

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Summary

Little is known about the energy costs of egg production in birds. We showed in previous papers that, during egg production, European starlings (*Sturnus vulgaris*) undergo a 22% increase in resting metabolic rate (RMR) and that the maintenance and activity costs of the oviduct are responsible for 18% of the variation in elevated laying RMR. Therefore, other energy-consuming physiological mechanisms must be responsible for the remaining unexplained variation in elevated laying RMR. Yolk precursor [vitellogenin (VTG) and very-low-density lipoprotein (VLDL)] production is likely to be costly because it signifies a marked increase in the biosynthetic activity of the liver. We documented the pattern of yolk precursor production in response to daily injections of 17β-estradiol (E_2) in zebra finches (*Taeniopygia guttata*). Based on this pattern we carried out an experiment in order to evaluate the metabolic costs of producing VTG and VLDL. Our E_2 treatment resulted in a significant increase in plasma VTG and VLDL levels within the natural breeding range for the species. Although RMR was measured during the period of active hepatic yolk precursor production, it did not differ significantly within individuals in response to the treatment or when comparing E_2-treated birds with sham-injected birds. This could mean that yolk precursor production represents low energy investment. However, we discuss these results in light of possible adjustments between organs that could result in energy compensation.

Key words: egg production, energy cost, resting metabolic rate, RMR, vitellogenin, very-low-density lipoprotein, zebra finch, *Taeniopygia guttata*, yolk precursor production.

Introduction

Physiological mechanisms underlying one of the major assumptions of life history theory, namely that an increased effort in current reproduction may have a negative impact on future reproductive success, are not well understood (Stearns, 1992). One reason for this lack of knowledge is that, in avian systems, most of the research attention has been focused on manipulations of reproductive effort at the incubation or chick-provisioning stages (Monaghan and Nager, 1997) and, thus, the potential physiological costs incurred earlier in breeding, i.e. during follicle development and egg production, have received very little attention. Recent research, however, has shown that the energy cost of egg production in birds may be significant (Nilsson and Raberg, 2001; Vézina and Williams, 2002). We recently showed that the physiological process of egg formation in female European starlings (*Sturnus vulgaris*) is responsible for a 22% increase in resting metabolic rate (RMR) in laying individuals (Vézina and Williams, 2002). We further demonstrated that 18% of the variation in elevated laying RMR was explained by the maintenance and activity cost of the working oviduct (Vézina and Williams, 2003) and emphasized that this organ is probably costly enough that selection has led to a very tight size–function relationship, explaining its rapid pattern of recrudescence and regression. However, 82% of the variation in laying RMR remains unexplained, suggesting that other energy-consuming physiological mechanisms must be responsible, at least in part, for the metabolic cost of egg production (Vézina and Williams, 2003).

Another component of egg production that is likely to be energetically costly is the increased liver activity involved in protein and lipid production for oogenesis. During the process of egg formation, the hypothalamus initiates a hormonal cascade by releasing gonadotropin-releasing hormone (GnRH), which induces the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary gland (Williams, 1998; Scanes, 2000). These hormones stimulate the ovary to produce estrogens (Williams, 1998), which then trigger the production of the egg-yolk precursors, vitellogenin (VTG) and yolk-targeted very-low-density lipoprotein (VLDLy), by the liver (Bergink et al., 1974; Deeley et al., 1975; Wallace, 1985; Walzem, 1996; Williams, 1998), which are then secreted into the blood. During rapid yolk development, plasma VTG and VLDLy are taken up by the ovary and are processed within the follicles into yolk, the nutrient and energy source for the developing avian embryo

both precursors have been reported to respond similarly to E_2 injections in zebra finches (Williams and Martyniuk, 2000). For this experiment, 32 male zebra finches were used. The birds were divided into five groups, and all birds received a daily E_2 injection (30 μl, i.m.) over four consecutive days (days 1–4; Fig. 1A). Starting from the day following the first injection, one group of birds was blood sampled each day until two days following the last injection (sample sizes were 4, 9, 5, 10 and 4 for days 2–6, respectively; Fig. 1A). All birds were blood sampled only once from the brachial vein. We also repeated this experiment using females (*N*=23) to confirm that both sexes responded the same way to the estradiol treatment.

Experiment 2: metabolic costs of yolk precursor production

For this experiment, we used 32 males randomly assigned to one of two

from the first chamber was sampled for 55 min. Then the system switched back to baseline for 10 min before changing again to the second, third and fourth chambers. Preliminary analysis showed that measuring RMR using this protocol did not generate a time effect (*sensu* Hayes et al., 1992) on RMR ($F_{3,15}$=0.48, P=0.7). The birds stayed in their chambers for approximately 5 h. After RMR measurement, the birds were weighed for a second time and the average of first and second masses was used in subsequent analysis. To calculate RMR, a running mean representing 10 min of recording was passed through the data for each bird, with the lowest mean taken as RMR.

Yolk precursor analysis

In order to measure circulating levels of VTG and VLDL, blood samples were centrifuged at 2200 ***g*** for 10 min, and the plasma portion of each sample was isolated. Plasma samples were then assayed for vitellogenic zinc (Zinc kit; Wako Chemicals, Richmond, VA, USA) using the method developed for the domestic hen (Mitchell and Carlisle, 1991) and validated for passerines (Williams and Christians, 1997; Williams and Martiniuk, 2000; Challenger et al., 2001; Salvante and Williams, 2002). The concentration of vitellogenic zinc is proportional to circulating levels of VTG (Mitchell and Carlisle, 1991). The overall inter-assay coefficient of variation for the vitellogenic zinc assay (calculated from repeated analyses of a reference sample) was 14.2% (N=9 assays).

Circulating VLDL was assessed by measuring plasma triglyceride levels (Triglyceride E kit; Wako Chemicals) according to the method of Mitchell and Carlisle (1991). Plasma triglyceride has commonly been measured in non-domesticated birds as an index of total plasma VLDL, which consists of both the generic and yolk-targeted forms of VLDL (Williams and Christians, 1997; Williams and Martyniuk, 2000; Challenger et al., 2001). Despite the marked increase in circulating lipid levels reported during egg production (Griffin and Hermier, 1988; Walzem et al., 1994, 1999; Walzem, 1996), *in vivo* studies on laying poultry hens have detected only low circulating levels of intermediate-density and low-density lipoproteins, both by-products of the metabolism of generic VLDL, suggesting that VLDLy is resistant to metabolism by laying hens (Hermier et al., 1989; Walzem et al., 1994; Walzem, 1996). These studies provide evidence that the marked increase in total VLDL during avian egg production or following estrogen administration is the result of increased synthesis of the estrogen-dependent VLDLy component of total VLDL. The overall inter-assay coefficient of variation for the triglyceride assay (calculated from repeated analyses of a reference sample) was 10% (N=5 assays). All assays were run using 96-well microplates and measured using a Biotek 340i microplate reader (Winooski, VT, USA).

chambers, we were not able to control for the time effect (i.e. the birds were not consistently put in the same chambers). We do not believe that this introduced a systematic bias. As mentioned earlier, the only detectable time effect was recorded between measurements for chambers 1 and 4 at pre-treatment. However, only seven of 32 birds were measured in these two chambers over the two measurement periods. Out of these seven, only four individuals had their RMR measured in chamber 4 at pre-treatment and in chamber 1 at mid-treatment, which would artificially increase their RMR. Data are reported as means ± S.E.M.

Results

Experiment 1: pattern of yolk precursor plasma levels in response to daily E_2 injections

Administration of estradiol triggered a significant increase in circulating VTG in male and female zebra finches (two-way ANOVA, treatment effect, $F_{4,49}$=5.60, P<0.005), and there was no significant difference in the pattern of response to E_2 treatment between the sexes (sex effect, $F_{1,49}$=2.08, P=0.2; no significant interaction term). Therefore, we pooled the samples to obtain a more accurate picture of the pattern of VTG production (treatment effect $F_{4,50}$=5.31, P<0.005; Fig. 2). In response to daily E_2 injections, plasma VTG increased to reach a peak of 3.2±0.7 μg ml^{-1} on the day following the last injection (day 5 in Fig. 2). This VTG level is higher than previously reported values for breeding female zebra finches at the one-egg stage (1.68 μg ml^{-1}; Williams and Christians, 1997) but well within the normal physiological breeding range

VTG levels in both groups were low and representative of typical non-breeding birds (Williams and Christians, 1997). Therefore, this difference is not biologically relevant. At mid- and post-treatment, E_2 birds exhibited significantly higher levels of VTG than sham individuall f E

administration in the form of a gradual increase in plasma VTG and that this response did not differ from that of non-breeding females undergoing the same treatment. This is consistent with

This experiment was specifically designed to measure the potential costs of yolk precursor production. However, the influence of other aspects of egg formation on overall energy expenditure should also be examined. For example, egg yolk mass is related to the rate of yolk precursor uptake at the ovary and is potentially limited by the number of VTG/VLDL receptors and their rate of recycling (Christians and Williams, 2001). Therefore, the very active process of rapid yolk development may also result in substantial energy investment. These aspects of egg formation cannot be assessed using males or even non-breeding females due to the lack of developing ovarian follicles. Thus, more investigation is needed to explain the remaining variation in elevated laying RMR (Nilsson and Raberg, 2001; Vézina and Williams, 2002) not accounted for by the maintenance and activity costs of the oviduct (Vézina and Williams, 2003).

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