Effects of corticosterone on the proportion of breeding females, reproductive output and yolk precursor levels

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Abstract

In this study we investigated the role of corticosterone (B) in regulating the proportion of laying females, timing of breeding, reproductive output (egg size and number), and yolk precursor levels in chronically B-treated female zebra finches (*Taeniopygia guttata*). Corticosterone treatment via silastic implant elevated plasma B to high physiological (stress-induced) levels (24.1 ± 5.3 ng/ml at 7-days post-implantation). B-treated females had high plasma levels of very-low density lipoprotein (VLDL) but low levels of plasma vitellogenin 7-days post-implantation, suggesting that corticosterone inhibited yolk precursor production and perhaps shifted lipid metabolism away from production of yolk VLDL and towards production of generic (non-yolk) VLDL. Only 56% of B-treated females ($n = 32$) initiated laying, compared with 100% of sham-implanted females ($n = 18$). In females that did breed, corticosterone administration delayed the onset of egg laying: B-treated females initiated laying on average 14.5 ± 0.5 days after pairing compared to 6.4 ± 0.5 days in sham-implanted females. B-treated females that laid eggs had significantly higher plasma B levels at the 1st-egg stage (45.9 ± 9.0 ng/ml) than did sham-implanted females (7.9 ± 6.8 ng/ml). Despite this there was no difference in mean egg mass, clutch size, or egg composition in B-treated and sham-implanted females. These results are consistent with the idea that elevated corticosterone levels inhibit reproduction, but contrast with studies of other oviparous vertebrates (e.g., lizards) in relation to the role of corticosterone in regulating egg and clutch size.

1. Introduction

Avian egg production is dependent on dramatic changes in protein and lipid metabolism. Hepatic protein and lipid production increases substantially with the production of the yolk precursors, vitellogenin (VTG) and yolk-targeted, very-low density lipoprotein (VLDLy) (Gruber, 1972; Walzem et al., 1999). Concurrently, estrogens and progesterone stimulate the newly re-grown oviduct to synthesize egg albumen, which is predominantly composed of protein (Burley and Vadehra, 1989; Yu et al., 1971). Consequently, modulation of reproductive effort may be achieved by altering protein and lipid availability. Corticosterone (B), a glucocorticoid hormone produced in the adrenal cortex, is well known for its regulatory role in modulating protein and lipid metabolism. Plasma B levels increase rapidly (within 2–3 min) from baseline to 'stress-induced' levels in response to a variety of stressor stimuli (e.g., handling, capture, food deprivation, extreme temperatures, etc.; Harvey et al., 1984; Wingfield, 1988). Elevated plasma B stimulates behaviors associated with positive energy balance, such as increased foraging, increased fat deposition as energy storage for later use, and mobilization of energy reserves to meet energetic demands via an increase in gluconeogenesis, resulting in maintenance of elevated blood glucose levels (Gray et al., 1990; Ramenofsky et al., 1999; Rebuffe-Scrive et al., 1992; Wilson and Wingfield, 1992). However, the specific role of corticosterone in regulating female reproductive physiology, and in particular individual variation in reproductive output (e.g., egg size and number), remains unclear (but see Sinervo and DeNardo, 1996).

Studies on oviparous vertebrates have reported seasonal peaks in basal plasma corticosteroid (cortisol (F) in fish; B in amphibians, reptiles and birds) levels coincident with periods of egg production and seasonal

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peaks in female reproductive condition (fish, Lamba et al., 1983; Pickering and Christie, 1981; amphibians, Pancak and Taylor, 1983; Zerani and Gobbetti, 1993; reptiles, Wilson and Wingfield, 1992; birds, Silverin and Wingfield, 1982; Wingfield, 1994; Wingfield and Farner, 1978). In contrast, many studies have documented an association between elevated levels of corticosteroids and *suppression* of reproductive behavior (Kamel and Kubajak, 1987; Moore and Zoeller, 1985; Suter and Schwartz, 1985; Suter et al., 1988). Consequently, it is not clear if the reported elevations in plasma B during egg formation are indicative of involvement of corticosterone in metabolic changes associated with egg production, or if they reflect a response to the energetic stress of reproduction (Bry, 1985).

In this paper we investigate the relationship between corticosterone and reproduction experimentally by manipulating plasma B levels in female Zebra Finches (*Taeniopygia guttata*) prior to, and during, egg formation. Specifically, we describe the effect of elevated plasma B on: (a) the proportion of laying females and timing of clutch initiation, and (b) egg mass and clutch size and the potential 'trade-off' between these two life history traits (sensu Sinervo and DeNardo, 1996). Finally, since yolk precursor production is key to egg formation, we measure changes in plasma VTG and total VLDL in relation to B-treatment, as a potential mechanism for B-related modulation of egg production.

2. Materials and methods

2.1. Animals and husbandry

Zebra Finches (*T. guttata*) were maintained in controlled environmental conditions (temperature 19–23 °C, humidity 35–55%, constant light schedule of 14L: 10D, lights on at 07:00). All birds received a mixed seed diet (Panicum and white millet, 50:50; approximately 12.0% protein, 4.7% lipid; Jameson's Pet Food, Vancouver), water, grit, and cuttlefish bone (calcium) ad lib. Birds also received a multivitamin supplement in the drinking water once per week. When not paired for breeding, the birds were housed in same-sex cages, but were not visually or acoustically isolated from the opposite sex. All experiments and animal husbandry were carried out under a Simon Fraser University Animal Care Committee permit (No. 558B) following guidelines of the Canadian Committee on Animal Care.

2.2. Hormone administration

Corticosterone was administered in medical grade silastic tubing (Dow Corning) that was placed subcutaneously in the lower back. This technique has been used widely in a range of other organisms, such as dark-eyed juncos and side-blotched lizards (Schoech et al., 1998; Sinervo and DeNardo, 1996). Crystalline corticosterone (Sigma Chemical) was packed in either 1.00-cm ($n = 5$) or 0.75-cm ($n = 27$) lengths of silastic tubing (0.058 mm i.d. $\times$ 0.077 mm o.d.) that were sealed at only one end with 0.25-cm of silastic adhesive (Dow Corning) to enhance diffusion. Sham females received empty implants ($n = 18$). The 1.0 and 0.75 cm B implants did not differ in their effectiveness at elevating plasma B in the short-term, 7 days after pairing ($F_{1,29} = 0.00$, $P > 0.90$), or in the long-term, 15 days after pairing ($F_{1,28} = 0.01$, $P > 0.90$). The difference in corticosterone between days 7 and 15 was also not related to implant length ($F_{1,28} = 0.00$, $P > 0.95$). Therefore, data from 1.0 to 0.75 cm B-implanted females were combined for all analyses.

Randomly chosen, non-breeding, female Zebra Finches were weighed (± 0.1 g) and then anesthetized via an intra-muscular injection of 20 µl ketalean and xylazine solution (50:50 by volume; Associated Veterinary Products, Abbotsford) and implanted with either one corticosterone-filled implant or one empty implant. All surgical procedures were completed between 08:30 and 10:30. Females were allowed to recover under a heat lamp overnight.

2.3. Breeding conditions

On the morning after implantation, females were paired with randomly chosen experienced males. Breeding pairs were housed individually in cages ($61 \times 46 \times 41$ cm) equipped with an external nest box ($11.5 \times 11.5 \times 11.5$ cm). Males and females were weighed (± 0.1 g) at the time of pairing. Pairs were provided with an egg-food supplement (20.3% protein: 6.6% lipid) daily between pairing and clutch completion. Data on laying interval and egg and clutch size were obtained by checking the nest boxes daily between 09:00 and 11:00. All eggs were weighed (to 0.001 g) on the day they were laid, and the second egg of each clutch was collected for egg composition analysis. Clutches were considered complete if no new eggs were laid over two days, and the pair was returned to non-breeding cages after removal of the female's implant. Females that failed to lay eggs within 20 days of pairing were classified as non-breeders; their implants were removed, and they were returned to non-breeding cages.

All females that initiated laying were weighed (± 0.1 g) and blood sampled (~ 200 µl from the brachial vein) at the one-egg stage and at clutch completion. In unmanipulated females, initiation of laying generally occurs five to seven days after pairing (6.6 ± 0.3 days, Williams, 1996; 6.1 ± 0.3 days, Williams and Martyniuk, 2000). Females that did not initiate laying by the seventh day after pairing were blood sampled and weighed (± 0.1 g) on day seven. If laying was still not

initiated by 15 days after pairing, females were blood sampled and weighed ($\pm 0.1\,g$) again on day 15. All blood samples were collected between 09:00 and 11:30 and within 2 min of capture. Females were divided into three groups according to their implant and breeding status: (1) sham-implanted females that bred (ShamBr, $n = 18$), (2) corticosterone-implanted females that bred (CortBr, $n = 18$), and (3) corticosterone-implanted females that did not breed (CortNBr, $n = 14$).

Mean sampling day for the first blood sampling period of the laying cycle was 6.7 ± 0.8 days after pairing. This sampling period will be referred to as 'Day 7.' During this time all 18 ShamBr females and 3 of 18 CortBr females (17%) bred and were sampled on the day of their first egg (5–9 days after pairing), while 15 of the 18 CortBr females (83%), which eventually bred about 8 days later, and 13 of the 14 CortNBr females were sampled at 7 days after pairing (i.e., not the day of their first egg). For all 'Day 7' statistical analyses, 'CortBr females' include only samples from the 15 non-laying, B-implanted females sampled at 'Day 7.'

Mean sampling day for the second blood sampling period was 14.9 ± 2.1 days after pairing. This sampling period will be referred to as 'Day 15.' During this time 15 of the 18 (83%) CortBr females initiated laying and were sampled on the day that their first egg was laid, while all 18 of the ShamBr females (only 8 of which were sampled) and 3 of the 18 CortBr females (17%) were at clutch completion (13–18 days after pairing). Of the 14 B-treated females that did not initiate laying, 13 were sampled at 15 days after pairing. For all 'Day 15' statistical analyses, 'CortBr females' include only samples from the 15 B-implanted females sampled at the 1st-egg stage. Statistical comparisons were made between CortBr and CortNBr females at 'Day 15'; ShamBr females were not included in statistical comparisons because they were already at clutch completion.

2.4. Analysis of yolk precursors and corticosterone

Plasma samples were assayed for VTG and total VLDL using the zinc and triglyceride methods, respectively, developed in the domestic hen (Zinc and triglyceride kits—Wako Chemicals, Virginia, USA; Mitchell and Carlisle, 1991), and validated for passerines (see Challenger et al., 2001; Williams and Martyniuk, 2000). Total plasma zinc and plasma VTG-Zn were highly correlated ($r^2 = 0.69$, $P = 0.0001$). Therefore, total zinc was used as an index of VTG-Zn when there was insufficient plasma to carry out the depletion step necessary to measure VTG-Zn. Inter-assay coefficient of variation was 13.9% for vitellogenic zinc using a laying hen plasma pool ($n = 11$). Inter-assay coefficient of variation was 7.9% for total triglyceride using a 19-week hen plasma pool ($n = 6$). This method measures total VLDL, which is made up of non-laying VLDL and es-

trogen-dependent, yolk-targeted VLDL (VLDLy). All assays were run using 96-well microplates, and measured using a Biotek 340i microplate reader.

Plasma samples were analyzed for corticosterone using a specific radioimmunoassay, modified (without chromatography) from the method described by Wingfield and Farner (1975). Each sample was equilibrated overnight at 4 °C with 2000 cpm [^{3}H]corticosterone (NEN) to measure the percentage of recovery following the extraction procedure. Redistilled dichloromethane (4 ml) was added to each sample to extract the endogenous and [^{3}H]corticosterone. Extracts were dried at 40 °C under a stream of nitrogen gas and reconstituted with phosphate buffer. Aliquots of each sample (100 µl) were placed into scintillation vials (with 4.5 ml of scintillation fluid). The cpm of tritium in these vials provided an estimate of percent recovery following extraction for each sample. Aliquots of the reconstituted extracts (200 µl) were then placed into duplicate assay tubes, and were incubated overnight at 4 °C with 10,000 cpm [^{3}H]corticosterone (for competitive binding) and 100 µl corticosterone antiserum (Endocrine Sciences). Dextran-coated charcoal (0.5 ml) was then added to remove any unbound corticosterone (endogenous and [^{3}H]). After 10 min the mixture was centrifuged at 2000 rpm for 10 min at 4 °C in a Beckman TJ-6 refrigerated centrifuge. The supernatants (containing bound cpm) were decanted into scintillation vials, and 4.5 ml of scintillation fluid were added to each vial. Samples were allowed to equilibrate for 4 h before being counted for bound radioactivity in a Beckman LS3500 system (for 10 min or 2% accuracy). The corticosterone concentration of each sample was determined from a standard curve that ranged in concentration from 7.8 to 1000 pg. All samples were adjusted for percentage recovery of the internal standard; recovery values for all samples ranged from 80.2 to 97.6%. Plasma corticosterone concentrations were calculated as nanograms per milliliter.

2.5. Egg composition analysis

The second egg in each clutch was subjected to protein and lipid composition analysis following the method of Balzer and Williams (1998). Within 6 h of being laid, the collected eggs were boiled for 3 min, frozen, and then later thawed and separated into shell, albumen, and yolk and dried to constant weight in a 50 °C drying oven. Lipid was removed from the dry yolks by sohxlet extraction for 8 h with petroleum ether as the solvent (Dobush et al., 1985). Lipid-free yolks were then weighed to the nearest 0.0001 g (lean dry mass). Lipid composition of the yolks was determined by subtracting lean dry yolk mass from dry yolk mass. Dry albumen mass and lean dry yolk mass were assumed to be approximately 88% protein (Burley and Vadehra, 1989). The protein and lipid composition of the eggs of corti-

costerone-treated mothers were compared to those of sham-implanted mothers.

2.6. Data analysis

All statistical analyses were performed using SAS (SAS Institute, 1989). All non-normal variables (including total zinc, corticosterone, and VLDL) were log transformed (log 10) for analyses (although some non-transformed values were used for graphical purposes). Changes in body mass over time and comparisons of body mass between groups were assessed using repeated-measures ANOVA using only females that were weighed at all three weighing periods (i.e., implantation and 1st and 2nd blood sampling periods). The relationships between body mass and reproductive traits (precursor levels, egg size, etc.) were examined by regression of the trait values against body mass. Mass-dependent traits were corrected for body mass by taking the residual values from the regression analyses. To determine the relationships between plasma corticosterone, circulating levels of the yolk precursors, and reproductive traits, ANOVA, ANCOVA, and regression analyses were performed, using Bonferroni adjustment of significance level for multiple comparisons tests when needed. The overall significance level for multiple comparisons tests was retained as 0.05; each of the three comparisons was tested at a significance level of (0.05/3) or 0.017. ANOVA was used to analyze egg data from sham- and B-implanted females to assess the affects of B-administration on the size and composition of the second egg in each clutch. All values are given as least-squared means ± SE (unless otherwise stated), all tests are two-tailed, and the overall significance level is $P < 0.05$.

3. Results

3.1. Corticosterone levels

Plasma B levels differed significantly between groups at 'Day 7' ($F_{2,42} = 6.35$, $P < 0.005$), with CortNBr females having significantly higher circulating corticosterone than ShamBr and CortBr females (Table 1, Fig. 1a). Plasma corticosterone at 'Day 15' did not differ between CortBr and CortNBr females ($F_{1,25} = 1.48$, $P > 0.20$; Table 1, Fig. 1b). At the 1st-egg stage, CortBr females (measured at 'Day 15') had significantly higher circulating B levels than ShamBr females (measured at 'Day 7') ($F_{1,29} = 11.45$, $P < 0.005$; Table 1; Fig. 1c). In non-manipulated, breeding female Zebra Finches (sampled at the 1st-egg stage) plasma corticosterone increased from basal levels of 3.3 ± 2.5 to 18.1 ± 12.6 ng/ml in response to a 30-min standardized stress (sensu Wingfield et al., 1992; T.D. Williams, un-

Table 1
Comparison of body mass, plasma corticosterone, and plasma levels of the yolk precursors during the 'Day 7' and 'Day 15' sampling periods for B-implanted non-breeders and breeders, and sham-implanted breeders

Trait	Corticosterone non-breeders		Corticosterone breeders		Sham breeders	
	Day 7	Day 15	Day 7	Day 15 (1st egg)	Day 7 (1st egg)	Day 15 (CC)
Mass at blood sampling (g)	$16.57 \pm 0.45(13)_{a}$	$15.92 \pm 0.43(13)_{A}$	$16.58 \pm 0.42(15)_{a}$	$15.83 \pm 0.31(15)^{a}_{A}$	$15.47 \pm 0.38(18)^{a}_{a}$	$14.59 \pm 0.38(18)_{B}$
Plasma corticosterone (ng/ml)	$31.58 \pm 7.72(13)_{a}$	$29.02 \pm 12.40(13)_{A}$	$17.70 \pm 7.18(15)_{b}$	$45.89 \pm 8.99(14)^{a}_{A}$	$7.86 \pm 6.75(17)^{b}_{b}$	$4.23 \pm 15.81(8)$
Plasma VTG (µg/ml, zinc)	$0.06 \pm 0.51(5)_{a}$	$0.55 \pm 0.59(4)_{A}$	$-0.19 \pm 0.51(5)_{a}$	$1.98 \pm 0.32(10)^{a}_{B}$	$2.28 \pm 0.38(9)^{a}_{a}$	—
Total plasma zinc (µg/ml)	$1.27 \pm 0.39(12)_{a}$	$2.11 \pm 0.46(10)_{A}$	$1.89 \pm 0.36(14)_{a}$	$3.92 \pm 0.45(13)^{a}_{B}$	$4.45 \pm 0.33(17)^{a}_{b}$	$1.71 \pm 0.84(3)$
Plasma VLDL (mg/ml, triglyceride)	$29.69 \pm 7.24(13)_{a}$	$20.49 \pm 6.71(12)_{A}$	$29.90 \pm 6.97(14)_{a}$	$30.00 \pm 7.08(13)^{a}_{A}$	$18.30 \pm 6.15(18)^{a}_{a}$	$7.63 \pm 9.49(6)$

Values are least-squared means ± SE with sample sizes in parentheses. Plasma corticosterone, total zinc, and plasma VLDL were logged, and VLDL was corrected for mass at blood sampling for statistical analyses. Similar subscript and superscript letters represent groups that do not differ ($P > 0.05$) in comparisons between groups within sampling periods and between sampling periods, respectively. Lower case and capital subscripts represent comparisons within the 'Day 7' and 'Day 15' sampling periods, respectively.
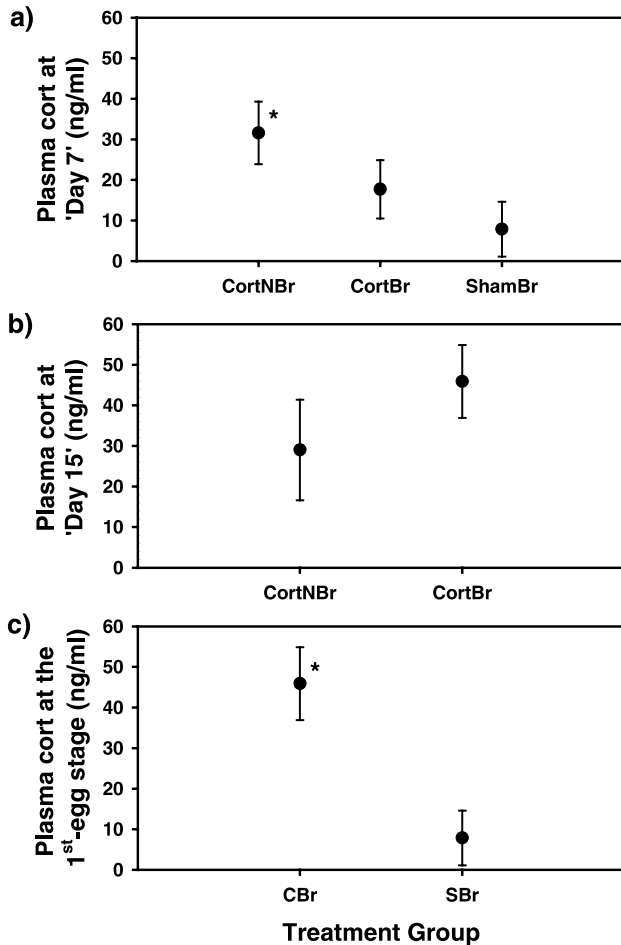
Fig. 1. Comparison of plasma cortisterone: (a) at 'Day 7' in B-implanted non-breeders (CortNBr), B-implanted breeders (CortBr), and sham-implanted breeders (ShamBr), (b) at 'Day 15' in CortNBr and CortBr females, and (c) at the 1st-egg stage in CortBr and ShamBr females. Asterisks indicate significantly different values.
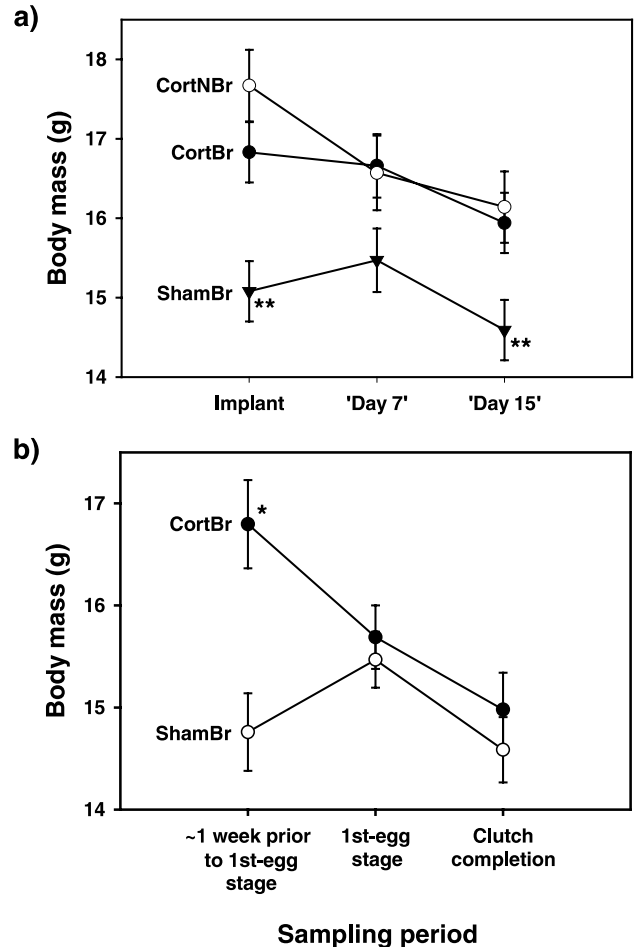

Fig. 2. Female body mass: (a) of B-implanted non-breeders (CortNBr), B-implanted breeders (CortBr) and sham-implanted breeders (ShamBr) at various sampling periods throughout the study and (b) of CortBr and ShamBr females throughout the laying cycle. Masses indicated by one asterisk are significantly different from one of the other masses within the same sampling period. Masses indicated by two asterisks are significantly different from both of the other masses within the same sampling period.

published data). In the present study, therefore, corticosterone treatment by silastic implant elevated plasma corticosterone to high physiological levels.

3.2. Mass effects

Female body mass declined significantly over the three mass sampling periods (i.e., implantation, 'Day 7' and 'Day 15'; repeated-measures ANOVA: $F_{2,47} = 9.10$, $P < 0.0005$; Fig. 2a). The three groups of females (i.e., CortNBr, CortBr, and ShamBr), however, did not differ in their pattern of mass decline throughout the experiment (repeated-measures ANOVA; time $*$ group interaction: $F_{4,90} = 2.07$, $P > 0.09$). There was, however, a group effect on female body mass within sampling periods (repeated-measures ANOVA: $F_{2,46} = 7.60$, $P < 0.002$). ShamBr females were significantly lighter than both groups of B-implanted females at implantation and at 'Day 15' (Table 1; Fig. 2a). In contrast, body

masses during 'Day 7' did not differ between groups (Table 1; Fig. 2a).

Comparison of ShamBr and CortBr females through the laying cycle showed that the pattern of body mass change between pre-laying and clutch completion differed between the two groups (repeated-measures ANOVA; time $*$ group interaction: $F_{2,29} = 7.29$, $P < 0.005$; Fig. 2b). ShamBr females gained mass from pairing (about 1 week prior to the 1st-egg stage) to the 1st-egg stage, and then lost mass from the 1st-egg stage to clutch completion (repeated-measures ANOVA: $F_{2,16} = 11.02$, $P < 0.001$), while CortBr females lost mass consistently from pre-laying (i.e., 'Day 7') through to clutch completion (repeated-measures ANOVA: $F_{2,12} = 8.25$, $P < 0.01$; Fig. 2b). There was also a treatment effect on female body mass within laying stages (repeated-mea-
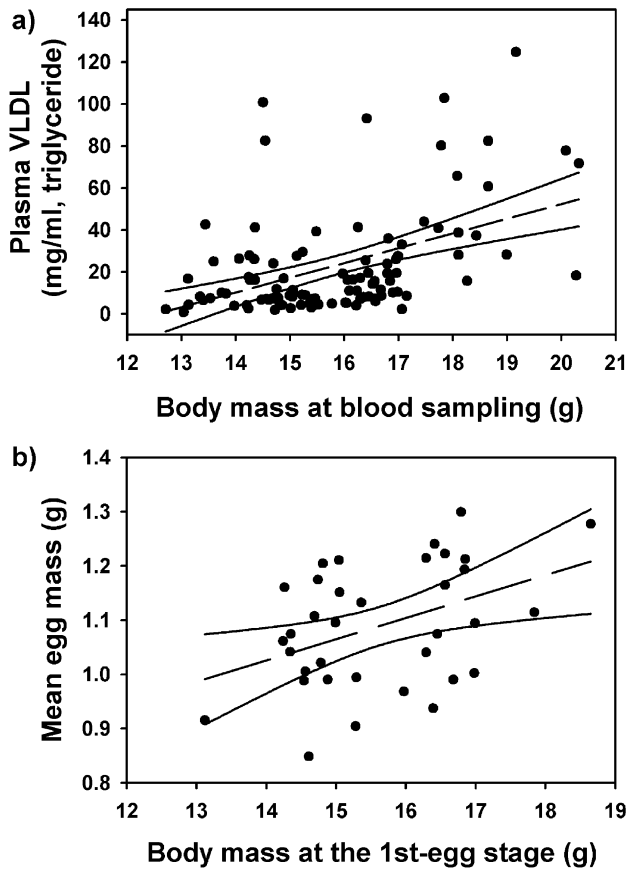
Fig. 3. Relationships between: (a) body mass at blood sampling and plasma VLDL and (b) body mass at the 1st-egg stage and mean egg mass. Log-transformed VLDL was used for statistical analyses.

sures ANOVA: $F_{1,30} = 4.47$, $P < 0.05$). Pre-laying CortBr females were significantly heavier than pre-laying ShamBr females. In contrast, body mass at the 1st-egg stage and at clutch completion did not differ between CortBr and ShamBr females.

Plasma VLDL levels were positively correlated with female body mass at the time of blood sampling ($F_{1,97} = 10.67$, $P < 0.0001$, Fig. 3a). In contrast, circulating levels of B, VTG, and total zinc were independent of body mass at blood sampling ($P > 0.50$ in all cases). Mean egg mass was also positively related to female body mass at the 1st-egg stage ($F_{1,30} = 7.82$, $P < 0.01$, Fig. 3b), but clutch size, number of days skipped during laying (without an egg being laid), and laying interval were all independent of mass on the day of the first egg ($P > 0.60$ in all cases).

3.3. Proportion of breeding females and timing of laying

Elevated corticosterone markedly decreased the proportion of females that initiated egg laying: only 18 of 32 B-implanted females laid (56%), compared with 18 of 18 sham-implanted females (100%, $\chi^2 = 6.1$, df = 1, $P < 0.02$). In females that did breed, corticosterone ad-

ministration delayed the onset of egg laying, as most of the B-treated females that bred (15 out of 18) initiated laying an average of 8 days later than sham-implanted females (14.5 ± 0.8 days compared to 6.4 ± 0.5 days; $F_{1,34} = 50.30$, $P < 0.0001$). All further analyses on reproductive effort will compare the 15 'delayed' CortBr females to the 18 ShamBr females.

3.4. Egg size, number, and composition

Although most CortBr females initiated laying an average of 8 days later than ShamBr females, residual mean egg mass, controlling for body mass, clutch size, and number of days skipped during the laying cycle did not differ between CortBr and ShamBr females ($P > 0.40$). Egg composition of the second eggs in each clutch was similarly independent of hormone treatment; CortBr and ShamBr females did not differ in fresh egg mass, yolk protein, total protein (i.e., protein in albumen and yolk), yolk lipid, or dry masses of egg shell, albumen, or yolk, ($P > 0.40$ in all cases).

3.5. Plasma yolk precursor levels

There was a significant treatment effect on total plasma zinc and plasma VTG at 'Day 7'; sham breeders had significantly higher total plasma zinc ($F_{2,40} = 20.56$, $P < 0.0001$, Table 1, Fig. 4a) and plasma VTG ($F_{2,16} = 10.12$, $P < 0.0025$, Table 1) than CortNBr and CortBr females. Plasma VLDL ($F_{2,42} = 0.32$, $P > 0.70$) did not differ between groups at 'Day 7' (Table 1, Fig. 4a). Plasma VLDL and VTG at 'Day 15' did not differ between CortBr and CortNBr females (VLDL: $F_{1,23} = 0.24$, $P > 0.60$; VTG: $F_{1,12} = 4.28$, $P = 0.0609$; Table 1, Fig. 4b). In contrast, CortBr females had significantly higher total plasma zinc than CortNBr females ($F_{1,21} = 8.45$, $P < 0.01$, Table 1, Fig. 4b). At the 1st-egg stage, CortBr and ShamBr females had comparable plasma levels of VTG, total zinc, and VLDL ($P > 0.30$ in all cases, Table 1, Fig. 4c).

4. Discussion

Corticosterone treatment via silastic implant elevated plasma B to high physiological levels (24.1 ± 5.3 ng/ml in all B-implanted females at 'Day 7') and decreased the percentage of laying birds by over 40%. In B-treated females that did lay, initiation of laying was delayed by about 8 days. However, even though corticosterone-treated breeders had significantly higher levels of plasma B at the 1st-egg stage, there was no effect of corticosterone on circulating levels of the yolk precursors at the 1st-egg stage, egg size, clutch size, laying rate, or egg composition.
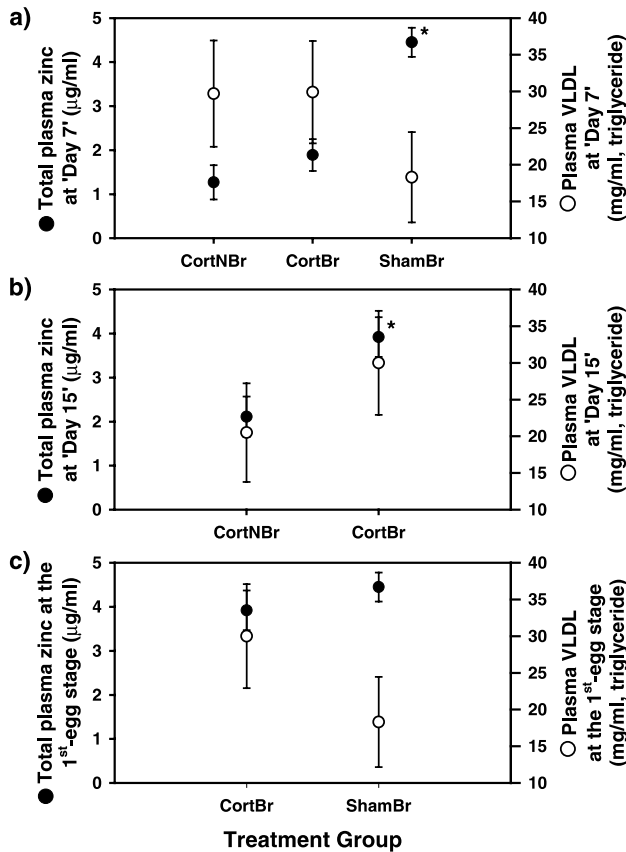
Fig. 4. Comparison of circulating levels of total plasma zinc (●) and plasma VLDL (○): (a) in B-implanted non-breeders (CortNBr), B-implanted breeders (CortBr) and sham-implanted breeders (ShamBr) at 'Day 7,' (b) in CortNBr and CortBr females at 'Day 15,' and (c) in CortBr and ShamBr females at the 1st-egg stage. Asterisks indicate significantly different values.

Sham-implanted females showed a pattern of mass change during the laying cycle similar to that previously reported (Williams and Martyniuk, 2000). Body mass increased on average 0.71 g from pre-laying to the 1st-egg stage, predominantly due to the growth of the oviduct and ovary (average combined wet mass 0.823 ± 0.187 g; Williams and Martyniuk, 2000). Body mass then decreased on average by 0.88 g between the 1st-egg stage and clutch completion; a decrease comparable to that expected from the regression of the reproductive organs. In contrast, we observed steady mass loss in CortBr females from pre-laying (about 1 week prior to the 1st-egg stage) to clutch completion. Given that the reproductive organs of CortBr females were also growing between pre-laying and the 1st-egg stage (since they laid eggs) an increase in body mass comparable to that of ShamBr females during this time period would be expected. However, 'non-reproductive' mass loss in corticosterone-treated birds between pre-laying and the 1st-egg stage (i.e., [the decrease in total body mass] + [the average mass of the oviduct and the ovary, taken from Williams and Martyniuk, 2000]) averaged

1.93 g or 12% of initial body mass (cf. 0.11 g or 1% in ShamBr females). This might have been caused by protein catabolism effects of corticosterone since we noted a decrease in flight muscle profile. However, even if corticosterone did decrease muscle mass this did not cause any subsequent change in reproductive output.

The results of the present study clearly support the hypothesis that chronically elevated levels of plasma corticosterone suppress or delay reproduction, at least in some individuals. Previous studies, mostly in mammals, have found inconsistent relationships between corticosteroids and reproduction (for review see Brann and Mahesh, 1991). This is probably due to studies assessing the effects of corticosteroids on reproduction at different physiological levels. For example, at the level of the hypothalamus, chronically elevated levels of corticosteroids have been found to suppress the release of gonadotropin-releasing hormone (GnRH) in rhesus monkeys (Dubey and Plant, 1985) possibly via a corticosteroid regulatory element in the GnRH gene (Radovick et al., 1988), suggesting a potential regulatory role of the stress hormones in GnRH synthesis or secretion. In contrast, at the pituitary level, acute and chronically elevated levels of corticosterone may enhance follicle-stimulating hormone (FSH) synthesis and secretion but suppress luteinizing hormone (LH) (Kamel and Kubajak, 1987; Suter and Schwartz, 1985; Suter et al., 1988). Since there is no effect of corticosterone administration on the number or affinity of GnRH receptors in the anterior pituitary (Suter et al., 1988), corticosterone might suppress LH release through an inhibitory effect of corticosterone on the coupling between GnRH receptors in the anterior pituitary to second messenger systems in the intra-cellular cascade leading to LH secretion (Brann and Mahesh, 1991). With regard to acute vs. chronic exposure, Brann and Mahesh (1991) concluded that the effects of elevated plasma corticosterone on reproduction are dependent on: (a) the length of exposure to the hormone, and (b) a history of estrogen priming; acute exposure to elevated corticosterone in estrogen-primed females results in stimulation of reproductive effort, while acute exposure to B in non-estrogen primed females and chronic (i.e., long-term) exposure to B results in suppression of reproductive behaviors (but see Sinervo and DeNardo, 1996). Acute elevations of plasma corticosteroids have been shown to increase hypothalamic GnRH levels (Brann et al., 1991) and stimulate LH and FSH secretion from the pituitaries of estrogen-primed rats (Brann et al., 1990).

While several studies have reported elevated basal levels of corticosterone coincident with periods of egg production, ovulation, or oviposition in various species of passerine birds (e.g., Hegner and Wingfield, 1986; Silverin and Wingfield, 1982; Wingfield and Farner, 1978), these studies did not manipulate plasma cortico-

sterone levels nor did they examine the functional relationship between plasma B and avian egg formation or laying. It is only in laying hens that there is experimental evidence linking elevated plasma B with egg production. Corticosterone infusion in laying hens for 14 days via subcutaneously implanted mini-osmotic pumps chronically elevated plasma B to high physiological levels, decreased plasma LH and plasma estradiol levels, and induced ovarian regression and cessation of laying (Etches et al., 1984; Williams et al., 1985).

The results of our study are consistent with previous findings that chronically elevated plasma B is associated with elevated plasma triglyceride levels and increased fat deposition (Gray et al., 1990; Rebuffe-Scrive et al., 1992). Hepatic synthesis of both VTG and VLDLy are estrogen-dependent (Bergink et al., 1974; Deeley et al., 1975; Wallace, 1985; Walzem, 1996; Williams, 1998) and circulating levels of the two yolk precursors generally increase simultaneously in response to elevated plasma estradiol. At 7 days post-implantation, corticosterone birds had high plasma VLDL levels even though they were not forming eggs. These elevated levels of total VLDL in CortNBr and CortBr females were most likely composed of generic (non-laying) VLDL particles, as both measures of plasma VTG, total plasma zinc and plasma VTG-Zn, were low at this time. Therefore, the elevated levels of total plasma VLDL in CortNBr and CortBr females, coincident with low levels of total plasma zinc and plasma VTG suggest that CortNBr and CortBr females did not breed because elevated plasma B inhibited yolk precursor production, possibly through suppression of LH release (Etches et al., 1984; Williams et al., 1985). Without concurrent stimulatory increases in circulating LH and FSH, increased production and secretion of estradiol associated with reproduction would not occur, thereby preventing development of the ovary and oviduct and inhibiting production of the yolk precursors, VTG and VLDLy. In addition, corticosterone may have caused a shift in lipid metabolism away from VLDLy production, with increased non-yolk VLDL production (sensu Walzem 1996).

Corticosterone treatment also significantly delayed initiation of laying by an average of 8 days in CortBr females compared to ShamBr females. This is consistent with the finding that chronic administration of corticosterone and FSH to female lizards (*Mabuya carinata*) during the quiescent phase of the ovarian cycle suppressed FSH-induced ovarian and oviductal recrudescence, estradiol secretion, hepatic growth and VTG production, and vitellogenic growth of ovarian follicles (Najagal and Yajurvedi, 1999). However, in contrast to the increase in egg mass found in chronically B-treated side-blotched lizards (Sinervo and DeNardo, 1996), mean egg mass, clutch size, and laying rate did not differ between B-implanted and sham-implanted Zebra Finches. This was despite the fact that CortBr females had

significantly higher circulating levels of B at the time of egg production (1st-egg stage) compared with ShamBr females (45.9 ± 9.0 and 7.9 ± 6.7 ng/ml, respectively). In birds at the 1st-egg stage, circulating levels of total zinc, VTG, and VLDL were not different between CortBr and ShamBr females, despite the high levels of plasma corticosterone in the former birds. Since all of the females that bred during this study had comparable circulating levels of yolk precursors at the time of egg production, it is not surprising that there was a lack of a treatment effect on mean egg mass, clutch size, laying rate, and egg composition. Thus, it appears that B-treated females that bred while exposed to elevated plasma B levels were somehow able to modulate their response (i.e., inhibition of reproduction) to chronically elevated corticosterone. This effect might be mediated by: (a) changes in plasma levels of corticosterone binding globulins (CBGs) which bind to corticosterone in the bloodstream (Wingfield et al., 1992), (b) down-regulation of B-receptors, or (c) decreasing receptor affinity to the hormone.

Finally, Sinervo and DeNardo (1996) showed that chronically elevated plasma corticosterone in female side-blotched lizards was accompanied by an increase in egg size, as measured by egg mass, independent of any change in clutch size. The results of our study have shown that chronically elevated plasma corticosterone in female Zebra Finches had no effect on egg or clutch size, or the trade-off between these two traits. The contradictory results of these studies suggest that corticosterone may play different roles in mediating egg size and clutch size and the relationship between these reproductive traits in different oviparous, vertebrate taxa.

Acknowledgments

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