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Effects of corticosterone treatment on responses to fasting in Japanese quail

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ABSTRACT

Plasma concentrations of corticosterone, a glucocorticoid hormone, have been reported to increase during fasting in some species of birds. Although Japanese quail are widely used in laboratory studies, corticosterone responses to fasting have not been described for this species. We therefore investigated the effects of 24 h of fasting on plasma corticosterone concentrations in quail. Previous work has shown that the corticosterone response to handling in quail may be affected by treatment with corticosterone, so we also measured corticosterone responses to 24 h of fasting in quail treated with corticosterone in their drinking water, and determined the effects of corticosterone on food intake immediately after a fast. Plasma corticosterone concentrations were unaffected by 24 h of fasting in controls and in three out of four groups of birds with varying corticosterone intakes. Fasting in birds can be divided into three phases, and our results suggest that quail remained in phase I during the 24 h fast. Food intake in the 3 h immediately after fasting was higher in four groups of birds with varying corticosterone intakes than in the control group, and was greatest in quail with the highest corticosterone intake. The current results suggest that corticosterone can stimulate food intake in quail following a period of food deprivation.

1. Introduction

Stressors are stimuli that activate the hypothalamo-pituitary-adrenal (HPA) axis and lead to the secretion of glucocorticoid hormones (Cockrem, 2007). Corticosterone is the primary glucocorticoid secreted in birds (Carsia and Harvey, 2000), and elevated plasma concentrations of corticosterone can be associated with increased protein breakdown and fat deposition (Gray et al., 1990; Hayashi et al., 1994), suppression of reproductive behaviour (Silverin et al., 1997), heightened fear behaviour (Jones et al., 1988) and increased locomotor activity (Breuner et al., 1998). Corticosterone may also stimulate food intake in birds, although relationships between corticosterone and food intake remain unclear. Treatment of chickens (*Gallus gallus domesticus*) with corticosterone for example, increased food intake in some studies (Siegel and Van Kampen, 1984; Nasir et al., 1999; El-Lethey et al., 2001), but not others (Davison et al., 1983; Simon, 1984; Williams et al., 1985; Kafri et al., 1988).

Fasting can be a potent stressor in birds (Freeman, 1985; Mench, 1991), and there are reports of significant increases in plasma corticosterone after 24 h of food deprivation in immature (Nir et al., 1975; Freeman et al., 1980; Harvey et al., 1983; Geris et al., 1999) and adult (Scanes et al., 1980) chickens. Corticosterone responses to fasting however, vary among individuals and may depend on energy reserves and past experience (Webster, 2003). Deprivation of food and water for 12 h elevated plasma corticosterone in mature Japanese quail (*Coturnix coturnix japonica*; Scott et al., 1983), but the effects of food withdrawal alone on corticosterone have not been investigated in quail.

The effect of corticosterone on food intake in birds may be dose-dependent. Food intake increased in chickens delivered higher doses of corticosterone but not in chickens given lower doses (Petitte and Etches, 1991; Covasa and Forbes, 1995), and corticosterone stimulated food intake in chickens fed high protein but not low protein diets (Bartov, 1985). Furthermore, delivery of corticosterone to white-crowned sparrows (*Zonotrichia leucophrys gambelii*) did not affect food intake in otherwise unmanipulated birds (Astheimer et al., 1992). Following 24 h food deprivation however, the intensity of feeding was much greater in birds with corticosterone implants than in untreated controls, suggesting corticosterone may stimulate food intake in birds following a fast.

In the present study, corticosterone was delivered to Japanese quail in their drinking water to generate a range of plasma corticosterone concentrations. It has previously been found that corticosterone responses of birds to a handling or restraint stressor can be affected by this treatment (Hull et al., 2007; Muller et al., 2009). Birds were subjected to a 24 h fast then given free access to food for the following 3 h. The aims of the study were to determine in quail the effects of corticosterone treatment on corticosterone responses to fasting, and to determine the effects of corticosterone on food intake immediately after a fast.

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2. Materials and methods

2.1. Animals and husbandry

Wild-type female Japanese quail (*Coturnix coturnix japonica*) were purchased at five weeks of age from our usual supplier (Canter Valley Farm, Christchurch). The birds had been raised in mixed sex groups under a long day photoperiod (15 h light: 9 h dark) at air temperatures between 20 and 25 °C. Each bird was identified with a coloured, numbered leg band and housed in an individual cage measuring 35×20×24 cm (length×width×height). Each cage had individual troughs in which fresh food (NRM meat bird crumbles) and water were provided *ad libitum*. Quail were held on a long day photoperiod (16 h light: 8 h dark; lights on from 0600 to 2200 h). An extractor fan provided ventilation for each room and a Carrier temperature control unit maintained the air temperature at 20±2 °C. Light was provided by two 75 W incandescent light bulbs controlled by a 24 h/seven day time switch (HPM Excel Light Switch and Timer). Quail were given four weeks to acclimatise to housing conditions and were nine weeks of age when the experiment began.

2.2. Experimental design

Quail were randomly assigned to four groups of 20 birds on day −8 (day −8 was 8 days before corticosterone treatment began). All quail in this study were part of a larger experiment in which additional data were collected in the week before corticosterone treatment began (day −7 to day −1) and during the three week treatment period (day 0 to day 20). Corticosterone treatment began at 0800 h on day 0, then quail were subjected to a 24 h fast beginning between 1230 and 1430 h on day 14 of corticosterone treatment. Corticosterone (Sigma-Aldrich) was dissolved in 99% ethanol and then diluted in water to concentrations of 0.0077, 0.0154 and 0.0231 mg/mL. Previous work in our laboratory had shown that daily corticosterone intakes were approximately 0.4, 0.8 and 1.2 mg/bird in quail drinking water containing corticosterone at these concentrations (Hull et al., 2007). Groups of quail were provided with water (controls) or with water containing 0.0077, 0.0154 or 0.0231 mg/mL of corticosterone. A measured volume of water was added to the individual water trough of each bird at the beginning of the treatment period. Water troughs were located on the outside of cages, and birds had access to water through a small opening in their cage. The volume of water added each day was recorded so the total volume consumed over the treatment period and the mean daily water intake could be calculated. Mean daily water intakes were multiplied by corticosterone concentrations to give mean daily corticosterone consumptions for each bird. Daily water intakes were greater than expected, so beginning at 1700 h on day 4 of treatment the concentrations of corticosterone were reduced to 0.0058, 0.0115 and 0.0173 mg/mL, respectively, in order to maintain the planned daily corticosterone intakes. The relative corticosterone intakes of quail remained similar when corticosterone concentrations in the drinking water were reduced on day 4 of treatment. Birds were assigned to treatment groups on the basis of their calculated daily corticosterone intakes between 1700 h on day 4 and when treatment finished at the end of day 20. The groups were 0 (control group, $n=18$), 0.31–0.60 ($n=20$), 0.61–0.90 ($n=13$), 0.91–1.50 ($n=19$) or >1.51 ($n=6$) mg corticosterone/bird/day. Two control birds and two treated birds died before day 14 and were not included in these groups.

2.3. Experimental procedures

Blood samples were collected immediately before the 24 h fast began on day 14 (starting at 1230 h), after 24 h of fasting, and 3 h after food presentation. Birds were bled in the same order on each occasion. Body mass was measured immediately after each blood sample was collected at the beginning and end of the fast. Food intake was measured during the 3 h following the fast. Blood samples were collected and some measurements were made on the birds for another study before the fast. Blood samples for the measurement of corticosterone were collected on days −1 and 7. Body mass and cloacal diameter were measured on days −1 and 7, and cloacal diameter was measured on day 14. Food intake was measured over 24 h between day −6 and day −5, and between day 8 and day 9. Behavioural tests of fearfulness were conducted on days 6, 10, 11 and 12.

Blood samples were collected by puncture of a wing vein followed by withdrawal of up to 200 µL of blood into heparinised capillary tubes. All blood samples were collected in a separate room within 1 min from the time the bird was removed from its cage. Blood samples were kept cool on ice for up to 3 h until centrifugation at 1600 g for 10 min at 10 °C (Heraeus Christ Cryofuge 5000S). Plasma was removed after centrifugation and frozen at −20 °C. Plasma corticosterone concentrations were subsequently measured by radioimmunoassay. Body mass (±0.1 g) was measured using an electronic balance. Food intake measurements began when each quail was returned to its cage following blood sample collection after the fast. Individual empty food troughs were removed and filled with 30 g of food and placed back on the cage. Food troughs were located on the outside of cages, and birds had access to food through a small opening in their cage. The remaining food in each trough was weighed (±0.1 g) 3 h later.

2.4. Corticosterone radioimmunoassay

Each plasma sample was thawed, transferred to a 1.5 mL microcentrifuge tube and centrifuged for 10 min at 18,000 g to separate lipids from plasma. Clear plasma from below the lipid layer was transferred to another tube and diluted in phosphate buffer with saline and gelatine (PBSG) for the measurement of corticosterone by radioimmunoassay using an assay validated for the measurement of corticosterone in quail plasma (Hull et al., 2007). Iodinated corticosterone, antiserum against corticosterone and a second antibody were obtained from MP Biomedicals. The sensitivity of the corticosterone assay was determined as the hormone concentration at the mean −2 standard deviations from the zero hormone point on the standard curves. The assay sensitivity, expressed as ng steroid/mL plasma, was 0.35 ng/mL. Solutions of corticosterone in PBSG at concentrations that gave approximately 80, 50 and 20% binding on the standard curve were used as low, medium and high quality controls in every assay. The intra-assay coefficients of variation were 8.8%, 4.3%, and 13.5% and the inter-assay coefficients of variation 16.0%, 8.3% and 16.9% for low, medium and high solutions respectively.

2.5. Statistics

A high corticosterone value for one bird in the lowest treatment group was identified as an outlier using Grubb's test (Barnett and Lewis, 1994), and this bird was omitted from statistical analyses. Statistical analyses were performed using Systat (Systat Software). Plasma corticosterone concentrations were transformed to logarithms, and Levene's tests confirmed homogeneity of variances for these data. Changes in plasma corticosterone concentrations and body mass were compared between groups using repeated measures ANOVAs with time (before and after 24 h of fasting, and 3 h after food presentation for corticosterone concentration; before and after 24 h of fasting for body mass) and group (control or corticosterone treatment) as the grouping factors. *Post hoc* comparisons were made between times for control and treatment groups and between control and treatment groups for each time using univariate *F* tests. Differences in food intake in the 3 h following food presentation were compared between groups using one way ANOVA. *Post hoc*