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RESEARCH ARTICLE

Developmental stress has sex-specific effects on nestling growth and adult metabolic rates but no effect on adult body size or body composition in song sparrows

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SUMMARY

Variation in the prenatal and postnatal environments can have long-term effects on adult phenotype. In humans and other animals, exposure to stressors can lead to long-term changes in physiology. These changes may predispose individuals to disease, especially disorders involving energy metabolism. In addition, by permanently altering metabolic rates and energy requirements, such effects could have important fitness consequences. We determined the effects of early-life food restriction and corticosterone (CORT) treatment on growth and adult body size, body composition (assessed *via* quantitative magnetic resonance) and metabolic rates in the song sparrow, *Melospiza melodia*. Nestlings were hand-raised in captivity from 3 days of age. Treatments (*ad libitum* food, food restriction or CORT treatment) lasted from day 7 to day 60. Both experimental treatments had sex-specific effects on growth. In the nestling period, CORT-treated males weighed more than controls, whereas CORT-treated females weighed less than controls. Food-restricted males weighed the same as controls, whereas food-restricted females weighed less than controls. Both experimental treatments also had sex-specific effects on standard metabolic rate (SMR). Females exposed to food restriction or CORT treatment during development had higher SMRs in adulthood than control females, but neither stressor affected SMR in males. There were no effects of either treatment on adult body size, body composition (lean or fat mass) or peak metabolic rate. Therefore, early-life stress may have sex-specific programming effects on metabolic rates and energy expenditure in song sparrows. In addition, both treatments affected nestling growth in a manner that exaggerated the typical sex difference in nestling mass, which could provide male nestlings with a competitive advantage over their sisters when developing in a poor-quality environment.

Key words: aerobic capacity, basal metabolic rate, bird, glucocorticoid, metabolic scope, peak metabolic rate, plasticity, songbird, standard metabolic rate, corticosterone.

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INTRODUCTION

Variation in the prenatal and postnatal environments can lead to long-term variation in adult phenotype, a process often referred to as developmental programming (Mcmillen and Robinson, 2005). In particular, exposure to stressors early in life, such as nutritional restriction, infection or elevated gluocorticoid levels, can alter development, leading to permanent changes in physiology (Mcmillen and Robinson, 2005; Rinaudo and Wang, 2012; Welberg and Seckl, 2001). In humans, these early-life events alter fetal or infant growth and may predispose individuals to disease, especially those involving energy metabolism. For example, low birth mass in humans is associated with increased risk of obesity, type II diabetes and impaired lipid metabolism in adulthood (Barker et al., 1993; Rinaudo and Wang, 2012). Individuals exposed to famine *in utero* have higher indices of obesity (Ravelli et al., 1999) and impaired glucose tolerance (Ravelli et al., 1998), suggesting that nutritional restriction during development may be a particularly important risk factor for disease in later life. In support of this, rats exposed to a low protein diet *in utero* or during the early postnatal period exhibit altered postnatal growth and long-term changes in glucose metabolism and insulin resistance (Zambrano et al., 2006). In mammals, the specific physiological effects of a stressor often depend on the stage of development in which exposure occurred (Painter et al., 2005).

In addition to changes in energy metabolism, studies in birds have shown important links between variation in the early rearing environment and variation in metabolic rates. For example, zebra finches (*Taeniopygia guttata*) raised in experimentally enlarged broods had higher standard metabolic rates (SMRs) in adulthood compared with those raised in smaller broods (Verhulst et al., 2006). In the same species, treatment with the glucocorticoid hormone corticosterone (CORT) during the nestling period increased overnight variability in SMRs; however, this effect was seen only during the treatment period and not in adulthood (Spencer and Verhulst, 2008). In both of these studies, the effect of the stressor on metabolic rates was more severe in females than in males, suggesting that early-life stressors could have sex-specific programming effects on energy expenditure. Variation in metabolic rates could in turn have important fitness consequences. For example, individuals with higher metabolic rates have higher energy

requirements and may have to spend more time foraging for food or be less likely to survive food shortages. High resting metabolic rates have also been linked to decreased longevity (Manini, 2010; Speakman, 2005). In addition, basal metabolic rates (BMRs) are positively correlated to reproduction, such that species with high BMRs often have higher reproductive rates (Hennemann, 1983). Therefore, at the interspecific level, variation in metabolic rates may mediate important tradeoffs between reproduction and survival. However, whether variation in metabolic rates is related to reproduction and survival within a species is less clear.

The physiological mechanisms underlying the effects of early-life stressors on energy metabolism and metabolic rates involve many processes (Rinaudo and Wang, 2012). The stressor may directly alter the development of an organ, resulting in permanent changes in organ morphology or function. For example, prenatal and postnatal protein restriction in rats reduces the growth of the pancreas, spleen, muscle and liver (Desai et al., 1996). Changes in organ size could be due to reductions in cell number or cell size. In rats, early-life protein restriction decreases beta cell proliferation and the size of islets in the pancreas (Snoeck et al., 1990). A variety of stressors may also increase fetal or neonatal glucocorticoid exposure, which also affects offspring growth and development (Fernandez-Twinn and Ozanne, 2006; Welberg and Seckl, 2001). Food restriction can increase baseline and stress-induced glucocorticoid levels in birds (Kempster et al., 2007; Kitaysky et al., 2001a), amphibians (Crespi and Denver, 2005) and mammals (Lesage et al., 2001). In turn, early-life glucocorticoid exposure has many of the same detrimental effects as nutritional restriction, including growth retardation (Spencer et al., 2003), impaired brain development (Buchanan et al., 2004) and altered energy metabolism (Harris and Seckl, 2011; O'Regan et al., 2004). In addition, stressors during development can alter typical patterns of somatic growth, which can also be detrimental. A stressor may initially retard growth but be followed by a period of rapid growth acceleration once the stressor subsides (catch-up growth) such that there are no long-term effects on body size. Although beneficial in the short-term, catch-up growth may negatively affect health and fitness (Hales and Ozanne, 2003; Metcalfe and Monaghan, 2001). For example, catch-up growth results in long-term increases in resting metabolic rates in zebra finches (Criscuolo et al., 2008) and decreases longevity in rats (Jennings et al., 1999).

We examined the effects of early-life food restriction and treatment with exogenous CORT on: (1) nestling growth and (2) adult body size, (3) adult body composition and (4) adult metabolic rates in song sparrows, *Melospiza melodia* (Wilson 1810). We used CORT treatment to determine whether glucocorticoids have effects similar to those of food restriction on growth and physiology. Because a variety of stressors increase glucocorticoid levels, this allowed us to determine whether a number of different stressors might affect growth and metabolism *via* CORT in song sparrows. We monitored nestling growth during and after the treatment period to determine whether birds exhibited catch-up growth and to evaluate the long-term effects of each treatment on adult body size. We also used quantitative magnetic resonance (QMR) analysis to examine body composition, to determine whether developmental stress has long-term effects on lean and fat mass. Last, we investigated the effects of food restriction and CORT treatment on metabolic rates, specifically SMRs and peak metabolic rates (PMRs). Although past studies on birds have examined the effects of variation in the early rearing environment on SMRs, no studies have examined PMR to determine whether early-life stress could affect the ability of an animal to perform intense exercise. Because the ability to perform intense exercise might be necessary for birds to forage, escape predators and complete annual migrations, changes in PMR could have important fitness consequences.

MATERIALS AND METHODS

Study subjects and rearing conditions

Song sparrow nests were located near Newboro, Ontario, Canada (44°38′N, 76°20′W), during May and June 2010. Nests were monitored to determine the day-of-hatch. All nests hatched between 9 May and 7 June 2010 and represented the first brood for the pair that year. The territorial male associated with each nest was caught using mistnets and conspecific song playback, and morphological measurements were collected (see below) from these males prior to nests hatching, in April and May 2010. Because extra-pair paternity is infrequent in this study population [consistently below 10% of nestlings (Potvin and MacDougall-Shackleton, 2009; E.A.M.-S., unpublished data)], the resident male was presumed to be the genetic father of nestlings hatching on the territory. We did not catch the female associated with each territory (the presumed mother) because we did not want to interfere with egg laying or incubation, which may increase the chance of nest predation or desertion. A total of 47 nestlings from 15 broods were used for this study. Of these, 43 were brought into captivity at 3–4 days post-hatch (days 3–4), and four were brought in at ~day 7 (mean ± s.e.m.=3.44±0.16 days; Table 1).

Nestlings were kept warm using heat lamps and electric heating pads until they developed feathers (~day 7), and were transported to The University of Western Ontario, London, Ontario, Canada, and housed at the Advanced Facility for Avian Research for the remainder of the experiment. Nestlings were housed in a cage with their siblings until they began eating independently (~day 25), at which point they were housed individually. Birds were kept on a long day photoperiod (16 h:8 h light:dark) until 16 August 2010, and then switched to short days (10 h:14 h light:dark) for the remainder of the experiment. Sex of nestlings was determined using PCR amplification of genes on the sex chromosomes (Griffiths et al., 1998). Amplification and electrophoresis conditions are described elsewhere (Potvin and MacDougall-Shackleton, 2010).

Table 1. Age and mass of song sparrow nestlings at the start of the experiment

	Control		Food restriction		CORT	
	Male	Female	Male	Female	Male	Female
Sample size	9	7	8	8	6	9
Age at capture (d)	3.56±0.44	3.71±0.57	3.25±0.16	3.63±0.50	3.17±0.17	3.44±0.44
Mass at capture (g)	8.98±1.18	9.45±1.13	9.60±0.69	9.18±1.36	10.03±0.55	8.88±1.12

Age at capture and mass at capture represent the age and mass of nestlings the day they were brought into captivity.
Values are means ± s.e.m.
CORT, corticosterone.

Experimental treatments

Within each brood, nestlings were assigned to one of the three treatment groups: control (*ad libitum* food), food restriction or CORT treatment. This was done using block randomization, such that if there were three or more nestlings in a brood, at least one nestling was assigned to each treatment. This method of randomization was used instead of true randomization to ensure that we had similar sample sizes for each treatment group. In addition, this procedure allowed us to ensure that there were never more than two nestlings from a given brood in a treatment, therefore allowing us to control for nest of origin as best as possible. In total, there were 16 control subjects (nine males, seven females), 16 food-restricted subjects (eight males, eight females) and 15 CORT-treated subjects (six males, nine females; Table 1). Food restriction and CORT treatment lasted from day 7 to day 60 (see Fig. 1 for timeline).

All nestlings received a standard hand-rearing diet administered *via* 1 ml syringes. The diet consisted of ground Mazuri Small Bird Maintenance diet (catalogue number 56A6, Brentwood, MO, USA), hard-boiled chicken eggs (shells removed), wheat germ, water and Prime avian vitamin supplement (Rolf C. Hagen, Montreal, QC, Canada). We followed a food restriction protocol that has been used for a variety of songbird species (Nowicki et al., 2002; MacDonald et al., 2006). Briefly, for each brood, the control and CORT-treated birds were first fed *ad libitum*. We calculated the average amount of food eaten by nestlings in these two groups and then fed 65% of this amount to the food-restricted siblings. Nestlings were fed every 30 min during daylight hours until day 18. At this time, we added food dishes to the cages and slowly lengthened the feeding interval to encourage birds to eat independently. Once feeding independently, birds were fed a 50:50 mix of ground Mazuri Small Bird Maintenance Diet and premium budgie seed (Rolf C. Hagen). To continue the food restriction stressor into the fledgling period, we removed food cups for 3 h per day until day 60 for this treatment group. The start of this 3 h period was randomized each day. This protocol has been used in European starlings and affects adult body size, immune function, song production and spatial learning (Buchanan et al., 2003; Farrell et al., 2011).

For CORT treatment, CORT was dissolved in peanut oil and administered orally to birds. This non-invasive technique results in a transient increase in CORT similar to that experienced in response to an acute stressor, and in nestling zebra finches has been shown to affect nestling growth, brain development and song learning (Buchanan et al., 2004; Spencer et al., 2003). We used a dose of $0.87\,\mu g\,g^{-1}$ body mass, which was determined during pilot studies (see below). CORT was fed to nestlings twice per day, once in the morning and once in the evening. Control and food-restricted birds were fed peanut oil alone. Once birds were eating independently, CORT was first injected into wax worm larvae and then fed to birds once per day in the morning until day 60 (Breuner et al., 1998). Control and food-restricted birds were fed wax worm larvae injected with oil only.

We conducted a pilot study to verify that orally administering CORT resulted in a transient increase in CORT similar to that observed in song sparrows in response to restraint stress (MacDougall-Shackleton et al., 2009; Newman et al., 2008). We injected CORT into wax worm larvae (dose=$1\,\mu g\,g^{-1}$ body mass) and fed the worms to captive song sparrows. Blood samples were collected 0, 10 or 30 min post-ingestion of the worm. CORT levels were low 0 min post-ingestion ($4.16\pm2.38\,ng\,ml^{-1}$, N=4), peaked 10 min post-ingestion ($173.13\pm51.40\,ng\,ml^{-1}$, N=3) and had begun to decrease after 30 min ($61.58\pm9.35\,ng\,ml^{-1}$, N=4). Because peak CORT levels were slightly higher than post-restriction CORT levels in our population (MacDougall-Shackleton et al., 2009; Schmidt et al., 2012), we used a slightly lower dose of $0.87\,\mu g\,g^{-1}$ body mass for our experiment. In studies using a similar manipulation in white-crowned sparrows, CORT levels peaked 7 min post-ingestion of the worm, were still elevated 30 min post-ingestion and had returned to baseline after 60 min (Breuner et al., 1998). Therefore, this method of administration results in a transient increase in CORT that is very similar to the increase observed after exposure to an acute stressor.

To verify that the CORT treatment was effective during the experiment, we collected blood samples (~30 µl) on days 10 and 45, 10 min after administration of CORT or vehicle to determine plasma CORT levels. CORT was quantified in unextracted plasma using a radioimmunoassay (07-120103, MP Biomedicals, Santa Ana, CA, USA) that has been previously validated in song sparrows (Newman et al., 2008). Three separate assays were conducted and samples from all subjects were randomly assigned to an assay such that each treatment was equally represented in each assay. The lower limit of detectability ranged from 1.8 to $2.6\,ng\,ml^{-1}$. Inter-assay variation was 5.5% for a low control ($39\,ng\,ml^{-1}$) and 4.1% for a high control ($179\,ng\,ml^{-1}$). Intra-assay variation was 9.4% for the low control and 3.9% for the high control.

Body measurements

Body mass was measured using a spring scale to the nearest 0.1 g. We measured nestling body mass daily as soon as the lights came on (05:30 h) until day 25. Thereafter, we measured body mass every 5 days until day 60. Adult body mass (at ~7 months) was measured the evening prior to and the morning following SMR measurements and prior to PMR measurements. To compare adult masses across treatments, we used masses recorded the morning after SMR measurements when birds were in the post-absorptive state. We also measured the length of the wing chord and tarsus to the nearest 0.1 mm using dial calipers on days 25 and 45, and during adulthood prior to SMR measurements.

Body composition analysis

We determined lean and fat mass using QMR analysis (Guglielmo et al., 2011) the morning following SMR determination when birds were still in the post-absorptive state. The QMR unit (Echo-MRI-B, Echo Medical Systems, Houston, TX, USA) was custom-designed for use with small birds and bats. The QMR was calibrated daily using 5 and 94 g canola oil standards. To use the QMR, birds (when awake) were placed into plastic holding tubes and inserted into the QMR analyzer and scanned using the 'small bird' and 'two

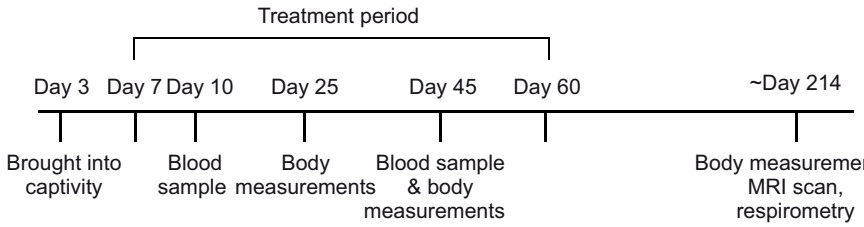

Fig. 1. Experimental timeline used to determine the effects of early-life food restriction or corticosterone treatment on nestling growth and adult body size, body composition, and metabolic rates in song sparrows.

accumulation' settings of the Echo MRI software. Fat and lean mass measurements were reported to the nearest 0.001 g. Fat and lean mass measurements were slightly adjusted to improve accuracy using calibration equations developed from house sparrows and zebra finches [fat mass: raw value × 0.94; lean mass: raw value × 1.021 (Gerson and Guglielmo, 2011; Guglielmo et al., 2011)]. A validation study conducted previously showed that the coefficients of variation for fat and lean mass are 3 and 0.5%, respectively, and relative accuracies are ±11 and ±1%, respectively (Guglielmo et al., 2011).

Respirometry

SMR

Metabolic rates were measured using open-circuit respirometry. We measured the SMR of birds between December 2010 and January 2011 when birds were ~7 months old (mean ± s.e.m.=214±0.88 days), which was ~5 months after the end of the stress treatments. Beginning at 20:00 h, body measurements were taken and birds were placed into one of five stainless-steel chambers. Chambers were placed in a temperature-controlled cabinet at 30°C, which is within the thermoneutral zone for other species of songbirds that are similar in size to song sparrows (Root et al., 1991). Four birds were individually placed into the chambers every night and the remaining chamber was used for baseline measurements. Birds fasted in the chambers for 3 h and then O_2 consumption was measured in the remaining 9 h of the overnight period. Thus measurements were taken during the inactive period, in the post-absorptive state and while birds were housed on short days and thus in non-breeding condition. However, the exact temperature range of the thermoneutral zone for song sparrows is unknown, so we refer to our measurements as SMR instead of BMR. Incurrent air was scrubbed of CO_2 and water vapor using soda lime and Drierite (W. A. Hammond Drierite Company, Xenia, OH, USA), respectively. The five sealed chambers received a constant flow of 450 ml min^{-1}. Excurrent air was sub-sampled at 150 ml min^{-1} and passed through a Drierite column to the CO_2 analyzer (catalogue number CA-2A, Sable Systems Las Vegas, NV, USA) and the O_2 analyzer (Sable Systems FC-1B), with CO_2 and water scrubbing between the two gas analyzers. Gas analyzers were calibrated daily using a standard containing 20.9% O_2 and 2% CO_2 balanced with N_2 (Praxair, London, ON, Canada). Using a multiplexer (Sable Systems), one chamber was measured at a time for 10 min before switching to the next chamber. In total, each bird was measured 12 times throughout the night for 10 min at a time. All instruments were connected to an analog-to-digital converter (UI-2 model, Sable Systems), which was connected to a laptop computer. Data were analyzed using Warthog Systems Lab Analyst software (M. A. Chappel, University of California Riverside, Riverside, CA, USA). SMR values reported were calculated as the minimum 10 min mean of O_2 consumption throughout the measurement period. We calculated the rate of O_2 uptake ($\dot{V}_{O_2}$) [based on eqn 10.6 in Lighton (Lighton, 2008)] and converted $\dot{V}_{O_2}$ to watts. The equation that we used to calculate $\dot{V}_{O_2}$ used the data for both O_2 consumption and CO_2 production (Lighton, 2008). The following morning, birds were weighed, analyzed for body composition using QMR and returned to their home cage.

PMR

The same flow system used to determine SMR was used to determine the PMR of each bird. After measuring SMR, birds were left undisturbed in their home cage for one full day. We measured PMR the afternoon of the following day (39–42 h after the start of SMR measurement). PMR was measured using an enclosed running wheel modified for use with flying birds (Pierce et al., 2005; Price and Guglielmo, 2009). The wheel (16×24 cm, width × diameter) was made of acrylic plastic and was lined with rubber. Three ping-pong balls were placed in the wheel to prevent birds from walking. Air flowed into the wheel at a rate of 4000 ml min^{-1} and was sub-sampled as described above for measurements of SMR. Food dishes were removed 3 h before testing to ensure that birds were in the post-absorptive state. Beginning at 11:00 h, and no later than 14:00 h, birds were weighed and placed into the flight wheel. The flight wheel was covered and birds were allowed to acclimate for 10 min. The wheel was then spun manually to initiate exercise. The wheel was kept in constant motion so that birds were forced to hop and hover until PMR was reached (always occurred within 15 min). This method provides a significant aerobic challenge and has been used to estimate PMR in previous studies of flying birds (Pierce et al., 2005; Price and Guglielmo, 2009). In all cases, after PMR was reached O_2 consumption decreased and then stabilized. The PMR of an individual was calculated as the maximum mean of O_2 consumption over a 1 min period. Data are expressed as watts and we calculated the metabolic scope (PMR/SMR) of each individual, which provides an estimate of intensity of exercise (Pierce et al., 2005).

Data analysis

Statistical analyses were conducted using SPSS version 19 (IBM, Armonk, NY, USA). For CORT levels, we conducted linear mixed models using restricted maximum likelihood models. Subject identity was added as a random factor with unstructured covariance. Age, treatment and sex were included as fixed effects. Significant main effects of treatment were analyzed using least significant difference (LSD) pairwise comparisons.

We also used linear mixed models to analyze nestling growth data. We conducted two separate analyses to reflect the two different parts of the treatment period. The first analysis involved the mass of nestlings from day 9 to day 18, that is, throughout the hand-rearing period. We expected the treatments to most strongly affect growth during this period because this is when the food restriction stressor was most severe and was also when CORT-treated birds were fed CORT twice per day instead of once. The second analysis involved the mass of nestlings from day 19 to day 60, the period in which birds began feeding independently up to the end of the treatment period. For both analyses, age was added as a repeated factor with first-order autoregressive covariance structure (West, 2009). Sex, treatment and age were added as fixed effects. Significant sex × treatment interactions were further analyzed by conducting linear mixed models for each sex with treatment and age as fixed factors. Significant main effects of treatment were analyzed using LSD pairwise comparisons. Paternal body mass and hatch date were included as covariates and nest identity (the natal brood nestlings came from) was included as a random factor. For nest identity, each nest was assigned a nominal value so that all siblings shared the same value but had a different value than individuals from other nests. This variable was coded as a nominal variable and was selected as a random factor in all analyses. The mass of nestlings the day they were brought into captivity, and thus before the treatments begun, was also included as a covariate in order to control for chance variation in mass or condition. One initial model was conducted for each age period (days 9–18 and days 19–60) that included the fixed factors (treatment, sex, age), the random factor (nest identity) and the covariates (hatch date, paternal mass, initial nestling mass). If the covariates or random factor were not significant, they were removed from the analysis in order to create the simplest model possible.

Table 2. Principal component analysis for morphological measurements of song sparrows

PC1	Eigenvalue	Variance explained (%)	Factor loadings		
			Mass	Tarsus length	Wing length
Day 25	1.71	56.86	0.83	0.74	0.69
Day 45	1.71	56.89	0.77	0.74	0.75
Adult	1.83	61.02	0.75	0.87	0.72

At each age, principal component analyses revealed one principle component (PC) with an eigenvalue greater than 1.

To compare the effects of the treatments on body size, we analyzed mass, tarsus length and wing length using a principal component analysis (PCA) at each age (day 25, day 45, adulthood), as these three measures were highly correlated. Data were log-transformed before being entered into the PCA. At all three ages, the PCA revealed one component with an eigenvalue greater than 1 (Table 2). We interpreted this component as representing overall body size. The resulting PC scores were then analyzed using two-way ANOVAs with treatment and sex as between-subjects factors. Significant main effects of treatment were compared using LSD pairwise comparisons. Hatch date was included as a covariate and nest identity was included as a random factor. At each age, the initial model included the fixed factors (treatment and sex), the random factor (nest identity) and the covariate (hatch date). If the covariate or random factor were not significant, they were removed from the analysis.

Body composition (fat, lean mass, adult mass) and metabolic rates (SMR, PMR, metabolic scope) were analyzed using two-way ANOVAs with sex and treatment as between-subjects factors. Significant sex × treatment interactions were further analyzed by conducting ANOVAs for each sex with treatment as a fixed factor. Significant main effects of treatment were analyzed using LSD pairwise comparisons. Hatch date was added as a covariate and nest identity as a random factor for analyses of both metabolic rates and body composition, and body mass was included as a covariate for analyses of metabolic rates. The initial models included the fixed factors (treatment and sex), the random factor (nest identity) and the covariates (hatch date, body mass). If the covariates or random factor were not significant, they were removed from the analysis.

Finally, total adult body mass and lean body mass of the hand-raised birds was directly compared with the mass of their fathers using simple linear regressions. All tests were two-tailed and were considered significant at $P \leq 0.05$. Data are presented as means ± s.e.m., adjusted for significant covariates where applicable.

RESULTS

CORT levels

The exogenous CORT treatment was effective in significantly elevating plasma CORT levels (main effect of treatment: $F_{2,41.77}=84.79$, $P<0.001$). CORT levels 10 min post-administration of CORT or vehicle were higher in CORT-treated birds (day 10, 136.64±15.64 ng ml^{-1}; day 45, 143.35±14.48 ng ml^{-1}) than in controls (day 10, 6.76±1.70 ng ml^{-1}; day 45, 18.88±3.69 ng ml^{-1}; $P<0.001$) or food-restricted birds (day 10, 4.19±0.62 ng ml^{-1}; day 45, 28.24±4.45 ng ml^{-1}; $P<0.001$). Control and food-restricted birds did not differ significantly in plasma CORT levels ($P=0.71$). Therefore, our method of oral CORT administration was effective at increasing circulating CORT, and levels reached those typically observed in wild song sparrows subjected to an acute stressor (MacDougall-Shackleton et al., 2009; Schmidt et al., 2012). We also detected a significant main effect of age ($F_{1,42.11}=7.51$, $P=0.01$), as CORT levels were higher at day 45 than at day 10. No significant main effect of sex was detected ($F_{1,41.79}=1.06$, $P=0.31$), nor were any of the interaction terms significant ($P>0.40$ in all cases).

Nestling growth

To compare mass between nestlings at the start of the treatment period (day 7), we conducted an ANOVA with treatment and sex as fixed factors. The main effect of treatment was not significant at day 7 ($F_{2,47}=0.60$, $P=0.56$). Neither the main effect of sex ($F_{1,47}=2.86$, $P=0.10$) nor the treatment × sex interaction ($F_{2,47}=1.67$, $P=0.20$) were significant.

For the hand-rearing period (days 9–18), both the treatment × sex ($F_{2,40.07}=6.24$, $P=0.004$) and age × sex ($F_{9,182.601}=2.12$, $P=0.03$) interactions were significant (Fig. 2A,B). However, neither the

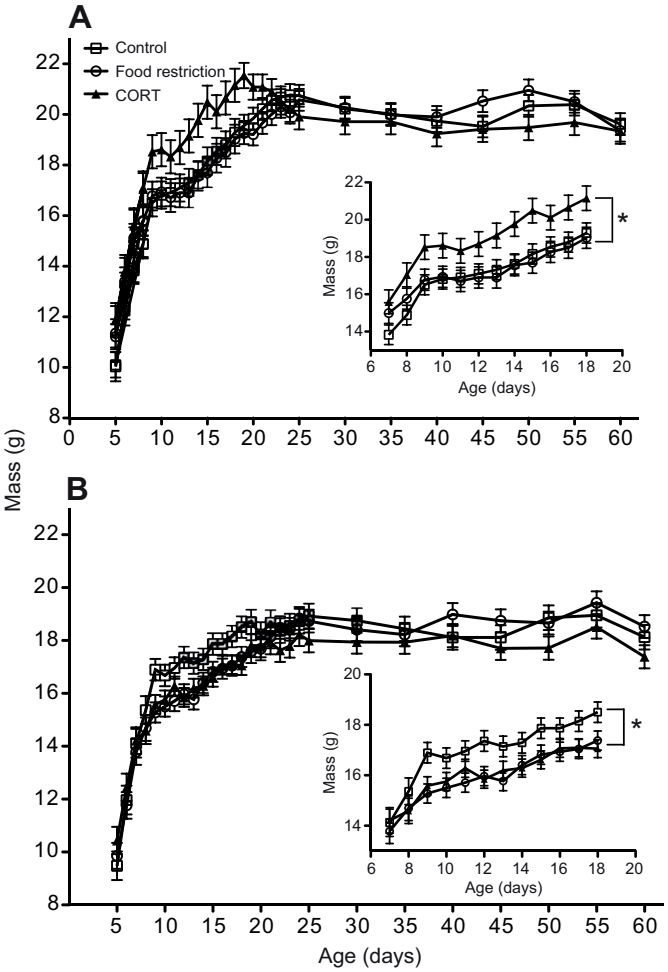

Fig. 2. The effect of food restriction or corticosterone (CORT) treatment on nestling growth rates in (A) male and (B) female song sparrows. Insets show mass of nestlings during the hand-rearing period (days 9 to 18), when treatments were most intense. The total treatment period (hand-rearing and post-fledging treatment) lasted from 7 to 60 days of age. *$P<0.05$.

treatment × sex × age nor the treatment × age interactions were significant (P>0.66 in both cases). The mass of nestlings prior to the treatment period was positively related to mass during the hand-rearing period ($F_{1,39.94}$=7.19, P=0.01, estimate of fixed effect=0.16, s.e.m.=0.06). To explore the treatment × sex interaction, we conducted linear mixed models for each sex with treatment and age as fixed factors. For males, the main effect of treatment was significant ($F_{2,19.02}$=3.98, P=0.04; Fig. 2A): CORT-treated males weighed more than control (P=0.03) and food-restricted (P=0.02) males, but control and food-restricted males did not differ (P=0.80). The mass of males prior to the treatment period was positively related to mass during the hand-rearing period ($F_{1,18.98}$=4.24, P=0.05, estimate of fixed effect=0.25, s.e.m.=0.12). For females, similar to males, the main effect of treatment was significant ($F_{2,20.08}$=4.58, P=0.02; Fig. 2B). However, control females weighed more than both food-restricted (P=0.01) and CORT-treated (P=0.02) females. Food-restricted and CORT-treated females did not differ (P=0.81). The mass of females prior to the treatment period was positively related to mass during the hand-rearing period ($F_{1,19.94}$=4.24, P=0.05, estimate of fixed effect=0.11, s.e.m.=0.05).

The second analysis examined the latter part of the treatment period (days 19–60), after food cups had been added to cages and birds began to feed independently. During this period, neither the treatment × age × sex interaction nor any of the two-way interactions were significant (P>0.10 in all cases). There was a significant main effect of sex ($F_{1,37.75}$=47.31, P<0.001): males were larger than females (Fig. 2A,B). The main effect of age was also significant ($F_{13,266.257}$=4.87, P<0.001). The main effect of treatment was not significant ($F_{2,37.78}$=0.86, P=0.43). The mass of nestlings prior to the treatment period was positively related to the mass of nestlings during the latter part of the treatment period ($F_{1,35.94}$=4.55, P=0.04, estimate of fixed effect=0.10, s.e.m.=0.05). Hatch date was also positively related to mass during this period ($F_{1,35.92}$=4.67, P=0.04, estimate of fixed effect=0.05, s.e.m.=0.02). Finally, paternal body mass was also a significant covariate ($F_{1,35.92}$=4.04, P=0.05, estimate of fixed effect=0.26, s.e.m.=0.13): heavier fathers had heavier offspring.

Body size

On day 25, after 18 days of experimental manipulation, the main effect of treatment on body size (PC scores) was not significant ($F_{2,47}$=1.23, P=0.30), nor was there a significant treatment × sex interaction ($F_{2,47}$=0.22, P=0.80). However, the main effect of sex was significant ($F_{1,47}$=31.93, P<0.001): males were larger than females (Fig. 3A). On day 45, after ~5 weeks of manipulation, the main effect of treatment on body size was significant ($F_{2,45}$=3.53, P=0.04): CORT-treated birds were smaller than control (P=0.02) and food-restricted birds (P=0.002), but control and food-restricted birds did not differ (P=0.37). Again, we observed a main effect of sex ($F_{2,45}$=21.64, P<0.001) such that males were larger than females (Fig. 3B), but the treatment × sex interaction was not significant ($F_{2,45}$=0.82, P=0.45). Last, in adulthood, the main effects of treatment ($F_{2,27}$=0.81, P=0.46) and sex ($F_{1,27}$=3.16, P=0.09; Fig. 3C) were not significant, nor was the treatment × sex interaction ($F_{2,27}$=0.37, P=0.69). Nest identity was significantly related to adult body size ($F_{14,27}$=3.16, P=0.005). Thus, the effects of our treatments on body size were limited to a period following rapid growth (day 45) and were no longer apparent by adulthood.

Relationship with paternal mass

Despite the fact that the experimental treatments altered nestling growth, we observed no long-term effects on adult body size,

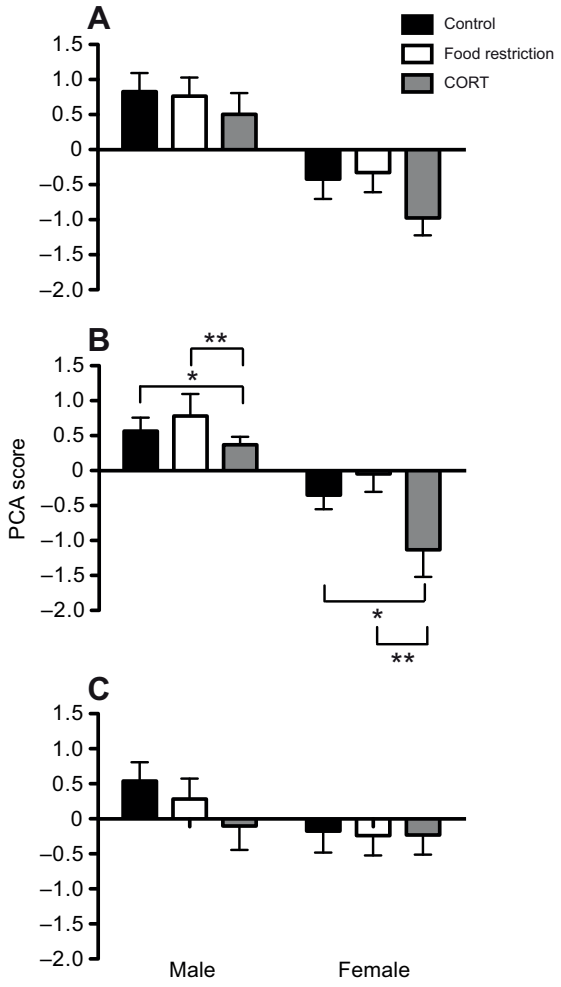

Fig. 3. The effect of food restriction or corticosterone (CORT) treatment on structural body size of song sparrows at (A) 25 days of age, (B) 45 days of age and (C) in adulthood. Body size scores are the results from principal component analysis (PCA) that included measures of body mass, tarsus and wing length. Results from the PCA can be found in Table 1. Treatments lasted from 7 days of age to 60 days of age. *P<0.05, **P<0.01.

suggesting that variation in final adult body size may primarily be due to heritable factors in song sparrows. To explore this possibility, we asked whether the adult mass of study subjects was related to the mass of their fathers. Paternal body mass was positively and significantly related to offspring body mass (r^2=0.11, P=0.03; Fig. 4A) and lean mass (r^2=0.23, P<0.001; Fig. 4B).

Body composition

For adult total body mass (Fig. 5A), the main effects of treatment ($F_{2,27}$=1.45, P=0.25) and sex ($F_{1,27}$=0.70, P=0.41) were not significant, nor was the treatment × sex interaction ($F_{2,27}$=0.78, P=0.47). Nest identity was significantly related to adult total body mass ($F_{14,27}$=3.51, P=0.003). For adult lean body mass (Fig. 5B), there was no significant main effect of treatment ($F_{2,27}$=1.50, P=0.24). However, the main effect of sex was significant ($F_{1,27}$=5.36, P=0.03): males had a higher lean mass than females (Fig. 5B). The treatment × sex interaction was not significant ($F_{2,27}$=1.23, P=0.31). Nest identity was significantly related to adult lean mass ($F_{14,27}$=2.11, P=0.05). For adult fat mass, the main effect of treatment was not significant ($F_{2,27}$=1.20, P=0.32; Fig. 5C). The main effect of sex was significant ($F_{1,27}$=5.73, P=0.02): females had a

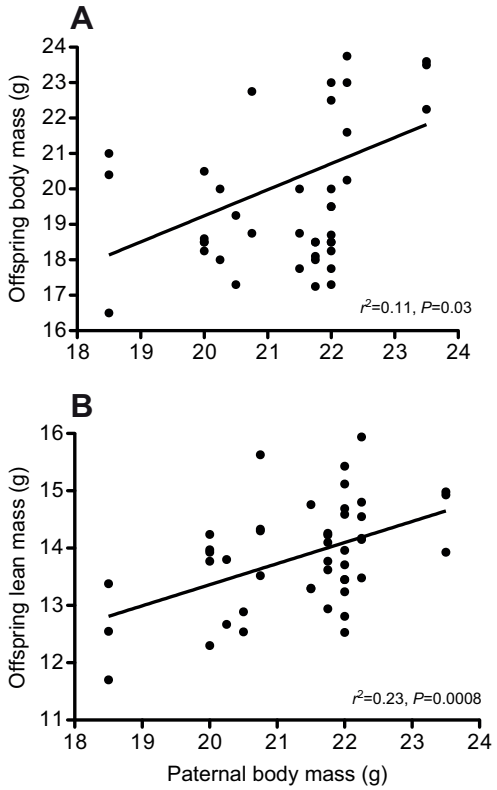

Fig. 4. Simple linear regressions showing the relationship between paternal body mass and (A) body mass and (B) lean mass of the experimental birds in adulthood. The father was assumed to be the resident male bird on the territory where a nest was located, and was caught prior to hatching.

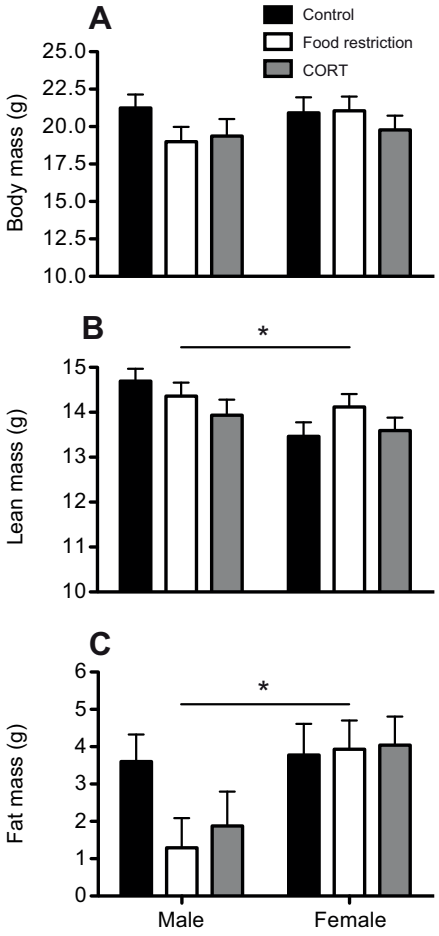

Fig. 5. The effect of food restriction or corticosterone (CORT) treatment on body composition of song sparrows, including (A) total body mass, (B) lean mass and (C) fat mass. Treatments lasted from 7 days of age to 60 days of age. Body composition analysis was conducted using quantitative magnetic resonance analysis when birds were ~7 months of age. *P<0.05.

higher fat mass than males (Fig. 5C). The treatment × sex interaction was not significant ($F_{2,27}$=1.06, P=0.36). Again, nest identity was significantly related to adult fat mass ($F_{14,27}$=3.87, P=0.001).

Metabolic rates

For SMR (Fig. 6A), body mass was a significant covariate ($F_{1,26}$=26.13, P<0.001) and nest identity was a significant random factor ($F_{14,26}$=2.19, P=0.02). The treatment × sex interaction was significant ($F_{2,26}$=4.36, P=0.02). To further analyze this interaction, we conducted ANOVAs for each sex with treatment as a fixed factor. For males, the main effect of treatment was not significant ($F_{2,8}$=0.72, P=0.52). For females, the main effect of treatment was significant ($F_{2,8}$=5.81, P=0.03). Control females had lower SMRs than food-restricted (P=0.009) and CORT-treated (P=0.04) females. The SMRs of food-restricted and CORT-treated females did not differ (P=0.34). For PMR (Fig. 6B), the main effects of treatment ($F_{2,26}$=0.92, P=0.41) and sex ($F_{1,26}$=0.35, P=0.56) were not significant, nor was the treatment × sex interaction ($F_{2,26}$=0.14, P=0.87). Nest identity was significantly related to PMR ($F_{14,27}$=2.11, P=0.05). For metabolic scope (Fig. 6C), the main effects of treatment ($F_{2,47}$=0.88, P=0.42) and sex ($F_{1,47}$=1.26, P=0.27) were not significant, nor was the treatment × sex interaction ($F_{2,47}$=0.05, P=0.96).

DISCUSSION

Food restriction affected growth and metabolic rates without increasing CORT

CORT levels did not differ between food-restricted and control subjects in our study. Therefore, food restriction might affect growth and metabolic rates independently of CORT, for example by directly altering organ morphology or cell number (Rinaudo and Wang, 2012). However, we cannot rule out the possibility that food restriction affects development by altering stress physiology. First, we only measured CORT levels at two ages (days 10 and 45). It is possible that food restriction affected CORT levels during a time in the treatment period when blood samples were not collected. Second, we only measured baseline plasma CORT levels. In European starlings (*Sturnus vulgaris*), exposure to an unpredictable food supply increased stress-induced CORT levels but not baseline levels (Buchanan et al., 2003). Last, there are many other factors that can influence the exposure of tissues to CORT, such as the level of corticosteroid binding globulins in the blood and the expression of corticosteroid receptors or enzymes that metabolize CORT in tissues (Schmidt et al., 2008).

CORT levels were manipulated for a relatively long period of time in our study (53 days). However, whereas other methods of hormone manipulation (e.g. silastic implants) constantly elevate hormone levels throughout the treatment period, our method of daily manipulation was transient and CORT levels began to decrease 30 min post-administration (determined during pilot study; see Materials and methods, Experimental treatments). In addition, in white-crowned sparrows, CORT levels returned to baseline 60 min

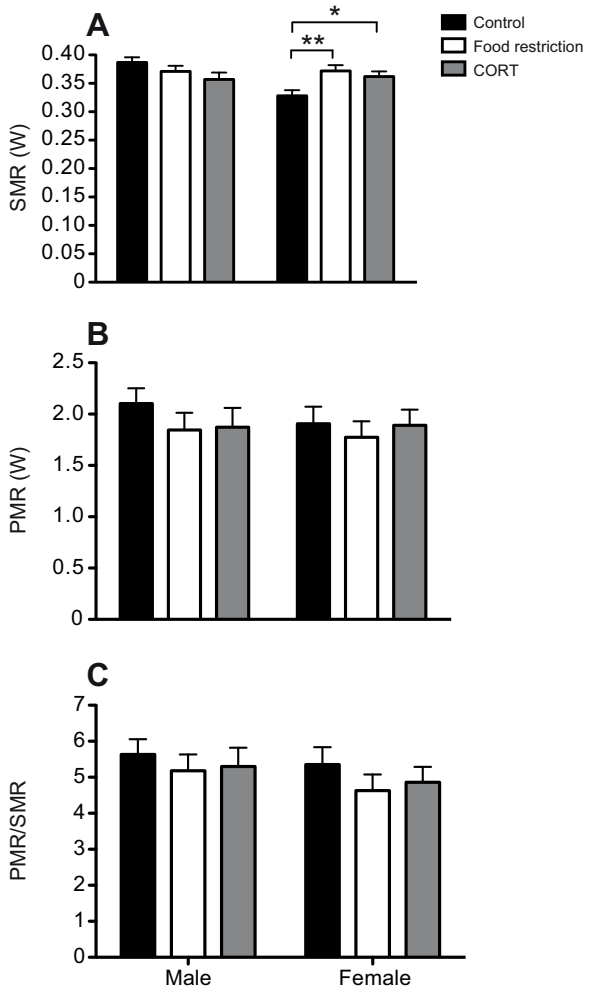

Fig. 6. The effect of food restriction or corticosterone (CORT) treatment on (A) standard metabolic rate, (B) peak metabolic rate and (C) metabolic scope of song sparrows. Treatments lasted from 7 days of age to 60 days of age. Metabolic rates were assessed when birds were ~7 months of age. *P<0.05, **P<0.01.

post-administration using a similar technique (Breuner et al., 1998). Therefore, total exposure to elevated CORT was limited to ~2 h per day in the hand-rearing period and ~1 h per day in the latter part of the treatment period. Our method of manipulation would thus be comparable to an individual living in an environment where they are frequently exposed to acute stressors, such as temporary food shortages or frequent encounters with predators. Frequent exposure to acute stressors may become chronically stressful to an individual over time (Clinchy et al., 2004). Indeed, a common paradigm for experiments looking at the physiological effects of chronic stress is to expose individuals to daily acute stressors over several days (e.g. Rich and Romero, 2005).

Developmental stress had sex-specific effects on nestling growth

There were profound sex differences in the effects of developmental stress on nestling growth rates. First, CORT-treated males weighed more than food-restricted and control males throughout the hand-rearing period. This finding is surprising because most studies have found that exposure to elevated glucocorticoid levels during development retards growth (Seckl, 1994; Spencer et al., 2003), although differences in the dose of CORT or method of administration might explain some of the variation between studies. This weight advantage disappeared shortly after nestlings begun feeding independently. Because CORT administration can increase begging rates in nestling birds (Kitaysky et al., 2001b) and we fed both control and CORT-treated birds to satiation, CORT-treated males may have begged more and been fed more throughout the hand-rearing stage of the experiment. Alternatively, instead of altering behavior and food intake, CORT may have increased anabolic processes. For example, in European starlings, CORT treatment *in ovo* accelerates pectoral muscle development, leading to enhanced flight performance (Chin et al., 2009). Glucocorticoids can also increase fat deposition (Asensio et al., 2004). If CORT accelerates growth in male nestlings and increases flight performance, it might decrease the age at which nestlings can fledge. Consistent with this, CORT increases locomotor activity (Breuner et al., 1998) and CORT levels increase prior to fledging or dispersal in many species (e.g. Belthoff and Dufty, 1998; Kern et al., 2001). If nestlings are raised in a poor-quality environment, premature fledging may be beneficial because it would allow a young bird to escape a stressful nest environment, for example if there was intense sibling competition in the nest or an abundance of ectoparasites. Similarly, environmental stressors, including food restriction and pond desiccation, have been shown to accelerate metamorphosis in spadefoot toads, *Scaphiopus hammondii* (Denver et al., 1998). In contrast to males, CORT-treated females in the present study weighed less than controls throughout the hand-rearing period. Similarly, early-life glucocorticoid exposure has been shown to retard growth in zebra finches (Spencer et al., 2003; Spencer and Verhulst, 2007) and humans (Seckl, 1994). Thus, it appears that the effects of glucocorticoids on growth rates are sex and age dependent.

Second, there were also sex differences in the effect of food restriction on nestling growth. Food-restricted males weighed the same as control males; however, food-restricted females weighed less than control females. This is in contrast to past studies in song sparrows (Kempster et al., 2007) and zebra finches (Spencer et al., 2003) in which food restriction decreased growth in both sexes. However, our results are consistent with a study of zebra finches that also found that food restriction decreased growth in females but not males (Martins, 2004). Thus, there may be sex differences in the amount of resources males and females allocate to body growth when exposed to early-life stressors. Males may allocate more resources to body growth at the expense of other systems (e.g. brain, immune system) in order to ensure survival to the fledgling stage. We are currently conducting studies to look at the effects of food restriction and CORT treatment on other physiological systems, which will hopefully shed light on the different trade-offs and strategies used by males and females when developing in a poor-quality environment.

Last, because larger nestlings may be fed more by parents and be more likely to fledge (Price and Ydenberg, 1995), the sex-specific effects of food restriction and CORT treatment on nestling growth could provide males with a competitive advantage over their female siblings when raised in a stressful environment (Zanette et al., 2005).

Body size in song sparrows may be a canalized trait

There were no effects of food restriction or CORT treatment on body size at day 25, but by day 45 CORT-treated birds were smaller than food-restricted and control birds. This was true for both females and males, despite the mass advantage that CORT-treated males exhibited during the hand-rearing period. Our PCA for body size

included three morphological measures: mass, wing length and tarsus length. Therefore, we interpret these PCA scores as measures of overall body size, but all three measures might not have been equally affected. CORT-treated birds may be structurally smaller because glucocorticoids can decrease bone formation (Delany et al., 1994). In addition, wing length is related to feather development, and CORT administration has been shown to impair feather growth in European starlings (Romero et al., 2005). Despite the effect on body size during the treatment period, there were no effects of either treatment on adult body size. Because our treatments lasted until day 60, this suggests that a young song sparrow may compensate for a bad rearing environment by accelerating growth once a stressor subsides even very late during development, well after full adult body size is normally attained. Adult body size may be a canalized trait in song sparrows, showing a large amount of stability even in the face of early-life perturbations [referred to as developmental homeostasis (Mitton and Grant, 1984)]. Therefore, variation in adult body size in song sparrows may be largely determined by variation in genotype, with less influence from environmental factors. In support of this, both adult body mass and lean mass of the experimental birds were significantly related to their father's body mass, and nest identity (natal brood of origin) was significantly related to adult body size. Because we hand-reared nestlings from day 3, the relationship between their mass and their father's mass would be largely due to a common genotype and not a common environment, although we cannot rule out the possibility that the environment before day 3 had strong carryover effects on offspring body size. This is in contrast to past studies that have found long-term effects of early-life stress on adult body size (e.g. Searcy et al., 2004). However, our results are consistent with findings from a wild population of song sparrows where morphological measurements of offspring were strongly related to their genetic parents, but not their foster parents (Smith and Dhondt, 1980; also see Merila and Sheldon, 2001).

Developmental stress did not alter body composition

There were no long-term effects of food restriction or CORT treatment on body composition (total body mass, lean mass or fat mass), despite the fact that both treatments altered nestling growth. In contrast, in humans, prenatal exposure to famine increases the risk of obesity (Ravelli et al., 1999) and a low birth rate is positively associated with obesity (Rinaudo and Wang, 2012). Catch-up growth may be a particularly important risk factor. For example, rat pups exposed to protein restriction *in utero* but then transferred to a high quality diet during the post-partum period exhibit rapid catch-up growth, resulting in a larger body mass and a higher percentage of body fat (Desai et al., 2005). In our study, both food-restricted and CORT-treated females exhibited growth retardation during the hand-rearing period, followed by a period of rapid growth during the latter stage of the treatment period. However, despite experiencing this period of rapid growth, we observed no effect on final body composition. We did observe sex differences in body composition. Males and females had similar total body mass in adulthood, but males had higher lean mass whereas females had higher fat mass.

Developmental stress had sex-specific effects on metabolic rates

The SMRs of birds in the present study were similar to those obtained for house sparrows, *Passer domesticus* (Buchanan et al., 2001), which are similar in size to song sparrows. The average PMR of flying birds is 16 times higher than the BMR (Hinds et al., 1993). Past studies in both red-eyed vireos, *Vireo olivaceus* (Pierce et al., 2005), and house sparrows (Chappell et al., 1999) using similar exercise wheels have obtained PMR values that were ~10 times higher than BMR. In the present study, PMR values were only approximately six times higher than SMR values. However, the former studies used wild-caught birds, not hand-reared birds, and prolonged periods of captivity can decrease aerobic capacity in birds (Buttemer et al., 2008). Alternatively, the fact that we may have measured SMR and not true BMR could also explain why metabolic scope was lower in the present study.

Both food-restricted and CORT-treated females had higher SMRs than control females. However, SMRs did not differ between males in the three treatment groups. This suggests that developmental stress has sex-specific effects on metabolic rates in song sparrows. Similarly, past studies in birds have found that variation in the rearing environment more strongly affects the metabolic rates of females than males. For example, zebra finch nestlings raised in experimentally enlarged broods have higher SMRs in adulthood, and this effect is stronger in females (Verhulst et al., 2006). In this species, individuals that experience catch-up growth are more likely to experience long-term effects on metabolic rates. For example, nestling zebra finches reared on a low protein diet during the early phase of the nestling period, but then transferred to a high protein diet for the latter part of the nestling period, exhibit catch-up growth and have higher SMRs in adulthood (Criscuolo et al., 2008). In this study, zebra finches reared on a low protein diet throughout the nestling period did not exhibit catch-up growth or an increase in metabolic rates. This suggests that variation in growth patterns during development may contribute to variation in metabolic rates in adulthood. In our study of song sparrows, both food restriction and CORT treatment decreased growth in females; however, in adulthood, there was no difference in body size or mass between the three treatment groups. Therefore, it is possible that the stress treatments had long-term effects on the SMRs of females because they altered normal growth patterns of females. In contrast to SMR, there was no effect of either experimental treatment on PMR or metabolic scope. Nest identity was significantly related to both PMR and SMR, suggesting that genetic factors also influence variation in metabolic rates in song sparrows. In the present study, time constraints prohibited us from taking more than one measurement of SMR or PMR. However, zebra finches exposed to CORT during development exhibited higher variability in SMR [although only during the treatment period (Spencer and Verhulst, 2008)]. Therefore, it may be of interest in future studies to look at the effects of developmental stress on variability in SMR or PMR.

Conclusions

In many species, variation in the early rearing environment can have profound effects on adult phenotype. In particular, exposure to stressors during development can permanently alter physiology and may predispose individuals to disease and negatively affect fitness (Mcmillen and Robinson, 2005; Monaghan, 2008). In the present study, both food restriction and CORT treatment had long-term effects on SMR in females but not males, suggesting that the long-term effects of early-life stress on physiology and fitness may be sex specific. This finding supports past research in zebra finches showing that females are more susceptible to early-life stressors than males (Verhulst et al., 2006; Martin, 2004). In addition, both food restriction and CORT treatment had sex-specific effects on nestling growth rates that exaggerated normal sex differences in nestling mass. This could give males a competitive advantage over their female siblings when being reared in a poor-quality environment

(e.g. Zanette et al., 2005). Future studies looking at the effects of developmental stress on other physiological systems (e.g. immune system, endocrine system) will help elucidate how males and females differentially allocate resources to growth and development when raised in a poor-quality environment.

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REFERENCES

Asensio, C., Muzzin, P. and Rohner-Jeanrenaud, F. (2004). Role of glucocorticoids in the physiopathology of excessive fat deposition and insulin resistance. *Int. J. Obes. Relat. Metab. Disord.* **28 Suppl. 4**, S45-S52.

Barker, D. J., Hales, C. N., Fall, C. H., Osmond, C., Phipps, K. and Clark, P. M. (1993). Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. *Diabetologia* **36**, 62-67.

Belthoff, J. R. and Dufty, A. M., Jr (1998). Corticosterone, body condition and locomotor activity: a model for dispersal in screech-owls. *Anim. Behav.* **55**, 405-415.

Breuner, C. W., Greenberg, A. L. and Wingfield, J. C. (1998). Noninvasive corticosterone treatment rapidly increases activity in Gambel's white-crowned sparrows (*Zonotrichia leucophrys gambelii*). *Gen. Comp. Endocrinol.* **111**, 386-394.

Buchanan, K. L., Evans, M. R., Goldsmith, A. R., Bryant, D. M. and Rowe, L. V. (2001). Testosterone influences basal metabolic rate in male house sparrows: a new cost of dominance signaling? *Proc. R. Soc. Lond. B* **268,** 1337-1344.

Buchanan, K. L., Spencer, K. A., Goldsmith, A. R. and Catchpole, C. K. (2003). Song as an honest signal of past developmental stress in the European starling (*Sturnus vulgaris*). *Proc. R. Soc. Lond. B* **270,** 1149-1156.

Buchanan, K. L., Leitner, S., Spencer, K. A., Goldsmith, A. R. and Catchpole, C. K. (2004). Developmental stress selectively affects the song control nucleus HVC in the zebra finch *Proc. R. Soc. Lond. B* **271,** 2381-2386.

Buttemer, W. A., Warne, S., Bech, C. and Astheimer, L. B. (2008). Testosterone effects on avian basal metabolic rate and aerobic performance: facts and artefacts. *Comp. Biochem. Physiol.* **150A**, 204-210.

Chappell, M. A., Bech, C. and Buttemer, W. A. (1999). The relationship of central and peripheral organ masses to aerobic performance variation in house sparrows. *J. Exp. Biol.* **202**, 2269-2279.

Chin, E. H., Love, O. P., Verspoor, J. J., Williams, T. D., Rowley, K. and Burness, G. (2009). Juveniles exposed to embryonic corticosterone have enhanced flight performance. *Proc. R. Soc. Lond. B* **276,** 499-505.

Clinchy, M., Zanette, L., Boonstra, R., Wingfield, J. C. and Smith, J. N. M. (2004). Balancing food and predator pressure induces chronic stress in songbirds. *Proc. Biol. Sci.* **271**, 2473-2479.

Crespi, E. J. and Denver, R. J. (2005). Roles of stress hormones in food intake regulation in anuran amphibians throughout the life cycle. *Comp. Biochem. Physiol.* **141A**, 381-390.

Criscuolo, F., Monaghan, P., Nasir, L. and Metcalfe, N. B. (2008). Early nutrition and phenotypic development: 'catch-up' growth leads to elevated metabolic rate in adulthood. *Proc. R. Soc. Lond. B* **275,** 1565-1570.

Delany, A. M., Dong, Y. and Canalis, E. (1994). Mechanisms of glucocorticoid action in bone cells. *J. Cell. Biochem.* **56**, 295-302.

Denver, R. J., Mirhadi, N. and Phillips, M. (1998). Adaptive plasticity in amphibian metamorphosis: response of *Scaphiopus hammondii* tadpoles to habitat desiccation. *Ecology* **79**, 1859-1872.

Desai, M., Crowther, N. J., Lucas, A. and Hales, C. N. (1996). Organ-selective growth in the offspring of protein-restricted mothers. *Br. J. Nutr.* **76**, 591-603.

Desai, M., Gayle, D., Babu, J. and Ross, M. G. (2005). Programmed obesity in intrauterine growth-restricted newborns: modulation by newborn nutrition. *Am. J. Physiol.* **288**, R91-R96.

Farrell, T. M., Weaver, K., An, Y. S. and MacDougall-Shackleton, S. A. (2011). Song bout length is indicative of spatial learning in European starlings. *Behav. Ecol.* **23**, 101-111.

Fernandez-Twinn, D. S. and Ozanne, S. E. (2006). Mechanisms by which poor early growth programs type-2 diabetes, obesity and the metabolic syndrome. *Physiol. Behav.* **88**, 234-243.

Gerson, A. R. and Guglielmo, C. G. (2011). House sparrows (Passer domesticus) increase protein catabolism in response to water restriction. *Am. J. Physiol.* **300**, R925-R930.

Griffiths, R., Double, M. C., Orr, K. and Dawson, R. J. (1998). A DNA test to sex most birds. *Mol. Ecol.* **7**, 1071-1075.

Guglielmo, C. G., McGuire, L. P., Gerson, A. R. and Seewagen, C. L. (2011). Simple, rapid, and non-invasive measurement of fat, lean, and total water masses of live birds using quantitative magnetic resonance. *J. Ornithol.* **152**, 75-85.

Hales, C. N. and Ozanne, S. E. (2003). The dangerous road of catch-up growth. *J. Physiol.* **547**, 5-10.

Harris, A. and Seckl, J. (2011). Glucocorticoids, prenatal stress and the programming of disease. *Horm. Behav.* **59**, 279-289.

Hennemann, W. W. (1983). Relationship among body mass, metabolic rate and the intrinsic rate of natural increase in mammals. *Oecologia* **56**, 104-108.

Hinds, D. S., Baudinette, R. V., MacMillen, R. E. and Halpern, E. A. (1993). Maximum metabolism and the aerobic factorial scope of endotherms. *J. Exp. Biol.* **182**, 41-56.

Jennings, B. J., Ozanne, S. E., Dorling, M. W. and Hales, C. N. (1999). Early growth determines longevity in male rats and may be related to telomere shortening in the kidney. *FEBS Lett.* **448**, 4-8.

Kempster, B., Zanette, L., Longstaffe, F. J., MacDougall-Shackleton, S. A., Wingfield, J. C. and Clinchy, M. (2007). Do stable isotopes reflect nutritional stress? Results from a laboratory experiment on song sparrows. *Oecologia* **151**, 365-371.

Kern, M., Bacon, W., Long, D. and Cowie, R. J. (2001). Possible roles for corticosterone and critical size in the fledging of nestling pied flycatchers. *Physiol. Biochem. Zool.* **74**, 651-659.

Kitaysky, A. S., Kitaiskaia, E. V., Wingfield, J. C. and Piatt, J. F. (2001a). Dietary restriction causes chronic elevation of corticosterone and enhances stress response in red-legged kittiwake chicks. *J. Comp. Physiol. B* **171**, 701-709.

Kitaysky, A. S., Wingfield, J. C. and Piatt, J. F. (2001b). Corticosterone facilitates begging and affects resource allocation in the black-legged kittiwake. *Behav. Ecol.* **12**, 619-625.

Lesage, J., Blondeau, B., Grino, M., Bréant, B. and Dupouy, J. P. (2001). Maternal undernutrition during late gestation induces fetal overexposure to glucocorticoids and intrauterine growth retardation, and disturbs the hypothalamo-pituitary adrenal axis in the newborn rat. *Endocrinology* **142**, 1692-1702.

Lighton, J. R. B. (2008). *Measuring Metabolic Rates: A Manual for Scientists*. New York: Oxford University Press.

MacDonald, I. F., Kempster, B., Zanette, L. and MacDougall-Shackleton, S. A. (2006). Early nutritional stress impairs development of a song-control brain region in both male and female juvenile song sparrows (*Melospiza melodia*) at the onset of song learning. *Proc. R. Soc. Lond. B* **273,** 2559-2564.

MacDougall-Shackleton, S. A., Dindia, L., Newman, A. E., Potvin, D. A., Stewart, K. A. and MacDougall-Shackleton, E. A. (2009). Stress, song and survival in sparrows. *Biol. Lett.* **5**, 746-748.

Manini, T. M. (2010). Energy expenditure and aging. *Ageing Res. Rev.* **9**, 1-11.

Martins, T. L. F. (2004). Sex-specific growth rates in zebra finch nestlings: a possible mechanism for sex ratio adjustment. *Behav. Ecol.* **15**, 174-180.

Mcmillen, I. C. and Robinson, J. S. (2005). Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. *Physiol. Rev.* **85**, 571-633.

Merila, J. and Sheldon, B. C. (2001). Avian quantitative genetics. In *Current Ornithology*, Vol. 16 (ed. V. Nolan Jr and C. F. Thompson), pp. 179-198. New York: Plenum Press.

Metcalfe, N. B. and Monaghan, P. (2001). Compensation for a bad start: grow now, pay later? *Trends Ecol. Evol.* **16**, 254-260.

Mitton, J. B. and Grant, M. C. (1984). Associations among protein heterozygosity, growth rate, and developmental homeostasis. *Annu. Rev. Ecol. Syst.* **15**, 479-499.

Monaghan, P. (2008). Early growth conditions, phenotypic development and environmental change. *Philos. Trans. R. Soc. Lond. B* **363**, 1635-1645.

Newman, A. E., Pradhan, D. S. and Soma, K. K. (2008). Dehydroepiandrosterone and corticosterone are regulated by season and acute stress in a wild songbird: jugular versus brachial plasma. *Endocrinology* **149**, 2537-2545.

Nowicki, S., Searcy, W. A. and Peters, S. (2002). Brain development, song learning and mate choice in birds: a review and experimental test of the "nutritional stress hypothesis". *J. Comp. Physiol. A* **188**, 1003-1014.

O'Regan, D., Kenyon, C. J., Seckl, J. R. and Holmes, M. C. (2004). Glucocorticoid exposure in late gestation in the rat permanently programs gender-specific differences in adult cardiovascular and metabolic physiology. *Am. J. Physiol.* **287**, E863-E870.

Painter, R. C., Roseboom, T. J. and Bleker, O. P. (2005). Prenatal exposure to the Dutch famine and disease in later life: an overview. *Reprod. Toxicol.* **20**, 345-352.

Pierce, B. J., McWilliams, S. R., O'Connor, T. P., Place, A. R. and Guglielmo, C. G. (2005). Effect of dietary fatty acid composition on depot fat and exercise performance in a migrating songbird, the red-eyed vireo. *J. Exp. Biol.* **208**, 1277-1285.

Potvin, D. A. and MacDougall-Shackleton, E. A. (2009). Parental investment amplifies effects of genetic complementarity on growth rates in song sparrows, *Melospiza melodia*. *Anim. Behav.* **78**, 943-948.

Potvin, D. A. and MacDougall-Shackleton, E. A. (2010). Paternal song complexity predicts offspring sex ratios close to fledging, but not hatching, in song sparrows. *Wilson J. Ornithol.* **122**, 146-152.

Price, E. R. and Guglielmo, C. G. (2009). The effect of muscle phospholipid fatty acid composition on exercise performance: a direct test in the migratory white-throated sparrow (*Zonotrichia albicollis*). *Am. J. Physiol.* **297**, R775-R782.

Price, K. and Ydenberg, R. (1995). Begging and provisioning in broods of asynchronously-hatched yellow-headed blackbird nestlings. *Behav. Ecol. Sociobiol.* **37**, 201-208.

Ravelli, A. C., van der Meulen, J. H., Michels, R. P., Osmond, C., Barker, D. J., Hales, C. N. and Bleker, O. P. (1998). Glucose tolerance in adults after prenatal exposure to famine. *Lancet* **351**, 173-177.

Ravelli, A. C., van Der Meulen, J. H., Osmond, C., Barker, D. J. and Bleker, O. P. (1999). Obesity at the age of 50 y in men and women exposed to famine prenatally. *Am. J. Clin. Nutr.* **70**, 811-816.

Rich, E. L. and Romero, L. M. (2005). Exposure to chronic stress downregulates corticosterone responses to acute stressors. *Am. J. Physiol.* **288**, R1628-R1636.

Rinaudo, P. and Wang, E. (2012). Fetal programming and metabolic syndrome. *Annu. Rev. Physiol* **74,** 107-130.

Romero, L. M., Strochlic, D. and Wingfield, J. C. (2005). Corticosterone inhibits feather growth: potential mechanism explaining seasonal down regulation of corticosterone during molt. *Comp. Biochem. Physiol.* **142A**, 65-73.
Root, T. L., O'Connor, T. P. and Dawson, D. R. (1991). Standard metabolic level and insulative characteristics of eastern house finches, *Carpodacus mexicanus*. *Physiol. Zool.* **64**, 1279-1295.
Schmidt, K. L., Pradhan, D. S., Shah, A. H., Charlier, T. D., Chin, E. H. and Soma, K. K. (2008). Neurosteroids, immunosteroids, and the Balkanization of endocrinology. *Gen. Comp. Endocrinol.* **157**, 266-274.
Schmidt, K. L., Furlonger, A. A., Lapierre, J. M., MacDougall-Shackleton, E. A. and MacDougall-Shackleton, S. A. (2012). Regulation of the HPA axis is related to song complexity and measures of phenotypic quality in song sparrows. *Horm. Behav.* **61**, 652-659.
Searcy, W. A., Peters, S. and Nowicki, S. (2004). Effects of early nutrition on growth rate and adult size in song sparrows *Melospiza melodia*. *J. Avian Biol.* **35**, 269-279.
Seckl, J. R. (1994). Glucocorticoids and small babies. *Q. J. Med.* **87**, 259-262.
Smith, J. N. M. and Dhondt, A. A. (1980). Experimental confirmation of heritable morphological variation in a natural population of song sparrows. *Evolution* **34**, 1155-1158.
Snoeck, A., Remacle, C., Reusens, B. and Hoet, J. J. (1990). Effect of a low protein diet during pregnancy on the fetal rat endocrine pancreas. *Biol. Neonate* **57**, 107-118.
Speakman, J. R. (2005). Body size, energy metabolism and lifespan. *J. Exp. Biol.* **208**, 1717-1730.
Spencer, K. A. and Verhulst, S. (2007). Delayed behavioral effects of postnatal exposure to corticosterone in the zebra finch (*Taeniopygia guttata*). *Horm. Behav.* **51**, 273-280.
Spencer, K. A. and Verhulst, S. (2008). Post-natal exposure to corticosterone affects standard metabolic rate in the zebra finch (*Taeniopygia guttata*). *Gen. Comp. Endocrinol.* **159**, 250-256.
Spencer, K. A., Buchanan, K. L., Goldsmith, A. R. and Catchpole, C. K. (2003). Song as an honest signal of developmental stress in the zebra finch (*Taeniopygia guttata*). *Horm. Behav.* **44**, 132-139.
Verhulst, S., Holveck, M. J. and Riebel, K. (2006). Long-term effects of manipulated natal brood size on metabolic rate in zebra finches. *Biol. Lett.* **2**, 478-480.
Welberg, L. A. and Seckl, J. R. (2001). Prenatal stress, glucocorticoids and the programming of the brain. *J. Neuroendocrinol.* **13**, 113-128.
West, B. T. (2009). Analyzing longitudinal data with the linear mixed models procedure in SPSS. *Eval. Health Prof.* **32**, 207-228.
Zambrano, E., Bautista, C. J., Deás, M., Martínez-Samayoa, P. M., González-Zamorano, M., Ledesma, H., Morales, J., Larrea, F. and Nathanielsz, P. W. (2006). A low maternal protein diet during pregnancy and lactation has sex- and window of exposure-specific effects on offspring growth and food intake, glucose metabolism and serum leptin in the rat. *J. Physiol.* **571**, 221-230.
Zanette, L., MacDougall-Shackleton, E. A., Clinchy, M. and Smith, J. N. M. (2005). Brown-headed cowbirds skew host offspring sex ratios. *Ecology* **86**, 815-820.