J Exp Biol Advance Online Articles. First posted online on 12 June 2012 as doi:10.1242/jeb.068965
Access the most recent version at http://jeb.biologists.org/lookup/doi/10.1242/jeb.068965

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

Kim L Schmidt[1, 2*], Elizabeth A MacDougall-Shackleton[1], Scott A MacDougall-Shackleton[1, 2, 3]

[1] Department of Biology, University of Western Ontario, London, Ontario, Canada
[2] Advanced Facility for Avian Research, University of Western Ontario, London, Ontario, Canada
[3] Department of Psychology, University of Western Ontario, London, Ontario, Canada

Short title: Effect of developmental stress on growth and metabolism

[*] Corresponding Author:
Kim Schmidt
kschmi5@uwo.ca
Dept. of Biology, Advanced Facility for Avian Research
University of Western Ontario
London, ON, N6A 5B8
Phone: 519-661-2111 ext 84646

Summary

Variation in the pre- 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 a songbird, the song sparrow (*Melospiza melodia*). Nestlings were hand-raised in captivity from 3 days of age (d3). Treatments (*ad libitum* food, food restriction, or CORT-treatment) lasted from d7–d60. 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 rates (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 rates. 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.

Keywords: aerobic capacity, basal metabolic rate, bird, body composition, glucocorticoid, metabolic scope, peak metabolic rate, plasticity, songbird, standard metabolic rate, stress

1. Introduction

Variation in the pre- 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, 2011; 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 weight 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, 2011). 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 what stage of development 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 to 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 these studies, the effect of the stressor on metabolic rates was more severe in females than 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 inter-specific level, variation in metabolic rates may mediate important tradeoffs between reproduction and survival. However, whether or not 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, 2011). The stressor may directly alter the development of an organ resulting in permanent changes in organ morphology or function. For example, pre 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 affect 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., 2001), 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 i) nestling growth and adult ii) body size, iii) body composition, and iv) metabolic rates in song sparrows (*Melospiza melodia)*. We used CORT treatment to determine whether glucocorticoids have similar effects as food restriction on growth and physiology. Since a variety

of stressors increase glucocorticoid levels, this allowed us to determine if 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 if 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 if 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 standard metabolic rates (SMR) and peak metabolic rates (PMR). 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 if 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.

2. Methods

2.1 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 May 9th – June 7th 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 had morphological measurements collected (see below) prior to nests hatching, in April and May 2010. Since extra-pair paternity is infrequent in this study population (consistently below 10% of nestlings; Potvin and MacDougall-Shackleton, 2009; EAMS 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 (d3-d4), and 4 were brought in at ~d7 (mean=3.44 days, SEM=0.16; Table 1).

Nestlings were kept warm using heat lamps and electric heating pads until they developed feathers (~d7), 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 (~d25), at which point they were housed individually. Birds were kept on a long day photoperiod (16L:8D) until August 16th, 2010, then switched to short days (10L:14D) for the remainder of the experiment. Sex of nestlings was determined using polymerase chain reaction (PCR) amplification of genes on the sex chromosomes (Griffiths et al., 1998). Amplification and electrophoresis conditions are described elsewhere (Potvin and MacDougall-Shackleton, 2010).

2.2 Experimental Treatments

Within each brood, nestlings were assigned to one of the three treatment groups (control, 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 and therefore to control for nest of origin as best as possible. In total, there were 16 control subjects (9 males, 7 females), 16 food-restricted subjects (8 males, 8 females) and 15 CORT-treated subjects (6 males, 9 females; Table 1). Food restriction and CORT treatment lasted from d7-d60 (see Fig 1 for timeline).

All nestlings received a standard hand-rearing diet administered via 1mL syringes. The diet consisted of ground Mazuri Small Bird Maintenance diet (56A6), hard-boiled chicken eggs (shells removed), wheat germ, water, and Prime avian vitamin supplement (Rolf C. Hagen Inc, Montreal, QC). 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 d18. 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 (catalogue number 56A6) and premium budgie seed (Rolf C. Hagen Inc, Montreal, QC). In order to continue the food restriction stressor into the fledgling

period, we removed food cups for 3 h per day until d60 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 orally administered 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 affects nestling growth, brain development, and song learning (Buchanan et al., 2004; Spencer et al., 2003). We used a dose of 0.87 μg/g body weight, 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 d60 (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 μg/g body weight) 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 (n=4, 4.16 ± 2.38), peaked 10 min post-ingestion (n=3, 173.13 ± 51.40 ng/mL) and had begun to decrease after 30 min (n=4, 61.58 ± 9.35 ng/mL). Because peak CORT levels were slightly higher than CORT levels post-restraint in our population (MacDougall-Shackleton et al., 2009; Schmidt et al., 2012), we used a slightly lower dose of 0.87 μg/g body weight 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 d10 and d45, 10 min after administration of CORT or vehicle to determine plasma CORT levels. CORT was quantified in unextracted plasma using a radioimmunoassay (MP Biomedicals, 07-120103) 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 – 2.6 ng/mL. Inter-assay variation was 5.5% for a low control and 4.1% for a high control. Intra-assay variation was 9.4% for the low control and 3.9% for the high control.

2.3 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 (5:30 AM) until d25. Thereafter, we measured body mass every 5 days until d60. Adult body mass (~ 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 d25, d45, and during adulthood prior to SMR measurements.

2.4 Body Composition Analysis

We determined lean and fat mass using quantitative magnetic resonance (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, Texas) was custom-designed for use with small birds and bats. The QMR was calibrated daily using 5 g and 94 g canola oil standards. To use the QMR, awake birds were placed into plastic holding tubes and inserted into the QMR analyzer and scanned using the “small bird” and “two 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 x 0.94; lean mass: raw value x 1.021, Gerson and Guglielmo, 2011; Guglielmo et al., 2011). Validation studies conducted previously show 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).

2.5 Respirometry

Standard Metabolic Rates

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=214 days, SEM=0.88), which was about 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 5 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 standard metabolic rates (SMRs) instead of basal metabolic rates (BMRs). Incurrent air was scrubbed of CO_2 and water vapor using soda lime and Drierite, respectively. The five sealed chambers received a constant flow of 450 mL/min. Excurrent air was sub-sampled at 150 mL/min and passed through a Drierite column to the CO_2 analyzer (catalogue number: CA-2A; Sable Systems Las Vegas, NV) 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). Using a multiplexer (Sable Systems), one chamber was measured at a time for 10 minutes before switching to the next chamber. In total, each bird was measured 12 times throughout the night for 10 minutes 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 analysis was done using Warthog Systems Lab Analyst software (M.A. Chappel, University of California Riverside). SMR values reported were calculated as the minimum 10 min mean of O_2 consumption throughout the measurement period. We calculated VO_2 (based on calculations in Lighton, 2008; p 112, equation 10.6) and converted VO_2 to watts (W). The equation that we used to calculate VO_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.

Peak Metabolic Rates

The same flow system used to determine SMRs 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 (width=16 cm; diameter=24 cm) 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 and was sub-sampled as described above for measurements of SMR. Food dishes were removed 3 h before testing to insure birds were in the post-absorptive state. Beginning at 11:00, and no later than 14:00, 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 several 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 of each individual (PMR/SMR), which provides an estimate of intensity of exercise (Pierce et al., 2005).

2.7 Data Analysis

Statistical analyses were conducted using SPSS version 19. For CORT levels, we conducted linear mixed models using restricted maximum likelihood (REML) 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 d9-d18, that is, throughout the hand-rearing period. We expected the treatments to most strongly affect growth during this period since 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 d19-d60, 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 x 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 (d9-d18 and d19-d60) 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.

To compare the effects of the treatments on body size, we analyzed mass, tarsus, and wing length using a principal component analysis (PCA) at each age (d25, d45, adulthood), since 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 x 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 to the mass of their fathers using simple linear regressions. All tests were two-tailed and were considered significant for $p \leq 0.05$. Data are presented as mean ±SEM, adjusted for significant covariates where applicable.

3. Results

3.1. 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 (d10=136.64 ± 15.64; d45 = 143.35 ± 14.48) than controls (d10=6.76 ± 1.70; d45=18.88 ± 3.69; $p<0.001$) or food-restricted birds (d10=4.19 ± 0.62; d45=28.24 ± 4.45; $p<0.001$). 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 d45 than d10. 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).

3.2 Nestling Growth

To compare mass between nestlings at the start of the treatment period (d7), we conducted an ANOVA with treatment and sex as fixed factors. The main effect of treatment was not significant at d7 ($F_{2,47}=0.60$, $p=0.56$). Neither the main effect of sex ($F_{1,47}=2.86$, $p=0.10$) nor the treatment x sex interaction ($F_{2,47}=1.67$, $p=0.20$) were significant.

For the hand-rearing period (d9-d18), the treatment x sex ($F_{2,40.07}$=6.24, p=0.004) and the age x sex ($F_{9,182.601}$=2.12, p=0.03) interactions were significant (Fig 2A and 2B). Neither the treatment x sex x age nor the treatment x 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.=0.06). To explore the treatment x 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. 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.=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.=0.05).

The second analysis examined the latter part of the treatment period (d19-d60), after food cups had been added to cages and birds began to feed independently. During this period, neither the treatment x age x 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 and 2B). 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.=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.=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.=0.13); heavier fathers had heavier offspring.

3.3 Body Size

On d25, 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 x

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 d45, after about 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). 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 no treatment x sex interaction ($F_{2,45}$=0.82, p=0.45). Last, in adulthood neither the main effect of treatment ($F_{2,27}$=0.81, p=0.46) nor sex ($F_{1,27}$=3.16, p=0.09; Fig 3C) were significant. We observed no significant treatment x 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 (d45) and were no longer apparent by adulthood.

3.4 Relationship to Paternal Mass

Despite the fact that the experimental treatments altered nestling growth, we observed no long-term effects on adult body size, suggesting that variation in final adult body size may primarily be due to heritable factors in song sparrows. To explore this possibility, we asked if 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).

3.5 Body Composition

For adult total body mass (Fig 5A), neither the main effect of treatment ($F_{2,27}$=1.45, p=0.25) nor sex ($F_{1,27}$=0.70, p=0.41) was significant, nor was the treatment x sex interaction significant ($F_{2,27}$=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 x 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 (Fig 5C), the main effect of treatment was not significant ($F_{2,27}$=1.20, p=0.32). The main effect of sex was significant ($F_{1,27}$=5.73, p=0.02); females had a higher fat mass than males (Fig 5C). The treatment x 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).

3.6 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 x 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 PMRs (Fig 6B), the main effects of neither treatment ($F_{2,26}$=0.92, p=0.41), nor sex ($F_{1,26}$=0.35, p=0.56) were significant. The treatment x sex interaction was also not significant ($F_{2,26}$=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), neither the main effect of treatment ($F_{2,47}$=0.88, p=0.42), nor sex ($F_{1,47}$=1.26, p=0.27) were significant. The treatment x sex interaction was also not significant ($F_{2,47}$=0.05, p=0.96).

4. Discussion

4.1 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, 2011). 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 (d10 and d45). 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 (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

456 elevate hormone levels throughout the treatment period, our method of daily manipulation was
457 transient and CORT levels begun to decrease 30 min post-administration (determined during
458 pilot study, see Methods Section 2.2). In addition, in white-crowned sparrows CORT levels
459 returned to baseline 60 min post-administration using a similar technique (Breuner et al., 2008).
460 Therefore, total exposure to elevated CORT was limited to about 2 h per day in the hand-rearing
461 period and about 1 h per day in the latter part of the treatment period. Our method of
462 manipulation would thus be comparable to an individual living in an environment where they are
463 frequently exposed to acute stressors, such as temporary food shortages or frequent encounters
464 with predators. Frequent exposure to acute stressors may become chronically stressful to an
465 individual over time (Clinchy et al., 2004). Indeed, a common paradigm for experiments looking
466 at the physiological effects of chronic stress is to expose individuals to daily acute stressors over
467 several days (e.g. Rich and Romero, 2004).

468 ***4.2 Developmental Stress had Sex-Specific Effects on Nestling Growth***

469 There were profound sex differences in the effects of developmental stress on nestling
470 growth rates. First, CORT-treated males weighed more than food-restricted and control males
471 throughout the hand-rearing period. This finding is surprising because most studies have found
472 that exposure to elevated glucocorticoid levels during development retards growth (Seckl, 1994;
473 Spencer et al., 2003), although differences in the dose of CORT or method of administration
474 might explain some of the variation between studies. This weight advantage disappeared shortly
475 after nestlings begun feeding independently. Because CORT administration can increase begging
476 rates in nestling birds (Kitaysky et al., 2001b) and we fed both control and CORT-treated birds to
477 satiation, CORT-treated males may have begged more and been fed more throughout the hand-
478 rearing stage of the experiment. Alternatively, instead of altering behavior and food intake,
479 CORT may have increased anabolic processes. For example, in European starlings (*Sturnus*
480 *vulgaris*), CORT treatment *in ovo* accelerates pectoral muscle development leading to enhanced
481 flight performance (Chin et al., 2009). Glucocorticoids can also increase fat deposition (Asensio
482 et al., 2004). If CORT accelerates growth in male nestlings and increases flight performance, it
483 might decrease the age at which nestlings can fledge. Consistent with this, CORT increases
484 locomotor activity (Breuner et al., 1998) and CORT levels increase prior to fledging or dispersal
485 in many species (e.g. Belthoff and Duffy, 1998; Kern et al., 2001). If nestlings are raised in a
486 poor quality environment, premature fledging may be beneficial since 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, accelerate metamorphosis in spadefoot toads (*Scaphiopus hammondii;* Denver et al., 1998). In contrast to males, CORT-treated females weighed less than controls throughout the hand-rearing period. Similarly, early-life glucocorticoid exposure retards 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, since 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).

4.3 Body Size in Song Sparrows may be a Canalized Trait

There were no effects of food restriction or CORT treatment on body size at d25, but by d45 CORT-treated birds were smaller than food-restricted and control birds. This was true for both females and males, despite the weight advantage that CORT-treated males exhibited during the hand-rearing period. Our PCA for body size included three morphological measures (mass, wing, tarsus). 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 impairs 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. Since our treatments lasted until d60 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. Since we hand-reared nestlings from d3, 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 d3 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 (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 review by Merila and Sheldon, 2001).

4.4 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, 2011). 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

549 rapid growth during the latter stage of the treatment period. However, despite experiencing this
550 period of rapid growth, we observed no effect on final body composition. We did observe sex
551 differences in body composition. Males and females had similar total body mass in adulthood,
552 but males had higher lean mass, while females had higher fat mass.

553 ***4.5 Developmental Stress had Sex-Specific Effects on Metabolic Rates***

554 The SMRs of birds in the current study were similar to those obtained for house sparrows
555 *(Passer domesticus;* Buchanan et al., 2001), which are similar in size to song sparrows. The
556 average PMR of flying birds is 16 times higher than the BMR (Hinds et al., 1993). Past studies in
557 both red-eyed vireos (*Vireo olivaceus;* Pierce et al., 2005) and house sparrows (Chappell et al.,
558 1999) using similar exercise wheels have obtained PMR values that were ~10 times higher than
559 BMR. In the current study, PMR values were only ~6 times higher than SMR values. However,
560 the former studies used wild-caught birds, not hand-reared birds, and prolonged periods of
561 captivity can decrease aerobic capacity in birds (Buttemer et al., 2008). Alternatively, the fact
562 that we may have measured SMR and not true BMR could also explain why metabolic scope was
563 lower in the present study.

564 Both food-restricted and CORT-treated females had higher SMRs than control females.
565 However, SMRs did not differ between males in the three treatment groups. This suggests that
566 developmental stress has sex-specific effects on metabolic rates in song sparrows. Similarly, past
567 studies in birds have found that variation in the rearing environment more strongly affects the
568 metabolic rates of females than males. For example, zebra finch nestlings raised in
569 experimentally enlarged broods have higher SMRs in adulthood, and this effect is stronger in
570 females (Verhulst et al., 2006). In this species, individuals who experience catch-up growth are
571 more likely to experience long-term effects on metabolic rates. For example, nestling zebra
572 finches reared on a low protein diet during the early phase of the nestling period, but then
573 transferred to a high protein diet for the latter part of the nestling period, exhibit catch-up growth
574 and have higher SMRs in adulthood (Criscuolo et al., 2008). In this study, zebra finches reared
575 on a low protein diet throughout the nestling period did not exhibit catch-up growth nor an
576 increase in metabolic rates. This suggests that variation in growth patterns during development
577 may contribute to variation in metabolic rates in adulthood. In our study of song sparrows, both
578 food restriction and CORT treatment decreased growth in females, however in adulthood there
579 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 current 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 SMRs or PMRs.

4.6 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 current 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.

Acknowledgements

We thank Alex Gerson and Brendan McCabe for technical assistance, Ainsley Furlonger, Janet Lapierre, and Shawn Kubli for help with fieldwork, Dr. Elizabeth Hampson for assistance with radioimmunoassays, and Dr. C. Guglielmo and two anonymous reviewers for comments on the manuscript. We are grateful to Dr. Alexandra Hernandez for advice on hand-rearing nestlings.

Finally, we thank The Queen's University Biological Station for allowing us to access the field site.

Funding

This research was supported by grants from The Natural Sciences and Engineering Research Council of Canada (NSERC) to S.A.M.-S. and E.A.M.-S. and a NSERC Canada Graduate Scholarship and a Queen Elizabeth II Graduate Scholarship in Science and Technology to K.L.S.

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,** S45-52.

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. Biol.* **268,** 1337-1344.

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. Biol.* **271,** 2381-2386.

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. Biol.* **270,** 1149-1156.

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. A. Mol. Integr. Physiol.* **150,** 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. Biol.* **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. R. Soc. Lond. B* **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. A. Mol. Integr. Physiol.* **141,** 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. Biol.* **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. Regul. Integr. Comp. Physiol.* **288,** 91-96.

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*. In press

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. Regul. Integr. Comp. Physiol.* **300,** 925-930.

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.* **75,** 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., Breant, 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 Mannual for Scientists.* Oxford University Press. New York, N.Y., USA, 2008.

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. Biol.* **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.

Martin, 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.

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

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.

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. *Phil. Trans. R. Soc. Lond. Biol.* **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. Endocrinol. Metab.* **287,** 863-870.

Painter, R.C., Roseboom, T.J., 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. (2010). Paternal song complexity predicts offspring sex ratios close to fledging, but not hatching, in song sparrows. *Wilson J. Ornithol.* **122,** 146-152.

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.

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. Regul. Integr. Comp. Physiol.* **297,** 775-782.

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. Regul. Integr. Comp. Physiol.* **288**, 1628-1636.

Rinaudo, P. and Wang, E. (2011). Fetal programming and metabolic syndrome. *Annu. Rev. Physiol* **74**: 3.1-3.24, 2011.

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. A. Mol. Integr. Physiol.* **142,** 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., Furlonger, A.A., Lapierre, J.M., MacDougall-Shackleton, E.A., 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.

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 endocriniology. *Gen. Comp. Endocrinol.* **157**, 266-274.

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., 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.

Spencer, K. A., Buchanan, K. L., Goldsmith, A. R. and Catchpole, C. K. (2004). Developmental stress, social rank and song complexity in the European starling *(Sturnus vulgaris*). *Proc. R. Soc. Lond. Biol.*. **271 Suppl 3,** S121-S123.

Spencer, K. A., Evans, N. P. and Monaghan, P. (2009). Postnatal stress in birds: a novel model of glucocorticoid programming of the hypothalamic-pituitary-adrenal axis. *Endocrinology* **150,** 1931-1934.

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.

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., Deas, M., Martinez-Samayoa, P. M., Gonzalez-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.

Figure Legends

Figure 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.

Figure 2. The effect of food restriction (Food Res) or corticosterone (CORT) treatment on nestling growth rates in male (A) and female (B) 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 days of age to 60 days of age. *$p<0.05$

Figure 3. The effect of food restriction or corticosterone (CORT) treatment on structural body size of song sparrows at 25 days of age (A), 45 days of age (B) and in adulthood (C). Body size scores are the results from principal component analyses (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. *$p<0.05$, **$p<0.01$.

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

Figure 5. The effect of food restriction or corticosterone (CORT) treatment on body composition of song sparrows including total body mass (A), lean mass (B), and fat mass (C). 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. *$p<0.05$

Figure 6. The effect of food restriction or corticosterone (CORT) treatment on standard metabolic rates (A), peak metabolic rates (B) and metabolic scope (C) 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. *$p<0.05$, **$p<0.01$

Table 1. The Age and Mass of 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

Note: Age at capture and mass at capture represent the age and mass of nestlings the day they were brought into captivity. Values represent means ± SEM. CORT = corticosterone

Table 2. Principal Component Analysis for Morphological Measurements

	Eigenvalue	% Variance Explained	Factor Loadings		
			Mass	***Tarsus***	***Wing***
day 25 PC1	1.71	56.86	0.83	0.74	0.69
day 45 PC1	1.71	56.89	0.77	0.74	0.75
Adult PC1	1.83	61.02	0.75	0.87	0.72

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

Figure 1

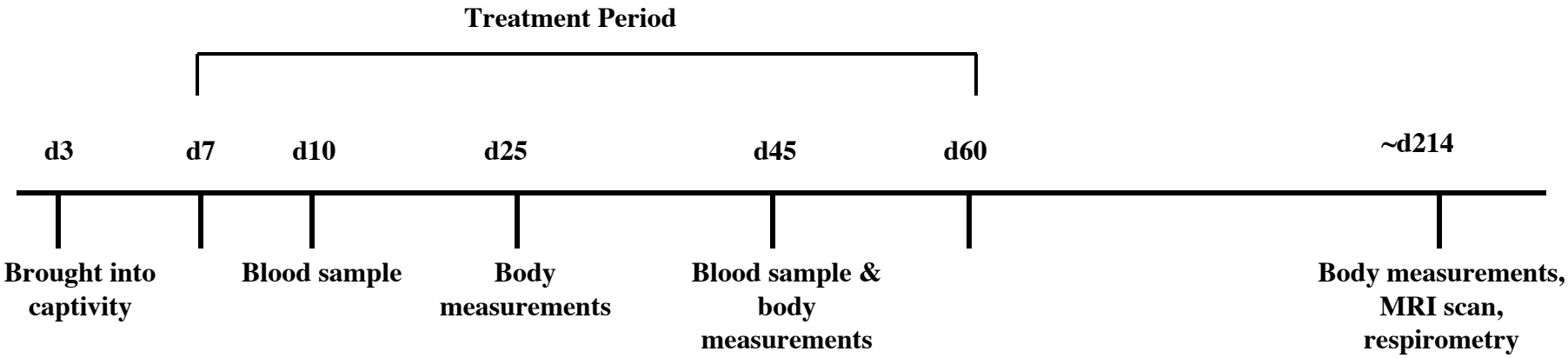


Figure 2

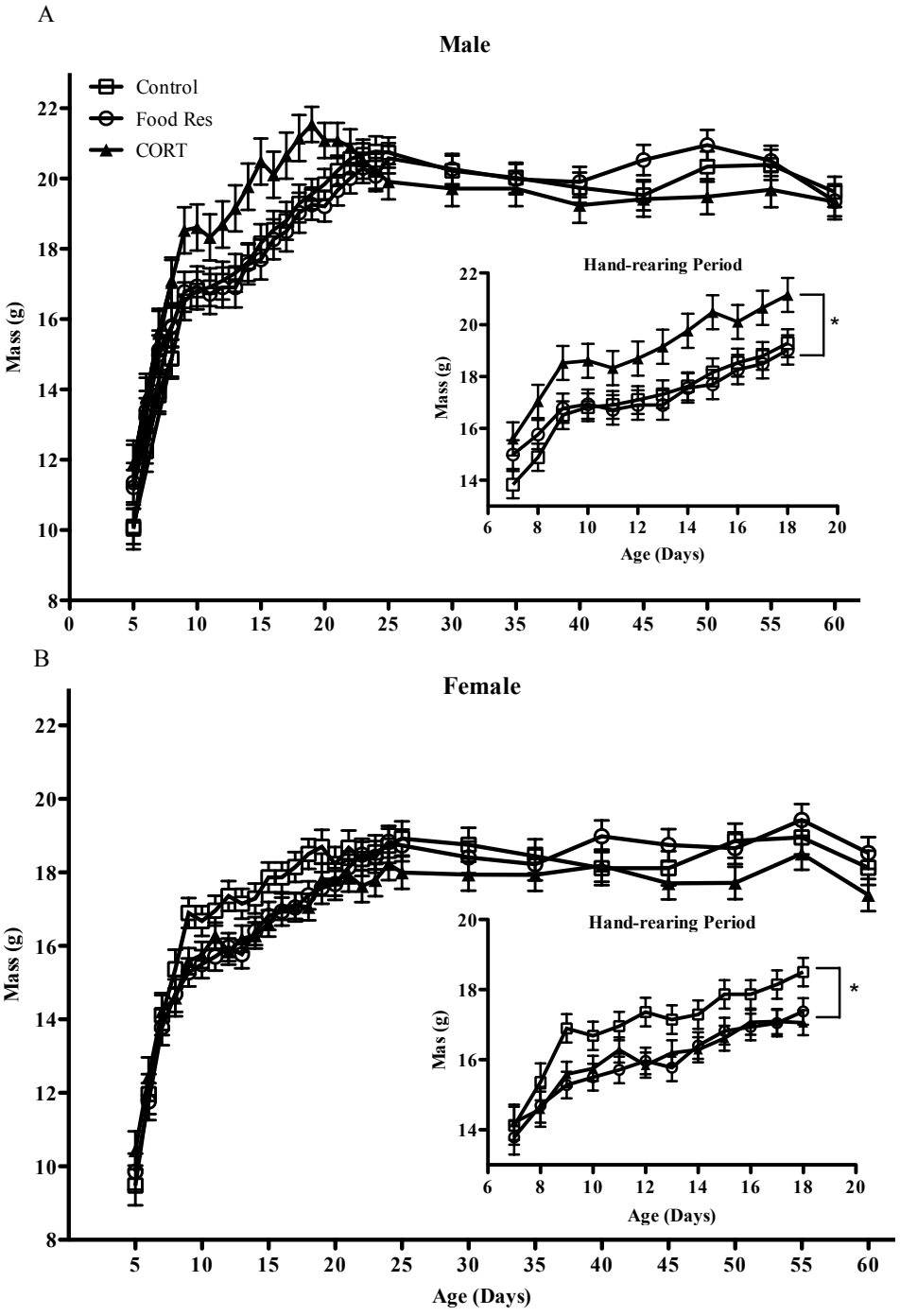


Figure 3

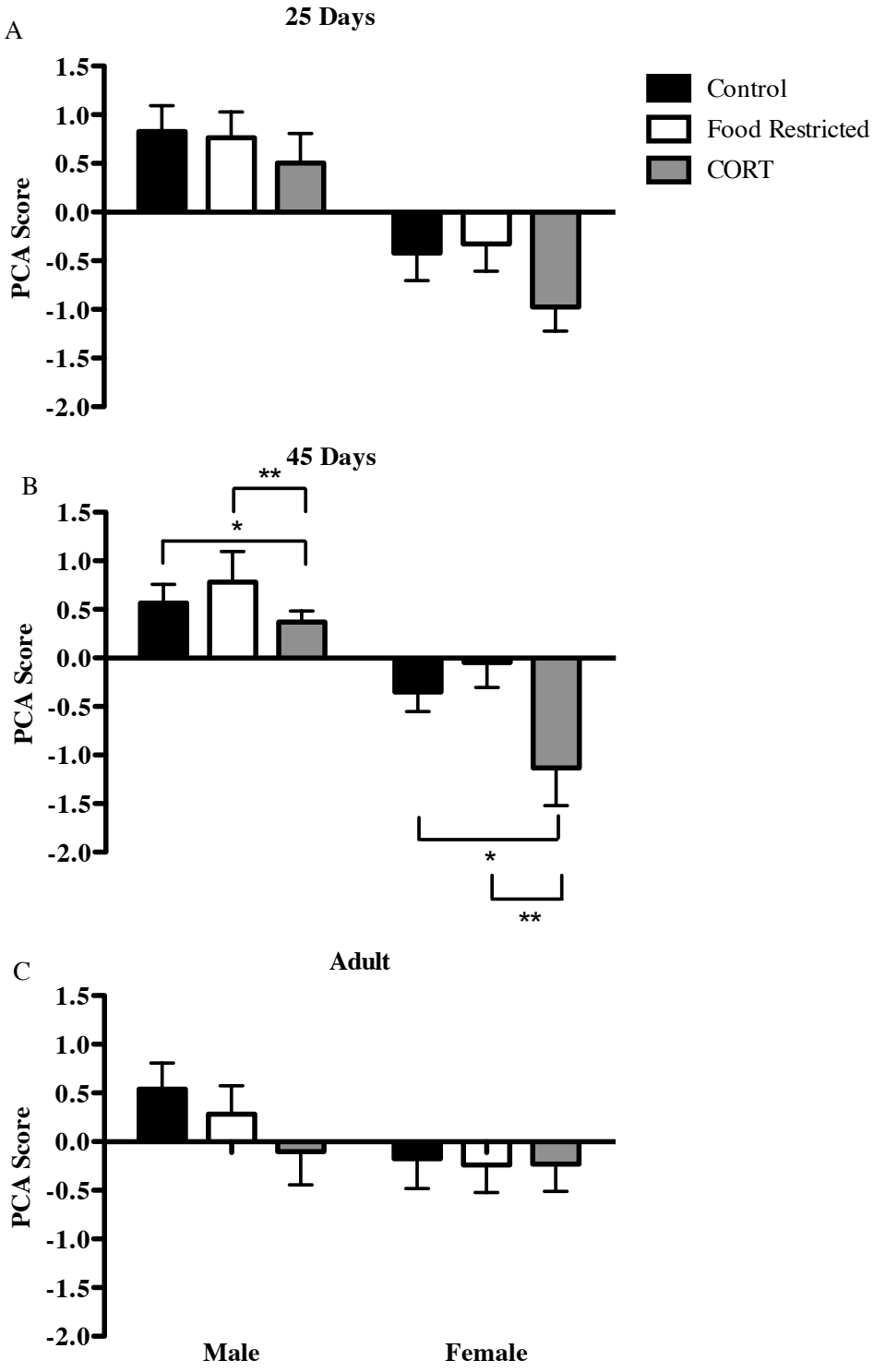
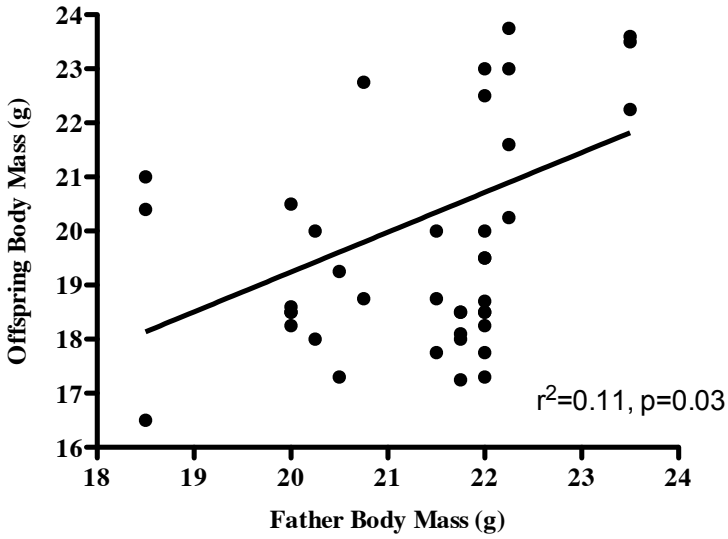


Figure 4

A



B

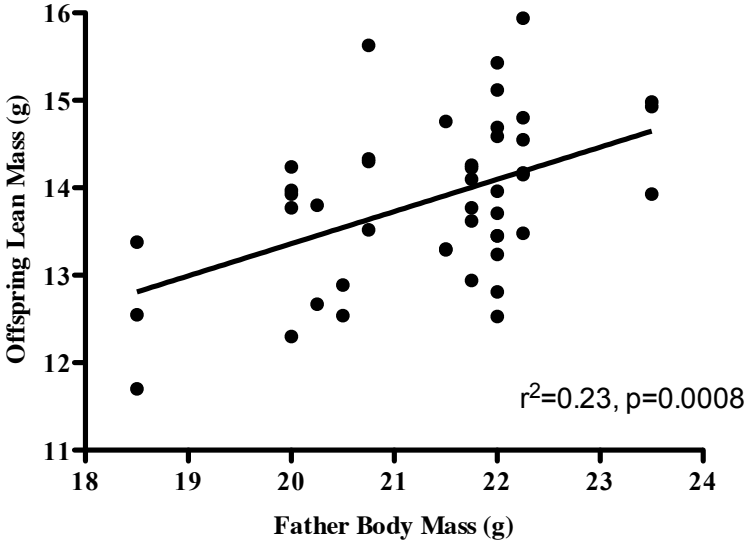


Figure 5

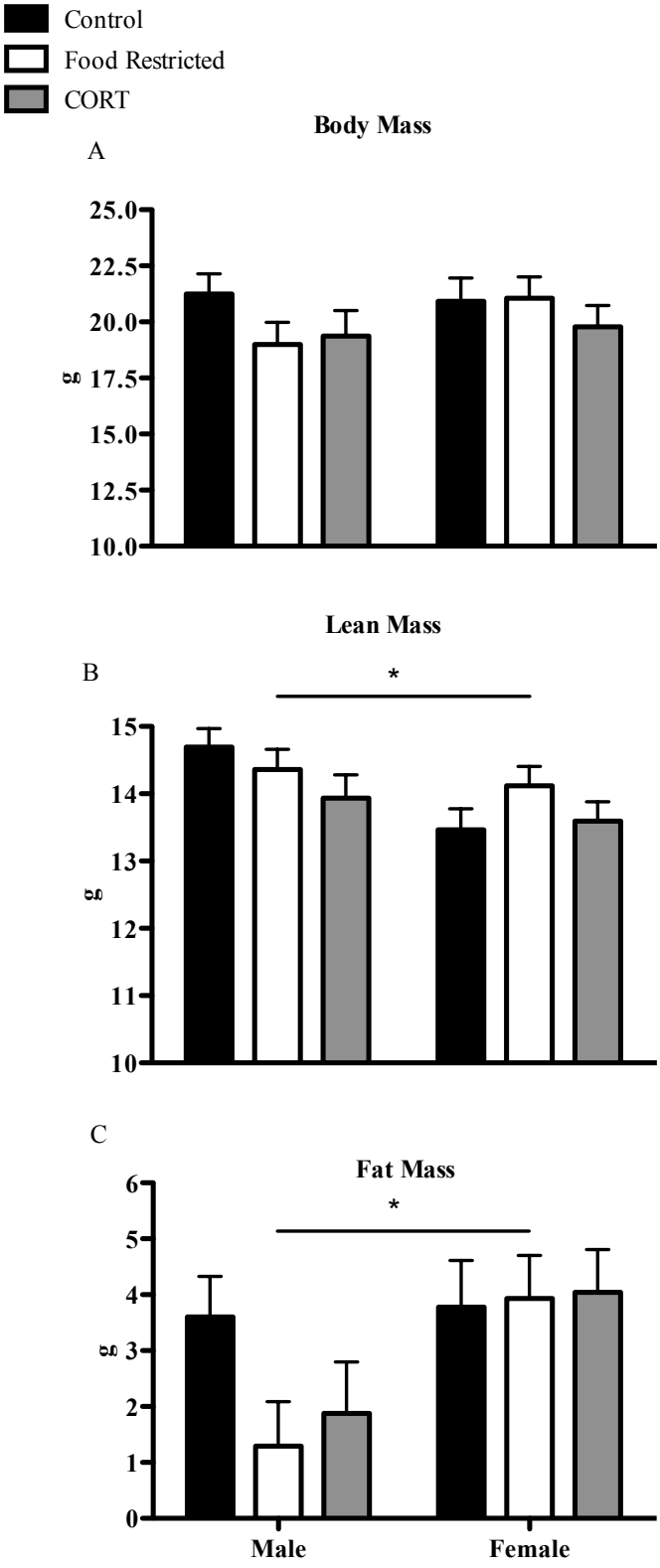


Figure 6