RESEARCH ARTICLE

Growth and development of house sparrows (*Passer domesticus*) in response to chronic food restriction throughout the nestling period

Tess L. Killpack[1],* and William H. Karasov[2]

[1]Department of Zoology, University of Wisconsin, A229 Russell Laboratories, 1630 Linden Drive, Madison, WI 53706, USA and
[2]Department of Forest and Wildlife Ecology, University of Wisconsin, 1630 Linden Drive, Madison, WI 53706, USA

*Author for correspondence (tkillpack@wisc.edu)

SUMMARY

Birds have evolved phenotypic plasticity in growth and developmental patterns in order to respond to fluctuating environmental conditions and to mitigate the impact of poor feeding on fitness. Chronic food shortage can occur during chick development in the wild, and the responses of altricial birds have not been thoroughly studied. House sparrow (*Passer domesticus*) nestlings were raised in the laboratory on age-specific meal sizes (controls) or meal sizes 25% less than age-specific amounts (food-restricted) and analyzed at 6, 9 and 12 days post-hatch for differences in growth and development. Food-restricted birds had significantly reduced body mass and body temperature, but skeletal growth was maintained with respect to controls. Muscle mass was significantly reduced and muscle water content was slightly, though not significantly, higher in food-restricted birds, which may reflect slight developmental immaturity. Assimilation organ masses, summed enzymatic capacity of the intestine and lipid content of the liver were significantly reduced in food-restricted birds. Findings from this study indicate that altricial birds experiencing chronic, moderate food restriction throughout the nestling period may allocate resources to structural growth through energy-saving reductions in mass of assimilation organs and body temperature.

Key words: developmental plasticity, diet restriction, digestive organs, digestive enzymes, body composition, skeletal growth.

INTRODUCTION

Chronic, moderate food shortage can occur during development of altricial birds in the wild because of fluctuating environmental conditions, inadequate parental delivery of food to the nest or sibling competition for the delivered food within the nest. Additionally, climate change is affecting the phenologies of breeding birds and their prey, causing asynchrony between the timing of nestling needs and the availability of the resources on which they depend (Thomas et al., 2001; Visser et al., 2006). Nestling altricial birds are featherless and immobile at hatch, and rely solely on parental delivery of food to the nest for up to several weeks prior to fledging. The nestling period of altricial birds is an energetically demanding time period during which rapid anatomical growth and considerable maturation in physiology and biochemical digestive capacity occurs (Caviedes-Vidal and Karasov, 2001). Altricial birds undergo more rapid growth and generally reach asymptotic size in a shorter period of time during the post-hatch period compared with precocial birds, which hatch with more mature tissues and thermoregulatory and locomotive abilities (Ricklefs, 1973; Ricklefs, 1979). Consequently, food shortage experienced during the energetically demanding nestling period may have a considerable impact on growth and development prior to fledging and on future survival and fitness of altricial birds in the wild.

Evolved plasticity in growth and developmental patterns allows birds to respond to fluctuating environmental conditions and to mitigate the impact of poor feeding conditions on chick survival. A growing bird's response to food shortage may depend on the magnitude, duration and frequency of the shortage, as well as the timing of the shortage with respect to the course of the bird's growth

(Schew and Ricklefs, 1998). If growth and development cannot be maintained at the normal pace, given the available food intake and energy stores, birds may slow or arrest growth and maturation until sufficient resources become available. Alternatively, they may preferentially allocate resources to body parts that are more crucial to survival and future success at the expense of other less crucial parts (Schew and Ricklefs, 1998). Studies of acute food restriction (lasting for less than 1 week during the nestling period) and chronic food restriction (lasting for a substantial part of the nestling period) can be used to examine this plasticity in growth and developmental patterns in altricial birds.

Organs of assimilation, such as the intestine and liver, process food to fuel growth and thus represent a large proportion of the total body mass at hatch and early in development when rapid body growth occurs (Starck, 1996). Some studies of food restriction in growing birds have demonstrated maintenance of assimilation organ masses and intestinal enzymatic capacity during food shortage (Konarzewski and Starck, 2000; Moe et al., 2004; Takenaka et al., 2005; Fassbinder-Orth and Karasov, 2006), which allows birds to maintain digestive efficiency to meet the demands of growth (Karasov, 1996). However, maintenance of assimilation organ size during food shortage can be energetically costly, given the high metabolic activity and cell turnover rates of these organs (Ricklefs et al., 1998). Thus, other studies have shown that growing birds downregulate the size and function of some assimilation organs (Konarzewski et al., 1996; Burness et al., 2000; Brzęk and Konarzewski, 2001; Dahdul and Horn, 2003) and also reduce body temperature and resting metabolic rates (Kitaysky, 1999; Burness et al., 2000; Konarzewski and Starck, 2000; Brzęk and Konarzewski,

2001; Moe et al., 2004) in response to energy limitations encountered during acute and chronic reductions in food intake. Assimilation organ processing capacity is therefore relatively flexible and can be adjusted to meet changing energy demands and nutrient flow during growth.

Slowed maturation (i.e. acquisition of adult function) because of food restriction may be apparent in measurements of water, lean and lipid content of the soft tissues. Muscle water content of lean tissue is a commonly used index of functional maturity of muscle tissue, because water content declines with age in parallel with increasing functional lean content such as contractile proteins and metabolic machinery (Marsh and Wickler, 1982; Choi et al., 1993). Indeed, the age-related decline in muscle water content is inversely correlated with increasing metabolic response to cold stress in hatchling passerine birds (Ricklefs, 1967; Ricklefs and Webb, 1985), supporting the use of this tissue composition measure as an index for muscle maturity. Additionally, tissue lipid content can indicate maturity, as lipid stores are low at hatch and allocation of resources to lipid accumulation typically occurs only after substantial growth of internal organs and skeletal structures has been attained (Reid et al., 2000). Decreases in body lipid content and lean tissue mass occur in altricial passerines that are food-restricted through brood manipulation during the nestling period (Burness et al., 2000). One study of moderate, chronic energy restriction in semi-precocial elegant terns showed that reductions in lipid content prevented reductions in lean tissue matter in restricted birds (Dahdul and Horn, 2003), indicating allocation of limited food energy in favor of maintenance and growth over storage. Thus, measurements of lean dry mass as well as water and lipid content can reflect patterns of maturation and trade-offs in allocation in growing birds experiencing food shortage.

Skeletal growth during food shortage appears to be less flexible than patterns of soft tissue growth. A number of studies have shown that altricial (Lepczyk and Karasov, 2000; Moe et al., 2004), semi-altricial (Negro et al., 1994; Reid et al., 2000) and semi-precocial (Takenaka et al., 2005) birds that are food-restricted during the nestling period maintain skeletal growth despite reductions in the mass of the whole body and soft tissues. Some studies of chronic food restriction show that if birds receiving inadequate quantities of food during the nestling period decrease structural growth, then decreased competitive ability within the nest, delayed fledging and reduced recruitment and survival after fledging may result (Cruz and Cruz, 1990; Emlen et al., 1991; Emlen and Wrege, 1991; Searcy et al., 2004; Mock et al., 2009; Miller, 2010). Thus, prioritizing growth of skeletal structures during the growth period may prevent long-term negative effects of food shortage.

Few studies have examined the effects of chronic food restriction imposed early post-hatch and lasting throughout the majority of the nestling period in passerines. Of the studies that have been performed, body mass and skeletal growth measures were tracked throughout the restriction period yet measurements of the impact of food restriction on various internal tissues were not performed until the end of the chronic restriction period, near fledging age (Burness et al., 2000; Searcy et al., 2004). Given that altricial passerines can commonly experience food restriction while undergoing rapid growth during their brief nestling period (Thomas et al., 2001; Torok et al., 2004), it is of interest to understand the patterns of allocation to growth of various body tissues at several time points throughout chronic restriction. In this study, we imposed chronic 25% food restriction spanning the nestling period of house sparrows [*Passer domesticus* (Linnaeus 1758)] through hand rearing in the laboratory. Chronic restriction began at 3 days post-hatch,

and birds were sampled at 6, 9 and 12 days post-hatch (2–3 days prior to fledging) to examine the patterns of growth and maturation of assimilation organs, muscles and the skeleton during the course of growth. We expected that in response to chronic, moderate restriction, nestling birds would reduce assimilation organ size and enzyme activity to match reduced food intake and decrease lipid storage and body temperature to free energy resources for maintenance of skeletal growth.

MATERIALS AND METHODS
Study site and bird collection

House sparrow nest sites, both wooden nest boxes and natural nests, were located in dairy barns on the University of Wisconsin–Madison campus. In early May 2010, all potential nesting locations in the barns were checked weekly to note the onset of egg laying. From mid-May to mid-July, we visited the 23 known active nesting sites daily, between 08:30 and 10:30 h CST, to ensure accurate and consistent aging of nestlings. At each visit, we counted eggs and noted the number of nestlings in each nest (average brood size=four nestlings). We recorded the date that a new nestling was found as 'day 0' of age for that nestling. Nestlings were marked with colored permanent marker and returned to the nest so that each bird could be distinguished from its nest mates on later dates. Nestlings were collected from the nest on day 3 post-hatch and transported to our laboratory. Nestlings were placed in round tissue-lined plastic containers (445 cm^3) and housed in an environmental chamber under constant conditions of 15 h:9 h light:dark photoperiod (lights on 06:00 h), 35°C chamber temperature and 40–45% relative humidity. All experimental procedures were accepted by the University of Wisconsin, Madison, Ethics Committee (permit no. RARC A-01396-6-29-09).

Feeding protocol

A total of 60 nestlings were raised in the laboratory beginning on day 3 post-hatch, with 10 nestlings assigned to each treatment group (control and food-restricted at 6, 9 and 12 days post-hatch). Nestlings from the same brood were randomly assigned to different age and feeding treatment groups. Birds were hand-fed a synthetic starch-containing diet developed by E. Caviedes-Vidal (Lepczyk et al., 1998), which has been shown in previous studies to provide adequate nutrition and hydration for growing house sparrows (Lepczyk et al., 1998; Brzęk et al., 2009). Nestlings were hand-fed either age-specific meal sizes (control group) or meal sizes 25% less than age-specific amounts (food-restricted group) based on the assigned treatment group. Age-specific and food-restricted meal sizes were based on previous studies (Lepczyk et al., 1998; Brzęk et al., 2009) and preliminary hand-feeding trials in house sparrows. Beginning at 06:30 h, nestlings were removed from the environmental chamber hourly and fed using a 0.50 or 1.0 ml syringe (depending on meal size) for a total of 15 meals per day. Food-filled syringe mass (±0.01 g) was recorded prior to feeding and weighed following feeding to calculate the exact meal mass delivered.

Tracking body mass, skeletal lengths and body temperature

Body mass and body temperature, using a thermocouple inserted approximately 1 cm into the bird's cloaca, were recorded daily before the first feeding at 06:30 h. Skeletal lengths [skull (head+beak), tarsus and wing (radius)] to the nearest 0.10 mm were also measured daily using digital calipers.

Assimilation organ and muscle measurements

On day 6, 9 or 12 post-hatch (between 13:30 and 15:30 h), nestlings were euthanized with CO_2. Blood samples were taken for another

study. Birds were then dissected to remove the small intestine, liver, pancreas and gizzard. Wet masses of internal organs were recorded (±0.10 mg). Intestines were flushed with ice-cold avian Ringer solution, cut into three sections corresponding to proximal, middle and distal regions, blotted dry, massed and measured for section area calculations. Intestinal sections were immediately stored in cryovials and preserved in liquid nitrogen for later measurement of digestive enzymes (see Intestinal enzyme assays, below). Livers were dried to a constant mass in a 60°C drying oven for tissue composition analyses (see Tissue composition analyses, below). Dissected carcasses were immediately placed in the freezer in sealed plastic bags until further analysis.

At a later date, the dissected bird carcasses were removed from the freezer, and allowed to only partially thaw to facilitate dissection but reduce water loss from the tissue. The flight muscles (pectoral and supracoracoideus muscles) and leg muscles (muscles of the femur and tibiotarsus) of the right side were then dissected. Dissected muscles were placed into tared aluminum weigh boats and covered with a damp paper towel until the wet muscle mass was measured (±0.1 mg). Wet muscles and containers were dried in a 60°C drying oven to a constant mass until analyzed for composition (see Tissue composition analyses, below).

Intestinal enzyme assays

We measured the disaccharidase and aminopeptidase-N activity of membrane-bound enzymes in whole-tissue homogenates of the proximal, medial and distal regions of the intestine. Enzyme parameters for a given bird were all measured on the same day. The enzyme assays of all experimental birds were run over several days, with birds from different experimental groups run on each day to avoid bias. We assayed maltase activity using a modification of the colorimetric method developed by Dahlqvist (Dahlqvist, 1984). Assays are described in detail elsewhere (Caviedes-Vidal and Karasov, 2001; Fassbinder-Orth and Karasov, 2006; Brzęk et al., 2009). Briefly, a small piece of intestinal tissue from each section was randomly sampled, thawed at 4°C and homogenized (Omni 5000 homogenizer, Omni International, Waterbury, CT, USA; 30 s, setting 6) in 350 mmol l^{-1} mannitol in 1 mmol l^{-1} Hepes-KOH, pH 7.0. Gut homogenates (30 μl) diluted 1:250 with 350 mmol l^{-1} mannitol in 1 mmol l^{-1} Hepes-KOH were incubated with 30 μl of maltose substrate (56 mmol l^{-1} maltose in 0.1 mol l^{-1} maleate-NaOH buffer, pH 6.5) at 40°C for 20 min. Next, 400 μl of a stop-develop reagent (GAGO-20 glucose assay kit; Sigma-Aldrich, St Louis, MO, USA) was added to each tube, vortexed and incubated at 40°C for 30 min. Lastly, 400 μl of 12 mol l^{-1} H$_2$SO$_4$ was added to each tube, and the absorbance at 540 nm was measured. Each gut homogenate was assayed in triplicate for maltase activity, and the average absorbance from the triplicate assays is reported. The mean coefficient of variation (=s.d./mean) of the triplicate assays of maltase activity across all samples was 0.066±0.006.

We used L-alanine-p-nitroanilide as a substrate for aminopeptidase-N assays. To start the reaction, we added 10 μl of the undiluted homogenate to 1 ml of assay mix (2.0 mmol l^{-1} L-alanine-p-nitroanilide in one part 0.2 mol l^{-1} NaH$_2$PO$_4$/Na$_2$HPO$_4$ buffer, pH 7 and one part deionized H$_2$O) previously heated to 40°C. The reaction solution was incubated for 20 min at 40°C and then stopped with 3 ml of ice-cold 2 mol l^{-1} acetic acid, and absorbance was measured at 384 nm. Each gut homogenate was assayed in triplicate for aminopeptidase-N activity, and the average absorbance from the triplicate assays is reported. The mean coefficient of variation of the triplicate assays of aminopeptidase-N activity across all samples was 0.080±0.005.

Enzyme activities of the randomly sampled pieces from each intestinal section (of known mass) were calculated based on absorbance measurements from the average of the triplicate assays and on glucose and p-nitroanilide standards. Activities are expressed as micromoles substrate converted per minute per gram of wet mass of tissue ('mass-specific activity'). Mean mass-specific enzyme activities were calculated by averaging the mass-specific activity of the proximal, medial and distal intestine sections for a given bird. We calculated the 'summed enzymatic capacity', an index of the total hydrolysis activity of the entire small intestine, by multiplying average activity per gram tissue in the sample from each intestinal section by their respective whole section masses, and summing over the three sections.

Tissue composition analyses

Dry masses of the liver and of the combined muscle (flight + leg) samples were recorded and water mass (g wet tissue–g dry tissue) was calculated. Dried tissue samples were individually wrapped in pre-dried filter paper, and lipids were extracted from the samples using diethyl ether in a modified Soxhlet side-arm extractor. Lean dry mass and tissue mass extracted (g dry tissue–g lean dry tissue), as well as indices of tissue water content (g water g^{-1} lean dry tissue) and tissue lipid content (g tissue extracted g^{-1} lean dry tissue), were calculated.

Statistical analyses

Results are given as means ± 1 s.e.m. (N=10 nestlings per age-treatment group unless otherwise noted). All tests were carried out using SYSTAT (Version 10) (Wilkinson, 2000) and R (R Development Core Team, 2008) statistical packages. Two-sample t-tests were performed to compare body mass and structural measurements between control and food-restricted birds on the initial day of the experiment (age=day 3 post-hatch). Daily food intake, body mass, body temperature and skeletal length measures (skull, wing and tarsus) of control and food-restricted birds throughout the experimental period were compared using a linear mixed-effects model fit by maximizing the restricted log-likelihood, with 'treatment' and 'day' as fixed effects and 'nestling' as a random effect. Wet and lean-dry organ and muscle masses were analyzed with analysis of covariance (ANCOVA), with feeding treatment as a factor and tarsus-cubed (an index of total body size) as a covariate. An alternative ANCOVA using organ-free body mass (body mass–assimilation organ mass) as the covariate gave consistent statistical results (data not shown). Tarsus-cubed (to link a linear skeletal measure to body size 'volume') was chosen as a covariate given that skeletal measures are related to structural body size (Sibly et al., 1987; Piersma and Davidson, 1991) and are less variable than mass measures. Summed enzymatic capacity was analyzed with ANCOVA, with feeding treatment as a factor and intestine mass as a covariate. To examine age-dependent effects on body components, we also performed analyses of final skeletal lengths, organ mass, muscle mass, mass-specific intestinal enzyme activity, summed enzymatic capacity and tissue composition measures (water content and lipid content) using two-way ANOVA, with age and feeding treatment as factors. Tukey's honestly significant difference (HSD) test was used for pairwise comparisons following significant two-way ANOVA results. Tests for normality and homogeneity of variance confirmed that assumptions for ANOVA/ANCOVA were met, and therefore no transformations of the data were performed. In all tests, the significance level was set at P<0.05 and 0.05<P<0.10 was considered to indicate a non-significant trend.

RESULTS
Growth and body temperature

Food-restricted birds were fed 0.24±0.01% less than the control birds' total daily food intake throughout the experimental period ($P<0.001$). At the initiation of the experiment (day 3 post-hatch), birds in control and food-restricted groups did not significantly differ in body mass ($P=0.952$) or length of the tarsus ($P=0.429$), wing ($P=0.982$) or skull ($P=0.657$). Body mass, tarsus, wing, skull and body temperature measures significantly increased with age ($P<0.001$ for all measures; Fig. 1, wing and skull data not shown). Beginning at day 6 post-hatch (after two full days of hand feeding in the laboratory) and through the end of the experimental period, body mass was consistently lower in food-restricted birds compared with controls by an average of 13% ($P<0.001$; Fig. 1A); however, lengths of the tarsus ($P=0.495$), wing ($P=0.182$) and skull ($P=0.701$) did not significantly differ between treatments throughout the experimental period (tarsus, Fig. 1B; skull and wing data not shown). Final skeletal measures of the tarsus, wing and skull significantly increased among the age groups ($P<0.001$; Table 1). There was no significant effect of treatment on final tarsus length ($P=0.201$) or final wing length ($P=0.069$; Table 1). Although there was a significant overall treatment effect on final skull length ($P=0.037$), post hoc analyses revealed that there were no significant pairwise differences in final skull length between control and food-restricted birds of the same age (Table 1). Body temperature was significantly reduced in food-restricted birds compared with controls throughout the nestling period ($P=0.024$; Fig. 1C).

Wet masses of assimilation organs and muscles

Body size (indexed by tarsus-cubed) was a significant covariate when examining wet mass of the intestine ($F_{1,56}=7.37$, $P<0.001$; Fig. 2A), liver ($F_{1,56}=36.76$, $P<0.001$; Fig. 2B) and pancreas ($F_{1,56}=17.74$, $P<0.001$; Fig. 2C), but not the gizzard ($F_{1,56}=0.05$, $P=0.819$; Fig. 2D). All assimilation organ masses were significantly reduced in food-restricted birds compared with controls, even after accounting for body size differences. Wet masses of the intestine ($F_{1,56}=11.65$, $P<0.001$; Fig. 2A), liver ($F_{1,56}=33.76$, $P<0.001$; Fig. 2B), pancreas ($F_{1,56}=9.76$, $P=0.003$; Fig. 2C) and gizzard ($F_{1,56}=5.08$, $P=0.028$; Fig. 2D) averaged 12, 26.5, 14 and 6% lower, respectively, in food-restricted birds compared with controls across all ages. Two-way ANOVA examining age-dependent differences in organ masses were consistent with ANCOVA analyses, except that age was a significant predictor of gizzard mass ($F_{2,54}=3.55$, $P=0.036$), though body size was not.
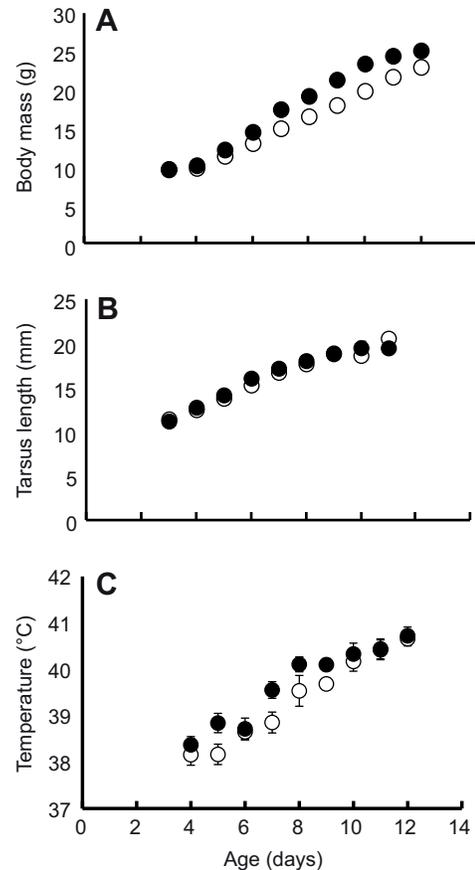

Fig. 1. Body mass (A), tarsus length (B) and body temperature (C) of house sparrows throughout the nestling period. Data are means ± s.e.m. (error bars are smaller than the circles in some cases) for control (filled circles) and food-restricted (unfilled circles) birds ($N=30$ per group for age 3–6 days, $N=20$ per group for age 4–9 days and $N=10$ per group for age 10–12 days). Body mass, tarsus length and body temperature significantly increased with age. Body mass and body temperature, but not tarsus length, were significantly reduced in food-restricted birds compared with controls.

Wet mass of the leg and flight muscles of food-restricted birds averaged 9 and 20.5% lower, respectively, than controls across all ages. After controlling for differences in body size (a significant covariate for both muscle groups), the trends of

Table 1. Skeletal measures and summary of results of ANOVA for the effects of age and feeding treatment on final tarsus, skull and wing length of control and food-restricted house sparrows in three age groups

Skeletal measure	Age (days)	Control	Restricted	Two-way ANOVA statistics			
				Factor	F	d.f.	P
Final tarsus length (mm)	6	17.385±0.5 (9)[a]	17.113±0.3 (10)[a]	Age	116.62	2,53	<0.001*
	9	20.843±0.3 (10)[b]	20.070±0.3 (10)[b]	Feeding treatment	1.68	1,53	0.201
	12	22.373±0.1 (10)[c]	22.357±0.2 (10)[c]	Age × Treatment	0.666	2,53	0.518
Final wing length (mm)	6	15.419±0.7 (9)[a]	14.797±0.3 (10)[a]	Age	234.10	2,53	<0.001*
	9	20.937±0.3 (10)[b,d]	19.953±0.2 (10)[b,c]	Feeding treatment	3.44	1,53	0.069
	12	22.237±0.2 (10)[d,e]	22.287±0.2 (10)[c,e]	Age × Treatment	1.20	2,53	0.309
Final skull length (mm)	6	20.367±0.4 (9)[a]	20.063±0.1 (10)[a]	Age	342.80	2,53	<0.001*
	9	24.537±0.1 (10)[b]	23.857±0.2 (10)[b]	Feeding treatment	4.57	1,53	0.037*
	12	26.553±0.1 (10)[c]	26.293±0.2 (10)[c]	Age × Treatment	0.47	2,53	0.625

Skeletal measure values are means ± s.e.m. Values in parentheses are number of individuals per age-treatment group.
Tukey's post hoc testing was performed following significant ANOVA results (denoted by asterisks). Values for a given skeletal measure that share the same superscript are not significantly different as determined by Tukey's post hoc tests.
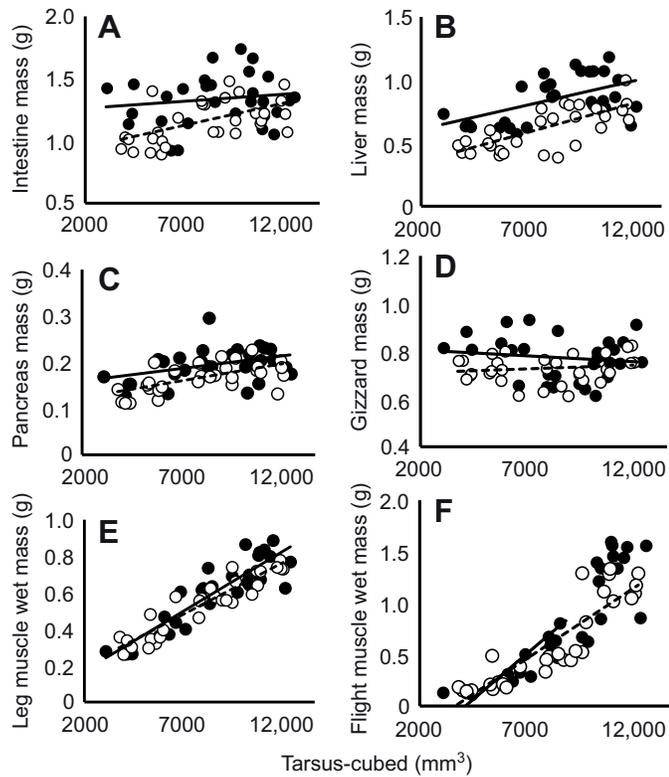
Fig. 2. Organ and muscle wet masses of control (filled) and food-restricted house sparrows (unfilled) with respect to body size (tarsus-cubed). Solid lines represent least-square linear trends for control birds and dashed lines represent linear trends for food-restricted birds. Body size was a significant covariate when examining wet mass of the intestine, liver, pancreas, leg muscle and flight muscle, but not the gizzard. All assimilation organ masses were significantly reduced in food-restricted birds compared with controls, even after accounting for body size differences, but reductions in leg wet mass and flight muscle wet mass in food-restricted birds were not statistically significant.

reduced leg wet mass ($F_{1,56}$=2.91, P=0.094; Fig. 2E) and flight muscle wet mass ($F_{1,56}$=3.62, P=0.062; Fig. 2F) in food-restricted birds were not statistically significant. For analysis of water and lean contents of these wet muscle masses, see Tissue composition, below.

Intestinal enzymes

Mass-specific aminopeptidase-N activity (activity per gram intestine) significantly increased with age in all intestinal sections (proximal, medial and distal), whereas age had a significant effect on mass-specific maltase activity only in the proximal and medial sections (Table 2, Fig. 3). Feeding treatment did not have a significant effect on mass-specific aminopeptidase-N or maltase activity in any of the intestinal sections (Table 2, Fig. 3).

Mean mass-specific enzyme activity (average activity per gram intestine of all three sections) of aminopeptidase-N and maltase significantly increased with age, but did not significantly differ between feeding treatment groups (Table 2, Fig. 4A). Summed enzymatic capacity for aminopeptidase-N and maltase significantly increased with age (Table 2, Fig. 4B). Summed enzymatic capacity of aminopeptidase-N and maltase were significantly higher in control birds compared with food-restricted birds at all ages (Table 2, Fig. 4B). After controlling for differences in intestine mass, there was no significant difference in summed enzymatic capacity of aminopeptidase-N between treatments ($F_{1,57}$=3.02, P=0.088), yet summed enzymatic capacity of maltase ($F_{1,57}$=5.32, P=0.025) was significantly higher in control birds compared with food-restricted birds at all ages.

Tissue composition

Lean dry mass of combined flight and leg muscles was 22% lower in food-restricted birds compared with controls, a significant treatment effect ($F_{1,55}$=229.75, P<0.001), even after accounting for significant body size differences ($F_{1,55}$=4.30, P=0.043). When examining lean dry muscle mass using two-way ANOVA, age and feeding treatment were significant factors, and there was a significant interaction of age and feeding treatment (Table 3). *Post hoc* analyses revealed that there was no significant difference in lean dry muscle mass between control and food-restricted 6-day-old birds, but a lower lean dry mass was observed in food-restricted birds compared with controls at days 9 and 12 post-hatch (Table 3). Water content (g water g^{-1} lean dry muscle) of combined flight and leg muscles significantly decreased with age and there was a trend for higher muscle water content in food-restricted birds compared with controls (Table 3). No significant difference in muscle lipid content (g extracted g^{-1} lean dry muscle) was observed with respect to age or treatment group (Table 3).

Lean dry mass of the liver was 25% lower in food-restricted birds compared with controls, a significant treatment effect ($F_{1,56}$=21.13,

Table 2. Summary of two-way ANOVA for effects of age and feeding treatment on aminopeptidase-N and maltase activity (proximal, middle, distal and mean mass-specific activity, total summed enzymatic capacity) in house sparrows

	Age			Feeding treatment			Age × Treatment		
	F	d.f.	P	F	d.f.	P	F	d.f.	P
Aminopeptidase-N									
Proximal	50.19	2,54	<0.001*	0.01	1,54	0.922	1.35	2,54	0.270
Medial	10.67	2,54	<0.001*	0.24	1,54	0.628	1.52	2,54	0.228
Distal	13.60	2,54	<0.001*	0.13	1,54	0.724	1.19	2,54	0.314
Mean (all sections)	29.04	2,54	<0.001*	0.05	1,54	0.820	1.84	2,54	0.169
Total (summed)	65.02	2,54	<0.001*	6.28	1,54	0.015*	0.42	2,54	0.659
Maltase									
Proximal	12.67	2,54	<0.001*	1.88	2,54	0.176	0.92	2,54	0.407
Medial	16.54	2,54	<0.001*	2.07	1,54	0.156	0.91	2,54	0.410
Distal	2.62	2,54	0.082	0.13	1,54	0.725	1.08	2,54	0.348
Mean (all sections)	14.50	2,54	<0.001*	2.22	1,54	0.142	0.74	2,54	0.483
Total (summed)	24.67	2,54	<0.001*	6.05	1,54	0.017*	0.77	2,54	0.467

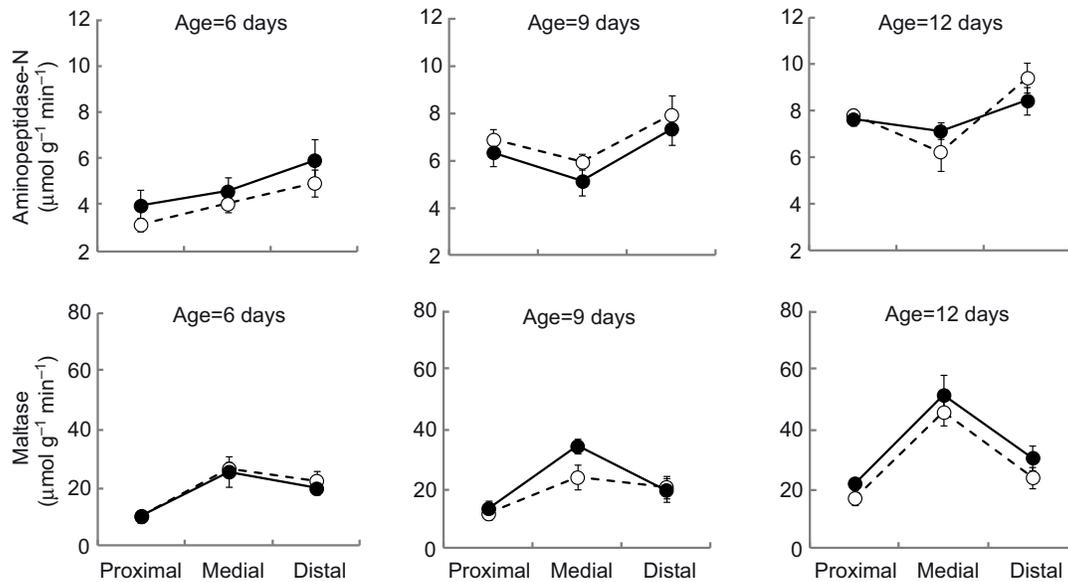Asterisks indicate signifigant ANOVA results at the α=0.05 level.

Fig. 3. Mass-specific intestinal enzyme activities in house sparrows as a function of intestinal section, age and feeding treatment. Aminopeptidase-N activity (top row) and maltase activity (bottom row) are expressed per gram wet mass of tissue in three intestinal positions. Data are means ± s.e.m. for control (filled circles) and food-restricted (unfilled circles) birds (N=10 per age-treatment group). Mass-specific enzyme activities significantly increased with age in all sections, except in the case of maltase activity in the distal intestine, where no age-related differences in activity were observed. Feeding treatment did not have a significant effect on mass-specific enzyme activity (see Table 2).

P<0.001), after accounting for significant body size differences ($F_{1,56}$=36.49, P<0.001). Two-way ANOVA analysis revealed that lean dry liver mass was significantly increased with age and was significantly reduced in food-restricted birds (Table 3). Liver water content (g water g^{-1} lean dry liver) significantly decreased with age, though there was no significant difference between treatment groups (Table 3). Lipid content of the liver (g extracted g^{-1} lean dry liver) was significantly higher in control birds compared with food-restricted birds by an average of 27% across all ages, though there was no significant change in lipid content of the liver with age (Table 3).

DISCUSSION
Beginning at 3 days post-hatch, we imposed a 25% food restriction to determine its impact on growth and development in nestling

Table 3. Tissue measures and summary of results of ANOVA for the effects of age and feeding treatment on tissue composition of muscles and liver of control and food restricted birds in three age groups

Tissue measure	Age (days)	Control	Restricted	Two-way ANOVA statistics			
				Factor	F	d.f.	P
Lean dry muscle mass (g)	6	0.091±0.012 (10)[a]	0.074±0.004 (10)[a]	Age	477.47	2,54	<0.001*
	9	0.224±0.012 (10)[c]	0.168±0.007 (10)[b]	Feeding treatment	42.03	1,54	<0.001*
	12	0.458±0.014 (10)[e]	0.361±0.014 (10)[d]	Age × Treatment	7.07	2,54	0.002*
Muscle water content (g)	6	5.807±0.323 (9)[a]	6.088±0.295 (10)[a]	Age	63.63	2,53	<0.001*
	9	4.751±0.126 (10)[b]	4.969±0.082 (10)[b]	Feeding treatment	3.31	1,53	0.074
	12	3.776±0.087 (10)[c]	4.074±0.078 (10)[c]	Age × Treatment	0.03	2,53	0.972
Muscle lipid content (g)	6	0.095±0.027 (7)[a]	0.078±0.021 (8)[a]	Age	1.31	2,48	0.279
	9	0.071±0.006 (10)[a]	0.061±0.016 (9)[a]	Feeding treatment	1.37	1,48	0.248
	12	0.085±0.004 (10)[a]	0.075±0.006 (10)[a]	Age × Treatment	0.03	2,48	0.968
Lean dry liver mass (g)	6	0.140±0.003 (10)[a]	0.105±0.005 (10)[a]	Age	38.16	2,54	<0.001*
	9	0.207±0.014 (10)[b,c]	0.143±0.013 (10)[a]	Feeding treatment	36.36	1,54	<0.001*
	12	0.224±0.012 (10)[c]	0.184±0.007 (10)[b]	Age × Treatment	1.46	2,54	0.241
Liver water content (g)	6	3.328±0.092 (10)[a]	3.227±0.045 (10)[a]	Age	6.27	2,54	0.004*
	9	3.339±0.156 (10)[a]	3.155±0.055 (10)[a]	Feeding treatment	2.18	1,54	0.146
	12	3.020±0.054 (10)[b]	3.015±0.046 (10)[b]	Age × Treatment	0.62	2,54	0.541
Liver lipid content (g)	6	0.082±0.014 (10)[a]	0.065±0.004 (10)[b]	Age	1.20	2,54	0.310
	9	0.105±0.020 (10)[a]	0.074±0.008 (10)[b]	Feeding treatment	8.39	1,54	0.005*
	12	0.105±0.014 (10)[a]	0.072±0.003 (10)[b]	Age × Treatment	0.30	2,54	0.740

Tissue measure values are means ± s.e.m. Values in parentheses are number of individuals per age-treatment group.
Tukey's *post hoc* testing was performed following significant ANOVA results (denoted by asterisks). Values for a given tissue measure that share the same superscript letter are not significantly different as determined by Tukey's *post hoc* tests.
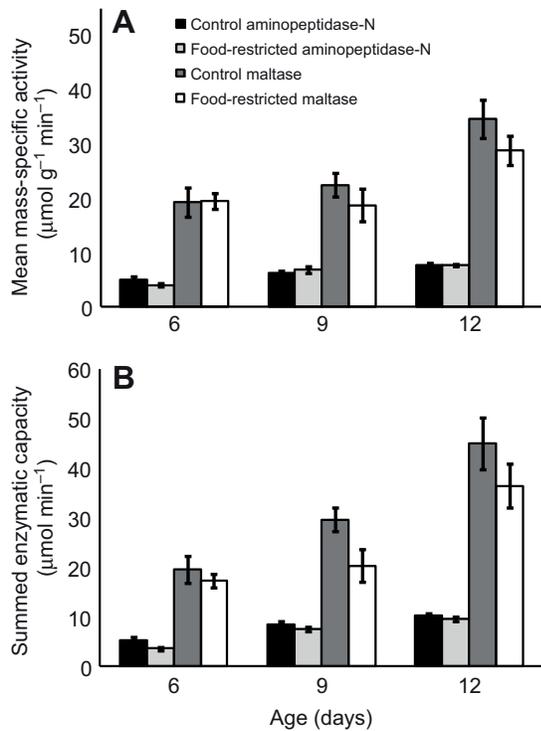
Fig. 4. Mean mass-specific activity (A) and summed enzymatic capacity (B) of aminopeptidase-N and maltase in house sparrows. Data are means ± s.e.m. for aminopeptidase-N and maltase (N=10 per age-treatment group). Mean mass-specific activities and summed enzymatic capacities significantly increased with age. Summed enzymatic capacities, but not mean mass-specific activities, were significantly reduced in food-restricted nestlings (see Table 2).

altricial house sparrows. This level of chronic, moderate food restriction is within the range that may be expected for a nestling that hatches asynchronously from sibling birds (Malacarne et al., 1994) or during a season of limited resource availability (Bertram et al., 1991; Thomas et al., 2001; Torok et al., 2004). We observed significant reductions in body mass 2 days after experimental food restriction began. These mass reductions persisted throughout the end of the experimental period, though structural growth was maintained. As we discuss below, reductions in the size and function of assimilation organs and muscles likely provided energy savings to allow food-restricted nestlings to maintain structural growth in line with well-fed controls.

Maintenance of structural growth

Long bone growth in endotherms is under strict endocrine control (Leach and Rosselot, 1992) and may be constrained to a critical window during development, given that levels of circulating growth hormones decline and growth-suppressing sex hormones increase with age (Scanes and Balthazart, 1981; Schew et al., 1996). Therefore, if growth is not maintained when circulating levels of growth hormones are high, negative and permanent consequences for bird fitness and survival may result. Reduced size because of food limitation has been shown to delay fledging time (Cruz and Cruz, 1990; Emlen et al., 1991; Searcy et al., 2004; Takenaka et al., 2005; Miller, 2010), decrease social rank (Richner et al., 1989) and decrease predicted recruitment and survival of birds in the wild (Emlen and Wrege, 1991). A study of mourning doves showed that,

overall, food-restricted nestlings fledged at later ages compared with controls (due to slower overall growth), yet the food-restricted birds that prioritized wing growth over growth of other body tissues mitigated the delayed fledging effects associated with their reduced size (Miller, 2010).

In both our control and food-restricted house sparrows, we observed rapid increases in tarsus length during the first week post-hatch, followed by slowed growth to day 12. Growth of the tarsus, skull and wing of food-restricted house sparrows was maintained in line with that of adequately fed controls, which is in agreement with previous studies showing prioritization of skeletal growth (relative to mass gain) in altricial young faced with acute or chronic, moderate restriction (Negro et al., 1994; Lacombe et al., 1994; Lepczyk and Karasov, 2000; Moe et al., 2004). An equivalent prioritization of skeletal growth was observed in our preliminary study of 25% chronic restriction lasting until 25 days post-hatch in house sparrows (Killpack et al., 2010). Compared with controls, food-restricted birds reached a significantly lower asymptotic body mass at day 12 post-hatch and had consistently lower body mass through day 25 post-hatch. However, food-restricted birds maintained skeletal growth in line with controls and fledged at the same age (Killpack et al., 2010). This pattern of reduced asymptotic body mass and maintenance of skeletal growth and fledging date has also been demonstrated with chronic 20–30% food restriction in captive American kestrels (Lacombe et al., 1994). Thus, maintenance of skeletal growth within the limitations imposed by chronic, moderate food restriction may allow birds to avoid the enduring negative consequences of reduced structural size on competitive abilities and survival within and outside of the nest.

Our experimental restriction of 25% was below the level that shifts resource allocation away from skeletal growth in altricial nestlings. Chronic restriction at more severe levels of 40–50% significantly reduced culmen and tarsus growth concomitant with body mass reductions in other studies of altricial birds (Searcy et al., 2004; Sears and Hatch, 2008). When these restricted birds were re-fed *ad libitum* for approximately 1 year after fledging, structural reductions persisted despite recovery of body mass (Searcy et al., 2004; Sears and Hatch, 2008). These data reinforce the notion that, although body mass reductions in our experimental nestlings may be reversible upon realimentation after fledging, skeletal growth patterns are less flexible to fluctuating food supply, and are constrained to critical windows of growth. Therefore, food-restricted birds must prioritize energy allocation to growth of long bones during the nestling period immediately post-hatch to prevent permanent stunting and negative impacts on fledging and success outside of the nest.

Energy-saving reductions in assimilation organ mass, activity and composition

In contrast to the inflexibility of skeletal growth in response to chronic, moderate food restriction, nestlings showed considerable plasticity in assimilation organ masses and intestinal enzymatic capacity. After only 3 days of imposed 25% food restriction (at day 6 post-hatch), food-restricted nestlings showed significant decreases in assimilation organ mass and enzymatic capacity that persisted for the duration of growth to asymptotic body mass attained at day 12 post-hatch. It has been proposed that metabolically active assimilation organs are expensive to maintain. It has been estimated that the digestive tract and liver of small mammals may account for 20–25% of the whole-animal metabolic rate (Martin and Fuhrman, 1955; Cant et al., 1996). Within rodent and avian species, increases

in size of the assimilation organs are associated with increases in basal metabolic rate (Konarzewski and Diamond, 1995; Piersma, 2002). Because of these metabolic costs, fitness advantages should lead to the size and performance of birds' digestive systems to be matched to food intake (Karasov et al., 2011). Reductions in assimilation organ size and enzymatic capacity have been observed with acute food restriction (from 50 to 70%) lasting several days in both full-grown adult birds (Karasov and Pinshow, 1998; Lee et al., 2002; Karasov et al., 2004) and growing altricial nestlings (Konarzewski et al., 1996; Brzęk and Konarzewski, 2001). Mass-specific enzyme activity did not significantly decrease with food restriction in this study, yet summed enzymatic capacity did, indicating that reduction in enzyme capacity can be attributed to mass loss of intestinal tissue (Lee et al., 2002). Because intestinal enzyme activity rates decline with decreasing temperature (Karasov and Hume, 1997), we may expect even larger reductions in enzymatic capacity in food-restricted birds *in vivo*, given that these birds generally had reduced body temperature compared with controls.

As a result of reductions in food intake, chronically food-restricted birds stored less energy as lipid, as indexed by the liver lipid content. This pattern was also observed in previous studies of both acute (Konarzewski and Starck, 2000; Brzęk and Konarzewski, 2001; Moe et al., 2004) and chronic food limitation in birds (Burness et al., 2000; Dahdul and Horn, 2003; Takenaka et al., 2005). It is important to note that reduction in lipid content does not account for the total reduction in liver wet mass observed in food-restricted birds; liver lean dry mass of food-restricted birds was also significantly reduced compared with controls at all ages. Thus, reductions in the size, function and stored energy content of assimilation organs to match chronically limited nutrient flow likely freed energy necessary for maintenance of skeletal growth.

In contrast to the inflexibility of skeletal growth, reductions in assimilation organ mass, enzymatic capacity and lipid content may be rapidly reversible upon realimentation, and therefore may not cause permanent issues for further growth and success of the nestling. Previous studies in young birds re-fed after restriction or fasting have shown recovery of reduced assimilation organ mass and lipid stores upon realimentation (Konarzewski et al., 1996; Lepczyk et al., 1998; Konarzewski and Starck, 2000; Fassbinder-Orth and Karasov, 2006). Future studies on the response of chronically food-restricted nestlings to realimentation would shed light on the extent to which reductions in assimilation organ mass, function and tissue lipid composition are in fact reversible.

Reductions in muscle mass but not maturity

There are competitive and fitness advantages to maintaining normal development of muscle size with respect to whole body size, given that muscles play an important role in sibling competition, flight and thermoregulation in altricial birds. Short-term fasting and food restriction in adult passerines results in muscle mass reductions proportional to body mass reductions (Karasov et al., 2004), and indeed, after total body size differences were accounted for, there was a non-significant trend for reduced wet muscle mass in our chronically food-restricted nestlings compared with controls. We observed no significant effect of food restriction on lean muscle mass in 6-day-old house sparrow nestlings. Nestlings at this age had combined flight and leg muscle mass accounting for less than 4% of total body mass and had minimal mobility and thermogenic capacity (Brzęk et al., 2009). In contrast, chronic food limitation did reduce functional lean

muscle mass in 9- and 12-day-old food-restricted birds compared with same-aged controls, which is in agreement with other studies of acute food restriction in growing birds (Brzęk and Konarzewski, 2001; Moe et al., 2004; Takenaka et al., 2005). Control birds at this age have a combined flight and leg muscle mass accounting for 6 or 9% of body mass, respectively, and show increased mobility and thermoregulatory independence compared with 6-day-old birds (Seel, 1969). Thus, food restriction had a more profound impact on functional muscle mass at those ages after which birds have begun prioritizing growth of muscles important for mobility and thermoregulation.

Relatively higher muscle water content can be used as an index for functional immaturity of muscle tissue in birds, and reduction in muscle water content coincides with increasing lean content and thermogenic capacity after cold stress in altricial starlings (Ricklefs and Webb, 1985). In agreement with previous studies, muscle water content of house sparrow nestlings declined with age (Ricklefs, 1967; Ricklefs and Webb, 1985; Konarzewski et al., 1996; Bech and Østnes, 1999; Konarzewski and Starck, 2000) but was not significantly altered with experimental nutrient limitation in growing birds (Konarzewski et al., 1996; Burness et al., 2000; Dahdul and Horn, 2003; Takenaka et al., 2005). The trend for higher muscle water content in restricted birds compared with controls ($P=0.069$) suggests that if chronic food restriction were imposed at a more severe level or for a longer duration, more significant differences in muscle water content in conjunction with lean muscle mass differences may be observed. Future studies must be performed to test this prediction.

Though chronic, moderate 25% food restriction does not appear to significantly alter muscle functional maturity as indexed by water content, we did observe reduced body temperature in food-restricted birds compared with controls, which may be indicative of reduced development of thermogenic capacity. Food restriction by experimental feeding reductions or brood enlargement has been associated with significant reductions in body temperature and size-specific resting metabolic rates in other growing birds (Boersma, 1986; Kitaysky, 1999; Burness et al., 2000; Konarzewski and Starck, 2000; Brzęk and Konarzewski, 2001; Moe et al., 2004). In this study, the greatest reductions in body temperature of food-restricted birds were observed during the period of rapid growth, when energy requirements are highest (5–9 days post-hatch), but not after day 9 post-hatch, when whole-body growth rate was decreasing. Thus, reduced body temperature in food-restricted birds may indicate facultative metabolic adjustments to decrease maintenance energy expenditure in the face of food limitation. Direct measurement of oxygen consumption rates would be necessary to verify this inference.

Alternatively, body temperature reductions in food-restricted birds may be a consequence of imposed reductions in assimilation organ and muscle size, and in effect, reductions in maintenance costs. Liver lean dry mass and lipid mass were positively correlated with resting metabolic rate in growing birds (Bech and Østnes, 1999; Moe et al., 2004). Significant reductions in these measures in food-restricted house sparrows could have resulted in decreased metabolic activity, thereby indirectly decreasing body temperature. Also, slight reductions in mass of the muscles, an important site of thermogenesis in birds, may have contributed to reductions in resting metabolic rate and body temperature (Moe et al., 2004). We cannot comment definitively on the impact of chronic food restriction on the development of thermoregulatory capacity in our experimental birds because no cold-challenge experiments were performed in the present study.

Conclusions

In summary, reductions in assimilation organ mass, intestinal enzymatic capacity and liver lipid storage were observed in growing altricial birds experiencing chronic 25% food restriction. It is possible that reallocation of energy saved by these reductions prevented decline in skeletal growth in the experimentally food-restricted birds. Evolution of such developmental plasticity allows altricial birds in the wild to mitigate the negative effects that fluctuating resource availability may have on growth and development during the nestling period. Future research should specifically test the physiological response of chronically food-restricted nestlings upon realimentation after fledging, as well as the impacts of chronic, moderate restriction on other aspects of maturity and fitness, such as thermoregulatory ability, immune function and fecundity. Also, investigation into the molecular regulatory controls in moderately *versus* severely restricted nestlings is of interest.

REFERENCES

Bech, C. and Ostnes, J. (1999). Influence of body composition on the metabolic rate of nestling European shags (*Phalacrocorax aristotelis*). *J. Comp. Physiol. B* **169**, 263-270.

Bertram, D., Kaisewr, G. and Ydenberg, R. (1991). Patterns in the provisioning and growth of nestling rhinoceros auklets. *Auk* **108**, 842-852.

Boersma, P. (1986). Body-temperature, torpor, and growth in chicks of fork-tailed storm petrels (*Oceanodroma furcata*). *Physiol. Zool.* **59**, 10-19.

Brzęk, P. and Konarzewski, M. (2001). Effect of food shortage on the physiology and competitive abilities of sand martin (*Riparia riparia*) nestlings. *J. Exp. Biol.* **204**, 3065-3074.

Brzęk , P., Kohl, K., Caviedes-Vidal, E. and Karasov, W. H. (2009). Developmental adjustments of house sparrow (*Passer domesticus*) nestlings to diet composition. *J. Exp. Biol.* **212**, 1284-1293.

Burness, G. P., McClelland, G. B., Wardrop, S. L. and Hochachka, P. W. (2000). Effect of brood size manipulation on offspring physiology: an experiment with passerine birds. *J. Exp. Biol.* **203**, 3513-3520.

Cant, J. P., McBride, B. W. and Croom, W. J. (1996). The regulation of intestinal metabolism and its impact on whole animal energetics. *J. Anim. Sci.* **74**, 2541-2553.

Caviedes-Vidal, E. and Karasov, W. H. (2001). Developmental changes in digestive physiology of nestling house sparrows, *Passer domesticus*. *Physiol. Biochem. Zool.* **74**, 769-782.

Choi, I., Ricklefs, R. E. and Shea, R. (1993). Skeletal-muscle growth, enzyme-activities, and the development of thermogenesis – a comparison between altricial and precocial birds. *Physiol. Zool.* **66**, 455-473.

Cruz, J. B. and Cruz, F. (1990). Effect of El Niño–Southern Oscillation conditions on nestling growth rate in the dark-rumped petrel. *Condor* **92**, 160-165.

Dahdul, W. M. and Horn, M. H. (2003). Energy allocation and postnatal growth in captive elegant tern (*Sterna elegans*) chicks: responses to high- *versus* low-energy diets. *Auk* **120**, 1069-1081.

Dahlqvist, A. (1984). Assay of intestinal disaccharidases. *Scand. J. Clin. Lab. Invest.* **44**, 169-172.

Emlen, S., Wrege, P., Demong, N. and Hegner, R. (1991). Flexible growth rates in nestling white-fronted bee-eaters: a possible adaptation to short-term food shortage. *Condor* **93**, 591-597.

Emlen, S. T. and Wrege, P. H. (1991). Breeding biology of white-fronted bee-eaters at Nakuru: the influence of helpers on breeder fitness. *J. Anim. Ecol.* **60**, 309-326.

Fassbinder-Orth, C. A. and Karasov, W. H. (2006). Effects of feed restriction and realimentation on digestive and immune function in the leghorn chick. *Poult. Sci.* **85**, 1449-1456.

Karasov, W. H. (1996). Digestive plasticity in avian energetics and feeding ecology. In *Avian Energetics and Nutritional Ecology* (ed. C. Carey), pp. 61-84. New York: Chapman and Hall.

Karasov, W. H. and Hume, I. D. (1997). The vertebrate gastrointestinal system. In *Handbook of Physiology, Section 13, Comparative Physiology, Vol. 1* (ed. W. H. Dantzler), pp. 407-480. New York: Oxford University Press.

Karasov, W. and Pinshow, B. (1998). Changes in lean mass and in organs of nutrient assimilation in a long-distance passerine migrant at a springtime stopover site. *Physiol. Zool.* **71**, 435-448.

Karasov, W. H., Pinshow, B., Starck, J. M. and Afik, D. (2004). Anatomical and histological changes in the alimentary tract of migrating blackcaps (*Sylvia atricapilla*): a comparison among fed, fasted, food-restricted, and refed birds. *Physiol. Biochem. Zool.* **77**, 149-160.

Karasov, W. H., Martínez del Rio, C. and Caviedes-Vidal, E. (2011). Ecological physiology of diet and digestive systems. *Annu. Rev. Physiol.* **73**, 69-93.

Killpack, T., Singh, N. and Karasov, W. (2010). Effect of chronic food restriction on gut morphology and digestive enzymes in nestling house sparrows. *Integr. Comp. Biol.* **50 (Suppl. 1)**, E90.

Kitaysky, A. (1999). Metabolic and developmental responses of alcid chicks to experimental variation in food intake. *Physiol. Biochem. Zool.* **72**, 462-473.

Konarzewski, M. and Diamond, J. (1995). Evolution of basal metabolic rate and organ masses in laboratory mice. *Evolution* **49**, 1239-1248.

Konarzewski, M. and Starck, J. (2000). Effects of food shortage and oversupply on energy utilization, histology, and function of the gut in nestling song thrushes (*Turdus philomelos*). *Physiol. Biochem. Zool.* **73**, 416-427.

Konarzewski, M., Kowalczyk, J., Swierubska, T. and Lewonczuk, B. (1996). Effect of short-term feed restriction, realimentation and overfeeding on growth of song thrush (*Turdus philomelos*) nestlings. *Funct. Ecol.* **10**, 97-105.

Lacombe, D., Bird, D. M. and Hibbard, K. A. (1994). Influence of reduced food availability on growth of captive American kestrels. *Can. J. Zool.* **72**, 2084-2089.

Leach, R. and Rosselot, G. (1992). The use of avian epiphyseal chondrocytes for *in vitro* studies of skeletal metabolism. *J. Nutr.* **122**, 802-805.

Lee, K. A., Karasov, W. H. and Caviedes-Vidal, E. (2002). Digestive response to restricted feeding in migratory yellow-rumped warblers. *Physiol. Biochem. Zool.* **75**, 314-323.

Lepczyk, C. A. and Karasov, W. H. (2000). Effect of ephemeral food restriction on growth of house sparrows. *Auk* **117**, 164-174.

Lepczyk, C. A., Caviedes-Vidal, E. and Karasov, W. H. (1998). Digestive responses during food restriction and realimentation in nestling house sparrows (*Passer domesticus*). *Physiol. Zool.* **71**, 561-573.

Malacarne, G., Cucco, M. and Bertolo, E. (1994). Sibling competition in asynchronously hatched broods of the pallid swift (*Apus pallidus*). *Ethol. Ecol. Evol.* **6**, 293-300.

Marsh, R. and Wickler, S. (1982). The role of muscle development in the transition to endothermy in nestling bank swallows, *Riparia riparia*. *J. Comp. Physiol. B* **149**, 99-105.

Martin, A. W. and Fuhrman, F. A. (1955). The relationship between summated tissue respiration and metabolic rate in the mouse and dog. *Physiol. Zool.* **28**, 18-34.

Miller, D. A. (2010). Morphological plasticity reduces the effect of poor developmental conditions on fledging age in mourning doves. *Proc. Biol. Sci.* **277**, 1659-1665.

Mock, D. W., Schwagmeyer, P. L. and Dugas, M. B. (2009). Parental provisioning and nestling mortality in house sparrows. *Anim. Behav.* **78**, 677-684.

Moe, B., Brunvoll, S., Mork, D., Brobakk, T. E. and Bech, C. (2004). Developmental plasticity of physiology and morphology in diet-restricted European shag nestlings (*Phalacrocorax aristotelis*). *J. Exp. Biol.* **207**, 4067-4076.

Negro, J. J., Chastin, A. and Bird, D. M. (1994). Effects of short-term food deprivation on growth of hand-reared American kestrels. *Condor* **96**, 749-760.

Piersma, T. (2002). Energetic bottlenecks and other design constraints in avian annual cycles. *Integr. Comp. Biol.* **42**, 51-67.

Piersma, T. and Davidson, N. C. (1991). Confusions of Mass and Size. *Auk* **108**, 441-443.

R Development Core Team (2008). *R: a Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. http://www.R-project.org.

Reid, K., Prince, P. A. and Croxall, J. P. (2000). Fly or die: the role of fat stores in the growth and development of grey-headed albatross *Diomedea chrysostoma* chicks. *Ibis* **142**, 188-198.

Richner, H., Schneiter, P. and Stirnimann, H. (1989). Life-history consequences of growth rate depression: an experimental study on carrion crows (*Corvus corone corone* L.). *Funct. Ecol.* **3**, 617-624.

Ricklefs, R. E. (1967). Relative growth, body constituents, and energy content of nestling barn swallows and red-winged blackbirds. *Auk* **84**, 560-570.

Ricklefs, R. E. (1973). Patterns of growth in birds. II. Growth rate and mode of development. *Ibis* **115**, 177-201.

Ricklefs, R. E. (1979). Patterns of growth in birds. V. A comparative study of development in the starling, common tern, and Japanese quail. *Auk* **96**, 10-30.

Ricklefs, R. E. and Webb, T. (1985). Water content, thermogenesis, and growth rate of skeletal muscles in the European starling. *Auk* **102**, 369-376.

Ricklefs, R. E., Starck, J. M. and Konarzewski, M. (1998). Internal constraints on growth in birds. In *Avian Growth and Development: Evolution Within the Altricial–Precocial Spectrum* (ed. J. M. Starck and R. E. Ricklefs), pp. 266-287. New York: Oxford University Press.

Scanes, C. G. and Balthazart, J. (1981). Circulating concentrations of growth hormone during growth, maturation, and reproductive cycles in ring doves (*Streptopelia risoria*). *Gen. Comp. Endocrinol.* **45**, 381-385.

Schew, W. A. and Ricklefs, R. E. (1998). Developmental plasticity. In *Avian Growth and Development: Evolution Within the Altricial–Precocial Spectrum* (ed. J. M. Starck and R. E. Ricklefs), pp. 288-304. New York: Oxford University Press.

Schew, W. A., McNabb, F. M. A. and Scanes, C. G. (1996). Comparison of the ontogenesis of thyroid hormones, growth hormone, and insulin-like growth factor-I in ad libitum and food-restricted (altricial) European starlings and (precocial) Japanese quail. *Gen. Comp. Endocrinol.* **101**, 304-316.

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.

Sears, J. and Hatch, S. A. (2008). Rhinoceros auklet developmental responses to food limitation: an experimental study. *Condor* **110**, 709-717.

Seel, D. C. (1969). Food, feeding rates, and body temperature in the nestling house sparrow *Passer domesticus* at Oxford. *Ibis* **111**, 36-47.

Sibly, R. M., Jones, P. J. and Houston, D. C. (1987). The use of body dimensions of lesser black-backed gulls *Larus fuscus* to indicate size and to estimate body reserves. *Funct. Ecol.* **1**, 275-279.

Starck, J. M. (1996). Intestinal growth in altricial European starling (*Sturnus vulgaris*) and precocial Japanese quail (*Coturnix coturnix japonica*). *Acta Anat.* **156**, 289-306.

Takenaka, M., Niizuma, Y. and Watanuki, Y. (2005). Resource allocation in fledglings of the rhinoceros auklet under different feeding conditions: an experiment manipulating meal size and frequency. *Can. J. Zool.* **83**, 1476-1485.

Thomas, D. W., Blondel, J., Perret, P., Lambrechts, M. M. and Speakman, J. R. (2001). Energetic and fitness costs of mismatching resource supply and demand in seasonally breeding birds. *Science* **291**, 2598-2600.

Török, J., Hegyi, G., Tóth, L. and Könczey, R. (2004). Unpredictable food supply modifies costs of reproduction and hampers individual optimization. *Oecologia* **141**, 432-443.

Visser, M. E., Holleman, L. J. M. and Gienapp, P. (2006). Shifts in caterpillar biomass phenology due to climate change and its impact on the breeding biology of an insectivorous bird. *Oecologia* **147**, 164-172.

Wilkinson, L. (2000). *SYSTAT Version 10 for Windows*. Chicago, IL: SPSS.