Ducklings Exhibit Substantial Energy-Saving Mechanisms as a Response to Short-Term Food Shortage

Børge Moe*
Einar Stølevik
Claus Bech
Department of Biology, Norwegian University of Science and Technology, NO-7491 Trondheim, Norway

Accepted 3/10/04

ABSTRACT

We investigated whether Pekin ducklings (*Anas platyrhyncos domesticus*) exhibited any energy-saving mechanisms that could lessen the detrimental effects of reduced food intake during early development. Further, we evaluated the role of body compositional changes behind such potential mechanisms and the consequences on thermoregulatory capacity. The ducklings exhibited substantial energy-saving mechanisms as a response to diet restriction. After 5 d of diet restriction, the resting metabolic rate (RMR) of 10- and 20-d-old ducklings was 16.4% and 32.1% lower, respectively, than predicted from body mass compared with ad lib. fed ducklings (controls). These reductions in RMR could have been adaptive responses in anticipation of a lasting food shortage, or they could have been consequences of the restricted diet and the lack of essential nutrients. We argue that the responses were adaptive. The low RMRs were not a consequence of depleted fuel stores because the diet-restricted ducklings exhibited substantial amounts of stored lipids at the end of the diet-restriction periods. Hypothermia accounted for ~50% of the reduction in RMR in the 10-d-old diet-restricted ducklings, but hypothermia did not occur in the 20-d-old diet-restricted ducklings. Diet restriction resulted in a reduced liver and intestine size and an unchanged size of the leg muscles and heart, while the length of the skull increased (compared with controls of a given body mass). However, changes in body composition were only minor predictors of the observed changes in RMR. Peak metabolic rate (PMR) was ~10% lower in the diet-restricted ducklings compared with the controls. We have interpreted the lower PMR as a consequence of the reductions in RMR rather than as a consequence of a decreased function of the thermoregulatory effector mechanisms.

* Corresponding author; e-mail: borge.moe@bio.ntnu.no.

Physiological and Biochemical Zoology 78(1):90–104. 2005. 1522-2152/2005/7801-3134$15.00

Introduction

Living organisms have to balance their energy allocation in such a manner that future survival and reproduction are ensured (Stearns 1992). This is particularly challenging for young birds, because they need to get through the vulnerable developmental period as rapidly as possible, while at the same time they should attain a fully developed adult size that ensures both survival and future reproduction (Schew and Ricklefs 1998).

Little is known about the effect of temporal changes in food availability on ontogenetic development in birds. As chicks grow from neonate to adult, they may encounter periods of low food availability that can affect their development (Schew and Ricklefs 1998). Poor feeding conditions can suppress normal growth, affect adult morphology (De Kogel 1997; Birkhead et al. 1999), and result in negative long-term consequences (Lindström 1999; Metcalfe and Monaghan 2001; Dufty et al. 2002).

In order to maximize their survival during poor feeding conditions, chicks should optimize the allocation of their ingested energy to growth and maintenance. At reduced levels of energy intake, the available energy for growth can be allocated to growth and functional maturation of different tissues and organs in the same relative proportions as during normal energy intakes (Konarzewski et al. 1996). Alternatively, it can be specifically allocated to favored organs and tissues at the expense of others (Øyan and Anker-Nilssen 1996; Schew and Ricklefs 1998).

Developmental plasticity is regarded as adaptive if the animal actively adjusts the ontogenetic processes to a change in the environment (Smith-Gill 1983). Modification of the basal level of energy expenditure could occur as an active response in anticipation of a lasting food shortage. By reducing the energy expenditure, the chicks could increase survival and enable more energy to be allocated to growth. Alternatively, any reduction of the basal level of energy expenditure could be a direct consequence of the lack of sufficient nutrients during food shortage. Also, the lack of nutrients could impose reductions in growth rate and in the size of energy-consuming organs, which, consequently, could cause reductions in the basal level of energy expenditure as a nonadaptive response. However, reductions

in the size of energy-consuming organs and in growth rate could also be an adaptive response in anticipation of a lasting food shortage.

Any reduction of the resting metabolic rate (RMR) may be associated with severe costs in terms of reduced capacity for peak metabolism (PMR). According to the aerobic capacity hypothesis (Bennett and Ruben 1979; Taigen 1983), the evolution of endothermy and high levels of sustained activity in birds and mammals was accompanied by an increase in the capacity of aerobic pathways of metabolism and in organ systems (digestion, respiration, circulation, and excretion) that support this capacity. Assuming a close coupling between RMR and PMR, as has been demonstrated interspecifically in adult birds (Dutenhoffer and Swanson 1996; Rezende et al. 2002) and intraspecifically in chicks (Konarzewski et al. 2000), a decrease in basal metabolism should be accompanied by a decrease in peak metabolism (i.e., thermogenic capacity). However, the functional relationship between basal metabolism and peak metabolism is not yet fully understood (Hayes and Garland 1995; Ricklefs et al. 1996).

Birds are generally believed to rely mainly on muscular shivering for heat production during cold exposure (Hohtola and Visser 1998). In contrast, visceral organs (especially the heart, liver, kidneys, and intestine) are believed to consume much of the energy used in basal metabolism (Daan et al. 1990). However, the specific organs and tissues that predict RMR or PMR differ among studies (e.g., Burness et al. 1998; Bech and Østnes 1999; Chappell et al. 1999). Hence, it is not fully understood how body composition functionally relates to RMR and PMR.

It is generally accepted that the energy budgets of chicks have responded to selection, but little consideration has been given to the evolution of the chicks' ability to modify the pattern of energy use and allocation. Precocial birds, like ducklings, are likely to encounter variable food availability during early development because of foraging inexperience, fluctuations in resources, adverse weather conditions, or dominance/sibling interactions. Ducklings are almost thermoregulatorily independent of their parents, and they depend on their own thermoregulatory capacity. In this study, we experimentally imposed short-term diet restriction on Pekin ducklings (*Anas platyrhynchos domesticus*) kept under laboratory conditions to shed light on the relationship between food availability, energy allocation, and metabolism during early development.

In this context, we ask whether ducklings exhibit any energy-saving mechanisms that can lessen the detrimental effects of reduced food intake during early development. Second, we ask whether a change in body composition is the physiological mechanism behind such a metabolic response and, third, whether any energy-saving mechanisms (i.e., lowered RMR) result in a negative effect on the thermoregulatory capacity (PMR) of growing chicks.

Material and Methods

Animal Housing and Experimental Design

Pekin duck eggs were obtained from a local farmer and hatched in the laboratory. After hatching (day 0), the ducklings were kept for ~12 h in the incubator at an ambient temperature (T_a) of 38°C. Thereafter, they were transferred to an enclosure (60 × 40 cm) with ad lib. access to food (poultry pellets) and water. A heat lamp provided a constant range of operative temperatures (Bakken 1992) of 24°–33°C within the enclosure. As the ducklings grew bigger, they progressively gained access to more space. The oldest ducklings (15–20 d old) were confined in an enclosure of 140 × 125 cm in groups of five to 10 ducklings.

Ducklings were randomly assigned to either diet restriction or control (ad lib.) treatment. Water was provided ad lib. to all ducklings in both groups. Diet restriction was imposed for 5-d periods on one group of ducklings from the age of 5 to 10 d and on a second group from the age of 15 to 20 d. The diet-restricted ducklings received small portions of food four to seven times a day to maintain a relatively stable body mass.

Metabolic measurements were made on each duckling at the end of the diet restriction period, at the age of 10 or 20 d, respectively. Ducklings fed ad lib. were used as controls, and metabolic measurements were made on four independent groups of controls at the age of 5, 10, 15, and 20 d, respectively. In total, we measured the metabolic rate (MR) of 55 ducklings, of which 33 were postabsorptive and 22 were not (see "Statistical Analyses"). Postabsorptive ducklings did not receive any food for >10 h before the metabolic measurement. The National Committee for Animal Research in Norway ("Forsøksdyrutvalget") approved the experimental protocols.

Metabolic Measurements

O_2 consumption rates were measured by open-flow respirometry (Withers 1977). A high-pressure air outlet in the laboratory facilitated atmospheric air. After drying over silica gel, the actual flow rates (0.85–3.7 L min^{-1}) entering the metabolic chamber were measured with a mass flow controller (Bronkhorst Hi-Tec, F-201C-FA-22-V). Excurrent air was dried over silica gel before a fraction of the air was directed to the O_2 analyzer (Servomex 1100A). The O_2 analyzer was calibrated with (*a*) dry atmospheric air (20.95%) before every experiment and with (*b*) pure N_2 after every ~10 experiments. Any changes from the pre- to the postexperiment readings of the O_2 content in dry atmospheric air were controlled for by assuming a linear drift. Measurements of the O_2 content in excurrent air (accuracy 0.01%) were stored, along with the temperature measurements, on a Squirrel data logger at 30-s intervals.

The MRs were calculated by using formula (1d) in Withers (1977), assuming a constant respiratory quotient of 0.79, and corrected for wash-out delays in the system by using the method

given by Niimi (1978). In this way, we obtained the instantaneous O_2 consumption rates. Values of the MR were calculated from the O_2 consumption rates using 5.5824 W as the caloric equivalent for 1 L O_2 h^{-1}.

RMR was defined as the lowest MR calculated with 25 min running average during exposure to thermoneutral conditions. The T_a for thermoneutral conditions was set between 33° and 26°C, depending on the ducklings' ages (Østnes and Bech 1997). The use of a running average over a 25-min interval was justified after plotting the minimum values of the MR, calculated in five randomly selected experimental runs using intervals that varied from 2 to 60 min. For a running average lower than 15 min, these curves revealed a very strong positive relationship between the minimum values of RMR and the length of the running average interval. Short intervals resulted in very low minimum values of RMR, thereby underestimating the RMR level. However, at a running average between 15 and 60 min, the minimum values of RMR changed relatively little (for a description of this procedure, see Meerlo et al. 1997).

Depending on the ducklings' ages, the metabolic chamber was 9 or 25 L. A surrounding climatic chamber (Heraeus Vötsch, type VEM 03/500) regulated the T_a inside the metabolic chambers. After 3–6 h exposure to thermoneutral conditions, the T_a was lowered at a constant rate of 0.7°C min^{-1} inside the 9-L and the 25-L chamber, respectively. The lowest T_a imposed in the metabolic measurements was −26.2°C. The ducklings' MR increased with decreasing T_a. After reaching a peak MR, MR and body temperature (T_b) showed a consistent decrease to a further decrease in T_a, and the experiment was terminated. PMR was defined as the highest 10-min running average MR during cold exposure. Some of the 20-d-old ducklings (four controls and four diet restricted) maintained a relatively stable MR and T_b at the lowest T_a's imposed by the climatic chamber. Hence, we cannot be sure that they reached the true PMR, and they were excluded from the PMR analyses. One 5-d-old duckling was also excluded because of a power supply failure during the PMR measurement. Each individual was used only once in the experiments. The ducklings were killed with ether immediately after the metabolic measurement and stored at −20°C for subsequent analysis of body composition. Body masses of the ducklings were weighed, to the nearest 0.1 g, before and immediately after each experiment. A linear decrease in body mass during the experiment was assumed when calculating the body mass at the time when RMR and PMR were obtained.

The T_a was measured with a thermocouple mounted inside the metabolic chamber. The T_b was measured during the entire metabolic measurement in the cloaca with a copper-constantan thermocouple (California fine wire, type 0.005) surrounded by polypropylene tubing (PP 50, Portex) and secured with adhesive tape over the cloaca. Depending on the duckling's age, the thermocouple was inserted 1.8–4.5 cm into the cloaca.

The minimal "wet" thermal conductance (MTC) was calculated according to the method originally described by Scholander et al. (1950). However, this method is valid only when T_b is kept constant. Since the T_b of the ducklings decreased during cold exposure, we had to include a correction factor to account for the fall in T_b (see also Visser and Ricklefs 1993). Thus, the following formula was used to calculate MTC:

$$\mathrm{MTC} = \frac{\mathrm{PMR} + A}{T_b - T_a}, \tag{1}$$

where A is the correction factor for the decrease in energy content (W kg^{-1}). The calculation of the correction factor was based on the rate of fall in T_b recorded during the last 10-min period before PMR was attained and on a specific heat of 3.45 J g^{-1} °C^{-1} (Hart 1951). Thermal conductance (TC) during thermoneutral conditions was calculated according to the following formula:

$$\mathrm{TC} = \frac{\mathrm{RMR}}{T_b - T_a}. \tag{2}$$

Body Composition

Dissection was performed on semithawed carcasses in order to reduce vaporization and improve organ separation. We removed heart, liver, kidney, gizzard, and intestines (small and large). The entire right breast muscle (*m. supracoracoideus* and *m. pectoralis*) was separated from the skeleton. Also, the entire right leg muscle was separated from the tibiotarsus-tarsometatarsus joint. Gizzard, intestine, and heart atrium were emptied of contents, while all organs and muscles were carefully trimmed of fat and weighed (± 1 mg, carcasses to ± 0.1 g). They were then dried to a constant mass at 56°C and reweighed. Fat content was thereafter removed in baths of petroleum ether, and the samples were again dried and reweighed. The lean dry fraction (LDF) of organs was calculated as the ratio of lipid-free dry organ mass to lipid-free fresh organ mass. The LDF of most organs and tissues increases during the ontogenetic development because of a build-up of proteins and functional components on the cellular level. Hence, the LDF is regarded as reflecting the functional maturity of an organ.

Statistical Analyses

We used a general linear model (GLM) procedure with the type III sum of squares to perform ANCOVA and ANOVA. The GLM was performed with the ENTER method, in which we excluded insignificant interaction terms, factors, or covariates one by one from the null model. We inspected all variables graphically to ensure linearity before performing GLM. MR and organ mass show allometric relationships to body mass. Hence, $\log_{10}$ transformation was used to linearize these variables before examination.

We analyzed the relationship between organ mass and MR as well as the relationship between PMR and RMR by including body mass as a covariate to remove the effect of body mass (i.e., body mass is held constant; Hayes and Shonkwiler 1996). In order to avoid possible effects of part-whole correlation, we subtracted organ mass from the body mass variable, before examination, when organ mass and body mass were included in the same analysis (Christians 1999).

Typically for precocial species, the ontogenetic development of RMR follows a biphasic pattern in relation to body mass. A biphasic pattern also exists for the ontogenetic development of many organs. Hence, we have defined age group as a factor, in which 5–10-d-old and 10–20-d-old ducklings constitute two different age groups. When we have performed statistical analyses on each age group separately, a two-level factor for treatment (1 = controls, 2 = diet-restricted ducklings) is used. When performing analyses including both age groups, age group is included as a factor in the model, and the two-level factor treatment or the three-level factor treatment group (1 = controls, 2 = 10 d old diet restricted, 3 = 20 d old diet restricted) is included in the model and specified in the text.

When two regressions (eq. [3]) with $\log_{10}$-transformed variables (e.g., MR on body mass [BM]) have the same regression coefficient (β) but have different intercepts (α and $\alpha + \gamma$), we have calculated the percentage difference (X) between the nontransformed regressions according to formula (4):

$$\begin{aligned} \log_{10}\mathrm{MR}_{(1)} &= \beta \log_{10}\mathrm{BM} + \alpha, \\ \log_{10}\mathrm{MR}_{(2)} &= \beta \log_{10}\mathrm{BM} + \alpha + \gamma, \end{aligned} \tag{3}$$

$$\begin{aligned} X &= 100 \times \left(1 - \frac{\mathrm{MR}_{(2)}}{\mathrm{MR}_{(1)}}\right) \\ &= 100 \times \left(1 - \frac{\mathrm{BM}^{\beta} \times 10^{\alpha} \times 10^{\gamma}}{\mathrm{BM}^{\beta} \times 10^{\alpha}}\right) \\ &= 100 \times (1 - 10^{\gamma}). \end{aligned} \tag{4}$$

We measured MR on postabsorptive and nonpostabsorptive ducklings. In order to find any effect of the "absorptive status" (i.e., postabsorptive or nonpostabsorptive) on MR (and T_b), we used the GLM procedure with MR as the dependent variable, body mass as covariate, and absorptive status and treatment as factors. This revealed that absorptive status and the interactions with body mass and treatment significantly affected MR. Consequently, we adjusted the MR of nonpostabsorptive ducklings downward to the postabsorptive levels. The parameter estimates from the GLM were used to make the appropriate equation for the adjustment for each treatment group. The $\log_{10}$-transformed MR of each nonpostabsorptive duckling was subtracted by ΔMR, which was calculated according to the following equation (5) within each treatment group:

$$\begin{aligned} \Delta\mathrm{MR} &= \text{predicted } \log_{10}\mathrm{MR}_{(1)} - \text{predicted } \log_{10}\mathrm{MR}_{(0)} \\ &= \beta_{(1)} \log_{10}\mathrm{BM} + \alpha_{(1)} - (\beta_{(0)} \log_{10}\mathrm{BM} + \alpha_{(0)}) \\ &= (\beta_{(1)} - \beta_{(0)}) \log_{10}\mathrm{BM} + \alpha_{(1)} - \alpha_{(0)}, \end{aligned} \tag{5}$$

where subscripts (1) and (0) denote parameters obtained from nonpostabsorptive and postabsorptive ducklings, respectively. BM is body mass, and α and β represent the intercepts and slopes, respectively. This correction to postabsorptive levels uses an average value (for each treatment group and controlled for body mass) for MRs to adjust nonpostabsorptive MRs. A variance in nonpostabsorptive RMR caused by a variation in heat increment of feeding will still exist after the correction to postabsorptive levels. However, we regard this "noise" as too weak to obscure the conclusions in this study.

We tested the effect of diet restriction on RMR (and PMR) by GLM models with RMR and lean dry body mass as dependent and independent variables, respectively. The effect of diet restriction on body composition was analyzed separately for each organ, with lean dry organ mass and lean dry body mass (minus organ mass) as the dependent and the independent variable, respectively. Treatment and age group were entered as factors in these models. The null models included the two interactions treatment × body mass and age group × body mass. The effect of diet restriction on LDF was analyzed separately for each organ and for each age group.

We analyzed the relationship between RMR (and PMR) and organ mass for each organ separately. Organ mass and body mass (minus organ mass) were entered as covariates, and treatment was entered as a factor. We performed separate analyses for each age group as well as analyses in which both age groups were included. In the latter analyses, we added age group and treatment group as factors.

Biometric measurements (wing length, tarsus length, skull length [head + bill]) were made on 5-, 10-, 15-, and 20-d-old ducklings. Growth rate was calculated as the daily growth during the last 5 d (mm d^{-1}). Hence, growth rates of structural elements were obtained for 10-, 15-, and 20-d-old ducklings and were not instantaneous growth rates at these specific ages. We used a principal component analysis to extract a factor score (PC1) of the growth rate of the wing, the tarsus, and the skull. Analyses of the relationship between growth rate and RMR were performed separately for each growth rate variable (wing, skull, tarsus, PC1, body mass). Body mass was included as a covariate, and age group was included as a factor, while treatment was included as a factor only for the analyses where both controls and diet-restricted ducklings were included.

The Bonferroni method was used for post hoc pairwise multiple comparisons ("pairwise comparisons" hereafter). It re-

ports adjusted P values that have been multiplied with the number of pairs tested. All statistical analyses were performed with SPSS v. 11.5.1 (2002).

Results

Body Mass

The diet restriction had a substantial effect on body mass growth (Fig. 1). The body mass of the diet-restricted ducklings was maintained at a relatively stable level during the periods of diet restriction; that is, they gained only 2.9 g d^{-1} between 5 and 10 d of age ($P < 0.001$) and 6.3 g d^{-1} between 15 and 20 d of age ($P < 0.001$). In contrast, the growth of the ad lib. fed ducklings (controls) followed a normal growth curve (Fig. 1).

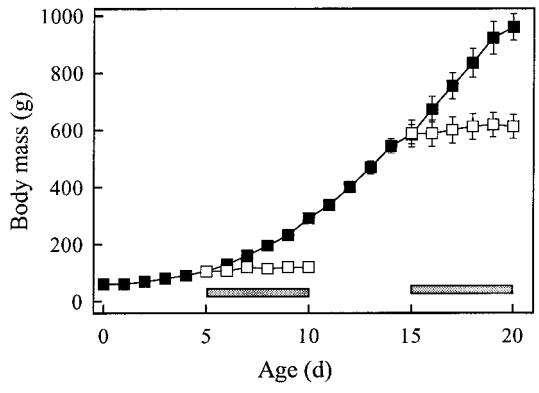

Figure 1. Body mass growth (g) of Pekin ducklings (*Anas platyrhynchos*). Ducklings fed ad lib. (controls) are shown as filled squares, and diet-restricted ducklings are shown as open squares. The gray horizontal bars indicate the time of the diet restriction periods. Means ± SE.

Metabolism

The development of RMR showed a biphasic pattern in relation to body mass (age group × body mass interaction, $F_{1,49} = 4.49$, $P < 0.05$; Fig. 2). RMR of 5–10-d-old ducklings scaled to body mass by the power of 1.01 (SE = 0.08), while RMR of 15–20-d-old ducklings scaled to body mass by the power of 0.82 (SE = 0.07). The treatment × body mass interaction was also significant ($F_{1,49} = 13.21$, $P < 0.001$), indicating that 10-d-old diet-restricted ducklings showed a different metabolic response to diet restriction compared with 20-d-old diet-restricted duckling. Hence, we removed the two-level treatment factor and entered treatment group as a factor with three levels (1 = controls, 2 = 10 d old diet restricted, 3 = 20 d old diet restricted). The treatment group × body mass interaction was not significant, while the intercepts of the regressions of the treatment groups were highly significantly different ($F_{2,49} = 56.89$, $P < 0.001$; Fig. 2). This shows that diet restriction had a substantial effect on RMR. The parameter estimates from the GLM and pairwise comparisons showed that the 20-d-old diet-restricted ducklings exhibited 32.1% lower RMR compared with controls ($P < 0.001$), while the 10-d-old diet-restricted ducklings exhibited 16.4% lower RMR compared with controls ($P < 0.001$).

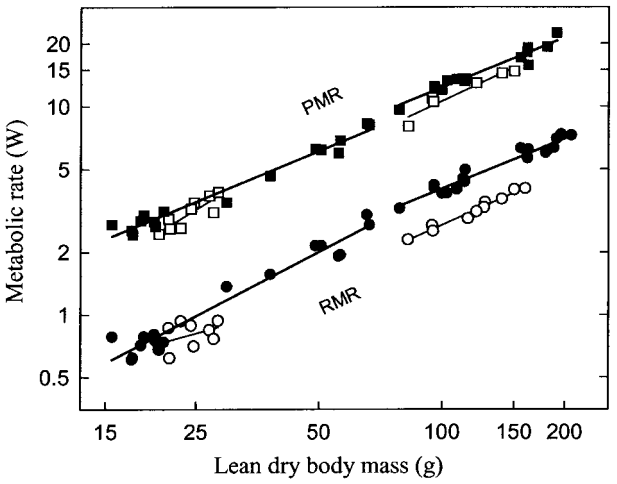

Figure 2. Resting and peak metabolic rate (RMR and PMR) of ad lib. fed (controls; *filled symbols*) and diet-restricted ducklings (*open symbols*). Metabolic rate in watts and lean dry body mass in grams. The axes are $\log_{10}$ scaled. Regression lines are shown separately for controls and diet-restricted ducklings within each of the two age groups (5–10-d-old and 15–20-d-old ducklings). The coefficient of determination (r^2) was 0.99 for both the RMR and the PMR model.

PMR scaled to body mass by the power of 0.82 (SE = 0.03, $F_{1,41} = 713.19$, $P < 0.001$; Fig. 2). The age group × body mass interaction was not significant, but for consistency with the RMR analysis, we included the three-level treatment group factor in the model. The main effect of treatment group was highly significant ($F_{2,41} = 10.95$, $P < 0.001$), while the interaction with body mass was not significant. Hence, PMR was lower in the diet-restricted ducklings compared with the controls. Pairwise comparisons showed that the PMR of the 20-d-old diet-restricted ducklings was 11.3% lower compared with controls ($P < 0.005$), while the PMR of the 10-d-old diet-restricted ducklings was 9.2% lower compared with controls ($P < 0.05$).

T_b measured at RMR increased with age (Fig. 3*A*). T_b of 20-d-old diet-restricted and 20-d-old controls were both 40.5°C. In contrast, T_b of 10-d-old diet-restricted ducklings was significantly lower compared with 10-d-old controls ($t = -4.55$, df = 15, $P < 0.001$). The relationship between $\log_{10}$-transformed T_b and lean dry body mass revealed that 10-d-old diet-restricted ducklings exhibited 0.7°C lower T_b than predicted from controls of the same body mass ($F_{2,51} = 11.03$, $P < 0.001$).

T_a measured at PMR showed a negative relationship to the age of the ducklings ($F_{1,40} = 23.47$, $P < 0.001$; Fig. 3*B*). The

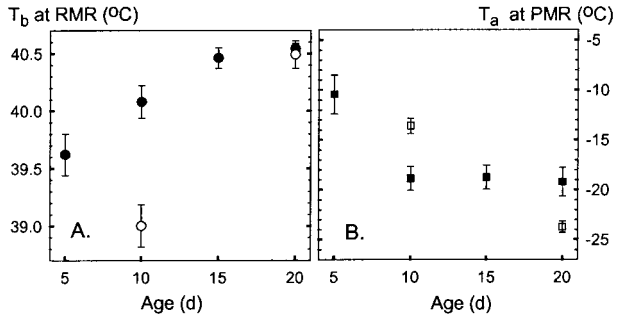

Figure 3. Body temperature (T_b) measured at RMR (*A*) and ambient temperature (T_a) measured at PMR (*B*) in ad lib. fed (controls; *filled symbols*) and diet-restricted ducklings (*open symbols*). Temperature in Celsius (°C) and age in days (d). Temperatures are given as means ± 1 SE.

age × treatment and age × age group interactions were significant. T_a measured at PMR for 10-d-old diet-restricted ducklings was 5.3°C higher compared with 10-d-old controls ($P<0.05$) but was not significantly different from 5-d-old controls. In contrast, T_a measured at PMR was lower for 20-d-old diet-restricted ducklings than that of 15-d-old controls ($P<0.05$), but not significantly different from that of 20-d-old controls. Hence, 20-d-old diet-restricted ducklings attained PMR at a lower T_a than expected from body mass ($F_{1,18}=9.1$, $P<0.001$), while 10-d-old diet-restricted ducklings attained PMR at a T_a as expected from body mass ($F_{1,21}=0.01$, $P>0.05$). T_b measured at PMR was not different between diet-restricted ducklings and controls ($F_{1,42}=0.02$, $P>0.05$). Average values for T_b at PMR were 37.2°C (±0.8), 37.4°C (±0.5), 39.0°C (±0.3), and 38.6°C (±0.4) for 5-, 10-, 15-, and 20-d-old controls, respectively, and 37.4°C (±0.4) and 38.1°C (±0.6) for 10- and 20-d-old diet-restricted ducklings, respectively.

MTC decreased with increasing body mass ($F_{1,40}=15.90$, $P<0.001$; Fig. 4*A*) and age (Fig. 4*B*). The slopes of the regressions between $\log_{10}$-transformed MTC and lean dry body mass were not significantly different between the treatment groups ($F_{2,37}=0.47$, $P>0.05$; Fig. 4*A*) or between the age groups ($F_{1,39}=3.46$, $P>0.05$). The main effect of treatment group was significant ($F_{2,40}=4.39$, $P<0.05$), and pairwise comparisons showed that the MTC of the 20-d-old diet-restricted ducklings was lower (by 15%) compared with controls ($P<0.05$), while the MTC of the 10-d-old diet-restricted ducklings was not.

The absolute scope (PMR minus RMR; i.e., the portion of PMR available for regulatory thermogenesis) showed no response to the diet-restriction treatment (Fig. 5*A*). The slopes of the regressions between $\log_{10}$-transformed absolute scope and lean dry body mass were not significantly different among the treatment groups ($F_{2,39}=2.79$, $P>0.05$; Fig. 5*A*), and the intercepts of these relationships were not significantly different ($F_{2,41}=0.90$, $P>0.05$). Body mass ($F_{1,41}=273.93$, $P<0.001$) and age group ($F_{1,41}=4.9$, $P<0.05$) were the only significant predictors of absolute scope. In contrast, the factorial scope (PMR/RMR) was significantly higher for 20-d-old diet-restricted ducklings compared with 15- and 20-d-old controls ($F_{2,18}=22.74$, $P<0.001$; Fig. 5*B*). The factorial scope was also different between 5- and 10-d-old ducklings ($F_{2,22}=11.00$, $P<0.001$; Fig. 5*B*), but the factorial scope of 10-d-old diet-restricted ducklings was significantly higher only compared with 10-d-old controls ($P<0.001$) and not with 5-d-old controls ($P>0.05$).

RMR showed a positive relationship to PMR in the 5–10-d-old duckling ($F_{1,22}=4.63$, $P<0.05$, $r=0.42$) and in the 15–20-d-old ducklings ($F_{1,18}=15.32$, $P<0.001$, $r=0.68$). Body mass was a significant covariate. Treatment and the treatment × RMR interaction were included in the null model, but they were not significant. Consequently, they were excluded from the final model. RMR also showed a positive relationship to PMR ($F_{1,42}=16.10$, $P<0.001$, $r=0.53$) in the analysis where both age groups were included.

Body Composition

Diet restriction affected organ size in three different ways. (1) There was no significant effect of the treatment (e.g., Fig. 6*A*). This was the case for the leg muscles ($F_{1,50}=0.46$, $P>0.05$; Fig. 6*A*), the heart ($F_{1,52}=0.25$, $P>0.05$), and the kidneys ($F_{1,52}=0.30$, $P>0.05$); diet-restricted ducklings and controls showed the same allometric relationship between organ mass and body mass. (2) There was a significant effect of the treatment but no significant treatment × body mass interaction (e.g., Fig. 6*B*). Diet-restricted ducklings had significantly lighter liver mass ($F_{1,50}=85.76$, $P<0.001$; Fig. 6*B*) and shorter intestine length ($F_{1,51}=6.61$, $P<0.001$) compared with controls. (3)

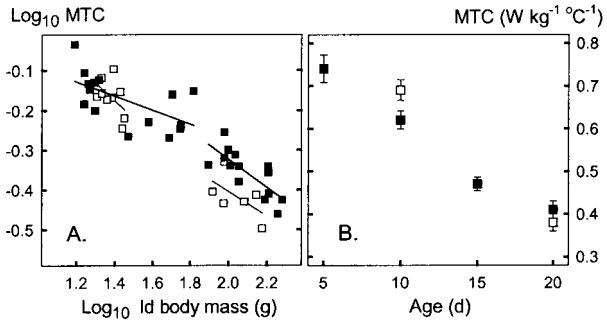

Figure 4. Minimal thermal conductance (MTC) in relation to lean dry body mass (*A*) and age (*B*) in ad lib. fed (controls; *filled symbols*) and diet-restricted ducklings (*open symbols*). MTC in watts per kilograms per degree Celsius (W kg^{-1} $°C^{-1}$), lean dry body mass in grams (g), and age in days (d). In *A*, the values are $\log_{10}$ transformed, and regression lines are shown separately for controls and diet-restricted ducklings within each of the two age groups (5–10-d-old and 15–20-d-old ducklings). MTC is given as means ± 1 SE in *B*.

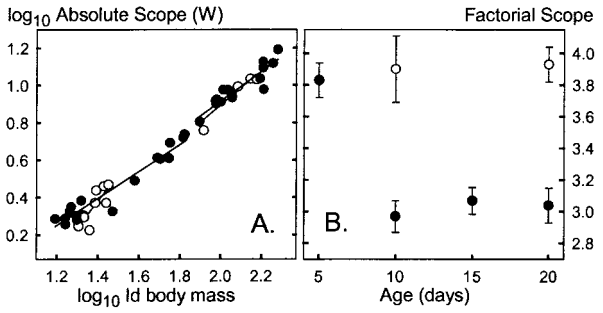

Figure 5. Absolute scope in relation to lean dry body mass (*A*) and factorial scope in relation to age (*B*) in ad lib. fed (*filled symbols*) and diet-restricted (*open symbols*) ducklings. Absolute scope was calculated as PMR − RMR, and factorial scope was calculated as PMR/RMR. In *A*, the values are $\log_{10}$ transformed, and regression lines are shown separately for controls and diet-restricted ducklings within each of the two age groups (5–10-d-old and 15–20-d-old ducklings). Absolute scope is given in watts (W), lean dry (*ld*) body mass in grams (g), and age in days (d). Factorial scope is given as means ± 1 SE.

There was a significant effect of the treatment, but the effect of the treatment depended on the body mass and was different between 10- and 20-d-old diet-restricted ducklings (i.e., significant treatment × body mass interaction; e.g., Fig. 6*C*, 6*D*). In these analyses, we entered treatment group as a factor in order to do pairwise comparisons between the intercepts (means controlled for body mass and age group) of each treatment group. This revealed that the lipid mass of 10-d-old diet-restricted ducklings was 30.8% lower compared with controls ($P < 0.001$), while the lipid mass of 20-d-old diet-restricted ducklings was 16.4% lower compared with controls ($P < 0.01$). In contrast, the gizzard mass of 10-d-old diet-restricted ducklings was 22.7% heavier compared with controls ($P < 0.001$), while the gizzard mass of 20-d-old diet-restricted ducklings was not significantly different from that of controls ($P > 0.05$). The mass of the pectoral muscles for 10-d-old diet-restricted ducklings was not significantly different compared with controls ($P > 0.05$), while the pectoral muscle mass of 20-d-old diet-restricted ducklings was 29.2% lighter compared with controls ($P < 0.001$). The intestine mass of 20-d-old diet-restricted ducklings was 21.3% lower compared with controls ($P < 0.001$), while the intestine mass of 10-d-old diet-restricted ducklings was 10.9% lower compared with controls ($P < 0.05$).

The structural size of the ducklings was also affected by diet restriction. The length of the skull (head + bill) of the diet-restricted ducklings was 6.0% longer compared with controls for a given mass ($F_{1,45} = 54.36$, $P < 0.001$; Fig. 6*F*). However, the age-specific growth of the skull was not fully sustained during the diet restriction. For a given age, the diet-restricted ducklings exhibited ~6% shorter skulls compared with controls ($F_{1,45} = 13.39$, $P < 0.001$). In the analysis of the length of the tarsus, there was a significant treatment × body mass interaction ($F_{1,46} = 4.89$, $P < 0.05$), indicating that diet restriction affected the tarsus of 10- and 20-d-old diet-restricted ducklings differently. However, after entering the three-level treatment factor, pairwise comparisons revealed that tarsus length did not vary among diet-restricted and control ducklings in either the 10- or the 20-d groups ($P > 0.05$). There was also a significant treatment × body mass interaction in the analysis of the length of the wing ($F_{1,44} = 13.18$, $P < 0.001$). After entering the three-level treatment factor, pairwise comparisons revealed that the length of the wing of 10-d-old diet-restricted ducklings was 15.6% shorter compared with controls ($P < 0.001$), while the

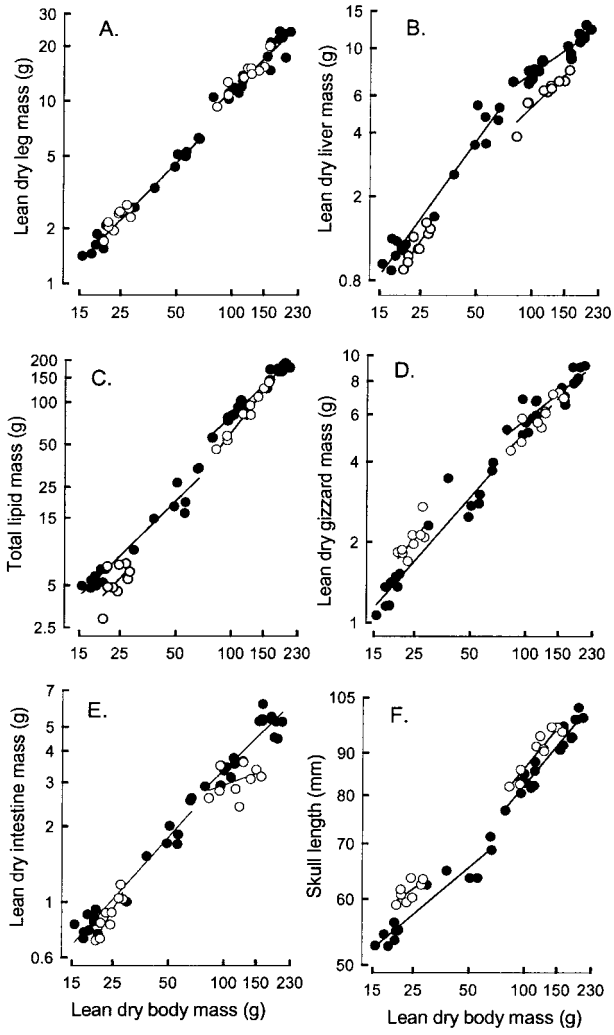

Figure 6. Body composition of ad lib. fed (controls; *filled symbols*) and diet-restricted ducklings (*open symbols*). The relationship of leg mass (*A*), liver mass (*B*), lipid mass (*C*), gizzard mass (*D*), intestine mass (*E*), and skull length (head + bill; *F*) to body mass. Regression lines are drawn separately for controls and diet-restricted ducklings within each of the two age groups (5–10 d old and 15–20 d old). Organ and body masses are lean dry masses in grams, and lipid mass is dry mass in grams. The skull length is given in millimeters. The axes are $\log_{10}$ scaled.

length of the wing of 20-d-old diet-restricted ducklings was not different compared with controls ($P > 0.05$).

The diet restriction also affected the LDF of organs. The LDF of the pectoral muscles was higher in 10-d-old ($F_{1,22} = 12.64$, $P < 0.005$) and 20-d-old diet-restricted ducklings ($F_{1,26} = 69.00$, $P < 0.001$) compared with controls (Fig. 7*D*). The LDF of the gizzard was also higher in 10-d-old ($F_{1,23} = 9.98$, $P < 0.005$) and 20-d-old diet-restricted ducklings ($F_{1,26} = 6.17$, $P < 0.05$) compared with controls (Fig. 7*B*). The LDF of the leg muscles was higher in the 10-d-old diet-restricted ducklings ($F_{1,22} = 5.31$, $P < 0.05$) but not in the 20-d-old diet-restricted ducklings ($F_{1,24} = 0.81$, $P > 0.05$) compared with controls (Fig. 7*A*). In contrast, the LDF of the liver was lower in 20-d-old diet-restricted ducklings ($F_{1,26} = 6.75$, $P < 0.05$) but not in 10-d-old diet-restricted ducklings ($F_{1,23} = 0.89$, $P > 0.05$) compared with controls. The LDF of the heart, the kidneys (Fig. 7*E*), and the intestine showed no significant differences between diet-restricted and controls.

Body Composition and RMR

No organ masses (main effects) were significant predictors of RMR in any of the two age groups of ducklings (Table 1). Body mass and treatment were strong predictors of RMR in all the analyses of any relationship between organ mass and RMR. However, there was a significant interaction with treatment for intestine length ($F_{1,21} = 6.74$, $P < 0.05$) and intestine mass ($F_{1,21} = 4.62$, $P < 0.05$) of 5–10 d old ducklings. For the controls, the parameter estimates showed a positive relationship between intestine length and RMR ($B = 1.1$, $r = 0.42$, $P < 0.05$; B is the partial regression coefficient) and a positive, but nonsignificant, relationship between intestine mass and RMR ($B = 0.41$, $r = 0.31$, $P = 0.16$). For the diet-restricted ducklings, the corresponding estimates were negative and nonsignificant (intestine length, $B = -0.37$, $r = -0.49$, $P > 0.4$; intestine mass, $B = -0.22$, $r = -0.42$, $P > 0.3$).

We also performed analyses in which both age groups were included. These analyses were consistent with the analyses in which we separated the age groups, except for one organ. The mass of the liver was a significant predictor of RMR ($F_{1,50} = 8.54$, $P < 0.005$, $r^2 = 0.15$).

Growth Rate and RMR

In the controls (ad lib. fed ducklings), the growth rate of the wings ($F_{1,22} = 10.20$, $P < 0.005$) and the growth rate of the body mass ($F_{1,33} = 10.42$, $P < 0.005$) were significant predictors of RMR. When we analyzed the controls and the diet-restricted ducklings together, the growth rate of the wings was still significant ($F_{1,22} = 10.20$, $P < 0.005$), but the growth rate of the body mass was not. PC1, the factor score extracted from a principal component analysis with wing, skull, and tarsus growth rate, showed a tendency toward a positive relationship to RMR in controls ($F_{1,22} = 3.07$, $P = 0.09$) and in controls and diet-restricted ducklings ($F_{1,38} = 3.20$, $P = 0.08$).

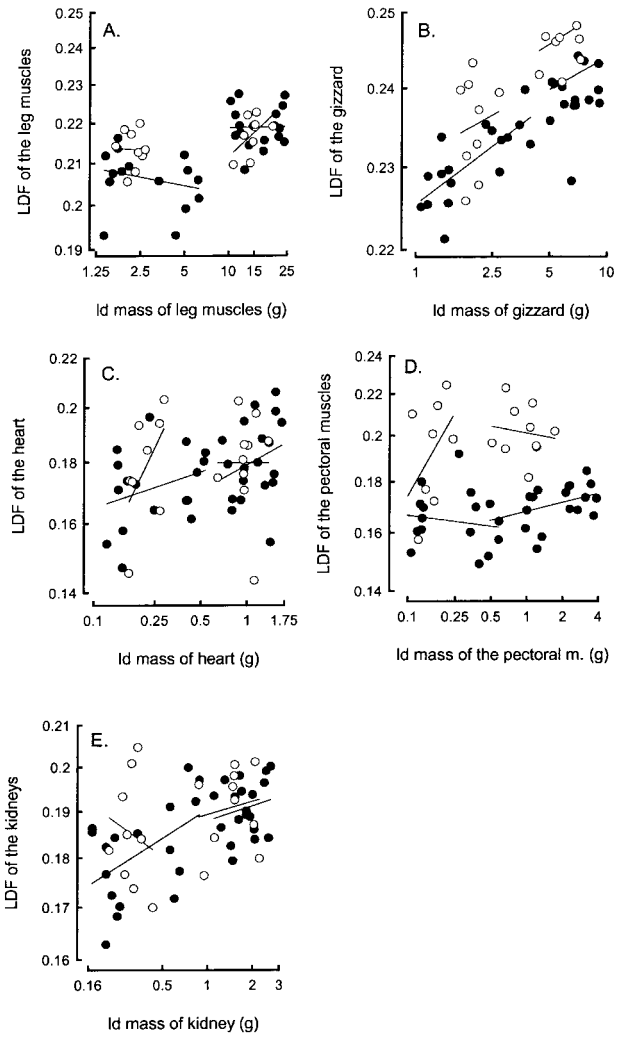

Figure 7. Lean dry fraction (LDF) of leg muscles (*A*), gizzard (*B*), heart (*C*), pectoral muscles (*D*), and kidneys (*E*) in relation to lean dry (*ld*) organ mass in ad lib. fed (controls; *filled symbols*) and diet-restricted (*open symbols*) ducklings. Regression lines are drawn separately for controls and diet-restricted ducklings within each of the two age groups (5–10-d-old and 15–20-d-old ducklings). LDF was calculated as lipid-free dry organ mass/lipid-free fresh organ mass. Organ masses in grams (g). The axes are $\log_{10}$ scaled.

Body Composition and PMR

A general trend was apparent for the analyses of any relationship between organ mass and PMR. The treatment factor and body mass were strong predictors of RMR in almost all the analyses. In addition, some organ masses were significant predictors. In 5–10-d-old ducklings, the mass of the leg muscles ($F_{1,20} = 6.53$, $P < 0.05$; Table 2) and the total lipid mass ($F_{1,21} = 4.81$,

Table 1: Relationships between resting metabolic rate (RMR) and organ masses in controls and diet-restricted ducklings

	RMR of 5–10-d-Old Ducklings							RMR of 15–20-d-Old Ducklings						
	Main Effects				Organ Mass × Treatment Interaction			Main Effects				Organ Mass × Treatment Interaction		
	F	df	P	r^2	F	df	P	F	df	P	r^2	F	df	P
Breast muscle mass	.1	1, 21	NS	.00	1.5	1, 20	NS	.8	1, 25	NS	.03	.9	1, 24	NS
Leg muscle mass	.6	1, 21	NS	.03	2.5	1, 20	NS	.0	1, 25	NS	.00	.7	1, 24	NS
Heart mass	.1	1, 22	NS	.00	1.7	1, 20	NS	.3	1, 25	NS	.01	.5	1, 24	NS
Liver mass	1.0	1, 22	NS	.04	.6	1, 21	NS	.6	1, 25	NS	.02	.2	1, 24	NS
Gizzard mass	.2	1, 22	NS	.01	3.3	1, 21	NS	3.8	1, 25	.06	.13	.0	1, 24	NS
Kidney mass	.8	1, 22	NS	.04	3.8	1, 21	NS	1.1	1, 25	NS	.04	.0	1, 24	NS
Intestine mass	.2	1, 21	NS	.01	4.6	1, 21	<.05	.2	1, 25	NS	.01	.4	1, 24	NS
Intestine length	.8	1, 21	NS	.04	6.7	1, 21	<.05	1.5	1, 24	NS	.06	.2	1, 23	NS
Lipid mass	2.1	1, 22	NS	.41	1.8	1, 21	NS	.0	1, 25	NS	.00	.6	1, 24	NS

Note. The analyses were performed separately on each organ with a GLM on $\log_{10}$-transformed metabolic rate, lean dry body mass (minus organ mass), and lean dry organ mass. Treatment was included as a factor with two levels (1 = controls, 2 = diet restricted). The organ mass × treatment interaction was included in the null model. The treatment factor and body mass were strong predictors of RMR in all the analyses. In this table, we focus on the relationship between the organ masses and RMR. Hence, the statistics for treatment and body mass are not included in this table.

$P < 0.05$) were significant predictors of PMR. By using the residuals from these analyses, we found that the mass of the leg muscles was positively correlated to the total lipid mass ($r = 0.87$, $N = 24$, $P < 0.01$).

In 15–20-d-old ducklings, the mass of the liver ($F_{1,17} = 9.92$, $P < 0.01$) was a significant predictor of PMR. For the heart, the treatment × organ mass interaction was significant ($F_{1,16} = 7.25$, $P < 0.05$). The parameter estimates showed a positive relationship between organ mass and PMR in diet-restricted ducklings ($B = 0.46$, $r = 0.56$, $P < 0.05$) but no relationship between organ mass and PMR in controls ($B = 0.02$, $r = 0.05$, $P > 0.05$).

We also performed analyses in which both age groups were included. With these analyses, we found that the mass of the leg muscles was a significant predictor of PMR ($F_{1,37} = 8.20$, $P < 0.01$). The total lipid mass was also a significant predictor of PMR ($F_{1,41} = 10.07$, $P < 0.005$). Again, using the residuals from this analysis and from the analyses of the relationship between the mass of the leg muscles, we found that they were strongly correlated ($r = 0.87$, $N = 45$, $P < 0.01$).

The Relationship between LDF and MR (PMR and RMR)

There was a negative and nearly significant relationship between LDF of the leg muscles and PMR in 5–10-d-old ducklings, ($F_{1,20} = 4.08$, $P = 0.057$). In 15–20-d-old ducklings, there was no relationship between LDF of the leg muscles and PMR ($F_{1,15} = 0.73$, $P > 0.05$). There were no significant relationships between the LDF of any of the other organs and PMR in any of the two age groups.

In 5–10-d-old ducklings, there was a positive and significant relationship between LDF of the kidneys and RMR ($F_{1,22} = 12.89$, $P < 0.005$). In 15–20-d-old ducklings, there was no relationship between LDF of the kidneys and RMR ($F_{1,25} = 0.74$, $P > 0.05$) or between LDF of any of the organs and RMR.

In 5–10-d-old ducklings, the LDF of the leg muscles × treatment interaction was a significant predictor of RMR ($F_{1,20} = 5.15$, $P < 0.05$). There was a significant positive relationship between LDF of the leg muscles and RMR in the diet-restricted ducklings ($B = 2.6$, $r = 0.45$, $P < 0.05$), while a negative significant relationship existed between LDF of the leg muscles and RMR in the controls ($B = -2.2$, $r = -0.46$, $P < 0.05$).

Discussion

In this study, we asked three main questions. (1) Will ducklings show any energy-saving mechanisms that lessen the detrimental effects of reduced food intake during early development? (2) Are changes in body composition the physiological mechanism behind such a metabolic response? (3) Will any energy-saving mechanism (i.e., lowered RMR) result in a negative effect on the thermoregulatory capacity (PMR) of growing chicks?

The results of this study reveal that ducklings exhibit a substantial energy-saving mechanism as a response to short-term diet restriction. After 5 d of diet restriction, the RMR of 10- and 20-d-old duckling was 16.4% and 32.1% lower, respectively,

Table 2: Relationships between peak metabolic rate (PMR) and organ masses in controls and diet-restricted ducklings

	PMR of 5–10-d-Old Ducklings							PMR of 15–20-d-Old Ducklings						
	Main Effects				Organ Mass × Treatment Interaction			Main Effects				Organ Mass × Treatment Interaction		
	F	df	*P*	r^2	*F*	df	*P*	*F*	df	*P*	r^2	*F*	df	*P*
Breast muscle mass	.1	1, 20	NS	.00	2.4	1, 19	NS	.5	1, 17	NS	.03	.2	1, 16	NS
Leg muscle mass	6.5	1, 20	<.05	.25	1.7	1, 19	NS	.3	1, 17	NS	.02	2.7	1, 16	NS
Heart mass	.0	1, 21	NS	.00	2.1	1, 20	NS	4.0	1, 16	.062	.20	7.3	1, 16	<.05
Liver mass	.2	1, 21	NS	.01	.9	1, 20	NS	9.9	1, 17	<.01	.37	.2	1, 16	NS
Gizzard mass	.6	1, 21	NS	.03	.8	1, 20	NS	.8	1, 17	NS	.04	.3	1, 16	NS
Kidney mass	.3	1, 21	NS	.01	.2	1, 20	NS	3.4	1, 17	.083	.17	.2	1, 16	NS
Intestine mass	1.9	1, 21	NS	.08	.0	1, 20	NS	2.3	1, 17	NS	.12	1.6	1, 16	NS
Intestine length	.0	1, 21	NS	.00	.0	1, 20	NS	.6	1, 17	NS	.03	1.3	1, 16	NS
Lipid mass	4.8	1, 21	<.05	.19	1.1	1, 20	NS	.4	1, 17	NS	.02	2.4	1, 16	NS

Note. The analyses were performed separately on each organ with a GLM on $\log_{10}$-transformed metabolic rate, lean dry body mass (minus organ mass), and lean dry organ mass. Treatment was included as a factor with two levels (1 = controls, 2 = diet restricted). The organ mass × treatment interaction was included in the null model. The treatment factor and body mass were strong predictors of PMR in almost all the analyses. In this table, we focus on the relationship between the organ masses and PMR. Hence, the statistics for treatment and body mass are not included in this table.

compared with ad lib. fed ducklings (controls). The results revealed that diet restriction induced changes in the body composition of the ducklings. The liver, the intestine, and the lipid mass were substantially lower than predicted from the body mass. Our analyses of the relationship between RMR and organ masses suggested that the liver mass partly explained the low RMR in diet-restricted ducklings. The results also revealed that diet restriction affected PMR. Diet-restricted ducklings (10 and 20 d old) exhibited ~10% lower PMR than predicted from the body mass.

Adaptive Metabolic Responses to Food Shortage

This study clearly demonstrates developmental plasticity in the ontogenetic development of the ducklings. Developmental plasticity is regarded as adaptive if the animal actively adjusts the ontogenetic processes to a change in the environment (Smith-Gill 1983). Schew and Ricklefs (1998) suggested that temporal changes in the metabolic processes of chicks in response to variation in food intake could be adaptive because it might facilitate survival of chicks during food shortages. We regard the low RMR of the diet-restricted ducklings as an adaptive response because the reductions in RMR occurred before the stored lipid mass was severely depleted. After 5 d of diet restriction, the ducklings still exhibited a substantial amount of stored lipid (lipid mass/lean dry mass, 10 d old, 18.4%; 20 d old, 40.9%), indicating a sufficient amount of stored nutrients to fuel the maintenance metabolism. Alternatively, the low RMR could have been nonadaptive. It could have been a consequence of the restricted diet, either through pathological changes in chick metabolism while near starvation (e.g., through depletion of other essential nutrients than lipids) or through reduced size of the visceral organs or reduced overall growth. Deleterious pathological changes most likely did not happen. We have unpublished results (from experiments with the same diet restriction protocols) that show that the ducklings immediately resumed normal growth at the start of realimentation. This also indicates that MRs were rapidly increased and that the cellular structures responsible for the metabolism were intact. Visceral organs and overall growth were reduced. These changes could have been a consequence of the restricted diet, but they also could have been adaptive changes in anticipation of a lasting food shortage. Nevertheless, changes in organ size or growth were not major predictors of the low RMR in the diet-restricted ducklings (also discussed later). A rapid response on diet restriction has been proposed to characterize an adaptive response to food shortage (Schew and Ricklefs 1998). We did not monitor the RMR changes (or the changes in body composition or growth) over the course of the diet-restriction periods, and therefore we cannot entirely rule out the RMR changes being nonadaptive.

During the last decade, adaptive metabolic responses of chicks to food shortage have been the subject of several studies. Schew (1995) demonstrated that Japanese quail (*Coturnix coturnix japonica*) exposed to diet restriction from the ages of 3 to 13 d old reduced MR by 40% by the second day of diet restriction and increased MR by 87% within the first day of realimentation. Similarly, male broilers (*Gallus gallus*) re-

sponded rapidly to diet restriction by decreasing MR and subsequently increasing it in response to realimentation (Zubair and Leeson 1994). Japanese quails exposed to diet restriction from the age of 20 to 30 d old also showed a rapid metabolic response, but the reduction in MR was very small compared with the response of the younger diet-restricted chicks. In contrast, neither young nor old chicks of European starling (*Sturnus vulgaris*) showed any metabolic response to 3 d of diet restriction (Schew 1995). Similarly, nestlings of another passerine species, the song thrush (*Turdus philomelos*), showed no metabolic response to diet restriction (Konarzewski and Starck 2000). Konarzewski and Starck (2000) suggested that a lack of frequent, unpredictable fluctuations in food availability or a strong selection for uniform adult phenotypes could prevent plasticity of the developmental program of nestlings.

Metabolic responses of chicks to food shortage have also been investigated in relation to inherent growth rate. Van der Ziel and Visser (2001) revealed that the level of plasticity of the metabolic development was not determined by the maximum inherent growth rate. Japanese quail chicks from a line selected for high postnatal growth rates did not show a different metabolic response to long-term undernutrition compared with chicks from a line not selected for high postnatal growth rates.

Of the above, it is possible that phylogenetic constraints and the ecological settings of the particular species may determine the metabolic responses of chicks to food shortage. Kitaysky (1999) conducted an experiment on chicks of four closely related alcid species that reduced MR by 24%–47% after 2 d of fasting. He highlighted the ecological settings, determining food provisioning, as an important selective factor. The piscivorous horned and tufted puffins (*Fratercula corniculata* and *Lunda cirrhata*), which rely on fluctuating food resources, showed greater metabolic responses to food shortage compared with the planktivorous crested and parakeet auklets (*Aethia cristatella* and *Cyclorhinchus psittacula*), which rely on continuously available food resources. However, this could also be a phylogenetic response, since the puffins behaved more similarly to each other than they did to the auklets.

Plasticity in the ontogenetic development of metabolism can be an adaptation to unpredictable fluctuating feeding conditions. The Pekin duck, the Japanese quail, and the broiler are domesticated species. It is unlikely that these species have been artificially selected for plasticity of the metabolic development. However, the ecological settings of their ancestors include factors that could select for such flexibilty. In nature, poor weather and foraging inexperience can impose short-term food limitations on self-feeding precocial chicks, as reported for the willow ptarmigan (*Lagopus l. lagopus*: Erikstad and Spidsø 1982; Erikstad and Andersen 1983), the black-tailed godwit (*Limosa limosa*: Beintema and Visser 1989*b*), and the northern lapwing (*Vanellus vanellus*: Beintema and Visser 1989*a*).

Body Composition and Explanations of the Variation in RMR due to Diet Restriction

The changes in body composition indicated that energy was allocated preferentially to parts of the skeletal structure (i.e., head + bill) to promote a higher growth of these parts relative to the growth in body mass. Energy was allocated to the leg muscles, heart, and kidneys in such a manner that these organs maintained normal size relationships to body mass. In contrast, the liver, intestine, and lipid mass were smaller than predicted from their normal allometric relationship to body mass. Liver tissue has a high intrinsic MR, while adipose tissue has a low intrinsic MR (Scott and Evans 1992). Our results showed that the liver size was a significant predictor of RMR when we analyzed the data where both age groups were included. In addition, the significant interaction between intestine mass (and length) and treatment in the RMR comparisons indicates that intestine differences might also play a role in the RMR differences. Statistically, the liver size (and the intestine size) was not a strong predictor of the variation in RMR. Body mass and the treatment factor were strong predictors in all organ mass–RMR analyses. By using 15.1 W kg^{-1} for liver and intestine MR, as measured for liver in vitro by Scott and Evans (1992), we calculated that the reductions in the liver and intestine size explained 15% and 18% of the reductions in the overall RMR in 10- and 20-d-old diet-restricted ducklings, respectively. However, such a quantitative value of the reduction in RMR should be treated carefully, since Scott and Evans (1992) measured the MR of liver samples from adult birds and in species different from ours.

The functioning of tissues may have changed during the diet restriction because of the changes in water content, measured as the LDF. The LDF is regarded to relate to the functional maturity of tissues, and in skeletal muscles the LDF is postively related to thermoregulatory abilities (Ricklefs et al. 1994). It is less clear how the LDF relates to the tissue RMR (Ricklefs et al. 1998). Despite the fact that the diet restriction treatment in this study imposed variation in water content in various tissues, LDF was not a good predictor of changes in RMR or PMR. Because neither organ size nor tissue water content was a major predictor of RMR in the diet-restricted ducklings, other mechanisms related to the intrinsic MR of organs or mechanisms related to the central control of the MR should be more important.

Hypothermia is a well-known energy-saving mechanism in small adult birds and mammals. In this study, we observed that 10-d-old diet-restricted ducklings used hypothermia (to a low degree), but the 20-d-old diet-restricted ducklings did not. They regulated their T_b 0.7°C below the expected value (predicted from body mass). By using the measured values for thermal conductance (0.95 W kg^{-1} $°C^{-1}$) and T_a (31.5°C) during thermoneutral conditions, we calculated that hypothermia accounted for ~50% of the observed reduction in RMR of 10-

d-old diet-restricted ducklings. Furthermore, a Q_{10} effect (assuming a Q_{10} of 2.5) explained 76% of the energy savings caused by hypothermia. Hypothermia is also reported for diet-restricted Japanese quail chicks (Schew 1995). They lowered the T_b by 2°–3°C during the diet-restriction period, and both small and large chicks exhibited hypothermia. An extreme example of hypothermia in young birds is reported for the fork-tailed storm petrel (*Oceanodroma furcata*), in which underfed and unattended chicks regulated the T_b at 10°C (Boersma 1986).

Organ size changes (15%) and hypothermia (50%) combined explained 65% of the observed reductions in RMR of 10-d-old diet-restricted ducklings. However, organ size changes were the only revealed energy-saving mechanism of the 20-d-old diet-restricted ducklings and explained only 18% of the observed reductions in RMR. Although we want to be careful in attributing a quantitative value to the reduction in RMR from the organ mass changes, we believe that we are left with a body of unexplained mechanisms behind the observed reductions in RMR of the 20-d-old diet-restricted ducklings.

The growth rate of wings and the growth rate of body mass was positively related to RMR in the controls (ad lib. fed ducklings). In addition, the factor score extracted from a principal component analysis with wing, skull, and tarsus growth rate showed a tendency ($P < 0.1$) toward a positive relationship with RMR. These results could support the proposed positive relationship between growth rate and RMR, in which RMR includes indirect costs of growth, in terms of costs of maintaining organs that support growth or represent a potential for growth (Drent and Klaassen 1989; Klaassen and Drent 1991). It could also suggest that variation in the direct costs of growth, that is, the costs of biosynthesis, is a significant source of the variation in RMR. However, only the growth rate of the wings (controlled for body mass) was significantly related to RMR when the controls and the diet-restricted ducklings were included in the analyses. Furthermore, the diet restriction only imposed reductions in growth rate of the wings in the 10-d-old diet-restricted ducklings (and not in those 20 d old). At that age, the growth of the wings is very low, and the potential savings in RMR must also be very low. Hence, variations in growth rate did not seem to be important predictors of the reductions in RMR of diet-restricted ducklings.

Diet Restriction and Thermoregulatory Abilities

The diet-restricted ducklings (both 10 and 20 d old) exhibited ~10% lower PMR compared with ducklings in the control group. In contrast, the absolute scope (PMR minus RMR) was not different between diet-restricted ducklings and controls. Hence, the portion of PMR available for regulatory thermogenesis was similar between the two groups. The leg muscles, which are regarded as the most important organs for shivering thermogenesis in young birds (Hohtola and Visser 1998), and the heart, which is important for maximum oxygen consumption (Chappel et al. 1999), were maintained at the normal size expected from the body mass during the diet restriction. The importance of the leg muscles for regulatory thermogenesis was also indicated by the positive relationship between leg muscle mass and PMR. However, liver mass showed a positive relationship to PMR in 15–20-d-old ducklings. The paradigm of muscular shivering as the only source of cold-induced thermogenesis has been thrown into debate (Duchamp et al. 1993; Marsh 1993). However, skeletal muscles, not the liver, have been targeted as the main site of a potential nonshivering thermogenesis. Hence, the statistical relationship between the liver mass and PMR is most likely an indirect relationship through a correlation with RMR. We showed a positive relationship between RMR and PMR and between the liver mass and RMR.

The lower PMR in diet-restricted ducklings could indicate a negative effect of the diet restriction on the thermoregulatory effector mechanisms or it could be a result of the lower RMR. The methods of describing the ducklings' capacity for regulatory thermogenesis, absolute scope, and factorial scope are based on different assumptions of the relationship between RMR and PMR, and they provide contrasting results (Fig. 5). While the absolute scope indicated a conserved capacity (Fig. 5*A*), the factorial scope indicated an increased age-specific capacity for regulatory thermogenesis of the diet-restricted ducklings (Fig. 5*B*). The calculation of factorial scope assumes a factorial relationship between RMR and PMR. In contrast, the calculation of absolute scope assumes that RMR is a fixed part of PMR (i.e., PMR = RMR + thermoregulation). RMR is predicted to correlate to PMR if RMR is a fixed part of PMR (Ricklefs et al. 1996), and we found such a positive relationship between RMR and PMR. Hence, we regard absolute scope to provide a better measure of the capacity for regulatory thermogenesis than factorial scope. Consequently, we think that the diet-restricted ducklings have conserved their capacity for regulatory thermogenesis, and we think the low RMR has entailed the lower PMR.

Although the diet-restricted ducklings exhibited lower PMR, they coped rather well with cold T_a (Fig. 3*B*) and attained PMR with T_b's not different from that of controls. The 20-d-old diet-restricted ducklings seemed to compensate for the lower PMR by increasing the insulation (probably increased down thickness), as indicated by the lower MTC (Fig. 4*A*). Consequently, they attained PMR at a T_a as expected from age. The 10-d-old diet-restricted ducklings did not compensate for lower PMR by decreasing MTC, and they attained PMR at a T_a as expected from body mass, that is, at a higher T_a compared with the 10-d-old controls (Fig. 3*B*).

Costs of Plasticity

Even small deviations from normal growth might be expected to produce fitness consequences (Gebhardt-Henrich and Richner 1998). The quantitative and qualitative nature of the food

restriction, as well as the duration and timing, determine these deviations (Schew and Ricklefs 1998). Generally, food restriction delays the schedule of mass and skeleton accretion (Øyan and Anker-Nilssen 1996; Lepczyk and Karasov 2000). Premature fledging (Kitasky 1999) and permanent stunting of external measurements (Boag 1987; Ohlsson and Smith 2001) have also been reported.

Plasticity of the developmental trajectory can be adaptive and lessen the detrimental effects of food stress during early development. However, plasticity may be associated with short- and long-term costs. The reduced PMR of the diet-restricted ducklings can be regarded as a short-term cost of the plasticity of the development of the RMR. Also, liver, intestine, and lipid masses can be energetically costly to restore after food stress. However, studies on growing young (Nir and Nitsan 1979; Schew 1995) and migrating adults (Piersma and Lindstrøm 1997; Piersma 1998) have reported that these organs and tissues can be rapidly rebuilt during sufficient realimentation. However, although body mass, external measurements, and visceral organs may fully recover during realimentation, long-term fitness consequences may appear later in life (Metcalfe and Monaghan 2001).

Conclusions

In this study, we have shown that ducklings exhibit substantial energy-saving mechanisms as a response to short-term food shortage. This physiological response entailed a negative effect on cold-induced PMR, but overall thermoregulatory abilities were nevertheless very well maintained. Changes in body composition were a minor predictor of the energy-saving mechanisms, and in 10-d-old diet-restricted ducklings, hypothermia was an important mechanism. Still, we are left with a body of unexplained mechanisms behind the metabolic responses to food shortage, especially for the 20-d-old diet-restricted ducklings. We argue that the observed energy-saving mechanisms are adaptive responses, but we cannot entirely rule out the responses being nonadaptive. Further investigation of the mechanisms behind physiological responses to food shortage as well as investigations of the long-term fitness consequences deserve attention in future studies.

Acknowledgments

We are grateful to the technicians, Odd Arne Indset and Bjørn Simensen, who skillfully constructed and maintained animal housing conditions. We thank Carolyn Baggerud for improving the English, and we thank two anonymous reviewers for constructive comments and suggestions on an earlier draft of this article. C.B. and B.M. were supported by a grant from the Research Council of Norway (138698/410).

Literature Cited

Bakken G.S. 1992. Measurement and application of operative and standard operative temperatures in ecology. Am Zool 32:194–216.

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

Beintema A.J. and G.H. Visser. 1989*a*. The effects of weather on time budgets and development of chicks of meadow birds. Ardea 77:181–192.

———. 1989*b*. Growth parameter in chicks of Charadriiform birds. Ardea 77:169–180.

Bennett A.F. and J.A. Ruben. 1979. Endothermy and activity in vertebrates. Science 206:649–654.

Birkhead T.R., F. Fletcher, and E.J. Pellatt. 1999. Nestling diet, secondary sexual traits and fitness in the zebra finch. Proc R Soc Lond B Biol Sci 266:385–390.

Boag P.T. 1987. Effects of nestling diet on growth and adult size of zebra finches (*Poephila guttata*). Auk 104:155–166.

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

Burness G.P., R.C. Ydenberget, and P.W. Hochachka. 1998. Interindividual variability in body composition and resting oxygen consumption rates in breeding tree swallows, *Tachycineta bicolor*. Physiol Zool 71:247–256.

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

Christians J.K. 1999. Controlling for body mass effects: is part-whole correlation important? Physiol Biochem Zool 72:250–253.

Daan S., D. Masman, and A. Groenewold. 1990. Avian basal metabolic rates: their association with body composition and energy expenditure in nature. Am J Physiol 259:333–340.

De Kogel C.H. 1997. Long-term effects of brood size manipulation on the morphological development and sex-specific mortality of offspring. J Anim Ecol 66:167–178.

Drent R.H. and M. Klaassen. 1989. Energetics of avian growth: the causal link with BMR and metabolic scope. Pp. 349–359 in C. Bech and R.E. Reinertsen, eds. Physiology of Cold Adaptation in Birds. Plenum, New York.

Duchamp C., F. Cohen-Adad, J.-L. Rouanet, E. Dumonteil, and H. Barré. 1993. Existence of nonshivering thermogenesis in birds. Pp. 529–533 in C. Carey, G.L. Florant, B.A. Wonder, and B. Horwitz, eds. Life in the Cold: Ecological, Physiological and Molecular Mechanisms. Westview, Boulder, CO.

Dufty A.M., Jr., J. Clobert, and A.P. Møller. 2002. Hormones, developmental plasticity and adaptation. Trends Ecol Evol 17:190–196.

Dutenhoffer M.S. and D.L. Swanson. 1996. Relationship of

basal to summit metabolic rate in passerine birds and the aerobic capacity model for the evolution of endothermy. Physiol Zool 69:1232–1254.

Erikstad K.E. and R. Andersen. 1983. The effect of weather on survival, growth rate and feeding time in different sized willow grouse broods. Ornis Scand 14:249–252.

Erikstad K.E. and T.K. Spidsø. 1982. Weather influence on food intake, insect prey selection and feeding behaviour in willow grouse chicks in northern Norway. Ornis Scand 13:176–182.

Gebhardt-Henrich S. and H. Richner. 1998. Causes of growth variation and its consequences for fitness. Pp. 324–339 in J.M. Starck and R.E. Ricklefs, eds. Avian Growth and Development. Oxford University Press, Oxford.

Hart J.S. 1951. Calorimetric determination of average body temperature of small mammals and its variation with environmental conditions. Can J Zool 29:224–233.

Hayes J.P. and T. Garland Jr. 1995. The evolution of endothermy: testing the aerobic capacity model. Evolution 49: 836–847.

Hayes J.P. and J.S. Shonkwiler. 1996. Analyzing mass-independent data. Physiol Zool 69:974–980.

Hohtola E. and G.H. Visser. 1998. Development of locomotion and endothermy in altricial and precocial birds. Pp. 157–173 in J.M. Starck and R.E. Ricklefs, eds. Avian Growth and Development. Oxford University Press, Oxford.

Kitaysky A.S. 1999. Metabolic and developmental responses of alcid chicks to experimental variation in food intake. Physiol Biochem Zool 72:462–473.

Klaassen M. and R.H. Drent. 1991. An analysis of hatchling basal metabolism: in search of ecological correlates that explain deviations from allometric relations. Condor 93:612–629.

Konarzewski M., A. Gavin, R. McDevitt, and I.R. Wallis. 2000. Metabolic and organ mass responses to selection for high growth rates in the domestic chicken (*Gallus domesticus*). Physiol Biochem Zool 73:237–248.

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

Konarzewski M. and J.M. Starck. 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.

Lepczyk C.A. and W.H. Karasov. 2000. Effect of ephemeral food restriction on growth of house sparrows. Auk 117:164–174.

Lindström J. 1999. Early development and fitness in birds and mammals. Trends Ecol Evol 14:343–348.

Marsh R.L. 1993. Does regulatory thermogenesis exist in birds? Pp. 535–538 in C. Carey, G.L. Florant, B.A. Wonder, and B. Horwitz, eds. Life in the Cold: Ecological, Physiological and Molecular Mechanisms. Westview, Boulder, CO.

Meerlo P., L. Bolle, G.H. Visser, D. Masman, and S. Daan. 1997. Basal metabolic rate in relation to body composition and daily energy expenditure in the field vole, *Microtus agrestis*. Physiol Zool 70:362–369.

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

Niimi A.J. 1978. Lag adjustments between estimated and actual physiological responses conducted in flow-through systems. J Fish Res Board Can 35:1265–1269.

Nir I. and Z. Nitsan. 1979. Metabolical and anatomical adaptations of light-bodied chicks to intermittent feeding. Br Poult Sci 20:61–71.

Ohlsson T. and H.G. Smith. 2001. Early nutrition causes persistent effects on pheasant morphology. Physiol Biochem Zool 74:212–218.

Østnes J.E. and C. Bech. 1997. Ontogeny of deep-body cold sensitivity in Pekin ducklings *Anas platyrhynchos*. J Comp Physiol B 167:241–248.

Øyan H.S. and T. Anker-Nilssen. 1996. Allocation of growth in food-stressed Atlantic puffin chicks. Auk 113:830–841.

Piersma T. 1998. Phenotypic flexibility during migration: optimization of organ size contingent on the risks and rewards of fueling and flight? J Avian Biol 29:511–520.

Piersma T. and Å. Lindstrøm. 1997. Rapid reversible changes in organ size as a component of adaptive behviour. Trends Ecol Evol 12:134–138.

Rezende E.L., D.L Swanson, F. F. Novoa, and F. Bozinovic. 2002. Passerines versus nonpasserines: so far, no statistical differences in the scaling of avian energetics. J Exp Biol 205:101–107.

Ricklefs R.E., M. Konarzewski, and S. Daan. 1996. The relationship between basal metabolic rate and daily energy expenditure in birds and mammals. Am Nat 147:1047–1071.

Ricklefs R.E., R.E. Shea, and I.H. Choi. 1994. Inverse relationship between functional maturity and exponential growth rate of avian skeletal muscle: a constraint on evolutionary response. Evolution 48:1080–1088.

Ricklefs R.E., J.M. Starck, and M. Konarzewski. 1998. Internal constraints on growth in birds. Pp. 266–287 in J.M. Starck and R.E. Ricklefs, eds. Avian Growth and Development. Oxford University Press, Oxford.

Schew W.A. 1995. The Evolutionary Significance of Developmental Plasticity in Growing Birds. PhD diss. University of Pennsylvania.

Schew W.A. and R.E. Ricklefs. 1998. Developmental plasticity. Pp. 288–304 in J.M. Starck and R.E. Ricklefs, eds. Avian Growth and Development. Oxford University Press, Oxford.

Scholander P.F., R. Hock, V. Walters, F. Johnson, and L. Irving. 1950. Heat regulation in some arctic and tropical mammals and birds. Biol Bull 99:237–258.

Scott I. and P.R. Evans. 1992. The metabolic output of avian (*Sturnus vulgaris, Calidris alpina*) adipose tissue liver and skeletal muscle: implications for BMR/body mass realtionships. Comp Biochem Physiol 103A:329–332.

Smith-Gill S.J. 1983. Developmental plasticity: developmental conversion versus phenotypic modulation. Am Zool 23:47–55.

SPSS. 2002. SPSS for Windows. Release 11.5.1. SPSS, Chicago.

Stearns S.C. 1992. The evolution of life histories. Oxford University Press, Oxford.

Taigen T.L. 1983. Activity metabolism of anuran amphibians: implications for the origin of endothermy. Am Nat 121:94–109.

Van der Ziel C.E. and G.H. Visser. 2001. The effect of food restriction on morphological and metabolic development in two lines of growing Japanese quail chicks. Physiol Biochem Zool 74:52–65.

Visser G.H. and R.E. Ricklefs. 1993. Temperature regulation in neonates of shorebirds. Auk 110:445–457.

Withers P.C. 1977. Measurement of $\dot{V}o_2$, $\dot{V}co_2$ and evaporative water loss with flow-through mask. J Appl Physiol 42:120–123.

Zubair A.K. and S. Leeson. 1994. Effect of early restriction and realimentation on heat production and changes in sizes of digestive organs of male broilers. Poult Sci 73:529–538.