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ORIGINAL PAPER

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Does food shortage delay development of homeothermy in European shag nestlings (*Phalacrocorax aristotelis*)?

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Abstract Nestlings seem to face a trade-off between reducing the basal level of energy metabolism, as an energy-saving response, and maintaining thermogenic capacity during temporal food shortage. In the present study we examined developmental responses to short-term diet restriction of 12–16 day old nestling European shags kept under laboratory conditions and tested whether temporal food shortage delay the development of homeothermy. During food shortage the European shag nestlings substantially reduced basal level of energy metabolism, resulting in significant energy savings. The reduction in basal level of energy metabolism corresponded with a reduction in peak metabolic rate. At the same time, the low peak metabolic rate of diet-restricted nestlings was offset by a lower mass-specific minimal thermal conductance, and an increased mass-specific absolute scope. Consequently, the insulation and the portion of peak metabolic rate available for regulatory thermogenesis seemed to develop normally, as expected from age, during the period of food shortage. Further, the degree of homeothermy, measured as the index of homeothermy, was not significantly lower in diet-restricted nestlings compared to controls at the same age. We conclude that temporal food shortage did not significantly delay the development of homeothermy in the European shag nestlings despite substantial reductions in basal level of energy metabolism and peak metabolic rate.

Keywords Development · European shag, *Phalacrocorax aristotelis* · Food shortage · Homeothermy · Nestlings

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Abbreviations M_b: body mass · GLM: general linear model · HI: index of homeothermy · LDF: lean dry fraction · MR: metabolic rate · MTC: mass-specific minimal conductance · PMR: peak metabolic rate · RMR: resting metabolic rate · T_a: ambient temperature · T_b: body temperature

Introduction

Avian development highly depends on food availability (Martin 1987). As chicks grow from neonate to adult, they may face periods of food shortage that can cause phenotypic changes from the normal ontogenetic development given by their genotype. Such phenotypic changes (arising from variation in food availability or other environmental conditions) are known as developmental plasticity (Schew and Ricklefs 1998; Schlichting and Pigliucci 1998). A number of recent studies have investigated how chicks can modify the pattern of energy use and allocation in response to food shortage during growth and development (Schew 1995; Kitaysky 1999; Konarzewski and Starck 2000; Brzek and Konarzewski 2001; Moe et al. 2005). These studies have revealed that growing birds may lower the resting metabolic rate (RMR) in response to food shortage. Such an energy saving response is considered to lessen the detrimental effects and enhance survival during temporal food shortage (Schew and Ricklefs 1998). Moe et al. (2005) demonstrated that a reduction in RMR negatively affected the capacity for maximum heat production, i.e. peak metabolic rate (PMR) in diet-restricted ducklings, but little is known about how food shortage affects the development of homeothermy in birds.

In this study we investigated the development of homeothermy in nestling European shags (*Phalacrocorax aristotelis*) subject to short-term food shortage. The European shag is very well suited for studying developmental responses to temporal food shortage because

of its life-history characteristics and its ecology. The European shag is a large altricial seabird, and nestlings exhibit high growth rates (Østnes et al. 2001) and compete with siblings for food (Amundsen and Stokland 1988; Velando et al. 1999, 2000). Consequently, the nestlings are highly dependent on successful food provisioning to follow the normal developmental trajectory. In this species, variable food provisioning during early development is reported to occur due to adverse weather conditions, which affects the foraging success of the parents (Velando et al. 1999).

The development of homeothermy in European shag nestlings has previously been studied by Østnes et al. (2001). They argued that the rapid increase of the European shag nestlings' homeothermic ability during the first 2–3 weeks of development was mainly due to a rapid increase in mass-specific RMR. We have performed experiments with nestling European shags subject to diet restriction (Moe et al. 2004), and the experiments have revealed that the nestlings substantially reduced the mass-specific RMR in response to the diet restriction. 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 (Bech and Østnes 1999; Konarzewski et al. 2000; Moe et al. 2005), a decrease in RMR should be accompanied by a decrease in PMR. Accordingly, one could expect a delayed development of homeothermy in nestlings subject to food shortage.

The functional relationship between RMR and PMR is not fully understood (Hayes and Garland 1995; Ricklefs et al. 1996). Klaassen and Bech (1992) advocated that a coupling between RMR and PMR in birds does not always imply causality. In arctic tern chicks (*Sterna paradisaea*) with varying growth rates, they found that RMR developed in pace with body mass, whereas PMR was more dependent on age. This relationship held true if the body mass of the chicks was not lower than 75% of that expected from their age. Accordingly, one could expect a sustained development of homeothermy in nestlings facing food shortage, if their body masses were not below a critical level.

Østnes et al. (2001) also argued that the homeothermic ability of the European shag nestlings was due to a substantial decrease in mass-specific minimal thermal conductance (MTC). Further, they argued that the decrease in MTC probably represented a passive effect of a decrease in the surface-to-volume ratio (causing an increased thermal inertia) rather than an increase in the insulation from growth of a down coating. In contrast, nestling European shags subject to diet restriction seemed to increase in the surface-to-volume ratio (Moe et al. 2004), due to the combination of a rather stable body mass (weight maintenance diet) and a continued structural growth (tarsus, wings and skull). Accordingly, one could expect a delayed development of homeothermy in nestlings facing food shortage.

In this study, we experimentally imposed short-term diet restriction on 12- to 16-day-old nestling European shags, kept under laboratory conditions, to shed light on the relationship between food availability and development of homeothermy during early development. In this context we tested the hypothesis that food shortage delays the development of homeothermy in altricial nestlings.

Materials and methods

Study area and animals

Data were collected during the 2001 breeding season (June and July) on Sklinna, a small group of islands situated ~50 km off the coast of central Norway (65°12' N, 11°00' E). In 2001 the breeding population of the study species, the European shag, consisted of 1,750 pairs (N. Røv, personal communication). We measured ambient temperature (T_a) in the colony with a temperature logger placed in the shade of a boulder.

Housing conditions, feeding protocols and treatment groups

A sample of 34 nestlings was brought to the laboratory at the age of 12 days (day of hatching termed day 0) for the purpose of metabolic measurements (RMR and PMR). They were kept, in groups of 4–8, in an enclosure (100×50 cm) with a heat lamp providing a constant range of operative temperatures (Bakken 1992) of 22–33°C. We randomly assigned 12 nestlings to a diet-restriction feeding protocol (hereafter 'diet-restricted nestlings') and 22 nestlings to a control group (hereafter 'controls'). Within the controls, 12 nestlings were subject to metabolic measurements at the age of 12 days, whereas 10 nestlings were subject to a control-feeding protocol. The diet-restricted and the control fed nestlings were hand fed with fillets of saithe (*Pollachius virens*) and cod (*Gadus morhua*), because these gadoids constitute 70% of the diet of shags breeding in the study area (Barrett et al. 1990). They were fed for 4 days, until they were 16 days old and metabolic rates were measured. The diet-restricted nestlings received small portions of food eight to ten times a day to maintain a relatively stable body mass, while the controls were fed every 2nd hour, allowing them to follow a normal body mass growth trajectory (Fig. 1). The daily food intake and the body mass growth of 16 day old controls were lower compared to that of the 15-day-old controls, because of fasting prior to the metabolic measurements.

This sample of nestlings ($n = 34$) is the same sample of nestlings from which the results of RMR are presented in Moe et al. (2004). Consequently, the housing conditions, the feeding protocols and the methods for RMR

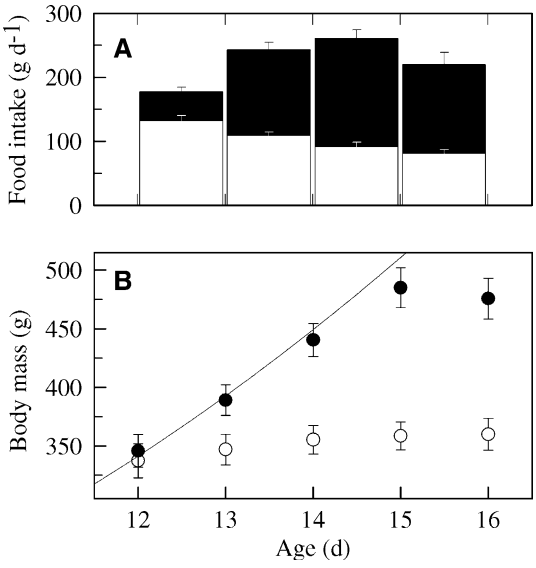

Fig. 1 Daily food intake (**a**) and body mass (M_b) (**b**) as a function of age in controls (*black bars and closed symbols*) and diet-restricted nestlings (*white bars and open symbols*) of European shags kept in the laboratory. The regression line of a logistic growth curve calculated from 1,645 M_b measurements of nestlings fed by their parents in the colony is shown for comparison in **b**. Food intake is given as fresh weight of gadoid fish fillets in grams per day. Error bars are 1SE

measurements are also described in details in Moe et al. (2004).

RMR and PMR measurements

The metabolic measurements were performed on post-absorptive nestlings. The lengths of fasting prior to the measurements were 6.4 ± 0.5, 7.3 ± 0.5 and 9.4 ± 0.4 h for 12-day-old controls, 16-day-old diet-restricted and 16-day-old controls, respectively. The longer length of fasting of the latter group was chosen due to presumed higher gut content. Diet-restricted nestlings and controls were randomly measured with respect to time of the day, but RMR showed no diurnal cycle.

O_2 consumption rates were calculated by using Eq. 1d in Withers (1977), assuming a constant RQ of 0.72 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 MR were calculated from the O_2 consumption rates using 5.4611 W as the caloric equivalent for 1 l O_2 h^{-1}, using gas exchange conversion factors from Schmidt-Nielsen (1990). RMR was defined as the lowest MR calculated with 25 min running average during exposure to thermoneutral conditions (29–31°C).

Outside air was dried using silica gel and pumped through a 10-l temperature controlled metabolic chamber with a flow rate of 3.3 l min^{-1}. The actual flow rates entering the metabolic chamber were measured with a calibrated mass flow controller (Bronkhorst Hi-Tec, Rurlo, Holland; type F-201C-FA-22-V). Excurrent air was again dried, before a fraction of the air was directed to the O_2 analyser (Servomex, Crowborough, East Sussex, UK; type 244A). The O_2 analyser was calibrated with dry atmospheric air (20.95%) and pure stock N_2. Any changes from the pre- to the post-experiment 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 0.001%) were recorded, along with the measurements of body and ambient temperatures (T_b and T_a; accuracy 0.1°C), on a data logger (Grant. Cambridge, UK; type Squirrel), at 30-s intervals.

The metabolic chamber was a water-jacketed vessel connected to a temperature controller (Grant Instruments, Royston, UK; type LT D G) that provided control of the T_a in the inner metabolic chamber. T_a was measured with a copper-constantan thermocouple mounted inside the metabolic chamber, and T_b was measured, during the entire metabolic measurement, in the cloaca with a copper-constantan thermocouple (California Fine Wire Company, Grover City, CA, USA; type 0.005) surrounded by a polypropylene tubing (outer diameter 0.96 mm). Depending on the nestling's size, the thermocouple was inserted 2–4 cm into the cloaca and secured with adhesive tape.

The PMR measurements were obtained as a continuation of the RMR measurements. After 3–5 h exposure to thermoneutral conditions, the T_a in the metabolic chamber was lowered at a constant rate of 0.3°C min^{-1}. The nestlings' MR increased with decreasing T_a. After reaching a peak MR, MR and T_b consistently decreased 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. One PMR measurement was excluded due to it being disturbed during the cold exposure part of the trial. Body masses of the nestlings 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. Each individual was only used once in the experiments to obtain independent measurements.

Minimal thermal conductance

The minimal 'wet' thermal conductance (MTC) was calculated according to the method originally described by Scholander et al. (1950). However, this method is only valid when T_b is kept constant. Since the T_b of the nestlings 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 mass-specific minimal thermal conductance:

$$\mathrm{MTC} = (\mathrm{PMR} + A)/(T_b - T_a) \quad (1)$$

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 assumed a specific heat of 3.45 J g^{-1} °C^{-1} for the chicks (Hart 1951).

Body composition

A sample of 28 nestlings were sacrificed immediately after the metabolic measurements and stored at –20°C, for subsequent analysis of body composition. These analyses produced information on organ masses and the LDF of the organs. The results on body composition are partly presented in Moe et al. (2004), and the methods for analysing body condition were described therein. However, in the present paper we focus on the aspects of body composition which relate to thermoregulatory ability.

Index of homeothermy

For the purpose of measuring the index of homeothermy (HI), another sample of 27 nestlings were brought to the laboratory at the age of 12 days. Housing conditions, including feeding protocols and fasting prior to the measurements, were identical to that of the nestlings subject to the metabolic measurements. The HI characterises the degree of homeothermy (Visser 1998), and it was obtained from cooling rates of 12-day-old control ($n = 8$), 16-day-old diet-restricted ($n = 12$) and 16-day-old control ($n = 7$) nestlings. Prior to the measurement of HI the nestlings were kept under a heat lamp to ensure normal T_bs, and subsequently, they were placed in a chamber in which T_a was maintained at 10°C (range 9.0–11.4°C), consistent to the mean T_a of the colony during June (10.2°C). The T_b of the nestlings and the T_a of the chamber were measured and stored as described above for the metabolic measurements. The nestlings were subject to cooling for 45 min, and the HI was calculated according to the Eq. 2 from Ricklefs (1987).

$$\mathrm{HI} = (T_f - T_a)/(T_i - T_a) \quad (2)$$

where T_f and T_i are final and initial body temperatures, respectively. The HI is equal to 1 if a nestling maintains its T_b throughout the entire cooling trial, and the HI is 0 if the T_b equals the T_a at the end of the cooling trial. After the HI measurements, the nestlings were brought back to their nest of origin or to a nest with foster parents where they were used for another study. In addition, 6 of the 34 nestlings that were subject to metabolic measurements were also brought back to a nest with foster parents.

Statistics

We used a general linear model (GLM) with the type III sum of squares to perform analyses of covariance and variance. We manually excluded insignificant interaction terms, factors or covariates one by one from the null model (ENTER method). All variables were inspected graphically to ensure linearity, and $\log_{10}$ transformation was used to linearize the variables (MR, body mass, organ mass) prior to examination.

We analysed the relationship between organ mass and MR, as well as the relationship between RMR and PMR (and absolute and factorial scope) 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, when organ mass and body mass were included in the same analysis (Christians 1999). Colinearity diagnostics were used to justify that LDF could be included as a covariate (together with body mass and organ mass) in the analyses of the relationship between organ mass and MR (tolerance > 0.3 for all variables).

When two regressions with $\log_{10}$-transformed variables (e.g. MR on body mass) have the same slope, but have different intercepts, we have calculated the percentage difference between the non-transformed regressions according to Eq. 4 in Moe et al. (2005). The Bonferroni method was used for post hoc pairwise multiple comparisons ('Post Hoc' hereafter). It reports adjusted P values that have been multiplied with the number of pairs tested. Means are reported with ±1SE. All statistical tests were performed with SPSS v. 11.5.1 (2002).

Results

RMR, PMR and T_b

The RMR was negatively affected by the diet restriction. With respect to body mass, the RMR of the diet-restricted nestlings was 36.5% lower than the controls ($F_{1,31} = 90.0$, $P < 0.001$) and scaled to body mass by the power of 0.84 (SE = 0.12, $F_{1,31} = 51.2$, $P < 0.001$). With respect to age, the mass-specific RMR was 11.6 ± 0.36, 11.1 ± 0.34 and 7.4 ± 0.37 W kg^{-1} for 12-day-old controls, 16-day-old controls and 16-day-old diet-restricted nestlings, respectively (for further details see Moe et al. 2004).

PMR was substantially affected by the diet restriction. With respect to body mass, the PMR of the diet-restricted nestlings was 16.4% lower than the controls ($F_{1,30} = 11.5$, $P < 0.002$; Fig. 2). PMR scaled to body mass by the power of 1.3 (SE = 0.13, $F_{1,30} = 99.1$, $P < 0.001$) in both groups (RMR×body mass interaction, $F_{1,29} = 0.0$, $P = 0.91$, but see Fig. 2 for separate linear regression equations). With respect to age, the mass-specific PMR was 14.5 ± 0.5, 17.2 ± 0.4 and 13.0 ± 0.7 W kg^{-1} for 12-day-old controls, 16-day-old controls and 16-day-old diet-restricted nestlings, respectively.

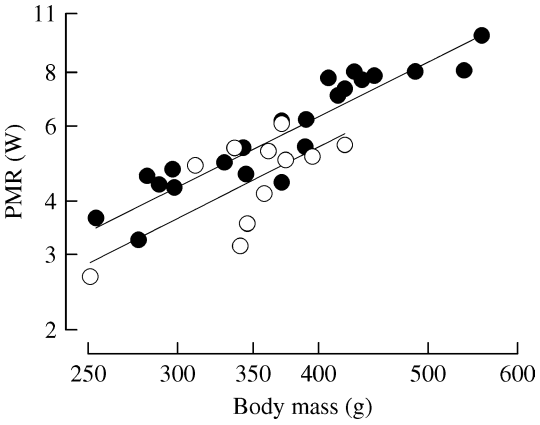

Fig. 2 Peak metabolic rate (PMR) as a function of body mass (M_b) in controls (*closed symbols*) and diet-restricted nestlings (*open symbols*) of European shags. The axes are log scaled, and linear regression lines are shown for each treatment group: log PMR controls = 1.30(±0.12)log M_b−2.56(±0.30); log PMR diet-restricted = 1.34(±0.45)log M_b−2.74(±1.15)

Diet-restricted nestlings exhibited a lower T_b compared to 16-day-old controls during thermoneutral conditions at RMR (Post Hoc, $P<0.001$, Fig. 3a) and during the cooling phase at PMR ($P<0.001$, Fig. 3a). However, they obtained PMR at the same T_a (P = 1.0, Fig. 3b).

Index of homeothermy

HI was measured in a different sample of nestlings ($n=27$) than the sample of nestlings ($n=34$) subject to the metabolic measurements (see Materials and methods). HI increased with body mass ($F_{1,24}=30.8$, $P<0.001$, Fig. 4a) and age ($F_{2,24}=6.7$, $P<0.005$, Fig. 4b). The effect of diet restriction on HI contrasted to that on PMR. With respect to body mass, the diet-restricted nestlings tended to exhibit a higher degree of homeothermy compared to the controls ($F_{1,24}=64.2$, $P=0.052$, Fig. 4a). The slopes of the regressions of HI on body mass were not significantly different (HI×body mass interaction, $F_{1,23}=1.0$, $P=0.32$). With respect to age, Post Hoc tests showed that the diet-restricted nestlings exhibited a HI (0.79 ± 0.02) not significantly different ($P>0.1$) to 16-day-old controls (0.84 ± 0.02), but they tended to exhibit a higher HI ($P=0.08$) compared to 12-day-old controls (0.72 ± 0.02).

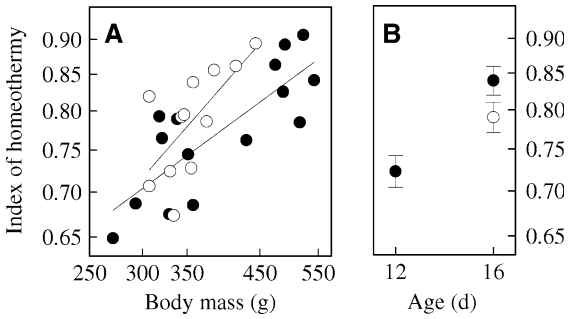

Fig. 4 Index of homeothermy (HI) as a function of body mass (**a**) and age (**b**) in controls (*closed symbols*) and diet-restricted nestlings (*open symbols*) of European shags. The axes are log scaled in **a** and **b**, and the linear regression lines are shown for each treatment group in **a**. Error bars are 1SE

Minimal thermal conductance

MTC calculated from the PMR measurements decreased with body mass ($F_{1,27}=12.1$, $P<0.002$, Fig. 5a) and age ($F_{1,27}=11.5$, $P<0.001$, Fig. 5b). The slopes of the regressions of MTC on body mass were not significantly different ($F_{1,26}=2.3$, $P=0.14$). With respect to body mass, the diet-restricted nestlings showed a 31% lower MTC compared to controls ($F_{1,27}=11.2$, $P<0.002$). With respect to age, the MTC was not different between controls and diet-restricted nestlings (Post Hoc, $P=1.0$).

Metabolic scope

The absolute scope (PMR−RMR, i.e. the portion of PMR available for regulatory thermogenesis) scaled to

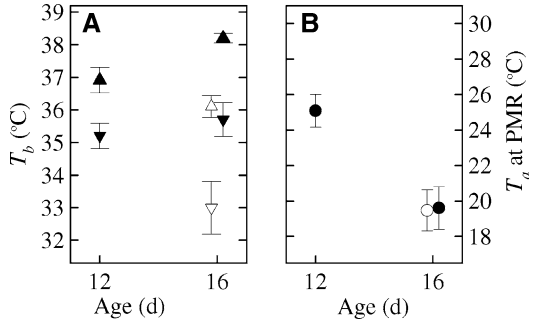

Fig. 3 Body temperature (T_b) at resting metabolic rate (RMR; upward triangles, **a**) and at peak metabolic rate (downward triangles, **a**) and ambient temperature (T_a) at peak metabolic rate (**b**) as a function of age in controls (*closed symbols*) and diet-restricted nestlings (*open symbols*) of European shags. Error bars are 1SE

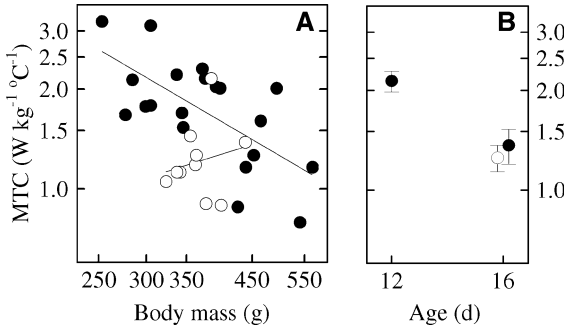

Fig. 5 Minimal thermal conductance (MTC) as a function of M_b (**a**) and age (**a**) in controls (*closed symbols*) and diet-restricted nestlings (*open symbols*) of European shags. The axes are log scaled in **a** and **b**, and the linear regression lines are shown for each treatment group in **a**. MTC is given in W kg^{-1} °C^{-1}. Error bars are 1SE

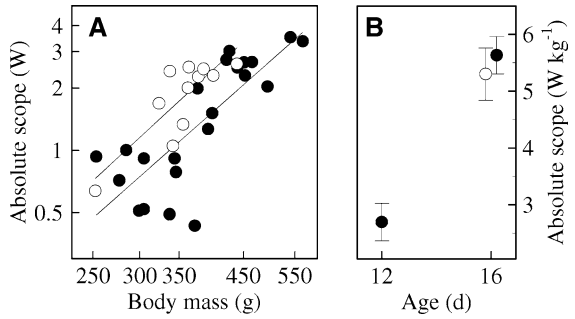

Fig. 6 Absolute scope (W) in relation to body mass (**a**) and mass-specific absolute scope (W kg^{-1}) in relation to age (**b**) in controls (*filled symbols*) and diet-restricted nestlings (*open symbols*) of European shags. Absolute scope was calculated as PMR−RMR. The axes in **a** are log scaled and linear regression lines are shown for each treatment group. Error bars are 1SE

body mass by the power of 2.5 (SE = 0.3, $F_{1,30} = 55.5$, $P < 0.001$, Fig. 6a) in both groups (interaction, $F_{1,29} = 0.0$, $P = 0.91$, Fig. 6a). With respect to body mass, the diet-restricted nestlings exhibited a 57% higher absolute scope compared to the controls ($F_{1,30} = 10.2$, $P < 0.003$). The allometric scaling exponent of 2.5 demonstrates a considerable increase in mass-specific absolute scope as a function of body mass. From Fig. 6b it is evident that this relationship was an effect of age. Mass-specific absolute scope increased from 2.7 ± 0.3 to 5.6 ± 0.3 W kg^{-1} in 12- and 16-day-old controls (Post Hoc, $P < 0.001$, Fig. 6b), and the mass-specific absolute scope was not significantly different between 16-day-old controls and diet-restricted nestlings ($P = 1.0$).

The factorial scope (PMR/RMR) increased with age ($F_{2,30} = 29.6$, $P < 0.001$), and the diet-restricted nestlings exhibited a higher factorial scope (1.75) compared to that of the 16-day-old controls (1.56; Post Hoc, $P < 0.05$) and the 12-day-old controls (1.25; Post Hoc, $P < 0.001$).

Body composition and organ maturation

With respect to body mass, the pectoral muscle mass ($F_{1,25} = 19.9$, $P < 0.001$, Fig. 7a) and the heart mass ($F_{1,25} = 18.2$, $P < 0.001$, Fig. 7c) of the diet-restricted nestlings was 18.9 and 17.4% lower compared to that of the controls, respectively. In addition, the leg muscle mass tended to be slightly lower in diet-restricted nestlings compared to controls ($F_{1,25} = 3.8$, $P = 0.06$, Fig. 7b). The total lipid mass, the liver mass, the gizzard mass and the kidney mass was also negatively affected by the diet-restriction, while the intestine mass was strictly maintained with respect to body mass (see Moe et al. 2004).

The lean dry fraction (LDF) increased significantly with age in all organs (e.g. pectoral and leg, $F_{1.25} > 22.3$, $P < 0.001$, Fig. 7d, e) except for the liver, the intestine and the heart (e.g. heart, $F_{1.25} = 0.9$, $P = 0.39$, Fig. 7f), indicating that the latter organs had already reached a high degree of functional maturation. The LDF was not different between 1- day-old diet-restricted and 16-day-old controls in any organ or muscles (e.g. pectoral muscles, leg muscles and heart; Post Hoc, $P > 0.1$, Fig. 7d, e, f), except for the intestine. The LDF of the intestine was lower in diet-restricted nestlings compared to controls (Post Hoc, $P < 0.05$).

Relationships between RMR, PMR, absolute and factorial scope

We tested for any relationship between RMR and PMR. RMR was a significant predictor of PMR ($F_{1,28} = 39.8$, $P < 0.001$), but the positive correlation between RMR and PMR was stronger in the diet-restricted nestlings compared to the controls (interaction, $F_{1,28} = 5.2$,

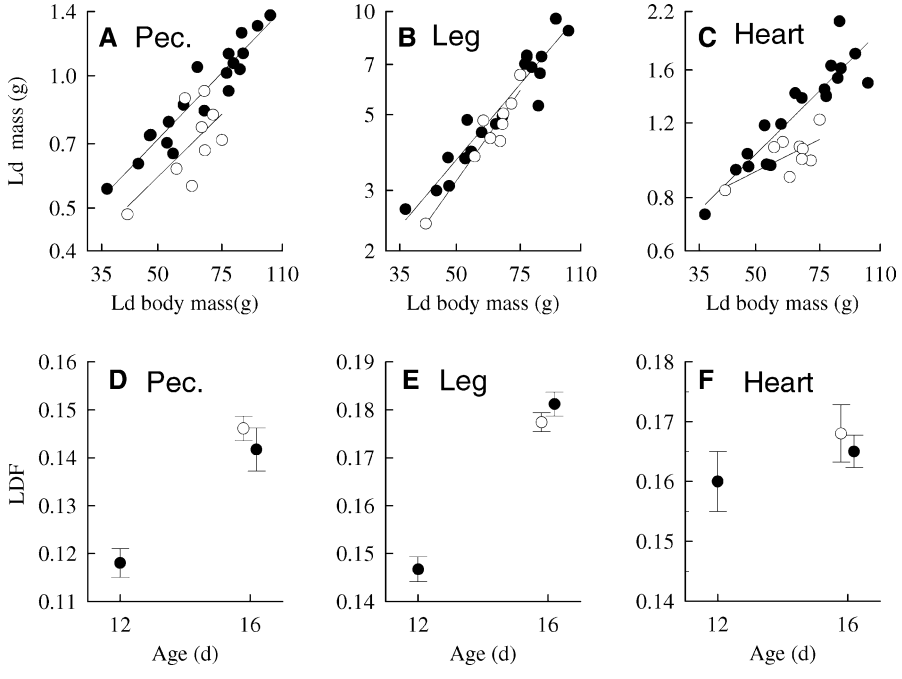

Fig. 7 The relationship of lean dry (Ld) pectoral muscle mass (**a**), leg muscle mass (**b**) and heart mass (**c**) to Ld body mass, and the relationship of lean dry fraction (LDF) of the pectoral muscles (**d**), the leg muscles (**e**) and the heart (**f**) to age in controls (*filled symbols*) and diet-restricted nestlings (*open symbols*) of European shags. The axes are log-scaled, and the linear regression lines are shown for each treatment group in **a**, **b**, and **c**. LDF was calculated as lipid-free dry organ mass/lipid-free fresh organ mass. Error bars are 1SE

$P < 0.05$). In contrast, RMR was not related to absolute scope ($F_{1,29} = 0.0$, $P = 0.87$, body mass and treatment were significant covariate and factor, respectively). Factorial scope was positively related to PMR within both treatment groups ($F_{1,29} = 7.0$, $P < 0.05$). Factorial scope related differently to RMR between the treatment groups (interaction, $F_{1,28} = 6.1$, $P < 0.05$). It was negatively related to RMR within the controls ($r = -0.47$, $F_{1,19} = 5.4$, $P < 0.05$), while no significant correlation existed for the diet-restricted nestlings ($r = 0.04$, $F_{1,8} = 0.0$, $P = 0.90$).

Correlations between organ mass, LDF and metabolic rate

The diet restriction had a substantial effect on body composition. In order to evaluate whether changes in body composition could explain any of the differences in metabolic rates between the treatment groups, we tested whether organ masses correlated with RMR, PMR, absolute scope or factorial scope. In these analyses, we controlled for organ LDF, body mass (minus organ mass) and treatment by including them in the models.

The mass of the liver, the pectoral muscle mass and the lipid mass were significant predictors of RMR ($r > 0.4$, $P < 0.05$, Table 1). It might be expected that the same organs also correlated to PMR, as RMR was a major predictor of PMR. However, for these organs and for the heart mass, the interaction term (with treatment) was significant, and the organ masses and the intestine length positively correlated to PMR in the diet-restricted nestlings only (Table 1). No main effects of organ masses were significant predictors of absolute or factorial scope, but the leg mass tended to be significantly correlated to absolute and factorial scope ($r > 0.37$, $P = 0.07$, Table 1). The interaction term (with treatment) was significant for the liver mass and the lipid mass, and these variables correlated negatively to factorial scope in the controls only. In addition, the intestine length was positively correlated to factorial scope in the diet-restricted nestlings only.

We did not reveal any significant statistical relationships between the LDF of the thermoregulatory effector organs (i.e. the leg muscles, the pectoral muscles and the heart) and PMR, absolute or factorial scope. However, for the LDF of the leg muscles, the results depended on how we specified the final model in the GLM analysis. If we excluded treatment from the final model, the LDF of the leg muscles tended to be a significant predictor of absolute scope ($F_{1,23} = 4.3$, $P = 0.051$). Treatment was not a significant factor in the final model ($F_{1,22} = 0.9$, $P = 0.36$), but the fit of the final model was slightly better when treatment was included ($r^2 = 0.75$ versus $r^2 = 0.74$). However, the relationships between LDF of organs and absolute scope seem to depend on age. We specified a model, in which only the 16-day-old nestlings were included. This model indicated that absolute scope was positively related to the LDF of the leg muscles ($F_{1,13} = 4.3$, $P = 0.059$), the leg muscle mass ($F_{1,13} = 3.9$, $P = 0.071$), treatment ($F_{1,13} = 4.4$, $P = 0.057$) and body mass ($F_{1,13} = 5.1$, $P < 0.05$).

Table 1 Correlations (r values) between lean dry organ mass and metabolic performance in controls and diet-restricted European shag nestlings (RMR resting metabolic rate, PMR peak metabolic rate). Separate GLM analyses were performed for each organ and each dependent variable (RMR, PMR, absolute scope and factorial scope). The null models included organ mass, lean dry fraction (LDF) and lean dry body mass (minus organ mass) as covariates, treatment as factor and the interactions organ mass×treatment and LDF×treatment

	RMR	PMR	Absolute scope	Factorial scope
Pectoral	0.50**	[a]	0.28	0.04
Leg	−0.05	0.29	0.37 (0.07)	0.38 (0.07)
Heart	0.17	[a]	0.05	−0.08 [b]
Liver	0.64***	[a]	0.03	
Gizzard	−0.26	−0.20	−0.13	0.12
Kidney	0.09	−0.05	0.05	−0.13
Intestine	0.25	0.27	0.08	0.03 [b]
Lipid	0.44*	[a]	-0.09	

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (for cells with $0.1 > P > 0.05$, the P level is given in parentheses)

[a]Significant interaction between treatment and organ mass. Significant positive correlation between organ mass and PMR in diet-restricted nestlings only

[b]Significant interaction between treatment and organ mass. Significant negative correlation between organ mass and factorial scope in controls only

Discussion

Food shortage and homeothermic abilities

The diet-restricted nestlings exhibited a lower PMR compared to that of the controls, with respect to body mass and age. The low PMR corresponded with a low RMR and T_b. In a study of ducklings, Moe et al. (2005) also found that PMR was inferior in ducklings subject to food shortage compared to that of ducklings fed ad libitum.

The absolute metabolic scope was substantially higher in diet-restricted nestlings compared to the controls with respect to body mass (Fig. 6a), and the mass-specific absolute scope did not differ between the treatments with respect to age (Fig. 6b). This indicates that the capacity for heat production at low T_a improved along with age in diet-restricted nestlings.

The MTC improved during the period of diet restriction despite an apparent increase in the surface-to-volume ratio in the diet-restricted nestlings (Moe et al. 2004), and MTC was not different between 16-day-old diet-restricted nestlings and controls. Consequently, the improved MTC must have occurred as a result of growth of down or improved vasomotor control or both. We observed, but did not measure, that the thickness of the down coating grew and that it did not seem to differ between controls and diet-restricted nestlings. It is, however, difficult to assess whether the

growth of the down coating was sufficient, alone, to account for the improvement in MTC.

Despite a substantial reduction in PMR, the food shortage did not significantly delay the development of homeothermy in the European shag nestlings. With respect to body mass, the diet-restricted nestlings tended to exhibit a higher HI compared to controls, and with respect to age, the HI was not significantly different between 16-day-old diet-restricted nestlings and controls. To our knowledge, this is the first study to measure the index of homeothermy in nestlings subject to food shortage. Consequently, comparative data is not available.

We interpret the lower PMR as a consequence of the lower RMR and T_b, rather than a consequence of reduced thermogenic capacity. Moe et al. (2005) also found that the low PMR of diet-restricted ducklings corresponded with a low RMR and T_b, and argued that a lowered RMR rather than any decreased function of the mechanisms underlying regulatory thermogenesis caused the reduced PMR. RMR constitutes a major part of PMR in young shag nestlings, and in the present study we found that RMR was a significant predictor of PMR. The idea of Klaassen and Bech (1992) that RMR and PMR are uncoupled in chicks with body masses deviating from the normal growth trajectory, does not seem to apply to European shag nestlings. The strong correlation between RMR and PMR (after controlling for body mass) which we found within the controls as well as the diet-restricted nestlings, is not consistent with that view.

The nestlings obtained PMR at a lower T_b compared to the T_b at RMR, and this is a common feature of conventional cold-induced PMR measurements on young chicks. Ricklefs and Williams (2003) argued that the measured PMR does not represent the true thermogenic capacity of a chick, if it is measured at a low T_b. According to a suggested procedure of Ricklefs and Williams (2003), we used the simultaneous measurement of T_b to adjust every single value of MR during the entire cooling trail to calculate an adjusted PMR. This PMR was adjusted to a high reference T_b (40°C) with a Q_{10} of 2. This calculation showed that diet-restricted nestlings exhibited the same adjusted PMR as controls with respect to body mass. This finding supports the view that the low PMR in the diet-restricted nestlings was a consequence of the low RMR and T_b, rather than a consequence of a reduced thermogenic capacity.

Body composition, organ maturation and thermoregulation

The size of the pectoral muscles was significantly negatively affected by the diet-restriction, and the leg muscles tended to be slightly smaller in diet-restricted nestlings compared to controls of the same body mass. Hence, one could expect the thermogenic capacity also to be negatively affected in the diet-restricted nestlings (Hohtola and Visser 1998; Chappel et al. 1999). However, it has been shown for neonates that muscles are not capable of shivering thermogenesis unless their LDF is higher than 0.15 (Ricklefs and Webb 1985; Dietz et al. 1997), and the pectoral muscles are not regarded to participate significantly in shivering thermogenesis until the European shag nestlings are about 21 days old (Østnes et al. 2001). Therefore, a reduction in the mass of the pectoral muscles should not be crucial for thermogenic capacity in the diet-restricted nestlings. The mass of the leg muscles, on the other hand, should be expected to be important. A positive relationship between the mass of the leg muscles and PMR has been found in ducklings (Moe et al. 2005) and chickens (Konarzewski et al. 2000), but we found no such relationship in the present study. As the ability to show a metabolic response to a low T_a is poor in young altricial birds (i.e. low factorial scope), RMR rather than regulatory thermogenesis will constitute the major part of PMR. Consequently, the thermoregulatory effector organs should rather show a relationship to absolute scope or factorial scope in young altricial birds. In the present study, the mass of the leg muscles tended to correlate positively to absolute and factorial scope. However, despite the slight reduction in leg muscle mass with respect to body mass in the diet-restricted nestlings (Fig. 7b), the absolute scope or the factorial scope was not negatively affected of the diet restriction.

The LDF of the leg muscles increased in line with that of the controls during the time of diet restriction (Fig. 7e). The capacity for regulatory heat production during low T_a has been found to correlate with functional maturity of the skeletal muscles in altricial and precocial chicks (Ricklefs and Webb 1985; Choi et al. 1993; Dietz et al. 1997). Accordingly, we believe an increase in functional maturity of the leg muscles probably resulted in the high absolute scope of the diet-restricted nestlings.

The present study partly supports the idea of Klaassen and Bech (1992) that *'the maturation of the thermoregulatory system proceeds steadily with time even when body mass lags behind'*. Our results on the development of the LDF of the leg muscles and on the development of the mass-specific absolute scope are consistent with their view. However, their idea was based on the finding that PMR was more dependent on age and less dependent on body mass in Arctic tern chicks with varying growth rates. Rather than PMR per se, we suggest that the mass-specific absolute scope, i.e. the portion of PMR available for regulatory thermogenesis, proceeds steadily with time even body mass lags behind. Results on diet-restricted ducklings support this view (Moe et al. 2005). However, the thermoregulatory responses to food shortage could also depend on species-specific developmental priorities, but available comparative data is scarce.

Differences in growth and maintenance of different body components may be due to a competition for nutrients between various growing tissues (O'Connor 1977; Sedinger 1986). Resources should be allocated to

those tissues where they are most needed. During food shortage, the growth and maturation of the brain is reported to be strictly maintained (Schew 1995), and the lean dry mass of the heart is reported to be maintained in proportion to body mass (Schew 1995; Moe et al. 2005). Muscle tissue could serve as a crucial source of essential amino acids and energy for maintenance in nestlings during food shortage. However, drawing on stores of amino acids may negatively influence the function of a muscle. Surprisingly, we found that the mass of the heart of the diet-restricted European shag nestlings was 17% lower compared to controls with the same body mass. This corresponded with a substantial mass loss of the pectoral muscles (and all visceral organs except the intestine mass), while the mass of the leg muscles only tended to be lower in diet-restricted nestlings compared to controls. This indicates a high developmental priority of the leg muscles. If the leg muscles (peripheral organ), rather than the heart (central organ), limit maximum heat production in European shag nestlings, this result suggest that thermoregulatory ability was given a high developmental priority. However, it could also indicate a high priority of locomotor ability and competitive ability for sibling competition (Brzek and Konarzewski 2001).

Ecological correlates

The time and energy budgets of chicks and parents are interrelated (Beintema and Visser 1989; Coulson and Johnson 1993; Farner 2000; Moe et al. 2002). The development of homeothermy in the nestlings is a prerequisite for the parents to go on long foraging trips and leave the nestlings alone in the nest (Clark 1984; Tveraa and Christensen 2002). During food shortage, time needed for successful foraging increases. If food shortage delays the development of homeothermy in the nestlings, the need for continued brooding would constrain foraging time of the parents. In contrast, continued development of homeothermy would lessen the constraints on the time for foraging of the parents. The mean T_a was 10°C (range 6–18°C) in the shag colony in June, and the climate was typically windy and rainy. Continued development of homeothermy even during food shortage seems particularly adaptive for nestlings inhabiting harsh environments. Another explanation, though not mutually exclusive to the above, is that the observed pattern of continued development of homeothermy may be a typical pattern for seabird species receiving regular feeds with less probability of facing protracted fasting (e.g. inshore feeders). In species receiving more irregular feeds (e.g. pelagic feeders), periods of protracted fasting might induce substantial hypothermia of unattended nestlings (e.g. Boersma 1986). However, Bech et al. (1991) and Weathers et al. (2000) found no evidence that nestling Antarctic fulmarine petrels normally experience hypothermia in the field, and suggested that the cold climate precludes these species from using substantial hypothermia. More studies are needed to assess whether inshore and pelagic feeders exhibit different adaptive patterns of development of homeothermy and whether the prevailing climatic conditions interact with these patterns.

In conclusion, we have shown that short-term food shortage did not significantly delay the development of homeothermy in the European shag nestlings. The PMR was negatively affected by the food shortage, but the mass-specific absolute scope (i.e. the portion of PMR available for regulatory thermogenesis) and minimal thermal conductance were improved during the period of food shortage. However, the duration and the magnitude of the food shortage should be a crucial factor. The nestlings in the present study seemed to be close to a physiological limit for keeping up the development of homeothermy. The functional capacity of the organs most important for regulatory thermogenesis could fail during a longer and a more severe food shortage.

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