Catabolic capacity of the muscles of shorebird chicks

Krijgsveld, K.L.; Olson, J.M.; Ricklefs, R.E.

Published in:
Physiological and Biochemical Zoology

DOI:
10.1086/319655

Publication date:
2001

Catabolic Capacity of the Muscles of Shorebird Chicks: Maturation of Function in Relation to Body Size

K. L. Krijgsveld[1,*]
J. M. Olson[2]
R. E. Ricklefs[3]
[1]Zoological Laboratory, Biological Centre, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands; [2]Department of Biology, Villanova University, 800 Lancaster Avenue, Villanova, Pennsylvania 19085-1699; [3]Department of Biology, University of Missouri—St. Louis, 8001 Natural Bridge Road, St. Louis, Missouri 63121-4499

Accepted 10/31/00

ABSTRACT

Newly hatched precocial chicks of arctic shorebirds are able to walk and regulate their body temperatures to a limited extent. Yet, they must also grow rapidly to achieve independence before the end of the short arctic growing season. A rapid growth rate may conflict with development of mature function, and because of the allometric scaling of thermal relationships, this trade-off might be resolved differently in large and small species. We assessed growth (mass) and functional maturity (catabolic enzyme activity) in leg and pectoral muscles of chicks aged 1–16 d and adults of two scolopacid shorebirds, the smaller dunlin (*Calidris alpina*: neonate mass 8 g, adult mass 50 g) and larger whimbrel (*Numenius phaeopus*; neonate mass 34 g, adult mass 380 g). Enzyme activity indicates maximum catabolic capacity, which is one aspect of the development of functional maturity of muscle. The growth rate–maturity hypothesis predicts that the development of catabolic capacity should be delayed in faster-growing muscle masses. Leg muscles of both species were a larger proportion of adult size at hatching and grew faster than pectoral muscles. Pectoral muscles grew more rapidly in the dunlin than in the whimbrel, whereas leg muscles grew more rapidly in the whimbrel. In both species and in both leg and pectoral muscles, enzyme activities generally increased with age, suggesting increasing functional maturity. Levels of citrate synthase activity were similar to those reported for other species, but L-3-hydroxyacyl-CoA-dehydrogenase and pyruvate kinase (PK) activities were comparatively high. Catabolic capacities of leg muscles were initially high compared to those of pectoral muscles, but with the exception of glycolytic (PK) capacities, these subsequently increased only modestly or even decreased as chicks grew. The earlier functional maturity of the more rapidly growing leg muscles, as well as the generally higher functional maturity in muscles of the more rapidly growing dunlin chicks, contradicts the growth rate–maturity function trade-off and suggests that birds have considerable latitude to modify this relationship. Whimbrel chicks, apparently, can rely on allometric scaling of power requirements for locomotion and the thermal inertia of their larger mass to reduce demands on their muscles, whereas dunlin chicks require muscles with higher metabolic capacity from an earlier age. Thus, larger and smaller species may adopt different strategies of growth and tissue maturation.

[1]Corresponding author; e-mail: k.l.krijgsveld@biol.rug.nl.

Physiological and Biochemical Zoology 74(2):250–260. 2001. 1522-2152/2001/7402-0016$03.00

Introduction

Shorebird chicks growing on the arctic tundra are faced with a climatically variable environment and a short season to complete their development. Being precocial, the chicks forage by themselves and, thus, are strongly affected by environmental conditions. Their thermoregulatory capacities develop rapidly (Visser and Ricklefs 1993*b*; J. B. Williams and R. E. Ricklefs, unpublished data), but during the first days after hatching, the chicks can produce heat and maintain their own body temperatures only to a limited extent. Thus, as small chicks forage they lose body heat, and their parents must brood the chicks at regular intervals to rewarm them. Time devoted to brooding cannot be used for foraging by either the chick or the parent. For chicks, the reduced time for foraging can depress growth rate, especially during adverse weather conditions (Beintema and Visser 1989*a*). If chicks could increase their thermoregulatory capacities at a given age or size, they would increase the proportion of the day available for feeding.

Thermoregulatory capacity in birds depends on the functional maturity of the skeletal muscles, which, apparently, are the primary source of heat production in response to cold stress in both adult (Dawson 1975; Hohtola and Stevens 1986) and young (Olson 1994; Marjoniemi and Hohtola 1999) birds. However, development of functional maturity also imposes costs. Several authors have suggested that a trade-off exists between functional capacity and growth rate (Dawson and Evans 1957; Ricklefs 1979; Ricklefs and Webb 1985; Olson 1992;

Choi et al. 1993; Ricklefs et al. 1994; Dietz and Ricklefs 1997; Pearson 1998; Starck and Ricklefs 1998). For example, Shea et al. (1995) showed that functional maturity, indicated by dry matter content and pyruvate kinase (PK) activity of muscles, was lower in a line of Japanese quail (*Coturnix coturnix japonica*) chicks selected for higher growth rate than in nonselected chicks. To the extent that energy, nutrients, and tissues allocated to mature function cannot be allocated to growth, investment in mature function reduces growth rate.

If a trade-off existed between growth rate and functional maturity, it is plausible that this compromise would be resolved differently by chicks of small and large species. The body size of a precocial neonate influences its relationship to the thermal environment during the growth period (Visser and Ricklefs 1993*a*), and adult body size is also correlated with the rate of postnatal growth and development (Ricklefs 1979; Starck and Ricklefs 1998). Chicks of smaller species have high surface-to-volume ratios and, therefore, lose heat rapidly to their surroundings. Chicks of larger species have more favorable thermal relationships to their environments but, typically, have relatively long developmental periods. Small species could increase functional maturity to increase foraging time, but it is unclear whether the resulting increased energy intake would more than balance their increased energy expenditure for locomotion and body temperature regulation. Large species could decrease functional maturity to accelerate growth, but this might impose a cost in that it reduces foraging time and, therefore, energy intake more than it reduces the energy requirement of growth. Measurements of mass increase show that relative growth rates of shorebird chicks vary inversely with adult body mass (Ricklefs 1968; Beintema and Visser 1989*b*). Accordingly, we would predict that smaller species would exhibit lower functional maturity, having resolved the growth rate–maturity trade-off in favor of rapid growth and increased dependence on parental care for thermoregulation early in development.

In this study, we explored functional maturity and growth rate in chicks of two species of shorebirds that differ in size. We selected one small and one large shorebird, both belonging to the family Scolopacidae: the dunlin (*Calidris alpina*: neonate mass 7.7 g [K. L. Krijgsveld, unpublished data]; adult mass 50 g [Jehl and Murray 1986]) and the whimbrel (*Numenius phaeopus*: neonate mass 33.5 g [own data], adult mass 380 g [Dunning 1993]). We assessed ontogenetic changes in the functional maturity of muscle by measuring the activities of three key catabolic enzymes: citrate synthase (CS), L-3-hydroxyacyl-CoA-dehydrogenase (HOAD), and PK. Activities of catabolic enzymes indicate the capacity of muscle to generate ATP, which is necessary for sustained shivering thermogenesis and locomotion (Marsh and Wickler 1982; Newsholme and Crabtree 1986; Olson 1990; Bishop et al. 1995). We also weighed chicks and their principal muscle groups and related measures of muscle biochemistry and size to the metabolic scope of chicks in response to cold stress. Thus, we could characterize and relate functional maturity of skeletal muscles, growth rate, and whole-organism function. In the general context of the growth rate–maturity hypothesis, we also address the use of each catabolic pathway at different times during development, the roles of leg and pectoral muscles in the development of endothermic capacity, and the influence of body size on the course of maturation in skeletal muscle function.

Material and Methods

Animals

Whimbrel and dunlin eggs were collected on the tundra near Churchill, Manitoba, Canada, in June–July 1996 and 1997. After hatching in an incubator, chicks were kept in boxes fitted with lightbulbs to provide a range in air temperatures between 25° and 38°C. Thus, the chicks could select a preferred thermal environment. When the chicks were homeothermic, they were kept at ambient room temperature (18°–25°C). Chicks stood on a 0.5 cm^2 wire-mesh cloth floor elevated above a layer of sawdust placed on the floor of the boxes for hygiene and easy cleaning. All chicks were provided food and water ad lib. Food consisted of pheasant starter type 2 mix (Spelderholt, Beekbergen, The Netherlands; 1996) or a comparable turkey prestarter mix (Puratone, Niverville, Manitoba; 1997), supplemented with a mix of canned tuna, boiled eggs, and freshly caught mosquitoes. Adult birds were trapped or shot in July near Churchill and were sampled immediately. Collecting was carried out under permit from the Canadian Wildlife Service. Husbandry and laboratory procedures were approved by the Institutional Animal Care and Use Committee of the University of Missouri—St.Louis.

Growth Analyses

Estimates of growth parameters were obtained by nonlinear curve fitting of the Gompertz equation. Parameters for body mass growth were based on daily weighings of 54 chicks that were pen reared in Churchill from 1995 to 1998 (K. L. Krijgsveld, unpublished data). Mean body masses for each day of age were log transformed and fitted by nonlinear regression to the logarithmic form of the Gompertz equation:

$$\ln [W(t)] = \ln A - \exp [-K(t - t_i)],$$

where $W(t)$ is the mass (g) at age t (d), A is the asymptote, or growth plateau (g), K is the growth rate constant (d^{-1}), and t_i is the inflection point of the growth curve (d). The Gompertz equation fit the data more closely than either the von Bertalanffy or logistic growth models (Ricklefs 1967). In this analysis, asymptotic body masses were set at 50 g for the dunlin (Jehl and Murray 1986) and 380 g for the whimbrel (Dunning 1993).

Growth parameters for pectoral and leg muscle growth were based on the fresh muscle masses of the 12 whimbrel and 12

dunlin chicks that were killed (see below). Parameters were estimated by fitting one growth curve to all data, with standard errors being asymptotic values returned by the NLIN procedure of SPSS (version 7.5 for Windows).

In addition, we calculated exponential growth rates (EGR) for pectoral and leg muscles over each age interval as the logarithmic growth increment divided by the age interval:

$$\text{EGR} = \frac{[\ln(W_f) - \ln(W_i)]}{(f - i)},$$

where W_i is the initial mass (g), W_f is the final mass (g), and $(f - i)$ is the interval (d) between samples. Average values were used at each age. Poor growth in one of the whimbrel chicks at 12 d induced a large variance in the exponential growth rate. Accordingly, we averaged the growth increment from 8 to 16 d in both muscles. The standard error of EGR was estimated as the square root of the variance in EGR, which is equal to $[\text{var}(W_f) + \text{var}(W_i)]/(f - i)$, divided by the square root of the sample size. According to the Gompertz model of growth, the exponential growth rate decreases linearly with the logarithm of size, that is, $[\ln(W_i) + \ln(W_f)]/2$. Thus, the Gompertz growth constant is the slope relating the exponential growth rate to $\ln(W/A)$ (Ricklefs et al. 1994; see also Fig. 3). Due to small sample sizes, differences in EGR could not be tested statistically.

Tissue Preparation

We collected muscle samples from 1-, 2-, 4-, 8-, 12-, and 16-d-old chicks and adult dunlin and whimbrel, with a sample size of 2 at each age. We restricted our measurements to the first 16-d posthatch because of the high relative growth rates and the large changes in thermoregulatory capacities during this period. All birds were killed by cervical dislocation or CO_2 asphyxiation. Birds were weighed to the nearest 0.1 g and transferred immediately to a glass plate chilled on ice. For the biochemical measurements, the left pectoral muscle (*Musculus pectoralis pars thoracicus*) and all of the muscles of the left leg below the hip were dissected, carefully removing nonmuscle tissue as much as possible. Excised tissues were weighed to the nearest 0.1 mg. These masses were multiplied by 2 to obtain the total masses of the muscles. After weighing, tissues were returned to a second glass plate maintained on ice, minced, and stored in tightly sealed 1.0 mL InterMed NUNC cryotubes. All tissue samples were frozen in liquid nitrogen within 15–25 min of death. Samples were shipped to Villanova University on dry ice and stored at −80°C until assayed. Independent measurements established that the activities of at least CS and HOAD are unaffected by the typical handling time in this study (O'Connor and Root 1993) or by storage at −80°C for periods of up to 4 mo (Olson et al. 1988).

Assays of Enzymatic Activities

We measured the activity of CS (EC 4.1.3.7), HOAD (EC 1.1.1.35), and PK (EC 2.7.1.40) under saturating substrate concentrations. All three are regulatory, nonequilibrium enzymes and were used to indicate the capacity for flux through the major catabolic pathways (Newsholme and Crabtree 1986). The activities of the enzymes indicate the maximum capacity for flux and not the actual flux through the respective catabolic pathways. CS catalyzes the condensation of acetyl CoA and oxaloacetate in the tricarboxylic acid (TCA) cycle. CS activity reflects the capacity for flux through the TCA cycle and, therefore, provides an index of aerobic capacity (Fitts et al. 1975; Hochachka et al. 1977). HOAD catalyzes the formation of ketoacyl CoA in the β-oxidation pathway for the breakdown of fatty acids, and HOAD activity indicates the capacity for lipid catabolism (Bass et al. 1969). PK catalyses the conversion of phosphoenolpyruvate (PEP) to pyruvate in the glycolytic pathway. The activity of this enzyme provides an index of the glycolytic capacity (Newsholme and Leech 1983).

On the day of the assay, the minced muscle was thawed on ice, weighed, and homogenized in 10 volumes (unless a limited amount of tissue required a higher dilution) of 100 mM potassium phosphate buffer with 2 mM EDTA (pH 7.3 at 0°C) using a glass-glass homogenizer. For the assays of CS and HOAD activity, homogenates were sonicated with a Branson Sonifier equipped with a microtip for three 15-s intervals separated by 45-s pauses. All samples and homogenates were maintained on ice during homogenization and sonication and until assayed to prevent thermal denaturation.

The activities of CS, PK, and HOAD were assayed spectrophotometrically on a Gilford response spectrophotometer at 25.0°C. Each assay was replicated at least once. All assays were performed in a final medium of 1 mL. Absorbance was measured for 6 min before adding the final substrate to allow time for thermal equilibration and for a control rate to be measured. Subsequently, the absorbance was measured for 5 min. CS activity was measured at 412 nm, according to a modification (as used by Olson [1990]) of the protocol of Srere (1969). The reaction medium contained 200 mM Tris-HCl, 5 mM EDTA, 0.1 mM 5,5′-dithiobis (2-nitrobenzoic acid), 0.2 mM s-acetyl CoA, 0.5 mM cis-oxaloacetate, and 0.1 mL diluted homogenate, pH 7.3 (at 25°C). The reaction was started by adding the oxaloacetate. HOAD activity was measured at 340 nm, according to a modification (Olson 1990) of the method of Bass et al. (1969). The reaction medium contained 100 mM triethanolamine-HCl, 5 mM EDTA, 0.225 mM NADH, 0.1 mM s-acetoacetyl CoA, and 0.1 mL diluted homogenate, pH 7.0. The reaction was started by adding the s-acetoacetyl CoA. PK activity was measured at 340 nm. The reaction medium contained 80 mM Tris-HCl, 100 mM KCl, 10 mM $MgCl_2$, 2 mM PEP, 5 mM adenosine 5′-diphosphate, 0.15 mM NADH, 0.10 mM D-fructose 1,6-diphosphate trisodium salt, 5 units LDH, and 0.1

mL diluted homogenate. The reaction was begun by adding the PEP. Throughout the text, activities are expressed as micromoles of substrate converted per minute per gram fresh tissue, or international units per gram.

Statistics

All data on adult birds were excluded from statistics. Statistics were performed using SPSS statistical software version 7.5. Tests were performed as much as possible within a single model, using general linear models (GLM). Age was entered in the models as a covariate or as a random factor, depending on the linearity of the data, and body mass was entered as covariate. Species and tissue type were entered as fixed factors. For all tests, individual data were used.

Results

Growth

Dunlin chicks grew more rapidly than whimbrel chicks. At 16 d of age, the dunlin chicks had nearly reached adult body mass, whereas the whimbrel chicks were only half grown. As a result, the growth rate constant (K) for body mass in dunlin chicks exceeded that for whimbrel chicks by 50% (t-test: $t_{52} = -10.7$, $P < 0.0001$), and the inflection point for growth (t_i) occurred correspondingly earlier in the dunlin (t-test: $t_{52} = -13.7$, $P < 0.0001$; Fig. 1; Table 1). The pectoral muscles of both species were relatively small at hatching, and as with body mass, the growth constant of the dunlin was more than 100% higher than that of the whimbrel. At 16 d of age, pectoral muscles in both species were still growing rapidly. The growth constant of the leg was 50% higher in the whimbrel than in the dunlin. Compared to the pectoral muscles, growth was rapid, and at 16 d of age, the leg muscles had already reached adult size (Fig. 1; Table 1).

The size of the pectoral muscle as a proportion of body mass started out low and at a similar value for both species (Fig. 2). In the dunlin, this value increased rapidly over the next 16 d, whereas in the whimbrel it remained low. The size of the leg muscle as a proportion of body mass started out higher than that of the pectoral muscle and changed little over the first 16 d of age. The two species showed a similar pattern in development of proportional leg muscle size. In adult birds, pectoral muscles were similar proportions of body mass in both species, but the leg muscles of adult whimbrels were smaller compared with those of adult dunlins, in contrast with the relative sizes of the leg muscles in the chicks.

The relative growth rates obtained from fitting Gompertz equations are reflected in the EGR. As shown in Figure 3, EGRs of the pectoral muscle were generally higher in the dunlin than in the whimbrel. After an initial low value, the EGR of the dunlin increased rapidly to a higher level than that of the whimbrel and remained relatively high almost until adult mass was achieved. EGRs of leg muscle were higher in the whimbrel early in the growth period.

Using a criterion of nonoverlapping asymptotic standard errors, statistically significant differences in growth rate are summarized as follows: WHleg ≥ DNleg > DNpect > WHpect. The growth rate for whimbrel leg muscle may be inflated because

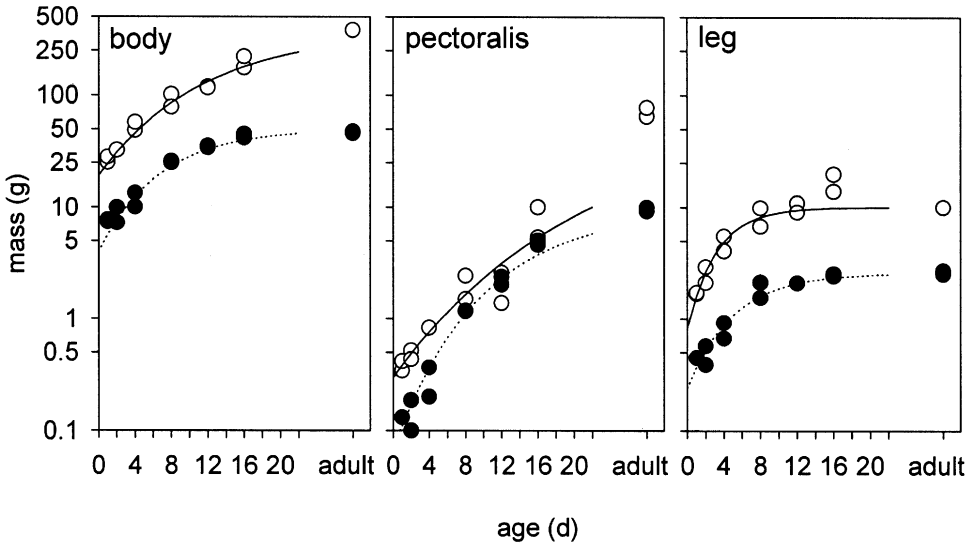

Figure 1. Body mass, leg muscle mass, and pectoral muscle mass in chicks of dunlin (*filled circles*) and whimbrel (*open circles*) shown as a function of age. The lines denote the average growth curves obtained by nonlinear curve fitting of the Gompertz equation.

muscle mass exceeded the adult level during the growth period and because adult mass was used to estimate the Gompertz growth rate.

Development of Catabolic Enzyme Activity

The developmental course of muscle mass-specific enzyme activity is shown in Figure 4. For all tissues and enzymes, adult levels were similar in the whimbrel and dunlin, and enzyme activities were higher in pectoral than in leg muscle.

The most striking aspects of the development of CS activity were its relatively high level in the leg muscles compared to the pectoral muscles of young chicks and its more rapid development toward adult levels in the dunlin compared to the whimbrel. CS activity of pectoral muscles was lower in whimbrel compared to dunlin chicks at all ages (ANOVA with species and age: DNpect > WHpect, $F_{2,21} = 38.47$, $P < 0.0001$). CS activity of leg muscles also was lower in whimbrel at all ages except 16 d (ANOVA with species and age: DNleg > WHleg, $F_{2,21} = 11.91$, $P < 0.0001$). A single statistical model for chicks showed that differences in CS activity could be attributed to species, tissue type, age, and the three-way interaction between these factors (GLM, age as random factor: intercept, $F_{1,5} = 47.91$, $P < 0.001$; species, $F_{1,16} = 19.85$, $P < 0.0005$; tissue type, $F_{1,16} = 9.63$, $P < 0.01$; age, $F_{5,16} = 4.66$, $P < 0.01$; species × tissue type × age, $F_{16,24} = 6.24$, $P < 0.0005$).

HOAD activity showed complex patterns of development (Fig. 4). HOAD activity was higher in the leg than in the pectoral muscles in both species, but this was not significant in the dunlin due to the high activity level measured in the pectoral muscle at day 8 (ANOVA with tissue type and age: WHleg > WHpect, $F_{2,21} = 13.03$, $P < 0.0001$). As with CS, HOAD activity was initially higher in the leg muscles than in the pectoral muscles. Combining these effects in a single statistical model showed that differences in HOAD activity in the chicks could be attributed to tissue type, age, and the interaction between species, tissue type, and age (GLM, age as random factor: intercept, $F_{1,5} = 74.83$, $P < 0.0005$; tissue type, $F_{1,17} = 6.28$, $P < 0.05$; age, $F_{5,17} = 3.27$, $P < 0.05$; species × tissue type × age, $F_{17,24} = 2.34$, $P < 0.05$). Differences between species were not significant.

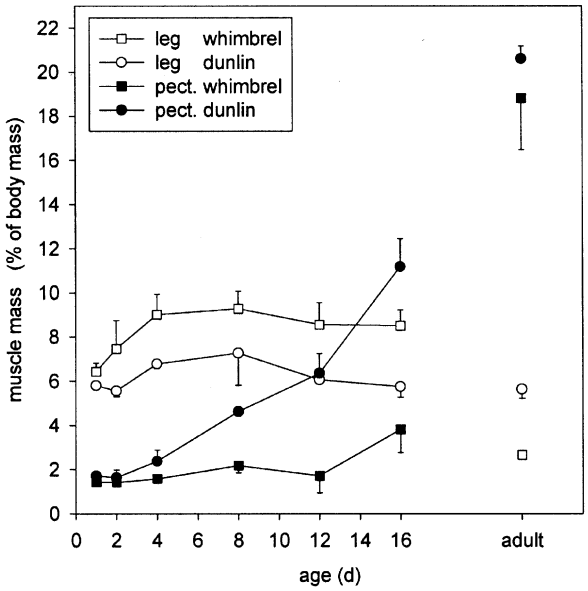

Figure 2. Muscle mass as percentage of body mass with standard deviations in relation to age, for the leg and pectoral muscles of dunlin and whimbrel chicks and adults.

As with CS and HOAD, PK activity was higher in the leg muscles than in the pectoral muscles of both species until 16 d of age (Fig. 4; ANOVA with tissue type and age: DNleg > DNpect, $F_{2,21} = 43.82$, $P < 0.0001$; WHleg > WHpect, $F_{1,22} = 25.05$, $P < 0.0001$; age is not significant). PK activity of dunlin pectoral and leg muscles increased rapidly until day 16 to levels

Table 1: Asymptotic size (A), growth constant (K), inflection point (t_i), coefficient of determination (r^2), and sample size (n) for dunlin and whimbrel chicks

	A (g)	K	t_i (d)	r^2	n
Body mass:					
Dunlin	50	.123 (.006)	6.68 (.387)	.927	24
Whimbrel	380	.075 (.002)	13.71 (.338)	.963	30
Leg muscle mass:					
Dunlin	2.6	.200 (.025)	4.37 (.368)	.953	12
Whimbrel	10.1	.312 (.079)	2.93 (.422)	.895	12
Pectoral muscle mass:					
Dunlin	9.6	.104 (.010)	15.42 (1.230)	.952	12
Whimbrel	71.5	.047 (.006)	36.35 (3.805)	.896	12

Note. Standard errors given in parentheses. Growth parameters were determined by Gompertz curve fitting. For details, see "Material and Methods."

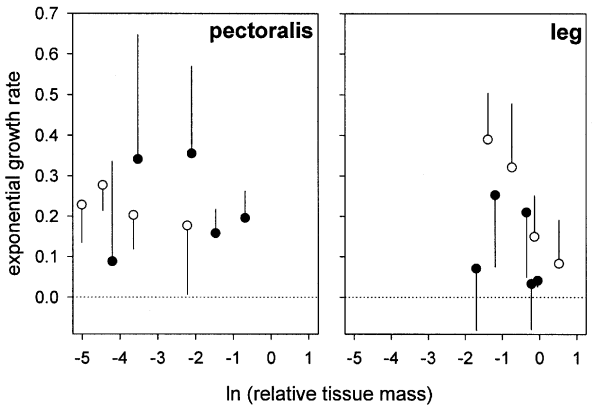

Figure 3. Relationship between the exponential growth rate and the natural logarithm of relative tissue mass (calculated as ln [tissue mass/adult tissue mass]), for pectoral and leg muscles of dunlin (*filled circles*) and whimbrel (*open circles*), with standard errors. Only growth increments up to 16 d of age were used.

similar to those of adults. PK activity in the whimbrel remained relatively low and constant compared to the dunlin until 16 d of age, after which it increased fivefold (pectoral muscles) or threefold (leg muscles) to adult levels. A single statistical model showed that PK activity was related to tissue type, the interaction between species and age, and the interaction between tissue type and age (GLM, age as random factor: intercept, $F_{1,6.8} = 19.30$, $P < 0.005$; tissue type, $F_{1,5} = 15.36$, $P < 0.05$; species × age, $F_{6,30} = 28.46$, $P < 0.0005$; tissue type × age, $F_{5,30} = 3.17$, $P < 0.05$).

Total Activity of the Pectoral and Leg Muscle

Multiplying the enzyme activity per unit mass by the mass of the muscle gives the total enzyme activity of the individual muscle (Fig. 5) and provides an estimate of the potential contribution of the muscle to the heat balance of the chick (see Choi et al. 1993). This estimate is more strongly influenced by variation in muscle mass than in mass-specific enzyme activity. In both species, leg muscle contributed most of the potential total enzyme activity early in development, although pectoral muscles predominated after 12 d in the dunlin and sometime after 16 d in the whimbrel. Total enzyme activities for leg muscle in the dunlin and whimbrel had similar allometric relationships. For pectoral muscles of the same weight, however, dunlin chicks consistently exhibited higher catabolic capacity.

Growth Rate versus Functional Capacity

The hypothesis that there is a trade-off between exponential growth rate and functional capacity predicts that the correlation between the two should be negative. This prediction is generally borne out by the data, especially for the leg muscles (Fig. 6). Since the sample size of age increments was small, these results allow us to form only a general concept of the relationship between enzyme activity and EGR. Statistics combining the data for the pectoral and the leg muscle in one model revealed that for both CS and PK the correlation between enzyme activity and EGR was significantly negative (GLM: CS, $F_{4,11} = 10.94$, $P < 0.001$, $r^2 = 0.80$; CS activity, $F_{1,11} = 35.18$, $P < 0.0001$; species, tissue type, and the interaction between tissue type and CS activity all contributed significantly to the model; PK, $F_{1,14} = 8.03$, $P < 0.05$, $r^2 = 0.37$; significant contribution of PK activity only). EGR showed no significant correlation with HOAD, although there appeared to be a negative relationship in the leg muscles.

Discussion

Growth

Growth rate was higher in the smaller dunlin than in the whimbrel (Fig. 1; Table 1). These values corresponded well with

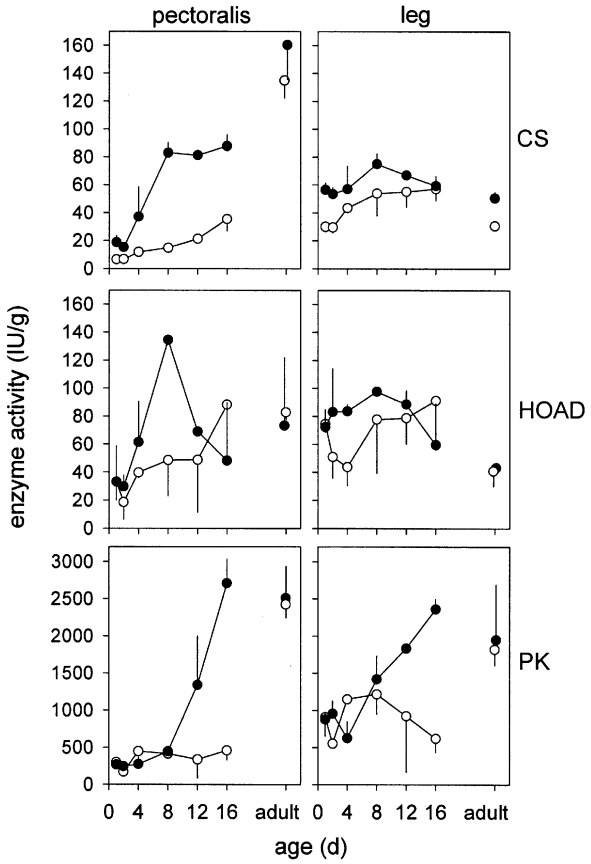

Figure 4. Activity of citrate synthase (*CS*), L-3-hydroxyacyl-CoA-dehydrogenase (*HOAD*) and pyruvate kinase (*PK*) with standard deviations of leg and pectoral muscles in dunlin (*filled circles*) and whimbrel (*open circles*) chicks (and adults), in relation to age.

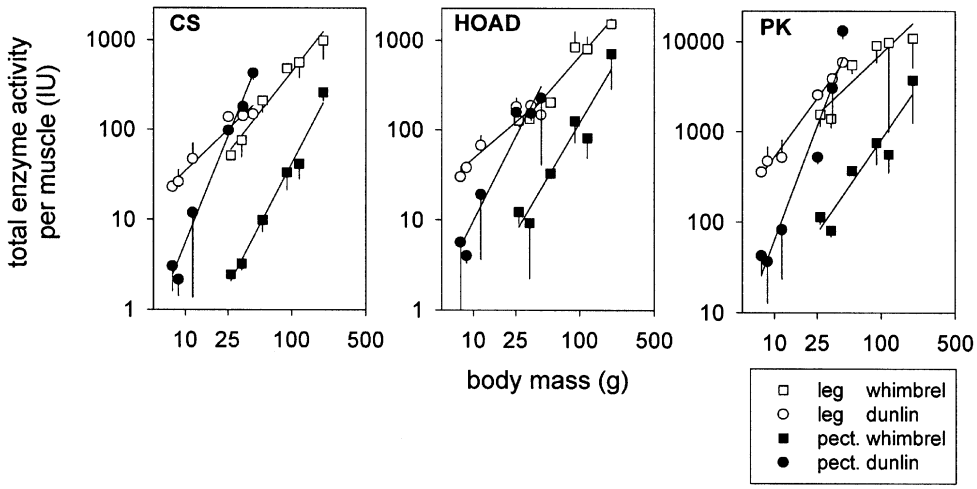

Figure 5. Total activity (enzyme activity multiplied by muscle mass) of citrate synthase (*CS*), L-3-hydroxyacyl-CoA-dehydrogenase (*HOAD*), and pyruvate kinase (*PK*), with standard deviations in relation to body mass for leg and pectoral muscles of dunlin and whimbrel chicks. The lines depict linear regressions.

growth rates calculated from other data on hand-reared chicks of these species (Visser and Ricklefs 1993*a*), and they are in line with the general inverse relationship of relative growth rate to body size (e.g., Ricklefs 1968; Starck and Ricklefs 1998). Muscle growth rate was higher in the leg than in the pectoral muscles in both dunlin and whimbrel (Fig. 1) and showed the importance of rapid development of the leg, which is needed for locomotion and early thermoregulation. This is also reflected in the high relative mass of the leg muscles (proportional to body mass) compared to the relative mass of the pectoral muscles in both species (Fig. 2). Pectoral muscles, in contrast, are small in young precocial chicks and are not able to contribute substantially to heat generation until the chicks are older and start to fly. Until this time, growth and functional development are delayed in these muscles (Barré et al. 1985; Dietz et al. 1997; Marjoniemi and Hohtola 1999). Accordingly, the absolute as well as the relative size of the pectoral muscles was low initially but increased to a size exceeding that of the leg muscles later in development. This happened sooner in the dunlin than in the whimbrel because growth was more rapid in the pectoral muscles of the dunlin than the whimbrel. This correlates with earlier development of flight in the dunlin (del Hoyo et al. 1996).

Development of Catabolic Capacity

Among adults, the catabolic profiles of the muscles were similar for the two species. The activities of all three enzymes were higher in the pectoral than in the leg muscles. In the chicks, enzyme activities in the leg muscles tended to be higher than in the pectoral muscles, at least early in development, and were close to or exceeded adult levels of enzyme activity in the leg muscles. The leg muscles are needed for locomotion shortly after hatching. Clearly, they also make up the bulk of the thermoregulatory capacity of the chick early in development. Enzyme activity in the pectoral muscles increased at later ages than in the leg muscles, although generally to higher levels. Since the dunlin grew more rapidly than the whimbrel, enzyme activities of dunlin chicks attained adult levels earlier, in some cases by 16 d of age, than did those of whimbrel chicks. Dunlin chicks also tended to have higher levels of enzyme activities than whimbrel chicks at any given age.

When we include the size of the muscles and consider the total enzyme activity (enzyme activity multiplied by the muscle mass), it becomes clear that the leg muscles of whimbrel and dunlin chicks had similar capacities relative to body mass, whereas the pectoral muscles of the dunlin had a higher capacity than those of the whimbrel. This indicates that whereas dunlin chicks rely more on catabolic capacity to generate heat, whimbrel chicks, to a considerable extent, can rely on the thermal inertia of their larger mass to reduce heat loss. This may explain why the whimbrel chick is homeothermic at only 3 d of age, whereas the dunlin does not achieve homeothermy until 8 d of age (Visser 1991).

Compared to altricial chicks, precocial chicks exhibit slow growth but have a high level of function. Enzyme activities can now be compared over a range of altricial species (bank swallow [*Riparia riparia*], Marsh and Wickler 1982; red-winged blackbird [*Agelaius phoeniceus*], Olson 1990; European starling [*Sturnus vulgaris*], Choi et al. 1993) and precocial species (Japanese quail [*Coturnix coturnix japonica*], Choi et al. 1993 and Shea et al. 1995; northern bobwhite [*Colinus virginianus*], Choi et

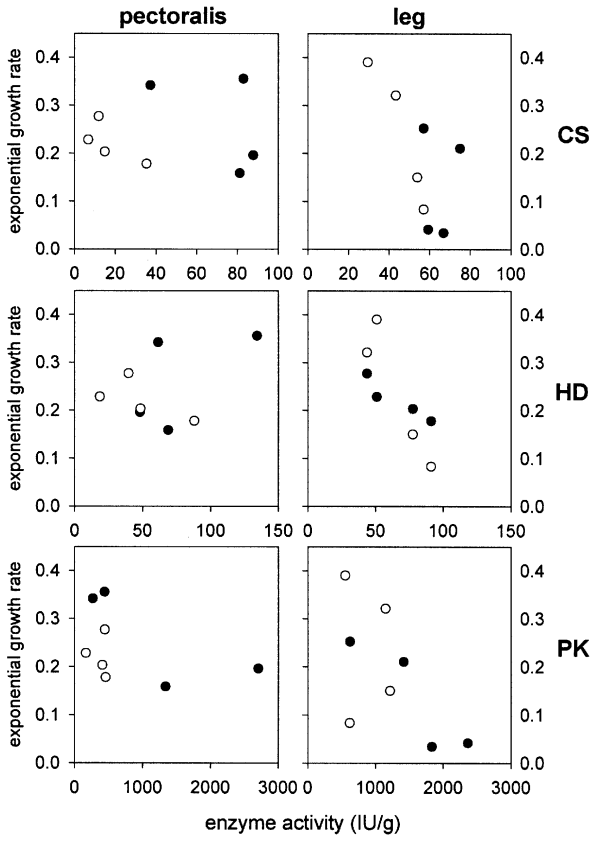

Figure 6. Exponential growth rate in relation to the activity of citrate synthase (*CS*), L-3-hydroxyacyl-CoA-dehydrogenase (*HOAD*), and pyruvate kinase (*PK*) for pectoral and leg muscles of dunlin (*closed circles*) and whimbrel (*open circles*). The lines depict linear regressions.

al. 1993; barnacle goose [*Branta leucopsis*], Bishop et al. 1995; dunlin and whimbrel, this study). These studies reveal that the aerobic capacity (CS) of pectoral muscles increases rapidly after hatching in altricial chicks and is generally higher than in precocial chicks. CS activities of leg muscles were similar in the two groups. Glycolytic capacity (PK) was much higher in precocial species from hatching on. Starlings exhibited the lowest glycolytic capacities, Japanese and bobwhite quail were intermediate, and dunlin and whimbrel had the highest capacities. This indicates that, besides aerobic catabolism, glycolytic catabolism plays an important role in ATP production and, therefore, presumably in heat production in chicks of precocial shorebird species, evidently more so than in chicks of altricial species. Shorebird chicks also have a relatively high capacity to break down fatty acids. For example, the levels of HOAD activity in the leg muscles are more than 10 times higher than in the chicks of the red-winged blackbird; in pectoral muscles, HOAD activities of shorebird chicks are about three times higher than those of red-winged blackbird chicks.

Both glycolysis and β-oxidation produce acetyl CoA, which would be available to the mitochondria as substrate for the TCA cycle. The ontogenetic changes in capacities observed here suggest that β-oxidation may be relatively more important as a source of acetyl CoA earlier in development than it is later. In pectoral muscle, for example, the large increase in HOAD activity in the dunlin at day 8, followed by the striking decrease in HOAD activity and simultaneous increase in PK activity after day 8, suggests a qualitative shift in the importance of these two pathways during development.

Catabolic Capacity as a Measure of Mature Function

To assess the suitability of catabolic enzyme activity as an estimate of functional maturity, we correlated total enzyme activities with the metabolic scope of shorebird chicks measured in a parallel study (J. B. Williams et al., unpublished data). Briefly, standard (SMR) and peak metabolic rates (PMR) of dunlin and whimbrel chicks were measured using an open-circuit respirometer. Temperature was initially maintained within the thermoneutral zone to obtain an estimate of SMR and then was decreased by about 0.5°C min^{-1} until metabolism reached PMR and subsequently began to decrease. Body temperature was monitored continuously throughout the trials. Metabolic scope was calculated as PMR − SMR. Metabolic measurements were obtained from chicks of similar age and body mass as those for which we measured enzyme activities.

The metabolic scope of dunlin and whimbrel chicks was significantly correlated with the total enzyme activity of the muscles (i.e., activity multiplied by muscle mass) for all three enzymes (Fig. 7), suggesting that the total catabolic capacity of the pectoral and leg muscles is a good predictor of heat generation in the chick (see also Choi et al. 1993; GLM: ln [metabolic scope] vs. ln [total enzyme activity]; CS: slope = 0.99, $F_{1,10} = 106.06$, $r^2 = 0.91$, $P < 0.0001$; HOAD: slope = 1.04, $F_{1,10} = 106.85$, $r^2 = 0.91$, $P < 0.0001$; PK: slope = 0.90, $F_{1,10} = 60.2$, $r^2 = 0.86$, $P < 0.0001$). However, this correlation was explained by variation in the mass of the muscles and not in enzyme activity itself. A statistical model with muscle mass and mass-specific enzyme activity as separate effects showed that enzyme activity was not significant (GLM: metabolic scope vs. mass of leg + pect and CS activity g^{-1} of leg + pect; all log transformed: $F_{2,9} = 19.92$, $P < 0.0001$, $r^2 = 0.82$; muscle mass, $P < 0.0001$; CS activity, $P > 0.05$; similar correlations were found for HOAD and PK). Why enzyme activity itself did not have an effect on metabolic scope can be explained partly by the fact that enzyme activity in the leg muscles changed relatively little during early development. Thus, the rapidly increasing mass of the leg muscle alone had an important effect on metabolic scope. In addition, the pathways that are used by the chicks to generate ATP change throughout development, as indicated by the ontogenetic changes in the relative activities of the three enzymes. Accordingly, levels of

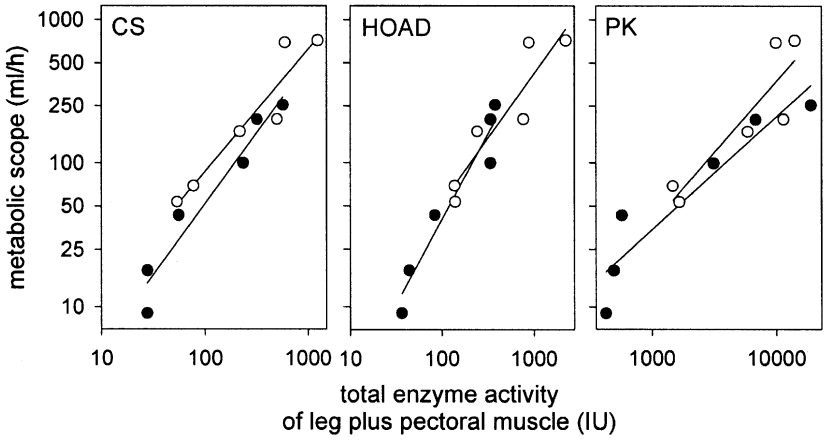

Figure 7. The relationship between metabolic scope and total enzyme activity summed for pectoral and leg muscle, plotted for citrate synthase (*CS*), L-3-hydroxyacyl-CoA-dehydrogenase (*HOAD*), and pyruvate kinase (*PK*) of dunlin (*closed circles*) and whimbrel (*open circles*) chicks. The lines depict linear regressions.

CS, PK, and HOAD activity not only increase with age but may also decrease as the need for a certain pathway diminishes. Because of this, the relationship between enzyme activity per gram tissue and metabolic scope and functional maturity is weakened, and thus, enzyme activity might not be a totally consistent indicator of muscle maturity.

Trade-Off between Growth Rate and Functional Maturity

Exponential growth rate was negatively correlated with CS activity per gram tissue (Fig. 6), which matches our expectations regarding a trade-off between growth rate and functional maturity. However, differences in levels of enzyme activities between the two species and between the leg and pectoral muscles indicate that processes other than this trade-off play important roles in determining catabolic capacity and growth rate. For example, dunlin chicks both grew more quickly and demonstrated relatively higher catabolic capacities than whimbrel chicks (Figs. 1, 3–5). Analyses using individual muscle groups lead to a similar conclusion. For example, the catabolic capacities in the leg muscles were initially higher than in the pectoral muscles even though growth rate of the leg muscles was higher than that of the pectoral muscles (Figs. 1, 4). These results can be related partly to qualitative differences in the catabolic capacities of the tissues, and they stress the importance of further studies on structural and functional properties of tissues. The results may also be explained simply by the heat-regulating effect of the mass of the chicks. The smaller dunlin chicks seem to rely more on the catabolic capacity of their muscles and invest more energy in rapid development of both pectoral and leg muscles than do the whimbrel chicks. The larger mass of the whimbrel chick apparently reduces the need for rapid functional maturation of the muscles. As the leg muscles of the whimbrel chick are crucial for locomotion and heat generation, a considerable amount of energy is invested in their rapid growth, but catabolic capacity is nonetheless relatively low compared to the dunlin. The pectoral muscles of the whimbrel chick both grow more slowly and have a lower enzyme activity than the pectoral muscles of the dunlin chick. As whimbrel chicks develop flight later than dunlin chicks (del Hoyo et al. 1996), development of the flight apparatus can be delayed, while resources are focused on, for example, the legs, skeleton, feathers, or the brain (Portman 1962; Carrier and Auriemma 1992).

The data presented in this article suggest that catabolic capacity together with body mass determine the functional output of the shorebird chick. A small chick needs to invest in both growth and mature function to its maximum capacity in order to stay warm during foraging. Because of its larger size, a larger chick is able to maintain its body temperature to a large extent. A high level of functional maturity seems to be less important in a larger chick, and consequentially, it may reduce its daily energy demand by lowering its level of functional maturity.

Acknowledgments

We kindly thank the Churchill Northern Studies Centre for their logistic support and housing. J. B. Williams provided unpublished data on whole-body metabolism in growing dunlin and whimbrel chicks. G. H. Visser supported the study throughout and made useful comments to the manuscript, as did S. Daan and J. M. Starck. We thank J. Spina, A. Smeglin, M. Hafey, and H. Simoes for assisting us with the assays and

data entry. This study was supported by National Science Foundation grant OPP-9453522 awarded to R.E.R. and G. H. Visser.

Literature Cited

Barré H., A. Geloen, J. Chatonnet, A. Dittmar, and J.-L. Rouanet. 1985. Potentiated muscular thermogenesis in cold-acclimated Muscovy ducklings. Am J Physiol 249: R533–R538.

Bass A., D. Brdiczka, P. Eyer, S. Hofer, and D. Pette. 1969. Metabolic differentiation of distinct muscle types at the level of enzymatic organization. Eur J Biochem 10:198–206.

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

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

Bishop C.M., P.J. Butler, S. Egginton, A.J. El Haj, and G.W. Gabrielsen. 1995. Development of metabolic enzyme activity in locomotor and cardiac muscles of the migratory barnacle goose. Am J Physiol 269:R64–R72.

Carrier D.R. and J. Auriemma. 1992. A development constraint on the fledging time of birds. Biol J Linn Soc 47:61–67.

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

Dawson W.R. 1975. Avian physiology. Annu Rev Physiol 37: 441–465.

Dawson W.R. and F.C. Evans. 1957. Relation of growth and development to temperature regulation in nestling field and chipping sparrows. Physiol Zool 30:315–327.

del Hoyo J., A. Elliott, and J. Sargatal. 1996. Handbook of the Birds of the World. Vol. 3. Hoatzins to Auks. Lynx, Barcelona.

Dietz M.W. and R.E. Ricklefs. 1997. Growth rate and maturation of skeletal muscles over a size range of galliform birds. Physiol Zool 70:502–510.

Dietz M.W., S. van Mourik, Ø. Tøyen, P.A. Koolmees, and M.H.G. Tersteeg-Zijderveld. 1997. Participation of breast and leg muscles in shivering thermogenesis in young turkeys and guinea fowl. J Comp Physiol 167:451–460.

Dunning J.B., Jr. 1993. CRC Handbook of Avian Body Masses. CRC, Boca Raton, Fla.

Fitts R.H., F.W. Booth, W.W. Winder, and J.O. Holloszy. 1975. Skeletal muscle respiratory capacity, endurance, and glycogen utilization. Am J Physiol 228:1029–1033.

Hochachka P.W., J.R. Neely, and W.R. Driedzic. 1977. Integration of lipid utilization with Krebs cycle activity in muscle. Fed Proc 36:2009–2014.

Hohtola E. and E.D. Stevens. 1986. The relationship of muscle electrical activity, tremor and heat production to shivering thermogenesis in Japanese quail. J Exp Biol 125:119–135.

Jehl J.R., Jr., and B.G. Murray. 1986. The evolution of normal and reversed sexual size dimorphism in shorebirds and other birds. Curr Ornithol 3:1–86.

Marjoniemi K. and E. Hohtola. 1999. Shivering thermogenesis in leg and breast muscles of galliform chicks and nestlings of the domestic pigeon. Physiol Biochem Zool 72:484–492.

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

Newsholme E.A. and B. Crabtree. 1986. Maximum catalytic activity of some key enzymes in provision of physiologically useful information about metabolic fluxes. J Exp Zool 239: 159–167.

Newsholme E.A. and A.R. Leech. 1983. Biochemistry for the Medical Sciences. Wiley, London.

O'Connor T.P. and T.L. Root. 1993. The effect of handling time and freezing on catabolic enzyme activity in house sparrow pectoralis muscle. Auk 110:150–152.

Olson J.M. 1990. Physiological and Biochemical Aspects of the Development of Endothermy in an Altricial Bird, the Red-Winged Blackbird. PhD diss. University of Michigan, Ann Arbor.

———. 1992. Growth, the development of endothermy, and the allocation of energy in red-winged blackbirds (*Agelaius phoeniceus*) during the nestling period. Physiol Zool 65: 124–152.

———. 1994. The ontogeny of shivering thermogenesis in the red-winged blackbird (*Agelaius phoeniceus*). J Exp Biol 191: 59–88.

Olson J.M., W.R. Dawson, and J.J. Camilliere. 1988. Fat from black-capped chickadees: avian brown adipose tissue? Condor 90:529–537.

Pearson J.T. 1998. Development of thermoregulation and post-hatching growth in the altricial cockatiel *Nymphicus hollandicus*. Physiol Zool 71:237–244.

Portmann A. 1962. Cerabralisation and ontogenese. Med Grundlagenforsch 4:1–62.

Ricklefs R.E. 1967. A graphical method of fitting equations to growth curves. Ecology 48:978–983.

———. 1968. Patterns of growth in birds. Ibis 110:419–451.

———. 1979. Patterns of growth in birds. V. A comparative study of development in the starling, common tern and Japanese quail. Auk 96:10–30.

Ricklefs R.E., 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 48:1090–1088.

Ricklefs R.E. and T. Webb. 1985. Water content, thermogenesis and growth rate of skeletal muscle in the European starling. Auk 102:369–376.

Shea R.E., R.E. Ricklefs, and I.-H. Choi. 1995. Growth rate and function of skeletal muscles in Japanese quail selected for four-week body mass. Physiol Zool 68:1045–1076.

Srere P.A. 1969. Citrate synthase. Pp. 3–11 in A.E. Renold and

G.F. Cahill, Jr., eds. Handbook of Physiology. Pt. 5. Adipose Tissue. American Physiological Society, Washington, D.C.

Starck J.M. and R.E. Ricklefs. 1998. Avian Growth and Development. Evolution within the Altricial-Precocial Spectrum. Oxford University Press, Oxford.

Visser G.H. 1991. Development of Metabolism and Temperature Regulation in Precocial Birds. PhD diss. University of Groningen, The Netherlands.

Visser G.H. and R.E. Ricklefs. 1993*a*. Development of temperature regulation in shorebirds. Physiol Zool 66:771–792.

———. 1993*b*. Temperature regulation in neonates of shorebirds. Auk 110:445–457.