

CHANGES IN PROTEIN AND ELECTROLYTE CONCENTRATIONS IN THE PECTORAL AND LEG MUSCLES DURING AVIAN DEVELOPMENT

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ABSTRACT.—The development of functional capacity of skeletal muscle proceeds more rapidly in precocial than in altricial birds and more rapidly in leg than in pectoral muscles. To determine whether protein accumulation and electrolyte differentiation proceeded in parallel with functional development, we examined changes in protein (lipid-free dry mass, LFDM), K⁺ concentrations, and Na⁺ concentrations in pectoral and leg muscles during postnatal development in altricial European Starlings (*Sturnus vulgaris*) and precocial Japanese Quail (*Coturnix c. japonica*) and Northern Bobwhites (*Colinus virginianus*). Proportion of LFDM in pectoral muscle had a similar pattern of increase in the three species during development, whereas the proportion in leg muscle was slightly higher in bobwhites than in the other two species during the first two weeks. Concentrations of K⁺ increased and Na⁺ decreased during development. [K⁺] exceeded [Na⁺] for the first time at 4 to 8 days for pectoral muscle and 2 to 8 days for leg muscle in starlings, and at about 5 days for pectoral muscle and 1 to 2 days for leg muscle in quail. Electrolyte concentrations approached adult levels at 13 to 16 days in starlings and 8 to 11 days in both quail. These ages preceded fledging in starlings and first flight in quail by 4 to 6 days. In neonates of each species, the proportion of LFDM was 3 to 5% higher in leg muscle than in pectoral muscle; adult levels of LFDM were achieved 3 to 7 days earlier in leg muscle. Differentiation of the two electrolytes (i.e. crossing of [K⁺] and [Na⁺] curves) occurred 2 to 4 days earlier in leg muscle than in pectoral muscle, especially for quail. These results indicate that compositional development of muscle parallels functional development of precocity during postnatal growth. Because the production and maintenance of proteins and the differentiation of electrolytes require metabolic energy expenditure, chemical maturity may compete with growth rate for allocation of energy and nutrients. Received 16 September 1996, accepted 13 May 1997.

PROTEINS AND ELECTROLYTES are important chemical bases for the contractile and conductive apparatus of skeletal muscle. Because animals exhibit significant functional development during their postnatal growth, concentrations of these chemicals must change with increasing functional capacity. Previous studies have revealed that protein contents and excitability of skeletal muscles increase dramatically during the early growth period in mammals and birds (Moulton 1923, Dickerson 1960, Hazlewood and Nichols 1969). For instance, in the pectoral muscle of cockerels (domestic fowl), fibrillar and sarcoplasmic proteins increase

more than 2-fold during the first month of growth (Dickerson 1960). Excitability of muscle fibers, indicated by resting membrane potential, increases more than 3-fold in the rat gastrocnemius (Hazlewood and Nichols 1969) and is known to be established by differential partitioning of electrolytes (e.g. potassium and sodium) across the cell membrane (Vernadakis and Woodbury 1964, Hazlewood and Nichols 1969, Park et al. 1981, Ward and Wareham 1985). The age at which concentrations of proteins and electrolytes reach mature levels has been called the point of "chemical maturation" (Moulton 1923). Although previous studies have described ontogenic patterns of the chemical composition of muscle tissue, few data relate variation in the processes of chemical maturation to variation in the rate of development of functional capacity.

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Birds provide good models for exploring this issue from a comparative perspective because they exhibit diverse rates of functional development in skeletal muscles (Ricklefs 1979). Physiological performance, measured by force generation, locomotory ability, and regulatory thermogenesis, is better developed in precocial than in altricial chicks (Choi and Bakken 1991, Ricklefs et al. 1994). Functional development also may vary among muscle groups; for example, pedal activity may appear as early as hatching in precocial chicks, whereas flight capability may not be acquired for several days or weeks (Ricklefs and Webb 1985; Choi and Bakken 1990, 1991). Thus, comparisons among different muscle groups of altricial and precocial species should reveal parallels between compositional and functional maturation. Here, we describe changes in total protein contents and electrolyte concentrations during posthatching growth in pectoral and leg muscles of the altricial European Starling (*Sturnus vulgaris*) and the precocial Japanese Quail (*Coturnix c. japonica*) and Northern Bobwhite (*Colinus virginianus*). Hereafter, we refer to these species as starling, quail, and bobwhite. If composition and functional capacity change in parallel, then simple measures of muscle composition could be used in comparative studies to indicate the development of functional capacity. In addition, compositional changes in muscle may provide a broader context for interpreting the observed inverse relationship between tissue growth rate and functional capacity (Ricklefs et al. 1994).

METHODS

Subjects.—Starling nestlings were collected from nest boxes at the Morris Arboretum farm in Montgomery County, southeastern Pennsylvania, between May and July 1991. The nestlings were transferred to our laboratory at each experimental age while being given enough food to maintain body mass. We purchased chicks of both quail species from a commercial farm and kept them in a brooder at 32 to 35°C with water and quail starter *ad libitum* (Choi et al. 1993).

Percent protein and concentrations of potassium and sodium.—Individual chicks were sacrificed by decapitation on ice. We sampled small portions of tissues (0.02 to 0.6 g) from the pectoral muscle (m. pectoralis and m. supracoracoideus) and leg muscle (all muscles combined) and weighed each tissue sample (± 0.0001 g). Samples were placed in tared and labeled

5-mL polypropylene tubes that were then dried to constant mass at 60°C, usually for 24 to 48 h. Lipids were removed from the dried samples by adding 3 mL of a 1:1 mixture of petroleum ether and chloroform in a hood followed by drying for 12 h at room temperature. We repeated this treatment and then recorded the final lipid-free dry mass (LFDM). We calculated fat mass as dry mass minus extracted mass; proportion LFDM = $100 \times \text{LFDM} / (\text{initial wet mass} - \text{lipid mass})$. Each dry, extracted sample was digested overnight in conc-HNO₃ (10 μ L per mg dry mass). The digested solution was then neutralized with conc-NH₄OH (approximately 1.7 NH₄OH:1.0 HNO₃) and 10 μ L phenol red to indicate pH. We measured potassium and sodium concentrations with an Instrumentation Laboratories IL 443 Digital Flame Photometer. Concentrations are reported as mmole per g wet mass.

Data analysis.—Differences between species for proportion of LFDM and changes in electrolytes were tested by ANOVA (with age up to 16 days, the oldest age for starlings). Differences in proportion of LFDM between pectoral and leg muscles within species were tested by paired *t*-tests. All procedures were performed with SPSS/PC+, and the statistical significance was examined at $P = 0.05$, unless otherwise noted. To determine the "age of chemical maturity" for each species, we followed Moulton's (1923) definition for the age at which the concentrations of proteins and salts become relatively constant in the fat-free cell; at the age of chemical maturation, the concentrations of the chemicals in question exceed 90% of adult (mature) levels.

RESULTS

Percent total protein.—The LFDM of pectoral muscle of neonates constituted about 13% for the three species and increased 1.7- to 2.1-fold during development (Fig. 1A). In leg muscle, the proportion of LFDM in starling neonates was 14% and increased 1.6-fold over the first 16 days; in quail and bobwhite, the proportion of LFDM was 18% initially and increased only about 1.2-fold during development (Fig. 1B). Compared with pectoral muscle, leg muscles of young chicks contained greater levels of LFDM in all three species, but especially for the quail and bobwhite (quail: $t = 4.92$, $n = 5$, $P = 0.008$ at 4 days; bobwhite: $t = 5.08$, $n = 5$, $P = 0.007$ at 2 days; $t = 4.89$, $n = 4$, $P = 0.02$ at 5 days). Comparing quail and bobwhite, the level of LFDM in leg muscles was 1 to 2% higher in bobwhite than in quail during the first 16 days ($F = 4.984$, $df = 1$ and 32, $P < 0.033$). Mature levels of LFDM (*sensu* Moulton 1923) were at-

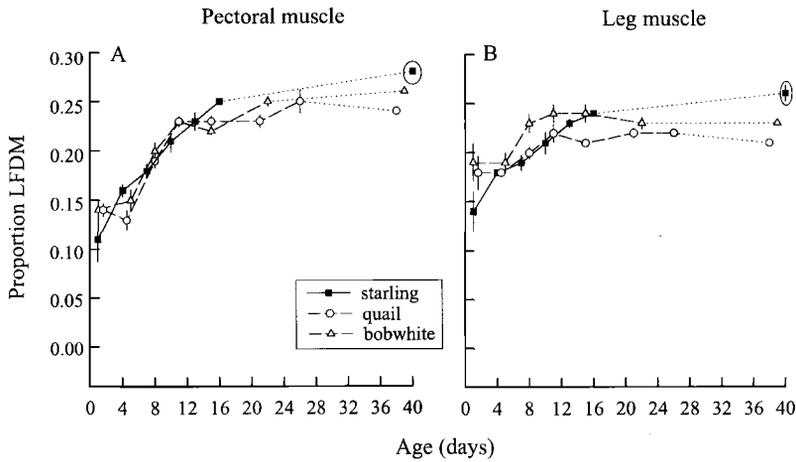


FIG. 1. Changes in the proportion of lipid-free dry mass for pectoral muscle (A) and leg muscle (B) as a function of age of chicks in European Starlings, Japanese Quail, and Northern Bobwhites. Each data point is the average of two to six values; vertical lines are ± 1 SE.

tained at 16 days for pectoral muscle and 13 days for leg muscle in starlings; in quail and bobwhite, chemical maturity was observed at 11 days for pectoral muscle and 4 to 8 days for leg muscle (Table 1).

Potassium and sodium ion concentrations.—In general, concentrations of sodium ion ($[Na^+]$) were higher than those of potassium ion ($[K^+]$) at hatching. As chicks grew, $[Na^+]$ decreased whereas $[K^+]$ increased in all three species (Fig. 2). This pattern appears to be quite general in birds (Vernadakis and Woodbury 1964, Park et al. 1981). In leg muscle, $[K^+]$ of neonatal starlings and quail were significantly lower than that of bobwhite hatchlings (one-way ANOVA and Scheffé's test, $P < 0.05$). Differentiation of electrolytes during the first 16 days differed significantly among species (species effect; for pectoral $[K^+]$, $F = 17.06$, $df = 2$ and 43 , $P < 0.001$; for pectoral $[Na^+]$, $F = 6.06$, $df = 2$ and 43 , $P < 0.001$; for leg $[K^+]$, $F = 18.05$, $df = 2$ and 43 , $P < 0.001$; for leg $[Na^+]$, $F = 9.20$, $df = 2$ and 43 , $P < 0.001$), particularly in that $[K^+]$ increased more slowly in the altricial starling than in the precocial quail and bobwhite.

The pectoral muscles of starling chicks exhibited a steady increase in $[K^+]$ and a steady decrease in $[Na^+]$ up to the age of 16 days, with little change or even a reversal between 4 and 8 days (Fig. 2A). The changing patterns of these electrolytes were nearly the same for the leg muscles. During development, crossing of the $[K^+]$ and $[Na^+]$ curves was poorly defined but

occurred sometime between the ages of 4 and 8 days for pectoral muscle and between 2 and 8 days for leg muscle (Fig. 2A and 2B). Near-adult values occurred at 16 days for both electrolytes in pectoral and leg muscles in starling chicks (Table 1).

For quail chicks, there was a rapid increase in $[K^+]$ and a rapid decrease in $[Na^+]$ up to the age of 11 days in pectoral and leg muscles (Fig. 2C and 2D). The concentrations of these electrolytes exceeded the mature levels at around 11 days for pectoral and leg muscles (Table 1, Fig. 2C and 2D). For bobwhite chicks, the patterns of these changes were similar (Fig. 2E and 2F), although $[K^+]$ values for the leg muscles of older chicks (11 to 22 days) were markedly lower than those of chicks at 39 days (Fig. 2F). Concentration of K^+ and Na^+ reached near-adult levels at 8 to 11 days in the pectoral and leg muscles. The $[K^+]$ and $[Na^+]$ curves crossed in both the quail and bobwhite at about 5 days for pectoral muscle (Fig. 2C and 2E) and at 1 to 2 days for leg muscles (Fig. 2D and 2F).

DISCUSSION

VARIABILITY IN CHEMICAL MATURATION AND DEVELOPMENT

Among species.—Protein and electrolyte concentrations of muscle during postnatal development clearly distinguish altricial and precocial birds. Precocial quail and bobwhite ne-

TABLE 1. Mass, % lipid-free dry mass (LFDM), and $[K^+]$ expressed as % of values of adults or mature chicks (38 or 39 days) for the pectoral and leg muscles of European Starlings, Japanese Quail, and Northern Bobwhites.

Age (days)	Body mass	Pectoral muscle			Leg muscle		
		Mass	% LFDM	$[K^+]$	Mass	% LFDM	$[K^+]$
European Starling							
1	15	1	48	47	6	62	51
4	38	3	57	60	18	71	75
7	67	10	65	60	39	75	66
10	90	21	74	73	60	81	71
13 ^a	94	35	83	83	69	88	81
16	103	57	88	93	77	93	89
Adult	100	100	100	100	100	100	100
Actual ^b	68.0	13.1	0.28	121.2	5.6	0.26	114.6
Japanese Quail							
1	6	1	17	46	3	85	55
4	9	1	55	66	5	89	85
8 ^a	11	2	78	77	5	98	88
11	35	22	94	120	24	107	111
16	24	11	96	104	15	105	105
21	41	27	95	108	29	103	107
26	72	60	102	91	58	105	110
38	100	100	100	100	100	100	100
Actual	126.4	27.6	0.24	118.0	19.1	0.21	112.6
Northern Bobwhite							
1	7	1	53	56	4	81	60
5	13	1	58	63	8	82	65
8 ^a	16	3	78	90	10	97	75
11	23	9	87	96	15	104	84
15	25	11	82	91	17	103	83
22	40	27	94	98	30	98	74
39	100	100	100	100	100	100	100
Actual	96.3	19.7	0.26	136.2	14.6	0.23	140.0

^a Age of chemical maturation is 13 to 16 days in starling and 8 to 11 days in quail and bobwhite.

^b Actual mean values for adult (starling) or mature chick (quail and bobwhite) body mass (g) and mass (g), LFDM (%), and $[K^+]$ (mmol g⁻¹) of muscles.

onates have higher proportions of lipid-free dry mass and more advanced differentiation of electrolytes, particularly in leg muscles, compared with altricial starlings (Figs. 1 and 2). Electrolyte differentiation in the leg muscle (crossing of the $[K^+]$ and $[Na^+]$ curves and ages of chemical maturation; Fig. 2) occurred 4 to 6 days earlier, and at a much lower proportion of adult mass (Table 1), in quail and bobwhites than in starlings. These results are consistent with the better development of pedal locomotion in young quail and bobwhite than in starling chicks during the early growth period (Ricklefs and Webb 1985, Choi and Bakken 1991). In contrast, all three species develop flight at about the same age (Choi and Bakken 1991, Choi et al. 1993), and there was correspondingly little variation in the pattern of

chemical maturation in pectoral muscle (Fig. 1A, and curve-crossing of electrolyte changes in Fig. 2A, 2C, and 2E). The fact that chemical maturation of the pectoral muscle comes several days before the time of fledging or powered wing-flapping of the chicks may indicate that genetic regulation of the development of muscular function precedes the behavioral demands of the young birds (Choi and Bakken 1991, Choi et al. 1993). Why the proportion of LFDM is greater in muscles of adult starlings than in quail and bobwhite chicks at 38 to 39 days (Fig. 1A and 1B) is not clear. Possibly, proteins and enzymes associated with the citric acid cycle and oxidative phosphorylation are more concentrated in adult starlings, which have a higher oxidative catalytic capacity (relative to glycolytic capacity) of both pectoral

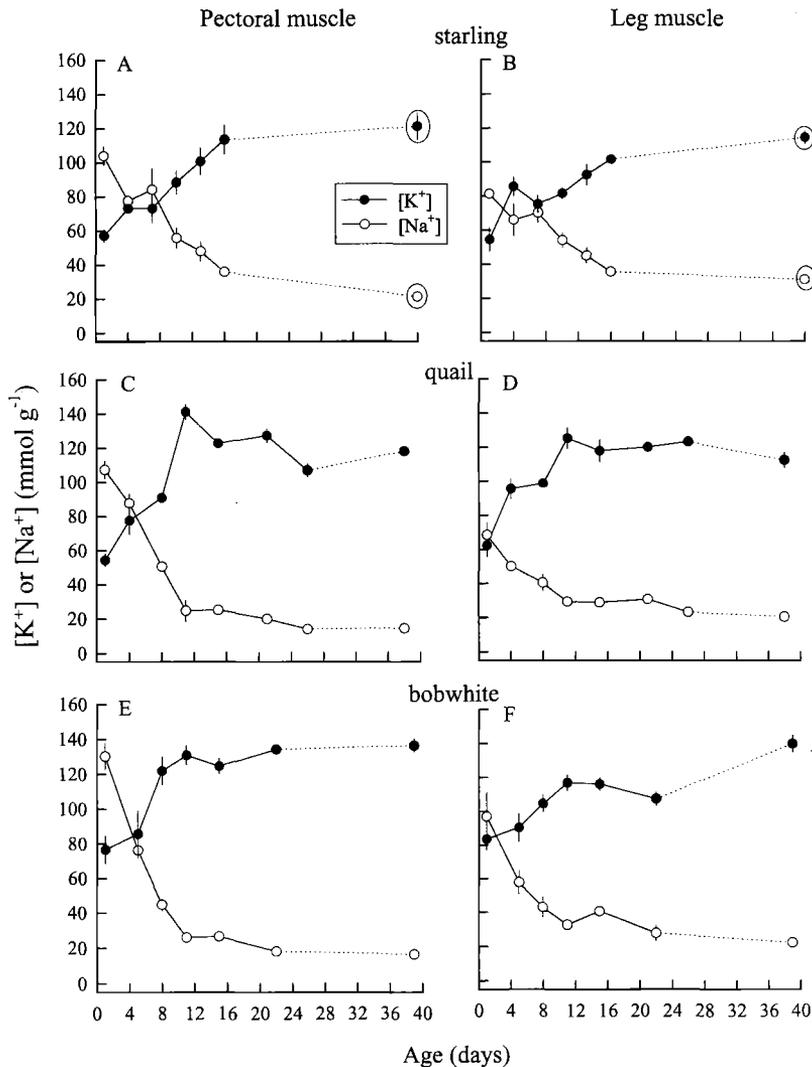


FIG. 2. Changes in concentration of potassium ($[K^+]$) and sodium ($[Na^+]$) ions for pectoral and leg muscles as a function of age of chicks in European Starlings (A, B), Japanese Quail (C, D), and Northern Bobwhites (E, F). Each data point is the average of two to six values; vertical lines are ± 1 SE.

and leg muscles than do quail or bobwhites (Choi et al. 1993).

Within species.—Chemical maturity was more advanced in leg muscle than in pectoral muscle for the early growth period of each species. Specifically, proportion of LFDM of the leg muscle was 3 to 5% higher at hatching and reached adult levels 3 to 7 days earlier than that of the pectoral muscle (Table 1, Fig. 1A and 1B). Electrolyte differentiation also began 2 to 4 days earlier in leg muscle than in pectoral muscle (Table 1, Fig. 2). These findings agree closely with general observations of the development

of avian pedal versus flight capability; i.e. the neonatal leg muscles function fairly well for begging in altricial birds and for walking and running in precocial chicks, whereas the pectoral muscles function poorly for the first several days or weeks of postnatal development (Ricklefs and Webb 1985; Choi and Bakken 1990, 1991).

Other species.—Previous studies on chemical maturation provide results comparable to those of our study. Moulton's early work (1923) on whole-body samples revealed that protein proportions (fat-free dry basis) in neonates

were 18% in cattle (relatively precocial); 12 to 14% in swine, dog, cat, rabbit, and human; and 10 to 11% in rat and mouse (relatively altricial). In rat hind-limb muscles, protein proportion increased progressively from 15 to 23% between days 5 and 45 of postnatal development (Park et al. 1981). This pattern of change is similar to that of starling leg muscle (Fig. 1). Protein proportions in the tissues of neonatal birds and mammals were correlated with the length of gestation period (or degree of precocity; Moulton 1923, Ricklefs 1983). For example, cattle have long gestation periods and are functionally well developed at birth, at which time they exhibit a high protein proportion. In contrast, neonatal rats and cats are poorly developed (altricial), and their tissues contain comparatively less protein (Moulton 1923). As noted above, we found that the proportion of LFDM in the leg muscles was slightly higher in quail and bobwhite hatchlings than in starling neonates.

Developmental patterns of change in ion concentrations are similar for most species of birds and mammals (Dickerson 1960, Dickerson and Widdowson 1960). In cockerel pectoral muscle, potassium levels increased from 46 mmole g^{-1} at hatching to 124 mmole g^{-1} at 15 days and 116 mmole g^{-1} at 32 days, whereas sodium levels decreased from 137 mmole g^{-1} at hatching to 19 mmole g^{-1} at 15 days and 20 mmole g^{-1} at 32 days (Dickerson 1960). These patterns for cockerel chicks were similar to those for our bobwhites at comparable ages, although the initial and mature values of $[K^+]$ in cockerels were about 60% and 87%, respectively, of those in bobwhites.

Concentrations of electrolytes at maturity differ between muscle fiber types; compared with white fibers (i.e. anaerobic, fast-twitch type), red fibers (i.e. aerobic, slow-twitch type) have lower $[K^+]$ and higher $[Na^+]$, resulting in a smaller ratio of $[K^+]$ to $[Na^+]$ (Sreter and Woo 1963, Luff and Goldspink 1970, Park et al. 1981). For instance, soleus (typically red fibers) of mature rats contained 98.1 mmole g^{-1} wet tissue of $[K^+]$ and 32.1 mmole g^{-1} of $[Na^+]$, and tibialis anterior (typically white fibers) contained 113.9 mmole g^{-1} of $[K^+]$ and 18.8 mmole g^{-1} of $[Na^+]$; hence, the ratio of $[K^+]$ to $[Na^+]$ is 3.06 for soleus and 6.06 for tibialis anterior (Sreter and Woo 1963). Previously, we found (Choi et al. 1993) that starling pectoral muscle

utilized primarily aerobic metabolism (a relatively high citrate synthase activity), whereas quail pectoral muscle emphasized anaerobic metabolism (a relatively high pyruvate kinase activity). The ratio of $[K^+]$ to $[Na^+]$ for the pectoral muscle examined from this study showed the same pattern as in other studies (Sreter and Woo 1963); the ratio was 3.6 for starlings (with more aerobic metabolism) and 5.5 to 6.3 for quail and bobwhite (with more anaerobic metabolism). The leg muscles of starlings, quail, and bobwhites showed a similar anaerobic activity at adult or mature ages (Choi et al. 1993), and the $[K^+]$ to $[Na^+]$ ratios of 5.7 for starling and 8.2 for quail and bobwhite compare well with values related to anaerobic metabolism.

MATURATION AND GROWTH RATE

Body mass of starling chicks increased 4 to 6 times faster than that of quail and bobwhite chicks during early postnatal development (Choi et al. 1993, Ricklefs et al. 1994). During this same period, masses of pectoral and leg muscles of starling chicks increased 2 to 3 times faster. These studies demonstrated an inverse relationship between exponential growth rate and functional maturity at the tissue level; exponential growth rate for both pectoral and leg muscles was negatively related to proportion of LFDM (contractile component) and $[K^+]$ (electrical potential).

In Table 1, we summarize masses, proportion of lipid-free mass, and $[K^+]$ in pectoral and leg muscles relative to those of adults (or old chicks) to compare the fraction of adult mass at which muscles achieve chemical maturation (i.e. least 90% of adult values). Quail and bobwhite chicks had reached only 11 to 35% of the body mass of 38- to 39-day-old chicks at the initiation of chemical maturation (8 to 11 days), whereas starling chicks had attained 94 to 100% of adult mass at chemical maturation (13 to 16 days). Masses of the pectoral and leg muscles in quail and bobwhites at chemical maturation were only about 2 to 25% of those of old chicks, compared with 35 to 77% of adult values in starlings. Thus, in terms of chemical maturity, 11-day-old quail and bobwhite chicks essentially are miniature adults with highly developed functional capacities. The slow growth of these species is consistent with a basic incompatibility between functional maturity and

embryonic development. As in other altricial birds, starling chicks achieve a rapid rate of growth during the first half of the nestling period when their body mass reaches the adult level, whereas chemical and other functional maturation is delayed until the end of nestling period (ca. 16 to 20 days).

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