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RESEARCH ARTICLE

Differential muscular myosin heavy chain expression of the pectoral and pelvic girdles during early growth in the king penguin (*Aptenodytes patagonicus*) chick

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SUMMARY

Continuous growth, associated with a steady parental food supply, is a general pattern in offspring development. So that young chicks can acquire their locomotor independence, this period is usually marked by a fast maturation of muscles, during which different myosin heavy chain (MyHC) isoforms are expressed. However, parental food provisioning may fluctuate seasonally, and offspring therefore face a challenge to ensure the necessary maturation of their tissues when energy is limited. To address this trade-off we investigated muscle maturation in both the pectoral and pelvic girdles of king penguin chicks. This species has an exceptionally long rearing period (1 year), which is prolonged when parental food provisioning is drastically reduced during the sub-Antarctic winter. Approximately 1 month post hatching, chicks acquire a functional pedestrian locomotion, which uses pelvic muscles, whereas swimming, which uses the pectoral muscles, only occurs 1 year later. We therefore tested the hypothesis that the MyHC content of the leg muscles reaches a mature state before those of the pectoral muscles. We found that leg muscle MyHC composition changed with the progressive acquisition of pedestrian locomotion, whereas pectoral muscle fibres reached their mature MyHC profile as early as hatching. Contrary to our predictions, the acquisition of the adult profile in pectoral muscles could be related to an early maturation of the contractile muscular proteins, presumably associated with early thermoregulatory capacities of chicks, necessary for survival in their cold environment. This differential maturation appears to reconcile both the locomotor and environmental constraints of king penguin chicks during growth.

Key words: development, bird, king penguin, skeletal muscle, myosin heavy chain.

INTRODUCTION

During growth, individuals have to develop capacities such as thermoregulation or locomotion in order to rapidly acquire their independence. A fast post-embryonic development is necessary, linked to the maturation of muscles and skeleton (Ricklefs, 1979; Olson, 2001; de Margerie et al., 2004). However, in birds, muscle growth rates differ between altricial and precocial species (Hohtola and Visser, 1998), in relation to variation in the degree of tissue maturation at hatching (Nice, 1962; Ricklefs, 1979; Starck and Ricklefs, 1998). In precocial species, the early development of the pelvic girdle and the delayed maturation of pectoral muscles allow young birds to perform pedestrian locomotion quickly after hatching but before acquiring flight capability (Starck and Ricklefs, 1998; Phillips and Hamer, 2000; Bennett, 2008). In contrast, offspring of altricial species are totally reliant on parental care and the leg and pectoral muscles are not well developed at hatching; they acquire terrestrial and aerial locomotion before fledging (Olson, 2001). As development and maturation of different organs and tissues are energetically costly, growth is generally associated with a steady parental food supply (Ricklefs, 1979). However, when the rearing period is long, parental food supply may show seasonal fluctuation (Cherel et al., 1993) and become a growth-limiting factor (Heath and Randall, 1985). Hence, during growth chicks may face periods of low food availability that can delay their development (Schew and Ricklefs, 1998). In this context, the specific strategies of energy allocation that are developed to ensure future survival and fledging of the offspring are not fully understood.

We addressed this question in the king penguin (*Aptenodytes patagonicus*) chick, a semi-altricial species showing an exceptionally long rearing cycle (~1 year) in the sub-Antarctic and interrupted by a period of severe food restriction during the two to three winter months (Stonehouse, 1960; Barrat, 1976). At hatching in summer, the chick totally depends on its parents for warmth and food. When 1 month old, thermogenic processes become sufficiently mature and chicks are then able to walk. Parents continue to forage intensively at sea to allow them to store sufficient body reserves before winter. From May to August, chicks mainly rely on their fat stores as an energy source and can lose half of their body mass and stop growing (Stonehouse, 1960; Barré, 1978). From September onwards, parental feeding rate increases allowing chicks to grow and moult before departing to sea and becoming independent (Barrat, 1976). The locomotor capacity and activity of the king penguin chick continuously changes throughout its growth until its independence. During the first weeks after hatching, chicks remain hidden in the parental brood patch and show limited locomotor activity. At 1 month old they use exclusively pedestrian locomotion. One year later, king penguin chicks switch from an exclusively terrestrial locomotion to a mixed terrestrial and aquatic locomotion. During their aquatic life, muscles of the pectoral girdle power the flippers,

which allows them to forage and dive to depths of 200–300 m (Barrat, 1976). In this way, the king penguin chick, with this distinctive growth cycle, is a useful model to investigate the impact of environmental conditions on skeletal muscle development.

In birds, each mode of locomotion (flapping flight, aquatic propulsion or terrestrial locomotion) and postural maintenance corresponds to different metabolic and contractile properties of muscles that are determined by their fibre types (Torrella et al., 1998). Fast-twitch oxidative-glycolytic (FOG) fibres provide a sustained rapid contraction, whereas fast-twitch glycolytic (FG) fibres contract more powerfully and fatigue rapidly (Sokoloff et al., 1998). In contrast, slow fibres are adapted for slow sustained contraction and are therefore numerous in postural muscles (Meyers and Mathias, 1997). In addition to the activities of their energy-generating enzymes, contractile properties of the muscle fibres also depend on their myosin heavy chain (MyHC) isoforms (Rosser et al., 1996). During muscular growth, a sequence of different MyHC isoforms is expressed in each avian fibre type (Bandman and Rosser, 2000) as shown in the FG fibres of gallineous birds such as the chicken (*Gallus gallus*) (Hofmann et al., 1988; Tidyman et al., 1997), the turkey (*Meleagris gallopavo*) (Maruyama et al., 1993) or the Japanese quail (*Coturnix japonica*) (Merrifield et al., 1989). Moreover, a differential expression of MyHC isoforms in the FOG and FG fibres during development of the pectoral muscle has been demonstrated in the domestic pigeon (*Columba livia*) (Rosser et al., 1998). Effective muscular contractions need an optimal myofibrillar ATPase activity, which has been related to myosin heavy chain composition (Rivero et al., 1996). Moreover, recent studies on king penguin chicks indicate that the developmental pattern of muscles and bones in the pectoral and pelvic limb are markedly different during the first weeks after hatching. High rates of periosteal bone tissue growth (de Margerie et al., 2004) and protein accretion (Erbrech et al., 2008) in the pelvic muscles allow nestlings to rapidly acquire an effective pedestrian locomotion that is essential for their survival. Conversely, the development of the pectoral girdle, which is required for aquatic locomotion, is delayed. Together with the acquisition of locomotion, muscular development has also been shown to be of a major importance in the ontogeny of thermogenic processes in penguin chicks (Duchamp et al., 2002).

In the present work, we therefore focused on muscle development during the first 2 months of growth of king penguin chicks, i.e. from hatching to the period when pedestrian locomotion and thermoregulation capacities are sufficiently developed. To validate the MyHC composition in king penguin muscles we first compared MyHC isoforms with those of the domestic chicken (*Gallus gallus*). Then, we tested the hypothesis that muscles of the pelvic girdle (gastrocnemius lateralis and iliotibialis cranialis) reach a mature state in their MyHC content before the pectoral girdle (pectoralis major), given that chicks acquire terrestrial locomotion far in advance of aquatic locomotion.

MATERIALS AND METHODS

Study area and specimens

Fieldwork was conducted in the Baie du Marin, Possession Island, Crozet Archipelago (46°26′S, 51°52′E; Indian Ocean) during the sub-Antarctic summer, from January to April 2006. Approximately 25,000 breeding pairs of king penguins are habituated to human presence because of its proximity to the Alfred Faure Scientific Station. The study protocol was approved by the French Ethic Committee of the Institut Polaire Paul-Emile Victor (IPEV) and by the Polar Environment Committee of the Terres Australes et Antarctiques Françaises (TAAF).

During daily surveys in the breeding colony, eggs with embryos close to hatching (N=4), chicks (N=25) and adult (N=5) king penguins (*Aptenodytes patagonicus* Miller, JF 1778) were collected immediately after being stolen and/or killed by predators [sub-Antarctic skuas (*Catharacta lonnbergi*) for eggs and chicks, or giant petrels (*Macronectes* sp.) for chicks and adults]. In a shelter close to the colony, embryos and birds were weighed to the nearest 0.1 or 1 g, depending on their absolute body mass. Within minutes after death, muscle samples from the pelvic and pectoral girdles were excised (~200 mg) and kept in crushed ice (less than 4 h) until myosin extraction. The length (accuracy ±0.5 mm) of the beak, foot and flipper was measured according to Stonehouse and the age of the embryo was determined from flipper length (Stonehouse, 1960). Chick age was estimated according to the appearance of the down body mass and behaviour (Verrier, 2003). Seven developmental stages were examined: six for chicks, and the adult (Table 1). To limit the impact of the experiments on the predator populations, the remaining parts of the carcasses were returned to the colony.

Muscle sampling

Three muscles were selected for the study: the pectoralis major (PM) from the pectoral girdle, which is involved in aquatic locomotion, the gastrocnemius lateralis (GL), and the iliotibialis cranialis (ITC) from the pelvic girdle, which is essential for pedestrian locomotion. The PM inserts on the deltoid crest of the humerus (George and Berger, 1966) and is recruited for the movement of flippers, allowing propulsion underwater. The ITC arises from the anterior iliac crest and inserts on the patellar ligament (George and Berger, 1966). It is recruited for hip flexion and knee extension (Smith et al., 2006). The GL takes has its origin on the proximal surface of the fibular condyle of the femur and ends on the most lateral part of the tendo achilis (George and Berger, 1966). This muscle is mainly recruited for ankle extension and knee flexion (Smith et al., 2006).

Myosin extraction and electrophoresis of MyHC analysis

Each muscle sample was weighed (±0.1 mg) and myosin was extracted in a specific high ionic strength buffer according to the method of D'Albis et al. (D'Albis et al., 1979). The extracts were kept at –80°C until analysis. Protein concentrations in the extracts were determined using the method described by Bradford (Bradford, 1976). Isoform separations were performed according to the method of Talmadge and Roy (Talmadge and Roy, 1993). The stacking and separating gels were respectively composed of 4% and 8% acrylamide-*N*,*N*′-methylene-bis-acrylamide (bis; 50:1). Mini-gels (0.75 mm thickness) were used in the Bio-Rad (Hercules, CA, USA) Mini-protean II Dual Slab Cell. Electrophoresis were carried out at a constant 70 V for 28 h in a cold room (+4°C). The amount of protein run on the gel was approximately 5 µg of total protein per lane. The gels were stained with Coomassie Blue R-250. The relative amounts of the different MyHC isoforms were measured using a Bio-Rad GS-800 integration densitometer, and the results analysed with the Quantity one 4.2.1 Program (Hercules, CA, USA). Only bands representing more than 1% of total MyHC were taken into account. Moreover, muscle samples obtained from an adult chicken (*Gallus gallus*) were used to compare the composition in MyHC isoforms of this species with that of adult king penguins, and to validate our extraction procedure.

Statistical analysis

Results are given as means ± s.e.m. Multiple comparisons were made using non-parametric Kruskal–Wallis ANOVAs, followed by

Table 1. Muscle sampling schedule of king penguin chicks in relation to age, thermoregulatory capacity, locomotor activity and plumage (following Barrat, 1976; Stonehouse, 1960; Verrier, 2003)

Developmental stage	Age	Body mass range	Thermoregulatory capacity	Locomotor activity	Plumage
Chick					
Stage A	Embryonic <1 week before hatching	125–160 g			Unfeathered
Stage B	Post hatching 1–3 days	<300 g	Heterotherm activity	Brooding phase: no locomotor	Unfeathered
Stage C	6–7 days	300–500 g	Acquisition of homeothermy	Brooding phase: no locomotor activity	Brown down
Stage D	7–15 days	0.5–1 kg	Homeotherm	Chicks sitting upright in front of their parent	Brown down
Stage E	3–4 weeks	1–2 kg	Homeotherm	End of brooding phase: chicks begin to move away from adults	Brown down
Stage F	1–2 months	2–3 kg	Homeotherm	Terrestrial locomotion: emancipated chicks wander alone on the colony	Brown down
Adult	>4 years	10–12 kg	Homeotherm	Aquatic locomotion: adults forage at sea and come on land to moult and breed	Feathers

Dunn's *post hoc* tests. Relative percentages were analysed after transformation to arcsin square roots. All statistical analyses were carried out using Statview 5.0. Statistical significance was set at $P<0.05$.

RESULTS

Validation of the extraction procedure

Pectoralis major

A single MyHC isoform was detected in the PM from both the domestic chicken and the adult king penguin (Fig. 1). However, the PM isoform of the king penguin had a lower mobility than that of the domestic chicken.

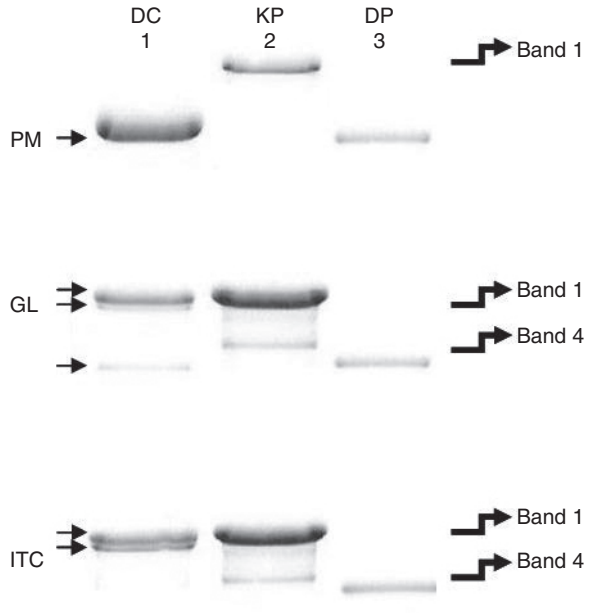

Fig. 1. Electrophoretic mobilities of myosin heavy chain (MyHC) isoforms found in pectoralis major (PM), gastrocnemius lateralis (GL) and iliotibialis cranialis (ITC) in the domestic chicken (DC, lane 1) and the king penguin (KP, lane 2). Lane 3 (DP) shows the mobility of porcine MyHC of a standard molecular mass (200 kDa) (Laemmli, 1970).

Gastrocnemius lateralis

The GL of the adult king penguin contained two MyHC isoforms (Fig. 1, bands 1 and 4), whereas three isoforms were detected in the domestic chicken. The slowest moving isoform in the two species had similar electrophoretic mobilities whereas the other MyHC isoforms all had different mobilities.

Iliotibialis cranialis

Two MyHC isoforms were present in the adult king penguin (Fig. 1, bands 1 and 4) and in the domestic chicken ITC samples. The slowest moving isoform in the two species had similar electrophoretic mobilities, but the second isoform from the chicken had a lower mobility than band 4 in the king penguin.

These results of isoform separations from PM and GL muscles in the domestic chicken are in accordance with previous studies that used immunoblotting and gene expression analyses (Hofmann et al., 1988; Tidyman et al., 1997), therefore validating our extraction protocol.

Differential foot and flipper growth in king penguin chicks

The foot and flipper lengths of king penguin chicks significantly increased during the first two months post hatching ($P\leq0.001$; Fig. 2). From stages A to F (within the first week of growth), they increased from 44.1±1.9 and 49.4±0.4 mm to 117.5±4.5 and 117.6±7.8 mm, respectively. In emancipated chicks (stage F), feet and flippers had reached 76.2% and 43.1% of their adult size, respectively.

Developmental expression of MyHC isoforms in the pelvic and pectoral girdles of king penguin chicks

A total of six different MyHC isoforms could be detected (Fig. 3). They were labelled as bands 1–6 according to their electrophoretic mobilities: bands 1 and 6 were, respectively, the slowest and the fastest migrating isoforms. However, only four MyHC isoforms (bands 1, 3, 4 and 6) that were more than 1% of the total MyHC, could be detected by the densitometric analysis. We therefore focused on these four isoforms (Fig. 3, Table 2).

Developmental expression of MyHCs in PM

The band 1 isoform was present in PM at growth stages A to F and in adults (Fig. 3). The band 3 isoform was only detected (5.6±1.7%) at stage E (3- to 4-week-old chicks; Table 2).

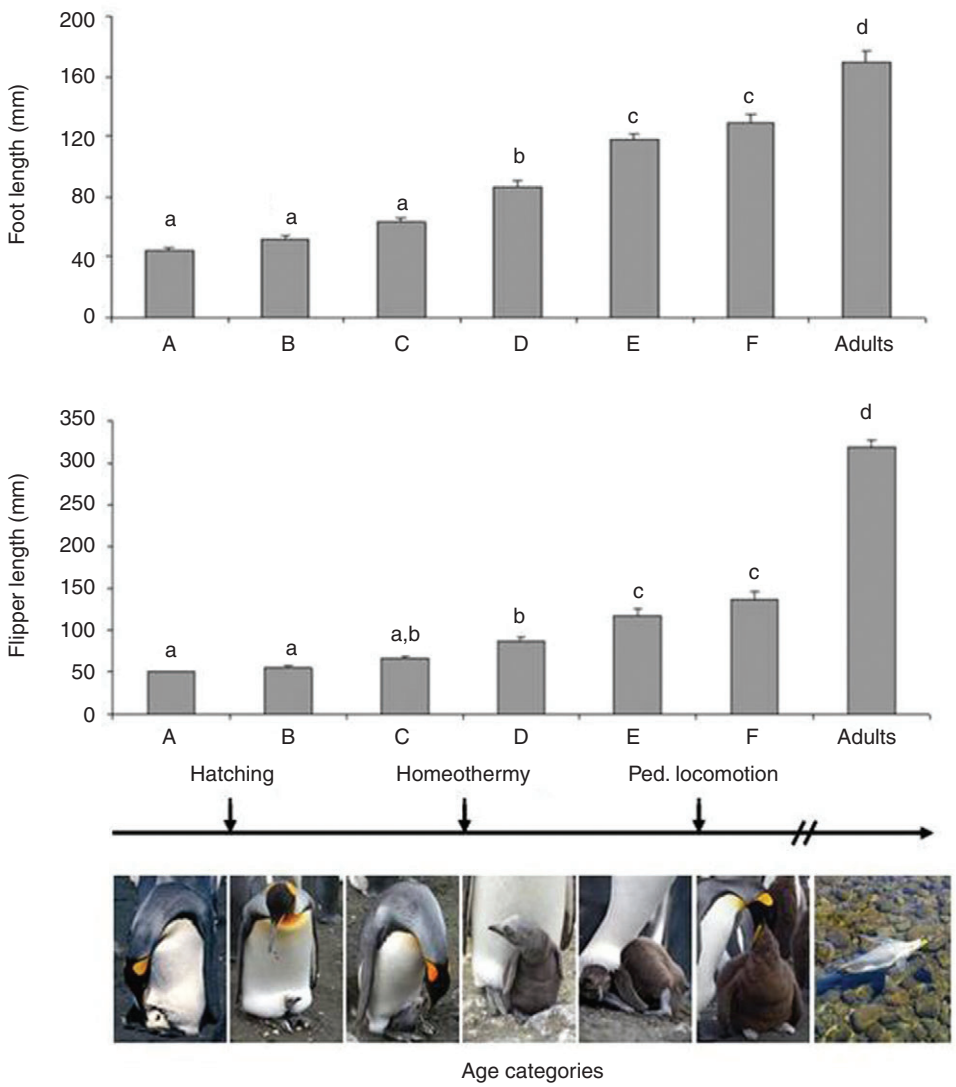

Fig. 2. Mean lengths (mm; ± s.e.m.) of the foot and flipper of chicks for the different age categories (A, <1 week before hatching; B, 1–3 days; C, 6–7 days; D, 7–15 days; E, 3–4 weeks; F, 1–2 months) and of adults. Different lowercase letters above the bars indicate significant differences between categories (P<0.05).

Developmental expression of MyHCs in GL

From stage A to D (up to 2-week-old chicks), the expression of three isoforms (bands 1, 4 and 6) was observed in the GL muscle (Fig. 3). Band 1 was the predominant isoform and its relative percentage (67–76%) did not vary significantly within this period (P=0.100; Table 2). The relative percentages of bands 4 and 6 were lower than band 1, and did not change significantly from stage A to C (P=0.087 and 0.350, respectively). At stage D (1- to 2-week-old chicks), the expression of the band 4 isoform was similar to that at stage A, whereas band 6 had decreased significantly (P=0.001; Fig. 3, Table 2).

At stages E and F (3- to 4-week-old and 1- to 2-month-old chicks), only bands 1 and 4 were detected. Their relative percentages were similar to those of the adult group (P>0.195). However, when the chicks were 1 to 2 months old, the band 1 isoform increased and reached its highest level (92.9±2.5%), but the variations in band 4 were not significantly different.

Developmental expression of MyHC isoforms in ITC

At stages A and B (1 week before hatching and 3 days post hatching), three MyHC isoforms (bands 1, 4 and 6) were detected in the ITC muscle (Fig. 3). Band 1 was the predominant isoform (~72%), while the relative proportions of bands 4 and 6 were lower (12–16%; Table 2).

At stage C (6 to 7 days old), the band 3 was detected (16.2±4.7%; Fig. 3, Table 2). The relative contribution of bands 1 and 4 were not significantly different from the previous two stages (P>0.118), but band 6 decreased significantly, by 1.6-fold in stage C compared with stages A and B (P=0.002; Table 2).

From stages D to F (1 week to 2 months old), bands 1 and 3 did not change significantly (P>0.359) whereas bands 4 and 6 disappeared (Table 2).

In the adult group, the contribution of band 1 did not vary significantly compared with the chick groups (P=0.380). Band 4 was present in adults but its expression (16.9±0.7%) was not significantly different from stages A–C (up to 1-week-old chicks; Table 2).

DISCUSSION

During the early life of king penguin chicks, their feet and flippers increased in size and reached respectively 76.2% and 43.1% of their average adult size at 2 months of age. At the end of the first period of growth, feet practically reached their adult size, whereas flippers reach their adult length later, at the end of the fledging period (Cherel et al., 2004). In parallel, it has been shown (de Margerie et al., 2004) that the pelvic girdle of king penguin chicks has the highest bone tissue growth rate during the first month after hatching. The fast structural development of the lower limb thus allows chicks to

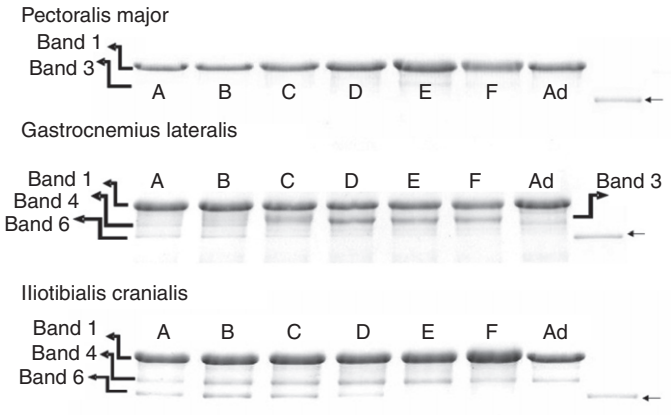

Fig. 3. Myosin heavy chain isoforms of the three different muscles: pectoralis major, gastrocnemius lateralis and iliotibialis cranialis, of king penguin chicks at different ages and adults identified by SDS-PAGE. A, <1 week before hatching; B, 1–3 days; C, 6–7 days; D, 7–15 days; E, 3–4 weeks; F, 1–2 months; Ad, adults. Small arrows indicate porcine myosin heavy chain of a standard molecular mass (200 kDa) (Laemmli, 1970).

rapidly acquire a bipedal posture and pedestrian locomotion (Verrier, 2003). The survival of chicks at the beginning of emancipation is indeed linked to their ability to escape predators and to chase their parents for food when they return from foraging trips.

Considering the PM, two isoforms could be detected from hatching to the full emancipation of king penguin chicks. Band 1 was the predominant isoform at each stage, whereas band 3 only appeared with a low relative percentage in chicks aged 3 to 4 weeks old. These results differ from those of the domestic chicken, in which five MyHC isoforms have been detected in the developing pectoral muscle (Tidyman et al., 1997): three embryonic MyHC isoforms are supplanted after hatching by a neonatal isoform that is in turn replaced by an adult isoform. In the domestic chicken, the PM has been reported to contain almost all FG fibres (Rosser et al., 1996); whereas only FOG fibres were found in king penguin chicks PM (A.E., R.G. and J.-P.R., unpublished data). As these two fibre types are linked to the expression of different MyHC isoforms during development (Rosser et al., 1996) the variation in MyHC content between chicken and king penguins could result from differences in the composition of the fibre types in this muscle. Surprisingly, muscle fibres of the PM of king penguin chicks expressed a MyHC profile similar to adults as early as hatching. This result reveals an early maturation of the PM contractile proteins, although in king penguin chicks of 3 to 4 weeks old, the cross-sectional area of the fast-twitch fibres is 30 times lower than in adults (Erbrech et al., 2008). These results are in contrast to those from the domestic pigeon (Rosser et al., 1998), in which PM fibres reach their adult MyHC composition and adult size after fledging. Because fibre size is a major determinant for the production of the mechanical force essential to locomotor activity (Olson, 2001), we suggest that despite the PM of king penguin chicks expressing a mature MyHC profile, its muscle fibres have not yet acquired the morphological characteristics necessary for the development of an efficient locomotor activity. Thus, in king penguin chicks, the delayed development of PM fibres compared with those of the leg muscles

Table 2. Percentage distribution of myosin heavy chain (MyHC) isoforms in three muscles (pectoralis major, gastrocnemius lateralis, iliotibialis cranialis) of king penguin chicks from hatching to emancipation

		MyHC isoforms				
Muscle	Developmental stage	Band 1	Band 3	Band 4	Band 6	N
Pectoralis major						
	Stage A	100.0	ND	ND	ND	4
	Stage B	100.0	ND	ND	ND	5
	Stage C	100.0	ND	ND	ND	2
	Stage D*	100.0	ND	ND	ND	7
	Stage E	94.4±1.7	5.6±1.7	ND	ND	6
	Stage F†	100.0	ND	ND	ND	4
	Adults	100.0	ND	ND	ND	5
Gastrocnemius lateralis						
	Stage A	67.0±3.5^{a}	ND	15.9±1.5^{a}	17.1±2.1^{a}	4
	Stage B	72.5±1.5^{a,b}	ND	14.0±0.7^{a}	13.5±1.1^{a,b}	5
	Stage C	67.1±1.9^{a}	ND	18.3±1.0^{a}	14.6±1.2^{a,b}	3
	Stage D*	75.6±2.6^{a,b}	ND	17.4±1.1^{a}	7.1±1.8^{b}	7
	Stage E	84.9±2.4^{b,c}	ND	15.1±2.4^{a}	ND	5
	Stage F†	92.9±2.5^{c}	ND	7.1±2.5^{a}	ND	4
	Adults	90.0±4.2^{c}	ND	10.0±4.2^{a}	ND	5
Iliotibialis cranialis						
	Stage A	71.9±5.1^{a}	ND	12.4±4.4^{a}	15.7±1.3^{a}	4
	Stage B	72.5±1.6^{a}	ND	15.0±1.0^{a}	12.5±0.7^{a}	4
	Stage C	69.5±2.1^{a}	16.2±4.7^{a}	6.4±3.2^{a}	7.9±0.3^{b}	3
	Stage D*	69.9±6.1^{a}	30.1±6.1^{a}	ND	ND	7
	Stage E	77.5±3.5^{a}	22.5±3.5^{a}	ND	ND	6
	Stage F†	76.4±1.8^{a}	23.6±1.8^{a}	ND	ND	4
	Adults	83.1±0.7^{a}	ND	16.9±0.7^{a}	ND	4

Stages: A, <1 week before hatching; B, 1–3 days; C, 6–7 days; D, 7–15 days; E, 3–4 weeks; F, 1–2 months.
*Start of homeothermy; †start of pedestrian locomotion.
ND: not detected.
Values are mean percentages of total myosin heavy chain, ± s.e.m.
For a given muscle and MyHC band, different letters indicate significant differences between groups (P<0.05).

(Erbrech et al., 2008) is not linked to the MyHC isoform type content.

In the pelvic girdle, we revealed a MyHC polymorphism in the GL and ITC muscles of king penguin chicks, from hatching to approximately 2 weeks of age. The composition of MyHC isoforms differentially changed in the GL and ITC with the progressive acquisition of terrestrial locomotion, in agreement with the fact that contractile activity is essential for the maturation of avian skeletal muscle fibres (Bandman and Rosser, 2000). Changes in the activity of muscle fibres are indeed linked to the expression of myosin isoforms, which can be delayed or induced as a function of the intensity of muscle activation (Salmons and Sreter, 1976; Brown et al., 1983; Cerny and Bandman, 1987). In the ITC muscle, band 4 and 6 isoforms disappeared and were replaced by band 3 isoform when the chick was 7 to 15 days old, corresponding to the time when chicks are emerging from the brood patch and are able to stand in front of their parents (Stonehouse, 1960; Barrat, 1976; Verrier, 2003) (personal observations). In the GL muscle, the proportion of the band 6 isoform decreased slightly until it disappeared at approximately 3 weeks of age, when chicks are starting to walk actively. Since the principal function of the ITC is to protract the femur (Torrella et al., 1998), this muscle should be recruited early to maintain a bipedal posture and to support the chick's body mass. In contrast, the GL muscle, which is involved in ankle extension and knee flexion, is essentially recruited for bipedal locomotion (Smith et al., 2006). At the time of emancipation (3–4 weeks), the GL muscle appeared to be already mature, containing similar MyHC isoforms to adults. In the contrast, the ITC muscle had not reached this state at the end of the brooding period: band 3 isoform, present in the emancipated chick, was replaced by the band 4 isoform in adults. This switch can possibly be explained by the less intensive use of this muscle in adults, in relation to their marine life. During the chick-rearing period, young birds possess an exclusively terrestrial locomotion and therefore use their ITC muscles more intensively than adults that spend 75% of their time at sea, alternating travels at sea and sojourns on land to moult and breed (Stonehouse, 1960; Barrat, 1976). However, to test this hypothesis, it would be necessary to evaluate the MyHC isoform composition in older chicks, particularly at the end of the second growth period, at approximately 1 year of age.

In addition to these mechanical factors being a possible explanation for the changes in myosin profiles, hormonal and thermal factors are also likely to be involved. Thyroid hormones (T3 and T4) in particular have been shown to induce changes in MyHCs during the development of muscle fibres (Maruyama et al., 1993; Gardahaut et al., 1992). In the domestic turkey, Maruyama et al. showed that the increase in thyroxine (T4) level in the plasma could support the transition from embryonic to neonatal MyHCs during the development of the breast muscle (Maruyama et al., 1993). Thus, in king penguin chicks, the progressive disappearance of the band 6 isoform in the GL muscle, the disappearance of band 6 and 4 isoforms, followed by the appearance of band 3 isoform in the ITC muscle, together with the detection of band 3 isoform in the PM muscle, may be related to the increase in T4 plasma level also observed by Cherel et al. in the same species during early growth (Cherel et al., 2004). Moreover, at hatching, king penguin chicks are essentially heterothermic, but the rapid improvement of thermoregulatory processes and thermal insulation during the first 2–3 weeks of life allows them to acquire thermal emancipation (Duchamp et al., 2002). Changes in plasma T4 levels (the major thermogenesis regulating hormone) also correspond to the period when chicks gain independent thermoregulation. Thermoregulatory capacity is crucial for chicks' survival during the sub-Antarctic winter when weather conditions deteriorate and parental food supply is restricted. Studies undertaken by Duchamp et al. on GL and PM muscles indicate that muscular shivering is the main thermogenic mechanism in growing chicks (Duchamp et al., 2002). Production of heat by repetitive muscular contraction therefore requires a rapid maturation of skeletal muscles. The development of endothermy in young birds requires the maturation of the neuromuscular system, an increased muscular oxidative capacity, as well as the development of myofibrillar ATPase in muscle fibres (Hohtola and Visser, 1998). Moreover, myofibrillar ATPase activity is linked to myosin heavy chain composition (Rivero et al., 1996). In the pectoral muscle of the domestic chicken, the contraction velocity of embryonic fast MyHCs were shown to be lower than the neonatal isoform (Lowey et al., 1993). In this context, changes in MyHC content occurring in king penguin chick leg muscles could be related to the progressive acquisition of homeothermy from their second week of life. Moreover, Duchamp et al. showed that shivering in king penguin chick PM, assessed by integrated electromyographic activity, occurred immediately after hatching, even though thermal insulation was not fully developed (Duchamp et al., 2002). The MyHC adult profile of the PM of young chicks could therefore indicate an early maturation of the contractile muscle proteins that would allow shivering as early as hatching. Furthermore, this muscle is known to be the major source of shivering and non-shivering thermogenesis in adults (Duchamp et al., 1989).

In order to rapidly acquire an effective pedestrian locomotion essential to their survival, early in their growth, chicks invest energy in the development of the pelvic girdle at the expense of the pectoral girdle (de Margerie et al., 2004; Erbrech et al., 2008). Considering the immature fibres size (Erbrech et al., 2008) and the mature MyHC content (this study) of the PM in young chicks, we also suggest that the PM is essential for thermoregulatory functions at this stage of development, whereas their locomotor function will be developed several months later before departing to sea. Together, these results from king penguin chicks illustrate the trade-off between muscle growth rate and functional capacity, as suggested for several bird species (reviewed by Krijgsveld et al., 2001). At the end of these first weeks of growth, king penguin chicks have acquired a functional pedestrian locomotion and are thermally emancipated. However, their growth will still last several months when they have to face a period of severe and prolonged under-nutrition during the sub-Antarctic winter, before finally departing to sea where they can forage independently. Throughout their winter fast, together with their locomotor and thermoregulatory functions, skeletal muscles may act as important protein reserves. One major task in future studies should therefore be to investigate the effect of this winter energy restriction on muscle development (fibre size and types, MyHC content) in both the pelvic and pectoral girdles when chicks are fully grown.

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