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STRUCTURAL AND METABOLIC CHARACTERIZATION OF THE MUSCLES USED TO POWER RUNNING IN THE EMU (*DROMAIUS NOVAEHOLLANDIAE*), A GIANT FLIGHTLESS BIRD

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Summary

The emu is a giant flightless bird, capable of sustained high-speed running. Anatomical, histochemical and biochemical properties of the lower leg muscles used to power running were investigated.

The gastrocnemius is the largest muscle in the emu leg. It has a short inelastic tendon and contains only fast fibres. It is the major power-producing muscle of the lower leg, with a greater capacity than the digital flexor muscles for bursts of high work output. In marked contrast, the digital flexors have long elastic tendons and contain both fast and slow muscle fibres. It is proposed that these muscles, rather than the gastrocnemius, are responsible for maintaining posture and that they facilitate elastic energy storage and retrieval in their tendons during running.

In comparison with equivalent muscles of flying and diving birds, emu lower leg muscles display features consistent with greater power output during both short burst and endurance running. The emu muscles are more massive relative to body size, and the gastrocnemii of other birds invariably contain slow fibres

This study illustrates some of the similarities as well as differences between muscles used during flying and running. Capacities for sustained high-energy work appear to be similar in flying birds and running emus as judged from (1) the muscle masses used during locomotion when expressed as a proportion of total body mass and (2) muscle fibre type compositions and their potential for fuel catabolism. The lower creatine kinase activity in emu leg muscles could be attributed to higher energy demands during the initial stages of lift-off for flight.

Introduction

Flying is the dominant form of locomotion for most birds, and the pectoralis and supracoracoideus, which power flight, display specializations associated with different flying behaviours (George and Berger, 1966; Pages and Planas, 1983; Rosser and George,

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1986; Rosser *et al.* 1987; Lundgren and Kiessling, 1988). At the structural and metabolic level these muscles range from predominantly anaerobic in the chicken (Patak and Baldwin, 1988) to highly aerobic in hummingbirds (Suarez *et al.* 1986). However, the muscles powering running in birds have received little attention. Differences between the structural and metabolic organization of muscles powering running and flying might be predicted on the following grounds: (a) the locomotory muscles of running birds have an important role in maintaining posture, whereas those used for flapping flight generally do not; (b) minimizing body mass, and therefore muscle mass, is more important in flying than in running. This latter point is illustrated by the observation that the largest flying birds are considerably smaller than the largest flightless birds.

The emu provides an extreme example of avian cursorial locomotion. The high aerobic capacity of the leg muscles is demonstrated by the ability of this bird to elevate oxygen consumption 11.4-fold above resting values during treadmill running (Grubb *et al.* 1983), an aerobic scope approaching that of flying pigeons (George and Berger, 1966; Brackenbury, 1984). We have investigated anatomical, histochemical and metabolic properties of major muscles used to power running in the emu and used these data to address the following questions. (1) How are the emu muscles used during locomotion? (2) How specialized are emu leg muscles relative to the leg muscles of flying birds? (3) Are differences between running and flying reflected in the structural and metabolic organization of emu leg muscles and the flight muscles of other birds?

Materials and methods

Collection of samples

Adult wild emus [*Dromaius novaehollandiae* (Latham): Aves: Struthioniformes: Dromaiidae] of both sexes (21–44kg) were shot in the field in Western Victoria, Australia, during a programme for the control of animals causing agricultural damage. Histochemical samples were taken from both superficial and deep parts of the widest portion of each muscle. Muscle blocks measuring $3mm^3 \times 3\,mm^3 \times 10mm^3$ were submerged for 10–30s in melting isopentane precooled in liquid nitrogen. Muscle samples for biochemical analysis were taken from the centre of the muscle in its widest part and freeze-clamped in liquid nitrogen. For anatomical studies, the weights of each muscle and of the whole bird were determined routinely. Three complete legs were dissected and approximately 14 lower legs were examined in less detail. The whole limbs had been frozen, whereas the lower legs were dissected fresh.

Histochemical methods

Preparation of sections

Transverse serial sections of frozen muscle blocks were cut in a cryostat (Reichert-Jung Cryocut E). Sections 8fm thick were picked up on warm (24˚C) subbed slides, and were air-dried for 1h before staining.

Sections were stained for myosin ATPase (mATPase) by a method modified from Doriguzzi *et al.* (1983) and Gollnick *et al.* (1983). Control sections, stained with

5 mmol l^{-1} sodium azide added to the incubation buffer, demonstrated that staining was not due to mitochondrial activity (Talesara and Goldspink, 1978; Gollnick *et al.* 1983).

For alkaline mATPase, sections were preincubated for 10min at 24°C in 0.1mol l^{-1} 2-amino-2-methyl-1-propanol (AMpro) buffer, pH10.3, containing 18mmol l^{-1} calcium chloride. This was followed by incubation for 5–30min at 37°C in 0.1mol l^{-1} AMpro buffer, pH9.4, containing 18mmol l^{-1} calcium chloride, 50mmol l^{-1} potassium chloride and 4mmol l^{-1} ATP. The sections were then rinsed with distilled water, stained in fresh 1 % (w/v) Azure-A for 10s, rinsed again, quickly dehydrated in graded ethanol, cleared in Histoclear and mounted in Euparal.

For acid mATPase, sections were preincubated for 2.5–3.5min at 15°C in 0.1mol l^{-1} acetate buffer pH4.2 or pH4.3, containing 18mmol l^{-1} calcium chloride and 50mmol l^{-1} potassium chloride. Incubation and staining was as for alkaline mATPase.

The method of Dubowitz and Brooke (1973) was used to stain sections for NADH–tetrazolium reductase (NADH-TR). Other sections were stained for phosphorylase a activity using Meijer's method (Pearse, 1972).

Absorbance measurements

Transverse sections stained for mATPase, NADH-TR and phosphorylase were analysed by measuring the absorbance of individual fibres using a Leitz Orthoplan microscope, fitted with a modified Leitz MPV-1 microscope photometer tube. An EMI multiplier no. 6904A acted as the photomultiplier, with an ultrastabilized high-voltage supply (Knott-Electronic, Munich, Germany). The current produced by the photomultiplier was measured with a Fluke 8000A digital multimeter. A stabilized Osram 150W xenon high-pressure lamp supplied the light, which was passed through a Leitz in-line monochromator and then through a 20/0.33 condensor before reaching the muscle section. The measurement area was adjusted with a variable diaphragm and ranged from 0.3×10^{-3} mm^2 to 1.1×10^{-3} mm^2 at 12.5× objective magnification.

Sections stained for mATPase and phosphorylase were measured using the two-wavelength method (Patau, 1952; Mendelsohn, 1966). Absorption of muscle fibres at a single wavelength was measured at 590nm for NADH-TR, with a 0.3×10^{-3} mm^2 measuring area positioned in the centre of the fibre (Swatland, 1984). Optical density was calculated with the Beer–Lambert law.

Fibre size

Fibre cross-sectional areas obtained from photographic negatives taken from transverse sections stained for mATPase, pH4.3, were measured using a Radio Shack TRS-80 digitizer on a Nikon profile projector (V-12 Nippon Kogaku Japan) interfaced with a SME Z-80 computer.

Analysis

Data were analysed using clustering methods (SPSS-X, Norusis, 1985). For a full account of the analysis, see Patak (1988).

Biochemical assays

Distribution of lactate dehydrogenase isoenzymes

Muscle was homogenized in ice-cold 40mmol l^{-1} sodium phosphate buffer, pH6.6 (1:9 w/v), and centrifuged at 17000 ***g*** for 30min at 4˚C. The supernatant was applied to cellulose acetate gel (Cellogel, Chemtron), which had been soaked for 20min in 50mmol l^{-1} Tris–EDTA–borate buffer, pH8.7. The gel was run in 100mmol l^{-1} Tris–EDTA–borate buffer, pH8.7, at 300V for 30min at 24˚C and stained for LDH activity (Markert and Ursprung, 1962). The gel was fixed in 5% acetic acid, and the relative proportions of the five possible isoenzymes were determined by densitometry (Gelman DCD16 digital computing densitometer).

Pyruvate inhibition of lactate dehydrogenase

Pyruvate inhibition ratios were determined at 25˚C as described by Wilson *et al.* (1963). The reaction mixtures contained 0.15mmol l^{-1} NADH, in 100mmol l^{-1} sodium phosphate buffer pH7.5, with 0.33 or 10mmol l^{-1} pyruvate. For comparison, the emu LDH homopolymers, LDH M_4 and LDH H_4, purified by affinity chromatography on oxamate-Sepharose as described by Yancey and Somero (1978), were included.

Myoglobin concentrations

Muscle myoglobin was determined spectrophotometrically by the method of Reynafarje (1963).

pH buffering capacities

Muscle pH buffering capacity (β) was determined as described by Castellini and Somero (1981).

Maximum activitPies of enzymes

Muscle samples were cleaned free of fat and connective tissue, weighed, minced with scissors and homogenized in ice-cold buffer (1:9 w/v) with an IKA-Ultra-Turrax. For all enzymes with the exception of phosphofructokinase (PFK), the homogenizing buffer contained 50mmol l^{-1} potassium chloride, 7mmol l^{-1} magnesium chloride and 5 mmol l^{-1} ethylenediaminetetraacetic acid (EDTA) in 50mmol l^{-1} imidazole–HCl buffer, pH7.4. The homogenizing buffer for PFK contained 5mmol l^{-1} magnesium sulphate, 5mmol l^{-1} dithioerithritol (DTT) and 1mmol l^{-1} EDTA in 50mmol l^{-1} Tris–HCl, pH8.2. The homogenate was sonicated and centrifuged at 3000 ***g*** for 20min at 4˚C and the supernatant was assayed immediately in triplicate. Phosphorylase (EC 2.4.1.1), hexokinase (EC 2.7.1.1), 6-phosphofructokinase (EC 2.7.1.11), pyruvate kinase (EC 2.7.1.40), lactate dehydrogenase (EC 1.1.1.27), 3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35), citrate (*si*)-synthase (EC 4.1.3.7) and malate dehydrogenase (EC 1.1.1.37) were assayed by the methods of Patak and Baldwin (1988). The reaction mixture for creatine kinase (EC 2.7.3.2) was 33mmol l^{-1} creatine, 4mmol l^{-1} ATP, 4mmol l^{-1} magnesium chloride, 5mmol l^{-1} phosphoenolpyruvate, 0.15mmol l^{-1} NADH and excess pyruvate kinase and lactate dehydrogenase in 100mmol l^{-1} glycine–NaOH, pH9.0.

Enzymes were assayed with a Unicam SP1800 recording spectrophotometer in which the cuvette temperature was maintained at 39°C with a circulating waterbath.

Statistical methods

F_{max} tests were used to assess the homogeneity of the data. Subject to favourable results from the F_{max} test, one-way analysis of variance was performed, which, if significant ($P<0.05$), was followed by Newman–Keuls tests (Dunnett, 1980).

Results

Myology

The muscles examined in this study are described in some detail, because adequate descriptions are not readily available in the literature (Gadow, 1880; Sudilovskaya, 1931; Patterson, 1983).

The anatomical arrangement of the lower leg muscles examined in this study is shown in Fig. 1, and muscle masses are given as a proportion of body mass in Table 1. The gastrocnemius is the largest and most superficial of the ankle extensors. Its bellies originate separately from the femur, knee joint and tibiotarsus and join to insert by a single, strong, short tendon on the tarsometatarsus.

The digital flexor muscles are much smaller than the gastrocnemius, and are closely associated with each other and with the gastrocnemius. As in other birds that lack the hallux, the tendon of insertion of the flexor digitorum longus muscle joins with that of the flexor hallucis longus. The joint tendon inserts on all three toes. The tendons of insertion of the digital flexor muscles are much longer than the gastrocnemius tendon.

The anatomy of the pelvic limb muscles is described in detail in Patterson (1983) and Patak (1988).

Table 1. *Masses of emu lower leg muscles expressed as a proportion of total body mass*

Muscle	Mass ($g100\,g^{-1}$ bodymass)
g lateralis	1.24±0.13
g caudalis and g int medialis	0.32±0.12
g medialis	1.56±0.18
fl pp III	0.18±0.04
fl p III	0.27±0.05
fl p IV	0.17±0.05
fl dig long	0.26±0.06

Values are expressed as the mean ± standard deviation. Sample size: 10–13 individual emus.

The g int caudalis and medialis were weighed together. Emu body masses ranged from 21 to 44kg. The muscles did not show a significant allometric scaling regression over this range of body masses.

Muscle abbreviations: g lateralis, gastrocnemius lateralis; g int caudalis, gastrocnemius intermedia caudalis; g int medialis, gastrocnemius intermedia medialis; g medialis, gastrocnemius medialis; fl pp III, flexor perforans et perforatus digiti III; fl p III, flexor perforans digiti III; fl p IV, flexor perforans digiti IV; fldig long, flexor digitorum longus.

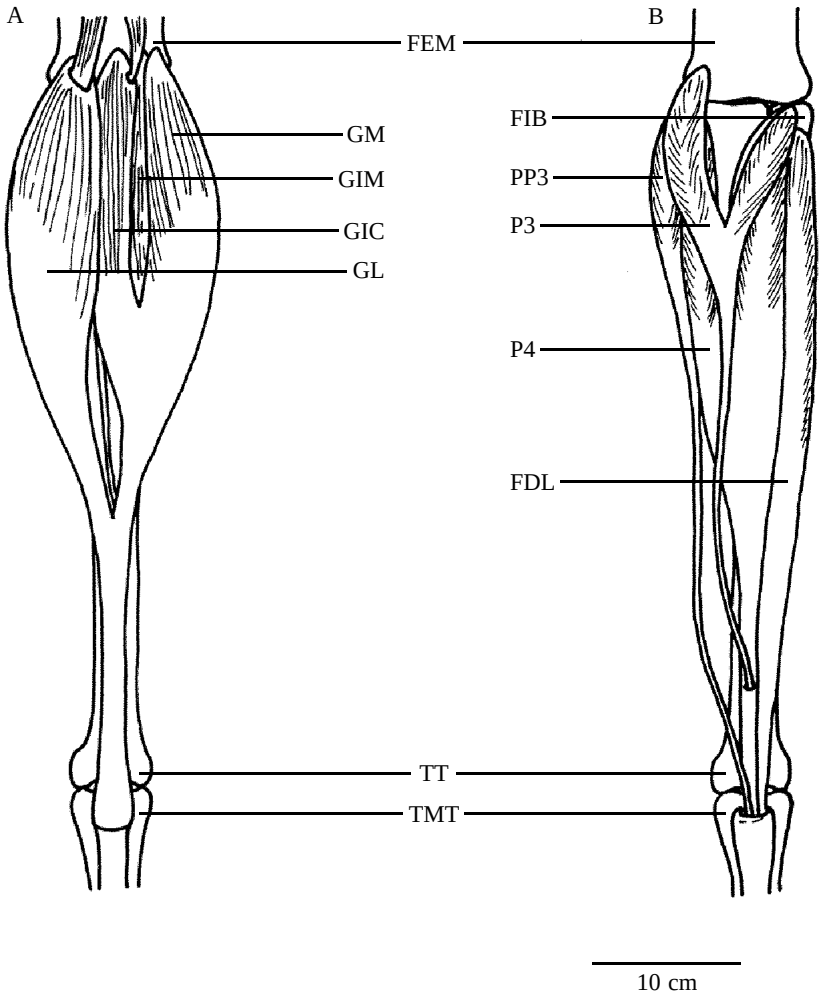


Fig. 1. Schematic diagram of the caudal view of the left leg of the emu, showing the muscles studied. (A) Gastrocnemius; (B) digital flexor muscles. FEM, femur; TT, tibiotarsus; FIB, fibula; TMT, tarsometatarsus; GL, gastrocnemius lateralis; GIC, gastrocnemis intermedia caudalis; GIM, gastrocnemius intermedia medialis; GM, gastrocnemius medialis; PP3, flexor perforans et perforatus digiti III; P3, flexor perforatus digiti III; P4, flexor perforatus digiti IV; FDL, flexor digitorum longus.

Muscle histochemistry

Using histochemical techniques, metabolic and mechanical characteristics of muscle fibre types can be determined. Muscle fibres differ in contraction speed, aerobic and anaerobic capacity and size. The contraction speed of muscle fibres depends on the activity of their myosin ATPase (mATPase), and fibres differ in mATPase isoenzyme composition. Staining muscle sections for mATPase visualizes differences in mATPase isoenzyme composition and, therefore, differences in contraction speed among fibres (Staron *et al.* 1987). The metabolic capacity of muscle fibres was displayed by staining

Table 2. *Fibre type distribution in fast fibre muscles*

Muscle	Fibre type (%) FO	FFG
g lateralis	45±15.3	55
g int caudalis	45±5.6	55
g int medialis	42±15.8	58
g medialis	28±8.2	72

Values are means of 5–6 muscle biopsies ± standard deviations. The biopsies were taken from a total of three emus.

Fibre type abbreviations are: FO, fast-oxidative-small; FFG, fastest-glycolytic-large.

Muscle abbreviations as for Table 1.

for phosphorylase (indicative of glycolysis) and NADH–tetrazolium reductase (NADH-TR, aerobic metabolism), and the cross-sectional area of muscle fibres was also measured.

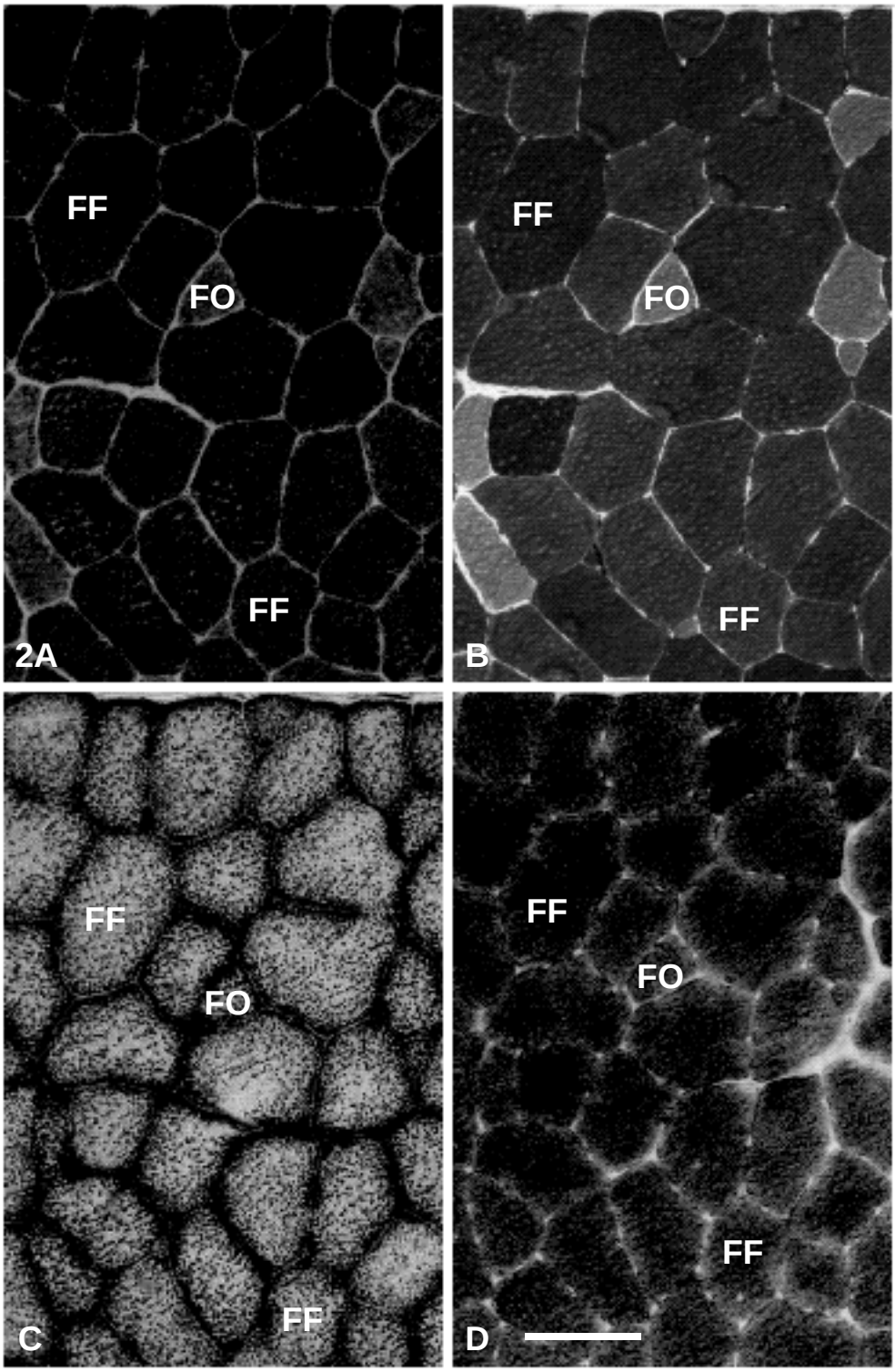
Examples of the range of staining patterns obtained in emu lower leg muscles are shown in Figs 2 and 3. Fibre types were not easily distinguished (see Fig. 2A,B). As has also been noted in various other birds and also in mammals, the fibres appeared to be continuously variable (Eisenberg and Kuda, 1977; Armstrong, 1980; Hintz *et al.* 1980; Staron *et al.* 1983; Essen-Gustavsson and Henriksson, 1984; Rosser *et al.* 1987). A method was therefore developed to assess each fibre objectively by measuring its absorbance in a semiquantitative way, and then using clustering analysis to group fibres into several types (see Patak, 1988).

The lower leg muscles were separated into two groups on the basis of mATPase staining reactions. The gastrocnemius was classed as a ‘fast’ muscle, as it had only fast fibres (Fig. 2). The digital flexor muscles were classed as ‘mixed’, as they possessed both fast and slow fibres (Fig. 3).

Fibres found in fast muscle were subdivided into fast-oxidative-small (FO) and fastest-glycolytic-large (FFG). Both fibre types were present in all biopsies, most often in approximately equal proportions (Table 2). In samples where one fibre type predominated, this was FFG. All mixed muscles, in contrast, had slow-oxidative (SO) as well as fast-glycolytic (FG) fibres (Table 3). SO fibres tended to make up about one-third of the fibre population of mixed muscles. In slightly less than half the muscle samples, an additional type of fast fibre was present, fastest-glycolytic [FFG(O)]. The fast fibre types from mixed muscles differed from those of fast muscles, in that their oxidative and glycolytic capacities did not vary consistently with mATPase staining.

Muscle biochemistry

The following biochemical variables were assessed in the emu lower leg muscles, and the results are presented as indicated: relative proportion of lactate dehydrogenase (LDH) M subunits, LDH pyruvate inhibition ratio, myoglobin content and intracellular pH buffering capacity (Table 4); maximum activities of enzymes associated with aerobic


FF
FO
FF
2A
FF
FO
FF
B
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FF
C
FF
FO
FF
D

Fig. 2. Staining patterns of serial sections of gastrocnemis medialis. (A) Alkaline mATPase; (B) acid mATPase; (C) NADH–tetrazolium reductase; (D) phosphorylase. Scale bar, 0.1mm. FF, fastest-glycolytic-large; FO, fastest-oxidative-small. In this biopsy the glycolytic capacities of the fibre types were equal.

ATP production (Table 5); maximum activities of enzymes associated with anaerobic ATP production (Table 6).

In comparing the variables associated with aerobic muscle work, the overall impression is one of great similarity among the lower leg muscles. Significant differences were not found in the activities of hexokinase, citrate synthase, 3-hydroxyacyl-CoA dehydrogenase or malate dehydrogenase, nor in myoglobin content.

With the anaerobic indices, creatine kinase activity was similar in all muscles, but the activities of glycolytic enzymes (phosphorylase, phosphofructokinase, pyruvate kinase, lactate dehydrogenase), intracellular pH buffering capacities, LDH M subunit percentages and pyruvate inhibition ratios consistently implied somewhat higher anaerobic capacities for the gastrocnemius and flexor digitorum longus than for the three flexor digiti muscles.

Discussion

Functions of the emu lower leg muscles during locomotion

The emu leg has about 39 separate muscle bellies which, in a 22kg emu, range in mass from a few grams to nearly 800g (Patak, 1988). Observations of running birds show that the more distal segments of the leg move through the greatest arc (Muybridge, 1957; Alexander *et al.* 1979). Thus, most of the work is done on the tarsometatarsus, or foot. This points to the muscles of the lower leg rather than the thigh being of major importance in power production, as it is the former which act on the tarsometatarsus. Although the ratio of the lower leg muscles to the thigh muscles is 0.8:1.0 in the emu (Patak, 1988), many thigh muscles are used in abduction or adduction of the leg, rather than in producing forward propulsion. In the lower leg the majority of the musculature is located posteriorly in the calf, which is composed of the gastrocnemius and digital flexors that act to extend the foot and flex the toes. The gastrocnemius spans two joints and potentially can extend the tarsometatarsus while keeping the knee at a constant angle. The digital flexor muscles are much smaller, with a combined mass about one-third that of the gastrocnemius (Patak, 1988). The former muscles all insert onto the toes and, therefore, can flex not only the toes but extend the tarsometatarsus as well. As their origins are closely associated with each other and with the gastrocnemius, all these muscles potentially could work in unison.

The energy output of locomotory muscles increases as animals progress from standing to walking, running and sprinting. Sequential recruitment of fibre types and activation of ATP-generating pathways are linked to these different exercise intensities (Hochachka and Somero, 1984; Armstrong and Laughlin, 1985; Vollestad and Blom, 1985). Muscle design features, such as fibre orientation and tendon properties, fibre type distribution and

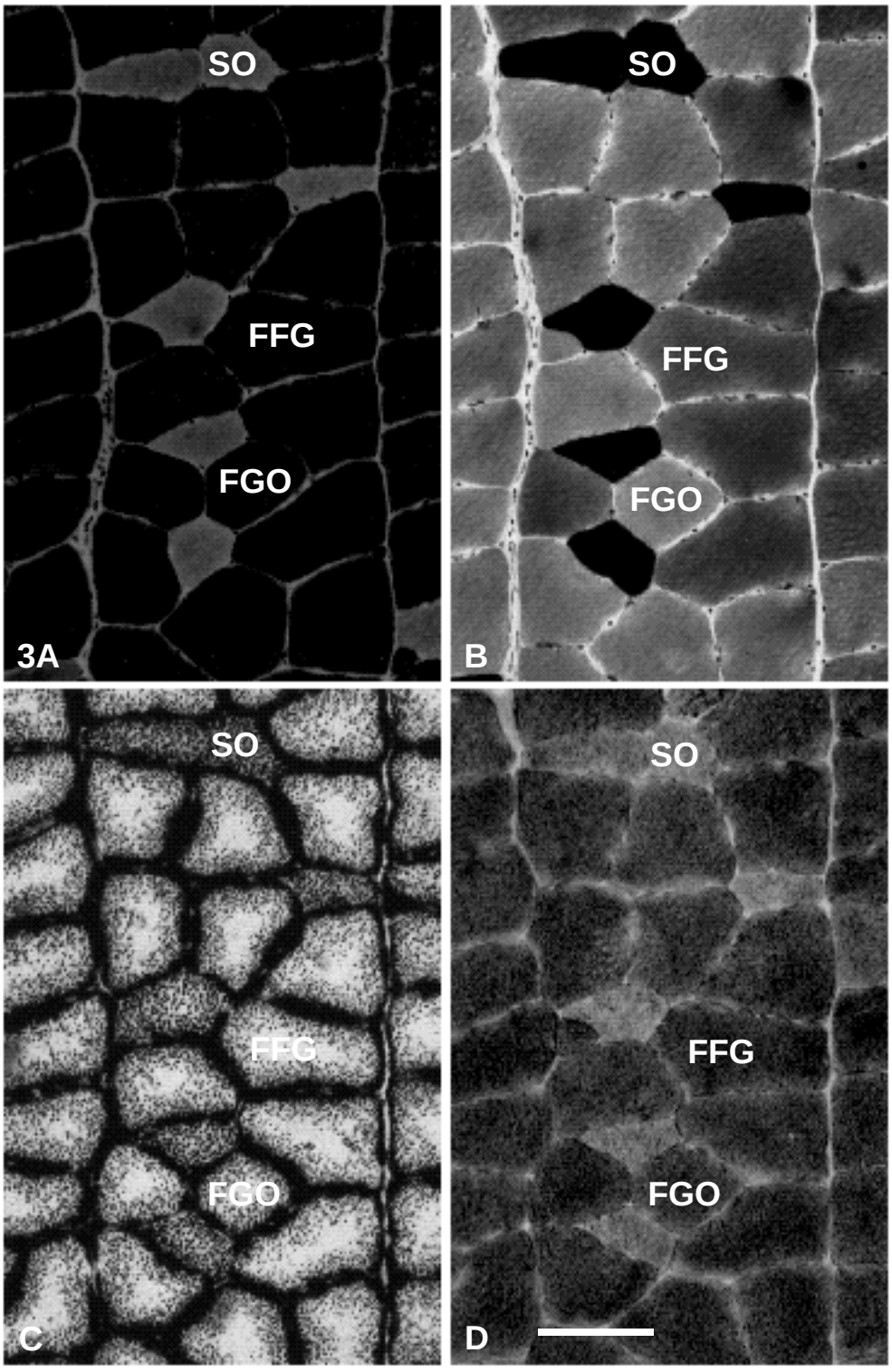
SO
FFG
FGO
3A
SO
FFG
FGO
B
SO
FFG
FGO
C
SO
FFG
FGO
D

Fig. 3. Staining patterns of serial sections of flexor digitorum longus. A–D and scale as in Fig. 2. FFG, fastest-glycolytic-large; FGO, fast with intermediate glycolytic and oxidative capacities and medium size; SO, slow-oxidative-small.

Table 3. *Fibre type distribution in mixed fibre muscles*

Muscle	*N*	Fibre type (%)		
		SO	FG(O)	FFG(O)
fl pp III	5	30±9.0	70	–
	1	38	34	28
fl p III	3	37±10.8	63	–
	3	30±4.6	29±13.1	41±8.7
fl p IV	5	37±7.7	63	–
	7	33±6.9	40±21.3	27±16.5
fl dig long	1	29	71	–
	3	19±2.3	41±8.7	40±8.5
	1	29	71	–

Values are means ± standard deviations and *N* is the number of biopsies examined. The biopsies were taken from a total of three emus.

Fibre type abbreviations are: SO, slow-oxidative; FG(O), fast-glycolytic-variable level of oxidative capacity; FFG(O), fastest-glycolytic-variable level of oxidative capacity.

Muscle abbreviations as for Table 1.

Table 4. *Lactate dehydrogenase (LDH) subunit content and pyruvate inhibition ratios, myoglobin content and pH buffering capacity (β) for emu lower leg muscles*

Muscle	LDH M subunits (%)	LDH pyruvate inhibition ratio	Myoglobin ($mg\,g^{-1}$ wetmass)	Buffering capacity, β ($\mu molpHunit^{-1}\,g^{-1}$)
g lateralis	96.2	0.76	8.1	49.9
	(88.6–100.0)	(0.74–0.77)	(5.6–10.8)	(49.3–50.9)
g int caudalis	100.0	0.77	8.7	49.6
	(–)	(0.75–0.80)	(4.9–12.9)	(43.3–53.0)
g int medialis	100.0	0.87	9.3	50.8
	(–)	(0.79–0.96)	(5.5–11.6)	(47.1–53.1)
g medialis	91.9	0.82	9.6	50.0
	(75.6–100.0)	(0.78–0.86)	(4.7–13.3)	(47.0–51.5)
fl pp III	92.1	0.83	9.4	43.6
	(76.2–100.0)	(0.78–0.92)	(6.1–11.9)	(37.7–47.8)
fl p III	73.3	0.96	9.1	44.2
	(48.9–90.5)	(0.81–1.10)	(5.8–12.1)	(41.7–47.5)
fl p IV	74.3	0.96	7.5	39.5
	(49.1–89.3)	(0.71–1.17)	(5.0–10.9)	(37.9–40.4)
fl dig long	88.9	0.72	11.0	50.6
	(76.4–100.0)	(0.69–0.73)	(7.4–15.0)	(49.9–51.2)
LDH M_4	100	0.65	–	–
LDH H_4	0	1.61	–	–

Values are means of three emus with ranges in brackets.

Muscle abbreviations as for Table 1.

Table 5. *Maximum activities of enzymes associated with aerobic ATP production in emu lower leg muscles*

	Enzyme activities (μmolsubstrate g^{-1} wetmassmin^{-1} at 39°C)			
Muscle	HAD	CS	MDH	HK
g lateralis	6.6	82.0	1094	0.79
	(2.9–9.2)	(69.5–91.5)	(919–1343)	(0.61–0.98)
g int caudalis	7.4	83.8	1153	0.42
	(3.9–11.6)	(69.5–109.1)	(1004–1251)	(0.24–0.77)
g int medialis	8.7	83.8	1334	0.70
	(6.3–10.6)	(43.0–126.8)	(1216–1529)	(0.49–0.83)
g medialis	5.3	58.0	1081	0.62
	(3.4–6.8)	(50.7–62.8)	(911–1235)	(0.53–0.71)
fl pp III	6.9	74.2	1374	0.67
	(5.7–7.7)	(51.8–92.6)	(1143–1498)	(0.53–0.85)
fl p III	6.6	61.0	1316	0.55
	(4.3–10.6)	(38.6–100.3)	(1004–1575)	(0.51–0.58)
fl p IV	7.1	58.4	1039	0.59
	(4.8–9.2)	(36.4–87.1)	(1004–1062)	(0.31–0.77)
fl dig long	7.7	77.2	1404	0.60
	(4.8–9.6)	(65.0–98.1)	(1312–1559)	(0.55–0.67)

Enzyme activities are means for three animals, with ranges in brackets.

Enzyme abbreviations are: HAD, 3-hydroxyacyl-CoA dehydrogenase; CS, citrate (*si*)-synthase; MDH, malate dehydrogenase; HK, hexokinase.

Muscle abbreviations as for Table 1.

biochemical variables, can be used to deduce conditions under which a particular muscle is designed to operate (Walmsley and Proske, 1981).

Most skeletal muscles have a heterogeneous population of fibres. At low work loads, slow-contracting aerobic fibres tend to be activated. As work load increases, faster-contracting aerobic fibres are recruited, and at the highest work loads, the fastest-contracting anaerobic fibres are activated (Armstrong and Laughlin, 1985; Vollestad and Blom, 1985). This arrangement enables a muscle with heterogeneous fibres to perform different types of work. In fact, it has been suggested that fibre composition is the most important muscle design factor in determining exercise performance (Parkhouse, 1988).

The fibre types found in emu muscle, SO and fast fibres ranging from FO to FFG, are similar to those found in the locomotory muscles of other birds (George and Berger, 1966; Talesara and Goldspink, 1978; Rosser *et al.* 1987; Butler, 1991).

In all terrestrial vertebrates, maintenance of posture is considered a major function of slow fibres (Simpson, 1979). In addition to their postural function, mammalian slow-oxidative fibres remain active during all types of exercise (Walmsley *et al.* 1978; Armstrong and Laughlin, 1985). If this also applies to birds, then the slow-oxidative fibres in the digital flexor muscles of the emu have a very important function in addition to maintenance of posture, namely in facilitating elastic energy storage in the tendons during locomotion. Slow fibres tend to have a greater short-range stiffness than fast fibres, possibly because they have a lower cross-bridge cycling rate (Proske and Rack, 1976).

Table 6. *Maximum activities of enzymes associated with anaerobic ATP production in emu lower leg muscles*

	Enzyme activities (μmolsubstrate g^{-1} wetmassmin^{-1} at 39°C)				
Muscle	Pase	PFK	PK	LDH	CrK
g lateralis	58.7	49.9	1608	2436	131.5
	(43.4–82.0)	(36.2–62.7)	(1423–1809)	(1978–2798)	(107.1–174.6)
g int caudalis	56.2*	48.7	1616	2243	143.8
	(47.8, 64.6)	(35.0–60.3)	(1495–1713)	(2002–2605)	(111.0–186.2)
g int medialis	27.5	35.8	1520*	2183*	110.6
	(15.9–39.1)	(25.3–48.2)	(1471, 1568)	(2098, 2267)	(104.2–114.6)
g medialis	49.0	83.2	1504	1809	102.6
	(37.1–69.0)	(36.2–111.0)	(1303–1688)	(1471–2098)	(65.6–170.8)
fl pp III	34.6	30.9	760*	1480	115.1
	(27.5–46.3)	(14.2–47.0)	(675, 844)	(1110–2123)	(92.6–157.3)
fl p III	30.9	23.7	852	1270	85.2
	(16.9–55.5)	(20.5–28.9)	(651–1013)	(892–2002)	(50.2–134.1)
fl p IV	25.9	15.7	828	917	84.0
	(19.3–32.8)	(12.1–21.7)	(675–917)	(772–1134)	(55.0–111.0)
fl dig long	45.0	59.9	1439	1737	110.0
	(31.8–63.7)	(51.9–66.3)	(1182–1737)	(1471–2171)	(100.3–121.6)

Enzyme activities are means for three animals, with ranges in brackets except for values marked with an asterisk, which are means of two animals with individual activities in brackets.

Enzyme abbreviations are:Pase, phosphorylase; PFK, 6-phosphofructokinase; PK, pyruvate kinase; LDH, lactate dehydrogenase; CrK, creatine kinase.

Muscle abbreviations as for Table 1.

This greater stiffness enables slow fibres to counter disturbing forces more effectively than do fast fibres. Thus, more of the stretch during locomotion goes into the tendons, which are storage sites for elastic energy. The presence of slow-oxidative fibres in the digital flexor muscles of the emu would therefore increase savings in muscle energy expenditure by facilitating elastic energy storage and retrieval in the very long tendons of these muscles during running.

The relative size and structure of the gastrocnemius indicates that this muscle contributes most of the propulsion generated by the lower leg during locomotion. It is devoid of slow fibres, with FFG and FO fibres being present in approximately equal numbers. Thus, the gastrocnemius is assumed to make little contribution to postural maintenance. As fast fibres are generally recruited only at higher exercise levels, the gastrocnemius appears to be specialized for powering high-speed running and fast acceleration such as occurs during rapid take-off. This is consistent with the presence of a short inelastic tendon giving efficient energy transfer from the contracting muscle to the limb. The biochemical data imply that while its ability for submaximal sustained aerobic work during running is similar on a weight basis to that of the other calf muscles, the gastrocnemius is better suited for short bursts of maximal anaerobic work during rapid acceleration.

Although many of the data suggest considerable functional separation of the gastrocnemius and the digital flexor muscles, there is some evidence that they can act

together to some extent. In addition to the muscle origins being closely associated, the biochemical profile of the flexor digitorum longus is more similar to that of the gastrocnemius than to that of the other digital flexor muscles.

Comparisons between emu leg muscles and the leg muscles of other birds

Muscle mass when expressed as a proportion of total body mass provides a useful index of the relative contributions of avian muscles during locomotion (Hartman, 1961), and the extreme cursoriality of the emu is highlighted when such comparisons are made between the leg muscles of emus and of other birds. The pelvic limb muscles of the emu constitute 25% of total body mass (Patak, 1988), which is higher than the 2–20 % reported by Hartman (1961) for the equivalent muscles of 70 families of flying, swimming and diving birds. Interestingly, the emu may be exceeded only by the ostrich, a much larger flightless bird with a leg muscle to body mass value of 29% (Alexander *et al.* 1979).

Comparisons of the fibre type distributions of leg muscles also serve to emphasize the emu's specialization for high-speed running. Unlike the emu, at least parts of the gastrocnemius, in addition to the digital flexors, of flying, swimming and diving birds possess both slow-oxidative and fast fibres (Kruger and Gunther, 1958; Cracraft, 1971; Talesara and Goldspink, 1978; McGowan, 1979; Narang and Talesara, 1979; Maier, 1981; Barnard *et al.* 1982; Rosser and George, 1986; Turner and Butler, 1988). Consequently, any postural role the gastrocnemius has in these birds is greatly reduced or even absent in the emu. Conversely, the presence of only fast fibres in the emu gastrocnemius implies greater specialization for power output during locomotion.

With the exception of myoglobin, biochemical comparisons between the leg muscles of the emu and other birds are limited by a lack of comparable data. The myoglobin content of the emu lower leg muscles exceeds that of most flying birds (1–5mg g^{-1} wetmass; Pages and Planas, 1983), with the exception of some species that use their legs for swimming and diving (6–8mg g^{-1} wetmass; Davis and Guderley, 1987; Turner and Butler, 1988). The leg muscles of the emu can therefore maintain high rates of aerobic metabolism to power locomotion.

Comparisons between emu leg muscles and muscles used to power bird flight

During exercise, emus display an aerobic scope similar to those of flying birds (Grubb *et al.* 1983; Brackenbury, 1984; Butler, 1991), and when the masses of muscles used to power locomotion are expressed as a percentage of total body mass, comparable values are obtained for flight muscles and emu leg muscles (10–35% and 25% respectively; Hartman, 1961; Patak, 1988). The fibre compositions of the emu gastrocnemius, which provides most of the propulsion, and the pectoralis of flying birds also are similar in lacking slow fibres (Talesara and Goldspink, 1978; Rosser and George, 1986; Butler, 1991). In addition, the biochemical correlates of aerobic and anaerobic muscle work in the emu lower leg muscles are intermediate between those of the flight muscles of chickens and hummingbirds, which are extreme examples of high-power output anaerobic burst and aerobic endurance flight, respectively (Smith *et al.* 1985; Suarez *et al.* 1986; Johansen *et al.* 1987; Patak and Baldwin, 1988).

In contrast to their ability to generate energy from glycolysis and respiration, the creatine kinase activity of the muscles of the emu is orders of magnitude lower than that of the pectoralis of the chicken [1247 μmol g^{-1} wet mass min^{-1} at 25°C, Smith *et al.* (1985)] and hummingbird [2848 μmol g^{-1} wet mass min^{-1} at 39°C, Suarez *et al.* (1986)]. The lower assay temperature in the chicken effectively increases the difference even more (possibly threefold), as all enzymes in emu muscles were measured at the physiological temperature of 39°C. Thus, although emus and flying birds have (a) a similar scope for activity, (b) similar muscle masses used during locomotion relative to body mass, (c) a similar fibre type composition and (d) a similar ability to generate ATP *via* catabolism of fuels, the muscles of the emu have a much lower capacity for phosphagen hydrolysis. This difference may be explained by differences in energy demand during the initial stages of take-off between terrestrial and aerial locomotion. Such a difference in power production is supported by the presence of slow-oxidative fibres in some of the lower leg muscles of runners such as the emu, whereas the pectoralis of most flying birds contains only fast fibres (Rosser and George, 1986; Butler, 1991).

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