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MYOFIBRE TYPES IN EIGHT SKELETAL MUSCLES FROM THE EASTERN GREY KANGAROO (*MACROPUS GIGANTEUS*)

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SUMMARY

A selection of muscles from male Eastern Grey kangaroos was assessed for myofibre type. Myofibres were classified as slow-oxidative (type I), fast-oxidative-glycolytic (type IIA), fast-glycolytic (type IIB) and intermediate types (IIC and IIAB) based on staining with monoclonal antibodies specific to myosin heavy chain isoforms. Classification using these antibodies was validated with myofibrillar adenosine triphosphatase, nicotinamide adenosine dehydrogenase, and ∝-glycerophosphate dehydrogenase staining. The most abundant myofibre type in the muscles studied was type IIB-fast-glycolytic, with the exception of *M. psaos minor*. Staining characteristics for myofibres were similar to other mammalian species apart from type IIA, which did not react with the type II (fast) antibody used in this study.

Keywords: myofibres, muscle fibre type, muscle, macropod

Abbreviations: MHC, myosin heavy chain; mAbs, monoclonal antibodies; mATPase, myofibrillar adenosine triphosphatase; NADH, nicotinamide adenosine dehydrogenase; GPD, ∝-glycerophosphate dehydrogenase

INTRODUCTION

Skeletal muscle is an extremely heterogeneous tissue composed of a variety of fast and slow fibre types and subtypes. Muscle fibres can be classified according to their metabolic, contractile and colour properties and there exists an array of classification schemes and nomenclature. According to Pette and Staron (2000), the most informative methods to delineate muscle fibre types are based on specific myosin profiles, especially the MHC isoform complement. The classification of kangaroo muscle has been previously carried out by Dennington and Baldwin (1988) and Zhong *et al*. (2001) using histochemical techniques and mAbs, respectively. Collectively, these papers classified the *M. tibialis cranialis* and the major extensor muscles of the macropod tibio-tarsal articulation.

In the present study a selection of antibodies against MHC isoforms typically used to study muscle in livestock species was chosen to facilitate the classification of kangaroo muscle fibres as type I, IIC, IIA, IIAB and IIB. These findings were validated using mATPase, NADH, and GPD staining. Six hindquarter muscles (*M. gluteus medius, M. vastus lateralis, M. biceps femoris, M. adductor, M. semitendinosus, M. semimembranosus*), one tail muscle (*M. sacrocaudalis dorsalis lateralis*), and one supportive lumbar muscle (*M. psaos minor*), were examined. These muscles were studied because of their potential economic importance for meat production.

MATERIALS AND METHODS

Animals, muscles and sample preparations

Five male Eastern Grey kangaroos were field harvested between 23:00 and 02:00h over two nights using an accredited commercial kangaroo harvestor. The dressed weight (bodyweight minus the contents of the gastrointestinal tract) ranged from 16 to 30kg, with a mean (±SD) weight of 23±5kg. Eight muscles were sampled from each of the 5 animals approximately 7 hrs post-mortem. For each muscle, a specific sampling site was chosen (Table 1). Muscle blocks (1 to 2cm^3) were trimmed of epimysium and adipose tissue and mounted on cork tiles using 5% gum tragacanth (w/v in ddH_20). They were immediately frozen in isopentane, which was frozen in liquid nitrogen, and then stored at -70^oC.

Transverse 10μm serial sections were cut from the frozen muscle blocks using a cryostat microtome at -25^oC, and mounted on glass slides. Sections were air dried at room temperature and stored at -20^oC.

Table 1. Descriptions of the sampling sites of the 8 muscles dissected from the carcase

Muscle	Site of sampling within the muscle
M. *psaos minor* (PM)	Central portion
M. *sacrocaudalis dorsalis lateralis* (SAC)	Dorsal portion from the sacral part of the muscle
M. semitendinosus (ST)	Proximal portion
M. *vastus lateralis* (VL)	Central portion
M. *biceps femoris* (BF)	Proximal portion
M. *adductor* (AD)	Central portion
M. gluteus medius (GM)	Cranio-distal portion
M. *semimembranosus* (SM)	Central portion

Immunohistochemistry

Immunohistochemical analysis was carried out on serial sections using mAbs against MHC (Picard *et al*. 1998). Anti-slow MHC mouse monoclonal antibody (clone MHCs; Novacastra, Newcastle-upon-Tyne) was used to identify type I fibres; MY-32 anti-fast MHC antibody (Sigma Chemical Co. St. Louis, Missouri) for type II fibres; and mAb S5 8H2 (gift from Dr Brigitte Picard, INRA, Thiex, France) for the identification of type I and IIB myofibres. Antibodies were detected using a labeled-strept-avidin-biotin (LAB-SA) system with the substrate-chromagen DAB (Zymed Laboratories, South San Francisco, California). Individual myofibres were compared across serial sections and classified as type I, IIC, IIA, IIAB or IIB (Picard *et al*. 1998). Table 2 summarises the classification system used based on staining characteristics for kangaroo muscle.

Table 2. Differential staining using three monoclonal antibodies. ++ indicates strong staining, + indicates intermediate staining, and - indicates no staining.

Type MHC	Clone	I	IIC	IIA	IIAB	IIB
Anti-I	MHCs	++	+	-	-	-
Anti-II	MY-32	-	+	-*	++	++
Anti-I+IIB	S5 8H2	++	+	-	+	++

*No staining, but for sheep (Greenwood *et al*. 2000) and cattle (Greenwood, unpublished results) a strong reaction occurs for type IIA MHC.

Histochemistry

Histochemical analyses were undertaken to validate MHC immunostaining classification. The contractile properties of myofibres were determined by staining for mATPase activity (Padykula and Herman 1955; Guth and Samaha 1970). This activity was revealed at pH 9.4 following acid pre-incubations (range pH 4.1 to 4.8 at increments of 0.1 pH units) for five minutes, or alkaline pre-incubation (range pH 10.2 to 10.4) for 10 minutes. Myofibres that showed mATPase activity at an optimal acidic pH value of 4.3 were classified as slow-twitch fibres, those reacting strongly at an optimal alkaline pH value of 10.3 or 10.4 were classified as fast-twitch fibres, and fibres reacting positively following acid or alkali pre-incubation as type IIC. The metabolic properties of myofibres were determined using staining characteristics for NADH (Novikoff *et al*. 1961) and GPD (Wattenburg and Leong 1960).

Image Analysis

Muscle sections were viewed with a Leica compound microscope (DMLB, Germany) using bright field microscopy. Images were captured using a Spot RT colour camera (Diagnostic Instruments, Michigan) and analysed using Spot RT Software v3.1 (Diagnostic Instruments, Michigan). The prevalence of each fibre type as a percentage of myofibres was measured manually from the serial sections using two randomly selected regions, each containing a minimum of 100 cells.

Statistical analysis

The statistical package SAS v8.2 (SAS Institute) was utilised. The differences between muscles within fibre type were analysed using a mixed model procedure with muscle as the fixed effect and animal as a random effect. These differences were then analysed using the differences of least squares means.

RESULTS AND DISCUSSION

Myofibre staining characteristics

Myofibre staining characteristics are presented in Figure 1.

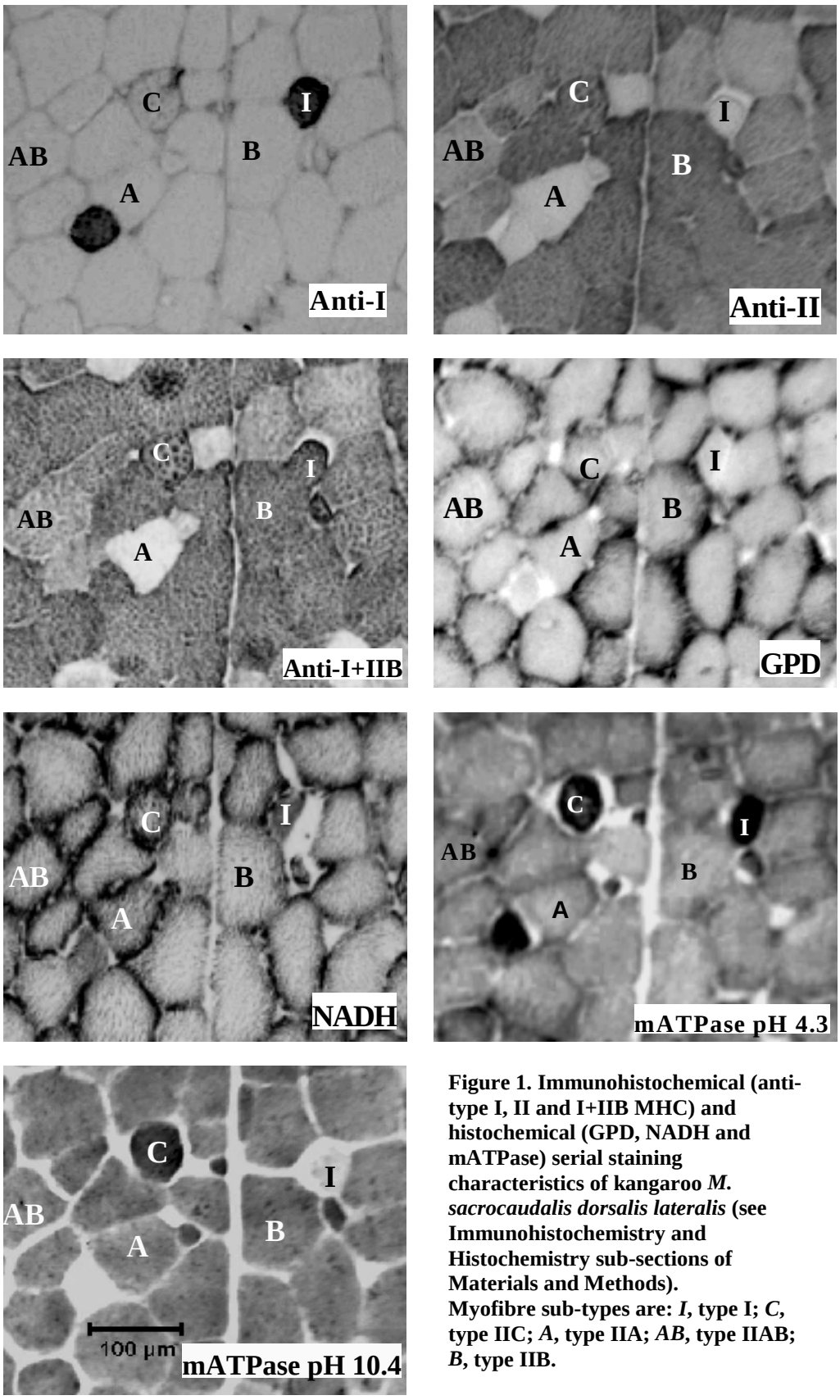

Figure 1. Immunohistochemical (anti-type I, II and I+IIB MHC) and histochemical (GPD, NADH and mATPase) serial staining characteristics of kangaroo *M. sacrocaudalis dorsalis lateralis* (see Immunohistochemistry and Histochemistry sub-sections of Materials and Methods). Myofibre sub-types are: *I*, type I; *C*, type IIC; *A*, type IIA; *AB*, type IIAB; *B*, type IIB.

Type I myofibres stained positively for the anti-type I MHC and anti-types I+IIB MHC. Type IIC stained positively for all three mAbs. Type IIB stained positively for the anti-type II and type I+IIB MHC, as did type IIAB myofibres but with reduced staining intensity. Type IIA myofibres were negative for all 3 mAbs and required histochemical staining profiles to prescribe functionality to this fibre type. This class of myofibre showed weak GPD staining and a strong reaction to the NADH stain, which is indicative of strong oxidative potential. These fibres also showed weak reactivity following acidic pre-incubation but strong reactivity following basic pre-incubation, thereby

characterising them as fast-twitch myofibres. Hence, these myofibres were classified as type IIA. The finding of non-specificity of the fast MHC antibody suggests a unique type IIA isoform in macropod muscle compared to other mammalian species including livestock. This finding is not consistent, however, with those of Zhong *et al*. (2001), who showed that kangaroo limb muscles express the same fast MHC sub-types as the cat, baboon, and rodent species. Irrespective, the findings of the present study highlight the importance of validating immunostaining with histochemical techniques.

Myofibre Type Distribution

Table 3 shows the fibre type distribution for each muscle. The eight muscles studied contained predominantly type IIB fibres followed by type IIA; the exception was the *M. psoas minor*. This indicates that kangaroos display considerable potential for both aerobic and anaerobic ATP production. The *M. psoas minor* displayed a significantly higher proportion of type I fibres ($p<0.01$) compared to the other muscles analysed in this study. It therefore has a higher oxidative potential, consistent with this muscle being a supportive/postural muscle in the kangaroo, as muscles involved in posture are more oxidative than those involved in movement (Totland and Kryvi 1991).

Table 3. Muscle fibre type percentages in 8 kangaroo muscles. Values are means ± SD for 5 animals.

Muscle[1]	Fibre type (%)				
	I (SO)[2]	IIC (FOG/SO)	IIA (FOG)	IIAB (FOG/FG)	IIB (FG)
PM	31.9 ± 3.2	—	30.2 ± 7.5	3.0 ± 3.4	34.9 ± 3.6
SAC	8.1 ± 2.0	0.1 ± 0.1	35.3 ± 14.2	10.7 ± 6.8	45.9 ± 14.0
ST	7.2 ± 1.1	—	28.0 ± 3.3	5.3 ± 3.1	59.5 ± 4.2
VL	6.1 ± 2.4	0.3 ± 0.6	27.0 ± 9.5	10.6 ± 6.2	56.1 ± 13.4
BF	4.4 ± 2.6	0.5 ± 1.2	34.8 ± 13.2	11.4 ± 4.5	48.9 ± 12.9
AD	2.8 ± 1.2	—	32.6 ± 10.0	12.4 ± 3.0	52.3 ± 12.3
GM	2.1 ± 0.8	0.1 ± 0.2	24.5 ± 7.2	10.8 ± 5.0	62.5 ± 11.0
SM	1.5 ± 1.2	0.1 ± 0.2	32.0 ± 10.1	7.5 ± 3.9	59.0 ± 11.8

[1]See Table 1 for details of muscles studied; [2]SO=Slow-oxidative, FOG=Fast-oxidative-glycolytic, and FG=Fast-glycolytic

CONCLUSION

The results from this study add to the knowledge base on muscle metabolism in macropods by providing a detailed classification of kangaroo muscle fibre types in an array of muscles of potential economic importance. This information will be important in understanding factors that impact on post-mortem metabolism, and hence, eating quality of kangaroo muscle. This study also highlights the importance of validating classification techniques when determining myofibre characteristics using MHC antibodies.

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