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The fibre type composition of the rabbit latissimus dorsi muscle

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ABSTRACT

The fibre type distribution has been mapped in the latissimus dorsi muscle of the Dutch rabbit. Using the myosin ATPase stain, a distinct border was found to run in a cranial to caudal direction, which effectively divided the muscle into 2 segments of different fibre type proportions. Although both segments contained mostly fast twitch fibres, the medial areas were found to contain approximately 10–20% slow (i.e. type I) fibres while the lateral portions contained very few, if any, slow fibres. Significantly fewer type IIa fibres were also found in the lateral areas of the muscle. These histochemical findings were confirmed by the use of the reverse transcriptase polymerase chain reaction, which demonstrated that more messenger RNA of the slow myosin heavy chain was found in the medial regions compared with the lateral segment. These results demonstrate the importance of choosing well defined sampling sites when evaluating regimes designed to transform this heterogeneous muscle for use in subsequent myoplasty procedures.

Key words: Muscle histochemistry; myosin ATPase; myosin heavy chain.

INTRODUCTION

The latissimus dorsi (LD) is a large flat muscle located on the dorsal area of the thorax. In man it can be transposed, with the neurovascular bundle intact, in a procedure known as dynamic cardiomyoplasty (Carpentier et al. 1991). The LD muscle is wrapped around the ventricles of a patient suffering from heart failure and stimulated to contract in synchrony with ventricular systole, thus augmenting cardiac output. This procedure is a potential alternative to heart transplantation, but in order to be able to work continuously like the heart, this muscle must be transformed, i.e. trained so that its usual mixed fibre type composition is converted into one containing more oxidative and fatigue-resistant fibres.

Although the anatomy of the LD muscle has been studied in several species, i.e. man (Sola et al. 1990), dog (Sola et al. 1990, 1992), sheep (Sola et al. 1992), monkey (Sola et al. 1992) and pig (Sola et al. 1992), no comprehensive study of the rabbit LD muscle has been published. This is perhaps surprising since the rabbit is a popular species for studies on muscle transformation. The above studies covering different species have shown that the distribution of fast and slow fibres in the LD is not homogeneous. For example, in man the muscle consists of 3 distinct segments, the most superior of which contains more slow fibres than the other 2 segments (Sola et al. 1990). In the same study the LD of the dog was shown to have a similar segmental pattern to that in man, but all 3 segments contained fewer slow fibres than the human muscle. No published study has distinguished between fast glycolytic (type IIb or FG) and fast oxidative/glycolytic (type IIa or FOG) fibres. Hence the oxidative status of these muscles has not been clearly defined.

Our long term aim is to monitor the changes in the proportions of the fibre types and oxidative status of the rabbit LD muscle as a function of time in response to various training regimes. However, in this study we aim to establish the normal fibre type composition and distribution, including distinction between type IIa and IIb fibres and the oxidative status of the rabbit LD, before any experimental procedure is undertaken. This is important because in the experimental animals

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we will be using histological, biochemical and molecular biological analytical techniques on small samples of muscle. We need to be certain that any changes observed after training are real and not simply due to variations in sampling sites within a heterogeneous muscle. To that end, this study presents in detail the normal fibre type distribution in this muscle, as determined by standard histochemical procedures, complemented by measurements of the mRNA concentrations of the slow myosin heavy chain (MHC).

METHODS

Dutch rabbits of both sexes with body weights between 1 and 1.5 kg were used. They were allowed access to food and water ad libitum and subjected to a 12 h light (06.00–18.00)–dark cycle. The animals were killed with a single injection of 180 mg of Sagatal (pentobarbitone sodium)/kg administered via an ear vein, and the LD muscles from both sides dissected out immediately and weighed. A total of 5 muscles from 3 different rabbits were analysed in detail.

Histochemical techniques

Five 3 mm wide strips, labelled A–E, were cut across the muscles in a medial to lateral direction at approximately 1.5 cm intervals from the humeral tendon (Fig. 1). Several blocks were taken from each of these strips and numbered from 1 to 5 commencing on the medial edge. Strips from the midmuscle region contained more blocks than those from the ends close to the tendons, where the muscle is narrower. The blocks were mounted on cork discs, covered in OTC compound (Tissue Tek) and frozen in isopentane, which was precooled in liquid nitrogen. Blocks were stored at −70 °C. Sections, 10 μm thick, were cut in a cryostat at −20 °C and stained for either myosin adenosine triphosphatase (ATPase) or succinic dehydrogenase (SDH).

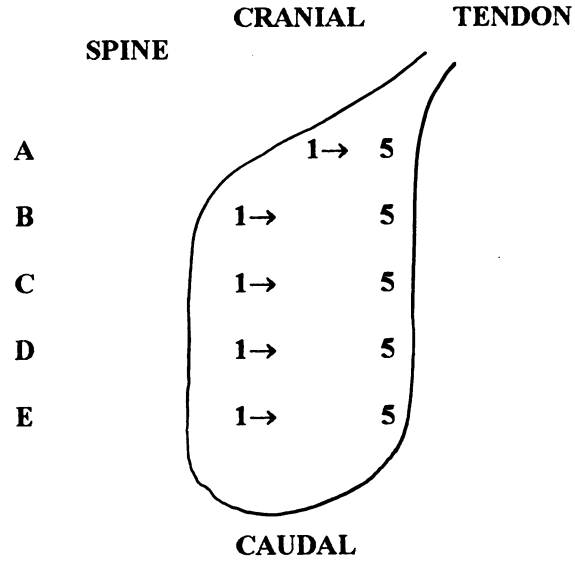

Fig. 1. Sampling sites from the LD muscle.

The method used to stain for myosin ATPase was based on that of Padykula & Herman, (1955). Minor modifications were made in the pH of the preincubation buffer to optimise the differentiation between fibre types in rabbit tissue. Serial sections were subjected to either an acid preincubation solution (0.2 M acetic acid/0.2 M sodium acetate, pH 4.2) for 4.5 min at 20 °C, or an alkaline preincubation solution (0.02 M glycine and 0.02 M calcium chloride, pH 10.4) for 20 min at 20 °C. All slides were then washed in distilled water and incubated in a solution of 0.1 M glycine, 0.05 M $CaCl_2$ and 3.3×10^{-3} M ATP (pH 9.4) for 30 min at 37 °C. Thereafter slides were washed in 3 changes of 1% $CaCl_2$, incubated in 2% cobalt chloride for 3–4 min at 20 °C and placed in 2% ammonium polysulphide for 30 s. Finally, sections were washed, dehydrated, cleared and mounted. These sections were analysed using a Seescan Solitaire image analyser. Approximately 300 fibres were counted in each section and assigned to the appropriate class of fibre types. Muscle fibre areas were determined for a representative region of each of the muscles.

The stain for the mitochondrial enzyme SDH was based on the method of Nachlas et al. (1957). Thawed 10 μm sections were placed in a solution made up of 0.67 M disodiumhydrogen orthophosphate, 0.67 M potassium dihydrogen orthophosphate, 0.2 M sodium succinate (pH 7.0) and 2.5 mM nitroblue tetrazolium. Sections were incubated at 37 °C for 1 h and fixed in 10% phosphate buffered formalin for 10 min. They were then rinsed in 0.9% saline, dehydrated, cleared and mounted.

Slow myosin heavy chain (MHC) mRNA

Total RNA was extracted from the muscle samples by the method of Chomczynski & Sacchi (1987). The RNA pellet was washed twice in 70% alcohol, dried in a Savant speed vac concentrator and resuspended in RNase free water. The quality of the RNA was determined by electrophoresis through a 1% agarose gel in TRIS acetate EDTA buffer (Sambrook et al. 1989). Ethidium bromide (0.5 μg/ml) was added to allow the discrete 18 and 28 S ribosomal RNA bands to be visualised on a UV transilluminator.

All samples of RNA were quantified in a Beckman

DU 70 spectrophotometer using a wavelength of 260 nm. A 2 μg aliquot of total RNA was reverse transcribed into cDNA in a volume of 20 μl using AMV reverse transcriptase (2 units), RNasin (ribonuclease inhibitor, 20 units) and oligo (dT) as supplied by Promega. The resultant cDNA samples were made up to 150 μl with RNase free water.

PCR amplifications were carried out on a Techne PHC-3 thermal cycler, using 96 well microtitre plates. All reagents were supplied by Promega unless otherwise stated. 10 μl of the above diluted cDNA mixture were added to 20 μl of PCR cocktail, which consisted of 3 μl of buffer (10 mM Tris HCl, 1.5 mM $MgCl_2$, 50 mM KCl and 0.7 μg gelatin/μl), forward and reverse primers for the slow MHC, deoxyribonucleoside triphosphates (dNTPs) and Taq DNA polymerase (0.4 units). The amplification programme involved an initial denaturation at 95 °C for 5 min, followed by 22 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 45 s and synthesis at 72 °C for 30 s. Serial dilutions of known quantities (0.005–10 pg) of rabbit slow myosin cDNA were run alongside the samples to provide a standard curve. Each sample and standard were amplified in duplicate. The amplification products were separated on a 2% agarose gel, which contained 0.5 μg/ml ethidium bromide.

The gels were photographed with Polaroid type 665 film and the bands of the slow MHC cDNA quantified from the film negative using an LKB scanning laser densitometer. A standard curve was constructed from the absorbance readings obtained from the serial dilutions of the standard MHC cDNA. From this, the message levels of the slow MHC derived from the different areas of the muscle were calculated and compared with each other and expressed semiquantitively as 'fold differences'.

Statistics

Statistical analysis was carried out using the Statview program for Macintosh. Student's t test (unpaired) was used to compare the fibre type distributions of different parts of the muscle. A paired t test was used to compare weights of muscles from the right and left sides of rabbits. The correlation between body and muscle weights was carried out by regression analysis.

RESULTS

The wet weight of the LD muscles from the left and right sides of the same animal did not differ significantly (Table 1). The weights of 25 untreated LD muscles were correlated with the body weights of the animals. As expected LD weights increased with increasing body weights (Fig. 2), this being highly significant with a regression coefficient of $P = 0.01$. Nonetheless, there were still some variations in this relationship. For example, 2 animals with near identical body weights of 1281 and 1295 g possessed

Table 1. *Weights of the LD muscles in Dutch rabbits*

LD muscle wet weight (g)		
Left side	Right side	Body weight (g)
7.7	8.1	1985
4.7	4.7	1510
5.5	5.2	1461
4.6	4.7	1295
5.7	5.4	1125

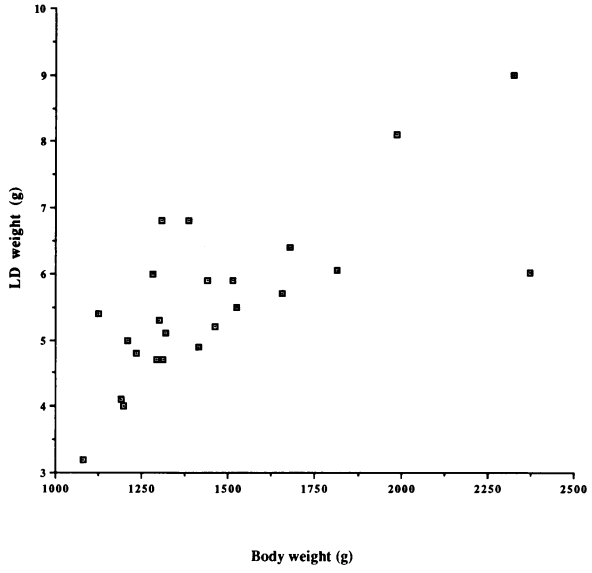

Fig. 2. Correlation between LD weight and body weight in Dutch rabbits.

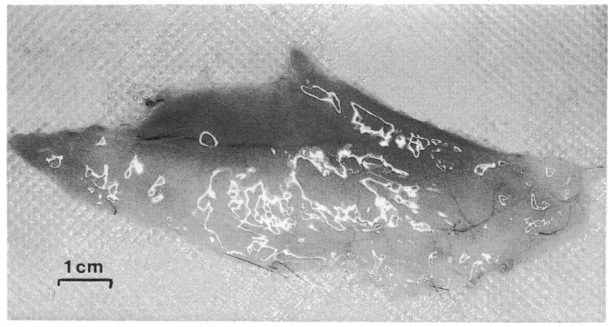

Fig. 3. The gross appearance of the rabbit LD muscle. The humeral tendon is on the right. The medial area (top) is clearly darker than the lateral.

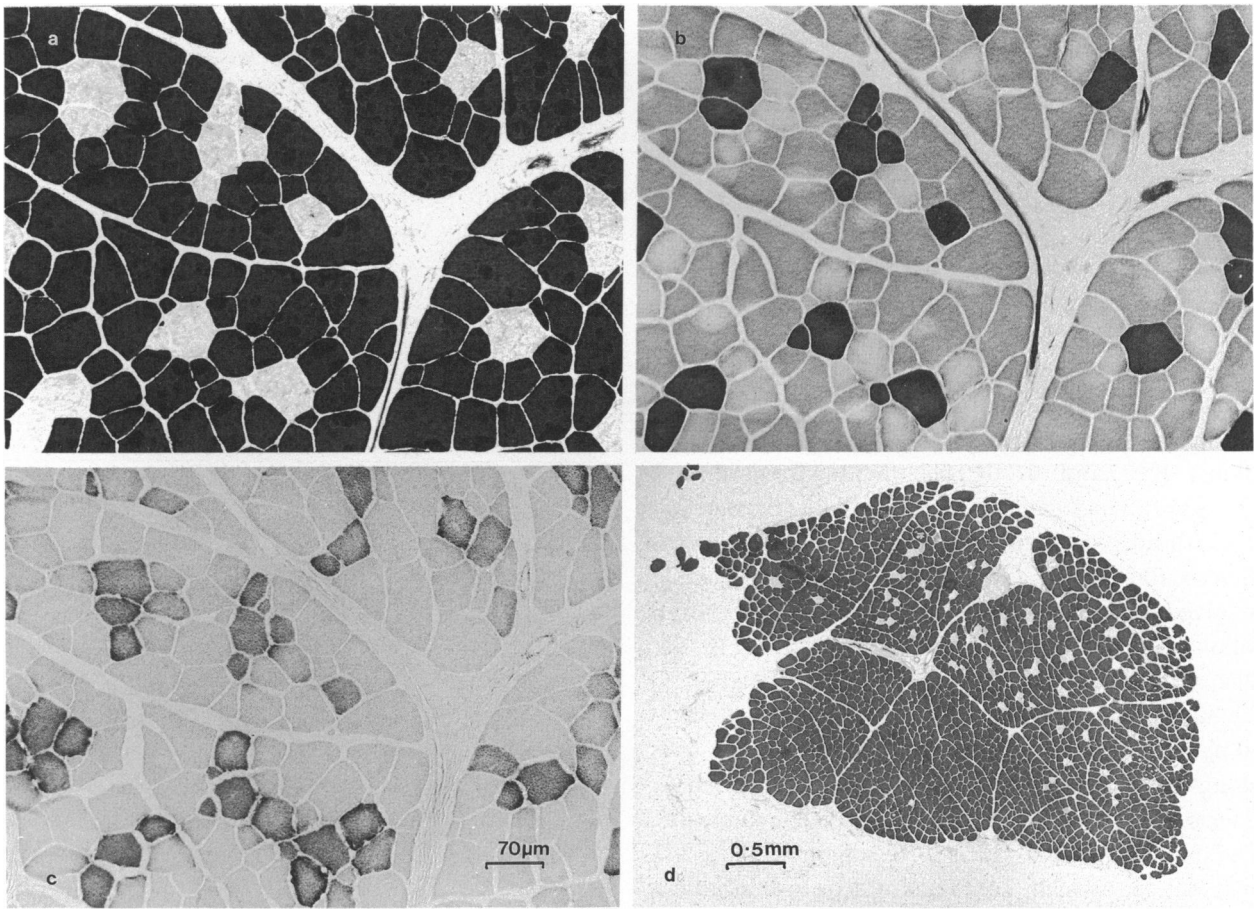

Fig. 4. Histochemical analysis of the rabbit LD muscle. Photographs show transverse sections from the medial segment of the muscle which were stained for myosin ATPase at either pH 10.4 (*a*) or pH 4.2 (*b*). A section stained for SDH is also shown (*c*). The border that was observed in some sections is clearly shown in (*d*) (myosin ATPase, pH 10.4).

LD muscles that weighed 6 and 4.7 g respectively, i.e. a difference of 28%.

Histochemical studies

The gross appearance of the rabbit LD muscle is shown in Figure 3. The most medial area of the muscle was visibly more red than the remainder, which indicates the presence of a richer blood supply and more myoglobin and cytochromes.

In the sections that were preincubated at pH 10.4 only 2 fibre types were seen (Fig. 4*a*), with the type II appearing dark compared with the pale type I fibres. Detailed fibre type analysis from 17 different parts of the LD muscle (Table 2) showed that the majority of fibres were type II. It was clear from sections preincubated at pH 10.4 that there were consistently more fast fibres in the extreme lateral regions, when compared with the most medial regions. However, these differences were statistically significant only in strip B (Table 2).

With acid preincubation (Fig. 4*b*), the myosin ATPase stain allowed the differentiation of 3 fibre types, i.e. the type I slow oxidative (SO) fibres which were stained darkly, and the distinction of the fast fibres into type IIa fibres (light in colour) and type IIb (stained an intermediate brown). It can be seen (Table 2) that the most lateral part of the muscle (e.g. B4) has significantly more type IIb fibres than the medial parts (e.g. B1 and B2). Conversely, more of the type IIa and type I fibres were present in the medial regions. In order to show the medial to lateral variations in fibre type proportions more clearly Figure 5 presents the data for strip B of Table 2 in graphical form. The type I fibres make up 13–20% of the fibres in the medial part of the muscle (Table 2), but were totally absent in 4 of the 9 samples taken from the lateral edge of the muscles studied. Although this decrease in slow fibres was apparent in all strips, it was statistically significant only in strips A and B.

The classification based on the myosin ATPase after acid preincubation was supported by serial sections which were stained for the mitochondrial enzyme SDH (Fig. 4*c*). The density of the blue tetrazolium salt in the SDH reaction was greatest in the type I fibres, slightly less blue in the type IIa and

Table 2. *Fibre type proportions on different regions of the rabbit LD muscle*

	Percentage of each fibre type			
	Type II (pH 10.4)	Type I (pH 4.2)	Type IIa (pH 4.2)	Type IIb (pH 4.2)
A1	79.3±2.0	20.0±1.2	33.5±3.5	47.9±3.9
A2	83.7±1.2	15.0±2.5*	18.8	68.8
A3	92.4±5.3	7.6±5.3	14.3±8.9	72.9±21.8
B1	84.2	13.0±0.2	24.7±1.4	62.2±1.4
B2	82.6±2.6**	18.0±0.7***	31.5±3.6***	52.3±5.3***
B3	91.9±8.1	6.1±5.5	19.6±6.0	71.3±14.0
B4	100±0.0	0±0.0	5.2±0.2	94.8±0.2
C1	86.4±0.2	14.4±2.7	29.7±1.7	56.0±1.1
C2	84.5±1.7	18.6±0.7	26.2±1.9	57.1±1.8
C3	86.2±2.9	15.6±2.7	20.4±1.8	64.0±3.3
C4	90.1±1.5	6.7±3.5	15.7±4.0	82.8±7.2
D1	82.5±1.4	14.2±4.08	30.5±0.9	55.2±4.0
D2	88.8±0.3*	13.3±0.82***	21.9±1.9***	64.8±1.1*
D3	95.4±2.4	6.8±2.77	15.1±2.3	79.2±5.3
E1	84.3±1.5	15.9±3.07	34.0±2.8	50.8±8.0
E2	87.6	15.2±1.99	35.6	54.2
E3		5.3±2.75		34.3

A simple distinction between fast and slow fibres was achieved using an alkaline preincubation (pH 10.4). Further differentiation of fast fibres into IIa and IIb was possible using an acid preincubation (pH 4.2). All values are mean±S.E.M. of histological sections from 3 to 5 different muscles. Where the difference in fibre types between medial and lateral parts of each strip is significant (e.g. A1 compared with A3, B1 compared with B4, etc) this is indicated accordingly $*P < 0.05$, $**P < 0.01$ and $***P < 0.005$.

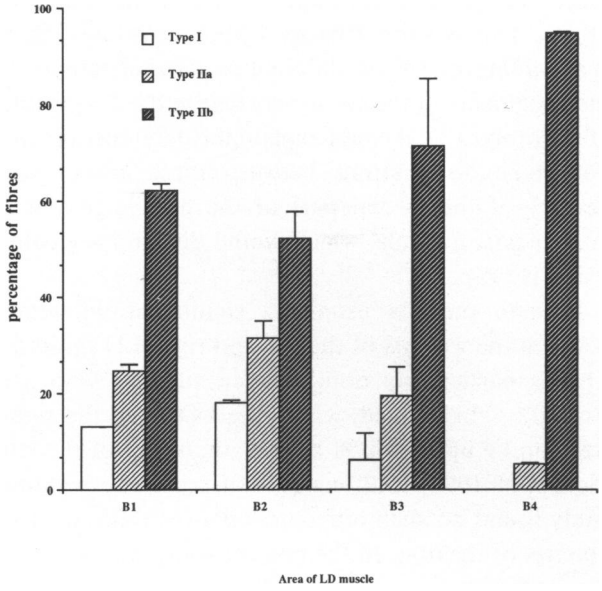

Fig. 5. Fibre type proportions in 4 different areas from a medial to lateral strip across the LD muscle (strip B, Table 2). Data from transverse sections of 5 separate muscles stained for myosin ATPase (pH 4.2).

almost absent in the type IIb fibres, which therefore appeared pale. This stain showed that the total proportions of SDH positive fibres was approximately equal to the sum of the type I and type IIa fibres (Table 3), and that the medial portion of the muscle was more oxidative than the lateral.

Table 3. *Muscle fibre area and fibre type proportions from the medial part of the rabbit LD muscle*

Muscle fibre type	Type I	Type IIa	Type IIb	SDH positive
Fibre area μm^2	1942±808**	1512±669**	2312±1336**	
Fibre proportions (%)	13±2	24±7	63±8	40±7

All measurements were made on sections stained with the myosin ATPase method with a preincubation of pH 4.2, n = 5. **Significantly different from other fibre types, $P = 0.001$. The number of SDH positive fibres from sections taken from the same area of the muscles is also given (n = 3). All values are means±S.D.

An interesting observation was that there was a distinct border running in a cranial to caudal direction which demarcated an abrupt change in fibre types (Fig. 4*d*). Very few type I fibres could be found lateral to this border, which further supports the profiles shown in Figure 5 and Table 2. In addition, there were no significant differences in the fibre type proportions when the muscles were analysed along the long axis, suggesting 2 distinct segments.

The mean cross sectional area of the different fibre types was determined using sections from areas B1 or 2 and C1 or 2. Type IIb were found to be the largest fibres and type IIa the smallest (Table 3). A similar pattern has been noted both in the tibialis anterior (Hudliká et al. 1982) and the adductor magnus (Hämäläinen & Pette, 1993) of the rabbit.

Studies on the mRNA concentrations of slow MHC

The PCR primers specific for the slow MHC were prepared on an Applied Biosystems DNA synthesizer. They were designed and produced to flank a 294 bp fragment within the nontranslated 3′ end of the rabbit slow myosin sequence, as published by Brownson et al. (1992). The forward and reverse primers were both 23 boxes in length. Restriction cleavage of the PCR product by the endonuclease Pst-I produced 2 fragments, which when run alongside DNA molecular weight markers were shown to be 107 and 187 bases in length, i.e. the sizes predicted from the sequence. This suggested that the amplified product was indeed the slow MHC.

PCR was carried out under optimal conditions (see Methods) on samples prepared from several different

Table 4. *Slow MHC message levels in different areas of the rabbit LD*

Medial/ Lateral comparisons		Lateral comparisons		Medial comparisons	
A1:A3	180	B1:A1	3	D4:A3	3
B1:B4	234	A1:D1	2	D3:A3	4
D1:D3	307	B1:D1	4	B4:D3	3
Mean	240.3±36.8	Mean	3.0±0.6	Mean	3.3±0.3

Results are shown as ratios of message level comparing samples from the 2 distinct segments of the muscle (i.e. a medial to lateral direction). Ratios are also shown for the comparisons made between samples from within the medial segment and within the lateral segment (i.e. in a cranial to caudal direction). The average value for the ratios in each set of comparisons is shown as means±S.E.M.

regions of the LD muscle (Table 4). These corresponded to the areas A1, A3, B1, B4, D1 and D3 in Table 2. The mRNA levels for the slow MHC were an average of 240 fold greater in the medial areas (i.e. A1, B1, D1) than the equivalent lateral regions (i.e. A3, B4, D3). When the lateral areas were compared with each other (i.e. along the long axis), only a maximum 4-fold difference in levels was observed. The same trend was true when the medial areas were compared with each other. Hence, large differences in the message levels were found only across the muscle (i.e. medial to lateral) and not along its length.

DISCUSSION

The histological findings of this study clearly show that the rabbit LD is predominantly a fast twitch muscle, and that the small percentage of slow fibres are mostly confined to the medial area of the LD (Table 2, Fig. 5). The rabbit LD is therefore appreciably faster and more glycolytic in nature than that of man and dog (Sola et al. 1990). These histochemical observations were supported by the unequal distribution of the mRNA for the slow MHC (Table 4). These observations, together with the evidence of a distinct border (Fig. 4*d*) and no differences in fibre type proportions in the longitudinal direction, collectively suggest the presence of 2 separate segments running along the length of the muscle.

Although the data on slow MHC are largely in agreement with the histochemical findings, some slow MHC message was found even in the lateral areas where no slow fibres were identified histochemically. However, PCR is a very sensitive technique, which can detect very small quantities of mRNA. It is also known that muscle fibres can simultaneously express different myosin isotypes both during differentiation and in normal adult tissues (Lutz et al. 1979). Thus in this study those fibres identified as fast by myosin ATPase staining may actually be expressing low levels of slow myosin mRNA which can only be detected by PCR.

Clearly a physiological explanation must exist for the differences in fibre type distribution within the muscle. The most obvious would be a difference in innervation and/or activity pattern of the medial and lateral segments. In the rabbit the thoracodorsal artery, which supplies most of the LD muscle, enters the muscle in close proximity to the humeral tendon. It then divides into 3 branches. One of these, the internal artery, supplies the medial part of the muscle, while the other 2 branches supply the lateral area (Suarez et al. 1985). The thoracodorsal nerve accompanies the artery and branches accordingly. It seems likely, therefore, that the medial part of the muscle may have a separate innervation to the remainder. In 50% of humans the most medial of the 3 segments of the LD fuses with the teres major, a muscle that arises from the lateral border of the scapula and inserts on the humerus alongside the LD. In 4% of these cases the superior segment of the LD inserts into the humerus by a separate tendon from the other 2 segments (Sola et al. 1990, 1991). It is possible, therefore, that in man the superior segment of the muscle has a different physiological function than the others. This is supported by EMG recordings (Sola et al. 1991) which show different patterns of activity in the 3 segments. If the same were true in the 2 segments of the rabbit LD, it could explain the differences in the fibre type distributions between these areas, particularly if the medial part of the muscle played a greater postural role, which would demand a greater oxidative capacity.

In man there is usually a significant difference between the weights of the left and right LD muscles. This is particularly noticeable in subjects who are strongly right handed, where the LD from the right side can be up to 100% heavier than that of the left (Sola et al. 1990). Although not unexpected, the same study found no such differences between the two LD muscles of the dog. In the present study we have also shown that no contralateral differences exist in rabbits (Table 1). However, the size of the muscles can vary even between rabbits of very similar body weights. This could simply relate to the fact that body weight is not a particularly stable measurement, since it varies with the proportion of body fat, a full or empty stomach and even with moulting. It is therefore important to use contralateral muscles as internal

controls where possible, since even weight-matched external control animals may have muscles which are different in size from those in the experimental animal.

Internal control muscles are also valuable in many situations as they allow for possible interanimal variations in nutritional and endocrine status, etc. However, some experimental regimes, such as electrical stimulation or passive stretch, may also induce changes within the contralateral muscle via either reflex activity or changes in the animal's posture or gait. Such changes in internal control muscles can only be assessed by reference to external controls. However, even external control muscles may need to undergo sham operations to allow for possible effects induced by the anaesthetics and/or surgical trauma. This is especially important when monitoring changes at a molecular level.

In our future studies we intend to examine the effects of various electrical and mechanical stimuli on muscle fibre size and fibre type composition of the LD muscle. Before embarking on any procedure designed to change the contractile and/or metabolic properties of a muscle it is important to acquire sufficient morphological knowledge of the muscle in question. It is clear from the present study that the rabbit LD is a heterogeneous muscle that consists of at least 2 segments with some possible divergence of physiological function. Because of the variations in fibre types and MHC isoform distributions between these segments, the effectiveness of any manipulative procedure may be either under or over-estimated due to inconsistencies in the sampling procedure. Since the medial part contains more type I and type IIa fibres than the lateral, it is initially more oxidative in nature. Therefore, the overall conversion of fibre types, in response to stimuli designed to increase the oxidative capacity and fatigue resistance, would clearly be less in this area of the LD. We therefore conclude that the sampling sites chosen should be from both the medial and lateral areas of this muscle.

This study serves two important functions. First, it provides new, fundamental anatomical information relating to the fibre type proportions and their distribution in the LD of the rabbit. Secondly, it stresses the need for consistent and reproducible biopsy sampling when comparing the effect of any training regimes on a heterogenous muscle.

REFERENCES

Brownson C, Little P, Jarvis JC, Salmons S (1992) Reciprocal changes in myosin isoform mRNAs of rabbit skeletal muscle in response to the initiation and cessation of chronic electrical stimulation. *Muscle and Nerve* **15**, 694–700.

Carpentier A, Chaques JC, Grandjean P (eds) (1991) *Cardiomyoplasty*. New York: Futura Publishing Company.

Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Analytical Biochemistry* **162**, 156–159.

Hämäläinen N, Pette D (1993) The histochemical profiles of fast fibre types IIB, IID and IIA in skeletal muscles of mouse rat and rabbit. *Journal of Histochemistry and Cytochemistry* **41**, 733–743.

Hudlická O, Dodd L, Renkin EM, Gray SD (1982) Early changes in fibre profile and capillary density in long-term stimulated muscles. *American Journal of Physiology* **243**, H528–H535.

Lutz H, Weber H, Billeter R, Jenny E (1979) Fast and slow myosin within single skeletal muscle fibres of adult rabbits. *Nature* **281**, 142–144.

Nachlas M, Tsou KC, Desouza E, Cheung CS, Seligman AM (1957) Cytochemical demonstration of succinate dehydrogenase by the use of a new p-nitrophenyl substituted ditetrazole. *Journal of Histochemistry and Cytochemistry* **5**, 420–436.

Padykula H, Herman E (1955) The specificity of the histochemical method for adenosine triphosphatase. *Journal of Histochemistry and Cytochemistry* **3**, 170–195.

Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning, a Laboratory Manual* 2nd edn. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

Sola OM, Haines LC, Kakulas BA, Ivey T, Dillard DH, Thomas R et al. (1990) Comparative anatomy and histochemistry of human and canine latissimus dorsi muscle. *Journal of Heart Transplantation* **9**, 151–159.

Sola OM, Kakulas BA, Thomas R (1991) Anatomy of the latissimus dorsi muscle II. Segmental anatomy and function. *Cardiomyoplasty* (ed. A. Carpentier, J. C. Chaques, & P. Grandjean), pp. 68–71. New York: Futura Publishing.

Sola OM, Herring S, Zhang G, Huang X, Hayashida N, Haines LC et al. (1992) Significance of the biopsy site of the latissimus dorsi muscle for fibre typing. *Journal of Heart and Lung Transplantation* **11**, 5315–5319.

Suarez CN, Martinez A, Garcia MJ, Garcia PB, Comis JE (1985) Latissimus dorsi myocutaneous flap; vascular anatomy and experimental viability. *Journal of Otolaryngolgy* **14**, 394–398.