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Dimensions of the Rabbit Tenuissimus Muscle

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ABSTRACT

A study of the physical dimensions of the rabbit tenuissimus muscle is described and compared with previous work on the same muscle from the cat. The muscle in the rabbit was found to be larger than in the cat, but was consistent in its dimensions and suitable for use as a model for microvascular research.

INTRODUCTION

The cat tenuissimus muscle has been analysed by Eriksson & Myrhage (2). A similar study is described of the rabbit tenuissimus muscle.

Data are presented which show the rabbit muscle to be more substantial than its counterpart in the cat, and reasons are suggested for differences found in muscle fibre diameter.

MATERIAL AND METHODS

New Zealand White rabbits fed on a standard diet (Teknosan pellets, AB Ferrosan, Malmö, Sweden) were used in this study. Tenuissimus muscle from the legs of 40 rabbits (mean weight 1.0 kg±0.3 kg s.d.) (Table I) were examined, after the rabbits were anaesthetised with urethane and the tenuissimus muscle exposed.

Examination of the muscle was carried out in several ways. The muscle length from origin to insertion and its width were measured *in vivo* with vernier calipers and its thickness with a micrometer eyepiece ×16 on a stereomicroscope (Leitz). Before removal from the rabbit, some muscles were fixed either by bathing with 3% glutaraldehyde (buffered with 0.075% sodium cacodylate at pH 7.2) or by cannulation of the aorta and intra-arterial perfusion of the muscle with this solution. Other muscles were perfused with Indian ink (Pelikan) after cannulation of the main artery supplying the muscle 1 cm before it entered the muscle. In this way all sections of the vascular bed were filled with carbon particles, making possible the measurement of vascular lengths and diameters by microscopy. Attempts were made to ensure maximal vasodilatation in some of these muscles by electrical stimulation or by adding papaverine to the ink.

After removal from the rabbit, each muscle was prepared for measurement of vascular dimensions by fixation for a further 15 hours in 3% glutaraldehyde and dehydration with ethanol. Some of the muscles which had been perfused with ink were then placed in a mixture of 85% benzylbenzoate and 15% wintergreen oil (6, 8). By this procedure the muscle becomes quite transparent and ink-filled vessels can be visualised. The diameters of ink-filled vessels, including capillaries, were measured under a microscope using a measuring eyepiece (×12.5, Leitz) and ×6.5 ×12.5 or ×23 objective lenses. The eyepiece was calibrated by viewing a micrometer slide.

The lengths of vessels were measured by moving the microscope table under a cross-wire eyepiece, the table being connected to a potentiometer calibrated over a micrometer slide so that the distance the table moved was indicated as vascular length.

The rest of the ink-perfused muscles, and those which had not been perfused, were embedded in epoxy resin (Shell Epon 812) for sectioning (4). 3 μm thick sections were cut with a glass microtome knife and stained with toluidine blue. Wax embedding was found to give inadequate support to the muscle which disintegrated when sectioned. For estimation of the distribution of capillaries relative to muscle fibres, photomicrographs (Leitz Orthomat) were taken of these sections through ×2.5 or ×10 (Leitz) microscope objectives. Areas of muscle sections were measured from enlargements of these photographs with a digitizer (HD9107 A, Hewlett Packard).

RESULTS

The muscle was found to be 6.4 cm long in the left leg (±0.9 cm S.D.) with a variation in length per kg body weight of from 4.0 cm to 9.3 cm. Its greatest thickness was found to be 1.1 mm (±0.2 mm S.D.) and the muscle was shaped like an aerofoil with its narrower angle anterior (Fig. 1). The central vessels were located in the thickest part of the muscle running parallel to its edges (Fig. 2). The width of the muscle was found to vary, being greater at the distal end of the muscle (mean 4.4 mm±0.8 mm S.D.) than at its proximal end (mean 4.2 mm±0.7 mm S.D.) or mid-way along it (mean 4.2

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Table I. *Summary of experimental findings*

Measurements of length, width and thickness of the muscle were made before removal from the rabbit

	No. of muscles	Mean value
Rabbit body weight, kg		1.0±0.3 S.D.
Tenuissimus length		
Left leg, mm	34	64.4±8.8 S.D.
Left leg, mm/kg b.wt.		
Tenuissimus length		
Right leg, mm	26	65.1± 8.9 S.D.
Right leg, mm/kg b.wt.	26	65.1±11.9 S.D.
Muscle width		
Left leg, Proximal, mm	26	4.2±0.8 S.D.
Left leg, Mid-level	46	4.2±0.8 S.D.
Left leg, Distal	42	4.4±1.1 S.D.
Right leg, Proximal	25	4.3±0.8 S.D.
Right leg, Mid-level	37	4 4±0.7 S.D.
Right leg, Distal	34	4.5±0.8 S.D.
Muscle width, mm/kg b.wt.		
Left leg, Proximal	25	4.3±1.0 S.D.
Left leg, Mid-level	46	4.3±1.0 S.D.
Left leg, Distal	42	4.5±1.1 S.D.
Right leg, Proximal	25	4.4±1.1 S.D.
Right leg, Mid-level	37	4.5±1.1 S.D.
Right leg, Distal	35	4.5±1.1 S.D.
Distance between arterior muscle edge and central vessels, mm		
Left leg, Proximal	25	2.5±0.7 S.D.
Left leg, Mid-level	46	2.4±0.6 S.D.
Left leg, Distal	42	2.7±0.7 S.D.
Right leg, Proximal	25	2.5±0.7 S.D.
Right leg, Mid-level	37	2.6±0.6 S.D.
Right leg, Distal	35	2.6±0.7 S.D.
Ratio of anterior section to whole muscle width		
Left leg, Proximal	25	0.58±0.1 S.D.
Left leg, Mid-level	46	0.59±0.1 S.D.
Left leg, Distal	42	0.60±0.08 S.D.
Right leg, Proximal	25	0.60±0.07 S.D.
Right leg, Mid-level	37	0.60±0.08 S.D.
Right leg, Distal	33	0.58±0.08 S.D.
Tenuissimus thickness, mm	14	1.1 ±0.2 S.D.
Cross-sectional area of each muscle fibre, μm²	4	1 022±50 S.D.
Calculated muscle fibre diameter=36μm		

mm±0.8 mm S.D.) The width of the muscle was not significantly related to the weight of the rabbit, varying from 2.1–8.0 mm/kg body weight. There was no significant difference between the dimensions of muscles from left and right legs. The ratio of the part of the muscle lying anterior to the central vessels to the part lying posterior to them was calculated and found to be 0.58 at its upper end, 0.59 at mid-level and 0.60 at the distal end of the muscle.

The density of 10 muscles was calculated after weighing each and determining its volume by displacement of saline in a 2 ml pipette. The mean density was found to be 1.20 g/cc (±0.1 S.D.).

Areas of muscles from 2 rabbits were measured from photographs, as described above, to determine the cross-sectional area of muscle fibres (Table II). The cross-sectional area of each fibre was calculated to be 1022 μm^2 (±50 S.D.) corresponding to 978 muscle fibres per mm^2 and a fibre diameter of about 36 μm.

COMMENT

In all its physical dimensions, the rabbit tenuissimus muscle was found to be more substantial than its counterpart in the cat. At its thickest part it was nearly twice as thick (0.8–1.4 mm compared with 0.3–0.6 mm). Like the cat muscle, however, it had an anterior part, accounting for just over half its width, which tapered to become less than 0.1 mm thick at the anterior edge. This was clearly the most suitable area for blood flow studies as transillumination of the muscle would be unlikely to be satisfactory towards the thickest part. An illustration of the shape of the muscle cross section is given in Fig. 1.

The width of the muscle was found to vary along its length between 4.2 and 4.4 mm (mean values) compared with 3–5 mm for the cat.

Measurements of length, thickness and width

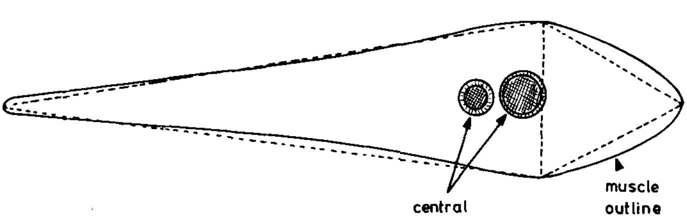

Fig. 1. Diagram of a cross-section of the tenuissimus muscle. In calculations the area of this cross-section was approximated to that of the two triangles (shown as dotted lines).

Table II. *Data from 2 rabbits for calculation of cross-sectional area of muscle fibres and calculation of the area of muscle cross-section served by each capillary*

Muscle area measured (μm^2)	Number of muscle fibres	Number of capillaries	Calculated cross sectional area of muscle per fibre (μm^2)	per capillary (μm^2)
53 187	54	29	985	1 834
37 225	38	17	980	2 190
23 893	22	14	1 086	1 707
13 436	13	9	1 035	1 493
		Mean	1 022 (±50 S.D.)	1 806 (±292 S.D.)

were made in unfixed muscles *in situ* so that no distortion occurred.

Some difficulty was experienced in obtaining satisfactory measurements for muscle fibre diameter. It is reported by Brånemark & Eriksson (1) that shrinkage or swelling of muscle fixed in 3% glutaraldehyde and embedded in epoxy resin is less than 5%, but this was not the experience of this study. The change in muscle width during preparation varied from a decrease of 1.7 mm to an increase of 1.2 mm, representing an average change of width of 22% (Table III). A further problem was that there was sometimes shrinkage of muscle fibres leading to their separation along connective tissue planes.

These changes did not occur with every muscle, however, and it was possible to calculate fibre diameter from muscles that showed no change in overall dimensions during fixation and whose fibres showed no separation because of shrinkage. Calculation of average fibre diameter showed this to be 36 μm. Eriksson & Myrhage (2) found the diameter of Type A fibres in the cat tenuissimus to be 55 μm compared with 41 μm for Type B fibres and 26 for Type C fibres. The mean diameter, taking the proportion of each fibre type into account was found to be 44 μm. This value is higher than that found for the rabbit in this study and probably represents a species difference in fibre size as well as in the proportion of each fibre type present. No attempt was made in this study to differentiate between muscle fibres types.

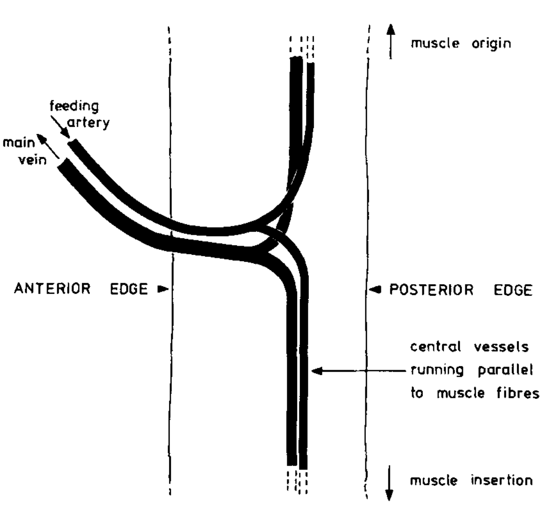

Fig. 2. Diagram showing the main artery and vein supplying the muscle at about the mid-point in its length.

It has been suggested (2, 3, 5) that connective tissue makes up between 20 and 30% of the bulk of skeletal muscle. Photographs of sections of muscle in this study suggested, however, that connective tissue in the tenuissimus formed a much smaller proportion of the total bulk than this. If muscle fibre diameter is calculated, from the data obtained from the rabbit, on the assumption of an 8:2 ratio between muscle tissue and connective tissue plus vascular tissue, the diameter of each fibre decreases to 32 μm.

In their study of skeletal muscle fibre density in the gastrocnemius muscle (Table IV), Schmidt-Nielsen & Pennycuik (7) found that this muscle contained only 14% of Type B fibres, with a total fibre density of 518 fibres mm^{-2}. Eriksson & Myrhage (2) found that the cat tenuissimus contained 48% of the smaller Type B fibres but did not calculate the density per square millimeter. In this study muscle fibre density was found to be 978 mm^{-2} and suggests that the relatively high percentage of Type B fibres found by Eriksson & Myrhage (2) in the cat

Table III. *Mid-level muscle width changes with preparation*

Each pair of values is from a separate rabbit

Muscle width		
Before preparation (μm)	After preparation (μm)	Change in width (μm)
4.1	2.4	−1.7
2.8	3.7	+0.9
4.1	4.9	+0.8
5.7	3.2	−1.5
4.2	3.6	−0.6
4.5	4.4	−0.1
3.6	4.8	+1.2
4.4	3.9	−0.5
4.1	4.2	+0.1
4.0	3.8	−0.2
5.0	3.3	−1.7
5.1	3.1	−2.0
3.7	4.0	+0.3
4.2	2.8	−1.4
Mean, 4.3 μm		Mean, 0.93 μm

tenuissimus is also present in the rabbit and accounts for the greater number of muscle fibres per unit area. It is also likely that a species difference also accounts for the lower value for fibre diameter of 36 μm found in this study. It is not likely that fibre shrinkage during preparation is responsible because great care was taken to ensure that the overall dimensions of the muscles actually used was unchanged by preparation and that there was no microscopic evidence of muscle fibre separation in the areas measured.

In the aerofoil shape of the rabbit tenuissimus muscle, cross section is treated as two triangles joined at their bases, its area can be estimated. Calculated from the mean values found for width and thickness (Table I) the cross-sectional area is 2.31 mm^2. With a muscle fibre density of 978 mm^{-2} a cross section would contain 2 259 fibres reflecting the rabbit muscle's greater size than the cat tenuissimus where Eriksson & Myrhage found 1 375 fibres in a cross section.

CONCLUSION

This study of the rabbit tenuissimus muscle provides information for comparison with already published data for the cat. No attempt has been made at fibre-typing the muscle, although the results suggest it to be of a similar mixed type to that

Table IV. *Distribution of fibre types in the tenuissimus muscle of the cat (Eriksson & Myrhage, 1972) (2)*

Type of muscle fibre	A	B	C	Mean value
Distribution of fibres, %	38	48	14	
Muscle fibre diameter, μm	55	41	26	44
Number of capillaries surrounding one muscle fibre	3.5	3.6	3.8	3.6

in the cat but with a different distribution of fibre types. Although physically more substantial than its counterpart in the cat, the rabbit tenuissimus is confirmed as a muscle of sufficiently small dimensions to be of value in microvascular research.

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