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Muscle fibre size and number following immobilisation atrophy*

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INTRODUCTION

Muscle mass is a function of the size and number of the muscle fibres. Immobilisation atrophy results in a decrease in muscle mass (Booth & Kelso, 1973*a*; Eccles, 1944; Lippman & Selig, 1928; Thomsen & Luco, 1944). This decreased muscle mass must be the result of a decrease of one or both of fibre size and fibre number. Most of the studies investigating the mechanism of skeletal muscle atrophy have focused on the measurement of fibre area. These studies have concluded that the degree of atrophy is specific to fibre type and its location in different muscles or muscle regions (Boyes & Johnston, 1979; Herbison, Jaweed & Ditunno, 1978; Jaffe, Terry & Spiro, 1978; Tomanek & Lund, 1974). Few studies have investigated the effect of immobilisation atrophy on muscle fibre number (Booth & Kelso, 1973*b*; Boyes & Johnston, 1979; Cardenas, Stolov & Hardy, 1977). Using counts from histological sections, Booth & Kelso (1973*b*) reported a 24% decrease in fibre number of the soleus muscle as a result of atrophy produced by hindlimb immobilisation. Cardenas *et al.* (1977), however, reported no change in fibre number in the soleus muscle and concluded that the results of Booth & Kelso (1973*b*) were in error due to the method of fibre enumeration.

The purpose of this study was to investigate the effects of immobilisation atrophy on skeletal muscle fibre number and fibre area, to determine the contribution to muscle atrophy of each of the major components of muscle mass. Muscle fibre number was determined by the nitric acid digestion method (Gollnick, Timson, Moore & Riedy, 1981) to avoid the problems associated with fibre counts from histological sections (Clark, 1931; Gollnick *et al.* 1983; Maxwell, Faulkner & Hyatt, 1974).

MATERIALS AND METHODS

Twelve male Sprague–Dawley rats, weighing between 250–300 g, were used in the study. Animals were maintained on a 12 hours light–dark cycle and food and water were provided *ad libitum*.

Animals were anaesthetised with intraperitoneal sodium pentobarbitone (45 mg/kg). The right forelimb was held fully extended while strips of gauze soaked with plaster of Paris were wrapped around the limb, covering it from shoulder to the base of the paw. All casts were carefully checked to see that blood flow was maintained to

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Table 1. *Fibre number and area of triceps brachii muscles from control and immobilised limbs of rats*

	Control	Immobilised	Difference (%)
Muscle weight (mg)	1823±54	1124±45*	38·0
Fibre number	31396±753	31098±590	1·0
Muscle weight (mg)	1507±103	819±96*	45·7
Fibre area (um²)	3510±267	1918±330*	42·1

* $P < 0{\cdot}001$.

the paw and the cast was then taped to the side of the body to prevent chewing. Casts were inspected daily and were replaced at four weeks or when needed. The contralateral limb was used as the control.

Animals were killed by carbon dioxide asphyxiation following eight weeks of immobilisation. The long head of the triceps brachii (LHT) was removed from both limbs and immediately weighed (to 0·1 mg) using a Mettler balance. Muscles to be used for fibre number determination were stored at −80 °C until analysed. Sections from the belly of the remaining muscles were frozen in isopentane cooled to the temperature of liquid nitrogen and stored at −80 °C until analysed for fibre area.

Atrophy was determined by comparison of muscle wet weight between immobilised and control limbs and was expressed as percentage of control. Muscle fibre number was determined by the nitric acid digestion method (Gollnick *et al.* 1981). Muscles were placed in a 15% nitric acid solution and heated for 45 minutes. They were then rinsed in distilled water and the outer fibres were teased off the muscle under a dissecting microscope. The inner part of the muscle was again placed in the nitric acid solution until all the connective tissue was digested. All the fibres were then teased apart and individually counted under a dissecting microscope.

Fibre area was determined from muscle sections cut in a cryostat at −20 °C. The sections were stained for myofibrillar ATPase following pre-incubation at pH 10·2 (Padykula & Herman, 1955). Fibre area was determined using a Bioquant Hipad Digitiser connected to an Apple computer. Mean fibre area was determined from measurement of 300 fibres for each muscle.

The immobilised and the contralateral control limbs were compared for mean fibre number and fibre area using *t* tests for dependent means.

RESULTS

Mean muscle atrophy, fibre number and fibre area are presented in Table 1. Eight weeks of immobilisation produced a 38·0% atrophy, as estimated by wet muscle weight, in the LHT of the animals used for the fibre number study. Mean fibre number was 31098±590 and 31396±753 for the muscles from the immobilised and control limbs respectively. This difference of 1·0% was not significant.

Immobilisation produced a 45·7% atrophy in the LHT of the animals used for the fibre area study. Mean fibre area was 3150±267 and 1918±330 μm^2 for the muscles of the control and immobilised limbs respectively. This difference of 42·1% was significant ($P < 0{\cdot}001$).

DISCUSSION

The results of this study indicate that there is no change in fibre number in the LHT following eight weeks of immobilisation. These data support the conclusion of previous studies (Boyes & Johnston, 1979; Cardenas *et al.* 1977); however, they are in contradiction to the study of Booth & Kelso (1973*b*). It has been reported that the use of histological sections for determination of fibre number, in studies of muscle enlargement, is not valid for most muscles (Gollnick *et al.* 1981; Timson & Dudenhoeffer, 1984; Timson, Bowlin, Dudenhoeffer & George, 1985). Gollnick *et al.* (1981) using the data of Binkhorst & van't Hof (1973) proposed a model indicating that the increase in fibre angle, necessary to compensate for the increased girth of the fibres that must take place without a change in total muscle length, resulted in an increase in the number of fibres present in a histological section made from the belly of the muscle. It would be logical to assume that fibre angle would decrease with atrophy to accommodate the decreased fibre girth at constant muscle length. In this case fibre number appearing in a histological section would decrease due to the change in fibre angle as opposed to an actual loss of muscle fibres in muscles where all the fibres do not run through the belly of the muscle. It has been assumed that histological sections made of the soleus muscle would include all its fibres because of the parallel architecture of this muscle. Cardenas *et al.* (1977) proposed a model indicating that there is only a small portion in the middle of the soleus muscle where a section can be made that will include all the fibres. This model was supported by the work of Timson *et al.* (1985) using counts from histological sections made at measured points along the length of the soleus muscle. These studies indicate that there is a window in the middle of this muscle that will include all the fibres in it. The decrease in fibre angle, associated with atrophy, would result in a decrease in the size of the window from which a section including all the fibres in the muscle could be made. The size of the window would be a function of the degree of atrophy. It is conceivable that the window could disappear with a large degree of atrophy. This explanation could possibly account for the decrease in fibre number in the soleus muscle reported by Booth & Kelso (1973*b*). The nitric acid digestion method for fibre enumeration circumvents the problems associated with the use of histological sections. By this method true fibre counts can be made and would eliminate confusion as to whether or not fibres are lost during atrophy. This method has recently been used by Templeton *et al.* (1988) and demonstrated no change in soleus muscle fibre number following atrophy produced by hindlimb suspension.

Fibre area in this study was 42·1 % less in the atrophied muscles compared to the contralateral controls. This corresponds very well with the 45·7 % decrease in muscle mass. These data, indicating that decrease in muscle mass can be totally accounted for by decrease in mean fibre area, further support the concept that muscle fibre number does not decrease as a result of immobilisation atrophy.

Concern has been expressed about the use of the contralateral limb as a control in studies of immobilisation atrophy (Booth, Nicholson & Watson, 1982). It is possible that the muscle of the control limb may be hypertrophied because of increased work during normal movement of the animal about the cage. On the other hand, it may be that the muscle in the contralateral limb atrophies due to the total decrease in activity observed in rats with immobilised limbs. Because both these possibilities were of concern to this study, muscle weight to body weight ratios were determined for the LHT muscles from six normal rats in the same body weight range as the experimental animals. These data indicated that there was no difference in muscle weight to body

weight ratios between LHT muscles of normal rats and the same muscles from the control limbs of the experimental rats. Therefore, the muscles of the control limbs of the experimental animals neither hypertrophied nor atrophied during the immobilisation period.

There has been some discussion in the literature concerning the quantification of muscle atrophy (Appell, 1986). Some studies have quantified atrophy on the basis of muscle mass (Cooper, 1972; Lippman & Selig, 1928; Maier Eldred & Edgerton, 1972; Thomsen & Luco, 1944), whereas others have quantified it on the basis of fibre cross sectional area (Boyes & Johnston, 1979; Edgerton *et al.* 1975; Herbison *et al.* 1978; Tomanek & Lund, 1974). The reason for choosing quantification based on cross sectional area is that this parameter is not affected by oedema or relative increases in connective tissue (Fischer & Ramsey, 1946; Williams & Goldspink, 1984) but these would not be accounted for in a measurement of muscle mass. Measurement of fibre cross sectional area, however, is not free of possible errors that would result in less than precise estimates of muscle atrophy (Edstrom & Torlegard, 1969; Aniansson, Grimby, Hedberg, & Krotkiewski, 1981; Song, Shimada & Anderson, 1963). These errors include muscle cuts not made perpendicularly to the muscle fibres as well as alterations in fibre size caused by fixation and staining procedures. The results of this study indicate that quantification on the basis of muscle mass or fibre cross sectional area yield closely similar values and therefore both methods would serve equally well for the determination of muscle atrophy.

SUMMARY

Muscle fibre number and cross sectional area were studied in the response to immobilisation atrophy of the long head of the triceps brachii. Following eight weeks of immobilisation, fibre number of the muscle from the immobilised limb was compared to that of the contralateral control limb in six rats. Mean fibre cross sectional area of the LHT from the immobilised limb was compared to that of the contralateral control for another six animals. Atrophy, as estimated by a decrease in wet muscle weight, was 38·0% for the group used for fibre number estimations and 45·7% for the group used for fibre area. Fibre counts revealed no difference between muscles from immobilised and control limbs. Mean fibre area was 42·1% less for the muscle from the immobilised limb compared to the control limb. The results of this study indicate that atrophy of the LHT produced by immobilisation of the forelimb is the result of atrophy of the muscle fibres without a decrease in muscle fibre number.

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