J. Anat. (1990), **173**, pp. 33–36
Printed in Great Britain

Skeletal muscle fibre number in the rat from youth to adulthood*

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(*Accepted* 1 *May* 1990)

INTRODUCTION

It has been suggested that skeletal muscle fibre number in man decreases throughout life from birth to old age (Grimby & Saltin, 1983; Rice, Pettigrew, Noble & Taylor, 1988). Others, however, suggest that muscle fibre number does not begin to decrease until 60 years of age (Sato *et al.* 1984; Green, 1986). Similar reports in animals have indicated a decrease in skeletal muscle fibre number from youth to adulthood in rats (Rayne & Crawford, 1975; Caccia, Harris & Johnson, 1979), guinea-pigs (Faulkner, Maxwell, Brook & Lieberman, 1971), dogs (Ihemelandu, 1980), pigs (Staun, 1963) and cattle (Bendall & Voyle, 1967). Conversely, Rowe & Goldspink (1969) found no change in muscle fibre number from one day to six months of age in mice.

The likely explanation for the inconsistency in the results from these studies resides in the inability to determine accurately the total number of muscle fibres from muscle cross-sections. The problems associated with this and other methods of total fibre number estimation have been a topic of discussion for a number of years (Clark, 1931; Maxwell, Faulkner & Hyatt, 1974; Rayne & Crawford, 1975; Gollnick *et al.* 1983; Timson & Dudenhoeffer, 1984) but these problems have now been resolved with the advent of the nitric acid digestion technique by Gollnick, Timson, Moore & Riedy (1981). The purpose of the present study was to investigate the relationship of age to skeletal muscle fibre number during the period from youth to adulthood in the rat.

MATERIALS AND METHODS

Twelve male Sprague–Dawley rats were used in the study. At 25 days of age the animals underwent surgical removal of the soleus and tibialis anterior muscles from one hindlimb. Surgery was performed under sterile conditions with the animals anaesthetised with sodium pentobarbitone (45 mg/kg). A longitudinal incision was made along the lateral aspect of the lower hindlimb. The soleus muscle was isolated from beneath the gastrocnemius and plantaris muscles and was removed. The tibialis anterior muscle was removed from the anterior portion of the leg through the same incision. The muscles were weighed and stored at −80 °C until analysis. The incisions were closed with silk suture. Following recovery from the anaesthesia the animals were returned to their cages. They were housed in wire-bottomed cages on a 12 hourly light–dark cycle and food and water were provided *ad libitum*. At 180 days of age 6 of the animals were killed by cervical dislocation and the soleus and tibialis anterior

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muscles from the contralateral leg were removed and treated as described above. The same procedure was carried out on the remaining 6 animals at 365 days of age.

Fibre number for the soleus muscles was determined by the nitric acid digestion method (Gollnick *et al.* 1981). The muscles were placed in a 15% nitric acid solution for 4–6 hours to digest the connective tissue. They were then washed thoroughly and placed in a dish containing distilled water. Individual fibres were teased from the muscle under a dissecting microscope and counted. Fibre number for the tibialis anterior muscles was determined by the mean fibre dry weight estimation method (Timson & Dudenhoeffer, 1984) because of the much larger number of fibres in this muscle. Bundles of fibres were carefully separated from the muscles following nitric acid digestion and placed in groups of similar length. Approximately 400–500 fibres were counted from each group, as described for the soleus muscles. These fibres and the remaining bundles from each group were dried to a constant weight in an oven at 80 °C and weighed to 0·1 μg using a Cahn electrobalance. The mean fibre dry weight for each group was determined from the fibres that were counted. The number of fibres in the remainder of the group was estimated by dividing the mean fibre dry weight into the dry weight of the remainder of the group. Total fibre number was the sum of all the groups.

Fibre number differences for each muscle between 25 and 180 days of age and 25 and 365 days of age were analysed using paired *t* tests.

RESULTS AND DISCUSSION

Body weights and muscle weights and fibre numbers for the soleus and tibialis anterior muscles are shown for the 180 days old and 365 days old rats in Table 1. There was no difference in fibre number for either muscle between 25 days of age and 180 days of age or between 25 days of age and 365 days of age.

The significance of whether or not there is a reduction in skeletal muscle fibre number during the time period from youth to adulthood relates to the growth potential of the animal. Muscle size in the adult animal is primarily a function of muscle fibre number (Aberle & Doolittle, 1976; Luff & Goldspink, 1970); therefore, a decrease in fibre number would result in a decrease in growth potential of the animal. The results of this study support the work of Rowe & Goldspink (1969) indicating that there is no change in skeletal muscle fibre number from the early postnatal period until adulthood. It does not, however, support the findings of others indicating a decrease in fibre number during this period (Bendall & Voyle, 1967; Caccia *et al.* 1979; Faulkner *et al.* 1971; Ihemelandu, 1980; Rayne & Crawford, 1975; Staun, 1963).

A possible confounding factor in the interpretation of the data presented in these previous studies is the use of indirect methods for the determination of muscle fibre number. A major problem arises when fibre number is determined from counts of histological sections of skeletal muscle following periods of change in muscle size (Gollnick *et al.* 1983). The result is a decrease in apparent fibre number when there is muscle fibre atrophy (Nicks, Beneke, Key & Timson, 1989) and an increase in apparent fibre number when there is fibre hypertrophy (Gollnick *et al.* 1981) at constant muscle length. The reason for changes in the number of fibres in a histological section is that there is a change in the angle of the fibres relative to the long axis of the muscle resulting in more (hypertrophy) or fewer (atrophy) fibres appearing in the section. It is likely that during periods of rapid body growth, when the length of the muscle is increasing more rapidly than the cross-sectional area of the muscle fibres, the fibre angle relative to the long axis of the muscle would decrease. This would

Table 1. *Body weight, muscle weight and fibre number for* 180 *days and* 365 *days groups*

	Body weight (g)	Muscle weight (mg)		Fibre number	
		Soleus	Tib. ant.	Soleus	Tib. ant.
25 days	60·7 ±7·1	21·7 ±3·2	9·4 ±12·1	3035 ±130	17295 ±643
180 days	427·9 ±12·3	190·5 ±13·4	814·8 ±17·5	3069 ±123	17284 ±506
25 days	66·5 ±4·7	25·9 ±3·1	110·6 ±10·7	3179 ±66	17931 ±360
365 days	480·4 ±9·2	184·0 ±5·8	856·4 ±21·5	3173 ±66	18060 ±512

result in a decrease in the number of fibres appearing in a histological section of the muscle similar to that which occurs during muscle atrophy. The studies of Rayne & Crawford (1975) and Ihemelandu (1980) attempted to circumvent this problem by choosing muscles that would likely include all fibres in a histological section because of their architecture and small size. However, it cannot be certain that all fibres are included even in muscles of this type (Cardenas, Stolov, & Hardy, 1977; Timson, Bowlin, Dudenhoeffer, & George, 1985). The nitric acid digestion method of muscle fibre number determination used in this study eliminates the problems associated with the histological section method. Because of the utilisation of the nitric acid digestion method for muscle fibre number determination in this study the conclusion that muscle fibre number does not change from youth to adulthood in the rat can be made with a relatively large degree of confidence.

SUMMARY

The purpose of this study was to determine whether or not there was a decrease in skeletal muscle fibre number in the rat from the early postnatal period to adulthood. Twelve male Sprague–Dawley rats were used in the study. At 25 days of age the animals underwent surgical removal of the soleus and tibialis anterior muscles from one hindlimb. At 180 days of age, 6 of the animals were killed and the soleus and tibialis anterior muscles from the contralateral leg were removed. The same procedure was carried out on the remaining 6 animals at 365 days of age. Fibre number was determined by the nitric acid digestion method for the soleus muscles and the mean fibre dry weight method for the tibialis anterior muscles. There was no difference in fibre number for either muscle between 25 days of age and 180 days of age or between 25 days of age and 365 days of age. The results of this study indicate that there is no change in skeletal muscle fibre number from the early postnatal period until adulthood in the rat.

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