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Satellite cell content in muscles of large and small mice

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ABSTRACT

Quantitative analyses of the satellite cell content in the biceps brachii muscle of mice genetically selected for high (QL) and low (QS) bodyweight showed that selection alters the total number of satellite cell nuclei rather than the relative proportions of nuclei in the myofibre and satellite cell populations. These findings are in accordance with those previously published for other tissues of these mice and support the hypothesis that regulatory mechanisms remain unaltered by selection pressure. Size at birth, however, is a reflection of nutritional status as well as genetic background, and comparisons between differently sized littermates within each of the lines showed a significant increase in satellite cell density in larger compared with smaller individuals. These differences between littermates were not accompanied by any alteration in myofibre nuclear density. It is therefore suggested that whilst both genetic and nutritional factors exert their effects on muscle growth through an influence on satellite cell division, both do so at different stages during the programme of satellite cell differentiation.

INTRODUCTION

The role of nuclear proliferation during the postnatal growth of muscle was originally recognised through the work of Enesco & Puddy (1964) who showed that fibre hypertrophy was accompanied by an increase in the total number of nuclei. Extensive autoradiographic studies carried out both in vivo and in vitro have since identified the satellite cell as the sole source of nuclei added to the muscle fibre postnatally (Moss & Leblond, 1971; Bischoff, 1975, 1986*a*, *b*). Since between 50% and 99% of the total nuclear content of the muscle accumulates postnatally (Allen et al. 1979), and there is evidence to suggest that DNA accretion precedes protein accretion (Moss et al. 1968*a*, *b*), it seems that the satellite cell population represents some considerable potential for the postnatal growth of skeletal muscle.

Satellite cells characteristically lie wedged between the sarcolemma and basal lamina of the muscle fibre (Mauro, 1961). In young fast growing animals these cells display a high rate of division and may contribute up to 32% of the total myogenic nuclear population (Schultz, 1974; Cardasis & Cooper, 1975). However, during the early stages of postnatal growth, more than 50% of the cells produced by satellite cell division become incorporated into the muscle fibre, thereby successively reducing the satellite cell percentage to around the 4% level seen in the adult (Schultz, 1972, 1974; Ontell, 1974; Cardasis & Cooper, 1975). Whereas the quantitative differences associated with growth are well documented, the contribution made by this cell population to genetically determined differences in muscle mass has remained unclear.

The present study therefore set out to compare the satellite cell population in the biceps brachii muscle of Q strain mice genetically selected for high (QL) and low (QS) bodyweight (Falconer, 1973). These mice diverge in size around the time of birth (Rucklidge, 1981), after which their markedly different growth rates result in animals differing by as much as 63% in adult bodyweight. This pattern of growth suggests that comparison of high and low birthweight individuals both within and between each of the lines provide a situation for determining whether actual body size or genetic potential is more important in determining the satellite cell content of a given muscle. Biceps brachii was selected for such a study since fibre

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Table 1. *Transverse sectional area of muscle fibres in biceps brachii of newborn QL and QS mice*

Line	Littermate	Mean transverse sectional area*
QL	Large	71.67±5.06
QL	Small	73.06±11.6
QS	Large	79.16±4.31
QS	Small	84.24±11.8

* Figures represent the mean(±S.E.M.) of measurements taken from 4 individuals.

formation is complete by the time of birth, thereby avoiding any confusion between myoblasts, which will form new fibres, and satellite cells.

MATERIALS AND METHODS

The largest and smallest littermate, on the basis of weight, were selected at birth from each of 5 litters of high bodyweight (QL) and 5 litters of low bodyweight (QS). Animals were killed by decapitation and all limbs initially fixed in the resting position for 15 min in 0.1 M sodium cacodylate buffer, pH 7.3, containing 2% glutaraldehyde. The muscle was then dissected from the limb, fixed for a further 3 h in 2% glutaraldehyde, washed in cacodylate buffer for 1 h, fixed for 1.5 h in an aqueous solution containing 0.6% osmium tetroxide and 0.4% potassium ferrocyanide (Aguas, 1982), washed in distilled water, dehydrated through graded acetones up to 100%, infiltrated and embedded in Araldite resin (CY212) which was polymerised at 60 °C for 4–6 d.

Before comparisons could be made regarding satellite cell content, measurements of fibre transverse sectional area and nuclear lengths had to be made since any differences in these parameters would affect the estimations of satellite cell content.

Semithin (1 μm) sections were taken from the midbelly of the muscle and stained with 1% methylene blue. Measurements of transverse sectional area were made on 150 fibres selected across the girth of each sectioned muscle using a Kontron MOP Videoplan interactive image analysis system. Measurements of nuclear length in 1 littermate from each of the QL and QS lines were carried out on 200 nuclei sectioned in 1 μm longitudinal sections. Ultrathin transverse sections (90 nm) were taken from the midbelly portion of each muscle and collected on 100 mesh copper grids. Sections were stained in 1% uranyl acetate (40 min) followed by Reynolds (1963) lead citrate (5 min), and examined using a JEOL 1200X electron microscope operating at an accelerating voltage of 80 kV. Randomly selected areas of muscle were chosen and 200 fibres evaluated for the presence of myofibre or satellite cell nuclei. The criteria used for the identification of satellite cells were those employed by Ontell (1977), namely, the presence of a heterochromatic nucleus compared with that of the myofibre, enclosure within the basal lamina of the fibre, close apposition to the surface of the fibre and a complete absence of myofibrils (Fig. 1).

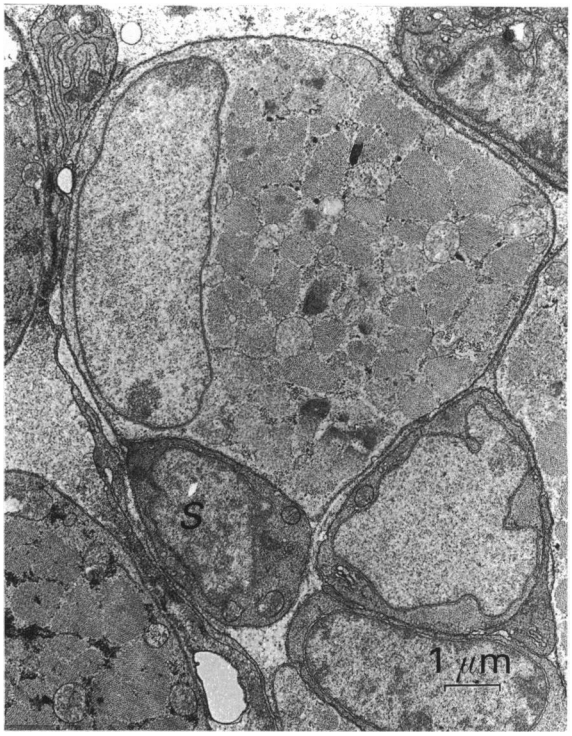

Fig. 1. Transverse section of a myofibre and associated satellite cell (S) in the newborn biceps brachii muscle.

RESULTS

Morphological description of satellite cells in the newborn QL and QS

Figure 1 shows a single mature myofibre with an attendant satellite cell typically found in the newborn mouse biceps brachii. The euchromatic myofibre nucleus, which is peripherally located within the fibre, contrasts with the more heterochromatic satellite cell nucleus. Both satellite cell and myofibre are enclosed within a common basal lamina, none of which penetrates between the closely opposed membranes of each cell. Also clearly identifiable is an immature myotube closely apposed to an adjacent myofibre. This myotube appears to be similar to the satellite myotubes previously described by Ontell & Dunn

Table 2. *Satellite cell content of biceps brachii in newborn QL and QS*

Line	Relative size of littermates	n	Bodyweight	Incidence of myofibre nuclei*	Number of satellite cell nuclei per 200 fibres	% Number of nuclei within the satellite cell population
QL	Large	5	1.8±0.07	0.36±0.02	25.60±2.3	26.2±1.3
	Small	5	1.4±0.1	0.31±0.03	17.46±1.8	22.4±2.5
QS	Large	5	1.42±0.03	0.35±0.03	21.98±2.76	23.6±2.8
	Small	5	1.26±0.04	0.33±0.02	12.38±1.48	16.2±1.7

* Population of myofibres containing a muscle nucleus in section.

(1978) in newborn rat muscle. The centrally located nucleus is indistinguishable from that of the adjacent myofibre, and myofibrils are scattered throughout the cytoplasm. The larger gap between this myotube and its parent myofibre, accentuated in this case by a cellular process, acts as a further feature which distinguishes the satellite myotube from the satellite cell.

Measurements of fibre transverse sectional area

A 2-way analysis of variance showed that fibre transverse sectional area was not significantly different between either the QL or QS or the large and small littermates. The means±S.E.M. are shown in Table 1.

Nuclear length measurements

Nuclear length in longitudinal section was not significantly different between the 2 lines as determined by a U test of the length distributions of 200 randomly selected nuclei (the mean values for the QL and QS were 10.24±0.19 and 9.824±0.21 μm respectively).

Comparisons of satellite content in the 2 lines could therefore be made assuming the absence of any bias due to differences in fibre transverse sectional area or nuclear length.

Table 2 summarises the nuclear content of biceps brachii for 5 large and 5 small individuals taken from each of the QL and QS lines. The incidence of myofibre nuclei (proportion of fibres displaying a fibre nucleus in transverse section) was not significantly different either between the QL and QS or between the large and small littermates (see Table 3).

The percentage of satellite cell nuclei relative to the number of myonuclei was not significantly different between the QL and QS, although there was a significant difference between large and small littermates (Tables 2, 3). Since there were no significant differences in the myonuclear population either between the lines or littermates this follows the same trend as the number of satellite cell nuclei per 200 fibres.

Table 3. *Results of 2-way analysis of variance to test for the effect of line (QL/QS) and littermate size (large/small) on nuclear content in biceps brachii in the newborn*

	QL/QS	Large/small
Incidence of myofibre nuclei	ns	ns
Number of satellite nuclei per 200 fibres	ns	Significant (1%)
% Number of nuclei within the satellite cell population	ns	Significant (2.5%)

ns, nonsignificant.

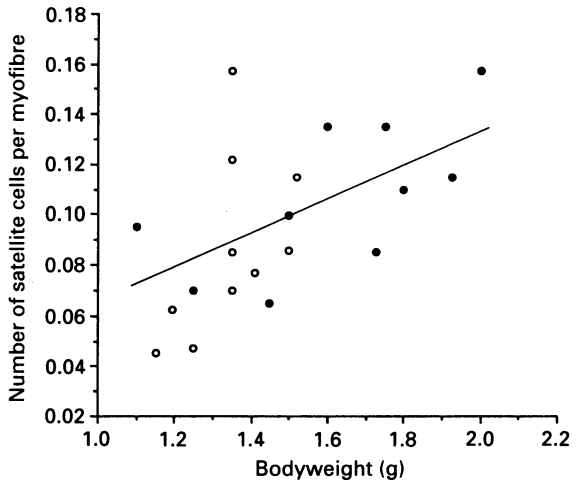

Fig. 2. Relationship between the number of satellite cells per myofibre and body weight in newborn QL (●) and QS (○) mice. The line is the calculated regression line for the relationship in the QL mice.

The number of satellite cell nuclei observed in a transverse section of 200 fibres was converted to the number of satellite cells per myofibre and presented graphically in Figure 2. The regression equations which describe the relationship between the number

Table 4. *Percentage number of satellite cell nuclei associated with myofibre nuclei*

Line	Relative size	% of satellite cell nuclei*
QL	Large	27.2±5.08
QL	Small	24.4±4.18
QS	Large	27.6±4.4
QS	Small	17.6±4.12

* Based on counts of 200 fibres.

Table 5. *Contingency table to test for the preferential association of satellite with myofibre nuclei*

	No. of myofibres without a myofibre nucleus	No. of myofibre with a myofibre nucleus	Total
No. of fibres with a satellite cell nucleus	266 [300]	95 [61]	361
No. of fibres without a satellite cell nucleus	2730 [2696]	509 [543]	3239
Total	2996	604	3600

Expected values assuming no association between the 2 populations of nuclei are shown in square brackets.

of satellite cells per myofibre (N) and birthweight (BW) were as follows:

QL: $N = -0.0087 \pm 0.0714\ BW$ Rsq 44.9%,
QS: $N = 1.17 \pm 2.02\ BW$ Rsq 34.6%.

A t test of the regression coefficients showed that these 2 parameters were significantly associated within the QL ($P = 0.034$) but not the QS ($P = 0.074$). Accordingly only the QL regression line is shown in Figure 2.

Association of satellite nuclei with myofibre nuclei

Table 4 shows the mean percentage number of satellite nuclei which were associated in the same section with a myofibre nucleus in 5 large and small littermates taken from each of the QL and QS lines. Analysis of these data (1-way ANOVA after angular transformation) showed there to be no significant difference between the number of satellite nuclei associated with myofibre nuclei in either of these groups. It was therefore considered appropriate to combine the data to test whether there was a preferential association between satellite and myofibre nuclei irrespective of animal group (Table 5). A χ^2 analysis carried out on the data was highly significant ($P < 0.001$), providing strong evidence of a preferential association between satellite and myofibre nuclei.

DISCUSSION

Comparisons between the QL and QS

During the early stages of postnatal growth the density of satellite cells may either increase or decrease, depending on the growth pattern of the muscle (Gibson & Schultz, 1983). This has led to the suggestion that each satellite cell division does not regularly result in one daughter cell fusing with the fibre and the other continuing as a satellite cell (Campion, 1984). Interestingly, selection for divergent body size seems not to have altered this pattern of fusion, since the proportion of muscle nuclei belonging to the satellite cell population was the same in both the QL and QS. Indeed, previous work in the high and control bodyweight lines of the Japanese quail found that during growth the number of satellite cells increased in proportion with the length of the muscle fibres, thereby effecting no change in the percentage of satellite nuclei between the lines at any age (Campion et al. 1982*b*). Nonetheless, the total nuclear content of the biceps brachii at birth in these mice does differ as a consequence of the divergence which they show in fibre number (Penney et al. 1983). This situation has been shown in the Japanese quail (*Coturnix coturnix japonica*) where selection for high body weight results in an increase in fibre number and length (Fowler et al. 1980) but not nuclear density which is unchanged (Campion et al. 1982*b*). It therefore seems that in both mice and quails a genetically determined increase in muscle weight is accomplished through an increase in the number of satellite cell segments (a single satellite cell plus the number of nuclei contained in the adjacent segment of fibre) rather than through any alteration in the satellite cell segment size.

A number of authors have noted an association between the nuclei of the myofibre and those of the attendant satellite cell (Ontell, 1974; Schmalbruch & Hellhammer, 1977). These muscle and satellite cell nuclei 'doublets' were suggested by Ontell (1974) to come about as a result of one daughter cell of a satellite cell division fusing with the fibre, and the other remaining within the satellite cell population. In the present study between 23% and 28% of the satellite nuclei were found to be associated with the nuclei of the principal fibre, a proportion which did not differ significantly between the QL or QS. Indeed, if the hypothesis of Ontell (1974) is correct, no

difference between the QL and QS would be expected, since the proportion of satellite cells which are incorporated into the muscle fibre after each round of division appears not to be altered by selection.

Cell proliferation plays an important role in muscle growth both prenatally, when the number of myoblasts available for fusion may determine the number of fibres that form (Penney et al 1983), and postnatally when DNA accretion becomes a fundamental process in fibre hypertrophy (Allen et al. 1979; Campion et al. 1982*b*; Purchas et al. 1985). The evidence presented here suggests that differences in fibre number and length between the QL and QS at birth must be the consequence of alterations in the rate of cell proliferation prenatally. However, confirmation of this awaits a study of prenatal development in these mice.

Comparisons between large and small littermates

Satellite cell density tended to be correlated with body weight although this was only significant in the QL line, with the largest (heaviest) littermate containing the highest density of satellite cell nuclei. Since both the QL and QS were inbred, thereby minimising genetic heterogeneity between littermates, a large proportion of this variation may be attributed to the intrauterine environment (McLaren, 1965). Such intrauterine effects are due to the variation in the blood supply at different positions within the uterine horn (McLaren & Mitchie, 1960), and appear to be manifested principally during the latter half of gestation when the nutritional demands of the fetus are greatest (Widdowson, 1980). The smallest littermate within an inbred line of mice may therefore be assumed to be small as a result of an inadequate plane of nutrition. It is consequently interesting to note that the effects of undernutrition appeared to be manifested principally in a reduction in the density of satellite cells, whilst the density of myonuclei remained unchanged. This latter observation might have been expected in view of the close parallel which has been shown to exist between DNA accretion and protein synthesis during muscle growth (Moss, 1968*a*; Allen et al. 1979). However, differences in fibre length (and therefore the total number of myonuclei) were evident between divergently sized littermates (personal observation), which implied that an alteration in the density of satellite cells had affected the number of nuclei able to fuse with the growing fibre. Whilst a reduction in DNA accretion as a response to an adverse nutritional environment has been noted by a number of other investigators (Moss, 1968*b*; Cheek et al. 1971; Campion et al. 1982*a*), a direct relationship between this response and the number of satellite cells has not previously been demonstrated.

The embryological origin of the satellite cell population remains unclear, although Cossu et al. (1988) claim to have identified this cell type at 16 d gestation in the mouse. Whilst such results require further confirmation, it is interesting to note that the appearance of this cell population during the later stages of gestation coincides with that period of fetal life which is most associated with nutritional constraint. It might therefore be envisaged that the reduced nuclear content of the smallest littermate came about as a direct consequence of a gradual reduction in the rate of satellite cell division during the later stages of gestation, a reduction which in itself was determined by the level of nutrition of each fetus.

Conclusions

The muscle mass attained by any given individual is dependent on both genetic and nutritional factors, both of which appear to operate through an effect on the availability of nuclei. Other factors such as mechanical activity, which may act postnatally, are not considered in this investigation. Quantitative analyses of the satellite cell content of mice which display genetically determined differences in bodyweight indicate that selection fails to alter the relative proportions of nuclei in the myofibre and satellite cell compartments. In contrast comparisons between differently sized littermates show an increase in the density of satellite cell nuclei within the larger individuals. This appears to suggest that adverse nutritional circumstances may alter the rate of cell division within the satellite cell compartment, although as the proportion of myonuclei sectioned within the fibres seems to show, this exerts no effect on the density of nuclei within the fibre itself. These findings are in general agreement with the hypothesis proposed by Falconer et al. (1978) which suggests that whilst selection for divergent bodyweight may act upon component cellular processes, such as the rate of cell division, the regulatory mechanisms, i.e. those which determine such factors as the proportion of nuclei which fuse with the fibre, remain unchanged.

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