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Muscle at birth in mice selected for large and small body size

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ABSTRACT

Selection for divergent body weight at 6 wk of age in the Q strain mouse has produced large (QL) and small (QS) mice which differ 2-fold in their adult body weight. The purpose of this investigation was to identify some of the cellular mechanisms which underlie the early divergence in size between the 2 lines. At birth, QL mice (for similar litter sizes) were 28% heavier and 6% longer than QS mice. This was reflected by measurements of longitudinal bone length which were greater in QL (tibia 6.2%, humerus 4.2%) compared with QS mice. Fibre number was found to be 18 and 17% greater in the biceps brachii and soleus muscles respectively of the QL mice. It was concluded that this was not a consequence of any alteration in the ratio of developing secondary to primary myofibres in either muscle. Fibre cross-sectional areas were only significantly different between the QL and QS for the soleus muscle, which might be explained by the relatively greater divergence in the length of its supporting bone (tibia) between the QL and QS compared with the humerus. Estimates of nuclear number showed that there were significantly more nuclei in biceps brachii muscle of QL than in the QS mice which could be attributed to the difference in fibre number, although no such differences were found for the soleus muscle. There was no apparent alteration in the proportion of nuclei found within the fibres of the biceps muscle. Overall the results indicate that selection in this situation has acted through the normal cellular processes of growth.

Key words: Q strain mice; growth.

INTRODUCTION

Selection for divergent body weight at 6 wk in the Q strain mouse has produced animals which differ 2-fold in their adult body weight (Falconer, 1973). Furthermore, it has been shown that this selection procedure produces the correlated response of an increase in growth rate both before and after weaning (Rucklidge, 1981). Whilst analyses of the body composition of these mice seem to indicate that the 2 lines show similar patterns of postnatal growth (Rucklidge, 1981), quantitative changes in total DNA and protein:DNA ratios fail to define the cellular mechanisms which underlie genetically determined differences in muscle mass.

We have shown that primary myotube number is significantly increased within the biceps brachii of QL compared with QS mice (Brown & Stickland, unpublished results). These observations, in the absence of any detectable difference in nuclear density, suggested that selection had altered the rate of myoblast proliferation thereby affecting the number of cells available for fusion. However, there is increasing evidence to suggest that primary and secondary myotubes are derived from separate myogenic lineages (Miller & Stockdale, 1986), each of which could respond differently to selection pressure. Indeed previous work in the pig seems to indicate that primary and secondary generation myotubes contribute unequally to genetically determined alterations in fibre number (Handel & Stickland, 1984).

Fibre number is not the only variable altered by selection for divergent body weight; alterations in muscle length and fibre transverse sectional area are also evident in mature mice (Hooper, 1978). Aspects of fibre size may, however, be very responsive to environmental influences and functional demand. Many of these influences are minimal up to the time of

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birth such that any fibre size differences seen at birth may be more indicative of direct genetic effects. The present investigation therefore concentrates on the relative importance of fibre and nuclear number, fibre transverse section area and fibre length in the newborn QL and QS, with the aim of identifying some of the cellular mechanisms underlying the early divergence in size between the 2 lines.

MATERIALS AND METHODS

Relationships between litter size and pup mean body weight in the 2 lines were assessed on the basis of measurements made from 11 QL and 29 QS newborn litters. Crown–rump length and body weight were specifically measured in 24 QL and 44 QS randomly selected littermates. For analyses of muscle transverse sectional area, fibre number and fibre size, the largest, average and smallest littermates, on the basis of weight, were selected at birth from each of 5 litters of high body weight (QL) and 5 litters of low body weight (QS).

Animals were killed by decapitation and all skinned limbs were initially fixed in the resting position for 15 min in 0.1 M sodium cacodylate buffer pH 7.3 containing 2% glutaraldehyde. Biceps brachii was dissected from the forelimb, 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 acetone up to 100%, infiltrated and embedded in Araldite resin (CY212) which was polymerised at 60 °C for 4–6 d. Lower hind limbs were processed intact but skinned due to the difficulty in dissecting the newborn soleus muscle.

Semithin sections (1 μm) were taken from the midbelly of the biceps brachii muscles and stained with 1% methylene blue. The midbelly region of each soleus muscle was assessed by sequential sectioning through a sample fore and hindlimb until the muscle displayed its maximal girth, at which point the relative position down the limb was noted together with the relative sizes of adjacent muscles. Subsequent limbs were then sectioned accordingly. In the absence of serial sectioning through each hindlimb this method was deemed to be the most accurate way of assessing the midbelly region of each soleus muscle. Ultrathin transverse sections (90 nm) taken from the same region of each muscle as the semithin (1 μm) sections were collected on 100 mesh copper grids. Sections were then 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.

Since individual fibres were difficult to distinguish in the centre of biceps brachii with the light microscope, low power (×250) electron micrographs of randomly selected areas were used to determine fibre and nuclear density. Total fibre and nuclear numbers were then calculated using total transverse sectional area of the muscles measured by light microscopy. Whilst this method may lead to inaccuracies as a consequence of the differential rate of stretching in the semithin and ultrathin sections, these were assumed to be less than those which might result from direct fibre counts on semithin sections. Moreover, any error incurred would be expected to be the same within the QL and QS. The proportion of nuclei in the myofibres was evaluated on electron micrographs of more than 200 fibres taken at ×800. The relative proportion of satellite to myofibre nuclei has previously been shown not to be significantly different between these 2 lines of mice (Brown & Stickland, 1993). Satellite cell nuclei were therefore not counted separately and due to their close apposition to the muscle fibre were included in the counts of myofibre nuclei. Capillary nuclei were not included in any estimations of nuclear number.

Fibre transverse sectional area measurements in biceps brachii were carried out on low power electron micrographs taken at ×400 and ×800 which were calibrated using a grid ruled at 1200 lines/mm. Measurements of areas of at least 200 fibres from each muscle were made using an Apple IIe computer (VIDS 11 analytical measuring system). Measurements of fibre transverse sectional area in soleus were carried out on light microscope images of 1 μm semithin sections using a Kontron MOP interactive image analysis system; 200 fibres were measured across the entire girth of each muscle.

Ossified bone length was assessed in the fore and hindlimb of randomly selected newborn QL (20 forelimbs and 17 hindlimbs) and QS (36 forelimbs and 19 hindlimbs) using microradiography. Radiographs of limbs previously fixed in formalin were made using a Hewlett Packard Faxitron x-ray machine with an exposure of 40 kV, 2.5 mA for 15 min onto Kodak Spectroscopic plates. Development was carried out in D19 (Kodak) for 10 min. Negatives were subsequently printed and the length of ossified bone measured using the Kontron MOP Videoplan image analysis system. A scale bar on the original radiograph provided the basis for calibration. Whilst this measurement did not represent the actual bone length, it was considered the most accurate assessment of this variable in view of the size of the specimens.

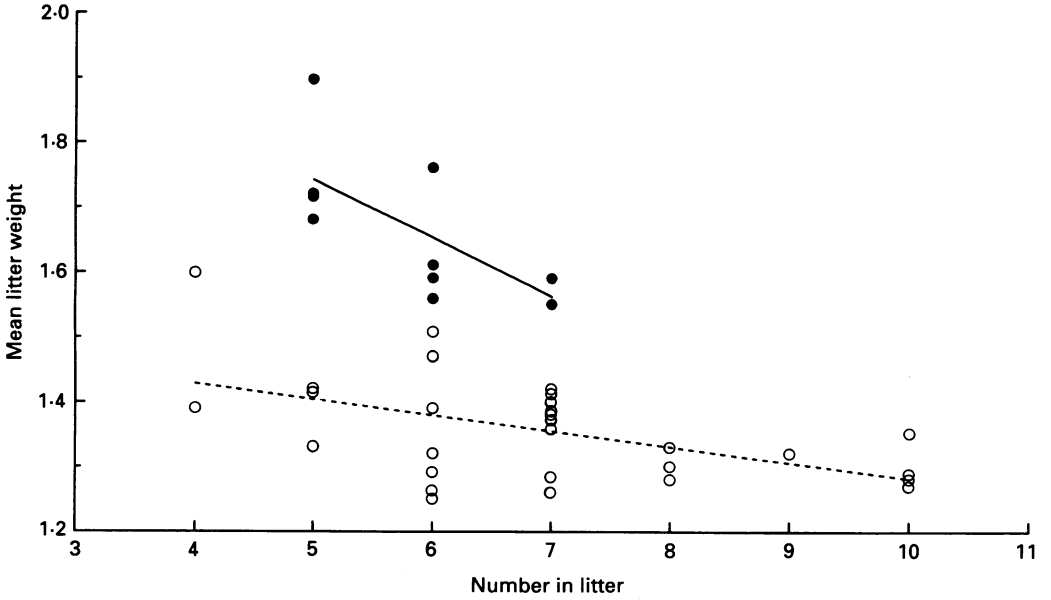

Fig. 1. Relationship between mean pup weight and number in the litter in the QL and QS mice. The regression equations for the QL and QS were $y = 2.19 - 0.09x$ ($r^2 = 0.5$) and $y = 1.5 - 0.02x$ ($r^2 = 0.3$), respectively. ●—●, QL; ○---○, QS.

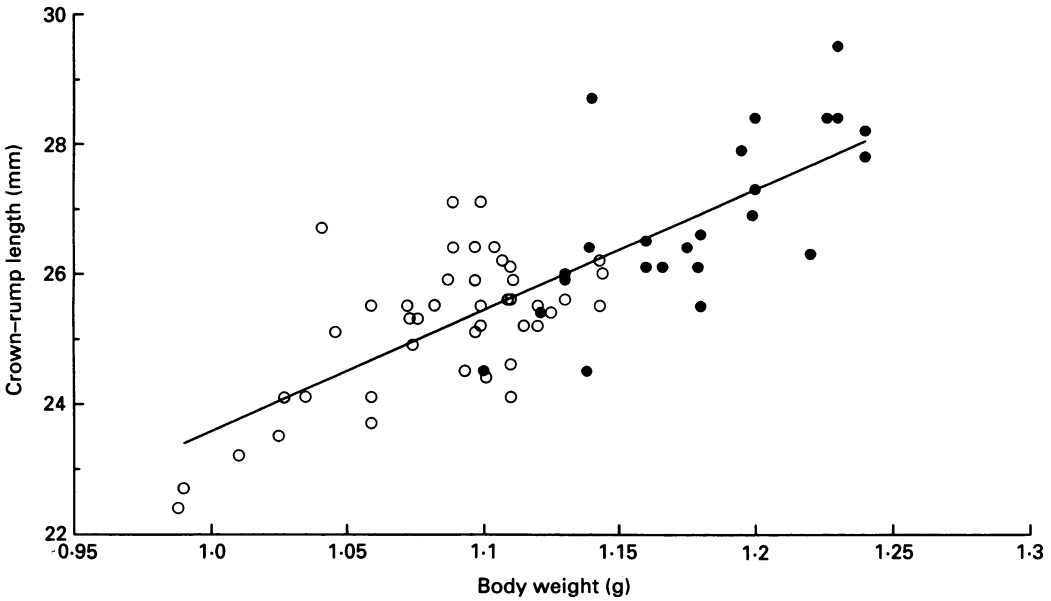

Fig. 2. Relationship between crown–rump length and body weight in the newborn QL and QS mice. The regression equations for the QL and QS were $y = -0.8 + 23.4x$ ($r^2 = 0.5$) and $y = 5.6 + 18.06x$ ($r^2 = 0.4$), respectively. ●, QL; ○, QS; —, QL/QS.

RESULTS

Birth weight in the QL and QS mice

There appeared to be no significant difference in gestational age between the QL and QS. Figure 1 shows the relationship between litter size and mean pup weight for 11 QL and 29 QS litters.

A comparison of randomly selected QL (n = 24) and QS (n = 44) showed that both crown–rump length and body weight were significantly different between the 2 lines at birth ($P \leqslant 0.001$). However, in view of the above correlation between litter size and mean litter weight, comparisons of crown–rump length and weight were carried out on 4 litters matched for litter size from each of the lines. These results confirmed the findings on randomly selected individuals ($P \leqslant 0.01$). Crown–rump length was increased by 6% in the QL relative to the QS, whilst the corresponding increase in body weight was 28%.

Crown–rump length in newborn QL and QS mice

Figure 2 shows the relationship between crown–rump length and body weight in newborn QL and QS mice.

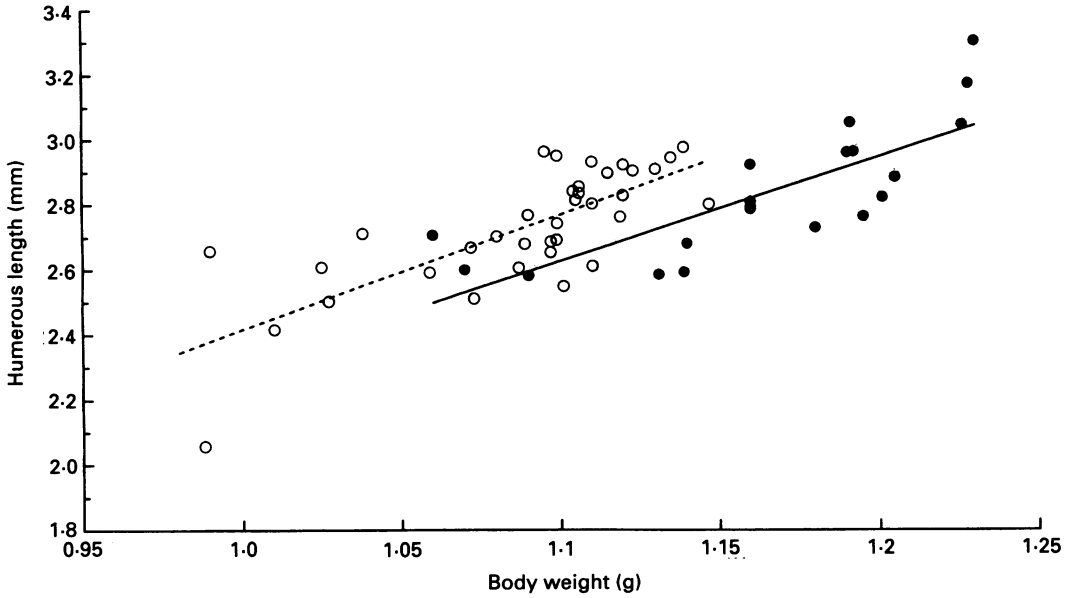

Fig. 3. Relationship between the length of the humerus and body weight in the newborn QL and QS mice. The regression equations for the QL and QS were $y = 0.9 + 3.2x$ ($r^2 = 0.6$) and $y = -1.11 + 3.5x$ ($r^2 = 0.6$), respectively. ●—●, QL; ○---○, QS.

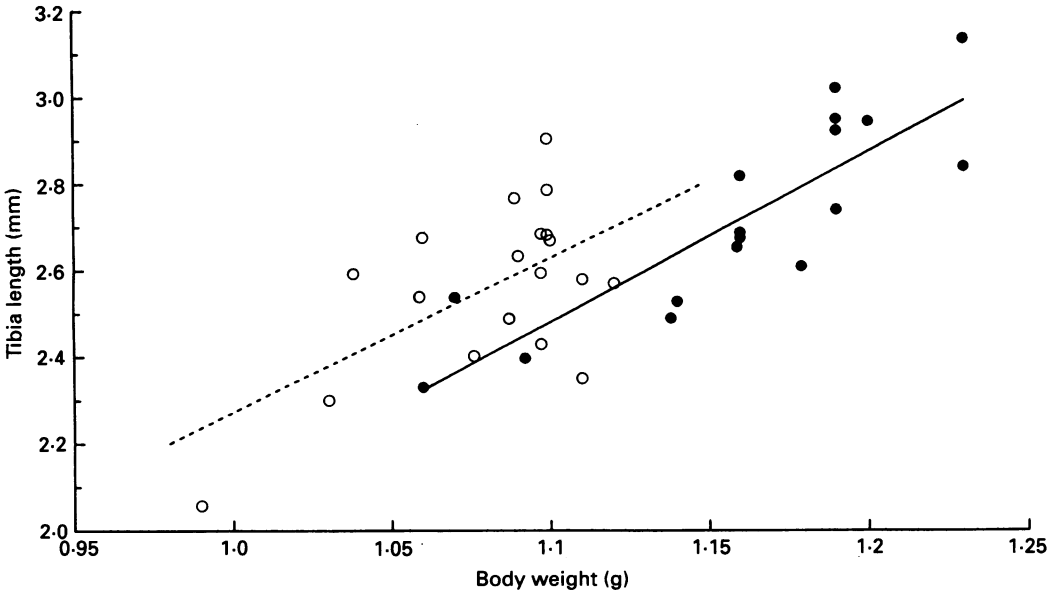

Fig. 4. Relationship between the length of the tibia and body weight in the newborn QL and QS mice. The regression equations for the QL and QS were $y = -1.8 + 3.93x$ ($r^2 = 0.7$) and $y = -1.3 + 3.6x$ ($r^2 = 0.4$), respectively. ●—●, QL; ○---○, QS.

These 2 variables were significantly associated in the QL ($P \leqslant 0.001$) and the QS ($P \leqslant 0.001$) mice (t test of the regression coefficients). A statistical comparison of the 2 lines showed no significant difference either in the slope or intercept.

Bone length in newborn QL and QS mice

Measurements of ossified bone length in the upper forelimb and lower hindlimb of the QL and QS mice were performed in order to obtain an approximation of muscle length in biceps brachii and soleus.

A comparison of the length of the humerus in randomly selected newborn QL and QS mice showed there to be a significant difference between the lines ($P \leqslant 0.05$). The percentage increase in the QL relative to the QS was 4.2. Figure 3 illustrates the relationship between the length of the humerus and body weight in the newborn QL and QS mice. These 2 variables were significantly associated in the QL ($P \leqslant 0.001$) and the QS ($P \leqslant 0.0001$) mice (t tests of the regression coefficients). A statistical comparison of these 2 lines with respect to slope and intercept indicated a significant difference in the intercept only ($P \leqslant 0.002$).

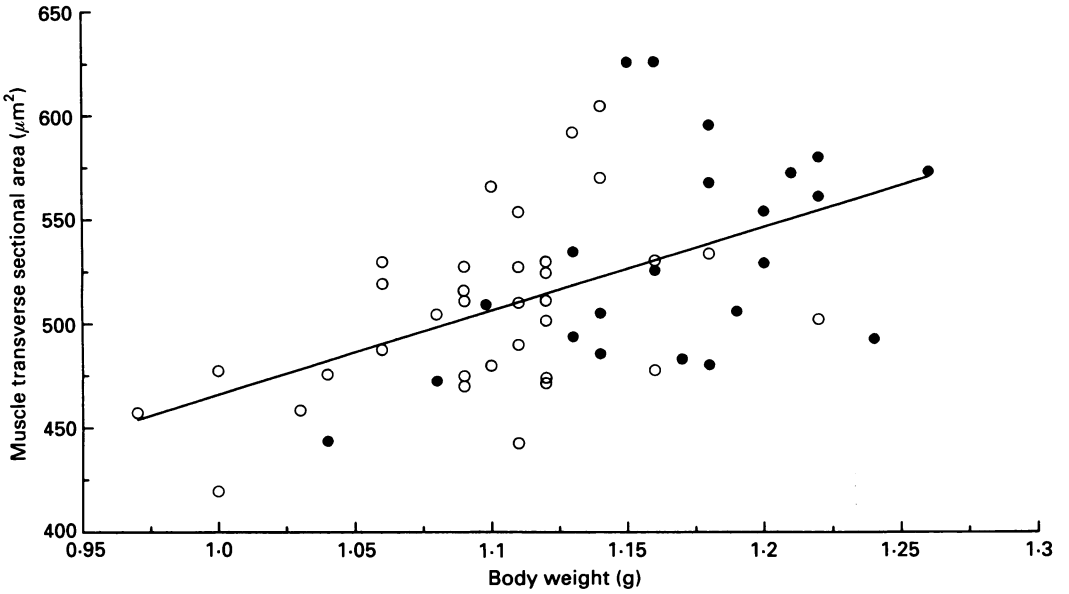

Fig. 5. Relationship between the transverse sectional area of biceps brachii and body weight in the QL and QS mice. The regression equation for the QL and QS data combined were $y = 65.1 + 401x$ ($r^2 = 28.2\%$). ○, QS; ●, QL; —, QL/QS.

Comparisons of bone length at equal body weight in the 2 lines showed bone length to be greater in the QS compared with the QL.

Tibial length was significantly greater in the QL relative to the QS mice at birth ($P \leqslant 0.05$). Analyses of the data show that the percentage increase in the QL relative to the QS was 6.2%. The relationship between the length of the tibia and body weight in the newborn QL and QS mice is shown in Figure 4. A t test of the regression coefficients showed a significant association between tibia length and body weight in both the QL ($P \leqslant 0.001$) and QS ($P \leqslant 0.001$). A comparison between these 2 lines showed a significant difference in the intercepts. As with the humerus, comparisons at equal body weight in the 2 lines indicated that the length of the tibia was greater in the QS mice.

Muscle transverse sectional area in the newborn QL and QS mice

Figure 5 shows the relationship between the transverse sectional area of biceps brachii relative to body weight in the QL and QS mice. A statistical comparison of these 2 lines showed there to be no significant difference between either the slope or intercept. A 2-way analysis of variance showed a significant effect of line ($P \leqslant 0.009$) and littermate size ($P \leqslant 0.006$) on the transverse sectional area of biceps brachii. The mean values showed that muscle transverse sectional area was increased by 13% in the QL relative to the QS mice.

A 2-way analysis of variance showed there to be a significant effect of line on the transverse sectional area of soleus (1% level), although the effect of littermate size was not significant. There was on average a 39% increase in the transverse sectional area of the QL soleus compared to the QS.

Fibre number

Fibre number was evaluated in both muscles in order to determine what proportion of the difference in muscle transverse sectional area between the lines was due to alterations in this parameter. A 2-way analysis of variance was carried out on the data for biceps brachii contained in Table 1 to compare the effect of line (QL/QS) and littermate size. This showed a significant difference between the QL and QS ($P \leqslant 0.025$) mice with respect to fibre number (18% greater in QL) but not between littermates.

In soleus (Table 1) there was a significant effect of both line and littermate size on fibre number ($P \leqslant 0.01$). The overall increase in fibre number within soleus was 17%. Comparisons between the large, average and small littermates of each line showed a 21, 24 and 3% increase, respectively.

Fibre transverse sectional area

Muscle transverse sectional area is mainly a consequence of both fibre number and fibre transverse sectional area. Fibre number was shown in the

Table 1. *Fibre number in biceps brachii and soleus**

	QL biceps	QS biceps	QL soleus	QS soleus
Large littermate	3301±209 (4)	3136±145 (4)	695±19 (3)	572±41 (3)
Average littermate	3302±173 (4)	2646±211 (4)	663±34 (3)	533±26 (3)
Small littermate	3293±252 (4)	2611±341 (4)	508±15 (3)	492±26 (3)
Total mean	3299±3	2798±169	622±58	532±23

* Means±S.E.M. The number of animals on which the mean for each sized littermate is based is shown in brackets.

Table 2. *Fibre transverse sectional area (μm^2) in biceps brachii and soleus**

	QL biceps	QS biceps	QL soleus	QS soleus
Large littermate	67.2±7 (6)	64.8±6 (5)	95.4±6 (3)	58.8±12 (3)
Average littermate	70.8±7 (6)	61.2±6 (5)	91.8±6 (3)	56.7±9 (3)
Small littermate	61.9±6 (6)	58.3±7 (5)	74.8±0.4 (3)	52.7±9 (3)
Total mean	66.6±3	61.5±2	87.3±6.4	56.1±2

* Means±S.E.M. The number of animals on which the mean for each sized littermate is based is shown in brackets.

Table 3. *Nuclear number in transverse sections of biceps brachii**

	Total no. nuclei	Fibre no.	Proportion of fibres with nucleus	No. fibre nuclei	% total nuclear population within fibres
QL mice					
Large littermate (4)	2195±90	3301±206	0.35±0.01	1145±109	52±3.2
Average littermate (4)	2073±187	3165±149	0.28±0.01	895±42	44±3.2
Small littermate (4)	1952±200	3092±216	0.26±0.02	806±9.3	43±4.7
QS mice					
Large littermate (4)	1750±376	3147±205	0.29±0.04	934±160	55±7.2
Average littermate (4)	1824±280	2597±291	0.28±0.01	739±97	40.7±1.5
Small littermate (4)	1478±218	2602±483	0.27±0.02	700±105	47.7±3.7

* Means±S.E.M. The number of animals on which the mean for each sized littermate is based is shown in brackets.

previous section to be increased in the QL relative to the QS mice. Table 2 shows fibre transverse sectional area for biceps brachii within the QL and QS mice. A 2-way analysis of variance showed there to be no significant differences either between the QL and QS ($P = 0.47$) or within the litters ($P = 0.45$). Furthermore, there was no significant correlation between fibre size and body weight in either line (correlation coefficients for the QL 0.28, n = 18 and QS 0.29, n = 15).

Table 2 gives mean fibre transverse sectional area in soleus. A 2-way analysis of variance of these data showed a significant effect of line ($P \leqslant 0.001$) but not littermate size ($P \leqslant 0.37$).

Nuclear number in transverse section

Fibre hyperplasia and hypertrophy are dependent on a supply of nuclei for fusion. The following calculations were therefore performed to determine whether selection had acted to alter the number of nuclei either within or outside of the fibres. Table 3 summarises the data on the total number of nuclei counted in transverse sections of biceps brachii at birth in the QL and QS mice. Column 1 contains the total number of nuclei counted in transverse section, column 2 the fibre number (for the mice used in this particular analysis), column 3 the proportion of fibres showing a nucleus in transverse section, column 4 the total number of fibre nuclei (calculated from the product of columns 2 and 3) and column 5 records the percentage number of the total nuclei contained within the fibres. Analyses of these data were performed using a 2-way analysis of variance to check for the effect of line (QL/QS) or the effect of littermate size within each line. The results of the statistical analyses are shown in Table 4.

Tables 3 and 4 show that the total number of nuclei in transverse section was significantly increased within

Table 4. *Results of a 2-way analysis of variance of the data contained in Table 3*

Comparison	Total no. nuclei	Fibre no.	Proportion of fibres with nucleus	No. fibre nuclei	% total nuclear population within fibres
QL/QS	**	**	n.s.	**	n.s.
Littermates	n.s.	n.s.	n.s.	*	**

* $P \leqslant 0.05$; ** $P \leqslant 0.025$.

the QL compared to the QS mice. Since there was no difference between the lines with respect either to the percentage number of myofibres sectioned with a nucleus (column 3) or to the proportion which this represented of the total nuclear population (column 5), this difference was attributed to the variation in fibre number between the lines. Indeed this conclusion is confirmed by the significant increase seen in the total number of myofibre nuclei of the QL compared with the QS (column 4).

There was no significant difference between littermates with respect to the total number of nuclei (column 1). However, the total number of myofibre nuclei (column 4) together with the proportion of the total nuclear population which this represented (column 5) was significantly reduced in the smaller individuals.

There was no significant effect either of line or of littermate size on the total number of nuclei in transverse sectional area of soleus. Further analyses of the compartmentalisation of the nuclei was not possible due to the method of counting nuclei in this muscle.

DISCUSSION

In agreement with the previous findings of Rucklidge (1982) birth weight was found to differ significantly between the QL and QS. However, birth weight is influenced by environmental as well as by genetic factors (McLaren, 1965) and a negative correlation between mean pup weight and litter size was observed in both the QL and QS mice. Previous work attributes this well known effect to haemodynamic factors within the uterus which effectively determine the level of nutrients delivered to each fetus (McLaren & Mitchie, 1960). Whilst it might be argued that such factors could act differentially in the QL and QS, thereby partly contributing to the differences seen between the lines at birth, the reciprocal transfer of fertilised eggs between high and low body weight Q strain mice has demonstrated that prenatal maternal effects are of limited importance and arise only from variations in litter size (Al-Murrani & Roberts, 1978). It may therefore be concluded that the differences seen in birth weight between the 2 lines, when matched for litter size, are a reflection of an alteration in fetal genotype, brought about as a direct consequence of selection pressure.

These observations do, however, contrast markedly with those previously reported by Penney et al. (1983) who found that body weight was not significantly different between the QL and QS until 10 d postpartum. This discrepancy may be explained in one of two ways. The first and most likely explanation relies upon the fact that both the QL and QS were formed from the combination of several of the replicate lines originally selected by Falconer. Falconer himself noted that selection had led to the fixation of different alleles within each of these replicate lines, although within each size category each has regulated to approximately the same body weight (Falconer, 1973). The combination of several of these replicate lines to form the QL and QS would therefore be expected effectively to increase genetic variation. Since several generations separate the present study and that of Penney et al. (1983) it might be envisaged that the segregation of minor genes, throughout this period, could have led to the observed differences in growth rate.

A second explanation is based upon the failure of Penney et al. (1983) to observe a negative correlation between mean litter weight and litter size in the 2 lines. As this is a well recognised effect in polytocous species such as the mouse (McLaren & Mitchie, 1960; McLaren, 1965; Wahlsten & Bulman-Fleming, 1987), it seems highly unlikely that the QL and QS would be exceptions. Instead, it may be suggested that the number of litters observed for each size category (this information was not reported) was insufficient to permit Penney et al. (1983) to carry out a proper evaluation of the effect of litter size on mean pup weight. The subsequent failure to take an effect of litter size into account when one existed may therefore have led to biased comparisons between the QL and QS.

The lengths of both the humerus and tibia were significantly greater in the QL. This might have been expected since alterations in both the length and diameter of the long bones are known to contribute to genetically determined differences in body weight (Hooper, 1978). However, perhaps a more interesting observation was that the tibia showed a greater percentage change in length than the humerus. This difference between the 2 bones may be related to the pattern of limb development which determines that the rate of growth of the more distal segments (lower limb) exceeds those which are more proximal (upper limb). The difference in the proportional change in length seen between the 2 bones therefore reflects the effect of selection, altering the rate of longitudinal bone growth prenatally.

At comparable body weights, bone length in the QL was less than in the QS. Similar results have previously been reported by Hooper (1978) postnatally, who found that bone length was greater in the QS when the 2 lines were compared at 25 g body weight, whilst the reverse trend was shown if the 2 lines were compared at the same age. These results were taken to imply a relationship between bone length and developmental age as opposed to body weight (Hooper, 1978). Indeed this would seem to apply to the data presented here, since comparisons between the QL and QS at equal birth weight necessarily involves comparisons between the largest QS and the smallest QL littermates. In view of the graded stages of morphological development often evident between fetuses in utero and assuming this is reflected in birth weight, it might very well be suggested that the lowest birth weight QL were developmentally earlier than the larger QS.

Transverse sectional area measurements of biceps brachii and soleus were significantly increased in the newborn QL compared with the QS mice. This increase, together with the changes in bone length discussed above, confirms the previous findings of Hooper et al. (1973) that selection for increased body weight is accompanied by corresponding changes in muscle mass. Muscle weight is determined by 3 parameters: fibre number, size (transverse sectional area and length) and the deposition of extracellular matrix. Since an analysis of fibre density and fibre transverse sectional area in biceps brachii failed to provide any evidence suggesting that the deposition of matrix was altered disproportionately between the lines, the following discussion will be confined to the relative contribution of fibre number and size.

Fibre number in both biceps brachii and soleus was significantly increased in the QL relative to the QS mice. These results are therefore in general agreement with a number of other studies which have shown an association between an increase in fibre number and selection for high body weight (Byrne et al. 1973; Hanrahan et al. 1973; Hooper, 1975; Penney et al. 1983; Stickland & Handel, 1986). However, there are a number of discrepancies with respect to both the pattern and magnitude of response between studies. For example Luff & Goldspink (1967) failed to record any alteration in fibre number within soleus of genetically large and small Q strain mice, whilst both the present study and that carried out by Byrne et al. (1973) reported a significant increase in this variable in soleus. Some diversity in the response of biceps brachii has also been demonstrated, with Byrne et al. (1973) reporting up to a 50% difference in fibre number and Penney et al. (1983) and the present study reporting a 30% and 15% divergence, respectively.

Reasons for the aforementioned variation in response could be severalfold. Previous work has indicated that the magnitude of response may be related to the number of generations of selection pressure which have been applied (Byrne et al. 1973). Hanrahan et al. (1973) and Byrne et al. (1973) both used mice which, whilst originating from the original Q strain, were the result of individual selection programs terminated at 10 and 14 wk, respectively. Others such as Penney et al. (1983) used lines directly descended from those which had been selected by Falconer (1973) for 23 generations. The variation in the number of generations of selection in each case may therefore underlie some of the differences apparent between studies.

A further factor to be taken into consideration is the genetic variation within the individual populations under investigation. Both Penney et al. (1983) and the present study used the QL and QS lines of mice which were originally derived from the combination of several of Falconer's replicate lines (see above discussion). However, the present study reported a 15% divergence in fibre number whilst that by Penney et al. (1983) observed up to a 30% divergence. These differences may only be accounted for by assuming that natural selection, through the number of generations which separate the 2 studies, had led to some alteration in the genetic determinants for fibre number.

The difference of 18% between QL and QS in fibre number of the biceps brachii muscle is similar to the difference found for primary myotube number (S. C. Brown & N. C. Stickland, unpublished observations). This therefore suggests that fibre number differences between the QL and QS in the newborn biceps brachii were due to alterations in primary myotube number

rather than any alteration in the ratio of secondary to primary myotubes. This is in contrast to the situation found by Stickland & Handel (1986) in the pig where genetically determined differences in fibre number were due to alterations in both primary myotube number and the secondary to primary myotube ratio.

Fibre formation and growth are dependent on a supply of nuclei since DNA accretion precedes or closely parallels protein accretion (Moss, 1968*a*, *b*). However, despite the increased growth potential of the QL mice there was no difference in the percentage number of nuclei either within or outside the fibres of biceps brachii at birth. These observations are of considerable importance since they indicate the absence of any alteration in the regulation of fusion within the early muscle, thereby lending support to the hypothesis that selection only acts through the normal cellular processes of growth (Falconer et al. 1978). However, due to the differences in fibre number and fibre length between the 2 lines, the total number of nuclei was increased within the QL biceps brachii compared with the QS. These results are therefore both in agreement with and also explain the biochemical analyses of Penney et al. (1983), who found an increase in the total number of nuclei but no alteration in the protein:DNA ratio between the lines.

Soleus in contrast showed no significant differences between the QL and QS mice with regard to the total number of nuclei in transverse sections of the muscle. This was somewhat surprising since fibre number was significantly increased in the QL compared with the QS, as in biceps brachii. However, since fibre transverse sectional area was also increased in the QL soleus compared with the QS, it might be suggested that this result was due to some change in the protein:DNA ratio incurred as a result of the rapidly diverging growth rates of soleus in the 2 lines. However, to confirm this, further data on the compartmentalisation of nuclei within this muscle would be required.

A comparison of nuclear number in biceps brachii and soleus of differently sized littermates showed no significant difference in either the QL or QS. However, a more detailed analysis in biceps brachii showed that the percentage number of nuclei incorporated into the fibres was significantly reduced in the smaller individuals. These results tend to imply that under conditions of nutritional constraint the fusion of nuclei into the fibre is affected prior to the rate of cell division. On the basis of these observations it seems that whilst genetically and environmentally induced difference in muscle growth may be implemented by separate mechanisms, the basis for both still relies on DNA accretion as was found previously by Moss et al. (1968*a*, *b*) in the chicken.

Selection for divergent muscle weight acts not only on fibre number but also on fibre size (Byrne et al. 1973). Fibre size has 2 main components: fibre transverse sectional area and fibre length. Fibre length in biceps brachii and soleus was estimated by the length of the ossified humerus and tibia respectively. At birth soleus but not biceps brachii showed a significant increase in fibre transverse sectional area, whilst of the 2 bones the tibia showed the greater proportional increase between the QL and QS lines. This would initially seem to lend support to the hypothesis that longitudinal bone growth promotes fibre hypertrophy (Hooper, 1978; Vandenburgh & Karlisch, 1989). However, comparisons between muscles which differ in their fibre type profile may be inherently unreliable in view of probable differences in their pattern of growth (Gibson & Schultz, 1983).

The main aim of the present investigation was to determine the cellular variables associated with the early divergence in size between the QL and QS. Muscle length (as determined by the length of the humerus and tibia) was significantly increased in both the biceps and soleus of the newborn QL compared with the QS. Both muscles showed a significant divergence in fibre number between the lines. Fibre cross-sectional areas were only significantly different between the QL and QS in soleus. This was suggested to be a reflection of the role played by the rate of longitudinal bone growth on fibre hypertrophy since the divergence in the length of the tibia was more marked than that of the humerus. However, further analyses of muscles with different fibre type profiles would be required to confirm this. Detailed analyses of the nuclear content of biceps brachii showed that alterations in fibre number had arisen without any alteration in the proportion of nuclei incorporated into the fibres. On the basis of these results it was concluded that selection pressure had altered fibre number through the normal processes of cellular growth.

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