J. Anat. (1986), **147**, pp. 181–189
With 3 figures
Printed in Great Britain

The numbers and types of muscle fibres in large and small breeds of pigs

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(*Accepted* 31 *October* 1985)

INTRODUCTION

The biphasic theory of muscle development is well established for the pig (Ashmore, Addis & Doerr, 1973; Swatland & Cassens, 1973; Beermann, Cassens & Hausman, 1978). Initially, a population of large primary myofibres forms within the presumptive muscle by fusion of myoblasts. The next phase of development is the formation of smaller, secondary, myofibres; these myofibres form by fusion of myoblasts which line up on the surface of the primary myofibres. In mixed fibre type muscles of pigs, it has been shown that primary myofibres take on slow-contracting characteristics whereas secondary myofibres acquire fast-contracting characteristics (Ashmore *et al.* 1973), although some secondaries become slow during late prenatal (Beermann *et al.* 1978) and postnatal growth (Davies, 1972). In postnatal porcine muscle these 'metabolic bundles', derived from one primary myofibre and its surrounding secondaries, can be readily identified (Fig. 1) using methods for the detection of myosin adenosine triphosphatase (myosin ATPase).

Restricted nutritional levels fed to pregnant animals of various species have been found to cause a significant reduction in myofibre number within muscles of the offspring (Everitt, 1968: sheep; Robinson, 1969: pigs; Aziz-Ullah, 1974: mice; Bedi *et al.* 1982: rats). It has been shown by Wigmore & Stickland (1983) that pigs which develop at disadvantaged sites in the uterus often develop fewer myofibres in their muscles and do so because fewer secondary myofibres form around each primary; the number of primary myofibres is not affected. The pig developing at a disadvantaged uterine site is analogous to the undernourished animals in the situations mentioned above.

The present study was carried out in order to determine whether genetically small animals develop fewer muscle fibres in their muscles by the same mechanism as in nutritionally small animals. It is known that selection for small body size in mice results in a decrease in the total number of muscle fibres in a given muscle (Luff & Goldspink, 1967; Hanrahan, Hooper & McCarthy, 1973). Smaller breeds of the same species also have fewer myofibres in their muscles than the larger breeds (Smith, 1963: chickens; Stickland & Goldspink, 1973: pigs). However, the developmental mechanisms responsible for these differences in myofibre number and the consequences upon the proportions of myofibre types in the adult are unknown. The pig provides an ideal model for investigating the proportions of fibre types in postnatal muscle and, for the reasons already discussed, the distribution pattern of myofibre types reflects the developmental stages of myogenesis in terms of primary and secondary myofibres. The numbers, ratios and distribution of muscle fibre

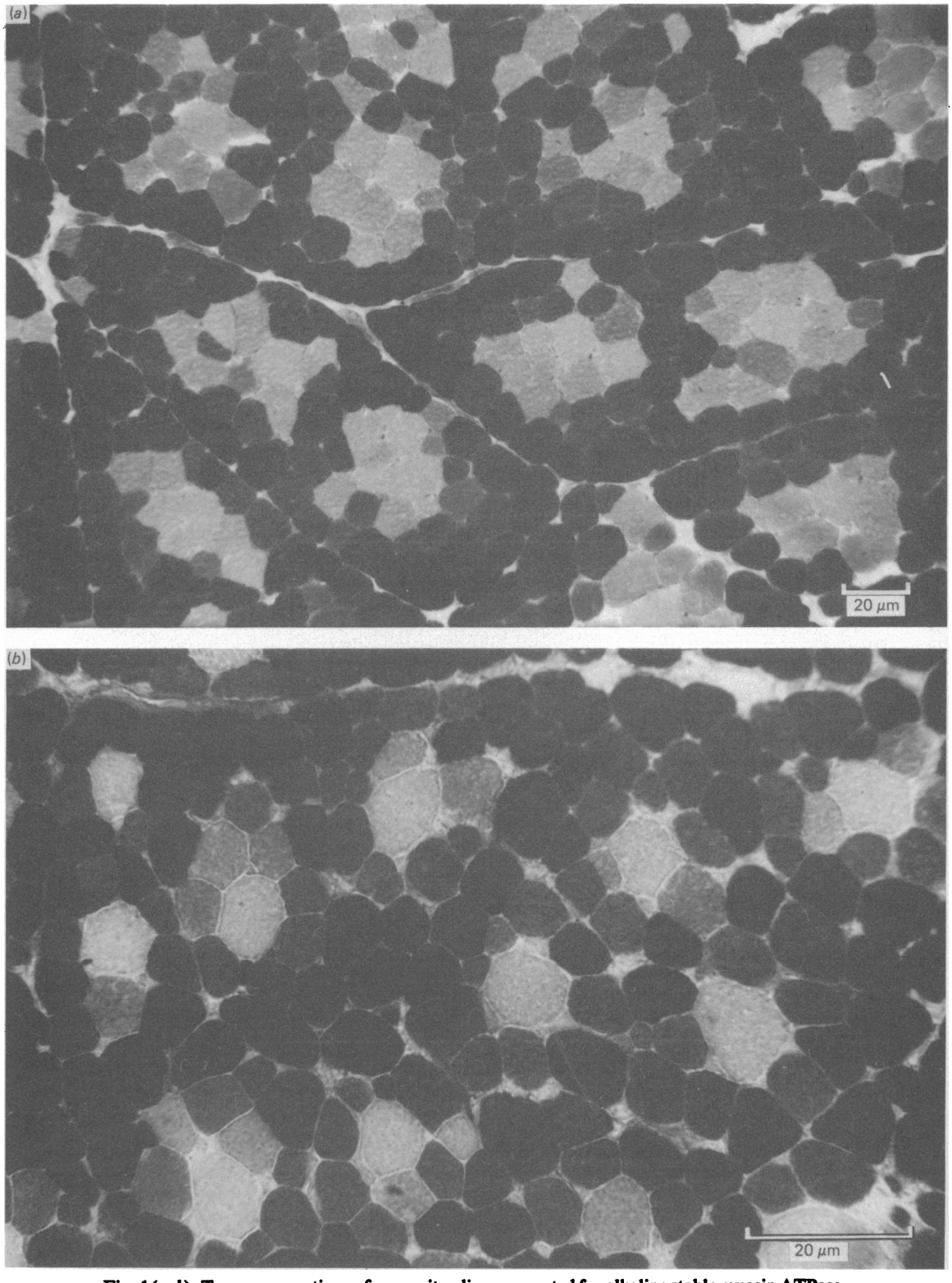

Fig. 1 (*a–b*). Transverse sections of m. semitendinosus reacted for alkaline stable myosin ATPase activity to show the total number of myofibres and the number of slow myofibres in each 'metabolic bundle' in (*a*) Large White pig, 64 days; (*b*) Miniature pig, 61 days.

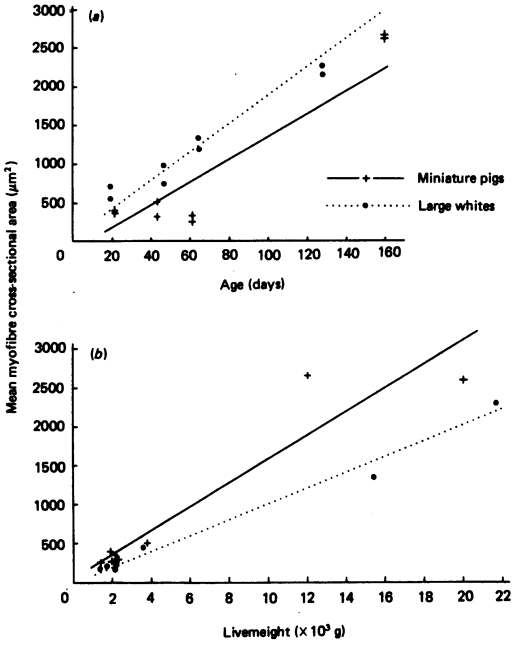

Fig. 2(*a–b*). Mean myofibre cross sectional area plotted against (*a*) age and (*b*) live weight for both miniature pigs and Large White pigs.

types (based mainly on myosin ATPase activity) were therefore investigated in Large White and in miniature pigs; at six months of age the latter are about one third of the body weight of the former, commercial, pigs.

MATERIALS AND METHODS

The miniature pigs were obtained from those reared at the Royal Veterinary College farm (Hawkshead, Herts) which are Göttingen miniature pigs (developed by Haring, Gruhn, Smidt & Scheven, 1966). Eight miniature pigs were used which ranged in age from 21 to 160 days; this corresponded to a weight range from about 2 to 20 kg. Sixteen Large White pigs (from a commercial, pedigree herd) were used, such that eight of them matched the live weights of the miniature pigs and eight matched their ages as closely as possible. The actual ages and live weights may be ascertained from Figure 2.

The pigs were killed by an intraperitoneal injection of pentobarbitone (Euthesate) followed by exsanguination. M. semitendinosus was dissected out from each animal and a complete transverse slice 3–4 mm thick was taken from the muscle, frozen in dichlorodifluoromethane (Arcton 12, ICI Ltd) cooled to its melting point of −158 °C with liquid nitrogen, and frozen sections 10 μm thick taken so that the constituent muscle fibres were cut transversely in a cryostat. A sample of m. trapezius was also taken from midway along the caudal border of its thoracic portion and transverse sections of its muscle fibres were made. Some sections for each muscle sample were treated by the method outlined by Guth & Samaha (1970) for the detection of both alkali-stable and acid-stable myosin ATPase, although the former was used for most of the analysis as it appeared to produce the best differential staining. Sections were,

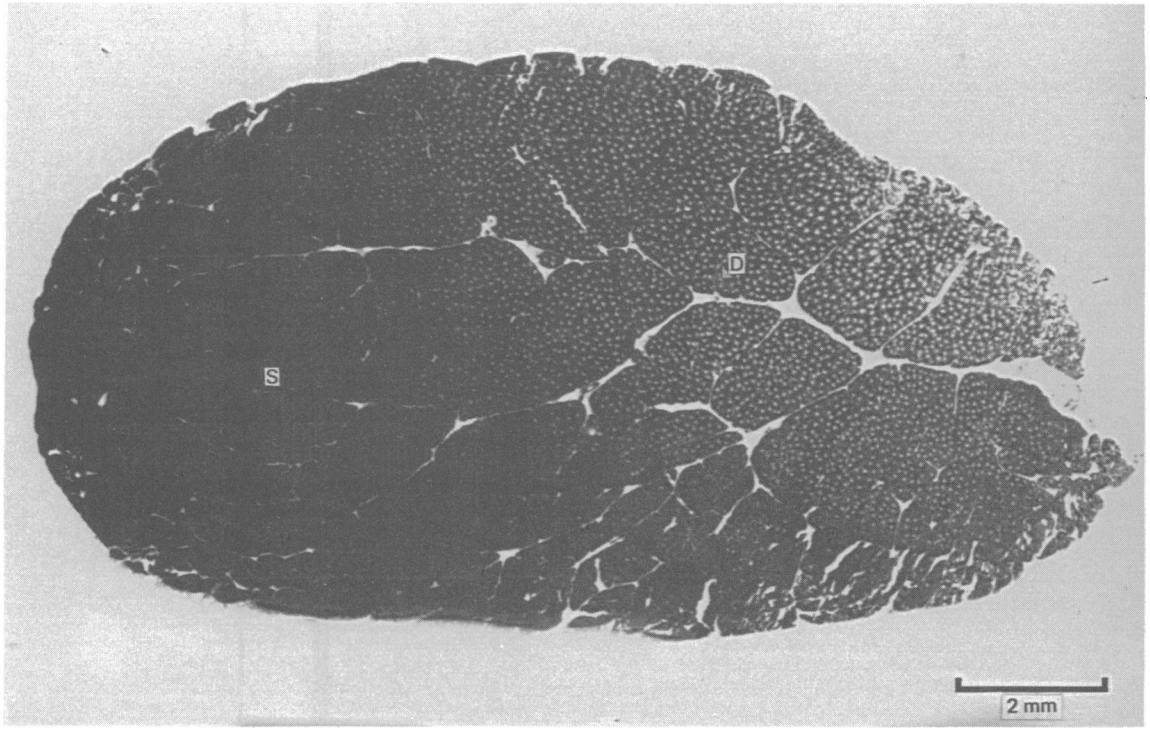

Fig. 3. Complete transverse section of m. semitendinosus reacted for alkaline stable myosin ATPase activity to show the superficial (S) and deep (D) regions of the muscle. Miniature pig, 61 days.

however, also tested for succinate dehydrogenase activity and glycogen phosphorylase activity by the methods of Nachlas *et al.* (1957) and Takeuchi (1956) respectively.

The sections were viewed and analysed using a microscope with camera linked to an image analysis system based on an Apple IIe computer with TV monitor and graphics tablet (VIDS II from Analytical Measuring Systems Ltd, Saffron Walden, Essex). Most of the analysis outlined below was carried out on the alkali-stable myosin ATPase reacted sections for the detection of slow and fast contracting myofibres, with other sections being used for confirmation. Measurements were made on five randomly selected areas of known size (each containing about 500 myofibres) from each muscle. For each area the total number of myofibres was noted as was the number of 'metabolic bundles' (indicated by the number of clusters of slow myofibres). These data were used to estimate the secondary: primary myofibre ratio (equivalent to the number of myofibres per 'metabolic bundle' minus one) for both m. semitendinosus and m. trapezius. In addition, the total cross sectional area of the whole m. semitendinosus was measured so that, for this muscle, the total number of primary myofibres (equal to the number of 'metabolic bundles') and of all myofibres per complete cross section could be estimated. The mean cross sectional area for all myofibres in each muscle was also estimated by measuring the area of fibres from complete 'metabolic bundles' taken from each of the five muscle areas selected such that 100 myofibres were measured for each muscle.

Under low magnification it was seen (Fig. 3) that m. semitendinosus could be divided into a distinguishable superficial region and a deep region; the former region

Table 1. *Numerical data on muscle fibres in the muscles of Large White (age controls) and miniature pigs (means* ± S.E.)

Number of animals	Large White 8	Miniature 8	Significance of difference
M. semitendinosus			
Total myofibre number	392159 (±13956)	143825 (±15286)	$P < 0.001$
Total 'primary' myofibre number	15454 (±791)	7184 (±632)	$P < 0.001$
Secondary:primary myofibre ratio	24·6 (±0·8)	18·9 (±1·0)	$P < 0.001$
M. trapezius			
Secondary:primary myofibre ratio	23·2 (±1·2)	18·3 (±1·1)	$P < 0.02$

contained larger clusters of slow myofibres. The relative proportions of these two areas were estimated for each of the m. semitendinosus sections. Within the deep area of the muscle the number of slow myofibres per 'metabolic bundle' was noted (mean for 100 bundles) as was their mean cross sectional area (based on measurements of 100 slow myofibres). The percentage of the muscle cross sectional area occupied by slow myofibres could therefore be calculated. The number of slow myofibres per 'metabolic bundle' was also noted for m. trapezius.

For most comparisons between the two breeds of pigs the age-control Large Whites were used. The weight-control Large Whites were only used for the analysis of myofibre size.

RESULTS

As there was no evidence of any change in myofibre numbers with growth, the data on cell numbers could be grouped as shown in Table 1. It can be seen from this Table that both total myofibre number and the total primary myofibre number in m. semitendinosus were greater (173 and 115% respectively) in Large White than in miniature pigs. The secondary:primary myofibre ratio was also greater in both muscles studied in the Large White pigs. However, the results indicate that the primary myofibre number difference was about four times more important than the secondary:primary myofibre ratio difference in bringing about the total myofibre number difference between the two breeds in m. semitendinosus. Although total myofibre number could not be estimated for m. trapezius, the secondary:primary myofibre ratio difference between Large White and miniature pigs was of the same order for m. trapezius (27%) as that for m. semitendinosus (30%). It therefore follows that, if the myofibre number difference was the same in m. trapezius, then there must have been a similar difference in primary myofibre number.

The relationship between the mean size of all myofibres and live weight and age are shown in Figure 2 and Table 2. No significant difference between Large White and miniature pigs could be demonstrated in the myofibre cross sectional area changes with age; at any given age mean myofibre areas were not significantly different between the two strains of pigs. However, when mean myofibre area was plotted against live weight the slope or regression coefficient was significantly larger ($P < 0.05$) for the miniature pigs; at any given live weight, the mean myofibre area in the miniature pigs was approximately 63% greater than in Large White pigs throughout the weight range studied.

For the age range under study there was no evidence of any change with increasing age in the number of slow myofibres per 'metabolic bundle'. The percentage of

Table 2. *Equations for the regression lines shown in Figure 2 of mean myofibre cross sectional area (μm^2) against (a) age and (b) live weight*

(*a*) Against age (in days)		
		SE_b
Miniature pigs	$Y = 14{\cdot}79X - 120$	1·66
Large White pigs	$Y = 18{\cdot}26X + 75$	0·88
(Neither slopes nor intercepts are significantly different.)		
(*b*) Against live weight (in g)		
		SE_b
Miniature pigs	$Y = 0{\cdot}155X + 52{\cdot}57$	0·016
Large White pigs	$Y = 0{\cdot}102X + 17{\cdot}62$	0·004
(Slopes significantly different, $P < 0{\cdot}05$.)		

Table 3. *'Slowness' of muscles of Large White (age controls) and miniature pigs (means* ± S.E.)

Number of animals	Large White 8	Miniature 8	Significance of difference
M. semitendinosus			
Deep area as % of whole	39·9 (±1·9)	48·4 (±3·3)	$P < 0{\cdot}05$
Number of slow myofibres per bundle in deep area	8·68 (±0·75)	1·67 (±0·28)	$P < 0{\cdot}001$
Total area of slow myofibres as % of whole muscle area	18·05 (±3·62)	6·18 (±0·77)	$P < 0{\cdot}01$
M. trapezius			
Number of slow myofibres per bundle	9·76 (±0·75)	4·73 (±0·51)	$P < 0{\cdot}001$

muscle cross sectional area occupied by slow myofibres was found to be about three times greater in the Large Whites than the miniature pigs (Table 3). This considerable difference was due to the significantly greater number of slow myofibres per 'metabolic bundle' in the Large White pigs (Fig. 1); it was not due to a difference in the proportion of the deep region (see above) which was in fact greater in the miniature pigs. The superficial region of m. semitendinosus contained very few slow myofibres; between 0 and 2 per 'metabolic bundle' in Large White pigs and negligible numbers (usually 0, but occasionally 1) in the miniature pigs. The numbers involved in the superficial region were relatively insignificant but if included could only accentuate the difference in slow myofibre content between the two strains. The number of slow myofibres per 'metabolic bundle' in m. trapezius was also significantly greater in the Large White pigs. However, although there was no significant difference in this parameter between the two muscles studied in the Large White pigs, for the miniature pigs there were significantly ($P < 0{\cdot}001$) more slow myofibres per 'metabolic bundle' in m. trapezius than in m. semitendinosus.

DISCUSSION

It is well established for many animals, including the pig (Staun, 1972; Stickland & Goldspink, 1973) that myofibre number exhibits no change with postnatal growth. It was therefore felt justified to group the numerical data as shown in Table 1. The results show that it is the difference in myofibre number which is totally responsible

for the muscle size difference between the Large White and miniature pigs; this was also noted by Stickland & Goldspink (1978). Myofibre size differences do not account for any muscle size difference. In fact, quite the converse was found in that, at any given live weight, miniature pigs have muscle fibres of greater cross sectional area (Fig. 2). This appears to be due to the fact that they are older for a given live weight because, at any given age, there was no significant difference between the two breeds with respect to myofibre cross sectional area (Fig. 2, Table 2). This is a very interesting result in that it appears to reflect some of the results found in pigs of the same breed but of varying age at the same slaughter weight. Hegarty & Allen (1978) and Powell & Aberle (1981) showed that runt pigs have myofibres of greater cross sectional area than their large littermates when slaughtered at the same body weight. Handel (1984) attributed this difference to the disparate ages of the littermates at similar slaughter weights. It would seem that, in the present investigation also, for the two breeds of pigs used, muscle fibre cross sectional area is more indicative of the age of the animal than its live weight. It could be assumed that the runt pigs mentioned above are a result of inadequate prenatal nutritional levels. It would appear, therefore, that pigs of disparate birth weights, due to either nutritional *or* genetic (present investigation) effects exhibit postnatal increases in myofibre size which are more related to age than to live weight. This relationship is clearly dependent on optimum postnatal growing conditions as it is well known that several factors, such as nutritional levels (Joubert, 1956), may affect muscle fibre size. It is normally assumed that muscle fibre size relates to body weight and that any relationship with age is secondary, but these results indicate that, in some situations for certain periods of growth, muscle fibre size is related more to the age of an animal than to its live weight.

For the reasons mentioned earlier, it is possible to extrapolate from the results the pattern of prenatal muscle development in terms of primary and secondary myofibres. In nutritionally smaller pigs of the same litter it has been shown (Wigmore & Stickland, 1983) that the reduction in overall myofibre number in given muscles is due to a reduction in the number of secondaries forming around each primary myofibre. Although this difference has also been found between the two breeds of pigs used here, the most important factor responsible for the overall myofibre number difference is the number of primary myofibres, which each form their own 'metabolic bundles'. This difference in primary myofibre number is not found in the nutritional situation except in some very severely runted pigs (Handel, 1984).

It is known that many locomotory muscles (Pullen, 1977; Bodine *et al.* 1982) contain relatively more slow myofibres in their deeper aspects which thereby take on a more postural function. This division is found in m. semitendinosus of both the Large White and miniature pigs used here. However, it has been shown very clearly that the miniature pigs contain a relatively much reduced slow myofibre content in m. semitendinosus which is approximately one third of that found in Large White pigs. This difference is due mainly to a difference in the number of slow myofibres per 'metabolic bundle' between the two breeds. This again contrasts with the 'nutritional' situation in the large and small birth weight littermates which show no difference in the number of slow myofibres per 'metabolic bundle' at later ages (Handel, 1984). The number of fast myofibres is, however, reduced in these situations and this has also been shown in rats after experimentally induced malnutrition during pregnancy (Bedi *et al.* 1982). It should be mentioned here that, although the

conversion of secondary myofibres to slow contracting characteristics begins prenatally, there is also some postnatal conversion (Davies, 1972) but there does not appear to be any significant conversion during the period under investigation here. It was therefore felt justifiable to group the data in Table 3 as shown. However, it is possible that heavier miniature pigs (similar in weight to the older age-control Large Whites) may contain more slow myofibres, although the range did extend beyond the 8 kg suggested by Handel (1984) as being the weight by which most slow fibre conversion had occurred in the Large White pig. The results for m. trapezius indicate that this muscle is significantly slower than the deep portion of m. semitendinosus for miniature pigs but comparable for Large Whites. M. trapezius is known to have an important postural role in helping to support the scapula on the trunk. However, despite some differences between muscles, it can still be clearly seen that the m. trapezius reflects the significant difference seen in m. semitendinosus between the breeds with respect to slow myofibre content.

One of the aims of commercial pig production has been to select animals of large body weight. The aim in miniature pig production has clearly been the reverse and has been achieved in the Göttingen stock partly by the re-introduction of wild pigs (Haring *et al.* 1966). The results suggest that the selection for large body weight, associated with commercial pig production, has resulted in the selection of muscles with more metabolic bundles containing more, thinner muscle fibres when compared on an equal weight basis. Stickland (unpublished observation) has also noted that wild wart hogs have significantly larger muscle fibres than commercial Large White pigs when compared at the same live weight. The selection for larger body size has also resulted in relatively more slow muscle fibres in given muscles which may be necessary to support the increased weight of the animal.

Taken overall, the results of this investigation appear to show that genetically small animals develop fewer muscle fibres in their muscles by a different mechanism to that exhibited by animals which are smaller due to nutritional deprivation *in utero*. There are also functional consequences of these differences reflected in the histochemical properties of the constituent muscle fibres.

SUMMARY

M. semitendinosus and m. trapezius (portion) were removed from eight miniature pigs ranging from 21 to 160 days of age and eight age-control as well as eight weight-control commercial Large White pigs. Complete transverse frozen sections were obtained for each muscle sample and stained for various enzyme activities including myosin adenosine triphosphatase activity which enabled the identification of 'metabolic bundles'. This in turn enabled conclusions to be made about the prenatal development of the muscle in terms of primary and secondary myofibres. The Large White pigs contained 173 % more muscle fibres in m. semitendinosus than did the miniature pigs. Primary myofibre number was found to be about four times more important than secondary to primary myofibre ratios in determining myofibre number in the two breeds of pigs. Both primary myofibre number and secondary to primary myofibre ratios were, however, significantly greater in Large White than in miniature pigs. When the age- and weight-control Large Whites were compared with the miniature pigs it was found that at any given live weight the miniature pigs had thicker myofibres whereas at the same age there was no significant difference. The total area of m. semitendinosus occupied by slow myofibres was about three

times greater in the Large White pigs; the functional aspects of this are discussed. It was concluded that genetically smaller animals develop fewer muscle fibres in their muscles by a different mechanism to that exhibited by animals which are smaller due to nutritional deprivation *in utero*.

The authors wish to thank Andrew Crook, Tricia Mescall, Glynn Hammond and Sue Evans for technical assistance. This work was supported by a grant from the Agricultural and Food Research Council.

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