J. Anat. (1987), **152**, pp. 107–119
With 3 figures
Printed in Great Britain

The growth and differentiation of porcine skeletal muscle fibre types and the influence of birthweight

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(*Accepted* 1 *July* 1986)

INTRODUCTION

Skeletal muscle is composed of a heterogeneous population of myofibres that differ in physiological (Burke *et al.* 1971) and biochemical (Sréter, Seidel & Gergely, 1966) properties. A myofibre's indigenous physiological and metabolic properties contribute to the effectiveness and efficiency of the functioning of the skeletal muscle with respect to the support and movement of the body. The most comprehensive system of nomenclature for myofibre 'typing' gives a full description of these physiological and metabolic capacities, based on the relative contraction speed of the fibre and its propensity for oxidative and glycolytic metabolism (Peter *et al.* 1972). Accordingly, these workers described three basic myofibre types in adult guinea-pig and rabbit limb muscles that are common to most mammalian muscles: (i) slow-twitch, oxidative metabolism (SO), (ii) fast-twitch, oxidative and glycolytic metabolism (FOG), and (iii) fast-twitch, glycolytic metabolism (FG). The histochemical staining patterns of myofibres are indicative of their physiological and biochemical properties.

Changes in the proportions of fast- and slow-contracting fibres occur naturally in the growing animal after birth. The growth process is associated with an increasing functional load due to liveweight gain and the way in which the muscle maintains a supportive role appears to be through an increase in the percentage of slow-contracting, fatigue-resistant fibres (Edström & Kugelberg, 1968; Davies, 1972). With growth the number of slow fibres in the muscle is augmented through the conversion of fast fibres since total muscle fibre number remains constant postnatally (Staun, 1963; Stickland & Goldspink, 1973).

The progenitors of the initial population of slow-contracting (acid-adenosine triphosphatase-stable) fibres in prenatal muscle are called primary fibres (Ashmore, Robinson, Rattray & Doerr, 1972*b*; Ashmore, Addis & Doerr, 1973). These fibres, once formed, act as a framework on which myoblasts align and fuse to form a population of secondary fibres. A proportion of the fast-contracting population of secondary fibres are the progenitors of slow fibres postnatally in mixed fibre and predominantly slow fibre type muscles. The lower ($P < 0{\cdot}001$) total muscle fibre number in low birthweight, runt pigs compared to their heavy birthweight littermates has been mainly attributed to a reduced secondary fibre population (Handel & Stickland, 1984). Low birthweight pigs also appear to suffer a consistently reduced postnatal liveweight relative to their heaviest birthweight siblings (Hegarty & Allen,

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Table 1. *Classification of myofibre types*

Histochemical staining pattern				Myofibre type classification
Acid ATPase	Alkaline ATPase	SDHase	GPase	
+	−	+	−	SO
+	/	+	−	IOa
+	+	+	−	IOb
/	/	+	−	IOc
/	+	+	−	IOd
−	/	+	−	FO
−	+	+	−	FO
−	/	+	+	FOG
−	+	−	+	FG

+, Positive staining reaction; /, intermediate staining reaction; −, negative staining reaction.

1978; Powell & Aberle, 1981). It was therefore considered valuable to investigate the effects that these phenomena might impose on the proportions and growth rates of the various muscle fibre types between littermates, and as a consequence the comparative physiological and biochemical properties of their muscles. In addition, a comprehensive study of this kind in pigs of disparate birthweight, and subsequently disparate liveweight gain, would enable the comparison of the muscle development of pigs of the same age (but different liveweights) or of equal liveweight (but different ages). This would therefore help to elucidate the separate effects that both age and liveweight might impose on postnatal muscle growth and development.

MATERIALS AND METHODS

Muscle samples

Muscle samples taken for this study were from 49 pigs, from a total of 17 litters of a Large White herd. Selection of littermates was by weight at birth. Where possible 3 animals were chosen from each litter; the largest male, smallest (not less than 1000 g) normal male, and the runt (950 g or less). The ages at which the litters were slaughtered were 0, 1, 2, 4, 6, 9, 12, 15, 19, 27, 33, 46, 64, 84, 100 and 128 days. Each pig was weighed and then slaughtered by exsanguination after electrical stunning. The two muscles chosen for investigation, the semitendinosus and the trapezius, were dissected free immediately after slaughter from the left side of the carcass. A complete midbelly transverse section was then taken from the semitendinosus while from the trapezius a strip, up to 2 cm in length, was cut from midway along the caudal edge of the thoracic portion. Serial sections of 10 μm thickness were cut at −22 °C. Four histochemical techniques were applied to the serial sections from each muscle sample. The techniques used were: the demonstration of (i) acid and (ii) alkaline stable adenosine triphosphatase (acid and alkaline ATPase) as outlined by Guth & Samaha (1970), (iii) the demonstration of succinate dehydrogenase (Nachlas *et al.* 1957) and (iv) the demonstration of glycogen phosphorylase as described by Takeuchi (1956; a modification of the method outlined by Takeuchi & Kuriaki, 1955).

Table 2. *Myofibre type percentages in 'mature' muscle of pigs* (n = 11) *over a liveweight of* 8·5 *kg*

	Mean % (S.D.)		
	Semitendinosus		
Fibre type	'Superficial'	'Deep'	Trapezius
SO	3·0 (2·6)	38·5 (6·0)	40·4 (4·6)
IO	—	1·0 (1·1)	1·0 (1·1)
FO	—	2·7 (2·7)	2·7 (1·4)
FOG	26·9 (7·1)	35·7 (4·1)	23·5 (6·6)
FG	71·3 (7·8)	23·8 (5·8)	32·4 (9·4)

Procedure of myofibre typing

A range of intensity was apparent in the acid and alkaline ATPase staining of muscle sections from young animals. Therefore, fibres were classified according to whether they showed a positive, negative or intermediate reaction for acid and alkaline ATPase. In addition, fibres were classified as succinate dehydrogenase-positive or negative. Although a continuous spectrum of activity of this enzyme ranging from weak to strong was apparent, fibres possessing weak activity were recorded as non-oxidative. Finally, fibres were recorded as either glycogen phosphorylase-positive, if staining was detectable, or negative, if staining was negligible. A total of 9 different combinations of histochemical staining patterns was recorded and categorised under 5 major titles (SO, transitional types IO and FO, FOG and FG), as displayed in Table 1. The histochemical (ATPase) fibre type staining pattern of the superficial portion of the semitendinosus (Table 2) was distinct from that of the deep portion as it possessed an almost negligible number of slow fibres and was therefore characterised as a 'fast' muscle area. The deep portion of the semitendinosus and the whole of the trapezius presented a mixed myofibre type composition (Table 2). Therefore, three different muscle type areas or 'sections' were obtained from the two muscles studied. Myofibre type percentages were estimated from two randomly selected fascicles from sites within each of these three sections of muscle. This amounted to an average of approximately 600 fibres classified for each section of muscle from each littermate. The two randomly selected muscle fascicles from each muscle section stained for alkaline ATPase were projected onto white card. Each myofibre within the chosen fascicle was outlined and the staining intensity for each of the four histochemical techniques recorded. The number of myofibre types from the two selected fascicles were then summated to determine percentages of each myofibre type for each of the three muscle sections. This procedure was performed for each of the 49 pigs in this study.

Porcine mixed muscle has a unique arrangement of fibres in that the slow (alkaline ATPase-labile, acid ATP-stable; Table 1) fibres are grouped together in bundles. These groups of slow fibres and their surrounding complement of fast fibres are termed 'metabolic bundles' (Fig. 1) in the present report. The numbers of alkaline ATPase-negative (slow, SO) fibres associated with a known number of (at least 150) metabolic bundles were counted to obtain an average value for the muscles showing mixed fibre types, i.e. the deep portion of the semitendinosus and the trapezius, from each animal.

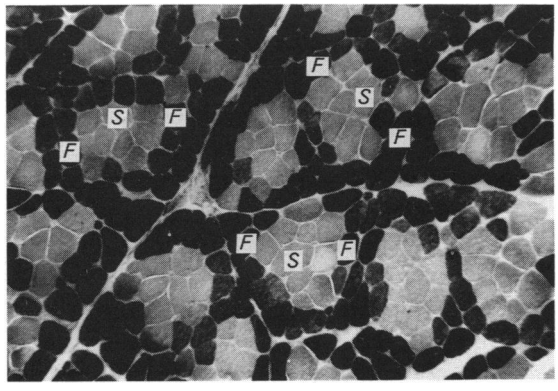

Fig. 1. Muscle fibres from the deep portion of the semitendinosus muscle of an 8·4 kg Large White pig stained for the demonstration of alkaline ATPase. Groups of slow-contracting, alkaline ATPase-positive (*S*) fibres and their surrounding complements of fast-contracting (*F*) fibres are termed 'metabolic bundles'. × 163.

Determination of the mean transverse sectional area of each myofibre type

The mean transverse sectional area (TSA) of 50 randomly selected fibres from each of three types classified as either SO, FOG (including FO) or FG were determined for the three muscle sections of each pig. TSA measurements were made of outlined fibres on a Reichert–Jung Videoplan. An estimate of the total TSA of slow muscle within the midbelly of the semitendinosus muscle from each littermate was made by multiplying the mean TSA values for slow fibres determined for both the deep and superficial portions of this muscle by the number of slow fibres estimated for each respective portion.

Analysis of data

The relative change in the histochemical properties and mean myofibre TSAs had to be quantified for a comprehensive analysis and a valid comparison between littermates. Regression equations were established for relationships between the measured parameters (i.e. slow fibres per metabolic bundle, proportions and TSAs of myofibre types) and both age and liveweight. The establishment of linear relationships between the TSAs of myofibre types and age or liveweight required logarithmic transformation of the X and Y variates. The resultant data enabled comparisons of changes in the number of slow fibres per metabolic bundle, and of changes in myofibre type percentages and TSA growth between littermate groups and also the analysis of differences in the TSA growth between fibre types. When two regression equations were established as having essentially equal coefficients, statistical comparison of their intercepts (Quenouille, 1969) enabled differences in their relative values of Y to be estimated for a given value of X; thus, when littermate groups showed similar rates of change with liveweight or age in any of the measured parameters, relative differences in the measured parameters between littermates of the same age or liveweight could be established.

RESULTS

Birthweights and liveweight gain

The mean birthweights (±S.D.) of the large, small and runt littermates were 1544 g ± 189 (n = 17), 1144 g ± 190 (n = 13) and 776 g ± 107 (n = 19), respectively.

The runt was, on average, about half the weight of the large littermate at birth, and the small littermate represented the median birthweight between that of the large and that of the runt. The difference in birthweight, calculated by a paired '*t*'-test, between each littermate category was highly significant ($P < 0.001$). Liveweight differences, evaluated by regression analysis, were maintained between large and runt ($P < 0.001$), and large and small ($P < 0.01$) littermates throughout the period studied.

Myofibre type differentiation

In the neonatal pig all the muscle fibres of the semitendinosus and the trapezius exhibited positive succinate dehydrogenase staining. Differential staining began at 6 days with a reduction in the intensity of staining in the superficial portion of the semitendinosus. This differential staining appeared to be specific to a liveweight of approximately 2·5 kg in the superficial portion of the semitendinosus while in its deep portion and in the trapezius differential staining was not apparent until the heavier liveweight of 8·5 kg was attained (these liveweights corresponded to the ranges in age of 6 to 19 days and 33 to 64 days, respectively). Once differentiation with respect to succinate dehydrogenase had occurred there was no significant change in the proportion of oxidative fibres.

At birth the glycogen phosphorylase activity in all myofibres was negligible. Activity of this enzyme was detected at a liveweight of 2·5 kg (6 to 19 days) in all muscle sections. The proportion of glycolytic fibres remained constant after the liveweight reached 8·5 kg. Glycogen phosphorylase activity in the myofibres of the superficial portion of the semitendinosus coincided with the loss of 100% succinate dehydrogenase staining. Therefore, fibres classified as FOG and FG could be identified in the superficial portion of the semitendinosus (a predominantly fast muscle) after the attainment of 2·5 kg liveweight, whereas in the deep portion of this muscle and in the trapezius (examples of mixed muscles), although FOG fibres appeared at this liveweight, FG fibres were not observed until about 8·5 kg.

Neonatal pigs presented poor alkaline ATPase differential staining in their muscles. However, one slow fibre per metabolic bundle was apparent in the deep portion of the semitendinosus in each littermate just after birth, while the trapezius possessed an average of $2{\cdot}4 \pm 0{\cdot}2$ slow fibres per metabolic bundle. On average, $3{\cdot}7 \pm 0{\cdot}6$ and $4{\cdot}5 \pm 0{\cdot}6$ (mean ± S.D.) fibres per metabolic bundle were stained positively for acid ATPase just after birth in the deep portion of the semitendinosus and in the trapezius, respectively. The disparity in the number of acid ATPase-positive and alkaline ATPase-negative fibres in the young pig indicated the presence of a population of transitional fibres (Table 1).

Transitional myofibres

In mixed muscle from the deep portion of the semitendinosus and from the trapezius a gradation of myofibre types was shown to exist between the mature SO and FOG fibres. As evident from Table 1, six of these so called 'transitional' fibre types were identified and allocated to two classes, IO and FO. All transitional fibres were oxidative and non-glycolytic, and possessed ATPase activities that ranged in staining intensity from alkaline ATPase-high and acid ATPase-low, to alkaline ATPase-intermediate and acid ATPase-high. Those fibres classified as FO were considered transitional fibres only after glycogen phosphorylase staining had been

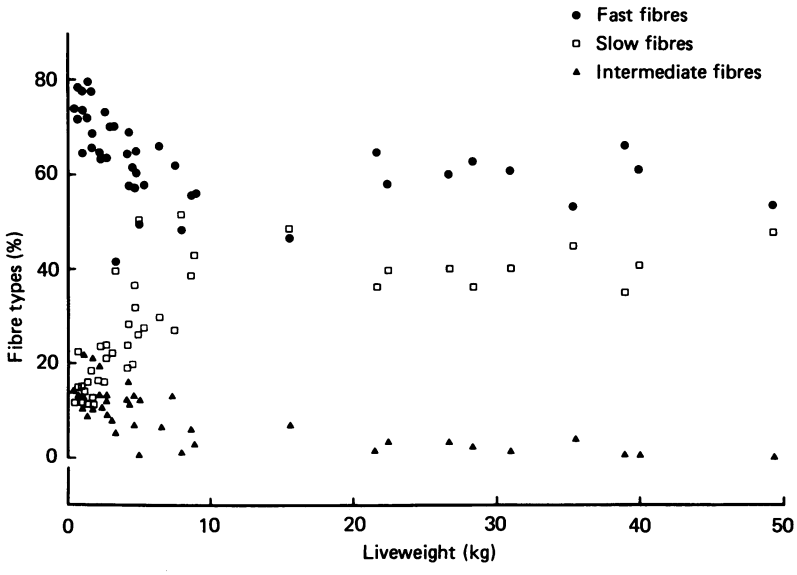

Fig. 2. Percentages of fast, slow and intermediate fibres (as classified in Table 1) in the trapezius muscle against liveweight.

detected in muscle sections; prior to this the majority were considered to be merely undifferentiated fibres and progenitors of the FOG fibre population.

The decrease in the percentage of transitional fibres with increasing liveweight appeared to be related to the attainment of the mature myofibre type ratios, especially those of the SO fibres. This is demonstrated by the myofibre type changes of the trapezius shown in Figure 2, which were similar to those seen within the deep portion of the semitendinosus. The negligible number of transitional fibres demonstrated in muscle from pigs heavier than 8·5 kg coincided with the constancy of the fibre type percentages that were considered representative of mature muscle (Table 2).

Changes in the proportions of myofibre types with growth

After birth the number of slow fibres per metabolic bundle, and therefore the percentage of slow fibres, in the deep portion of the semitendinosus and in the trapezius increased until a liveweight of approximately 8·5 kg was attained. Thereafter the number of slow fibres per metabolic bundle, and hence the percentages of slow fibres, were not shown to change significantly (Fig. 2). The superficial portion of the semitendinosus failed to show any slow fibres until 15 days postnatally but once slow fibres were established, their proportion remained constant. The percentages of slow fibres in the mature semitendinosus and trapezius muscles are given in Table 2. No significant differences existed between littermates of equal liveweight or age in the number of slow fibres per metabolic bundle of either the deep portion of the semitendinosus or of the trapezius. Likewise, the total percentages of slow fibres were not found to be significantly different between littermates of equal age in either of these muscles. However, on an equal liveweight basis the percentage of slow fibres in the trapezius was significantly greater ($P < 0{\cdot}05$) in the runt than the small littermate, while in the deep portion of the semitendinosus differences existed between the large and the runt ($P < 0{\cdot}001$) and the large and the small ($P < 0{\cdot}05$) littermate groups.

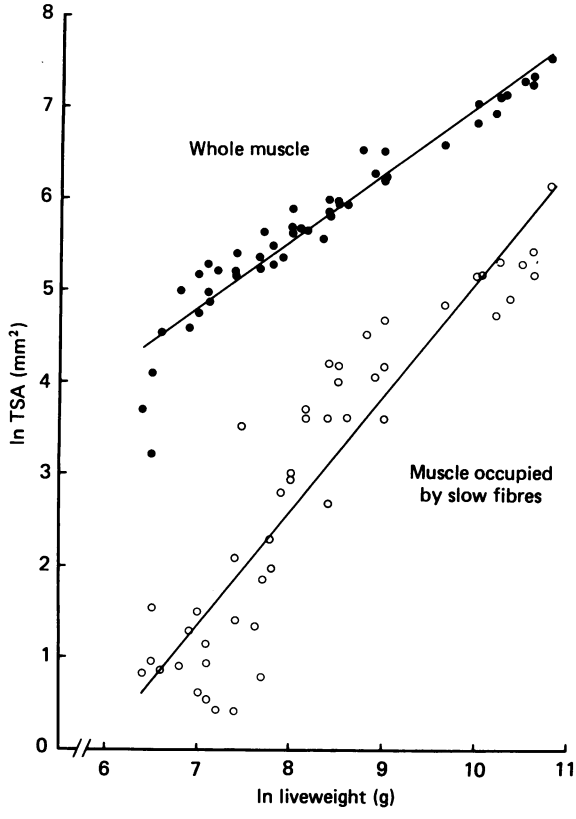

Fig. 3. Changes in the whole TSA of the semitendinosus muscle and the area of the muscle occupied by slow fibres with liveweight. Regression equations: Whole muscle, ln $Y = 0{\cdot}74$. ln $X - 0{\cdot}39$. Muscle occupied by slow fibres, ln $Y = 1{\cdot}23$. ln $X - 7{\cdot}26$.

Changes in the TSA of muscle occupied by slow myofibres

As shown in Figure 3, from birth to 128 days of age the TSA of the semitendinosus and the area occupied by slow fibres in the muscle increased with liveweight. The relationships established between the natural logarithms (ln) of these two parameters against both ln (liveweight) and ln (age) were essentially equal between littermates. The regression coefficient of the relationship between ln (semitendinosus TSA) and ln (liveweight) (Fig. 3) was not significantly different from 2/3, while the regression coefficient of ln (total area of slow muscle) and ln (liveweight) (Fig. 3) was significantly greater than 1·0 ($P < 0{\cdot}01$) and was best estimated at 5/4.

No significant difference existed in the area of slow muscle between littermates of the same age. However, the runt and small littermates both possessed a significantly ($P < 0{\cdot}001$) greater area of slow semitendinosus muscle than their respective large littermates on an equal liveweight basis.

Changes in the mean TSAs of myofibre types with growth and differences between littermates.

The regression coefficients of ln (myofibre mean TSAs) against both ln (age) and ln (liveweight) were not significantly different between littermate groups. This indicated that the rate of growth of myofibre TSAs was the same between litter-

Table 3. *Regression data for ln (myofibre type mean TSAs) against ln (liveweight)*

Muscle	Myofibre type	Regression coeffiients b (S.E.)†	Intercepts (ln) of regression lines			Significance of difference between intercepts		
			Large	Small	Runt	Large: Runt	Large: Small	Small: Runt
M ST* Deep Portion	SO	0·62 (0·04)	0·25	1·09	1·92	$P < 0·001$	$P < 0·001$	$P < 0·001$
	FO+FOG	0·82 (0·04)	−0·87	0·34	−0·77	$P < 0·05$	$P < 0·001$	$P < 0·001$
	FG	0·89 (0·04)	−1·52	−0·21	−1·26	NS	$P < 0·001$	$P < 0·05$
M ST* Superf. Portion	SO	0·58 (0·05)	0·82	1·89	1·86	$P < 0·001$	NS	NS
	FO+FOG	0·95 (0·03)	−2·30	−1·94	−1·87	NS	$P < 0·01$	$P < 0·001$
	FG	0·97 (0·05)	−2·25	−1·33	−1·97	$P < 0·001$	$P < 0·001$	NS
Trapezius	SO	0·67 (0·03)	0·18	0·03	0·58	$P < 0·01$	NS	$P < 0·001$
	FO+FOG	0·69 (0·03)	−0·37	0·79	−0·10	NS	$P < 0·001$	$P < 0·001$
	FG	0·76 (0·04)	−0·85	−0·37	−0·35	$P < 0·05$	$P < 0·001$	$P < 0·05$

*, Semitendinosus muscle. †, For large, small and runt TSAs combined.

mates. Comparisons of the intercepts of the regression lines for the natural logarithms of the various myofibre type mean TSAs and ln (age) for littermate groups revealed that littermates of the same age possessed myofibres of essentially equal TSAs. However, when a similar evaluation was made of specific myofibre type mean TSAs between littermates of the same liveweight they were not always found to be equal (see Table 3). Where significant differences existed, the myofibre types under consideration were larger in the lower birthweight littermate groups. It is interesting to note that the SO fibre mean TSAs in the deep portion of the semitendinosus were more significantly different ($P < 0·001$) between each littermate group than any other fibre type studied.

Regression coefficients for ln (myofibre mean TSAs) against ln (liveweight) for the SO myofibre types of the three muscle sections (as defined in Materials and Methods) and also for the FOG types of the trapezius (for all littermate groups) were not significantly different from 2/3. This suggested that the TSAs of these fibre types increased with the 2/3 power of the animal's liveweight. All the other types (i.e. the FOG and FG types of the deep and superficial portions of the semitendinosus and the FG types of the trapezius) grew at a significantly ($P < 0·025$) greater rate than the SO types. The TSAs of these other myofibres had the highest postnatal growth rates and in fact increased in direct proportion to liveweight.

DISCUSSION

The initial histochemical differentiation of muscle fibre types with respect to glycogen phosphorylase and succinate dehydrogenase was found to extend over a larger age range and up to a greater age (19 and 64 days, respectively) than has previously been determined for various muscles in the pig. The initiation of glycogen phosphorylase staining has been observed during the first week (in semitendinosus, Beermann, Cassens & Hausman, 1978) and up to 10 days (longissimus dorsi and the diaphragm, Davies, 1972) postnatally, while Ashmore, Addis & Doerr (1972*a*; cutaneous muscle and triceps brachii) found succinate dehydrogenase-negative (non-oxidative) fibres at 14 days and Davies (1972) demonstrated these fibres at 12 days

after birth. However, in the present investigation animals with a range in birthweights were specifically selected so that at any given liveweight the littermates of low birthweight were considerably older than those of a heavier birthweight. The results indicated that myofibre type differentiation, with respect to succinate dehydrogenase and glycogen phosphorylase, was weight, rather than age, specific, being more related to physiological than chronological age.

The process of fibre type conversion from fast to slow contractility, which was responsible for the increasing proportion of slow fibres in the growing muscle, involved the transitional myofibre types shown in Table 1. The transitional fibre types of the present study parallel the intermediate types documented by Suzuki & Cassens in pig (1980) and in sheep (1983) muscle. The population of FO fibres was apparently derived from fibres that had not yet adapted a combination of contraction speed and metabolic properties characteristic of a 'mature' fibre type. These fibres appeared to be the precursors of the transitional forms. Since the percentages of mature FOG and FG fibres were not attained in pigs until they had reached a liveweight of about 8·5 kg, after which the percentages of transitional fibre types were negligible (Table 2; Fig. 2), it seems that a contribution of FG or FOG fibres to the transitional myofibre population did not occur in either the semitendinosus or trapezius muscles of the pigs studied here.

Prior to the complete loss of alkaline ATPase stability the myosin ATPase of the FO fibres appeared to attain increasing degrees of acid stability (presented by Subtypes IOa, IOb, IOc and IOd; Table 1), a phenomenon documented by Kugelberg (1976) in rat soleus muscle, as well as by Suzuki & Cassens (1980) in pig muscles. These subtypes were already present in neonatal pig muscle which exhibited ratios of acid-stable to alkaline-labile fibres of between 1·6 and 4·9, illustrating the commencement of fibre type conversion prenatally (Beermann *et al.* 1978) in the pig. After a certain degree of acid stability had been achieved (demonstrated by Types IOb and IOd; Table 1) the alkaline stability of myosin ATPase appeared to decrease (seen in Types IOa and IOc; Table 1) until the acid and alkaline stability was characteristic of that demonstrated by SO fibres. Thus transitional fibres exhibiting a continuum of both acid and alkaline ATPase stabilities intermediate to FO and SO fibres were displayed on conversion to the latter fibre type.

The rates of change, with increasing liveweight, in the TSAs of the fast myofibres, especially the FG fibres, were generally greater (particularly in the semitendinosus) than those of the slow (SO) fibres (Table 3). It is interesting to relate these comparative differences in postnatal fibre size changes to the results of work carried out on the effects of undernutrition on the size of various muscle fibre types. A study conducted on protein-deprived rats from 6 to 25 weeks after birth (Oldfors, Mair & Sourander, 1983) shows that Type 1 (SO) and Type 2A (FOG) myofibres of the extensor digitorum longus muscle fail to grow while Type 2B (FG) fibres actually atrophy under this treatment. This dietary manipulation demonstrates the vulnerability of myofibres with a fast contraction speed to nutritional inadequacy. If postnatal undernutrition were to have similar effects in the pig as it does in the rat it would appear that relative postnatal myofibre growth rates are an indication of the comparative vulnerability of the different myofibre types to growth inhibition. The lack of differences in the relative growth rates of myofibre types between littermates of all three birthweight categories was verification of the adequate postnatal nutrition of even the most physically disadvantaged runt pigs.

The observation made, during the course of this work, that low birthweight pigs

have significantly larger myofibre TSAs in their semitendinosus and trapezius muscles (the degree of significance depending on the myofibre type and muscle, see Table 3) than their heavier littermates when considered at the same weight has also been noted by other authors. Hegarty & Allen (1978) found that, of the psoas major, semitendinosus, biceps brachii and longissimus dorsi muscles, the former two muscles contained fibres of greater ($P < 0{\cdot}05$) TSAs in runt pigs (average birthweight 810 g) than in their large littermates (average birthweight 1570 g) at 106 kg; the myofibre areas of the latter two muscles were not significantly different between siblings at the same liveweight. Likewise Powell & Aberle (1981) found that the fibre TSAs of the semimembranosus of runts (birthweight under 1000 g) were either equal to, or greater than, those of their heavier birthweight (over 1500 g) littermates at equal slaughter-weight. However, these authors did not attribute the fibre size differences to the disparate ages of similar slaughter-weight siblings of low and high birthweight. Since low birthweight pigs appear to maintain a significantly lower weight ($P < 0{\cdot}001$) through to 128 days they will be chronologically older at any specific weight. This highlights the very interesting and fundamentally important phenomenon that there must be an age-factor, unrelated to the growth-force of weight, influencing the TSA growth of myofibres. The relative differences in myofibre TSAs seen between littermates of the same liveweight is of particular significance in another aspect of muscle development, namely that concerning the TSA of slow muscle within the semitendinosus muscle which is discussed below.

The precise way in which the postural function of the semitendinosus adapts to the increasing liveweight of the pig through changes in the TSA of slow muscle was revealed in the present study. The increase was achieved, up to a liveweight of approximately 8·5 kg, by an increase in the percentage of slow fibres within the deep portion of the muscle together with an increase in the TSA of the individual fibres. The superficial portion of the semitendinosus contributed very few slow fibres (Table 2) and therefore a minimal supportive role to the muscle. Muscles, or parts of muscles concerned with a propulsive function, do not appear to show changes in their complement of slow fibres. This was exemplified by the superficial portion of the semitendinosus in the present study and in that of Sivachelvan & Davies (1981), and the biopsies (and therefore, presumably, the superficial portion) of the semitendinosus muscle of cattle in the work of Holmes & Ashmore (1972). Beyond a liveweight of 8·5 kg the only significant factor contributing to the increase in the area of slow muscle was the increasing TSA of the slow fibres. The results presented for the semitendinosus support the general hypothesis proposed by Davies (1972) that the TSA of a muscle varies, during growth, with the 2/3 power of liveweight while within the muscle the area occupied by slow fibres increases at a greater rate than the total TSA of the muscle. However, the differing regression coefficients determined for the longissimus dorsi (1·0; Davies, 1972) and for the semitendinosus in this paper (5/4) suggests that the slow muscle TSA increase, relative to liveweight, is a reflection of a specific muscle's unique adaptation to its comparative postural role within the body. This theory is supported by the relative values for the average alkaline ATPase-negative (slow) fibre type percentages for the two muscles; the longissimus dorsi of the mature Large White possesses less than half the number of slow fibres (18 %; Davies, 1972) possessed by the deep portion of the semitendinosus (38·5 %; Table 2). Consequently the supportive role of the former muscle is probably less than that of the latter.

The mechanism by which the muscle and its innervating neurons, which are

responsible for the muscle fibres' contractile properties (Close, 1965; Salmons & Sréter, 1976), acknowledge the need for an adaptation in the muscle's slow fibre compliment has yet to be elucidated. However, a notion of the way in which the supportive role of a muscle is maintained in accordance with the liveweight of an animal is given by the present findings. Littermates, when considered on a constant liveweight basis, had a similar number of slow fibres per metabolic bundle while possessing different slow fibre percentages. It therefore appears that it is possibly the number of slow fibres within a metabolic bundle, rather than the resultant percentage, that is regulated to maintain a constant relationship with liveweight. The percentage of slow fibres is a consequence of the number of fibres per metabolic bundle and the surrounding complement of fast fibres. The secondary/primary fibre number ratios, which are an indication of the total number of fibres per metabolic bundle, were significantly lower ($P < 0{\cdot}01$) in the low birthweight, runt littermates (Handel & Stickland, 1984) than in their heaviest birthweight littermates; the secondary/primary fibre number ratio, although lower in the small compared to large littermates, was not significantly different. Nevertheless, after acquisition of the number of slow fibres per metabolic bundle appropriate for the liveweight of the animal, both the small and runt littermates appeared to possess a lower complement of fast fibres and therefore a greater percentage of slow fibres (this produced a significant effect in the semitendinosus muscle). This, together with the greater mean TSA of the slow fibres within the deep portion of the semitendinosus of both small and runt littermates, contributed to the greater slow muscle TSA of these lower birthweight littermates relative to their large littermates. The resultant effect is indicative not only of a 'slower' semitendinosus muscle in the lower birthweight small and runt pigs but also higher oxidative and lower glycolytic capacities of this muscle (slow fibres were exclusively oxidative, Table 1).

The observations made as a result of this work indicate that relative weight at birth has a significant contributory effect on the subsequent comparative myofibre type composition of the muscle between pig littermates. It has also been revealed that age, in addition to liveweight, has an important influence on muscle growth and, likewise, its subsequent comparative myofibre composition. One of the cumulative effects of these phenomena is to produce significantly greater amounts of slow muscle in the smaller birthweight animals than in their larger siblings.

SUMMARY

Muscle growth and development was studied in 49 Large White pigs from a total of 17 litters. Representative large (mean birthweight of 1544 g), small (1144 g) and runt (776 g) littermates were selected and slaughtered at the same age, ages ranging from birth to 128 days. Fresh frozen, serial transverse sections taken from the semitendinosus and trapezius muscles of these animals were stained for the histochemical demonstration of acid and alkaline pre-incubated adenosine triphosphatase, succinate dehydrogenase and glycogen phosphorylase. Profiles of the muscle fibre types were compiled for each animal.

In both muscles the number of slow oxidative (SO) fibres, that were arranged together in groups within 'metabolic bundles', increased with growth. The transverse sectional area (TSA) of the semitendinosus muscle increased with the 2/3 power of liveweight whereas the area occupied by SO fibres increased at a rate significantly greater than 1·0 ($P < 0{\cdot}01$). Regression analysis revealed that the area of this muscle

occupied by SO fibres was greater ($P < 0.001$) in runt and small littermates relative to their large littermates when they were compared at an equal liveweight. This greater TSA of the semitendinosus classified as 'SO' in lower birthweight pigs was the result of a combination of higher percentages ($P < 0.05$) of SO fibres and significantly greater ($P < 0.001$) SO fibre mean TSAs. The mean TSAs of all myofibre types were similar between littermates of the same age but most types were of greater TSA in the lower birthweight littermates when compared (by regression analysis) at the same liveweight suggesting that fibre TSA was age- rather than weight-related.

The higher percentage of SO fibres in the low birthweight pigs, when compared at an equivalent liveweight to their large littermates, appeared to be related to their affected secondary/primary fibre number ratio. This phenomenon, plus the data on the number of slow fibres per metabolic bundle, indicated that it was apparently the number of slow fibres per metabolic bundle which was regulated with liveweight gain rather than the resultant percentage of slow fibres within the muscle.

The authors wish to acknowledge the technical assistance of Gordon Goodall. This work was supported by the funding of the Agriculture and Food Research Council.

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