J. Anat. (1992) **181**, pp. 373–376, with 2 figures. Printed in Great Britain

Short Report

Does the anatomical location of a muscle affect the influence of undernutrition on muscle fibre number?

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(*Accepted 21 July 1992*)

ABSTRACT

Nutritional restriction during muscle fibre number development invariably has a more detrimental effect on the biceps brachii than on the soleus. The difference may be due to the relative proportions of fibre types in the muscles or the anatomical location of the muscles. To distinguish between the effects of location and fibre type the biceps (fast, cranial and proximal), soleus (slow, caudal and distal), and extensor digitorum longus (EDL) (fast, caudal and distal) were examined from control and undernourished guinea pig neonates. A 40% reduction in maternal intake resulted in a reduction in neonate body and muscle weights ($P < 0.005$), biceps ($P < 0.05$) and EDL fibre numbers ($P < 0.005$), but did not affect soleus fibre number. At birth the ratio of fast fibres to slow was 7.5 for the biceps, 7.0 for the EDL, and 1.5 for the soleus. The effect of undernutrition on muscle fibre number therefore seems to be a function of the fibre types in that muscle.

INTRODUCTION

In the guinea pig, a 40% reduction in maternal feed intake has been shown to cause a decrease in muscle fibre numbers in the biceps brachii but not in the soleus of the progeny (Ward & Stickland, 1991). It seems that developing muscles with a relatively high slow-twitch content invariably suffer a smaller deficit in muscle fibre number than more fast-twitch muscles with maternal undernutrition (Howells et al. 1977; Bedi et al. 1982). This suggests that the effect of nutrition on muscle fibre development may be a function of the relative proportions of the fibre types. However, an alternative explanation may be the anatomical location of the muscles in the body. Mammalian development proceeds in a cephalocaudal and proximodistal direction with the head being most advanced, the trunk in advance of the limbs, and forelimbs in advance of the hindlimbs (Fleagle et al. 1975). In addition, a proximodistal gradient can be seen within the limbs. It is possible that, being a less mature muscle than the biceps, nutrients are preferentially directed towards the soleus such that muscle fibre numbers are maintained.

In order to determine whether muscle fibre number reduction is a function of fibre types or due to anatomical location, a third muscle, the EDL, was investigated. This muscle is located in the distal hind limb in close proximity to the soleus, in contrast to the biceps brachii in the proximal fore limb. The EDL is also involved in propulsive actions and hence is a predominantly fast muscle like the biceps brachii. Examination of this muscle should allow a differentiation to be made between the effects of muscle fibre types and anatomical location. This investigation was designed to test the hypothesis that the anatomical location of a muscle may be as important as the proportion of muscle fibre types (and thereby the proportions of secondary and primary fibres during development) in influencing the effect of prenatal undernutrition on muscle fibre number determination.

MATERIALS AND METHODS

This project involved 6 litters of neonate Dunkin–Hartley guinea pigs. Guinea pig dams were mated postpartum in a harem and assigned to a nutritional treatment on d 2 of gestation. The dams of 3 litters

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Table. *Comparison of gross body weights and lengths for control and restricted neonate guinea pigs*

Parameter	Control	40% restricted	% difference	P
Number	12	13		
Age (d)	67.3±1.20	66.3±1.33		n.s.
Body wt (g)	84.89±6.12	55.71±5.53	34.37	< 0.005
Liver wt (g)	4.646±0.447	2.946±0.447	36.59	< 0.05
Biceps wt (mg)	57.72±4.49	37.18±4.58	35.59	< 0.005
Soleus wt (mg)	26.10±1.52	16.44±2.03	37.01	< 0.005
EDL wt (mg)	25.13±1.97	16.19±1.99	35.58	< 0.005
CR length (cm)	11.02±0.16	9.16±0.30	16.88	< 0.001
Tibial length (mm)	27.23±0.46	23.56±0.75	13.48	< 0.001
Humeral length (mm)	21.53±0.36	19.03±0.51	11.61	< 0.001

were fed a standard diet at ad lib levels throughout gestation and formed the control group. The other 3 were pair fed to weight matched controls at a 40% restricted feed level.

Guinea pigs littered at a mean gestational age of 66.83±0.76 d. Mean litter sizes were 4.00 and 4.33 for control and restricted litters respectively. All neonates (n = 25) were killed by an intraperitoneal injection (1 ml/kg body wt) of pentobarbitone sodium (Euthesate). Body weight, liver weight, crown–rump length and the lengths of the humerus and tibia were recorded. The biceps brachii, soleus and extensor digitorum longus (EDL) muscles were removed, weighed, and rapidly frozen. Whole muscle transverse sections (10 μm) were cut and stained to demonstrate ATPase activity using a modification of the technique of Guth & Samaha (1970) at pH 10.3 and 4.6. Total fibre number counts were made for each muscle and animal using a Seescan image analyser (Seescan, Cambridge, UK) and the ratio of fast to slow fibres was determined. For the purposes of this study this was taken to be the mean ratio of fibres showing high myosin ATPase activity after preincubation at pH 10.3 to those showing little or no staining (referred to as the ratio of positive to negative basic ATPase activity). Differences between control and 40% restricted animals were determined by unpaired t tests.

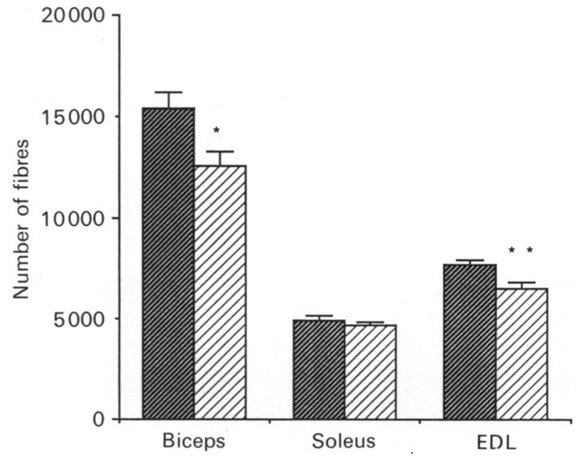

Fig. 1. Mean and S.E.M. of total fibre numbers for biceps, soleus and EDL from control (■) and 40% restricted (▨) guinea pig neonates.

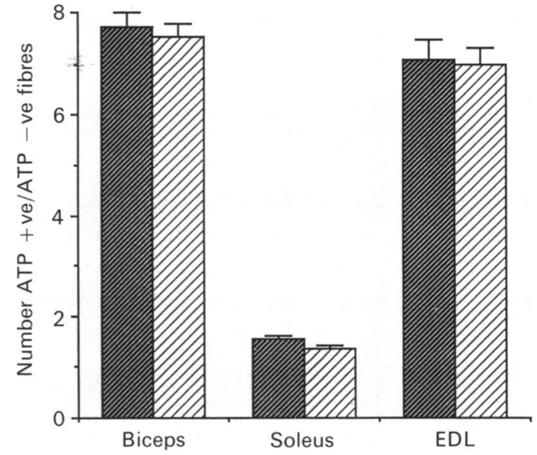

Fig. 2. Mean ratio of basic ATPase positive to basic ATPase negative fibres (fast- to slow-twitch fibres) for biceps, soleus and EDL from control (■) and 40% restricted (▨) neonates, Bars, S.E.M.

RESULTS

Body, liver and muscle weights and body and bone lengths are given in the Table. For all weight and length parameters measured, the control group was significantly larger than the restricted group, demonstrating the effects of the 40% reduction in maternal food intake throughout gestation. Body weight, liver weight and muscle weights were all reduced by approximately one third in the restricted group. Crown–rump, tibial and humeral lengths were reduced by 16.9, 13.5 and 11.6%, respectively.

Muscle fibre number data are shown in Figure 1. Biceps and EDL fibre numbers were significantly reduced in the restricted group by 18.6% ($P < 0.05$) and 15.4% ($P < 0.005$), respectively. The soleus fibre number showed a small decrease of 5.4% in the restricted group; this was not significant.

The ratio of fast to slow fibres (basic ATPase positive to negative) is shown in Figure 2. The soleus was a predominantly slow muscle with only 1.5 fast fibres per slow fibre. The biceps and EDL were both mixed muscles of a predominantly fast nature. The ratio of fast fibres to slow was approximately 7.5 in the biceps and 7.0 in the EDL (Fig. 2).

DISCUSSION

These results demonstrate the significant reduction in body and organ weights of neonates caused by a 40% restriction of maternal dietary intake throughout gestation. The weight of the biceps, soleus and EDL were all reduced by equal amounts (approximately one third). This was despite the known sparing of the soleus from the effects of undernutrition on muscle fibre number (Ward & Stickland, 1991). The location of the soleus and EDL, caudal and distal in relation to the biceps, did not appear to have any influence on the effect of undernutrition on muscle weight.

The most important results of this study was the finding that muscle fibre numbers in the biceps and EDL of restricted neonates were reduced but that muscle fibre number in the soleus was not significantly affected. This suggests, therefore, that the influence of muscle location in the body was not important in determining the effect of undernutrition on muscle fibre number. The percentage reduction in the EDL is slightly less than in the biceps (respectively, 15.4 and 18.6%) which suggests that anatomical location may play a small role. The biceps brachii and EDL, as fast type muscles, contained 7–7.5 fibres per slow bundle, whereas the soleus contained only 1.5. It seems, therefore, that the proportion of fibre types in a muscle is the most important determinant of the influence of nutritional restriction on total fibre number.

The disproportionate effect of nutrition on fast muscles, in comparison with slow muscles, seems to be due to the relationship between twitch speed of a fibre and its generative origin, i.e. slow-twitch fibres tend to be primary fibres in origin (Kelly & Rubenstein, 1986). This is true of the guinea pig soleus where the muscle is predominantly slow and primary fibres account for about 33% of the total fibre number (Ward & Stickland, 1991). This is in comparison with the biceps which is predominantly a fast-twitch muscle and where primary fibres account for less than 10% of all fibres (Ward & Stickland, 1991). Primary fibre numbers have been shown to be unaffected by maternal undernutrition (Wigmore & Stickland, 1983; Handel & Stickland, 1987; Wilson et al. 1988; Ward & Stickland, 1991) or by denervation (McLennan, 1983; Ross et al. 1987; Harris et al. 1989). All these studies, however, showed that the secondary fibre population was susceptible to maternal undernutrition or to denervation during muscle development, leading to a reduced secondary fibre number. Slow muscles, being composed of a relatively high proportion of primary fibres, are therefore less susceptible to environmental influences in terms of reduced secondary fibre numbers.

A more severe nutritional regime, e.g. 50% of the ad lib quantity in the guinea pig throughout gestation (Ward, 1989), 30% throughout gestation in the rat (Wilson et al. 1988), or 50% through gestation and lactation in the rat (Bedi et al. 1982), does cause a reduction in fibre number in the soleus. This reduction was, however, to a lesser degree than the fibre number reduction in fast muscles. The effect of nutrition on the 2 types of muscle seen in this study was not absolute, therefore, but reflected the lesser effect of nutrition on slow muscles.

In conclusion, this study has demonstrated that the effect of nutrition on muscle fibre number is a function of the fibre types in that muscle. Fast muscles suffer a disproportionate reduction in fibre number with undernutrition due to their relatively high contribution to total fibre number made by the secondary fibre population. Slow muscles, with a greater proportion of primary fibres, are less affected by undernutrition.

ACKNOWLEDGEMENT

This work was supported by a grant from the Agricultural and Food Research Council.

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