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The relationship between sarcomere length in the soleus and tibialis anterior and the articular angle of the tibia–calcaneum in cats during growth

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INTRODUCTION

In the postnatal growth of vertebrate skeletal muscles two results seem well established: firstly, the total number of sarcomeres increases along the individual fibres; secondly, the thick and thin filaments in the myofibril have the same length in the newborn and the adult animal (Goldspink, 1964, 1968). Changes in sarcomere length have also been reported, although Elliott & Crawford (1965) found that the length of the sarcomeres of the fully extended tibialis anterior muscle was the same in 3 weeks old and adult rabbits, Goldspink (1968) reported an increase in sarcomere length in the mouse biceps brachii. However, it is now not certain that the mouse muscles were fixed at equivalent lengths in the young and adult animals (Goldspink, personal communication). In addition, some authors have studied the length of sarcomeres at that muscle length at which tetanic tension is maximal. Calculation from Close's (1964) paper suggests that this sarcomere length decreases with age by about 30%. Edtjthehadi & Lewis (1974) re-investigated the problem with better immobilization of the muscle during fixation, and they concluded that in both the soleus and the flexor digitorum longus the sarcomeres were of identical length at all ages when measured at the length which gave the maximal tetanic tension.

The aim of the present work was to investigate the sarcomere length for different muscle lengths related to known articular angles of the tibia–calcaneum. The intention was to study whether, at any definite angle, the sarcomere length was related to the size of the cat, and whether this differed from the length previously measured in adult cats (Tabary *et al.* 1976). The shape of the length v. active tension curves was also studied in kittens of different ages and compared to that of curves obtianed from adult cats.

The two muscles used were the soleus and the tibialis anterior because it was felt that a comparison between a slow contracting muscle (soleus) and a fast contracting muscle (tibialis anterior) would be of interest.

METHODS

The 45 kittens used in this study were from 10 minutes to 5 months old. They were divided into six groups. In the first group (at birth) the animals were not yet using their muscles to support their weight. In the second group (2–5 days) the kittens were crawling. In the third group (10 days) they walked clumsily. In the last three groups

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(1, 2, 4–5 months) they walked and jumped as skilfully as adult animals. For histological study, 50 soleus and 42 tibialis anterior muscles from 32 cats were used. In most cases (25) both soleus and tibialis anterior were prepared in the same animal; sometimes the muscles of the two legs were used. For physiological study, 13 other animals were used, all belonging to groups 3–6. The length *v.* active-tension curves were established for 18 muscles.

The animals were anaesthetized with 40 mg pentobarbital sodium/kg body weight injected intraperitoneally. Soleus and tibialis anterior muscles were exposed, the animals were induced into a state of deep anaesthesia by a supplementary dose. The limbs were pinned out on a wooden board at an articular angle different in each case. However, these various angles did not exceed the articular range in which the muscle length bears a fairly linear relation to the articular angle (Tabary *et al.* 1976). The extension of the soleus was made from plantar-flexion (tibia–calcaneum angle 150°) to dorsiflexion (tibia–calcaneum angle 25°). The extension of the tibialis anterior was made from dorsiflexion (40°) to plantar-flexion (160°).

The animals were killed by intracardiac injection of pentobarbital, the limbs were detached from the animal and the wooden boards with the limbs on them were immersed in glutaraldehyde fixative (2·5 %) for 24 hours. The muscles were dissected out and washed in phosphate buffer at pH 7·2 for 24 hours, the angle of the tibia–calcaneum and the length of the fibula being measured. Further preparations were similar to those previously described (Tardieu *et al.* 1974; Tabary *et al.* 1976), the special arrangement of tibialis anterior fibres being taken into account. For each fibre studied the length of the fibre was measured, and the number of sarcomeres was counted for about 20 regions along the length of the fibre, each region containing about 50 sarcomeres. The average sarcomere length for the particular fibre was calculated, and the number of sarcomeres was then obtained by dividing the fibre length by the average sarcomere length. In all, six fibres were isolated from each muscle, and in addition the lengths of six other fibres were measured.

The methods used to obtain tension–extension curves were similar to those described in details for adult cats (Tardieu *et al.* 1974). After sectioning the sciatic nerve the anaesthetized animal was placed on an operating table with the tibia being firmly fixed to the table. The muscle was exposed, and the tendons of the other muscles of this leg and foot were cut. A mark was then made on the muscle which permitted the measurement of muscle length relative to the different angles of the tibia–calcaneum. Then the calcaneum was sectioned and the passive tension–extension and the tetanic tension–extension characteristics were measured. The active tension measurements were plotted against the different lengths of the muscle corresponding to known ankle positions. Some measurements were repeated at the end of each experiment to check that the tension had not diminished because of fatigue. For the soleus the curves of different sized animals were lined up so that the 90° angles on the abscissa coincided. The tibialis anterior muscle is slack at 90° when uncontracted, so the curves were lined up at the angle 180°, which allowed precise measurement. The range between complete flexion and complete extension was measured on the anatomical preparation because the measurement of this range *in vivo* was too imprecise in young kittens.

RESULTS

Sarcomere number

Figure 1 shows the means of the sarcomere number for the six groups and adult cats for the soleus and tibialis anterior. In Figure 1 (A) it is plotted against the body weight, in Figure 1 (B) against the length of the fibula, which was chosen as an alternative index of the size of the animal. A highly significant linear correlation was found between the sarcomere number and the length of the fibula, this correlation being essentially the same as that previously obtained for adult cats (Tabary *et al.* 1976). The length of the fibula is the most reliable criterion, particularly when the age of the animal is not precisely known.

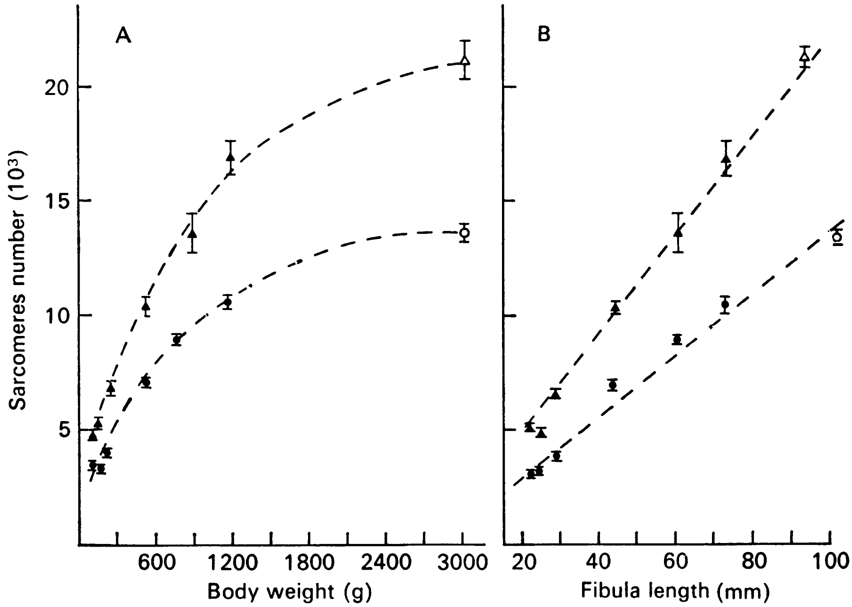

Fig. 1 (A). The mean sarcomere numbers (±S.E. of the mean) for the six groups of kittens and adult cats are plotted against the body weights. (B). The same mean sarcomere numbers are plotted against the lengths of the fibulae. The regression line is shown. In A and B the filled circles represent the soleus and the triangles the tibialis anterior.

Table 1. *Ratio of soleus/tibialis anterior sarcomere number*

Adult cats:	0·62±0·011	Group 1: 0·66±0·007	Group 4: 0·68±0·021
All kittens:	0·64±0 013	Group 2: 0·61±0·007	Group 5: 0·68±0·023
		Group 3: 0·59±0·021	Group 6: 0·62±0·009

The mean±S.E. of the mean are indicated. At the level $P = 0{\cdot}01$, no significant difference is found at any age.

The total sarcomere numbers for soleus and tibialis anterior are shown to differ markedly. The mean ratio between the soleus and tibialis anterior sarcomere numbers is 0·64±0·01 for the six groups, and this does not differ significantly ($P = 0{\cdot}01$) at any age (Table 1).

Mean sarcomere length

In Figures 2 and 3 the sarcomere length has been plotted against the tibia–calcaneum angles for the six groups. The linear regression line obtained for adult cats (Tabary *et al.* 1976) is drawn for comparison. The sarcomere lengths for kittens of all groups for the two muscles were found to be distributed on both sides of the regression line for adult cats.

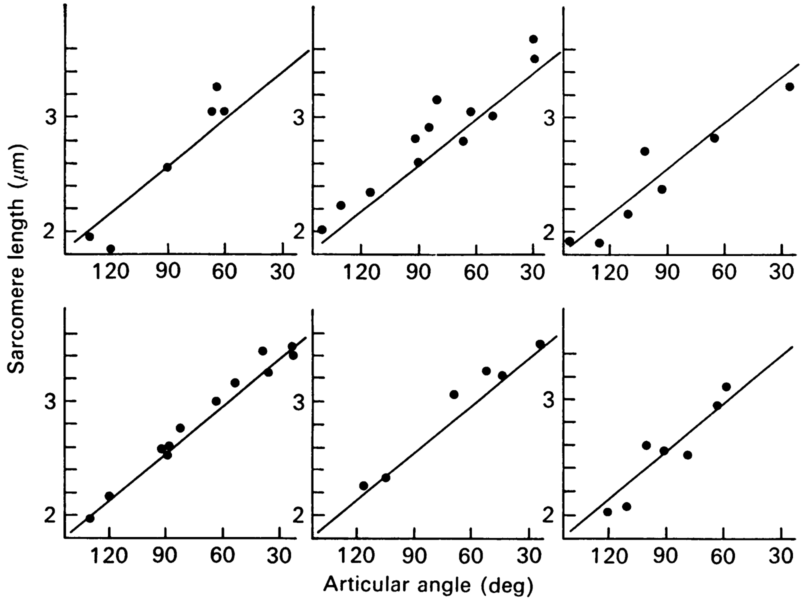

Fig. 2. Shows the sarcomere lengths of the soleus plotted against the tibia–calcaneum angles for the six groups of kittens. Each point represents the value of the sarcomere length of one muscle at a definite articular range. All the points are distributed on both sides of the regression line for adult cats drawn for each group.

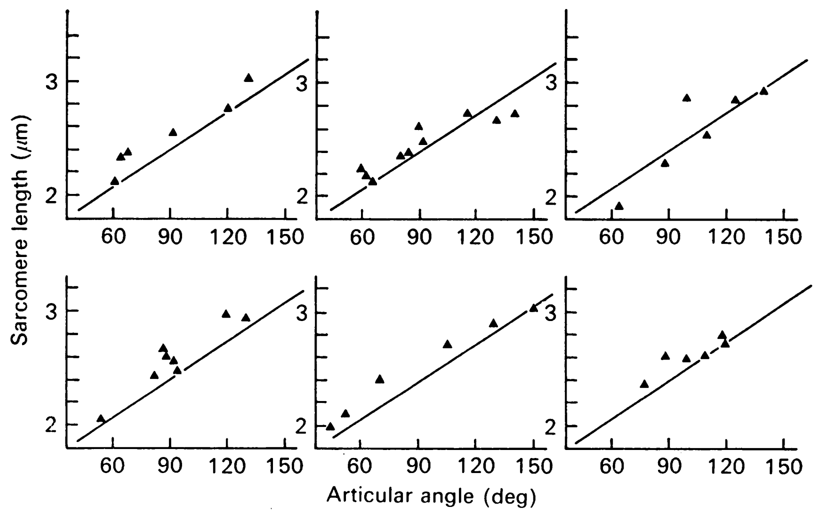

Fig. 3. Shows the sarcomere lengths of the tibialis anterior plotted against the tibia–calcaneum angle as in Fig. 2. The slope of the regression line for adult cats is smaller than in the case of the soleus.

Table 2. *Sarcomere length* v. *articular angle* (*linear regression*)

Groups	Age	Number of muscles studied	r	Slope × 10^2	Compar. with adult cats: t	Calculated sarc. length for 90° (μm)
			SOLEUS			
1	10 minutes	6	0·96	−2·06	2·33*	2·56
2	2–5 days	12	0·95	−1·36	0·01 NS	2·71
3	10 days	7	0·94	−1·25	0·54 NS	2·48
4	1 month	12	0·91	−1·30	0·30 NS	2·66
5	2 months	6	0·99	−1·44	0·64 NS	2·63
6	4–5 months	7	0·94	−1·62	0·87 NS	2·54
All kittens		50	0·98	−1·45	0·99 NS	2·59
Adult cats		25	0·95	−1·36	0·99	2·57
			TIBIALIS ANTERIOR			
1	10 minutes	6	0·98	1·14	1·83 NS	2·53
2	2–5 days	10	0·91	0·74	0·93 NS	2·45
3	10 days	6	0·89	1·32	1·30 NS	2·39
4	1 month	8	0·88	1·02	0·61 NS	2·51
5	2 months	6	0·98	0·95	0·67 NS	2·49
6	4–5 months	6	0·93	0·79	0·44 NS	2·52
All kittens		42	0·91	0·97	0·99 NS	2·48
Adult cats		21	0·94	0·87	0·99	2·40

The table shows, for kittens divided into six groups by age, for all kittens taken together, and for adult cats, the number of muscles studied, the correlation coefficients (r), the slopes of the regression lines. These slopes are negative for soleus because extension of the muscle corresponds to a diminution of the tibia–calcaneum angles. In the last column the sarcomere lengths for an angle of 90° are calculated from the equation of the regression line.

Table 2 shows the results for the six groups of kittens, for all kittens, and for adult cats. The slopes of the regression lines for the kittens are very close to the values found for adult animals, and in general the variations between the six individual groups are very small. A significant difference between the growing cats and adult cats was only found in Group 1 (10 minutes old) for the soleus. However, the numbers in this group were relatively low because of the difficulty in obtaining animals of this age. This result needs to be confirmed.

The slope of the linear regression line is greater for soleus than for tibialis anterior, and the difference is statistically significant. In other words, the sarcomere length of the fully extended soleus is greater than that of the fully extended tibialis anterior. This is true also for adult cats.

Length v. *active tension curves*

Figure 4 shows the length *v*. active tension curves for the soleus and tibialis anterior for cats of different ages. The tension has in every case been expressed as a percentage of the maximum active tension developed by the muscle. The extension is also expressed as a percentage of the articular range. From Figure 4 it will be seen that in all cases the kittens and the adult cats showed essentially the same curve. In particular, in kittens as in adult cats, when the two muscles are compared, it will be seen that the fully shortened tibialis anterior develops appreciably more tension than the fully shortened soleus. Also the tibialis anterior has a greater plateau than the soleus when it is extended.

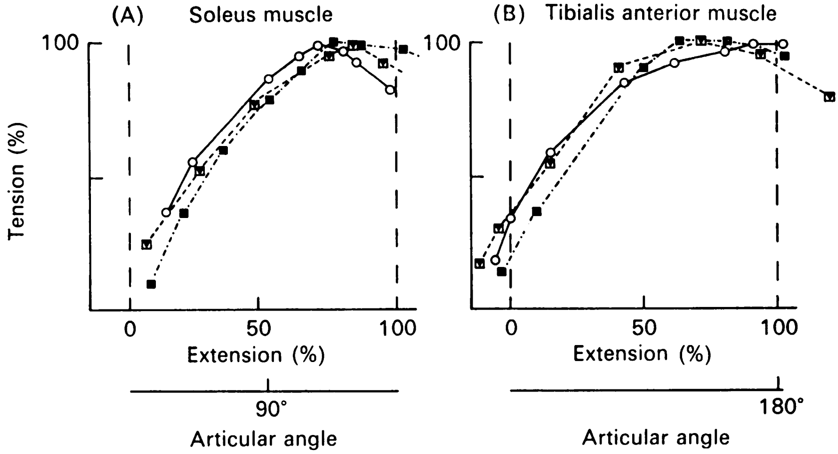

Fig. 4. Examples of length *v.* active tension curves for soleus and tibialis anterior. On the abscissae the extensions are expressed in percentage of the total articular range. One measured angle of the ankle is indicated also on the abscissae. On the ordinate, the tensions are expressed in percentage of maximum active tension. The open circles represent mean values obtained in the group of adult cats. Two examples of curves obtained from the kittens are shown: the filled squares for Groups 3 and 4, the triangle in the square for Groups 5 and 6.

DISCUSSION

No special comment is necessary concerning the increase of sarcomere number of soleus and tibialis anterior muscles in relation to increasing age, as this has been previously established (Goldspink, 1964, 1968; Close, 1964; Edjtehadi & Lewis, 1974). However, in this paper sarcomere number was plotted against fibula length, and this is considered to be a better index of size for the soleus and tibialis anterior than body weight.

The first point to be discussed relates to the mean sarcomere length. This measurement was made at different angles, all of them being within the limits of the articular range. Evidence was obtained that the values for kittens of all ages fitted on to the linear regression line previously calculated for adult cats (Tabary *et al.* 1976). In other words, the sarcomere length at any given articular angle is the same whatever the age of the cat. In general, this conclusion agrees with the results of Elliott & Crawford (1965) for the tibialis anterior of rabbits, but disagrees with those of Goldspink (1968) on the biceps brachii of mice. In both previous cases the measurements were made at the maximal body length. However, in the present paper, the situation regarding the *very* young animals (10 minutes of age) is still uncertain. If a difference is confirmed in such very young animals this might reflect the fact that they had not yet used their muscles to support their body weight.

A second point to be discussed is a difference between the soleus and the tibialis anterior muscles which is present from birth, and remains unchanged during the whole growth period to maturity, namely that sarcomere number measured on isolated muscle fibres is always lower in the soleus than in tibialis anterior, the ratio being very close to 0·63 in growing animals as well as in adults. For this reason the sarcomere length increases more with articular angle in the soleus than in the tibialis anterior. This at least partly explains the differences that these two muscles demonstrate in their length *v.* active tension curves.

When expressed as a percentage of the maximal active force and as a percentage of the articular range, tibialis anterior curves of the various groups of kittens superimpose quite well, as they do with those of the adults. No difference in the shape of the curves between young and adult muscles was observed as was reported for the biceps brachii of the mouse by Goldspink (1968). This latter muscle has, however, two heads, and its characteristics are probably different. On account of the high passive force developed by the soleus at its maximal articular extension the real active force was difficult to determine accurately. While the lower two-thirds of the active curves may be compared with confidence in growing muscle and adults, the tops of the curves have to be interpreted with care. It remains possible that the top of the curve might be displaced in the soleus of the youngest group. However, the opposite assumption is suggested by the findings of Edjtehadi & Lewis (1974) when measuring the sarcomere length in kittens at the muscle length at which the tetanic tension is maximal. Evidence is given that this sarcomere length remains constant during growth in the soleus and in the flexor digitorum longus. This is in complete agreement with our findings in the tibialis anterior.

The third point to be discussed is the difference between the relationship of the sarcomere number and the active curve for the soleus and tibialis anterior. The sarcomere number of the soleus is less than that of the tibialis anterior although the range of excursion is the same. The reason for this is not obvious and it might be assumed that this is related to the fact that the soleus is a slow muscle and the tibialis anterior a fast one. Actually, the sarcomere number in the tibialis anterior is already higher than in the soleus at birth, and both muscles are at this stage slow contracting muscles. Most probably, the difference in sarcomere number between these two muscles is related to the mechanical requirements of the lever systems involved (Tabary *et al.* 1976).

SUMMARY

Sarcomere number and sarcomere length were studied in six groups of kittens ranging in age from 10 minutes to 5 months and compared with those of adult cats. Although the soleus muscle is a slow contracting muscle and the tibialis anterior a fast contracting muscle, both have previously been shown to have the same range relative to ankle movement. For a given angle of articulation the sarcomere length was found to be the same at all ages except perhaps for the newly born. In contrast, the sarcomere number differed considerably, being much higher in the older animals. The relationship between active tension and muscle length was also measured, and again no difference was found between the muscles at any age, although the shape of the curves for the soleus and tibialis anterior was different.

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