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J. W. Heslinga · G. te Kronnie · P. A. Huijing

Growth and immobilization effects on sarcomeres: a comparison between gastrocnemius and soleus muscles of the adult rat

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Abstract The effects of growth and limb immobilization on muscle mass, total physiological cross-section (PC), the number of sarcomeres in series and the length of sarcomere components were investigated in the soleus muscle (SOL) and compared to previously obtained data on gastrocnemius (GM) muscles of rats between age 10 and 16 weeks. For SOL this period of growth was reflected in an increased muscle mass and PC. No such increases were found for GM. In contrast, immobilization caused severe atrophy of fibres of both muscles. Compared to the value at the start of the immobilization, it was found that the fast twitch muscle (GM) atrophied more than the typically slow twitch one (SOL). The number of sarcomeres in series within fibres increased after growth and decreased after immobilization of SOL. For fibres of GM no such changes were observed. Muscle architecture is proposed as an important factor for the explanation of the results concerning the number of sarcomeres in series and those arranged in parallel. Due to the difference in muscle architecture, GM being more pennate than SOL, during growth, it is thought that increases in bone length affect the length of fibres of SOL more than those of GM. During immobilization, atrophy of fibres of GM was sufficient for the muscle length adaptation to meet the muscle length change induced by immobilization but in SOL, atrophy had to be accompanied by decreases in the number of sarcomeres in series to achieve adequate muscle length adaptation.

Key words Atrophy · Growth · Immobilization Sarcomere · Skeletal muscle

G. te Kronnie
Department of Experimental Animal Morphology and Cell Biology, Zodiac, Landbouw Universiteit, Marijkeweg 40, Wageningen, The Netherlands

J. W. Heslinga[1] · P. A. Huijing (✉)
Vakgroep Functionele Anatomie,
Faculteit Bewegingswetenschappen, Vrije Universiteit,
v. d. Boechorststraat 9, 1081 BT Amsterdam,
The Netherlands

[1]*Present address:*
Laboratory for Physiology,
Institute for Cardiovascular Research (ICaR-VU),
Vrije Universiteit, v. d. Boechorststraat 7, 1081 BT Amsterdam,
The Netherlands

Introduction

Muscle will adapt to altered demands. During normal growth the muscles enlarge and produce more force. Examples of conditions when muscle wasting or atrophy occur are during long-term bedrest and zero gravity. Over the past years the latter has drawn much attention but it remains difficult if not impossible to assess human isolated muscle architecture and function. Although animals have been sent into space (e.g. Caiozzo et al. 1994; Martin et al. 1988), obvious restrictions of available space-time and finance have limited research so ground based animal models which mimic human muscle atrophy due to zero-gravity have been developed. Hindlimb suspension (Diffee et al. 1991; McDonald and Fitts 1993; Winiarski et al. 1987) and limb immobilization, e.g. by using plaster casts (Booth 1977; Heslinga and Huijing 1992; Tabary et al. 1972), are such models. Although both produce a comparable amount of muscle atrophy, differences exist in for example maximal unloaded velocity of shortening (Fitts et al. 1986). An obvious advantage of the limb immobilization model is the constant length at which the muscles are held. This enables a better comparison between muscles. Limb immobilization has been shown to change muscle architecture as well as functional characteristics (Heslinga and Huijing 1992; Williams and Goldspink 1978). For growth and immobilization, changes in optimal muscle length, i.e. length at which the muscle generates maximal active force, have been observed (Goldspink 1964; Heslinga and Huijing 1990, 1992; Witzmann et al. 1982; Woittiez et al. 1986). For immobilization during which the muscle is held at a short length, it has been

documented that optimal muscle length is adjusted to the immobilized short length (Williams and Goldspink 1978).

Within fibres, the number of sarcomeres in series and those arranged in parallel can change. Extensive loss of sarcomeres in series have been reported to occur mostly in the soleus muscle (SOL; Tabary et al. 1972, 1981) in contrast to the smaller changes found for other muscles (Spector et al. 1982; Witzmann et al. 1982). Recently, as a result of immobilization at short length, we have reported a similar difference between the gastrocnemius muscle (GM) and the SOL within the same immobilized limb (Heslinga and Huijing 1993). Fibre type composition, i.e. slow twitch compared to fast twitch, has been considered to be an important factor determining the immobilization effect (Bruce-Gregorios and Chou 1984; Herbison et al. 1978; Lieber et al. 1988; Maier et al. 1976). It seems reasonable to suppose that this vulnerability of the slow twitch muscle fibres is related to differences in metabolism: the half-life of muscle proteins in SOL is almost half of that of GM and immobilization has been found to cause different effects on protein synthesis of both muscle types (Booth et al. 1980). However, different protein turn-over rates cannot explain why in both type I and type II fibres of GM no atrophy has been found, whilst fibres of SOL have shown severe atrophy after hindlimb suspension (Sancesario et al. 1992). Therefore, the question needs to be addressed as to whether effects at the level of the whole muscle should be taken into account. Furthermore, through adaptation of the number of sarcomeres in series, fibre length can in principle be modified by sarcomere length. As it has been found that actual sarcomere length may vary along the fibre (Burton et al. 1989; Morgan 1990), we calculated optimal sarcomere length which only depends on the lengths of the constituent sarcomere components. After a period of growth as well as after immobilization, for GM it has been reported that no length changes of thin or thick myofilaments nor for Z-line and bare zone occurred (Heslinga and Huijing 1990, 1992). However this point has never been verified adequately for SOL.

The aim of the present work was to evaluate for SOL and GM the effects of growth and immobilization on the total physiological cross-section of the fibres and the number of sarcomeres in series, as well as sarcomere length. The initial values before onset of immobilization were also considered to gain more insight into the processes of adaptation. Estimates of total cross-section were derived mathematically from muscle volume and fibre length.

Methods

Animals and muscles used

For previous work we used a total of five groups to which the rats were randomly assigned. In the present study some data from these five groups (maximum of eight animals per group but individual sample size per parameter are indicated at the appropriate places) of young, adult male Wistar rats have been used. In a previous study (Heslinga and Huijing 1990), we have reported the results of three groups of rats aged approximately 10, 14 and 16 weeks, regarding length-force characteristics as well as the number of sarcomeres in series and the length of sarcomere components for GM. In the present study only this data has been used for the calculation of optimal fibre length (see below). In the remaining two groups, the immobilization effect was studied. At age 10 weeks the right hindlimb was immobilization for 4 or 6 weeks (IM4 and IM6) by means of a plaster cast which held the GM at minimal length (maximally flexed knee and plantar flexion of the ankle). For immobilized GM, the number of sarcomeres, length-force characteristics and muscle geometry at optimal muscle length have been described by Heslinga and Huijing (1992). In addition, the lengths of the sarcomere components of GM and the number of sarcomeres in series of SOL of the 14- and 16-week group have been a previous subject of study (Heslinga and Huijing 1993). In the present work, data on length parameters of sarcomere components of SOL during a period of growth and after immobilization are given. Combination of the complete sets of data of all five groups allows a comparison of growth and immobilization effects of SOL and GM at the muscle level (muscle mass and total physiological cross-section) as well as at the level of the muscle fibre (the number of sarcomeres in series and the length of sarcomere components).

Number of sarcomeres in series

The muscle was freed from its origin and mounted on a steel bar, after which it was stored in a 4% formalin solution for more than 3 days. To prepare bundles for determination of the number of sarcomeres in series, a method as has been described by Huijing (1985) was used. Previously, the same procedure has been followed to obtain results describing effects of growth on the number of sarcomeres in series (Heslinga and Huijing 1990). Single intact fibres were teased from the fibre bundles. For samples of four single fibres from the proximal and distal parts of the muscle (number of muscles indicated in Table 2), an estimate of mean sarcomere length was obtained by counting the number of sarcomeres at 10 to 15 places along the length of the fibre (microscope equipped with a video camera; total magnification $\times 4{,}000$). Of each fibre treated, fibre length was obtained on the projected images of these fibres (approximately 19.5 times life size), by means of a curvimeter. The number of sarcomeres in series was calculated from the ratio of these two variables (Dekhuijzen et al. 1986; Heslinga and Huijing 1990; Huijing 1985).

Lengths of sarcomere components

As with the GM (Heslinga and Huijing 1990), the stretched SOL was tied to a steel bar. Initial fixation was achieved by submerging the muscle for 15 min in a buffered solution (0.2 $mol \cdot l^{-1}$ sodium phosphate buffer, pH = 7.4) of paraformaldehyde (4%) and glutaraldehyde (5%). In addition, to achieve fast and adequate fixation, the muscle was injected with this fixation fluid. With the same solution the muscles were fixed for an additional 2 h at 4° C (van Lookeren Campagne et al. 1988). The electron microscopic preparation method has been described fully by van Lookeren Campagne et al. (1988). Ultrathin longitudinal sections were cut (< 60 nm). These sections were observed in a transmission electron microscope (Philips EM 400) and measurements were performed on photographs of the preparation. The features measured on the photographs are shown in Fig. 1. For each group, two muscles were used to determine the length of sarcomere components. The number of samples per muscle varied: on each micrograph two to four sarcomeres were used for measurements and for each muscle two to five micrographs were examined. The magnifications used, ranged from $\times 10{,}000$ to $\times 35{,}000$. We calculated the length of the different components of the sarcomeres

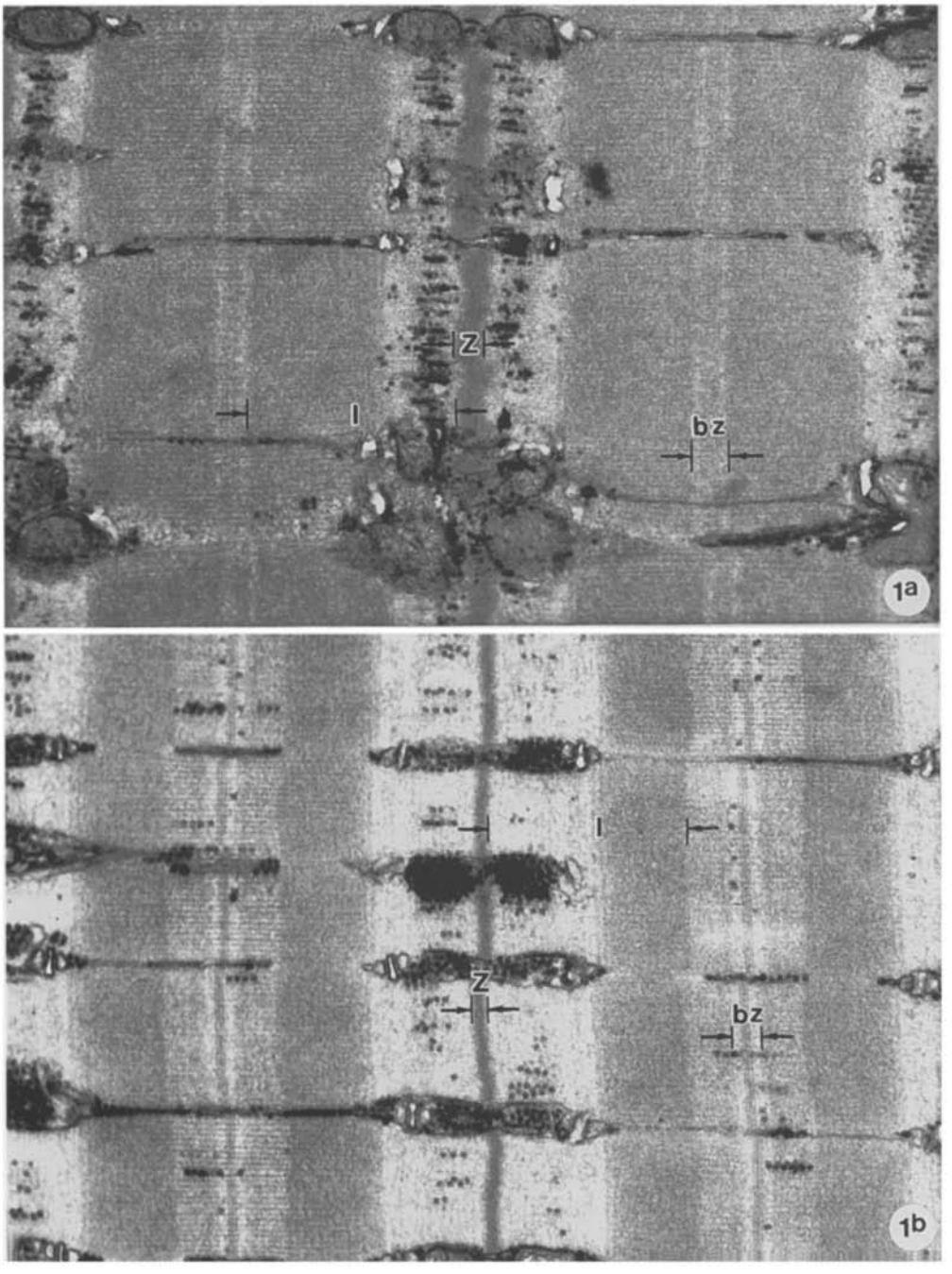

Fig. 1 Micrographs of two hindlimb muscles of the 14-week group. **1a** Soleus muscle, **1b** gastrocnemius muscle. Indicated are the length measurements: *Z* Z-line, *bz* bare zone, *I* thin filament length. Magnification ×32,000.

assuming the thick filament length to be 1.54 μm for both muscles and to remain unchanged during growth or immobilization. For some muscles of the different groups this assumption has been validated by calibration with an assumed tropomyosin/troponin periodicity of 38.5 nm (Huxley and Brown 1967). Comparison of length measurements on micrographs used in te Kronnie et al. (1987) with recent material showed similar thick filament lengths. Therefore, their data on filament length parameters were taken as representative for the 10-week group (see also van Lookeren Campagne et al. 1988).

As optimal force is produced when maximal overlap between the filaments occur, optimal sarcomere length ($l_{s,o}$) was estimated according to the equation:

$$l_{s,o} = 2l_{thin} + l_{bare} + l_z$$

where l_{thin} is the thin filament length, l_{bare} is the bare zone length, and l_z is the Z-line width. Note this length represents the maximal length at which maximal number of cross-bridges can be formed, equivalent to the right end of any sarcomere length-force plateau.

Other data collection

Body mass of the animals was determined on the day of the experiment. Weighing of the animals was done on a Mettler PC 440.

Muscle mass was measured for SOL and the mass of GM was calculated on the basis of volume measurements and an assumed density of 1.071 $mg \cdot mm^3$ (Gollnick et al. 1981). Wet mass of the muscles was measured (Mettler HK 160) just before fixation.

Length of the tibia was measured with a pair of compasses between the most proximal and distal margins of the bone along the lateral side. It should be noted that data for body mass, mus-

cle mass or volume and tibia length have been presented previously (Heslinga and Huijing 1990, 1992).

Cross-sectional area was used as a measure of atrophy. True cross-sectional area of all fibres can be calculated from the ratio of muscle volume and fibre length. In vivo fibre length is difficult to assess for different muscles under standard conditions, therefore we calculated sarcomere optimal length as indicated above and this parameter (mean value per group) times the number of sarcomers in series (mean of proximal and distal fibres per group) gives mean optimal fibre length. As a consequence, the standard deviations for physiological cross-section (PC) originate from muscle mass measurements for SOL and muscle volume measurements for GM. It is important to note that optimal sarcomere length and optimal fibre length depend only on anatomical differences between muscles and not on fixation artefacts such as shrinkage or muscle length at the time of fixation.

Statistics

Values for variable of the right and left leg of the three control groups (age 10, 14 and 16 weeks and not immobilized) were averaged per animal (Heslinga and Huijing 1990). Averaging of these individual values resulted in the total group mean.

Growth

One-way ANOVA followed by post-hoc Tukey tests were used to test differences in body mass and tibia length as well as for GM and SOL muscle mass and PC. The lengths of sarcomere components of SOL during growth were also tested for significant differences by using one-way ANOVA. For SOL, two-way ANOVA was used to test the number of sarcomeres in series for the 10-, 14- and 16-week groups for significant fixed time and fixed location (proximal compared to distal) effects. Post-hoc tests were used to locate significant differences. For GM these results have been reported previously (Heslinga and Huijing 1990).

Immobilization

Mean values of body mass of the immobilized animals were compared with that of the 10-week group and tested with a Dunnett test (Dunnett 1955). The results for GM regarding muscle mass, PC and the number of sarcomeres (proximal and distal separately) were compared only with the value obtained for the 10-week group and tested for significance with a Dunnett test.

For SOL, muscle mass, PC and the length of the sarcomere components of the IM4 group were compared to those of the 10-week group by using a Student's *t*-test. The number of sarcomeres in series in both IM groups was compared with the 10-week group and tested separately for the proximal and distal regions of the muscle. For all tests the level of significance was chosen at 5%.

Results

Growth

Although the growth period studied caused an increase in body mass (Table 1), the growth effect could not be shown to be above the significance level for GM muscle mass (Fig. 2) and PC (Table 1). In contrast, the results of SOL show significant growth effects for muscle mass (Fig. 2) and PC (Table 1). Tibia length was found to be significantly affected by growth. An increase of approximately 2.5 mm was found after 6 weeks of normal growth (Table 1). A significant increase in the number of sarcomeres was found for SOL. Post-hoc tests showed significantly more sarcomeres in proximal fibres of SOL of the 16-week group and for distal fibres of the 14 and 16-week group compared to those of the 10-week group (Table 2). The two-way ANOVA showed a significant interaction between time and location: more sarcomeres were added in distal fibres. Regarding lengths of sarcomere components, for SOL, thin filaments of the 16-week group were significantly longer than those of the 14-week group. This was also reflected in a greater optimal sarcomere length (Table

Table 1 Values for body mass and tibia length (from Heslinga and Huijing 1990, 1992) and muscle physiological cross-sectional area (PC). The mean ratio of muscle mass to body mass shows the failure of the muscles to increase their muscle mass. At age 10 weeks the right hindlimb was immobilized for 4 or 6 weeks (*IM4* group, *IM6* group, respectively). Numbers in brackets indicate group size

					Gastrocnemius muscle			Soleus muscle		
	Body mass (g) mean	SD	Tibia length (mm) mean	SD	Ratio	PC (mm^2) mean	SD	Ratio	PC (mm^2) mean	SD
10-week Group	303 (8)	9	39.8 (6)	1.1	2.74	60.76 (8)	6.26	0.41	8.88 (6)	0.72
14-week Group	378 (6)**	27	41.3 (6)	1.4	2.21	63.73 (6)	13.24	0.41	10.65 (2)**	0.35
16-week Group	432 (6)**	23	42.3 (5)**	1.1	2.31	70.90 (6)	9.27	0.39	10.11 (2)	0.36
IM4 group	304 (8)	20	41.1 (4)	1.8	1.67	36.35 (6)*	10.70	0.24	6.40 (5)*	1.30
IM6 group	318 (7)	30			1.56	37.48 (6)*	11.25			

* Significant difference compared to value of 10-week group

** Significant growth effect and difference compared to value of the 10-week group (ANOVA)

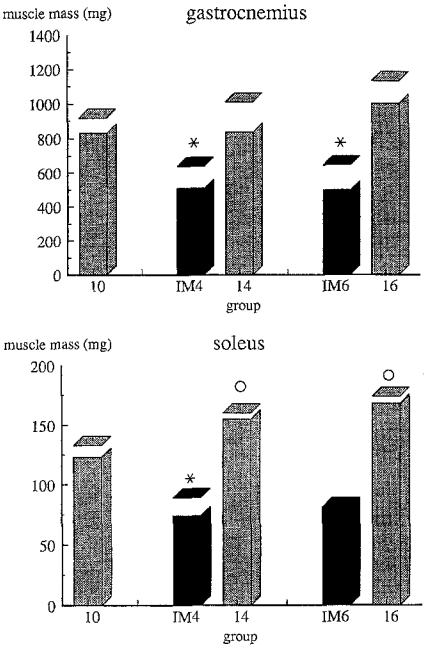

Fig. 2 Values of group means and standard deviations of muscle mass of gastrocnemius and soleus muscles. Significant growth effect and difference to the 10-week group are indicated by *o* (ANOVA). *Significant difference of an immobilized group compared to the 10-week group as shown by a Dunnett test (Dunnett 1955). For definitions see Table 2

3). The Z-line was wider in SOL than in GM (Table 3).

Immobilization

Table 1 shows that body mass remained at the level of the 10-week group. For tibia length a similar value as found for the 14-week group, was found for the IM4 group. From this it is concluded that immobilization did not affect bone growth.

For SOL and GM a lower muscle mass was found after immobilization. Compared to the 10-week group, the IM groups showed a 40% decreased muscle mass for both, SOL and GM (Fig. 2). Especially if expressed relative to body mass (Table 1), the decreases in muscle mass are clear; the ratio muscle mass:body mass decreased by 39% for GM and by 41% for SOL.

Expressed relative to PC of the 10-week group, the PC of GM was 40% smaller after immobilization, in contrast to a decrease of 28% found for SOL. In SOL after immobilization, a difference with the 10-week group of approximately 25% was observed in the number of sarcomeres in series in the proximal as well as distal fibres (Table 2). It should be noted that differences in GM were much smaller (Heslinga and Huijing 1992): after 4 weeks of immobilization a difference of only 12% with respect to the 10-week group was found in the distal part of GM exclusively (Table 2).

Although the number of sarcomeres in series was affected in SOL, length of sarcomere components (Table 3) was not affected by immobilization.

Discussion

It is important to emphasize the fact that although the animals were adults they were still growing, gaining body mass and increasing muscle mass. The results of the present study showed that growth induced in both muscles similar relative increases in PC, although the increase for GM could not be shown to be significant. However the increase in maximal active tetanic force found for the growth period (Heslinga and Huijing 1990) would seem to support the idea of hypertrophy.

The number of sarcomeres in series increased only for SOL starting in the distal region of the muscle after 4 weeks and then followed by the proximal region after 6 weeks of growth. Regarding the length of sarcomere components it was not surprising that the Z-line width was larger in the typical slow twitch SOL than in the

Table 2 Number of sarcomeres in series in whole fibres from the proximal and distal parts of the muscles (n = sample size). Results are mean values of groups; *10*, *14* and *16* refer to the three age groups; *IM4* and *IM6* refer to the 4 and 6 week immobilized group, respectively

Gastrocnemius muscle (from Heslinga and Huijing 1990, 1992)		10	14	16	IM4	IM6
Proximal	Mean	5196	5013	5388	5007	5075
	SD	582	418	360	391	931
Distal	Mean	5909	5818	5904	5202*	5603
	SD	334	304	227	390	354
	n	6	6	8	6	8
Soleus muscle						
Proximal	Mean	5656	5381	6000**	4347*	4455*
	SD	192	332	360	796	457
Distal	Mean	5296	5753**	5754**	4012*	4080*
	SD	260	337	370	925	331
	n	6	3	4	4	3

* Significant difference between experimental value and value of the 10-week group (Dunnett 1955)

** Significant time effect and difference to 10-week group (ANOVA)

Table 3 Results of measurements on sarcomere components. The mean values of the gastrocnemius muscle (GM) based on group mean values of the 10-, 14- and 16-week group from Heslinga and Huijing (1990) as well as that of the *IM4* and *IM6* groups (Heslinga and Huijing 1992). For definitions see Table 1

		Soleus muscle				Gastrocnemius muscle
	Group	10	14	16	IM4	
	n	2	2	2	2	
Ratio thin:thick	Mean	0.67	0.69	0.75	0.74	0.67
	SD	0.02	0.03	0.001	0.07	
Thin filament length (μm)	Mean	1.06	1.06	1.16*	1.13	1.03
	SD	0.01	0.03	0.00	0.11	
Z-line width (μm)	Mean	0.16*	0.16*	0.16*	0.15*	0.08
	SD	0.004	0.001	0.004	0.01	
Length bare zone (μm)	Mean	0.16	0.17	0.16	0.16	0.16
	SD	0.008	0.005	0.004	0.003	
Optimal sarcomere length (μm)[a]	Mean	2.36	2.44	2.64	2.58	2.29
	SD	–[b]	0.06	0.005	0.22	

[a] See Methods;
[b] missing SD because of calculation on mean value;
* Significant difference compared to *GM* (student's *t*-test)

fast twitch GM (Squire 1986). It should be noted that for SOL and GM only one type of fibre was sampled. However, the finding of longer thin filaments in SOL after 6 weeks of growth is puzzling it is uncertain as to whether this was a result influenced by the small sample size.

Because of the growth still apparent in the control animals, a comparison of immobilized groups and age-matched groups has been shown to yield information on a combination of true atrophy and impeded growth (Heslinga and Huijing 1992). The atrophy is best described when immobilized groups are compared to the initial group at the start of the immobilization. Compared to the PC of the 10-week group GM showed a greater relative decrease in PC than SOL, while relative muscle mass decreases were similar for both muscle groups. The latter agrees with the finding of Booth (1977). Therefore, measurement of PC, which circumvents architectural differences and fixation artefacts, revealed greater atrophy in the fast twitch muscle. This is in contrast to the idea that immobilization predominantly affects slow twitch muscles. Because slow twitch oxidative fibres are believed to function primarily as postural stabilizers, which function was prevented by the plaster cast, greater atrophy was expected for this type (Herbison et al. 1978; Lieber et al. 1988; Maier et al. 1976). This would agree with the greater change of level of use observed after immobilization for SOL compared to that for GM (Fournier et al. 1983; Hnik et al. 1985). The present results, therefore, add new doubt (see also Appell 1986b) to the hypothesis that slow twitch fibres are more susceptible to effects of changed demands than fast twitch fibres. More research in this field is needed, in which special attention should be paid to determine the cross-sectional area perpendicular to the fibres, as well as to the fact that measurements should be made at a standard length. The latter is important in that fibre length determines cross-section because the volume of a given muscle is constant. After immobilization, again only SOL muscles showed smaller numbers of sarcomeres in series.

A major result of the present work was the difference of adaptation of the number of sarcomeres in series of the two muscle types. Except for a small difference of 12% relative to the 10-week group in the distal fibres (Table 2), observed in GM due to immobilization, for GM no other adaptation of number of sarcomeres due to immobilization or growth, was encountered. In contrast, for SOL, increases were found after a period of growth and major decreases of 25% were found after a period of immobilization. This is surprising as for GM, (Heslinga and Huijing 1992) as well as SOL (Williams and Goldspink 1978), after short length immobilization, a decreased optimal muscle length has been found or can be inferred. Therefore, our present study showed that GM, in contrast to SOL, does not readily form (growth) or lose (immobilization) sarcomeres in series. As for example Tardieu et al. (1974) and Witzman et al. (1982) have pointed out, because the length at which the muscle is held during immobilization is an important parameter for the amount of lost sarcomeres, a closer examination of this length imposed on both muscles is appropriate.

Conditions imposed on fibres during growth and immobilization

Growth

During growth it has been shown that muscle length is increased as a result of traction caused by bone growth (Wheeler Haines 1932). Even during a short growth period as studied in the present work, tibia length increased. This increase was almost equal to the length increase of GM (Heslinga and Huijing 1990). Based on

the anatomical situation in the lower leg of the rat concerning origin and insertion of GM and SOL, it seems likely that GM and SOL would be exposed to similar longitudinal changes of the bones. To deduce the relative fibre length change imposed by tibial growth, one has to consider differences of muscle architecture. If it is assumed that contributions of changes of fibre and aponeurosis angles to length changes of muscle (Heslinga and Huijing 1990) are negligible for SOL, because of the relatively small value of the angle of pennation (Close 1964), changes of fibre length will be approximately equal to those of the muscle. However, due to a pennation angle of approximately 20° at optimal muscle length, this is clearly not the case for GM (Heslinga and Huijing 1990). Consequently, it is likely that during growth GM fibres are exposed to a smaller amount of extension as result of bone growth than fibres of SOL. Therefore, during growth, the occurrence of sizeable changes in the number of sarcomeres in series in the fibres of SOL and not of GM may be explained by the fact that the fibres were exposed to different conditions within the two muscles. This illustrates the importance of an estimation of fibre length changes induced by experimental conditions rather than of muscle length changes.

Immobilization

The relative length at which fibres are immobilized is considered important for the occurrence as well as the degree of atrophy and changes in the number of sarcomeres in series. In general, it is clear that the shorter the fibres are with respect to their optimal length, the greater the decrease in the number of sarcomeres in series (Spector et al. 1982; Tabary et al. 1972). For immobilized muscles the length change is brought about by changing ankle and knee joint angle, as a result of which the muscle is brought from optimal length to the minimal length possible (=immobilization length). Using data of Woittiez et al. (1985), this muscle length change can be estimated to be approximately 5 and 7 mm for SOL and GM, respectively. Using this value and data describing the relationship between fibre length change and muscle length change of the muscle in the initial situation (Fig. 4 in Heslinga and Huijing 1990), an estimate of actual fibre length at the start of the immobilization can be made for GM. Relative to optimal fibre length, it is estimated that fibre length of GM at the beginning of the immobilization was approximately 60% for both IM groups. For SOL, optimal fibre length for the 10-week groups was approximately 12.50 mm (Tables 2, 3). As SOL is considerably less pennate than GM, a 5-mm length change of muscle results in a 5-mm fibre length change. This also yields a relative fibre length of approximately 60%. It was concluded that there was no indication that GM and SOL fibres were immobilized initially at different relative lengths.

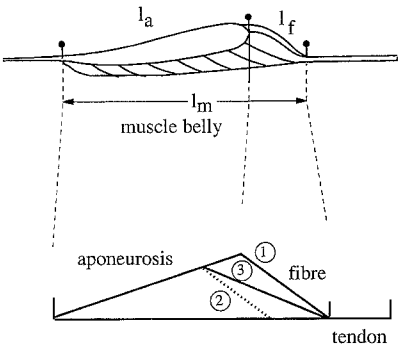

Fig. 3 Diagram of the geometric arrangement of the dorsal part of a pennate muscle (e.g. gastrocnemius). The initial situation at the start of immobilization at short length is shown (*1*). Atrophy of muscle fibres would lead to shortening of the muscle if the muscle is not kept at constant length (*2*). Therefore, to keep muscle length constant while muscle fibres atrophied, fibres of pennate muscles are exposed to a "lengthening stimulus" relative to the initial situation (*3*). Note that atrophy caused a shorter aponeurosis. l_a Aponeurosis length, l_f distal fibre length, l_m muscle length

Muscle architecture affects the imposed fibre length continuously during the process of adaptation to the immobilized length: as the duration of immobilization progressed, fibres of both muscles atrophied. Given the difference of architecture, GM being more pennate than SOL, it may be expected that this atrophy would lead predominantly to a sizeable decrease in muscle thickness for SOL and a change of muscle length for GM. At the start of the immobilization the muscle was slack and far below optimal muscle length. During the process of adaptation, optimal muscle length was shifted to this short length in agreement with Williams and Goldspink (1978) and Herring et al. (1984): regulation of the number of sarcomeres in series is such that optimal overlap of thick and thin filaments is obtained within sarcomeres (i.e. fibres at optimal length). Due to the difference of architecture, mentioned above, it is argued that for GM, the amount of atrophy related muscle shortening was sufficient to induce the optimal muscle length adaptation. A diagram of this situation is given in Fig. 3. In contrast, for SOL, atrophy must be accompanied by fibre length changes, i.e. adaptation of number of sarcomeres in series, to accomplish an adequate optimal muscle length change.

A different adaptation for number of sarcomeres in series may be found in slow and fast twitch muscle; however, it is hypothesized that differences in muscle architecture, rather than different metabolic characteristics, may explain the adaptation of the number of sarcomeres in series. This approach is rather novel in the understanding of the plasticity of muscle tissue and can also be extrapolated to human muscles in general. It means that in the evaluation of atrophy of human muscles and the effectiveness of countermeasures, a profile of the fibre type, as well as an estimate of the architecture of the muscle should be included. Animal experiments (Appell 1986a; Graham et al. 1989) as well as hu-

man data (MacDougall et al. 1980) have indicated that training programmes before and during an immobilization period are helpful to minimize the amount of atrophy. However, it should be noted that human SOL is rather different from rat SOL. Unpublished observations on cadaver material (Huijing) have indicated an angle between fibre and aponeurosis within the range of 20°–30° with extended knee and plantar flexed ankle joint with, compared to GM of the same individual, a similar number of sarcomeres in series within fibres (approximately 12000–20000). In contrast to rat SOL, human SOL is of mixed fibre composition comparable to human GM (SOL 70%:30% and gastrocnemius 50%:50% slow:fast twitch ratio: Edgerton et al. 1975; Johnson et al. 1973). Therefore, the human SOL should be considered a rather pennate muscle with at least a bipennate structure and mixed fibre composition. This implies that any atrophy which cannot be prevented by training will also for SOL lead to a decrease in force accompanied by a shift of optimal length to lower length without an adaptation of the number of sarcomeres in series.

It is concluded that SOL and GM muscles are affected differently during growth and immobilization. In the SOL, the number of sarcomeres arranged both in parallel and in series was adapted. In contrast, GM primarily showed adaptation in the number of sarcomeres arranged in parallel. To explain these differences of adaptation found during growth and immobilization, muscle architecture was considered important. For growth, different adaptation found for SOL and GM regarding the number of sarcomeres in series is related to differences of fibre length changes imposed by bone growth. Although for immobilization no difference was found for relative initial fibre length, the different muscle architecture can explain the difference of adaptation found for both muscle groups.

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