ELSEVIER

Mechanisms of Ageing and Development
78 (1995) 215-219

mechanisms of ageing
and development

Specific force of the rat plantaris muscle changes with age, but not with overload

H. Degens*, L. Hoofd, R.A. Binkhorst

Department of Physiology, Faculty of Medical Sciences, University of Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands

Received 27 July 1994; accepted 11 November 1994

Abstract

Age and overload effects on the specific force of the rat plantaris muscle were investigated. The specific force was affected by age, but not by overload, inducing a 30% hypertrophy, at any age. The relative amount of non-contractile tissue only minimally affected the conclusions.

Keywords: Specific force; Hypertrophy; Age

1. Introduction

We reported previously, that the maximal tetanic force was higher in plantaris muscles of 13-month-old than in those of 5- and 25-month-old rats and that it increased with hypertrophy at all ages [1]. The tetanic force/muscle weight ratio varied similarly with age, but remained unaffected by hypertrophy at all ages. We suggested that this indicated that the relative amount of non-contractile tissue was not significantly affected by overload.

In the present study, the relative amount of non-contractile tissue in normal and overloaded plantaris muscles of rats of different age was estimated and used to calculate the specific force of the contractile tissue.

* Corresponding author, present address: Department of Human Anatomy and Cell Biology, University of Liverpool, P.O. Box 147, Liverpool L69 3BX, United Kingdom.

SSDI 0047-6374(94)01538-W

2. Methods

Female Wistar rats aged 5, 13 and 25 months at termination were used in this study. Each age group consisted of 20 control and 20 overloaded rats. Overload of the m. plantaris was achieved by denervation of its synergists under pentobarbital anaesthesia (60 mg/kg i.p.). Six weeks later, in vivo isometric contraction measurements were performed by stimulation via the sciatic nerve as described previously [1]. The muscles were excised, weighed, and frozen onto cork at their L_0 (length at which the muscle produces its optimal twitch force) in isopentane cooled in liquid nitrogen and the animals were killed under anaesthesia by excision of the heart. Twelve-micrometre cross-sections were cut in a cryostat at −25°C and stained for alkaline phosphatase and dipeptidyl-peptidase-IV [2]. The amount of contractile and non-contractile tissue was evaluated in the deep region of the muscle (Bd), near the head of the tendon, and the superficial region (Bs), near a large blood vessel. The percentage of non-contractile tissue was estimated on a photograph using the following formula; 100% × (total area of the photograph − total area occupied by complete and incomplete muscle fibres) divided by total area of the photograph. The amount of non-contractile tissue of the whole muscle (B) was approximated by the average of its amount in the deep and superficial region. The cross-sectional area (A in cm^2) of the muscle was approximated as: $A = Mw/(1.072 \times L_0 \times 0.47)$ in which Mw = muscle weight (g), 1.072 is muscular density (in g/cm^3) [3] and 0.47 is taken as the correction factor for the angle of pinnation (set at 20° [4,5]), assuming one half of the fibres are found within the midbelly. Muscle weight and cross-sectional area, corrected for the amount of non-contractile tissue, were calculated as $Mcor = (100\% - B) \times Mw/100$ and $Acor = (100\% - B) \times A/100$, respectively. ANOVA was applied to test for age and overload effects. When age effects or interactions were found a Bonferroni corrected *t*-test was done to test differences between groups. Differences between the regions were determined by using a two-tailed paired *t*-test. Differences were considered significant at $P < 0.05$.

3. Results

Data on muscle length, relative amount of non-contractile tissue, muscle cross-sectional area and the specific forces are given in Table 1. The data on body and muscle weight, tetanic force and tetanic force per muscle weight are taken from [1]. Specific force is expressed as maximum tetanic force per muscle weight and as maximum tetanic force per muscle cross-sectional area, since both normalisations are used in the literature. No significant differences in the relative amount of non-contractile tissue between the superficial and deep regions of the muscle were detected. The muscles of the youngest group contained the highest relative amount of non-contractile tissue. The specific force was highest in the muscles of the 13-month-old rats, irrespective of whether it was normalised to muscle weight or muscle cross-sectional area and whether or not the amount of non-contractile tissue was taken into account. Overload resulted in a 30% hypertrophy at all ages.

Table 1
Morphological and force data in control (C) and overloaded (O) m. plantaris of the rat at different ages (in months)

	5C	5O	13C	13O	25C	25O
Body weight (g)	225 ± 6 (20)	215 ± 3 (18)[a]	303 ± 7 (20)[b]	285 ± 6 (19)[a]	307 ± 8 (17)[b]	296 ± 11 (5)[a]
Mw (mg)	329 ± 12 (19)	434 ± 13 (16)[a]	348 ± 14 (20)	446 ± 16 (18)[a]	357 ± 13 (16)	456 ± 19 (15)[a]
Lo (cm)	3.0 ± 0.04 (17)	3.0 ± 0.04 (13)	3.3 ± 0.03 (19)[b]	3.2 ± 0.05 (18)	3.2 ± 0.04 (17)[b]	3.3 ± 0.05 (15)
Bd (%)	7.1 ± 0.7 (15)	8.0 ± 1.0 (15)	3.4 ± 0.5 (20)[b]	3.5 ± 0.6 (19)	4.7 ± 0.8 (14)[b]	5.0 ± 0.7 (15)
Bs (%)	7.9 ± 1.0 (15)	6.8 ± 0.7 (13)	4.2 ± 0.7 (18)[b]	3.2 ± 0.5 (19)	3.6 ± 0.7 (15)[b]	3.8 ± 0.9 (15)
B (%)	7.6 ± 0.5 (13)	7.5 ± 0.8 (13)	3.8 ± 0.5 (18)[b]	3.3 ± 0.5 (19)	4.2 ± 0.6 (13)[b]	4.4 ± 0.6 (15)
A (cm^2)	0.215 ± 0.009 (16)	0.281 ± 0.010 (12)[a]	0.211 ± 0.008 (19)	0.272 ± 0.007 (17)[a]	0.225 ± 0.008 (14)	0.275 ± 0.010 (14)[a]
F (N)	5.81 ± 0.22 (17)	7.38 ± 0.19 (16)[a]	6.61 ± 0.25 (17)[b,c]	8.80 ± 0.28 (18)[a]	5.28 ± 0.28 (17)	7.11 ± 0.33 (14)[a]
F/Mw (N/g)	17.44 ± 0.69 (17)[c]	17.05 ± 0.71 (14)	19.02 ± 0.64 (17)[b,c]	20.14 ± 0.60 (17)	14.83 ± 0.82 (16)[b]	15.76 ± 0.61 (14)
F/Mcor (N/g)	17.70 ± 0.95 (10)[c]	19.05 ± 0.92 (11)	19.76 ± 0.75 (16)[c]	20.80 ± 0.63 (17)	15.92 ± 1.01 (12)	16.49 ± 0.64 (14)
F/A (N/cm^2)	26.6 ± 1.3 (14)	26.6 ± 1.2 (10)	31.3 ± 1.0 (16)[b,c]	32.4 ± 0.9 (16)	23.5 ± 1.3 (14)	26.9 ± 1.1 (13)
F/Acor (N/cm^2)	26.5 ± 2.1 (7)	29.4 ± 1.4 (9)	32.6 ± 1.2 (15)[b,c]	33.4 ± 0.9 (16)	25.3 ± 1.7 (10)	28.2 ± 1.2 (13)

Mw, muscle weight; L_0, muscle length; Bd and Bs, amount of non-contractile tissue in the deep and superficial region, respectively; B, amount of non-contractile tissue in the muscle; A, muscle cross-sectional area; F, tetanic force; F/Mw and F/Mcor, tetanic force: (corrected for B) muscle weight; F/A and F/Acor, tetanic force: (corrected for B) muscle cross-sectional area; number of animals in parentheses; values are mean ± S.E.M.

[a]Overload different from control.
[b]Different from 5-month-old rats.
[c]Different from 25-month-old rats.

Overload did not significantly affect the relative amount of non-contractile tissue, nor specific force in all groups.

4. Discussion

4.1. Ageing

In an earlier study, the specific force (expressed as force per muscle weight) was recorded as highest in rats from the 13-month-old and lowest in rats from the 25-month-old group, with the 5-month-old group intermediate between the two [1]. In the present study, the higher specific force, both as expressed per muscle weight and cross-sectional area, obtained from muscles in the 13-month-old compared to the 5-month-old group [1] could, at least partly, be explained by a decreased relative amount of non-contractile tissue during this period, since the difference in specific force disappeared when corrected for the relative amount of non-contractile tissue.

Between 13 and 25 months of age, the specific force decreases, irrespective of how it is expressed. This cannot be explained by a change in the relative amount of non-contractile tissue during this period. Supposedly, the cause may be an incomplete reinnervation of previously denervated fibres, during the denervation-reinnervation process at advanced age [6], or the occurrence of myofilament loss as found in human tibialis anterior muscle [7].

The difference in specific force, expressed as tetanic force/muscle weight, between m. plantaris of 5- and 25-month-old rats disappeared, when expressed as tetanic force per total muscle cross-sectional area, whether corrected for the relative amount of non-contractile tissue or not. Larsson and Edström [8] reported a decrease in tetanic force per muscle weight in tibialis anterior muscle of rats between 6 and 20–24 months of age, while the force per total muscle fibre cross-sectional area displayed no significant change. They suggested that this represented a gradual increase in the amount of adipose and connective tissue in relation to the number of muscle fibres. This suggestion could not be confirmed by data from the present study, but we could explain it by an age-related increase in muscle length, which is taken into account when normalising tetanic force to cross-sectional area, but not when normalised to muscle weight.

Thus, the present data on age-associated changes in specific force are largely in agreement with Larsson and Edström [8] showing that the force generating capacity of maintained contractile tissue is similar at 5 and 25 months of age. However, the force generating capacity of the contractile tissue of the muscles increased between 5 and 13 months, and decreased between 13 and 25 months of age.

4.2. Overload

The overload induced a 30% hypertrophy of the m. plantaris at all ages. This was accompanied by a proportional increase in the tetanic force [1]. In agreement with others [9], the specific force remained unaffected with overload at all ages. One might argue that possible changes in angle of pinnation with hypertrophy, as found by Binkhorst and van't Hof [4], may affect our calculations of specific force.

However, the relative overestimation of the force per cross-sectional area for the hypertrophied muscles, obtained by not taking into account changes in the angle of pinnation, will only be about 4%. As we suggested previously [1], the relative amount of non-contractile tissue was not significantly affected by overload. This corresponds to the similar muscle density in control and overloaded muscles [3] and the unchanged content of myofibrillar protein content throughout the overload period [10], suggesting a similar composition of both muscles. This indicates that the specific force of the contractile tissue remains unaffected by hypertrophy at all ages.

5. Conclusion

In conclusion, the specific force was highest in the 13-month-old, compared with the 5- and 25-month-old rats, irrespective of how specific force was expressed. Overload changed neither the specific force nor the relative amount of non-contractile tissue at any age. The conclusions are still valid if one takes into account the relative amount of non-contractile tissue.

Acknowledgements

The authors express their gratitude to Dr M.A. van't Hof for helping with the statistical analyses and Miss A.J. Craven for correction of the English text.

References

[1] H. Degens, Z. Turek and R.A. Binkhorst, Compensatory hypertrophy and training effects on the functioning of ageing rat m. plantaris. *Mech. Ageing Dev.*, *66* (1993) 299–311.

[2] H. Degens, Z. Turek, L.J.C. Hoofd, M.A. van't Hof and R.A. Binkhorst, The relationship between capillarisation and fibre types during compensatory hypertrophy of the plantaris muscle in the rat. *J. Anat.*, *180* (1992) 455–463.

[3] P.D. Gollnick, B.F. Timson, R.L. Moore and M. Riedy, Muscular enlargement and number of fibers in skeletal muscles of rats. *J. Appl. Physiol.*, *50* (1981) 936–943.

[4] R.A. Binkhorst and M.A. van't Hof, Force-velocity relationship and contraction time of the rat fast plantaris muscle due to compensatory hypertrophy. *Pflügers Arch.*, *342* (1973) 145–158.

[5] R.R. Roy, I.D. Meadows, K.M. Baldwin and V.R. Edgerton, Functional significance of compensatory overloaded rat fast muscle. *J. Appl. Physiol.*, *59* (1982) 473–478.

[6] L. Edström and L. Larsson, Effects of age on contractile and enzyme-histochemical properties of fast- and slow-twitch single motor units in the rat. *J. Physiol.*, *392* (1987) 129–145.

[7] F. Jakobson, K. Borg and L. Edström, Fibre-type composition, structure and cytoskeletal protein location of fibres in anterior tibial muscle. Comparison between young adults and physically active aged humans. *Acta Neuropathol.*, *80* (1990) 459–468.

[8] L. Larsson and L. Edström, Effects of age on enzyme-histochemical fibre spectra and contractile properties of fast- and slow-twitch skeletal muscles in the rat. *J. Neurol. Sci.*, *76* (1986) 69–89.

[9] R.N. Michel, A.E. Olha and P.F. Gardiner, Influence of weight bearing on the adaptations of rat plantaris to ablation of its synergists. *J. Appl. Physiol.*, *67* (1989) 636–642.

[10] R.W. Tsika, R.E. Herrick and K.M. Baldwin, Time course adaptations in rat skeletal muscle isomyosins during compensatory growth and regression. *J. Appl. Physiol.*, *63* (1987) 2111–2121.