FIBER NUMBER AND TYPE COMPOSITION IN MUSCLE OF AGING RATS

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SUMMARY

Skeletal muscle from young adult and senescent male Fisher 344 rats was characterized histochemically and mechanically. Extensor digitorum longus (EDL) and soleus (SOL) muscles were used. Histochemically, in the EDL, there were no differences in the percent of type I fibers among age groups, and there was no trend for change in percent type IIa or IIb fibers. For SOL, the percent of type I fiber was greater in the aged animals. The percent of type IIa was lower in the old animals and the percent of type IIb and IIc did not change. Maximal shortening velocity (V_{max}), determined by the slack test method, was unchanged in the EDL but was increased in the soleus muscle of young adult versus senescent rats. Isomeotric tension (P_o) was greater in SOL and was unchanged in EDL from senescent versus young rats.

INTRODUCTION

It has been reported that muscle performance, in regard to speed and strength, changes with senescence (Larson et al, 1979, Syrovy and Gutmann, 1977). Age-related changes may occur in the contractile proteins, or in other factors such as the neuromuscular junction or the calcium release and sequestering systems. Likewise, changes in fiber type percentages and fiber number have been reported (Silberman et al, 1983; Bass et al, 1975, Zelena and Hnik, 1963.)

One essential objective was therefore to describe histochemical and mechanical changes which occur in muscle as the animal ages. The rationale is to understand the changes as background information to apply in the field of Meat Science. It is conceivable that senescence related changes may be associated with factors controlling meat quality and functionality for further processing. Therefore the work was an exercise in fundamental science undertaken with the hope that the information would be useful to practical application.

Animal strain, age, nutrition, exercise and other factors may influence results. We therefore used rats raised specifically for aging studies. These Fisher 344 (F344) rats have a uniform genetic background and are reared under rigidly controlled conditions to minimize environmental effects.

MATERIALS AND METHODS

Rats were of ages 3, 8-10, 27-28 and 29-30 months for histochemical work and of ages 9 and 29-30 months for mechanical characterization. Upon receipt of animals they were kept in conventional housing, one per cage, for an average of 10 days before use. On the day of experiment the rat was anesthesized with sodium pentobarbitol and the EDL and SOL muscles were exposed and removed.

For histochemical experiments the EDL and SOL muscles were exposed, their in situ lengths (Lo) measured (with the foot at right angles to the lower leg), and they were cut free near the points of origin and insertion. Each muscle was gently blotted and weighed, and then placed in an oxygenated solution containing 137 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 3 mM HEPES, 11 mM glucose, pH 7.3, for 0.5-8 hr. This period of time was used to obtain measurements of shortening velocity and isometric tension of contralateral SOL and EDL muscles. The muscles were frozen at approximately rest length in isopentane cooled in liquid nitrogen, and were stored in liquid nitrogen until they were sectioned in a cryostat at -20°C. Transverse serial sections (8-10 µm thick) cut from the belly of each muscle were picked up on glass slides and stored for 24 hr or less in the cryostat until they could be stained. Half an hour before staining the slides were placed in a hood at room temperature to dry. The sections were stained for myosin ATPase (M-ATPase) using the method of Dubowitz and Brooke (1973) [with modifications by Suzuki (1976) and Suzuki and Cassens (1980)], which is based on the method of Padykula & Herman (1955). In the present work, the acid preincubation solution was adjusted to pH 4.35 and the alkaline to pH 10.25 just prior to use. The alkaline preincubation was for 20 min and the time in the incubation solution (step 2, Dubowitz and Brooke, 1973) was 45 min for the acid-preincubated slides and 30 min for the alkaline-preincubated slides. Following the second water rinse the slides were fixed in a 1:3 mixture of acetic acid and absolute ethanol for 4 min, followed by two 4-min changes in absolute ethanol, clearing, and mounting. Fiber type was distinguished solely from the acid preincubation sections using the nomenclature of Brooke and Kaiser (1970). Type I fibers stain dark following acid preincubation and light following alkaline preincubation. Type IIa fibers stain light following acid preincubation and dark following alkaline preincubation, and type IIb fibers stain intermediate following acid preincubation and dark following alkaline preincubation. The SOL is reported to have type IIc fibers rather than type IIb, although these fibers stained like type IIb fibers with the preincubations used in this study (Brooke and Kaiser, 1970). The alkaline preincubation slides were used to qualitatively verify the reversal of the staining pattern at this pH. The number of fibers of each type within the entire section were counted, with the sum of the fiber types being the total number of fibers in the cross section. Because of the anatomical organization of the EDL and SOL muscles, a cross section through the belly includes all the fibers in the case of the SOL and most of the fibers in the case of the EDL (Close, 1964).

The complete details of the preparation and apparatus used for making mechanical measurements on living fiber bundles and skinned fibers is given in Eddinger et al (1986).

RESULTS AND DISCUSSION

The histochemical fiber type composition and fiber number for the EDL and SOL muscles are summarized in Table 1. There was no change in the percentage of type I and type II (a and b) fibers in the EDL between the 3 and 30 month age groups. However, there were small changes in the percent of type II fibers between the 9 and 30 month age groups. The percent of type IIa fibers was found to decrease significantly between these age groups, whereas the percent of type IIb increased significantly. The number of fibers in the EDL did not change significantly with aging.

The SOL muscle showed a large increase in type I and a decrease in type II fibers (type IIa and c; according to the Brooke and Kaiser (1970) nomenclature the intermediate-staining fibers in the rat SOL are type IIc) with increasing age after 9 months. The percent of type I fibers increased significantly from the 3 and 9 month age groups to the 27 month age group and continued to increase to the 30 month age group. The percent of type IIa fibers showed a significant decrease from the 9 to the 30 month age group, whereas the percent type IIc

Table 1. Fiber percent and type composition of EDL and SOL muscles from F344 rats with aging[1]

Age (months)[2]	3	9	28	30
		EDL		
N	10	22	17	10
% Type I	3.4 ± 1.1[a]	3.1 ± 1.4[a]	3.2 ± 1.5[a]	3.0 ± 0.8[a]
% Type IIa	12.8 ± 2.7[a,b]	16.3 ± 5.1[b]	12.4 ± 3.8[a,b]	11.1 ± 5.8[a]
% Type IIb	83.8 ± 3.3[a,b]	80.6 ± 5.6[b]	84.4 ± 4.1[a,b]	85.9 ± 6.0[a]
Total fiber no.	3243 ± 439[a]	3152 ± 467[a]	2896 ± 441[a]	3071 ± 346[a]
		SOL		
N	10	26	17	12
% Type I	84.9 ± 5.7[a]	80.5 ± 5.0[a]	90.7 ± 7.1[b]	94.1 ± 3.5[b]
% Type IIa	9.2 ± 6.4[a,b]	11.3 ± 8.8[a]	6.2 ± 7.2[a,b]	2.8 ± 2.7[b]
% Type IIc	5.9 ± 5.5[a]	8.2 ± 7.6[a]	3.1 ± 3.9[a]	3.1 ± 3.1[a]
Total fiber no.	2751 ± 173[a]	2330 ± 277[b]	2161 ± 214[b]	2328 ± 205[b]

[1] All values given are means ± standard deviation; different superscript letters in the same row are significantly different ($p < 0.05$). Each superscript letter should be considered individually (i.e., for the EDL type IIa fibers, the only significant difference was found for values at 9 and 30 months of age).

[2] Group ages given are rounded off from mean ages.

fibers showed no statistically significant change. Fiber number decreased significantly from the 3 to 9 month age group and then remained constant through the 30 month age group.

In entire transverse sections through the belly of the EDL and SOL muscles, difference in fiber type composition were readily observed. The EDL had a nonuniform distribution of fiber types with the vast majority of the type I fiber population located on the medial and anterior side of the muscle corresponding to the proximal heads of the muscle. The SOL muscle had a more uniform distribution of fiber types.

Muscle bundles from the EDL and SOL showed characteristic differences in their optimal stimulus frequencies, twitch and tetanus time courses, and twitch-tetanus ratios. The EDL and SOL bundles generated maximal tetanic tension at stimulation frequencies of ~60 and 30 Hz, respectively. The EDL bundles showed a very rapid rise to peak tension, which then tended to decrease slightly over the remainder of the tetanus. The SOL bundles developed tension at a much slower rate, never actually reaching a steady plateau during a 1-s tetanus (values were >90% P_o). Differences were also apparent in the contraction times (CT) for EDL versus SOL muscles; although there were no significant age-related differences (senescent vs. control mean ± SD: SOL, 129 ± 17 vs. 113 ± 16 ms; EDL, 37 ± 2 vs. 39 ± 5ms). The CT values for SOL bundles were in each case approximately three times longer than those for the EDL bundles. These values are similar to those obtained by Close and Hoh (1980) from directly stimulated whole EDL (37.5 ms) and SOL (122 ms) muscles of a 4 wk-old female Wistar rats at 20°C.

The tension per cross-sectional area was higher in the EDL bundles than in the SOL bundles in the control group. There was a significant increase in P_o in the SOL bundles from the senescent rats vs. control, but no difference was found in P_o between the EDL bundles from the senescent and control rats (Table 2).

The force-velocity relationship was measured in several fiber bundles using the load-stepping technique, (Hill, 1938). The data from 7 to 11 muscle bundles for each age group were pooled to characterize the force-velocity relationships for both the EDL and SOL. Velocity values (V) obtained at a number of loads (P) less than P_o were plotted and were fitted with hyperbolas using Hill's equation: $(P + a)V = 6(P_o - P)$. The load-stepping results show a decrease in V_{max} (estimated by extrapolation) for the EDL bundles with senescence and no change in V_{max} for the SOL bundles.

To avoid reliance on extrapolation to zero load to estimate V_{max}, determinations of V_{max} were made directly using the slack test method. There was a significant increase in V_{max} in the SOL bundles of the senescent vs. control rats, with no significant difference in V_{max} between the EDL bundles of senescent and control rats. There was no correlation between the small changes in fiber type percentage and V_{max} values, with correlation coefficients ranging between 0.03 and 0.66 for the bundles and age groups examined.

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Complete details of the work described herein can be found in the manuscripts by Eddinger et al 1985 and 1986.

Table 2. Maximal isometric tension

	Bundles				Skinned Fibers			
	SOL*		EDL		SOL*		EDL	
	Control	Senescent	Control	Senescent	Control	Senescent	Control	Senescent
N	16	16	16	15	8	7	8	8
Po**	59.0±20.0	85.0±18.0	114.0±40.0	89.0±35.0	87.0±8.0	122.0±20.0	98.0±30.0	123.0±22.0

Values are means ± SD.
* Means for senescent rats significantly different from means for young adult rats, $P<0.05$.
** Maximal isometric tension (P_o) normalized to cross-sectional area (kN/m^2).

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