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Aging affects different human muscles in various ways. An image analysis of the histomorphometric characteristics of fiber types in human masseter and vastus lateralis muscles from young adults and the very old

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Summary. This study is an attempt to objectively evaluate age-related changes in human muscles by use of histomorphometric methods. Aging in humans induces dramatic transformations in the skeletal muscles but little is known as to whether or not the aging processes per se may affect all muscles equally. In this study aging of two human muscles with different functions, origin and nerve supply is compared.

Sections were cut from masseter and vastus lateralis muscles obtained from young adults aged 18-24 years and from the very old aged 90-102 years. Muscle fiber types were classified with the traditional myofibrillar ATPase staining. Various histomorphometric parameters of the different fiber types in human masseter and vastus lateralis muscle sections were obtained by image analyses to evaluate the age-related changes in the muscle fibers. The following variables were calculated: the number of each fiber type per photographed area; the area of each fiber and two indicators for the shape of the muscle fibers. In the aging muscles there was no relative preferential loss of a fiber type. High numbers of intermediate ATPase-stained fibers (IM fibers) were found in some old vastus muscles but were only sporadic in young vastus muscles. However, there was no change in the percentage distribution of intermediate ATPase-stained fibers when young and very old human masseter muscles were compared. Incubation of the sections with antimyosin antibodies showed that the IM fibers in old masseter and old vastus contained different myosin heavy chains. Thus ATPase activity and anti-myosin staining displayed a somewhat different pattern of fiber type distribution. The main changes in the shape and area indicated that type I fibers in the masseter became more circular while in the vastus they decreased significantly in size. The type II fibers in the vastus became very small and deviated significantly from circularity whereas the type II fibers in the masseter only exhibited a decrease in the size of the fibers. Histomorphometric measurements show that aging affects different human muscles in various ways.

Key words: Human, Skeletal muscle, Aging, Histomorphometry

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Introduction

Increasing human age leads to a reduction in muscle mass and at the age of 80 about half of the total muscle area is wasted (Lexell et al., 1988). The aging muscle atrophy causes decreased muscle strength and mobility often associated with pain from muscles and joints. The reduced functionality has been attributed to a number of changes taking place in the process of aging such as a gradual decrease in fiber diameter, degeneration of sarcoplasm and replacement of muscle fibers by fat and connective tissue (Larsson, 1995).

Most studies on aging human muscles have focused upon changes in the vastus lateralis muscle in which the age-related reduction in muscle mass is due to a reduction in both number and size of muscle fibers, mainly type II. This could, to some extent be caused by a slowly progressive neurogenic process (Lexell, 1995). However, not all muscles may be affected equally by the aging process (Jennekens et al., 1971; Coggan et al., 1992a). Changes in shape of muscle fibers are seen in some muscle diseases (Kaido et al., 1991; Nucci et al., 1996) and have occasionally been reported to also appear in aging muscles (Tomonaga, 1977). Regarding a

possible heterogeneity of age-related changes in muscle, and the involvement of different muscles in different functions, it seems appropriate to compare the aging processes in functional separate muscles (Dutta and Hadley, 1995). We here measure aging of muscle fiber types in human vastus and masseter muscles using the myosin ATPase staining as a marker for the different muscle fiber types to relate the present results with the prior studies on aging processes in muscle fiber types. Furthermore, the distribution of fast and slow fibers in young and old muscles as determined by ATPase staining was matched with the distribution of fiber types obtained after incubation with anti-myosin antibodies.

The main objectives of the present study were to compare age-related histomorphometric changes in two muscles with a different function, different embryological origin and different nerve supplies.

In this study we have measured fiber type distribution, area of single fiber types, and shape descriptors of fast and slow fibers in vastus lateralis and masseter muscles from young (18-24 years) and very old (90-102 years) humans. This was done in an attempt to objectify possible histomorphometric age-related changes in the muscle fibers. These two muscles fulfill separate functional demands (vastus lateralis is a limb muscle and masseter is a masticatory muscle). They have a different kind of nerve supply (spinal nerve in vastus lateralis and cranial nerve in masseter) and they are of different embryological origin (vastus lateralis is myotome-derived and masseter originates from the first branchial arch). A comparison of the age-related histomorphometric changes in the two muscles as we describe them in the present paper may thus contribute to the understanding of the effects of aging on human muscle.

Materials and methods

Muscle tissue

The material consisted of muscle samples from 40 adult Danes. Twenty individuals were at the age of 18-24 yr and the other twenty were at the age of 90-102 yr. Since the fiber type distribution varies depending on the location in the muscles (Eriksson, 1982; Lexell et al., 1988) the muscle samples were all taken from similar sites in the muscles: the superficial site of the anterior part of the masseter muscle and from the middle portion of the vastus lateralis of the quadriceps femoris muscle.

The material gained from the young adults was obtained as diagnostic biopsies from volunteers without known systemic disorders, and received with their informed consent. The vastus muscle biopsies were taken from young sedentary men, who were used as steady controls on an athletic program. The masseter muscle biopsies were taken from young females with natural dentition. None of the muscle samples contained signs of muscle or vascular disease based on histological examination.

The muscle samples from the old adults were obtained from autopsies from women less than 36 h post mortem. To explore the rationale of comparing histomorphometrical data obtained from biopsies and autopsies we further used biopsy samples from soleus muscle from five rats aged four months. Another five rats of the same age were killed by cervical dislocation and remained thereafter for 8 hrs at room temperature. The dead rats were then stored for 24 hrs at +4 °C, corresponding to the storage conditions as in the morgue of our institute, and the autopsy samples of soleus muscle were thereafter removed.

All muscle samples were frozen in isopentane cooled to -150 °C with liquid nitrogen and cut on a cryostat in 6-μm sections. Traditional myosin ATPase histochemistry with incubation at pH 9.4 was used to detect type I and type II muscle fibers in the sections (Brooke and Kaiser, 1970).

We wanted to examine the correlation between fiber typing based on activity for myosin ATPase and fiber typing based on staining after incubation with antibodies directed against myosin heavy chain isoforms. This was done to determine if immunohistochemical methods could substitute the traditional ATPase-based classification of muscle fiber types. For this purpose serial sections were incubated in the ATPase medium and with anti-myosins: Mab M4276, clone MY-32 (Sigma) directed against fast skeletal myosin and Mab A4.951 directed against slow skeletal myosin (Hughes and Blau, 1992; Hughes et al., 1993; the antibody was a generous gift from Dr. Hughes).

Some sections were incubated with a monoclonal antibody against laminin (Sigma L-8271) to outline the muscle fibers by detecting the basement membrane. The staining procedure with the monoclonal antibodies was performed according to Kirkeby (1996) using alkaline phosphatase-conjugated rabbit anti-mouse immunoglobulins (DAKO) and an alkaline phosphatase medium containing 5-bromo-4-chloroindoxyl phosphate (Sigma).

Photography and scanning

The ATPase-stained muscle sections were processed as a videoprint with a UP-910 Sony Videographic connected to a monitor to which the signal was transferred from a videocamera (KAPPA CF 11/2) mounted on a Leitz microscope using a x16 Leitz objective. The linear magnification was measured with a Wild micrometer photographed under the same conditions as the stained muscle sections. Suitable areas of each section containing transversely cut muscle fibers were photographed. The number of photographed fibers in a section was between 100-200.

The videoprints were scanned with a GT-Epson 6500 flat bed scanner to a TIFF-file (Tagged Image File Format) from which the images were loaded for image analysis with Image-Pro® Plus, version 3 for Windows™ (Media Cybernetics, Silver Spring, Maryland, USA).

Measurements

After retrieval of the image the outline of each muscle fiber was drawn manually with a stylus pen connected to a graphic tablet (HiSketch 1212 SP; Genius). The fiber area was filled with one of three preselected 8-bit grey scale values according to the muscle fiber type (type I fibers, pixel intensity set to 128; type II fibers, pixel intensity set to 0; type IM (intermediate fibers), pixel intensity set to 64). From the overlay the following variables were calculated:

a) The number of each fiber per photographed area.

b) The area of each fiber (fiber size in μm^2)

c) The radius ratio: defined as the ratio between the maximum radius and the minimum radius for each object. The radii are defined as the maximum respective to the minimum distance between the object's centroid pixel position and its perimeter.

d) The roundness is defined as: perimeter$^2/4\pi$ x area. This is the reciprocal of the formfactor as defined in Russ (1995). Objects with roundness 1 are circular; increasing values indicate deviation from circularity. Calibration was performed by the micrometer photograph.

Statistics

Since some of the variables deviated from normal distribution, median and ranges were calculated for each variable and nonparametric statistics were applied. The *Kruskal-Wallis one-way ANOVA* test was used to determine whether the samples were drawn from populations with the same medians. *Multiple comparisons with t-distribution* were performed amongst groups of unequal sizes in a way similar to Tukeys-HSD test using the mean ranks. When single pairs were compared the *Mann-Whitney U* test was used. $P<0.05$ was chosen as the level of significant differences. In the tables the mean and standard deviations are shown as well for comparison with figures in investigations, where these values are employed. Calculations were carried out with the *Unistat® Statistical Package.*

Results

Histochemistry

The three types of fibers described in the present study are defined on the bases of the ATPase reaction after preincubation of the sections at pH 9.4. Type I muscle fibers were unstained, type II were intensely black stained and the intermediate types (IM) stained in varying grades of grey. Examples of fiber typing in young and old muscles are shown in Fig. 1A-D.

In the sections from young vastus muscle the weakest ATPase-stained type I fibers reacted strongly with the antibody directed against slow myosin (Mab A 4.951) and were non-reactive after incubation with the antibody directed against fast myosin (M 4276). Most of the strongly ATPase-stained type II fibers were strongly stained after incubation with Mab M 4276 and unstained after incubation with Mab A 4.951. However some type II muscle fibers contained both slow and fast myosin since they were positive for both antibodies. In the old vastus muscle the type I and II fibers showed strong antibody reaction both in muscle fibers showing atrophy and in fibers with normal morphology. The intermediate ATPase-stained fibers in the old vastus muscles were positive for Mab M 4276 but negative for Mab A 4.951 (Fig. 2A-C).

The staining patterns for ATPase and anti-myosin antibodies were similar in young and old masseter muscles. The large type I fibers were stained only after incubation with Mab A 4.951 while the intermediate ATPase-stained fibers were positive for both Mab A. 4.951 and Mab M 4276. Incubation with anti-myosins showed that the type II fibers were reactive with Mab M 4276 and that a few type II fibers also were reactive with Mab for A 4.951 (Fig. 2D-F).

Atrophic and irregular fibers are frequent in the aging vastus. To ensure that these fibers were not the result of fragmentation of larger muscle fibers produced during tissue processing some serial sections were stained for ATPase activity and incubated with anti-laminin to detect the basement membrane. The small angulated, closely-packed muscle fibers were all encircled by an intact basement membrane (Fig. 3A,B).

Measurements

The descriptions refer to measurements on transverse sections of muscle fibers.

In the statistics: < refer to a median of a variable of a muscle fiber-type being significantly smaller than ($p<0.05$), and = refer to a median similar to ($p\geq 0.05$) the succeeding median.

Rat soleus muscle (Table 1)

No differences between the variables of biopsy and autopsy specimens were observed.

Human muscles

Fiber distribution (Table 2)

Between the young and the old muscles no changes were observed in the proportions of the various types of fibers. In the old and young masseter (MO and MY) the proportion of type II fibers was significantly higher than that of types I and IM (IM = I < II). In the old and young vastus lateralis (VO and VL) the proportion of type IM fibers was significantly lower than that of type I and II fibers (IM < I = II).

Fiber areas (Table 3)

Masseter. The only difference observed between the old

and the young fibers was a somewhat smaller area of the old type II fiber.

Vastus lateralis. The areas of all of the young fiber types were significantly larger than the areas of the old fibers.

Multiple comparison amongst all the fibers showed that the young vastus lateralis fibers were always significantly larger than the fibers of all of the other muscles (Type I and II: MO = MY = VO < VY; Type IM: MO = VO, MO < MY, MY = VO, VO < VY).

Radius Ratio and Roundness (Table 3).

Values higher than 1 indicate deviations from circularity.

Masseter. The values for type I fibers of the old muscles were significantly lower than the values for the fibers of the young. The same trend was true for the roundness of the IM fibers.

Vastus lateralis. Although the radius ratio of the type I

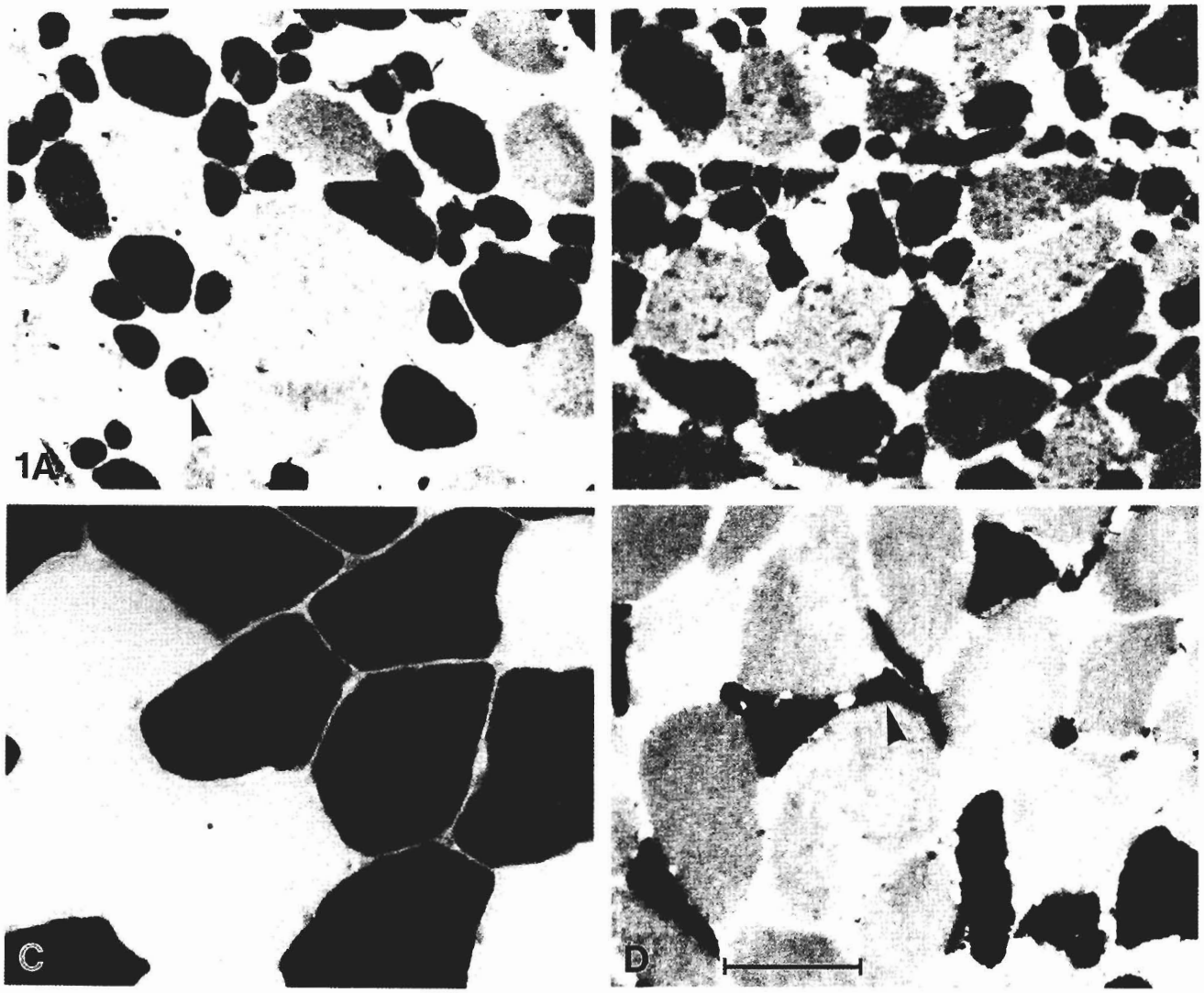

Fig. 1. ATPase staining of human muscle sections. **A.** Young masseter. Type I fibers are pale, type II fibers are dark, and type IM fibers are grey. Type I fibers are considerably larger than type II fibers. **B.** Old masseter. The muscle contains the same fiber types as those present in the young masseter. Most muscle fibers are regular in shape and the type II fibers are a little smaller than type II fibers in the young muscle. **C.** Young vastus. Large regular type I and II fibers. Part of an IM fiber is shown on this micrograph. **D.** Old vastus. Type I fibers are quite large and regular in shape while type II fibers are much smaller and some are irregular. As examples of the shape descriptors as used in this study one type II muscle fiber in Fig. A, and one type II muscle fiber in Fig. D has been marked with arrowheads. The roundness and radius ratio values for the fiber marked in young masseter (Fig. A) are 1.21 and 1.35 respectively, while the same measurements for the fiber marked in the old vastus (Fig. D) are 1.63 and 4.98 respectively. The type II fiber in the old vastus is thus more irregular in shape than the type II fiber in the young masseter. Scale bar: 70 μm.

Table 1. Histomorphometric measurements of fiber types in biopsy and autopsy of rat soleus muscle. Radius Ratio and Roundness = 1 corresponds to a circle, increasing values indicate irregularities. NS: Not statistically significant. Mann-Whitney's U test is carried out between autopsy and biopsy values.

	AUTOPSY		BIOPSY		MANN-WHITNEY
	Distribution	Area (μm^2)		Distribution	Area (μm^2)
Specimen	5		5		
Type I Fiber					
Mean		2091		2568	
Stand. Dev.		298		641	
Median		2111		2287	NS
Range		791-1914		1590-2192	
Mean	70%		67 %		
Stand. Dev.	9%		5%		
Median	68.8%		68.0%		NS
Range	57.8-83.4%		58.6-72.2%		
Type II fiber					
Mean		1696		1804	
Stand. Dev.		338		358	
Median		1544		1699	NS
Range		1387-2199		1489-2400	
Mean	24%		28%		
Stand. Dev.	11%		5%		
Median	26.9%		27.6%		NS
Range	9.1-37.8%		22.5-34.5%		
Type IM fiber					
Mean		1384		1551	
Stand. Dev.		234		832	
Median		1432		1412	NS
Range		1033-1613		527-2654	
Mean	6%		5%		
Stand. Dev.	2%		2%		
Median	4.4%		5.3%		NS
Range	4.3-8.1%		1.7-7.0%		
	RADIUS RATIO	ROUNDNESS	RADIUS RATIO	ROUNDNESS	
Specimen	5		5		
Type I fiber					
Mean	2.1168		2.2899		
Stand. Dev.	0.2234		0.1488		
Median	2.1048		2.3600		NS
Range	1.8795-2.4489		2.0513-2.4304		
Mean		1.4052		1.4564	
Stand. Dev.		0.0674		0.0368	
Median		1.4034		1.4602	NS
Range		1.3271-1.4909		1.4074-1.4955	
Type II fiber					
Mean	2.3700		2.5084		
Stand. Dev.	0.2294		0.3339		
Median	2.4461		2.3642		NS
Range	2.1129-2.6407		2.2434-3.0834		
Mean		1.4963		1.5251	
Stand. Dev.		0.0773		0.0604	
Median		1.5077		1.5094	NS
Range		1.3868-1.6004		1.4729-1.6248	
Type IM fiber					
Mean	2.2643		2.7193		
Stand. Dev.	0.3180		0.9120		
Median	2.2485		2.8603		NS
Range	1.9939-2.7765		1.3412-3.8883		
Mean		1.4840		1.6264	
Stand. Dev.		0.0686		0.1260	
Median		1.4836		1.6535	NS
Range		1.3789-1.5553		1.4152-1.7447	

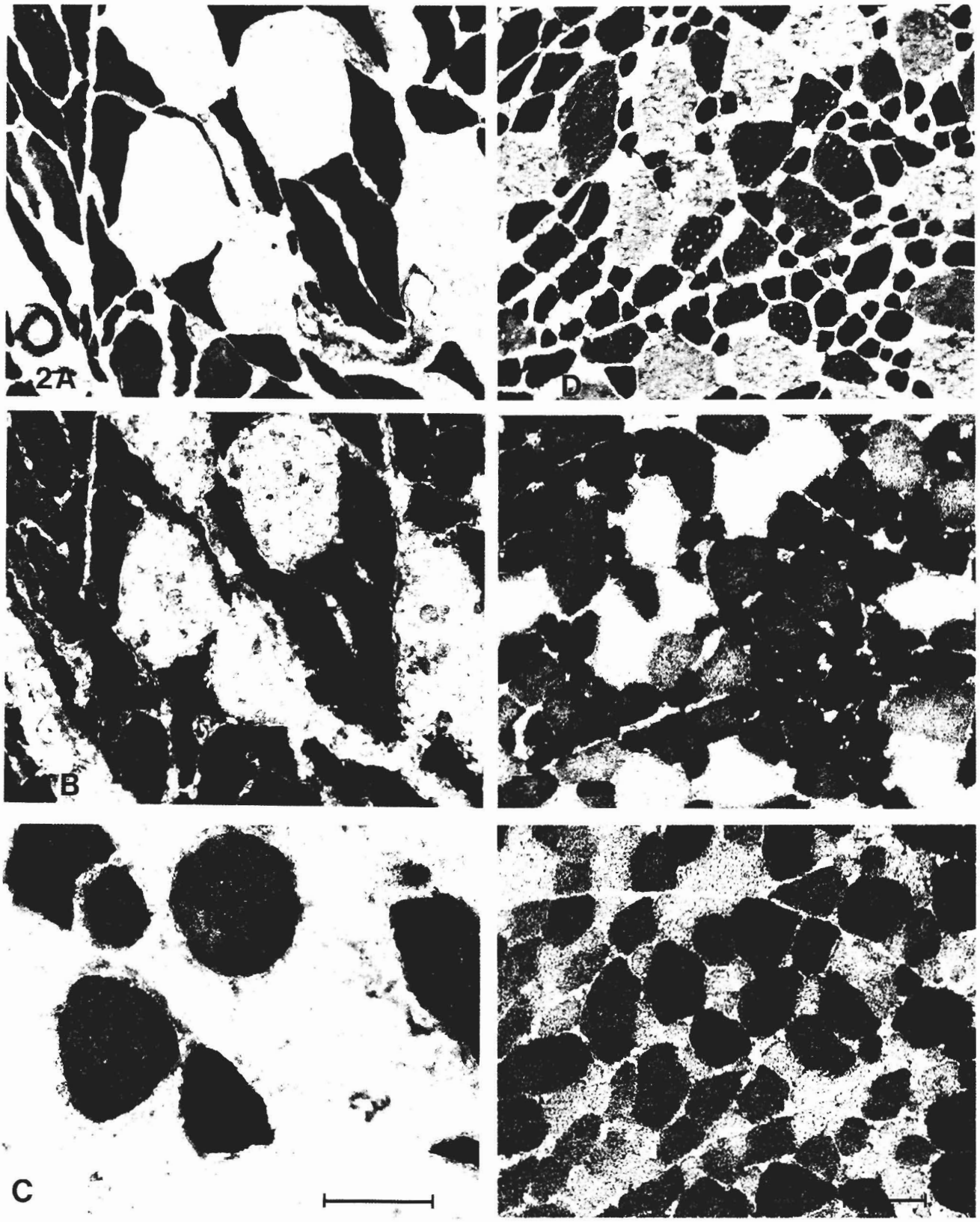

Fig. 2. A-C. Serial sections of an old vastus muscle stained for ATPase activity **(A)**, and incubated with antibodies directed against fast myosin **(B)**, and slow myosin **(C)**. Type I fibers are positive with anti-slow myosin while type II fibers and IM fibers are positive with anti-fast myosin. **D-F.** Serial section of an old masseter muscle stained for ATPase activity **(D)**, and incubated with antibodies directed against fast myosin **(E)** and slow myosin **(F)**. Type IM and type II fibers are positive with anti-fast myosin. Type I, type IM and some type II fibers are positive with anti-slow myosin. Scale bar: 70 μm.

fibers of the old muscles was significantly higher than that of the young, the most pronounced difference was observed in the increased radius ratio and roundness for type II fibers of the old muscles.

Multiple comparisons between the muscles showed that the radius ratio and the roundness of type I fibers in the old masseter was significantly lower than that of the other muscles (MO < MY = VO = VY). The values regarding the type II fibers of the old vastus lateralis were significantly increased compared to those of the other muscles (Radius ratio: MO = MY = VY < VO; Roundness: MO = MY, MO < VY, MY = VY, VY < VO) and the values of the IM fibers showed the same tendency (Radius ratio: MY = MO = VY < VO; Roundness: MO < MY = VY, VY < VO).

Discussion

The aging of muscles may be examined by various methods such as EMG, histochemistry, computed tomography, or biomechanics. We here use histomorphometric measurements of myosin ATPase-stained muscle fibers to describe and compare the age-related changes in a human masticatory muscle and a limb muscle. The muscle fibers were defined both after incubation with

Table 2. Distribution of muscle fibers in human masseter and vastus lateralis muscles. Mann-Whitneys's U test is carried out between old and young muscles. Kruskal - Wallis test is used amongst fiber types in the same muscle.

FIBER DISTRIBUTION	MASSETER			VASTUS LATERALIS		
	Old	Young	Mann-Whitney	Old	Young	Mann-Whitney
Specimens	10	10		10	10	
Type I Fiber						
Mean	25%	27%		42%	43%	
Stand. Dev.	17%	16%		12%	15%	
Median	18.3%	19.5%	NS	41.5%	42.4%	NS
Range	9.3-63.4%	9.4-55.0%		22.5-67.7%	20.6-60.2%	
Type II fiber						
Mean	60%	53%		48%	55%	
Stand.Dev	16%	19%		13%	14%	
Median	63.3%	53.7%	NS	51.5%	56.6%	NS
Range	24.4-76.5%	22.1-81.8%		28.3-67.5%	38.1-78.1%	
Type IM fiber						
Mean	15%	20%		10%	2%	
Stand. Dev.	6%	8%		13%	2%	
Median	16.8%	21.0%	NS	3.7%	1.1%	NS
Range	5.1-25.7%	8.8-31.5%		0.0-31.7%	0.0-8.4%	
Kruskal-Wallis	P = 0.0002	P< 0.002		P= 0.0001	P < 0.0001	

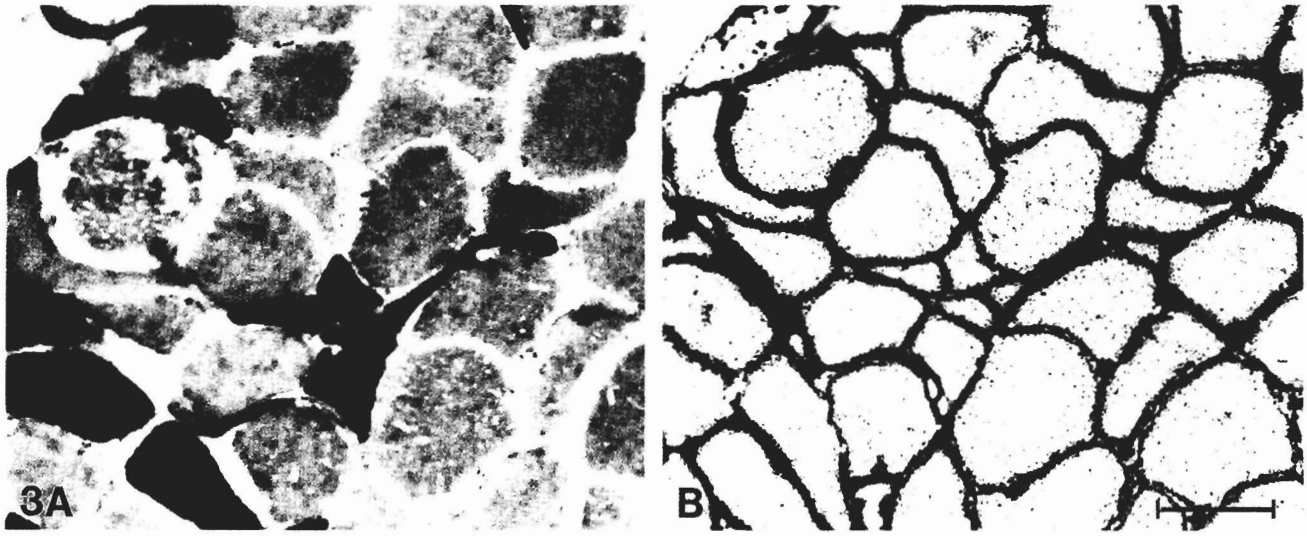

Fig. 3. Old vastus muscle. Serial sections showing activity for myosin ATPase **(A)** and staining after incubation with anti-laminin **(B)**. The small irregular type II fibers as shown in Fig. A are outlined after incubation with anti-laminin in Fig. B. Scale bar: 70 μm.

Table 3. Histomorphometric measurements of fiber types in human muscles. Radius ratio and Roundness = 1 correspond to a circle. Higher values indicate irregularities. NS: not statistically significant.

	MASSETER		VASTUS LATERALIS		KRUSKAL-WALLIS
	Old	Young	Old	Young	
AREA (μm2)					
Type I fiber					
Specimens	10	10	10	10	
Mean	1273	1475	1764	4452	
Stand. dev.	399	1101	769	1296	
Median	1255 (NS)[a]	1048	1802 (p=0.0001)[b]	4532	p=0.0001[c]
Range	775 - 1863	537-4140	821-3065	2507-6120	
Type II fiber					
Specimens	10	10	10	10	
Mean	217	384	440	4727	
Stand. dev.	72	185	315	1492	
Median	212 (p<0.02)	358	318 (p<0.0001)	4882	p<0.0001
Range	83-318	201-781	150-998	2014-7289	
Type IM fiber					
Specimens	10	10	6	6	
Mean	605	1039	882	4595	
Stand. dev.	332	657	506	2316	
Median	627 (NS)	874	761 (p<0.003)	5014	p<0.002
Range	227-1111	584-2854	475-1802	1922-7846	
RADIUS RATIO					
Type I fiber					
Specimens	10	10	10	10	
Mean	1.6517	1.9062	2.1301	1.8517	
Stand. Dev.	0.1286	0.2562	0.2559	0.1147	
Median	1.5922 (p<0.006)	1.9209	2.0589 (p<0.009)	1.8536	p=0.0003
Range	1.4968-1.9105	1.6056-2.2795	1.7786-2.4915	1.6325-2.0285	
Type II fiber					
Specimens	10	10	10	10	
Mean	1.9580	1.9670	4.2687	1.8954	
Stand. Dev.	0.3354	0.1384	2.0626	0.0970	
Median	1.9229 (NS)	1.9638	3.2840 (p<0.0001)	1.9069	p<0.0001
Range	1.6637-2.7863	1.7208-2.1319	2.9358-9.6926	1.7467-2.0327	
Type IM fiber					
Specimens	10	10	6	6	
Mean	2.0388	2.0095	3.0729	2.2427	
Stand. Dev.	0.2539	0.1667	1.1726	0.5597	
Median	2.0122 (NS)	1.9930	2.6655 (NS)	2.0656	p<0.02
Range	1.6833-2.4803	1.7120-2.2732	2.2018-5.3796	1.7611-3.3246	
ROUNDNESS					
Type I fiber					
Specimens	10	10	10	10	
Mean	1.2373	1.3438	1.4003	1.4092	
Stand. Dev.	0.0349	0.0909	0.0565	0.0992	
Median	1.2302 (p<0.003)	1.3254	1.4219 (NS)	1.3777	p=0.0001
Range	1.1811-1.3011	1.2314-1.5204	1.3049-1.4638	1.3245-1.6436	
Type II fiber					
Specimens	10	10	10	10	
Mean	1.2610	1.3075	1.9040	1.3945	
Stand. Dev.	0.0942	0.0518	0.4481	0.0331	
Median	1.2497 (NS)	1.2974	1.7685 (p<0.0001)	1.3843	p<0.0001
Range	1.1257-1.4745	1.2075-1.3851	1.5107-3.0105	1.3547-1.4444	
Type IM fiber					
Specimens	10	10	6	6	
Mean	1.3187	1.3662	1.7053	1.5128	
Stand. Dev.	0.0432	0.0439	0.3962	0.2400	
Median	1.3293 (p<0.02)	1.3737	1.6190 (NS)	1.4070	p<0.002
Range	1.2490-1.3710	1.2910-1.4232	1.3544-2.4660	1.3206-1.9682	

[a]: Mann-Whitney U test between old and young masseter muscles; [b]: Mann-Whitney U test between old and young vastus lateralis muscles; [c]: Kruskal-Wallis ANOVA. The multiple comparisons are described under results in the text.

anti-myosin antibodies and by their ATPase activity. The ATPase reaction was used as a marker for the muscle fibers because muscle fiber types are defined by their ATPase staining (Brooke and Kaiser, 1970) and in order to compare our results with the many previous observations on aging muscles based on myosin ATPase fiber typing.

Autopsies - biopsies

In the present study we have measured sections of muscle fibers from both autopsies and biopsies incubated for myosin ATPase. Although many studies on muscle histology and histochemistry have been performed on autopsy material the post mortem autolysis may in theory influence both the ATPase activity and the morphology of the fibers. Eriksson et al. (1980) have studied the reliability of using post mortem muscle samples with special reference to the influence of time and temperature and conclude that in specimens stored at +4 °C the muscle fibre typing could be reliably performed even after storage for several days. Also, the present study shows that the fiber typing could easily be accomplished and was comparable in rat soleus muscles taken by biopsy or necropsy. Oertel (1988) found that measurements of muscle fiber diameter obtained on post mortem material agrees with findings in normal muscles obtained at biopsy. Furthermore, in the present study a comparison on histomorphometrical data from biopsy and autopsy of rat soleus muscle showed no differences concerning distribution, area and shape of muscle fibers indicating that the measurements obtained from our post mortem muscle material are suitable.

Gender differences

This is a study of aging in muscles from very old subjects. We have compared the histomorphology of fiber types in vastus muscles from young men and old women, because vastus biopsies from young women were not obtainable and, at least in Scandinavia, very few males reach the age of 90 and above. The fiber-size in the vastus from young males has been reported to be about 10% larger than the fiber-size in the vastus from young females (Essén-Gustavsson and Borges 1986). The proportions of different fiber types in gastrocnemius muscles from sedentary subjects did not differ between young and old (60-70 years) or between men and women. In both sexes the type II areas were significantly smaller in the old subjects (Scelsi et al., 1980; Essén-Gustavsson and Borges, 1986; Coggan et al., 1992a,b). In the masseter muscle the values of size of the different muscle fibers do not differ significantly between males and females (Ringqvist, 1974). Using computed tomography the effect of aging on the cross-sectional area and density of human masseter muscle has been investigated and approximately the same changes were observed in male and female subjects (Newton et al., 1987). Thus, our material seems to be appropriate for the measurements employed in the present study since the gender differences appear to be of limited importance in the study of aging muscle atrophy.

The fiber type distribution

In a previous study on jaw closing muscles, Ringqvist (1974) measured the mean percentages for fiber distribution in the masseter from both sexes to be as follows: 28% type I; 57.2% type II; and 14.2% type IM fibers. In the masseter muscles from young females we found 27% type I; 53% type II; and 21% type IM fibers. In an autopsy study on vastus lateralis from young men aged 17-30 years 44% type I fibers and 60% type II fibers were noticed (Johnson et al., 1973). Likewise, our observation on vastus lateralis biopsies from young men showed 43% type I fibers and 55% type II fibers. The percentage distribution of fiber types in young masseter and vastus muscles as indicated in the present study is thus in accordance with previous measurements. Furthermore we observed no statistical differences between the distribution of fiber types in either young or old masseter muscles (types I, II and IM) or young and old vastus lateralis muscles (types I and II). We therefore conclude that in aging muscle there is on an average no predominant loss of a fiber type even when muscles with different function, origin and nerve supply are compared.

High numbers of intermediate ATPase-stained fibers (IM) were noticed in some of the old vastus muscles but were only sporadically observed in young vastus muscles. A similar increase of intermediate-stained muscle fibers during aging occurs in rats (Fujimoto et al., 1994). IM fibers in limb muscles may be related to denervation and reinnervation (Griffin and Pezeshkpour, 1988). In chronic denervating diseases IM fibers may be fibers that are switching their type because of reinnervation by a different type of motor neuron (Engel, 1974). In contrast intermediate-stained fibers are part of the fiber population in normal human masseter muscles (Ringqvist, 1974). It is noteworthy that on an average the present material showed no change in the distribution of intermediate ATPase-stained fibers in young and very old human masseter muscles.

The intermediate ATPase-stained fibers in vastus and masseter possess different myosin isoforms. In both young and old masseter muscle they express fast as well as slow myosin. The intermediate ATPase-stained fibres that develop in old vastus muscles were only stained with the anti-fast myosin. Thus, these fibres should be considered as a subgroup of type II fibers rather than transitory fibers changing between fast and slow forms. This suggestion is also supported by the observation made by Grimby et al. (1984) and confirmed by the present study namely that no change in relative fibre composition with age seems to take place.

Fiber type area

A selective reduction in size and area of type II fibers occurs when muscle strength is impaired

secondary to the neuropathic problems that occurs when the neural output to the muscle is reduced or lost. The same changes are, however, also common due to inactivity and immobilization (Lexell, 1993). In aging limb muscles selective Type II atrophy is frequent (Lexell et al., 1988; Brooks and Faulkner, 1993).

In the old vastus lateralis muscle the areas of both Type I and Type II fibers were significantly lower than in the young muscle. In the masseter there was some reduction in the area of type II fibers in the old muscle, while the area of Type I and IM fibers remained unchanged. Furthermore, the reduction in Type II fiber area was much more pronounced in the vastus lateralis than in the masseter muscle. These results suggest a milder and more selective age-dependent muscular atrophy in the masseter than in the vastus lateralis muscle.

Shape factors

Immobilization with or without pain of joints results in muscular disuse atrophy especially of Type II fibers. In this type of muscular atrophy the fibers are simply smaller than normal (Swash and Swartz, 1988). In contrast, a change in shape of the muscle fibers is often associated with neuropathic processes such as in chronic denervation. When human muscle fibers are deprived of their motor neurons they change in both size and shape. The fibers become atrophic and have an angular, sharply contoured configuration the so-called angulated fibers (Armbrustmacher, 1978; Griffin and Pezeshkpour, 1988). There are few reports on angulated fibers in histological investigations from old human muscle, while several studies using quantitative electromyography have reported a reduction in the number of functioning motor units in aging human muscles (Brooks and Faulkner, 1994; Carmeli and Reznick, 1994; Galea, 1996).

In the present study specific changes in the configuration of muscle fibers were observed in aging muscles. The most striking change is the increase in the shape descriptors roundness and radius ratio of Type II fibers in the vastus lateralis indicating irregularity and elongation in cross-sections of the fast contracting fibers in this limb muscle. Considering also the age-related decrease in the size of type II fibers in the vastus lateralis it seems that at old age these muscle fibers become both atrophic and angulated.

In the masseter muscle the measurements of shape factors showed that all muscle fibers in the old muscle were regular in shape since the roundness values were even lower in the old muscles than in the young. Furthermore, aging did not cause irregularity in the cross-sectioned shapes of the Type II in this muscle as judged from the shape factors. Thus while the shape of Type II fibers is drastically changed in old vastus lateralis resulting in atrophic muscle fibers with an angular configuration there is no age-related change in shape of Type II fibers in the masseter muscle. The atrophy of Type II fibers as measured in this study is restricted to a decrease in the size of the fibers.

Concluding remarks

Aging involves some degree of muscle atrophy, together with a reduction in voluntary strength, but there is still discussion concerning the nature of the cellular events involved (Galea, 1996). In a review on muscle fiber type transitions Pette and Staron (1997) suggest that age-related changes may be muscle specific. Monemi et al. (1998) suggest that while limb muscles decrease in functional demand during aging, the function of the masticatory muscles (chewing, swallowing, speech, etc.) remains relatively unchanged with aging. There is probably no simple explanation for the striking differences between the way human masseter and vastus lateralis muscles respond to aging as demonstrated in the present study. Factors such as use and disuse, innervation and origin of the muscles may be considered.

An important question is whether all human muscles are affected equally by the aging process. The purpose of this study was therefore to determine and compare age-related changes in a human limb muscle and a masticatory muscle. Using histomorphometric measurements of biopsies, taken from fixed well-defined parts of the muscles, we have shown that aging affects different human muscles in various ways.

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