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Fibre type classification and myosin isoforms in the human masseter muscle

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Abstract

Human masseter muscle is highly unusual since it contains relatively large numbers of fibres with variable myofibrillar ATPase staining as well as fibres that express neonatal and alpha-cardiac myosin heavy chain (MHC). These findings however, have not been organised together into a fibre type classification scheme. Biopsies from the anterior superficial area of masseter were collected from a large sample of healthy young adults. Biopsies were sectioned and stained for myofibrillar ATPase reactivity and the presence of MHC isoforms as detected by a series of antibodies. The MHC composition of the same biopsies was also analysed using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). A series of rectus abdominis muscle biopsies were analysed similarly to serve as a control for type I, IIA and IIB fibres and isoforms. From the histochemical, immunohistochemical and biochemical experiments we found the masseter to contain type I, IM, IIC, IIA and IIB fibres as previously classified, but in addition there were type neonatal, alpha-cardiac, and 'other' (three or more myosins including neonatal and alpha-cardiac). The percentage of each fibre type was highly variable in masseter biopsies, but generally type I fibres were most common, and the proportion of IIB, neonatal, alpha-cardiac and 'other' fibres was low. Even in biopsies that contained relatively large amounts of these last three fibre types, the amount of neonatal and/or alpha-cardiac MHC detected on SDS-PAGE was limited, suggesting that these MHCs are a minor component in the fibres in which they are expressed.

Keywords

ATPase; Human; Masseter; Myosin

1. Introduction

Healthy adult skeletal muscle fibres in humans have been classified by myofibrillar ATPase activity into three main groups: type I (slow-twitch), IIA and IIB (fast-twitch) (Brooke and Kaiser 1970). A small proportion of skeletal fibres, usually less than 1%, do not have myofibrillar ATPase reactivity characteristic of these three types (Dubowitz and Brooke 1973) and have been classified into two additional categories, type IIC (Brooke and Kaiser 1970; Dubowitz and Brooke 1973) and type IB (Karpoti et al. 1975), with both fibre types

containing variable amounts of type I and IIA myosin heavy chain (MHC) (Billeter et al. 1980; Schantz and Dhoot 1987). Myofibrillar ATPase studies of human jaw closing muscles have described type I, IIA and IIB fibres similar to other skeletal muscles; and two additional fibre types with unusual ATPase activity termed type IIC and IM (Ringqvist 1971, 1973a; Eriksson 1982).

In the past decade immunologic techniques have identified neonatal MHC and alpha cardiac MHC, in human masseter (Butler-Browne et al. 1988; Soussi-Yanicostas et al. 1990; Bredman et al. 1991); but these findings are not quantitative, or directly comparable to earlier ATPase classifications. Further, they have produced conflicting results with regard to fibre type percentages, probably by the use of very small sample sizes (Pedrosa-Domellof et al. 1992). The purpose of this work is to further describe the fibre types in the masseter muscle on a relatively large group of healthy adults, using staining methods used previously; and in addition, electrophoretic isolation of MHC isoforms to confirm immunohistochemical results. From this data a new fibre type classification is proposed that may be compared directly to earlier histochemical classifications of masseter as well as to fibres from other skeletal muscle.

2. Material and methods

Samples

Biopsies were obtained from the anterior superficial area of the masseter muscle of a mixed group of 58 healthy young adults. 27 biopsies were collected during orthognathic surgery involving mandibular osteotomy and 31 biopsies at the time of third molar extraction. There were 25 males and 33 females with a mean age of 24. For comparative purposes biopsies of the rectus abdominis muscle were taken from four adults, 3 males and 1 female with mean age of 59 undergoing abdominal surgery. Local ethics committee approval and informed consent was obtained for these patient groups. The size of the biopsies varied, but they were usually at least 0.5 cm in length. Upon excision, each biopsy was mounted onto a cork board with tissue-tek mounting medium, snap frozen in 2-methylbutane cooled by solid CO₂ to -70°C and cryostat-sectioned serially at 10/μm in an orientation that allowed transverse cutting through the majority of fibres. After sectioning was completed, a small portion of remaining muscle was placed in sample buffer and prepared for gel electrophoresis.

Histochemistry and immunohistochemistry

Serial sections were stained for myofibrillar ATPase reactivity after preincubation in specific pH buffers, or for the presence of MHC using a series of antibodies. The myofibrillar ATPase stain was similar to that described by Brooke and Kaiser (1970) with modifications given in Snow et al. (1982), method A, except that the incubation time in ATP was doubled to obtain optimum staining intensity. Immunohistochemical staining was by the indirect immunoperoxidase method after incubation in the following MHC antibodies type I polyclonal (Rowlerson et al. 1981), type IIA polyclonal (Carpene et al. 1982), neonatal polyclonal (Scapolo et al. 1991), fast monoclonal selective for all fast myosins including neonatal but not alpha-cardiac (Sigma U.K. clone MY-32), alpha-cardiac monoclonal (Sera lab U.K. clone F88112FF8) and type IIM (type II masticatory; Rowlerson et al. 1981).

Myosin preparation for electrophoresis

When sectioning was complete the remaining portion of biopsy was homogenised in relaxing buffer containing 0.1 M KCl, 5 mM EDTA and 1 mM dithiothreitol at pH 7.0. Samples were then centrifuged at 13000 rpm to precipitate myofibrillar material. Soluble proteins in the supernatant were discarded. This precipitation was repeated twice, with a small quantity of polyethylene(20) sorbitan monolaurate (Tween 20) (> 0.025%) used in the

second rinse. Following a third wash and spin the myosin-containing precipitates were resuspended and placed in electrophoresis sample buffer consisting of 0.15 M Tris-HCl, 4% SDS and 10% β -mercaptoethanol at pH 6.8 (Laemmli 1970). The protein samples were heated at 100°C for 5 min, then frozen at -24°C until used.

Discontinuous SDS-PAGE

SDS-PAGE for separation of MHC isoenzymes was conducted on 0.75 mm thick separating gels containing 30% glycerol (w/v) and 5%, acrylamide cross-linked with bisacrylamide. A pH of 8.8 was maintained by addition of Tris buffer. Before polymerization with ammonium persulphate and TEMED the gel solution was degassed. The stacking gel contained 3% acrylamide with Tris buffer at pH (6.8). The running buffer consisted of 0.3% Tris, 1.44% glycine and 0.1% SDS. It carried a constant current of 5 mA with a variable voltage and was cooled to about 8°C throughout the experiment. Proteins isolated in the gels were subsequently stained with silver using a modified version of the method of Morrissey (1981).

Morphometrics and classification scheme

For each biopsy a complete series of histochemical and immunohistochemical stains was evaluated. Only series with adequate reactions from all stains and acceptable morphology of most muscle fibres were used. All four rectus biopsies were included in the study, but from the 58 masseter biopsies only 28 met these criteria and were analysed as follows a specific area was identified on each of the serial sections which was clearly in transverse section, and had both adequate morphology and staining to type most of the fibres. This area was then photographed on all relevant stained sections and the reaction profile for each fibre identified. Eight fibre type categories were identified (see table 1). The area of each identified fibre on one stain of the series was measured using a VIDS-V image analysis system linked to a Nikon labophot microscope. On average two to three representative areas from each biopsy were analysed. Fibres of the same class from each of the 28 biopsies were analysed separately and then grouped together with all of the other biopsies for a description of mean fibre diameter, and percent of each fibre type. Diameter values were derived from the area measurements (diameter of a circle of equivalent area). Standard deviations were used to evaluate the variability present in the mean results and the Student's *t*-test to evaluate differences in the mean diameter of the eight fibre categories.

3. Results

3.1. Rectus abdominis muscle

Muscle fibre types—Only type I, IIA and IIB fibre types could be identified in biopsies of rectus muscle, and these had the same staining characteristics and composition as occurs in normal limb muscle (Dubowitz and Brooke 1973) (Fig. 1 and Table 1).

Fibre morphology and distribution—Average fibre diameters increased from type I ($46.72 \mu\text{m} \pm 10.55$), to type IIA ($63.17 \mu\text{m} \pm 11.67$), to type IIB ($76.47 \mu\text{m} \pm 13.58$). The percentage of IIA fibres was largest, and IIB the least (Table 2). The distribution of fibre types in rectus muscle was typical of the mosaic pattern found in limb and abdominal muscle of mixed fibre type (Brooke and Kaiser 1970; Dubowitz and Brooke 1973).

3.2. Masseter muscle

Muscle fibre types—Because myofibrillar ATPase staining was highly variable in a large portion of masseter fibres a classification scheme was developed using antibody and ATPase staining together. Eight fibre types could be identified (Table 1). The type I, IIA and IIB

fibres were found in addition to two other groups of fibres. The first group contained a mixture of IIA and I MHC which was divided into two fibre types – type IM and IIC – since this classification already exists in the literature (Ringqvist et al. 1982; Eriksson and Thornell 1983). Relative to type IIC the type IM fibres stained less intensely for the IIA and fast antibody and were less reactive for ATPase after alkali preincubation. Both had similar positive reactivity for the type I antibody, but after acid preincubation type IM fibres were more reactive for ATPase. IM fibres were consistently more reactive than type I fibres and less reactive than type IIA and IIB fibres after alkali preincubation (Table 1). The second group contained the neonatal, cardiac and ‘other’ fibre types identified almost solely on their antibody reactions. Type ‘neonatal’ contained fibres that stained positively for the neonatal antibody, type ‘cardiac’ contained fibres that stained positively for the alpha-cardiac antibody and type ‘other’ contained fibres that stained positively for both neonatal and alpha-cardiac antibody. It was impossible to describe these final three fibre type categories further using immunohistochemical staining since type I and/or type IIA MHCs were often present, but in highly variable proportions (Fig. 2 and Table 1). Type IIM fibres, type II masticatory fibres that stain positively for the IIM antibody, were never found.

3.3. Fibre morphology and distribution

Average fibre diameters ranged from smallest to largest as follows: type IIA, neonatal, ‘other’. IIC, atrial, IIB, IM and type I (see Table 3). The percent composition of masseter muscle fibres was highly variable in comparison to the rectus muscle. For example, the percent of type I fibres varied by up to 50.4% in the masseter biopsies but only by 27.3% in the rectus biopsies (Table 4). This percent variability in masseter fibres was greatest for the type IIB, neonatal, atrial and ‘other’ categories. Interestingly, only two biopsies in the original sample population of 58 were found to contain type IIB myosin. One of these biopsies was included in the group of 28 for full analysis, the other is presented in the SDS-PAGE results (Fig. 3). The distribution of fibre types was also highly unusual in the masseter. In many biopsies groups of 4 to 6 or more small diameter type IIA fibres could be found clustered together in groups rather than being interspersed between other fibre types (Fig. 2); and since our sample population was mostly healthy young adults we regard this distribution (which has also been observed by others, e.g. Ringqvist 1971; Eriksson and Thornell 1983) as normal for masseter muscle. There were no obvious differences in fibre morphology or distribution between patients undergoing surgical correction for malaligned jaws or dental extractions.

3.4. SDS-PAGE

SDS-PAGE isolated MHC into its specific isoforms. In rectus biopsies two large bands were present – a faster migrating band of type I MHC and a slower migrating band of type IIA MHC. A third minor band was often present containing the type IIB MHC (Fig. 4). The identification of these bands is based on the relative percent area of each fibre type in a given biopsy and the relative mobility of MHC isoforms given in other reports on human skeletal muscle (Biral et al. 1988). In the masseter samples type I and IIA bands were often present with the type I band usually of greatest intensity. Only one of the 28 biopsies contained the type IIB MHC and this correlated to the morphological presence of type IIB fibres in that biopsy. Foetal and/or neonatal MHC could be demonstrated biochemically only in a limited number of samples. When present these bands migrated intermediately between the type IIA and type I MHC bands (Fig. 4). Generally, the percent area of type neonatal, atrial or ‘other’ fibres had to be at least 12% for their MHC isoenzyme protein bands to be detected by SDS-PAGE. Further, the relative percent area of these fibre types did not always correspond well to the amount of protein present on gels. Biopsy 26, for example, had 24.4% type ‘other’ fibres, but only a thin band could be identified between the type IIA and type I MHC bands.

4. Discussion

4.1. MHC expression in masseter and rectus muscle

The anterior superficial area of masseter muscle contains fibres with marked heterogeneity of MHC and probably represents the area within the muscle with greatest number of different fibre types (Serratrice et al. 1976). We have classified the fibres here into 8 separate groups that may be identified by histochemical and immunohistochemical staining. We have obtained further support for our classification by isolating MHCs from masseter and comparing them to abdominal skeletal muscle. The rectus served as a good control muscle since it contained only the three MHCs characteristic of healthy adult limb muscle type I, IIA and IIB. SDS-PAGE of rectus biopsies allowed standardisation of MHC isoform bands in comparison to the relative migration speed of these isoenzymes in previous studies on human skeletal muscle (Biral et al, 1988). The intensity of rectus bands compared well to the percent of type I, IIA and IIB fibres identified from biopsy section staining. It was from comparison to these bands that we were able to identify the type and amount of MHC expressed in the masseter biopsies; and to subsequently confirm the histochemical and immunohistochemical results.

4.2. Staining characteristics Of masseter fibre types

Type I, IIA, IIB and IIC fibres in the masseter stained for myosin in a fashion consistent with Brooke and Kaiser's original description (Brooke and Kaiser 1970). Masseter IM fibres stained similar to IB fibres described in limb muscle (Karpati et al. 1975), and like IIC fibres represent cells containing varying degrees of type I and IIA MHC (Billeter 1980) (see Table 1).

An important difference in our study compared to others was the almost complete lack of type IIB fibres whereas in masseter IIA fibres were relatively common. Ringqvist et al. (1982) and Eriksson and Thornell (1983) found few IIA fibres and many more IIB fibres in anterior superficial masseter samples analysed by ATPase histochemistry only. We could confirm the presence of IIA myosin in our biopsies by both immunohistochemistry and SDS-PAGE, and our lack of IIB fibres was also confirmed by SDS-PAGE and by standardizing histochemical staining with the previously well-described type I, IIA and IIB fibres of abdominal muscle. It is possible that in the previous histochemical studies, classification of type IIA and IIB fibres (a difference in reactivity that must be detected over a change in acid buffer preincubation of pH 0.3) was difficult due the large number of ATPase intermediate staining fibres as well as the lack of limb control muscle for comparison.

The remaining three fibre types, neonatal, cardiac and 'other', could only be consistently characterised by antibody staining. These three fibre categories stained histochemically such that most of the fibres would have been classified as type IM or IIC fibres if antibody staining was not used. In addition, a few of the neonatal-positive fibres stained histochemically as IIA fibres, and often the larger diameter cardiac-positive fibres had reactivity after both acid and alkali buffer incubation (Fig. 3). These cardiac-positive fibres stained similar to the 'anomalous type IIC fibres' of rabbit masseter first reported by Rowlerson et al. (1988), that subsequently have been shown to contain alpha-cardiac MHC (Bredman et al. 1991).

4.3. Morphology of masseter fibre types

Although masseter type I, IIA and IIB fibres stained similarly to their counterparts in rectus, the morphology of the type II fibres was different. Type I fibres of masseter were of similar diameter to type I fibres of rectus: but the IIA and IIB fibre diameters were substantially

smaller on average. The neonatal, and 'other' fibre types were about the same size as masseter IIC fibres and the atrial fibres were intermediate in size between masseter IIC and IM fibres (Fig. 5).

Standard deviation as a percentage of the mean showed masseter fibre diameters to be more highly variable than in rectus. Masseter fibres could be thought of as falling into two groups: those with less variability in diameter ranging from about 25 to 27% of their means (types I, IM and cardiac) and those with a greater variability ranging from about 35 to 71% of their means (types IIC, IIA, neonatal and other), IIB fibres were not grouped since they were rarely found. Diameters for our type I, IM and IIC fibres were similar to those reported by Eriksson and Thornell (1983) for this portion of masseter, but our results for type IIA and IIB fibres were not comparable, probably due to differences in fibre type classification. Thus our IIA fibres (mean 23.24 μm) were about the same diameter as their IIB fibres (mean 20.3 μm). They reported almost the complete absence of IIA fibres from the anterior, superficial area of masseter.

4.4. Distribution and variability

Masseter fibres were often distributed such that small type IIA fibres could be found in clusters, a pattern which is common in the jaw closer muscles but resembles the type-grouping shown in limb muscles after reinnervation (Dubowitz and Brooke 1973). However, results of an EMG study by Stalberg et al. (1986) found no evidence for re-innervation as the cause of the pattern seen in masseter, and its frequency in our young healthy adult subjects also fails to support this possibility. There was a large variability in fibre percentages in the area of masseter we sampled. Type I fibres varied from about 78 to 22% (a range of 56%) for example, and the percent variability for all types was high. In contrast, the range in percentage of type I fibres in rectus was only about 27%. Given this variability it is difficult to characterise even this portion of the masseter as "fast" or "slow", but if IM fibres are assumed to have similar contractile properties to type I fibres, since IM fibres appear to have more type I than IIA MHC, then in most patients masseter could be regarded as containing a majority of slow-contracting fibres

The neonatal, atrial and 'other' fibre types were often completely lacking from biopsies, and when present never represented the majority of fibres. In biopsies that contained a large proportion of these fibres, usually only a small portion of neonatal or alpha-cardiac MHC could be detected by SDS-PAGE. Thus it is likely that neonatal and atrial isoforms are often a *minor* component in the fibres in which they occur, but further work on single fibres is needed to confirm this.

4.5. Expression of developmental and atrial myosins

Although recent work has found neonatal MHC, embryonic MHC, alpha-cardiac MHC and embryonic MLC (contractile proteins not present in healthy adult skeletal muscle) in the human masseter, the frequency and distribution of these proteins is still uncertain.

Butler-Browne et al. (1988) found neonatal MHC antibody staining in "3/5" of histochemically defined type I fibres, "3/5" of type IM fibres and "1/3" of type IIA and IIB fibres in a mixed group of only nine autopsy/biopsy samples. We report the presence of type I, IM, IIC, IIA and (rare) IIB fibres without neonatal or cardiac MHC in addition to fibres that do express neonatal and cardiac myosins together with other MHCs. In comparison, the total number of fibres containing neonatal MHC in our study was substantially smaller – with a maximum of 24.4% and a very common minimum value of 0%.

Previous reports on the number of fibres expressing alpha-cardiac MHC are conflicting. Bredman et. al. (1991) found between 20 and 45% alpha-cardiac positive fibres in masseter while Pedrosa-Domellof et al. (1992) found none or very few. Both of these values fall within the range we saw in our larger sample of patients (Table 4).

We did not use anti-embryonic antibodies in this study, but Soussi-Yanicostas et al. 1991 found embryonic MHC positive fibres in one adult biopsy. The presence of embryonic MLC, a MLC usually present only in developing skeletal muscle, has been identified in ATPase-defined type II fibres in adult masseter (Soussi-Yanicostas 1991), but even if present these isoforms would not be expected to influence our ATPase or antibody staining in any way.

4.6. Aetiology of unusual fibre types

With our classification scheme it is possible to classify fibres into those expressing atypical MHC isoforms (neonatal and alpha-cardiac) and those with MHC isoforms typical of adult skeletal muscle (I, IIA and IIB). The presence of neonatal and alpha-cardiac isoforms in masseter is difficult to explain, Neonatal MHC is found in some fibres in response to denervation and in all fibres during regeneration (Sartore et al. 1982 Schiaffino et al. 1986), but the morphology of masseter fibres is inconsistent with both conditions. For example, the small diameter fibres in masseter that express neonatal MHC are not typical of regenerating fibres since they have no central nuclei or basophilic. Likewise neonatal MHC may be found in fibres of all diameters which suggests this expression is not solely from denervation.

Alpha cardiac MHC is not expressed in limb skeletal muscle, but has been found in the masseter of rabbits. The effect of this isoform on contractile properties, however, remains unknown. The fibre types in mammalian jaw closing muscles are variable across species (Rowlerson 1990), which suggests highly modified functions in different animals. Experiments describing the expression and function of unusual myosins in masticatory muscles in other species could prove enlightening, and we are now beginning a study on contractile properties of the human masseter to advance our understanding of mastication.

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Fig. 1. Serial sections of rectus abdominis stained for myofibrillar ATPase activity after preincubation in buffer of pH 10.2 (A) and 4.6 (C. F) and for reactivity to anti-fast (B), anti-neonatal (D) and anti-alpha-cardiac (E) MHC antibodies. Arrow marks a type IIB fibre, star marks type IIA fibre and type I fibres are unmarked. Magnifications A–E. $\times 115$; F. $\times 57$.

Fig. 2. Serial sections of masseter stained for reactivity to anti-fast (A), anti-IIA (B), anti-I (C) and anti-neonatal (D) MHC antibodies; and for myofibrillar ATPase activity after preincubation in buffer of pH 10.4 (E), 10.2 (F), 4.6 (G) and 4.3 (H). Small arrow marks a type IIA fibre, large arrow a type IM fibre and arrow head a type IIC fibre. Magnification, $\times 95$.

Fig. 3. Serial sections of masseter stained for reactivity to anti-alpha-cardiac MHC antibody (A), and for myofibrillar ATPase activity after preincubation in buffer of pH 10.2 (B) and 4.3 (C). Arrows mark type cardiac fibres that contain alpha-cardiac MHC and are acid and alkali stable for ATPase. Magnification, $\times 115$.

Fig. 4. MHC separation with SDS-PAGE for individual biopsies by number, r = rectus abdominis, f = fetal muscle sample, x = a biopsy that contained IIB MHC. Lanes with two labels are comigrations of MHC from two biopsies. Speed of relative MHC migration from slow to fast was type IIB, IIA, alpha-cardiac/neonatal, I.

Fig. 5.
Mean fibre diameters.

Table 1

Fibre type staining

Antibody Reactivity of Fibre Types									
anti-	I	IM	IIC	IIA	IIB	Neo.	Atrial	Other	
I	+	+	+	-	-	(+)	(+)	(+)	
fast	-	(+)	(+)	+	+	+	+	+	
IIA	-	(+)	(+)	+	(+)	(+)	(+)	(+)	
Neo.	-	-	-	-	-	+	-	+	
cardiac	-	-	-	-	-	-	+	+	
ATPase Reactivity for Fibre Types									
pH*	I	IM	IIC	IIA	IIB	Neo.	Atrial	Other	
10.2	-	(+)	+	+	+	+	(+)/+	(+)/+	
4.6	+	+	(+)	-	(+)	(+)	(+)/+	(+)/+	
4.3	+	+	(+)	-	-	(+)	(+)/+	(+)/+	

* Myofibrillar ATPase reactivity after preincubation in buffer at specified pH.

Table 2

Rectus abdominis muscle

Mean diameter for each fibre type				Percent fibre type				
Bx.	I	IIA	IIB	n	I	IIA	IIB	
1.	54.4	78.7	86.4	1.	276	65.6	23.6	10.8
2.	40.1	57.7	64.5	2.	179	46.4	48.0	5.6
3.	49.9	63.2	64.4	3.	282	38.3	59.6	2.5
4.	39.9	60.4	73.6	4.	401	43.9	47.9	8.2
<i>x</i>	46.72	63.17	76.47					
S.D.	10.55	11.67	13.58					
% <i>x</i>	22.58	18.47	17.76					

Average fibre diameter and percentage of each fibre type as found in the four rectus abdominis biopsies (Bx.). *n* = number of fibres analysed.

Table 3

Masseter muscle. Mean Diameter in μm for each fibre type

Bx.	I	IM	IIC	IIA	IIB	Neo.	Atrial	Other
1.	47.28	0	40.55	14.06	0	0	0	0
2.	41.73	44.08	31.57	12.40	0	0	0	0
3.	46.78	0	26.28	0	0	27.57	34.54	0
4.	34.72	27.46	20.17	11.10	0	0	0	0
5.	46.02	0	37.26	3.57	0	0	0	0
6.	61.92	43.43	0	17.58	0	20.64	34.10	0
7.	45.82	41.30	35.87	22.60	0	0	0	0
8.	51.05	32.98	26.96	19.99	0	23.78	0	0
9.	61.70	49.96	37.42	24.88	0	0	0	0
10.	49.72	47.84	29.80	12.55	0	0	0	0
11.	36.94	23.63	25.68	14.05	0	21.92	24.52	24.95
12.	44.36	31.69	18.13	21.09	0	13.60	0	0
13.	55.40	0	20.77	26.66	0	43.71	25.96	17.16
14.	47.84	28.82	32.37	17.35	0	0	0	0
15.	47.25	34.23	32.88	21.32	0	25.09	33.74	34.10
16.	44.11	47.34	42.32	22.33	0	19.27	0	0
17.	47.84	35.86	0	23.59	0	15.42	0	0
18.	64.23	0	36.96	29.14	0	21.19	50.75	45.56
19.	45.82	41.30	35.87	22.60	0	0	0	0
20.	50.4	46.07	36.59	0	0	36.02	39.17	31.90
21.	41.63	41.06	28.74	11.62	0	28.84	29.14	51.29
22.	59.12	0	0	51.67	0	33.11	0	39.76
23.	41.54	0	42.13	6.31	0	26.27	40.80	0
24.	59.99	49.61	0	33.92	0	0	0	0
25.	46.12	0	26.55	23.55	0	0	0	0
26.	26.96	18.48	0	6.51	0	24.66	40.21	0
27.	43.30	49.79	35.70	23.57	0	28.43	0	0
28.	37.16	0	40.92	29.59	39.67	40.63	0	24.63
x	47.30	42.08	30.47	23.24	39.67	29.54	37.18	30.19

Bx.	I	IM	IIC	IIA	IIB	Neo.	Atrial	Other
S.D.	13.07	10.72	11.17	16.51		12.50	10.04	10.63
% x	27.63	25.48	36.66	71.04		42.32	27.00	35.21

Bx. = masseter muscle biopsy. Mean (x) and SD values for all biopsies together are also shown.

Table 4

Masseter muscle. Percent fibre type

Bx.	n	I	IM	IIC	IIA	IIB	Neo.	Atrial	Other
1.	90	72	0	6.7	13.3	0	0	0	0
2.	271	59.4	1.5	4.1	35.0	0	0	0	0
3.	139	77.7	0	6.5	0	0	3.6	9.4	2.8
4.	270	65.2	4.4	11.9	18.5	0	0	0	0
5.	201	54.2	0	12.7	33.1	0	0	0	0
6.	86	58.1	10.5	0	3.5	0	1.2	26.7	0
7.	153	57.5	13.0	19.0	10.5	0	0	0	0
8.	214	52.3	18.7	19.2	5.6	0	4.2	0	0
9.	100	63	17	19	1	0	0	0	0
10.	252	44.8	6.0	9.1	40.1	0	0	0	0
11.	334	55.2	2.8	12.9	13.7	0	0.5	12.7	2.2
12.	373	48.8	16.9	29.8	0.3	0	4.2	0	0
13.	200	54.0	0	1.0	9.5	0	19.0	14.0	2.5
14.	174	38.5	21.3	3.4	36.3	0	0	0	0
15.	493	41	1.0	0.5	45.8	0	8.7	2.2	1.0
16.	178	50.6	1.7	10.6	34.3	0	2.8	0	0
17.	329	43.2	16.1	0	38.6	0	2.1	0	0
18.	145	43.5	0	15.2	11.0	0	11.0	17.9	14
19.	322	51.2	25.8	19.9	3.1	0	0	0	0
20.	301	48.9	23.8	17.6	0	0	7.0	2.0	0.7
21.	542	47.0	0	0	41.3	0	11.1	0	0.5
22.	424	25.2	2.6	22.4	28.5	0	2.9	6.6	11.8
23.	320	39.7	0	18.1	18.1	0	23.1	0.9	0
24.	153	28.9	24.8	0	46.3	0	0	0	0
25.	400	65.5	0	3.5	31	0	0	0	0
26.	201	36.8	0.9	0	25.4	0	0	12.4	24.4
27.	119	27.7	26.9	26.9	15.1	0	3.4	0	0
28.	361	21.6	0	0.7	36.8	38.5	0.7	0	1.7

Percent composition for each fibre type in masseter muscle biopsies (Bx.).

n = number of fibres analysed.

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