# Myosin heavy chain composition of the human jaw muscles

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# MYOSIN ISOFORM COMPOSITION OF THE HUMAN MEDIAL AND LATERAL PTERYGOID MUSCLES

**Abstract** The medial and lateral pterygoid muscles are different in structure as well as in function. The medial pterygoid muscle is concentrically active during jaw closing, the superior head of the lateral pterygoid muscle is eccentrically active during jaw closing, while its inferior head is concentrically active during jaw opening. Architecturally, the medial pterygoid muscle can deliver higher forces than the lateral pterygoid muscle. We investigated whether these differences are reflected in the myosin heavy chain (MyHC) composition and the fibre cross-sectional area of these muscles. The pterygoid muscles from eight cadavers were investigated by means of monoclonal antibodies against different isoforms of MyHC. The proportions of pure MyHC type I fibres did not differ significantly among the muscles (32% in medial pterygoid, 34% in superior head, and 36% in inferior head of the lateral pterygoid), nor did the total proportions of pure MyHC type IIA and IIX fibres (16% in medial pterygoid, 26% in superior head, and 19% in inferior head of the lateral pterygoid). The mean fibre cross-sectional area of type I fibres was 1315 $\mu m^2$, which did not differ significantly among the muscles, and was significantly larger than the fibre cross-sectional area of type IIA fibres. The relative proportions of hybrid fibres, which expressed more than one MyHC isoform, were 52% in the medial pterygoid, 40% in the superior head, and 45% in the inferior head of the lateral pterygoid and did not differ significantly among the muscles. The most abundant hybrid fibre types found were fibres expressing MyHC-cardiac $\alpha$+IIA and MyHC-cardiac $\alpha$+I+IIA. Significant regional differences were found in the proportions of MyHC type I in the medial pterygoid muscle and in the inferior head of the lateral pterygoid. Although the

architecture and function of the muscles are different, we conclude that this is not reflected in their myosin isoform composition.

## Introduction

Depending on their physiological behaviour, muscle fibres are traditionally classified into three groups: type I fibres are slow contracting, fatigue resistant, and generate small forces; type IIA fibres are fast contracting, fatigue resistant, and generate larger forces; and type IIB fibres are fast contracting, fatigable, and generate the largest forces. The heavy chain of the myosin molecule (MyHC) largely determines the speed of contraction of the muscle fibre. The few existing studies on the fibre type composition of the human pterygoid muscles (Vignon *et al.*, 1980; Eriksson *et al.*, 1981; Eriksson and Thornell, 1983; Shaughnessy *et al.*, 1989) used ATPase histochemistry to classify muscle fibres into types I, IIA, IIB and into fibre types with an intermediate reactivity. This technique, however, does not give a complete image of the MyHC contents of fibres in the jaw muscles (Butler-Browne *et al.*, 1988; Zhang *et al.*, 1998).

Based on immunohistochemical techniques, which reveal different isoforms of MyHC, at least three MyHC isoforms can be distinguished in human limb and trunk muscles, namely type I, IIA, and IIX (Schiaffino *et al.*, 1989, 1994). Human jaw-closing muscle fibres, on the other hand, can also express two other MyHC isoforms, namely, MyHC-fetal, even at adult age (Butler-Browne *et al.*, 1988; Korfage and Van Eijden, 1999; Korfage *et al.*, 2000, 2001), and MyHC-cardiac $\alpha$ (Bredman *et al.*, 1991; Korfage and Van Eijden, 1999; Korfage *et al.*, 2000, 2001), a MyHC isoform which is normally expressed in the myofibrils of the atrium only. The MyHC contents of motor units and their physiological properties are correlated (Schiaffino *et al.*, 1988a; Kwa *et al.*, 1995b). There is a direct correlation between the ATPase histochemically defined fibre types and MyHC isoforms (Staron and Pette, 1986). In human muscle fibres, ATPase-classified type I fibres contain MyHC-I, and type IIA fibres contain MyHC-IIA, but ATPase-classified type IIB fibres contain a MyHC which is similar to MyHC-IIX found in rodents (Schiaffino *et al.*, 1989; Smerdu *et al.*, 1994; Schiaffino and Reggiani, 1996; Andersen *et al.*, 1999a). In the adult skeletal muscle, MyHC-fetal and MyHC-cardiac $\alpha$ cannot be distinguished by ATPase enzyme

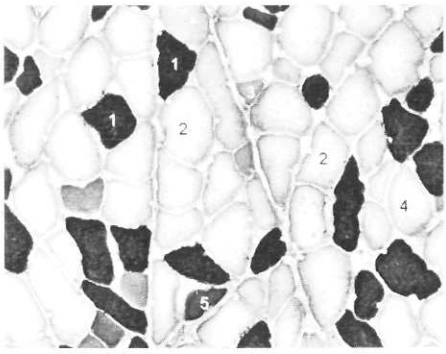
histochemistry. These MyHCs, however, could contribute to the different physiological properties of these fibres.

The human medial and lateral pterygoid muscles are anatomically and functionally different (Van Eijden *et al.*, 1995, 1997). The lateral pterygoid muscle can be divided into a superior and inferior head (Honée, 1972; Grant, 1973). The medial pterygoid muscle can be divided into a posterior and an anterior part. The medial pterygoid muscle is a multi-pennate muscle and consists of short muscle fibres. This muscle can produce large forces over a short distance only and is activated during the closing phase of the mandible. On the other hand, both heads of the lateral pterygoid muscle consist of long, parallel-arranged muscle fibres which can contract over a large distance but without much force. Electromyographic studies (McNamara, 1973; Juniper, 1981; Mahan *et al.*, 1983) suggest that the two heads of the lateral pterygoid muscle have different functions. The inferior head is supposed to be concentrically active during opening of the jaw. The superior head, which has a close relation to the articular disc of the temporomandibular joint, is supposed to be eccentrically active during closing of the jaw. The force this part delivers is believed to control the backward gliding of the articular disc.

In a study on the regional differences in the human temporalis (Korfage and Van Eijden, 1999) it was shown that the functionally different regions in this muscle consist of different proportions of MyHC-classified fibre types. Also, it was found that muscle fibres can express different MyHCs simultaneously. In the present study, we tested the hypothesis that the architectural and functional differences between the pterygoid muscles are reflected by their myosin isoform composition. Furthermore, since the muscles have a complex architectural design, we investigated whether there are regional differences in the distribution of fibre types within these muscles.

## Materials and Methods

In this study, we used the right unfixed pterygoid muscles (seven medial pterygoids and eight lateral pterygoids) from the cadavers of eight Caucasians, all of whom died naturally. Six cadavers had upper and lower dental prostheses, two were partially dentate. We used five males and three females (mean age ± S.D. = 71.6 ± 15.0 yrs). The muscles were obtained within 12 to 36 hours *post mortem*. The use of human

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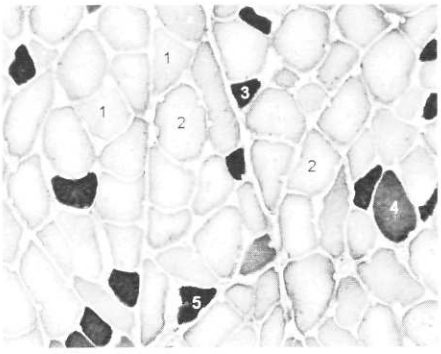


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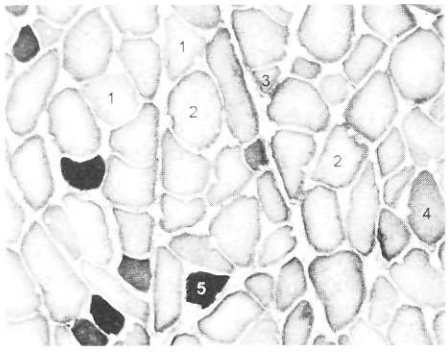


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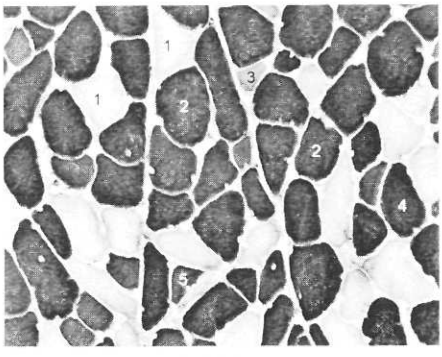


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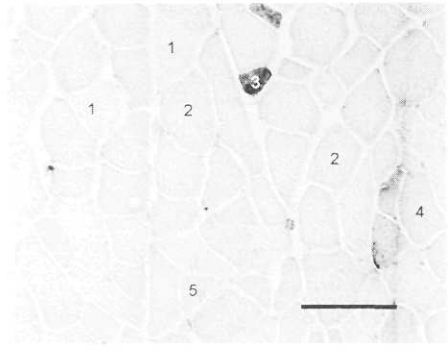


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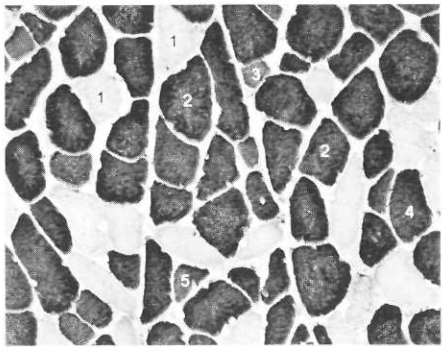


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**Figure 5.1**
Light micrographs of six consecutive sections of the medial pterygoid incubated with monoclonal antibodies against myosin heavy chains. Note the different hybrid fibres. 1 = MyHC-I, 2 = MyHC-IIX, 3 = MyHC-fetal+cardiac $\alpha$+IIX, 4 = MyHC-cardiac $\alpha$+IIX, and 5 = MyHC-cardiac $\alpha$+I+IIA.
Bar = 100 µm.

muscles conformed to a written protocol that was reviewed and approved by the Department of Anatomy and Embryology of the Academic Medical Center of the University of Amsterdam. After the muscles were exposed by removal of the coronoid

process and the adjoining part of the ramus of the mandible, they were cut from their attachment sites. The muscles were rapidly frozen in liquid nitrogen-cooled isopentane and stored at -80°C until required for further processing.

***Immunohistochemistry***

Serial transverse sections of 10 µm of the whole muscle were cut in a cryomicrotome (Model HM 500 M, Adamas Instruments BV, Leersum, the Netherlands). The sections of the muscles were taken approximately halfway through the muscle. They were cut perpendicularly to the main direction of the muscle fibres. The sections were mounted on microscope slides coated with AAS (3-aminopropyltriethoxysilane; Henderson, 1989). Consecutive sections were fixed overnight in a mixture of methanol:aceton:acetic acid:water (35:35:5:25) at -20°C (Wessels *et al.*, 1988) and incubated with monoclonal antibodies raised against purified myosin (Bredman *et al.*, 1991; Sant'Ana Pereira *et al.*, 1995a) (Table 2.1). The specificity and characterisation of these monoclonal antibodies against human MyHC isoforms have been described and demonstrated elsewhere (Wessels *et al.*, 1991; Sant'Ana Pereira *et al.*, 1995b, 1997). These studies showed that human ATPase type I and type IIA muscle fibres reacted with antibodies against, respectively, MyHC-I and MyHC-IIA, and that human ATPase-defined IIB muscle fibres contained an MyHC which was a homologue to the MyHC-IIX isoform of rodents. In the present study, we classified the fibres according to the MyHCs they express. Anti-fetal MyHC was purchased (Novocastra Laboratories Ltd, UK). We applied the indirect unconjugated immunoperoxidase technique (PAP-technique) to detect the specific binding of the different antibodies and used nickel-diaminobenzidine to visualise the staining (Hancock, 1982).

***Sample Method and Fibre Cross-sectional Area Measurements***

Six sample areas were analysed from the medial pterygoid muscle, three from the lateral side and three from the medial side, equidistant in anteroposterior direction. From the superior head of the lateral pterygoid muscle, three sample areas were taken at equal distance in lateromedial direction. From the inferior head of this muscle, six sample areas were taken, three from the lateral side and three from the medial side, equidistant in caudocranial direction.

In each sample area (about 0.6 - 0.4 mm) 75 - 300 fibres (average 175) were drawn, by means of a projection microscope (Carl Zeiss, Oberkochen, Germany) and a mirror table, onto a transparent sheet. Each fibre was classified by means of a series of six consecutive incubated sections. Fibres that were not recognised in each of the six sections (<1%) were omitted.

We measured the cross-sectional area of the fibres by reading the drawn sheets, together with a grade mark for correction of enlargement, via a flat-bed scanner (Hewlett-Packard, Scanjet 4c) into a personal computer. We then used a custom-made program, that converts the number of pixels into $\mu m^2$, to determine the cross-sectional area of each muscle fibre in $\mu m^2$. In total, more than 6700 fibres were analysed in the medial pterygoid muscle, and in the superior and inferior head of the lateral pterygoid muscle, more than 3300 and 9600 fibres, respectively, were analysed.

The total area of a muscle cross-section that was occupied by the fibres of a specific type was estimated according to the formula: (sum of cross-sectional areas of a specific fibre type) / (sum of cross-sectional areas of all fibres) x 100%.

| | Medial Pterygoid | | Lateral Pterygoid Superior Head | | Lateral Pterygoid Inferior Head | |
|---|---|---|---|---|---|---|
| | % | | % | | % | |
| MyHC fibre type | Mean | S.D. | Mean | S.D. | Mean | S.D. |
| I | 32.2 | 5.9 | 34.2 | 11.9 | 36.4 | 8.5 |
| IIA | 5.4 | 6.3 | 25.7 | 16.8 | 15.1 | 12.6 |
| IIX | 10.6 | 12.0 | 3.2 | 5.9 | 3.3 | 6.7 |
| Hybrid | 51.8 | 15.4 | 36.9 | 13.2 | 45.1 | 11.7 |

**Table 5.1**
Grand means and standard deviation of MyHC fibre type distribution (%).

### *Statistical Analysis*

For each muscle, the distribution and mean cross-sectional area of the different fibre types were determined. Mean and standard deviation values (S.D.) were calculated over the seven medial pterygoid muscles, and over the eight lateral pterygoid muscles, superior and inferior head. Variability of fibre cross-sectional area per individual was estimated by the coefficient of variation (cov = S.D./mean x 100%).

| | Medial Pterygoid | | | Lateral Pterygoid Superior Head | | | Lateral Pterygoid Inferior Head | | |
|---|---|---|---|---|---|---|---|---|---|
| Hybrid fibre type | Mean (%) | S.D. | n | Mean (%) | S.D. | n | Mean (%) | S.D. | n |
| I+IIA | 3.6 | 3.5 | 7 | 8.0 | 4.1 | 8 | 8.4 | 5.1 | 8 |
| I+IIX | 1.3 | 1.7 | 7 | 0.2 | 0.3 | 4 | 0.5 | 0.6 | 8 |
| I+Ia | 3.3 | 1.9 | 7 | 3.3 | 2.7 | 8 | 4.4 | 5.6 | 8 |
| fetal+I | 1.2 | 1.3 | 5 | 0.3 | 0.4 | 4 | 0.6 | 0.9 | 5 |
| fetal+IIA | 0.1 | 0.1 | 5 | 0.4 | 0.4 | 6 | 0.1 | 0.1 | 5 |
| fetal+IIX | 0.6 | 0.6 | 6 | 0.1 | 0.3 | 2 | 0.0 | 0.0 | 1 |
| fetal+I+IIA | 0.2 | 0.2 | 4 | 0.2 | 0.4 | 3 | 0.2 | 0.2 | 7 |
| fetal+I+IIX | 0.2 | 0.2 | 3 | 0.0 | 0.1 | 1 | 0.0 | 0.0 | 2 |
| fetal+I+Ia | 1.0 | 1.1 | 4 | 0.4 | 0.8 | 3 | 0.7 | 1.3 | 5 |
| cardiac $\alpha$+I | 4.6 | 7.2 | 7 | 1.8 | 2.9 | 8 | 1.8 | 3.2 | 8 |
| cardiac $\alpha$+IIA | 5.5 | 4.1 | 7 | 8.3 | 5.6 | 8 | 11.7 | 7.1 | 8 |
| cardiac $\alpha$+IIX | 2.2 | 3.3 | 7 | 0.2 | 0.3 | 3 | 0.2 | 0.2 | 5 |
| cardiac $\alpha$+I+IIA | 14.3 | 11.3 | 7 | 12.0 | 7.5 | 8 | 13.6 | 7.4 | 8 |
| cardiac $\alpha$+I+IIX | 3.6 | 3.4 | 7 | 0.3 | 0.3 | 4 | 0.3 | 0.7 | 6 |
| cardiac $\alpha$+I+Ia | 2.1 | 1.3 | 7 | 0.3 | 1.0 | 4 | 1.0 | 2.7 | 5 |
| fetal+cardiac $\alpha$+I | 2.5 | 3.9 | 7 | 0.1 | 0.2 | 3 | 0.1 | 0.3 | 4 |
| fetal+cardiac $\alpha$+IIA | 0.3 | 0.2 | 4 | 0.0 | 0.1 | 1 | 0.2 | 0.2 | 6 |
| fetal+cardiac $\alpha$+IIX | 0.6 | 0.7 | 6 | | | | | | |
| fetal+cardiac $\alpha$+I+IIA | 0.9 | 0.7 | 6 | 0.6 | 0.9 | 6 | 0.7 | 1.2 | 6 |
| fetal+cardiac $\alpha$+I+IIX | 1.0 | 1.0 | 5 | | | | | | |
| fetal+cardiac $\alpha$+I+Ia | 2.5 | 2.6 | 5 | 0.1 | 0.2 | 2 | 0.3 | 0.7 | 4 |

**Table 5.2**
Distribution of hybrid MyHC fibre types (mean ± S.D.) in the pterygoid muscles. n = number of muscles where the specific fibre was found

and mean and standard deviation of variability per muscle were calculated. Differences in fibre type distribution and in fibre cross-sectional area between muscles and muscle portions were analysed by the Wilcoxon ranking test for paired data. The level of significance was set at $P<0.05$.

## Results

### *Fibre Type Distribution*

Figure 5.1 shows an example of six consecutive areas in the medial pterygoid muscle incubated with the antibodies against MyHC. Table 5.1 lists the grand means and standard deviation values for the various muscle fibre types: the standard deviation values are a measure for interindividual variability. A distinction was made between

"pure" fibres, which expressed only one MyHC, and "hybrid" fibres, which expressed more than one MyHC. Proportions for hybrid fibres in each muscle are given in Table 5.2.

In both muscles, MyHC type I fibres were the predominant fibre type in every subject; the relative small S.D. values indicate that the frequency did not vary much among the subjects. The proportion of MyHC type IIA fibres differed significantly between the medial pterygoid muscle and superior head of the lateral pterygoid. More MyHC type IIA fibres were found in the superior head of the lateral pterygoid. In the medial pterygoid muscle, the proportion of fibres expressing MyHC-IIX was larger than that of MyHC type IIA fibres, while in both heads of the lateral pterygoid, the proportion MyHC type IIA fibres was significantly larger than that of fibres expressing MyHC-IIX. Both muscles showed that a relative large proportion of fibres were hybrid fibres which contained a great variety in the expression of MyHC (Table 5.2). The frequency of most hybrid fibres, however, was smaller than 5%. The most abundant

Medial Pterygoid

| | f-csa (μm²) | | cov (%) | | Total Area (%) | |
|---|---|---|---|---|---|---|
| Fibre type | Mean | S.D. | Mean | S.D. | Mean | S.D. |
| I | 1387.9 | 306.7 | 51.8 | 13.9 | 45.3 | 10.9 |
| IIA | 677.6 | 386.2 | 78.9 | 16.5 | 4.1 | 5.5 |
| IIX | 726.8 | 600.8 | 65.2 | 47.2 | 10.0 | 16.4 |
| hybrid | 659.8 | 291.0 | 83.4 | 16.7 | 39.6 | 14.1 |

Lateral Pterygoid, Superior Head

| | f-csa (μm²) | | cov (%) | | Total Area (%) | |
|---|---|---|---|---|---|---|
| Fibre type | Mean | S.D. | Mean | S.D. | Mean | S.D. |
| I | 1389.8 | 219.0 | 45.1 | 8.9 | 42.9 | 14.8 |
| IIA | 1082.1 | 356.5 | 46.3 | 7.7 | 20.3 | 12.1 |
| IIX | 1133.3 | 622.1 | 36.6 | 19.8 | 4.3 | 7.4 |
| hybrid | 1003.9 | 289.1 | 56.7 | 2.7 | 32.5 | 12.5 |

Lateral Pterygoid, Inferior Head

| | f-csa (μm²) | | cov (%) | | Total Area (%) | |
|---|---|---|---|---|---|---|
| Fibre type | Mean | S.D. | Mean | S.D. | Mean | S.D. |
| I | 1166.8 | 259.8 | 42.9 | 7.8 | 49.8 | 9.0 |
| IIA | 647.1 | 185.9 | 54.7 | 5.7 | 11.0 | 7.8 |
| IIX | 722.9 | 248.3 | 49.5 | 8.8 | 3.5 | 7.9 |
| hybrid | 760.1 | 137.7 | 60.0 | 12.3 | 35.7 | 9.3 |

**Table 5.3**
Fibre cross-sectional area (f-csa) and total area occupied by a specific MyHC fibre type.
cov = coefficient of variation.

| MyHC fibre type | Medial Pterygoid f-csa (μm²) Mean | S.D. | Lateral Pterygoid superior head f-csa (μm²) Mean | S.D. | Lateral Pterygoid inferior head f-csa (μm²) Mean | S.D. |
|---|---|---|---|---|---|---|
| I+IIA | 938.0 | 506.2 | 936.3 | 307.5 | 691.8 | 155.9 |
| I+IIX | 638.3 | 331.1 | 846.8 | 356.7 | 754.5 | 344.9 |
| I+Ia | 1048.2 | 321.9 | 1096.1 | 365.9 | 996.6 | 235.6 |
| fetal+I | 807.7 | 427.0 | 869.7 | 387.1 | 995.3 | 403.9 |
| fetal+IIA | 281.8 | 231.1 | 1150.2 | 618.9 | 722.2 | 588.9 |
| fetal+IIX | 344.9 | 281.8 | 1109.0 | 526.9 | 1267.0 | |
| fetal+I+IIA | 770.3 | 643.2 | 1147.7 | 406.6 | 815.8 | 276.9 |
| fetal+I+IIX | 946.9 | 75.7 | 567.0 | | 734.0 | 162.0 |
| fetal+I+Ia | 917.3 | 508.8 | 1075.0 | 330.5 | 859.7 | 235.3 |
| cardiac α+I | 956.6 | 301.7 | 1328.7 | 618.7 | 1075.1 | 317.5 |
| cardiac α+IIA | 503.1 | 259.1 | 708.7 | 235.5 | 504.0 | 163.8 |
| cardiac α+IIX | 451.5 | 423.2 | 460.3 | 141.8 | 564.7 | 241.8 |
| cardiac α+I+IIA | 700.9 | 303.9 | 868.9 | 412.5 | 571.8 | 133.6 |
| cardiac α+I+IIX | 620.9 | 207.5 | 908.4 | 580.2 | 448.4 | 178.3 |
| cardiac α+I+Ia | 811.6 | 317.2 | 1295.5 | 382.1 | 805.8 | 208.7 |
| fetal+cardiac α+I | 576.3 | 328.3 | 1362.0 | 421.5 | 721.2 | 275.7 |
| fetal+cardiac α+IIA | 149.5 | 53.9 | 323.0 | | 413.3 | 195.4 |
| fetal+cardiac α+IIX | 282.6 | 160.2 | | | | |
| fetal+cardiac α+I+IIA | 562.6 | 503.5 | 1288.4 | 929.2 | 913.6 | 720.7 |
| fetal+cardiac α+I+IIX | 649.6 | 411.1 | | | | |
| fetal+cardiac α+I+Ia | 687.6 | 442.6 | 1074.0 | 295.0 | 1114.7 | 348.1 |

**Table 5.4**
Fibre type cross-sectional area (f-csa) of the hybrid fibres.

hybrid fibre type in both muscles consisted of fibres expressing MyHC-cardiac α+I+IIA (12-14%), followed by fibres expressing MyHC-cardiac α+IIA (5-12%). When all hybrid fibres in each muscle that contained MyHC-fetal were combined, the medial pterygoid muscle had more hybrid fibres, 11%, containing MyHC-fetal than did the superior head of the lateral pterygoid, 2%, but this difference was not significant. Combining all MyHC-I positive fibres (*i.e.*, pure plus hybrid) revealed little difference between medial pterygoid, 74%, and the superior, 62%, and inferior, 69%, heads of the lateral pterygoid.

Some type I fibres reacted positively with antibody 332-3D4 (Table 2.1), which normally detects only MyHC-IIA and MyHC-IIX. These fibres showed no reaction with antibody 340-3B5, which also detects MyHC-IIA and MyHC-IIX in human muscle fibres. Electrophoretic studies showed that some pure type I fibres in the rabbit contained a novel MyHC isoform next to MyHC-I (Galler *et al.*, 1997a), which was

named MyHC-Ia. In the present study, MyHC-I fibres that also reacted with this antibody were dubbed type I+Ia.

The distribution of fibre types within the medial pterygoid muscle is depicted in Fig. 5.2. We observed a significant difference in the distribution of type I and type II fibres (*i.e.*, pure MyHC type IIA fibres plus pure MyHC type IIX fibres). More type I fibres were found in the anterior and lateral muscle portions than in the posterior and medial muscle portions (anterolateral portion, 47%; posteromedial portion, 18%), and the proportion of MyHC type II fibres was larger in the posterior and medial muscle portions than in the anterior and lateral muscle portions (posteromedial portion, 33%; anterolateral portion, 7%). There was no difference in the distribution of hybrid fibres among the muscle portions. No significant difference was found in the distribution of fibre types among the muscle portions of the superior head of the lateral pterygoid. In contrast, the distribution of fibre types within the inferior head, depicted in Fig. 5.2, showed that significant fewer pure MyHC type I fibres were found in the cranialmost, 32%, than in the caudalmost muscle portions, 40%; a significant difference between medial and lateral portions was not found.

***Fibre Cross-sectional Area***

Tables 5.3 and 5.4 list mean and standard deviation values of fibre cross-sectional area in the medial pterygoid, the superior head, and the inferior head of the lateral pterygoid; the standard deviation values are a measure for interindividual variability, and the coefficients of variation are a measure for intra-individual variability. A significant difference was found between the fibre cross-sectional area of MyHC type I fibres and the fibre cross-sectional area of type IIA, in all muscles studied, and of MyHC type IIX fibres, in the medial pterygoid and the inferior head of the lateral pterygoid. MyHC type I fibres in both muscles had the largest fibre cross-sectional area. MyHC types IIA and IIX and the hybrid fibres were approximately 35% larger in the superior head of the lateral pterygoid than in the medial pterygoid and inferior head of the lateral pterygoid. No significant differences were measured in fibre cross-sectional area between the different areas in both muscles.

Note that although the percentage of hybrid fibre types was the largest (Table 5.1), the total area of a muscle cross-section that was occupied by MyHC type I fibres was larger than the total area occupied by hybrid fibre types.

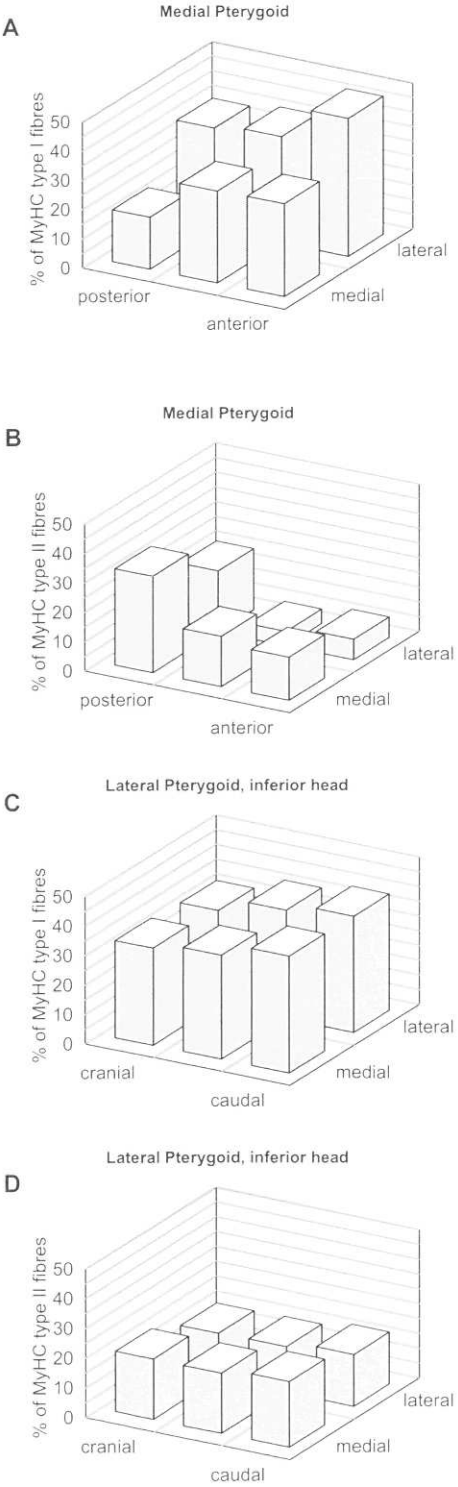


**Figure 5.2**
(A) Distribution of MyHC type I fibres in the medial pterygoid. (B) Distribution of MyHC type II fibres (sum of all pure MyHC type IIA and IIX fibres) in the medial pterygoid. (C) Distribution of MyHC type I fibres in the inferior head of the lateral pterygoid. (D) Distribution of MyHC type II fibres (sum of all pure MyHC type IIA and IIX fibres) in the inferior head of the lateral pterygoid.

## Discussion

The human jaw-closing muscles, like masseter and temporalis, have different fibre-type compositions and fibre cross-sectional areas compared with limb and trunk muscles (Ringqvist, 1974; Vignon *et al.*, 1980; Eriksson and Thornell, 1983; Korfage and Van Eijden, 1999; Korfage *et al.*, 2000). The immunohistochemical techniques for detection of different types of myosin heavy chains used in the present study showed that the pterygoid muscles contain a large proportion of hybrid muscle fibres. Most of the hybrid fibres co-expressed MyHC-cardiac $\alpha$ in combination with MyHC-I and/or MyHC-IIA. Hybrid fibres co-expressing MyHC-fetal were also found, although in a smaller proportion. Normally, MyHC-fetal is expressed only in limb and trunk muscles during development (Butler-Browne *et al.*, 1988; Soussi-Yanicositas *et al.*, 1990) or during regeneration (Sartore *et al.*, 1982; Schiaffino and Reggiani, 1996). However, studies on the fibre-type composition of the human jaw muscles (Butler-Browne *et al.*, 1988; Korfage and Van Eijden, 1999; Korfage *et al.*, 2000) indicate that MyHC-fetal expression is a normal feature for jaw-closing muscles.

The fibre type composition of the human pterygoid muscles has been described earlier in four studies where ATPase was used as a technique for classification (Vignon *et al.*, 1980; Eriksson and Thornell, 1981, 1983; Shaughnessy *et al.*, 1989). With that technique, it was possible for types I, IIA, IIB and two intermediate fibre types - namely, types IM, which is normally found only in the jaw-closing muscles, and IIC - to be classified. There is a direct correlation between the ATPase enzyme histochemically defined fibre types I and MyHC-I, and type IIA and MyHC-IIA. However, ATPase enzyme histochemically defined type IIB fibres contain an MyHC that is a homologue to MyHC-IIX in rodents (Schiaffino *et al.*, 1989; Smerdu *et al.*, 1994). Standard ATPase enzyme histochemistry does not make a distinction between type IIB and IIX fibres (Schiaffino *et al.*, 1989; Aigner *et al.*, 1993). The contents of ATPase histochemically defined IM and IIC are not completely clear. With the ATPase technique, it is also not possible to distinguish MyHC-fetal and MyHC-cardiac $\alpha$.

Table 5.5 summarises the results of various studies for the medial pterygoid. The proportions of fibre types differ between these studies and the present study. A decreasing proportion of type I fibres was noted in older subjects. A study by Monemi *et al.* (1998) showed that, in the human masseter, the proportion of type I fibres decreases during ageing, from 63 to 33%, which is in accordance with the data

shown in Table 5.5. In neither study mentioned was a proportion of hybrid fibres found as high as that in the present study. An explanation might be that the immunohistochemical technique detects more MyHCs which are not detected by ATPase enzyme histochemistry. The influence of gender in older persons on fibre type distribution is not yet clear. In young adults, however, it was found that the male masseter had a larger number of type II fibres, while the female masseter had a larger number of type I and IM fibres (Tuxen *et al.*, 1999).

| | Age (yrs) | I | II[a] | Intermediate | Hybrid |
|---|---|---|---|---|---|
| Eriksson and Thornell (1983)[b] | 19-25 | 54% | 36% | 10% | |
| Shaughnessy *et al.* (1989)[b] | 16-31 | 50% | 36% | 14% | |
| Vignon *et al.* (1980)[b] | 20-87 | 37% | 56% | 7% | |
| Present study[c] | 47-95 | 32% | 16% | | 52% |

**Table 5.5**
Comparison between age and percentages of fibre types in medial pterygoid found in literature and in the present study.
[a] Type II was the sum of all pure fast fibres.
[b] Fibre types defined by ATPase histochemistry.
[c] Fibre types defined by immunohistochemistry.

Eriksson and Thornell (1983), using ATPase histochemistry, found no type IIA fibres but only type IIB fibres as a fast fibre type. Type IIA fibres were found by Shaughnessy *et al.* (1989) and with immunohistochemistry in the present study. It is possible that, in young subjects, only one fast fibre type containing one fast MyHC, namely, MyHC-IIX, is expressed. In older subjects, MyHC-IIX could be downregulated in favour of MyHC-IIA. Such an age-related alteration was observed in a study of rat skeletal muscles (Sugiura *et al.*, 1992; Larsson *et al.*, 1993).

In the present study, we noticed that some MyHC type I fibres also reacted with antibody 332-3D4 but not with antibodies 333-7H1 and 340-3D5. We denoted these fibres as MyHC type I+Ia, according to a study by Galler *et al.* (1997a). A study by English *et al.* (1998) observed four different phenotypes among fibres containing a single MyHC slow/beta isoform in the rabbit masseter muscle. It might be that antibody 332-3D4, used in the present study, detects one of these phenotypes in human muscle fibres. Further tests are needed to clarify whether this is true.

The hybrid fibres displayed a large range of combinations of MyHCs, although fibres expressing MyHC-cardiac $\alpha$+I+IIA and fibres expressing MyHC-cardiac $\alpha$+I were the most frequent hybrid fibre types. The function and introduction of MyHC-

cardiac α in muscle fibres of jaw muscles is not understood at present. Generally, hybrid fibres are considered to arise during an alteration of movement pattern, *e.g.*, during training or long periods of non-inactivity, or during hormonal changes. The subjects used in the present study were relatively old, and most wore dental prostheses. This could be another explanation for the relatively high proportion of hybrid fibres found in the present study. It should be noted that hybrid fibres are a feature of jaw muscles in young as well as in old subjects. The contractile speed of hybrid muscle fibres lies between the contractile speeds of the MyHC isoforms they express (Pette and Staron, 1990; Kwa *et al.*, 1995b). The existence of different MyHC isoforms in a fibre makes a smooth transition possible of, for instance, speed regulation needed in movements of the jaw.

In an earlier study (Korfage and Van Eijden, 1999), we found that the temporalis contained more MyHC type I muscle fibres in areas that are more active, indicating an adaptation to a differential use. Since type I fibres are recruited first in a movement (Henneman *et al.*, 1965), and their innervation ratio is small, muscle portions with a high proportion of type I fibres are better equipped to regulate the magnitude of the produced force during chewing or biting than are muscle portions with a low proportion of type I fibres. These forces are needed not only during biting or chewing, but also, in humans, more often during speaking.

Within the medial pterygoid and the inferior head of the lateral pterygoid we observed that MyHC type I fibres are heterogeneously distributed. Eriksson and Thornell (1983) found, within the medial pterygoid muscle, a predominance of type I fibres in the anterior part. The present study confirms these findings, but we also found that the lateral muscle portions contained a larger proportion of MyHC type I fibres than the medial muscle portions. Since type I fibres are recruited first in a movement, and type I fibres occupy the largest total area, the heterogeneous distribution of fibre types within the medial pterygoid and the inferior head of the lateral pterygoid indicates that, with an increase of force, the line of action will shift from an area with a large proportion of type I fibres to the resultant line of action of all muscle fibres.

It was noticed that fibres in muscles of the masticatory system were smaller than those commonly found in limb and trunk muscles (Polgar *et al.*, 1973). In the present study, we observed that, in the pterygoid muscles, MyHC type II and hybrid fibres were smaller than type I fibres. This is in accordance with other studies of jaw muscle fibres (Ringqvist, 1974; Eriksson *et al.*, 1981; Eriksson and Thornell, 1983).

Compared with the mean cross-sectional fibre areas in the temporalis of the same group of subjects (Korfage and Van Eijden, 1999), the type I fibres in the pterygoid muscles are approximately 25% smaller. MyHC type II and hybrid fibres in the superior head of the lateral pterygoid are about the same size as in the temporalis, but in the medial pterygoid and inferior head of the lateral pterygoid, these fibres are approximately 50% smaller. This could indicate that the fibres in the pterygoid muscles are less intensively used than the fibres in the temporalis.

We conclude that, although medial pterygoid, and both heads of the lateral pterygoid differ in function and architecture, this is not reflected in their myosin heavy chain isoform composition and fibre cross-sectional area.