Published in final edited form as:
Arch Oral Biol. 2013 April ; 58(4): 435–443. doi:10.1016/j.archoralbio.2012.09.008.

Regional variation in IIM myosin heavy chain expression in the temporalis muscle of female and male baboons(*Papio anubis*)

Christine E. Wall[a,*], **Margaret M. Briggs**[b], **Emranul Huq**[c], **William L. Hylander**[a], and **Frederick Schachat**[b]

[a]Department of Evolutionary Anthropology, Duke University, Box 90383, Durham, NC 27708

[b]Department of Cell Biology, Duke University Medical Center, Durham, NC 27710

[c]International Doctoral Program in Anthropological Sciences, Stony Brook University, Stony Brook, NY 11794

Abstract

Objective—The purpose of this study was to determine whether high amounts of fast/type II myosin heavy chain (MyHC) in the superficial as compared to the deep temporalis muscle of adult female and male baboons(*Papio anubis*) correlates with published data on muscle function during chewing. Electromyograpic (EMG) data show a regional specialization in activation from low to high amplitude activity during hard/tough object chewing cycles in the baboon superficial temporalis (Wall et al., 2007, 2008). A positive correlation between fast/type II MyHC amount and EMG activity will support the high occlusal force hypothesis.

Design—Deep anterior temporalis (DAT), superficial anterior temporalis (SAT), and superficial posterior temporalis (SPT) muscle samples were analyzed using SDS-PAGE gel electrophoresis to test the prediction that SAT and SPT will show high amounts of fast/type II MyHC compared to DAT. Serial muscle sections were incubated against NOQ7.5.4D and MY32 antibodies to determine the breadth of slow/type I versus fast/type II expression within each section.

Results—Type I and type IIM MyHCs comprise nearly 100% of the MyHCs in the temporalis muscle. IIM MyHC was the overwhelmingly predominant fast MyHC, though there was a small amount of type IIA MyHC (≤5%) in DAT in two individuals. SAT and SPT exhibited a fast/type II phenotype and contained large amounts of IIM MyHC whereas DAT exhibited a type I/type II (hybrid) phenotype and contained a significantly greater proportion of MyHC-I. MyHC-I expression in DAT was sexually dimorphic as it was more abundant in females.

Conclusions—The link between the distribution of IIM MyHC and high relative EMG amplitudes in SAT and SPT during hard/tough object chewing cycles is evidence of regional specialization in fiber type to generate high occlusal forces during chewing. The high proportion of MyHC-I in DAT of females may be related to a high frequency of individual fiber recruitment in comparison to males.

Keywords

Myosin Type Composition; Mastication; Muscle; Anatomy

[*]Corresponding author. Tel.: +1 919 668 2543; fax: +1 919 684 8034; cw19@duke.edu.

Introduction

Mammalian chewing muscles express a variety of myosin heavy chain (MyHC) isoforms and fiber type phenotypes (English et al., 1998, 1999; Hoh, 2002; Rowlerson et al., 1981, 1983; Sciote et al., 2003; Tanaka et al., 2008). A number of workers have hypothesized that the differential distribution of fast/type II fibers within chewing muscles, which tend to be concentrated in the superficial parts of muscles such as the masseter and temporalis, is a specialization for the rapid production of high occlusal force during the power stroke of mastication (Anapol and Herring, 2000; Gibbs et al., 1983; Herring, Grimm, and Grimm, 1979; Nielsen and Miller, 1988). The high occlusal force hypothesis is consistent with the suggestion that expression of type IIM (masticatory) MyHC, because of its high force generating ability relative to other fast MyHCs, is often correlated with dietary specializations that require high occlusal forces during prey capture or hard/tough object feeding (Hoh, 2002; Hoh et al., 2006; Yamaguchi, 2007; Reiser et al., 2009; Toniolo et al., 2008).

The MyHC isoforms and fiber type phenotypes of the chewing muscles have been described for several primate species (Kang et al., 1994; Korfage et al., 2005a, b; Maxwell et al., 1979; Miller, 1991; Miller and Farias, 1988; Reiser et al., 2009; Rowlerson et al., 1983; Sciote et al., 1994; Stedman et al., 2004), most thoroughly for humans and macaques. The presence of type IIM MyHC in the jaw adductor muscles of nonhuman primates has long been known to distinguish them from humans (Rowlerson et al., 1983; Sciote and Morris, 2000).

A precursor of the studies on IIM MyHC was work by Maxwell et al. (1979) who analyzed the fiber type distribution in superficial anterior temporalis (SAT) and superficial posterior temporalis (SPT) of female and male macaques using enzyme histochemistry. They reported significantly more type I fibers in females (50%) as compared to males (20%) for the SAT, but no difference between females (9%) and males (10%) for the SPT. Miller and Farias (1988) found significantly more type I fibers in the deep anterior temporalis (DAT) compared to the SAT of macaques, but did not report differences between male and females in the proportion of type I fibers.

There are no descriptions of the MyHC isoforms present in the jaw muscles of baboons(*Papio anubis*), though baboons are a commonly used experimental model in studies of the kinetics and kinematics of the masticatory apparatus. The temporalis is the largest chewing muscle in baboons. The deep part is separated from the superficial part by a substantial tendon, and fiber orientation changes from a vertical and slightly anterior orientation in the anterior part to a strong posterior orientation in the posterior part (Wall et al., 2008). Gross dissections show that the fibers in the deep part have a red appearance whereas the fibers in the superficial part have a light pink appearance (Wall et al., 2008:Figure 1). This suggests that the deep and superficial parts differ in the distribution of fiber type phenotypes with type II fibers being more prevalent in the superficial temporalis.

The electromyographic (EMG) activity patterns of the chewing muscles of baboons are well-characterized (Hylander and Johnson, 1994; Hylander et al., 2000, 2005; Wall et al., 2006, 2007, 2008). In particular, Wall et al. (2007, 2008) found that the DAT in female and male baboons was recruited consistently and at similar amplitudes during both soft- and hard/tough object chewing cycles. By contrast, the SAT and SPT of both sexes showed weak activity during soft-object cycles and high relative EMG amplitudes during hard/tough object cycles. These data suggested that the superficial parts of temporalis are specialized for activity during the power stroke of hard/tough object chewing cycles, a time when the rapid production of high occlusal force is required.

Here we determine the major MyHC isoforms present in the DAT, SAT, and SPT of the EMG subjects. The results are used to evaluate the high occlusal force hypothesis that differential distribution of fast/type II fibers in superficial muscle layers are a specialization for the rapid production of high occlusal force during the power stroke of mastication (Anapol and Herring, 2000; Gibbs et al., 1983; Herring et al., 1979; Nielsen and Miller, 1988). Specifically, the high occlusal force hypothesis will be supported if the SAT and SPT contain a higher proportion of type II fibers than the DAT.

Materials and Methods

Animals

Three adult male (28.1 – 30.6 kg) and two adult female (13.6 and 15.6 kg) olive baboons(*Papio anubis*) were sacrificed after use in EMG studies. The Institutional Animal Care and Use Committee at Duke University reviewed and approved all procedures (DUIACUC Registry # A088-00-02-2).

Muscle removal, storage, and processing

After the subjects were euthanized with sodium pentobarbitol (150mg/kg), the bodies were transferred to a refrigerated storage room. The heads were transferred to a conventional freezer (−10°C) within 24 hours of death for 2 males and within 30 minutes of death for the remaining subjects. Muscle blocks were excised at variable times after the heads had been frozen. The heads were minimally thawed so that the skin could be incised and reflected from the temporalis muscle. Large still-frozen fiber blocks (approximately 1 cm wide × 5 mm deep × 1.5 cm long) were cut perpendicular to the long axis of the muscle fibers from the DAT and the SAT just posterior to the lateral orbital margin, and from the SPT just superior to the external auditory meatus. All subsequent sections were made perpendicular to the long axis of the muscle fibers in order to maximize the number of fibers sampled in transverse section.

To determine the distribution and relative abundance of type I (slow) and type II (fast) MyHCs, part of each fiber block was analyzed by SDS-PAGE gel electrophoresis. Frozen muscle samples were sliced and pulverized at liquid nitrogen temperature, and the muscle powder was extracted to prepare a myofibrillar protein fraction. The myofibrillar proteins were denatured in SDS buffer and analyzed using gel electrophoresis conditions modified from Talmadge and Roy (1993) as described in Briggs and Schachat (2000). The gels contain 7% acrylamide, and 35% glycerol, which optimize resolution of baboon MyHC types IIA, IIX, IIM, and I, as demonstrated in the human vastus lateralis and macaque temporalis control lanes of Figure 1. MyHC-IIM is well-separated from the other myosin bands, and present only in branchial arch I muscle (Rowlerson et al., 1981, 1983; Hoh 2002). The relative abundance of MyHCs was quantified by densitometry, performed as described in Briggs and Schachat (2000).

Because IIM MyHC accounted for virtually all of the type II myosin present, we did not quantify other MyHC isoforms (e.g., neonatal) using gel electrophoresis as their virtually undetectable levels made it unlikely they would contribute measureably to the predicted differential distribution of MyHCs.

To determine the breadth of type I versus type II expression within each section and to facilitate measurement of the areas of type I and type II expressing fibers, sections from each muscle block were stained with antibodies against MyHC-I (1:800 dilution, NOQ7.5.4D, Sigma) and MyHC-II (1:800 dilution, MY32, No. 4276, Sigma). The remainder of each frozen fiber block was transferred to the Immunohistochemistry Laboratory in the Department of Pathology and stored at −70°C. The frozen fiber blocks

were trimmed and 10 μm thick sections were serially cut with a cryostat at −25°C. The frozen sections were then mounted on glass microscope slides for immunostaining. Tissue damage (e.g., holes within the fibers), presumably due to slow freezing, was absent or minimal in most sections (Supplementary Material).

The MY32 antibody was used to identify fibers expressing type II MyHC because, in addition to reacting with all skeletal type II MyHCs (Sciote and Kentish, 1996), it was reported to react with IIM fibers in *Macaca fascicularis*, the long-tailed macaque (Stedman et al., 2004), and macaques and baboons are closely related to one another. Because the gels showed that IIM was almost the exclusive type II MyHC in baboon temporalis, we did not attempt a complete immunostaining profile, and so did not distinguish further among MyHC isoforms using more specific antibodies.

The muscle sections were saved as digital images at 20× magnification using a Zeiss axiocam digital camera attached to an Axioplan microscope and Axiovision 2.05 software. Microscope fields in serial and contiguous sections were matched by identifying specific muscle cells by their shape and size and using fiducial markers such as fascial planes and vessels. Images were printed at 600 dpi using a high resolution laser printer to facilitate identification of contiguous and serial sections. Fiber counts were made from the prints using a hand-held cell counter. Fibers were counted as strong, intermediate, weak, or no stain intensity. It was not possible to reliably assign fibers to either the strong or intermediate staining intensity, or to the weak versus negative staining intensity, across individuals and muscle parts. Therefore, strong and intermediate counts were combined as were weak and negative counts for subsequent statistical analysis (but see Supplementary Material, Table 1 for fiber count percentages for the four original categories). Fiber areas were measured with ImageJ software (version 1.24).

All statistical analyses were carried out using BIOMstat 3.3 (Exeter Software, Setauket, NY) or JMP 9 (SAS Institute, Inc., Cary, NC). Two-tailed Mann-Whitney U-tests were used to evaluate whether the muscle parts (DAT, SAT, and SPT) had different fractions of the MyHC-I and MyHC-IIM isoforms, and for each muscle part to test whether females and males differed in the fraction of MyHC-I isoform. A Model I log-linear test of independence using an intrinsic hypothesis to compute expected cell frequencies (Sokal and Rohlf, 2012) was used to test for three-factor, two-factor, and main effects associations of muscle part (DAT, SAT, and SPT), sex (Female and Male), and antibody reaction (React to Type I Only, React to Type II Only, Both I and II). The Kruskal-Wallis test was used to evaluate whether males and females showed significant differences in fiber area within muscle parts.

Results

Table 1 provides the quantitative results of the SDS-PAGE gel electrophoresis data for each subject. Figure 1 shows the SDS-PAGE results for each subject and includes control lanes that demonstrate the ability of the gel system to resolve the MyHC isoforms IIX, IIA, IIM, and I. Table 2 provides data on the fiber counts in the three antibody reaction categories (Type I Only, Type II Only, Both I and II) for each muscle and individual, and Table 3 provides the fiber area data. Figure 2 shows representative DAT and SAT immunostained cross-sections (see Supplementary Material for reprentative cross-sections for other individuals).

DAT contained a much lower fraction of MyHC-IIM than either SAT or SPT (Table 1). Two individuals (F3 and M2) showed a small fraction (≤ 5%) of MyHC-IIA in DAT. Compared to the males, the females had a much higher fraction of MyHC-I in DAT (U_S = 6.0, $p < 0.05$), whereas in SAT and SPT the fractions of MyHC-I were similar across the

sexes. The Mann-Whitney U-test on the gel data was significant ($U_s = 25$, $p < 0.0045$) for the DAT-SAT and the DAT-SPT contrasts, but was not significant for the SAT-SPT contrast ($U_s = 6.5$, $p < 0.458$).

Overall, there was a good correspondence between the levels of the type IIA, IIM, and I MyHCs determined by densitometry of SDS-PAGE and the distribution of fibers indicated by the immunostaining analysis. The densitometry revealed slightly higher levels of MyHC-I in SAT and SPT than indicated by the staining intensity data (Tables 1 and 2), but that was consistent with extremely weak reactions to the MyHC-I antibody in the large-diameter fibers (Figure 2 and Supplementary Material). Due to the weakness of the reaction, these large fibers were counted as “Type II only” (Tables 2 and 3). Many fibers in DAT showed a strong or intermediate reaction to both NOQ7.5.4D and MY32 though there was some variation across individuals (Table 2, Figure 2, and Supplementary Material). By contrast, the majority of fibers in SAT and SPT, and particularly the large-diameter fibers, reacted uniformly to MY32 and had a weak or negative reaction to NOQ7.5.4D in all individuals (Figure 2 and Supplementary Material).

The log-linear test of independence was highly significant for the three factor interaction of Muscle, Sex, and Reaction (Table 2, $G=249.63$, $p < 0.000001$). This means that the degree of association between any two factors (e.g., muscle and sex) depended on the category of antibody reaction (Type I only, Type II only, or Both). The tests of significance for the main effects and for each two-way interaction were also highly significant (Table 2). The major biological signals related to the distribution of MyHCs in these results were the concentration in DAT, as compared to SAT and SPT, of a large number of fibers that react strongly to NOQ7.5.4D, and the similarity of staining intensity frequencies in SAT and SPT in both males and females. The major biological signal related to sex-specific variation was the higher amount of fibers reacting to NOQ7.5.4D in the DAT of females as contrasted with the higher amount of fibers reacting only to MY32 in DAT of males.

Fiber area varied consistently in relation to sex, muscle part, and MyHC antibody reaction (Table 3). Sexual dimorphism in fiber area was absent in the fibers that reacted moderately or strongly to NOQ7.5.4D (DAT: $H = 5.9779$, ns; SAT: $H = 3.0179$, ns; and SPT: $H = 0.5526$, ns). The DAT in males and females also had similar fiber areas in the fibers that reacted to both NOQ7.5.4D and MY32 ($H = 4.3379$, ns). However, the SAT and SPT of males had significantly larger fiber areas in fibers that reacted only to MY32 (SAT: $H = 14.4945$, $p < 0.0001$; SPT: $H = 86.4322$, $p < 0.000001$).

Given the findings for the male-female contrasts, we did not test for statistically significant differences in fiber area between muscle parts or antibody reaction with the sexes combined (Table 3). The smallest fibers (means range from approximately 662 - 1485 μm^2) were found in the SAT and SPT of both males and females. These fibers frequently reacted moderately or strongly to both antibodies (Figure 2 and Supplementary Material). Intermediate sizes of fibers (means range from approximately 1577 – 2949 μm^2) were found in the DAT of both sexes where they often reacted to both antibodies (Figure 2 and Supplementary Material). Intermediate sizes were also observed in the SAT and the SPT of females and the SAT of M3. Very large fibers (means range from approximately 2830 – 7110 μm^2) are found in the SAT and the SPT of males where they reacted strongly to MY32 (Figure 2 and Supplementary Material).

Discussion

In both sexes, SAT and SPT express a fast/type II MyHC phenotype, whereas DAT expresses a type I/type II (hybrid) MyHC phenotype. The SAT and SPT of baboons are

distinguished by the high proportion of the IIM MyHC isoform, the lack of sexual dimorphism in EMG activity pattern, and the change in recruitment from low amplitude during soft-object chewing cycles to high amplitude during hard/tough object chewing cycles (Wall et al., 2007, 2008). Together, these observations provide strong evidence in support of the high occlusal force hypothesis to explain the differential distribution of fast/type II fibers in superficial temporalis (Anapol and Herring, 2000; Gibbs et al., 1983; Herring, Grimm, and Grimm, 1979; Nielsen and Miller, 1988).

MY32 does not react with IIM fibers in some mammals, including *Callithrix*, a New World monkey (Kang et al., 1994; Sciote and Rowlerson, 1998). However, MY32 does react with IIM fibers*in Macaca fascicularis*, a close relative of baboons (Stedman et al., 2004). In the baboons, reaction intensity to MY32 occurred along a continuum in DAT with the majority of fibers showing a strong reaction (Supplementary Material). Reaction intensity to MY32 was bimodal (present or absent) in SAT and SPT with close to 100% of fibers showing a reaction. Given that IIM comprises most (≥80%) of the MyHC present in SAT and SPT and large amounts of the MyHC present in DAT, we conclude that MY32 reacts with the type IIM MyHC in the baboons.

However, MyHC antibodies are known to display unusual patterns of co-reaction in mammalian jaw muscles (Hoh et al., 1988a,b, 1991; Widmer et al., 2002). Kang et al. (1994) found that slow (type I) fibers in temporalis and masseter of several species, including *Callithrix*, react to both MY32 and NOQ7.5.4D, suggesting an immunohistochemical difference between jaw and limb slow fibers. This interpretation is perhaps consistent with the observation that in the baboons NOQ7.5.4D often reacted weakly with the large fibers in SAT and SPT. The co-expression of small amounts of non-IIM MyHC in the large type II fibers of SAT and SPT does not alter the conclusion that SAT and SPT show a fast/type II phenotype. The data also suggest that there are numerous hybrid fibers in DAT, SAT, and SPT.

Hybrid fibers are common in skeletal muscle (Stephenson, 2001) and are known to occur in substantial proportions in the human temporalis muscle (Korfage and van Eijden 1999). Humans express many different MyHC isoforms in the masticatory muscles including fetal and neonatal (Eriksson and Thornell, 1983; Korfage et al., 2005a, b; Rowlerson et al., 1983; Sciote and Morris, 2000), but do not express MyHC-IIM (Rowlerson et al., 1983; Sciote et al., 1994; Stedman et al., 2004). Humans are also unusual in that the type II fibers of the jaw muscles are frequently smaller than the type I fibers (Eriksson and Thornell, 1983; Korfage and van Eijden, 1999; Ringqvist, 1974a, b; Sciote et al., 1994). Although the temporalis muscle of humans and baboons is similar in that both species express hybrid fibers, the data presented here suggest that the fiber type distribution and fiber area in the temporalis muscle of baboons are quite distinct from that of humans.

The SDS-PAGE results show that IIM is the major type II MyHC expressed in the baboon temporalis muscle. The physiological properties of IIM fibers in primates have not been measured. In the domestic cat and dog, IIM fibers are only moderately fast, but they are fatigue-resistant and capable of producing high isometric tension at the cost of high ATP turnover (Hoh, 2002; Reiser and Bicer, 2007; Saeki et al., 1987; Toniolo et al., 2008; Yamaguchi et al., 2010). The ability of IIM fibers to generate high isometric force, though recognized to be the ancestral condition for many mammalian species (Kang et al., 1994), is also thought to evolve in association with dietary specializations that require high occlusal forces (Hoh, 2002; Hoh et al., 2006; Rowlerson et al., 1983;Sciote et al., 2003; Yamaguchi, 2007; Reiser et al. 2009, 2010).

There was significant sexual dimorphism of fiber area in SAT and SPT with males having much larger fiber areas. This has been found in macaques (Maxwell et al., 1979; Miller and Farias, 1988), humans (Eriksson and Thornell, 1983), and rabbits (English et al., 1998, 1999), and may be due to the presence of androgen receptors on the muscles of the males (English et al., 1999;Lyons et al., 1986). It is interesting to note that one male baboon, M2, has extremely large fibers in SAT (Figure 2) and SPT and was also the dominant male in his social group.

The sexual dimorphism in fiber phenotype distribution in DAT observed here, in which the DAT fibers of the female baboons have a significantly higher proportion of MyHC-I in comparison to the males raises the possibility of an unexpected physiological correlate. The differences are not linked to variation in EMG amplitude during soft-object or hard/tough object chewing cycles (Wall et al., 2008). They may indicate sex differences in temporalis force at high levels of motor unit recruitment (English et al., 1999). A high proportion of type I fibers has a positive association with duration of daily EMG activity in the chewing muscles of humans, rabbits, and rats (Korfage et al., 2005a, b; Tanaka et al., 2008). This suggests that the high proportion of MyHC-I in the DAT of the female baboons may be related to increasing resistance to fatigue and/or controlling for cost caused by a high frequency of individual fiber recruitment in comparison to males, particularly if this recruitment occurs at sub-maximal muscle forces (Guelinckx et al., 1986). This might occur in a female over the course of day if a given fiber must be recruited more frequently or more fibers are recruited relative to total physiological cross-sectional area to generate occlusal force during chewing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors wish to thank two anonymous reviewers for their very helpful comments and suggestions for improving the manuscript. We also thank Dr. Kathleen Smith for the use of the microscope setup to image the muscle sections and her guidance in thinking about feeding muscle physiology. Mr. Wayne Terrell and Ms. Cleo Proctor of the Department of Pathology prepared the muscle sections. This work was sponsored by the National Institutes of Health (EY11377, PI - Schachat) and the National Science Foundation (BCS-0095522, PI - Wall).

References

Anapol F, Herring SW. Ontogeny of histochemical fiber types and muscle function in the masseter muscle of miniature swine. Am J Phys Anth. 2000; 112:595–613.

Briggs MM, Schachat F. Early specialization of the superfast myosin in extraocular and laryngeal muscles. J Exp Biol. 2000; 203(Pt 16):2485–94. [PubMed: 10903163]

Clark RW, Luschei ES. Histochemical characteristics of mandibular muscles of monkeys. Expl Neurol. 1981; 74:654–672.

English AW, Eason J, Pol M, Schwartz G, Shirley A. Different phenotypes among slow/beta myosin heavy chain-containing fibres of rabbit masseter muscle: a novel type of diversity in adult muscle. J Muscle Cell Res Cell Mot. 1998; 19:525–535.

English AW, Eason J, Schwartz G, Shirley A, Carrasco DI. Sexual dimorphism in the rabbit masseter muscle: myosin heavy chain composition of neuromuscular compartments. Cell Tiss Org. 1999; 164:179–191.

Eriksson PO, Thornell LE. Histochemical and morphological muscle-fiber characteristics of the human masseter, the medial pterygoid, and the temporal muscle. Arch Oral Biol. 1983; 28:781–795. [PubMed: 6227313]

Gibbs C, Mahan PR, Wilkinson TM. EMG activity of the superior belly of the lateral pterygoid muscle in relation to other jaw muscles. J Prosthet Dent. 1983; 51:691–701. [PubMed: 6587093]

Herring SW, Grimm AF, Grimm BR. Functional heterogeneity of a multipennate muscle. Am J Anat. 1979; 154:563–578. [PubMed: 433798]

Guelinckx P, Dechow PC, Vanrusselt R, Carlson DS. Adaptations in the temporalis muscles of rabbits after masseter muscle removal. J Dent Res. 1986; 65:1294–1299. [PubMed: 2959691]

Hoh JYH. 'Superfast' or masticatory myosin and the evolution of jaw-closing muscles of vertebrates. J Exp Biol. 2002; 205:2203–2210. [PubMed: 12110654]

Hoh JYH, Hughes S, Chow C, Hale PT, Fitzsimons. Immunocytochemical and electrophoretic analyses of changes in myosin gene expression in cat posterior temporalis muscle during postnatal development. J Musc Res Cell Motil. 1988a; 9:48–58.

Hoh JYH, Hughes S, Hale PT, Fitzsimons RB. Immunocytochemical and electrophoretic analyses of changes in myosin gene expression in cat limb fast and slow muscles during postnatal development. J Musc Res Cell Motil. 1988b; 9:30–47.

Hoh JYH, Hughes S, Walker ML, Kang LHD, Everett AW. Slow myosin heavy chains in cat jaw and limb muscles are phenotypically distinct: expression of jaw-specific slow myosin phenotype in regenerated and chronically stimulated jaw muscles. Basic Applied Myology. 1991; 1:285–294.

Hoh JYH, Kang LH, Sieber LG, Lim JHY, Zhong WW. Myosin isoforms and fibre types in jaw-closing muscles of Australian marsupials. J Comp Physiol [B]. 2006; 176:685–695.

Hylander WL, Johnson KR. Jaw muscle function and wishboning of the mandible during mastication in macaques and baboons. Am J Phys Anth. 1994; 94:523–547.

Hylander WL, Ravosa MJ, Ross CF, Wall CE, Johnson KR. Symphyseal fusion and jaw-adductor muscle force: An EMG study. Am J Phys Anth. 2000; 112:469–492.

Hylander WL, Wall CE, Vinyard CJ, Ross C, Ravosa MJ, Williams SH, Johnson KR. Temporalis function in anthropoids and strepsirrhines: An EMG study. Am J Phys Anth. 2005; 128:35–56.

Kang LHD, Hughes S, Pettigrew JD, Hoh JFY. Jaw-specific myosin heavy chain gene expression in sheep, dog, monkey, flying fox and microbat jaw-closing muscles. Basic Applied Myology. 1994; 4:381–392.

Korfage JAM, Van Eijden TMGJ. Regional differences in fiber type composition in the human temporalis muscle. J Anat. 1999; 194:355–362. [PubMed: 10386773]

Korfage JAM, Koolstra JH, Langenbach GEJ, van Eijden TMGJ. Fiber-type composition of the human jaw muscles – (part 1) origin and functional significance of fiber-type diversity. J Dent Res. 2005a; 84:774–783. [PubMed: 16109984]

Korfage JAM, Koolstra JH, Langenbach GEJ, van Eijden TMGJ. Fiber-type composition of the human jaw muscles – (part 2) role of hybrid fibers and factors responsible for inter-individual variation. J Dent Res. 2005b; 84:784–793. [PubMed: 16109985]

Lyons GE, Kelly AM, Rubinstein NA. Testosterone-induced changes in contractile protein isoforms in the sexually dimorphic temporalis muscle of the guinea pig. J Biol Chem. 1986; 261:13278–13284. [PubMed: 3759963]

Maxwell LC, Carlson DS, McNamara JA Jr, Faulkner JA. Histochemical characteristics of the masseter and temporalis muscles of the rhesus monkey (*Macaca mulatta*). Anat Rec. 1979; 193:389–402. [PubMed: 154857]

Miller, AJ. Craniomandibular Muscles: Their Role in Function and Form. Boca Raton: CRC Press; 1991.

Miller AJ, Farias M. Histochemical and electromyographic analysis of craniomandibular muscles in the rhesus monkey. J Oral Maxillofac Surg. 1988; 46:767–776. [PubMed: 3166047]

Nielsen IL, Miller AJ. Response patterns of craniomandibular muscles with and without alterations in sensory feedback. J Prosthet Dent. 1988; 59:352–361. [PubMed: 3162276]

Reiser PJ, Bicer S, Chen Q, Zhu L, Quan N. Masticatory ('superfast') myosin heavy chain and embryonic/atrial myosin light chain 1 in rodent jaw-closing muscles. J Exp Biol. 2009; 212:2511–2519. [PubMed: 19648394]

Reiser PJ, Bicer S, Patel R, An Y, Chen Q, Quan N. The myosin light chain 1 isoform associated with masticatory myosin heavy chain in mammals and reptiles is embryonic/atrial MLC1. J Exp Biol. 2010; 213:1633–1642. [PubMed: 20435813]

Reiser PJ, Bicer S. High force generation and moderate shortening velocity in jaw-closing muscle fiber expressing masticatory ("Superfast") myosin. Biophysical J Suppl. 2007; S:191A.

Ringqvist M. A histochemical study of temporal muscle fibers in denture wearers and subjects with normal dentition. Scand J Dent Res. 1974a; 82:28–39. [PubMed: 4132926]

Ringqvist M. Fiber types in human masticatory muscles, relation to function. Scand J Dent Res. 1974b; 82:333–355. [PubMed: 4137663]

Rowlerson AM, Pope B, Murray J, Whalen RB, Weeds AG. A novel myosin present in cat jaw-closing muscles. J Musc Res Cell Motil. 1981; 2:415–438.

Rowlerson AM, Mascarello F, Veggetti A, Carpene E. The fiber-type composition of the first branchial arch muscles in Carnivora and Primates. J Musc Res Cell Motil. 1983; 4:443–472.

Saeki Y, Kato C, Satomi M, Yanagisawa K. ATPase activity and tension development in mechanically-skinned feline jaw muscle. Arch Oral Biol. 1987; 32:207–210. [PubMed: 2959246]

Sciote JJ, Kentish JC. Unloading shortening velocities of rabbit masseter muscle fibres expressing skeletal or α-cardiac myosin heavy chains. J Physiol. 1994; 492:659–667. [PubMed: 8734979]

Sciote JJ, Morris TJ. Skeletal muscle function and fiber types: the relationship between occlusal function and the phenotype of jaw-closing muscles in human. J Orthod. 2000; 27:15–30. [PubMed: 10790441]

Sciote JJ, Rowlerson A. Skeletal fiber types and spindle distribution in limb and jaw muscles of the adult and neonatal opossum, *Monodelphis domestica*. Anat Rec. 1998; 251:548–562. [PubMed: 9713989]

Sciote JJ, Rowlerson AM, Hopper C, Hunt NP. Fiber type classification and myosin isoforms in the human masseter muscle. J Neurol Sci. 1994; 126:15–24. [PubMed: 7836942]

Sciote JJ, Rowlerson AM, Carlson DS. Myosin expression in the jaw-closing muscles of the domestic cat and American opossum. Arch Oral Biol. 1995; 40:405–413. [PubMed: 7639644]

Sciote JJ, Horton MJ, Rowlerson AM, Link J. Specialized cranial muscles: How different are they from limb and abdominal muscles? Cell Tiss Org. 2003; 174:73–86.

Sokal, RR.; Rohlf, FJ. Biometry. 4th. New York: Freeman; 2012.

Stedman HH, Kozyak BW, Nelson A, Thesler DM, Su LT, Low DW, Bridges CR, Shrager JB, Minugh-Purvis N, Mitchell MA. Myosin gene mutation correlates with anatomical changes in the human lineage. Nature. 2004; 428:415–418. [PubMed: 15042088]

Stephenson GMM. Hybrid skeletal muscle fibres: a rare or common phenomenon? Clin Exp Pharmacol Physiol. 2001; 28:692–702. [PubMed: 11473538]

Talmadge RJ, Roy RR. Electrophoretic separation of rat skeletal muscle myosin heavy-chain isoforms. J App Physiol. 1993; 75:2337–2340.

Tanaka E, Sano R, Kawai N, Korfage JAM, Nakamura S, Izawa T, Langenbach GEJ, Tanne K. Regional differences in fiber characteristics in the rat temporalis muscle. J Anat. 2008; 213:743–748. [PubMed: 19094190]

Toniolo L, Cancellara P, Maccatrozzo L, Patruno M, Mascarello F, Reggiani C. Masticatory myosin unveiled: first determination of contractile parameters of muscle fibers from carnivore jaw muscles. Am J Physiol Cell Physiol. 2008; 295:C1535–C1542. [PubMed: 18842829]

Wall CE, Vinyard CJ, Williams SH, Johnson KR, Hylander WL. Phase II occlusion in relation to jaw movement and masseter muscle recruitment during chewing in *Papio anubis*. Am J Phys Anth. 2006; 129:215–224.

Wall CE, Perry JMG, Briggs M, Schachat F. Mechanical correlates of sexual dimorphism in the jaw muscles and bones in baboons. Am J Phys Anth. 2007; 132:S44–242.

Wall, CE.; Vinyard, CJ.; Williams, SH.; Johnson, KR.; Hylander, WL. Specialization of the superficial temporalis muscle for hard/tough object feeding in baboons. In: Vinyard, CJ.; Ravosa, MJ.; Wall, CE., editors. Primate Craniofacial Function and Biology. New York: Springer Academic Publishers; 2008. p. 113-126.

Widmer CG, Morris-Wiman JA, Nekula C. Spatial distribution of myosin heavy-chain isoforms in mouse masseter. J Dent Res. 2002; 81:33–38. [PubMed: 11820364]

Yamaguchi M. Structure and function of masticatory (superfast) myosin. J Oral Biosci. 2007; 49:216–218.

Yamaguchi M, Takemori S, Kimura M, Tanishama Y, Nakayoshi T, Kimura S, Ohno T, Yagi N, Hoh JFY, Umazume Y. Protruding masticatory (superfast) myosin heads from staggered thick filaments of dog jaw muscle revealed by X-ray diffraction. J Biochem. 2010; 147:53–61. [PubMed: 19762343]

Abbreviations

DAT	Deep anterior temporalis
MyHC	Myosin Heavy Chain
SAT	Superficial anterior temporalis
SPT	Superficial posterior temporalis

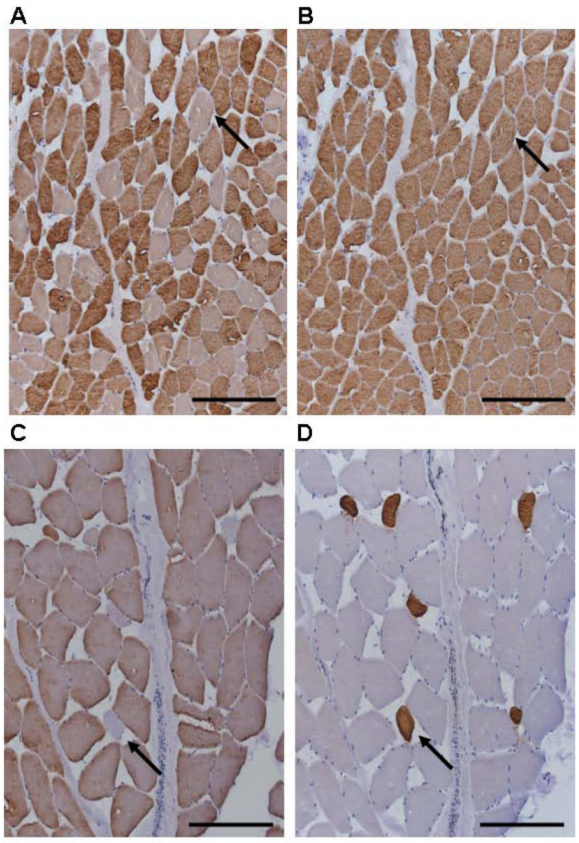

Figure 1.
SDS-PAGE results for the females (F2, F3) and the males (M1, M2, and M3) for DAT, SAT, and SPT. Control muscles from human vastus lateralis and macaque superficial temporalis identify the positions of MyHC isoforms IIX, IIA, IIM, and I.

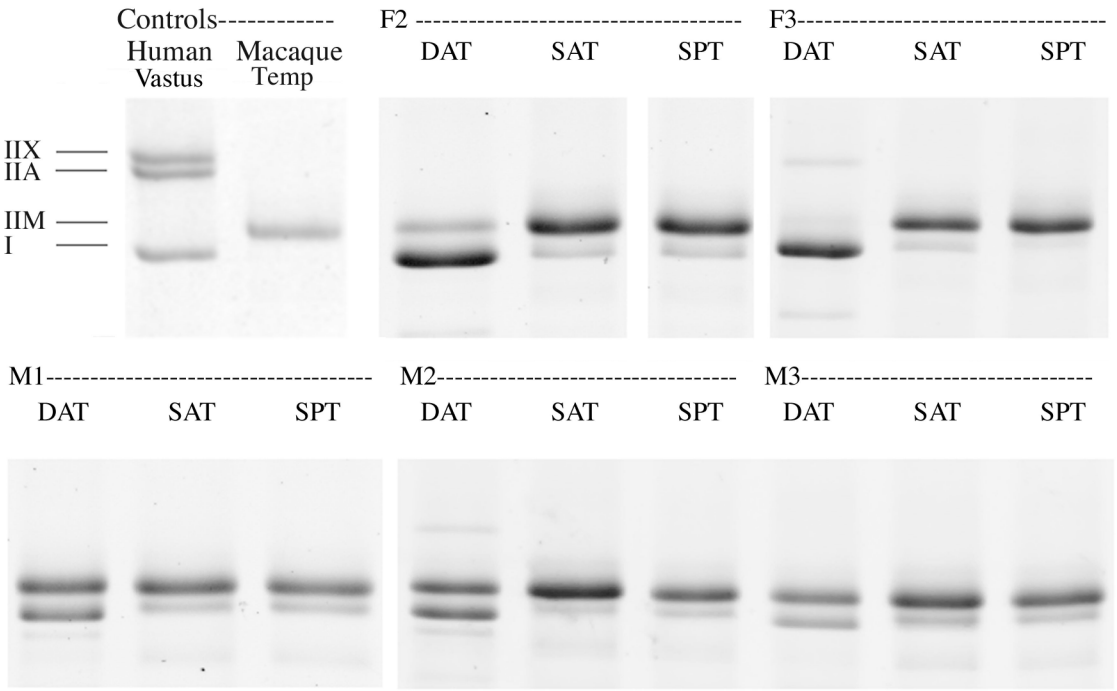

Figure 2.

Examples of antibody reactions in DAT and SAT in a male (M2). A and B illustrate the type I/type II (hybrid) phenotype of DAT. C and D illustrate the fast/type II phenotype of SAT and SPT. The scale bar is 0.2 microns. A, DAT, MY32 reaction. The black arrow points to a muscle cell that shows an intermediate reaction to the fast antibody. B, DAT, NOQ7.5.4D reaction. The black arrow points to the same muscle cell as noted in A that shows an intermediate reaction to the slow antibody. C, SAT, MY32 reaction. The black arrow points to a muscle cell that shows a negative reaction to the fast antibody. D, SAT, NOQ7.5.4D reaction. The black arrow points to the same muscle cell as shown in C that shows a dark reaction to the slow antibody.

Table 1

SDS-PAGE gel electrophoresis results for MyHC isoforms (expressed as a percentage of total MyHC) present in DAT, SAT, and SPT fibers in 2 female (F2 and F3) and 3 male (M1, M2, and M3) baboons. Mean and standard deviation (s) are provided for each sex. See text for discussion of statistical results.

DAT							
	F2	F3	Female Mean(s)	M1	M2	M3	Male Mean(s)
MyHC I	74.8	91.5	83.3(11.8)	38.4	44.1	31.8	38.1(6.2)
MyHC IIA	-	5.0	-	-	3.5	-	-
MyHC IIM	25.2	3.5	14.4(15.3)	61.6	52.4	68.2	60.7(7.9)
SAT							
	F2	F3	Female Mean(s)	M1	M2	M3	Male Mean(s)
MyHC I	14.3	8.4	11.4(4.2)	17.1	5.9	16.1	13.0(6.2)
MyHC IIA	-	-	-	-	-	-	-
MyHC IIM	85.7	91.6	88.7(4.2)	82.9	94.1	83.9	87.0(6.2)
SPT							
	F2	F3	Female Mean(s)	M1	M2	M3	Male Mean(s)
MyHC I	14.4	4.0	9.0(7.1)	20.3	11.5	12.7	14.8(4.8)
MyHC IIA	-	-	-	-	-	-	-
MyHC IIM	85.6	96.0	90.8(7.4)	79.7	88.5	87.3	85.2(4.8)

Table 2

Antibody reactions (Type I only, Type II only, Both I and II) in DAT, SAT, and SPT muscle fibers from 2 female (F2 and F3) and 3 male (M1, M2, and M3) baboons expressed as counts (corresponding row and column percentages italicized in parentheses). Column totals and proportions are listed for the female and male samples. Log Linear Model Statistical Results: Muscle × Sex × Reaction: G=249.63, p < 0.000001; Muscle × Sex given level of Reaction: G=211.25, p < 0.000001; Independence of Reaction from Muscle and Sex: G=5325.52, p < 0.000001; Muscle x Reaction given level of Sex: G=4289.53, p < 0.000001; Independence of Sex from Muscle and Reaction: G=635.48, p < 0.000001; Sex and Reaction given level of Muscle: G=411.49, p < 0.000001; Independence of Muscle from Sex and Reaction: G=5523.13, p < 0.000001; Complete Model (independence of Muscle, Sex, Reaction): G=5634.51, p < 0.000001.

Muscle	**Sex**	**Reaction**			
DAT	**Female**	**Type I only**	**Type II only**	**Both I and II**	**Total**
	F2	0(--)	0(--)	685(*100*)	685
	F3	0(--)	354(*63.4*)	204(*36.6*)	558
	Totals	0(--)	354(*28.5*)	889(*71.5*)	1243
	Male				
	M1	7(*.2*)	205(*46*)	227(*52*)	439
	M2	1(*.02*)	0(--)	417(*99.98*)	418
	M3	2(*.07*)	218(*77*)	64(*22.93*)	284
	Totals	10(*.9*)	423(*37.1*)	708(*62*)	1141
SAT	**Female**	**Type I only**	**Type II only**	**Both I and II**	**Total**
	F2	2(*.2*)	836(*96*)	33(*3.8*)	871
	F3	2(*.45*)	431(*98*)	7(*1.55*)	440
	Totals	4(*.3*)	1267(*96.6*)	40(*3.1*)	1311
SAT	**Male**	**Type I only**	**Type II only**	**Both I and II**	**Total**
	M1	1(*.3*)	331(*96.5*)	11(*3.2*)	343
	M2	3(*1.3*)	204(*88.7*)	23(*10*)	230
	M3	2(*.1*)	1339(*90.8*)	134(*9.1*)	1475
	Totals	6(*.3*)	1874(*91.5*)	168(*8.2*)	2048
SPT	**Female**	**Type I only**	**Type II only**	**Both I and II**	**Total**
	F2	4(*.6*)	624(*96.9*)	16(*2.5*)	644
	F3	2(*.4*)	507(*96*)	19(*3.6*)	528

Muscle	Sex	Reaction				
	Totals	6(*.5*)	1131(*96.5*)	35(*3*)		1172
	Male	**Type I only**	**Type II only**	**Both I and II**		**Total**
	M1	2(*.2*)	698(*98*)	12(*1.8*)		712
	M2	2(*.8*)	249(*97.2*)	5(*2*)		256
	M3	2(*.6*)	314(*97.2*)	7(*2.2*)		323
	Totals	6(*.45*)	1261(*97.7*)	24(*1.85*)		1291

Table 3

Mean (bold), standard deviation (in parentheses following the mean), and sample size (N) for cross-sectional areas (μm^2) of fibers that react to Type I, Type II, and Both I and II antibodies in the DAT, SAT, and SPT in 2 female and 3 male baboons. For DAT, fiber areas of F2 and M2 Type I and Type II could not be measured separately due to reaction of all fibers to both antibodies; fiber areas of F3 Type I could not be measured separately due to reaction of all fibers to MY32. For SAT and SPT, small numbers of fibers reacting strongly to NOQ7.5.4D limit sample size for Type I only and Both I and II categories.

Muscle	**Sex**	**Fiber Type**		
DAT	**Female**	**Type I only**	**Type II only**	**Both I and II**
	F2	-	-	**2379.8**(761.7)
		-	-	N=50
	F3	-	**2949.4**(505.9)	**2929.5**(620.8)
		-	N=30	N=30
	Male	**Type I**	**Type II**	**Both I and II**
	M1	**2813.3**(113.0)	**2911.4**(1280.4)	**2609.9**(719.4)
		N=5	N=50	N=25
	M2	-	-	**2498.5**(809.5)
		-	-	N=50
	M3	**2064.3**	**2301.5**(692.2)	**2012.2**(694.9)
		N=1	N=50	N=45
SAT	**Female**	**Type I**	**Type II**	**Both I and II**
	F2	877.1	2772.3(830.1)	1372.0(675.0)
		N=1	N=50	N=11
	F3	**785.4**(212.8)	**2740.1**(735.0)	**1243.6**(169.4)
		N=2	N=22	N=8
	Male	**Type I**	**Type II**	**Both I and II**
	M1	**855.6**	**3290.5**(1088.2)	**1249.4**(296.3)
		N=1	N=50	N=6
	M2	**2085.3**(378.5)	**5409.6**(1445.7)	**1154.2**(395.6)
		N=2	N=39	N=23
	M3	**404.0**	**2392.5**(426.0)	**662.4**(187.9)
		N=1	N=50	N=13
SPT	**Female**	**Type I**	**Type II**	**Both I and II**

Muscle	Sex	Fiber Type			
	F2	**634.9**	**2434.1**(541.2)	**806.5**(431.6)	
		N=1	N=50	N=11	
	F3	**935.9**	**2051.2**(933.0)	**1485.0**(498.7)	
		N=1	N=50	N=10	
	Male	**Type I**	**Type II**	**Both I and II**	
	M1	**1076.3**	**2827.3**(712.2)	**911.1**(374.0)	
		N=1	N=50	N=12	
	M2	**1411.3**	**7107.9**(2471.1)	**918.9**(115.5)	
		N=1	N=50	N=4	
	M3	**629.2**	**3232.7**(807.0)	**1311.7**(591.1)	
		N=1	N=50	N=4	