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Maximum Shortening Velocity and Myosin Heavy-chain Isoform Expression in Human Masseter Muscle Fibers

T. J. Morris[1], **C. A. Brandon**[1], **M. J. Horton**[1], **D. S. Carlson**[2], and **J. J. Sciote**[1,*]

[1]The Department of Orthodontics & Dentofacial Orthopedics, University of Pittsburgh, 3501 Terrace Street, Pittsburgh, PA 15261-1932

[2]Baylor College of Dentistry, Texas A&M University System Health Science Center

Abstract

While human masseter muscle is known to have unusual co-expression of myosin heavy-chain proteins, cellular kinetics of individual fibers has not yet been tested. Here we examine if myosin heavy-chain protein content is closely correlated to fiber-shortening speed, as previously reported in other human muscles, or if these proteins do not correlate well to shortening speeds, as has been demonstrated previously in rat muscle. Slack-test recordings of single, skinned human masseter fibers at 15°C revealed maximum shortening velocities generally slower and much more variable than those recorded in human limb muscle. The slowest fiber recorded had a maximum shortening velocity (V_0) value of 0.027 muscle lengths • s^{-1}, several times slower than the slowest type I fibers previously measured in humans. By contrast, human limb muscle controls produced V_0 measurements comparable with previously published results. Analysis by gel electrophoresis found 63% of masseter fibers to contain pure type I MyHC and the remainder to co-express mostly type I in various combinations with IIA and IIX isoforms. V_0 in masseter fibers forms a continuum in which no clear relationship to MyHC isoform content is apparent.

Keywords

V_0; slack test; skeletal muscle

INTRODUCTION

The precise relationship between muscle phenotype, based on myosin heavy-chain (MyHC) isoform content, and contractile properties is unclear. Where myosin heavy chains are expressed homogeneously within a single fiber, there is a strong association between the MyHC isoform and maximum unloaded shortening velocity (V_0), as for example in rats (Bottinelli *et al.*, 1991, 1994a; Thompson, 1999) and humans (Larsson and Moss, 1993; Harridge *et al.*, 1996; Bottinelli *et al.*, 1999), which can be ranked in order of V_0 as follows: I < IIA < IIX < IIB. However, these correlates of MyHC expression to physiological properties are often based on data with wide and overlapping variances, in particular for the fast isoforms. Single fibers may also co-express these proteins in the combinations I-IIA,

*corresponding author, jjs6@pitt.edu.

IIA-IIX, and IIX-IIB in rats (Bottinelli *et al.,* 1994b) and humans (Larsson and Moss, 1993) together with their corresponding transcripts (Smerdu *et al.,* 1994). Where there is heterogeneous MyHC expression, V_0 values may span the range of the individual isoforms. Some authors have demonstrated a positive relationship between V_0 and the relative content of fast MyHC isoforms in such fibers—for example, in the rabbit (Reiser *et al.,* 1985), the rat (Bottinelli *et al.,* 1994a), and humans (Larsson and Moss, 1993). Here also the high variability of V_0 strongly suggests that factors other than MyHC profile modulate contraction speed.

Mammalian jaw-closing muscles are of special interest. Varied jaw morphology and the diversity of functional requirements between species are complemented by equally diverse muscle-fiber type combinations. The jaw-closing muscles of carnivores and primates contain an additional fast-fiber type—with a notably rapid intrinsic shortening speed—termed IIM or ‘type II masticatory’ (Rowlerson *et al.,* 1983). Human jaw-closer muscles contain large relative amounts of type I in various combinations with IIA and IIX fiber types but, significantly, *no* IIM fibers. However, the fiber-type composition of these muscles is distinctive in other ways (Sciote *et al.,* 1994): Morphologically, human masseter type II fibers are atypically small in diameter compared with their type I neighbors, which are of normal size. The significance of this is not clear, but it runs contrary to the classic motor unit size principle, first proposed by Sherrington. Perhaps low masticatory forces required of modern diets have made type II fibers obsolescent (Ringqvist, 1974; Eriksson, 1982), so that, rarely recruited, they have simply atrophied. However, it is the high degree of fiber heterogeneity which is most conspicuous in human masseter: types I, IIA, and IIX MyHC isoforms can all be co-expressed in various combinations with neonatal, embryonic, and α-cardiac MyHCs (Bredman *et al.,* 1991). As yet unstudied, they are provocative subjects for physiological investigation.

In this paper we characterize skinned human masseter fibers. The range of recorded values of V_0 was very wide across fiber types. In particular, we have attempted to elucidate the relationship between V_0 and the relative percentage of MyHC expression as in single fibers. In homogeneously expressed fibers, which were mostly type I, the V_0 range was an order of magnitude larger than those typical skeletal type I fibers. In fibers where 2 or more MyHCs are co-expressed, there is no simple relationship between the proportion of fast and slow MyHC isoforms and V_0. These results indicate that human masseter fibers profiled on the basis of total MyHC content *per se* do not predict intrinsic shortening velocity.

MATERIALS & METHODS

After obtaining informed consent to a protocol that was reviewed by an appropriate Institutional Review Board, we took biopsies from the anterior superficial area of masseter muscles during orthognathic surgeries to reposition one or both jaws in conjunction with orthodontic treatment. Masseter subjects were generally healthy adults: four females aged 18, 18, 23, and 50 and four males aged 17, 32, 46, and 48 (n = 8). Control muscle was sampled from subjects undergoing radical amputation or limb resection, as follows (Male:Female/age in yrs): Triceps, M/33; gastrocnemius, F/40; vastus lateralis, M/46, F/24, and F/38 (n = 5). Control muscle results were grouped together, since there were no

detectable differences between age, sex, or muscle group. Muscle samples were taken from a disease-free normally functioning area of excised tissue. Portions of each biopsy from the various muscles sampled (area approximately 0.5 × 0.5 × 2 cm) were designated for single-fiber physiology or electrophoresis as described below. In addition, one sample was obtained from a human atrium, and one sample from fetal muscle (known to contain neonatal myosin heavy chain). These additional samples were processed for electrophoresis only, to act as control markers for α-cardiac and neonatal myosin heavy-chain species, respectively.

Slack-test Determination of Unloaded Shortening Velocity (V_0)

Single fibers (1.5–5 mm long) were dissected from skinned muscle samples, placed in relaxing solution (pCa 9), and mounted *via* small stainless steel clamps between a force transducer and the linear motor of the physiological test rig (Scientific Instruments, Heidelberg, Germany). Initial sarcomere length was adjusted to approximately 2.5 μm, as calculated by first-order laser diffraction pattern. Fiber diameter was measured with a dissecting microscope graticule, estimated as the mean of 3 measurements in fibers with non-uniform diameters. We carried out the slack-test protocol as described previously (Sciote and Kentish, 1996), to determine unloaded shortening velocities. For each length change (ΔL), the time it took the fiber to shorten and just redevelop force (ΔT) was measured. The graph of ΔL *vs.* ΔT was fitted with a straight line by least-squares regression, and V_0 was taken as the slope of this line. Data with $r^2 < 0.98$ were rejected.

Electrophoresis

Individual segments of single fibers (Sciote and Kentish, 1996) and samples of whole-muscle biopsies (Sciote *et al.*, 1994) were prepared for electrophoresis as described previously. Discontinuous SDS-PAGE was conducted on 0.75-mm-thick separating gels of 9% (w/vol) acrylamide cross-linked with bisacrylamide (200:1.0, acrylamide:bisacrylamide) and 6.0% glycerol, buffered with 0.75 M Tris at pH 8.8 (Sciote *et al.*, 2001b). Gels were run for approximately 24 hrs and 8000 volt hrs, and silver-stained according to the method of Morrissey (1981). To perform densitometry, we first converted gels into digital images by scanning them wet on a flat-bed scanner and saved as *.tif image files on a Pentium PC computer. Image band area and density were estimated by means of proprietary image analysis software (Quantity One, Bio-Rad Laboratories Inc., Hercules, CA, USA) and quantified as the relative percentage of all measured bands within a single lane. Equivalent amounts of a standard protein sample of myosin extracts from human vastus lateralis muscle were included in each gel. The relative proportions of myosin isoforms I, IIA, and IIX in each standard protein sample were determined by densitometry and hence acted as an internal control for comparison between and among gels.

RESULTS

Electrophoresis

Limb-muscle biopsies which served as controls had relative electrophoretic mobilities of MyHC type I > IIA > IIX isoforms. Whole-biopsy samples from masseter contained variable amounts of the typical isoforms I, IIA, and IIX (Fig. 1). Control markers of human atrium

and fetal muscle were used for the detection of α-cardiac and neonatal MyHC protein, respectively; however, these bands were not seen in masseter or limb-muscle controls. In our glycerol gel electrophoresis technique, the α species migrates more slowly than in other gel systems, as was seen in two previous publications (a,Sciote *et al.*, 2001b). Notably, all heterogeneous masseter fibers contained some proportion of the type I MyHC isoform.

V_0 and Myosin Isoform Content

Masseter fibers measured had a mean diameter (mean ± SD μm) of 53.2 ± 15.341 for type I and 42.7 ± 9.7 for heterogeneous, which differed significantly ($P < 0.05$) in an unpaired two-tailed *t* test. Human masseter fibers revealed highly variable V_0, with several fibers much slower than found in human limb muscle, both here and in previous studies (Fig. 2). Fig. 3 represents a slack test on one of the slowest type I masseter fibers, measured as 0 054 muscle lengths•s^{-1} (L/s). Sixty-five percent of masseter fibers tested were type I and had a similar mean V_0 but much larger variance than their controls (Table). These included 4 fibers with $V_0 < 0.1$ L/s (Fig. 2b), the slowest being 0.027 L/s— four times slower than previously measured in human limb-muscle fibers. The fastest fiber tested was also type I ($V_0 = 1.8$ L/s). Thus, type I human masseter fibers were both much faster and much slower than those from the human limb.

Heterogeneous masseter fibers contained IIA and/or IIX in various proportions with type I, so that about 97% of all masseter fibers sampled contained type I MyHC isoform to some degree. Some heterogeneous fibers co-expressed types I and IIX MyHC, a very rare combination of slow and fast MyHCs, 3 of which (Fig. 2c) were remarkably slow ($V_0 < 0.1$ L/s). Only one homogeneous fast masseter fiber was sampled, a type IIX with V_0 of 1.5 L/s. There was no detectable relationship between the proportion of fast to slow isoforms and V_0 in heterogeneous masseter fibers.

DISCUSSION

In the human masseter, we found type I fibers to have an unusually wide V_0 range and no discernible correlation between V_0 and the proportion of slow/fast MyHC isoforms in heterogeneous fibers, unlike previous mammalian investigations. In addition, some masseter fibers had shortening speeds much slower than the reported range of human type I fibers. The shortening speeds of our control fibers sampled from arm, leg, and abdominal muscles, however, were closely associated to MyHC composition, and also had V_0 similar to those reported previously from human quadriceps and soleus muscles (Larsson and Moss, 1993). Overall, subjects used in this study were relatively young and without effects of aging (Monemi *et al.*, 1999). For subjects from whom biopsies were obtained during jaw surgery, it has been demonstrated that human chewing muscles from this patient population show only modest variations in fiber type distribution and average fiber diameter related to craniofacial form (Daniel *et al.*, 2001) but no intrinsic differences in the fiber types or physiologic capacity of fibers. So, our data from the masseter represent a methodologically valid description of unloaded shortening velocity typical of this muscle, that was not biased by sampling, gender, or abnormal muscle. Yet more recent studies on human skeletal fibers have shown differences in V_0 within the same fiber type in relation to several factors,

including age and history of muscle activity (Bottinelli and Reggiani, 2000), so it would appear that fiber type is not entirely synonymous with function. Widrick *et al.*, (1997) have shown V_0 in gastrocnemius type I fibers to be significantly lower than that of soleus fibers, indicating potential function diversity within a fiber type between muscles. There are at least two explanations: (1) Structural differences in the myosin protein that modify function are not sufficiently resolved by gel electrophoresis; and/or (2) variation exists in other contractile components of the myofibril which affect V_0 to a significant degree. Amino acid sequences of MyHCs are highly conserved, and coding sequences for human I, IIA, and IIX isoforms are now known from samplings of cardiac, extra-ocular, and limb muscles (Weiss *et al.*, 1999). However, muscles that perform highly specialized activities, such as masseter and laryngeal, have not been investigated at the molecular level. The MyHC protein could be modified by alternative mRNA splicing or the expression of undiscovered genes. No evidence exists for the latter, and, in general, differential splicing is not regarded as a feature of the sarcomeric MyHC gene family. However, new research has shown alternate splicing in the 5′ end of the porcine perinatal MyHC gene, a region clustered with repeat elements (Da Costa *et al.*, 2000).

Aside from possible changes in the protein sequence, there is some evidence for differential post-translational modification of I/β murine MyHC based on sequential expression of at least 3 distinct epitopes during fetal development (Maggs *et al.*, 2000). Furthermore, distinctive myosin glycosylation is present in mammalian masseter in some species (Kirkeby, 1996).

Functional variability within fiber types may be due to differences in myosin light-chain (MLC) sequences. In fast rat fibers, the relative proportion of the alkali myosin light-chains MLC1 and MLC3 has been associated with V_0 (Bottinelli *et al.*, 1994b), but this association is less strong in human muscle (Larsson and Moss, 1993). The picture is more complex with masseter in which a large proportion of fibers expresses multiple MyHC isoforms. It is also known that masseter can express MLC1-embryonic (Butler-Browne *et al.*, 1988), although its pattern of association with various heavy-chain isoforms in adult skeletal muscle is unknown. It is therefore possible that MLC substitutions could modify contractile properties in masseter fibers, especially those co-expressing multiple MyHC isoforms.

In conclusion, our results suggest V_0 variability in adult human masseter fibers which does not correlate only to MyHC isoform content. Several fibers also displayed unusually slow V_0. Further investigation is needed for a fuller characterization of phenotypic differences in masseter compared with general skeletal muscle fibers, including profiles of myosin light-chain and protein components that regulate activation.

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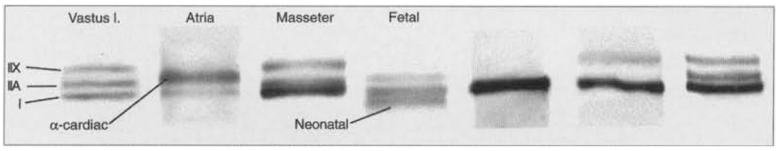

Figure 1.

Electrophoretic separation of MyHC isoforms stained with silver. The control marker samples were taken from the muscles indicated. Unmarked lanes are examples of single masseter fibers after slack tests showing a homogenous type I MyHC fiber and two other fibers with unusual MyHC co-expression. Densitometric scans of these protein bands indicate relative percent content of each myosin species in a given single fiber.

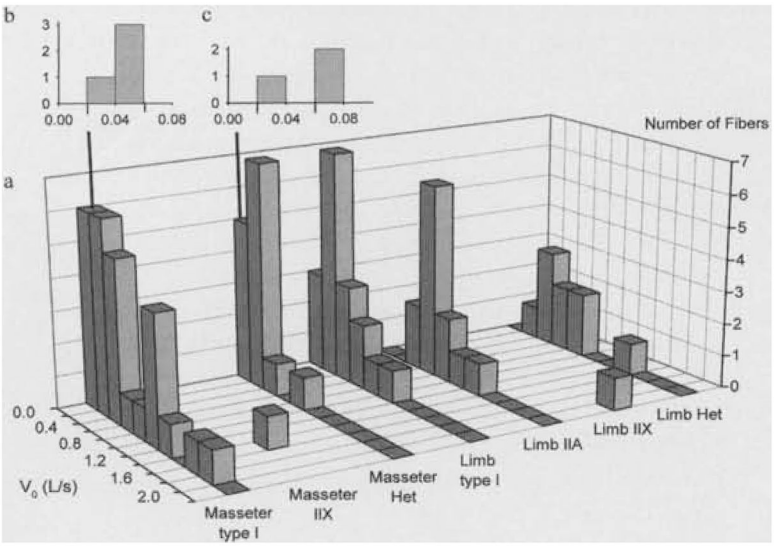

Figure 2.

Summary of V_0 measurements from single skinned fibers in relation to their myosin heavy-chain content: V_0 was determined by the slack-test method, and MyHC content was determined by SDS-PAGE. The 3D plot **(a)** shows V_0 values sorted into bins with an interval of 0.2 L/s. Insets represent the smallest interval (0–0.2) for masseter type 1 **(b)** and masseter heterogeneous **(c)**, further divided into bins of 0.02 L/s. Type I masseter fibers compared with the heterogeneous masseter fibers had significantly different shortening speeds ($P < 0.05$). Likewise, Type I limb fibers compared with type IIA and heterogeneous limb fibers had significantly different shortening speeds ($P < 0.001$). Two-tailed unpaired *t* test. Hence, biochemical and physiologic tests could classify fibers into types which were significantly different.

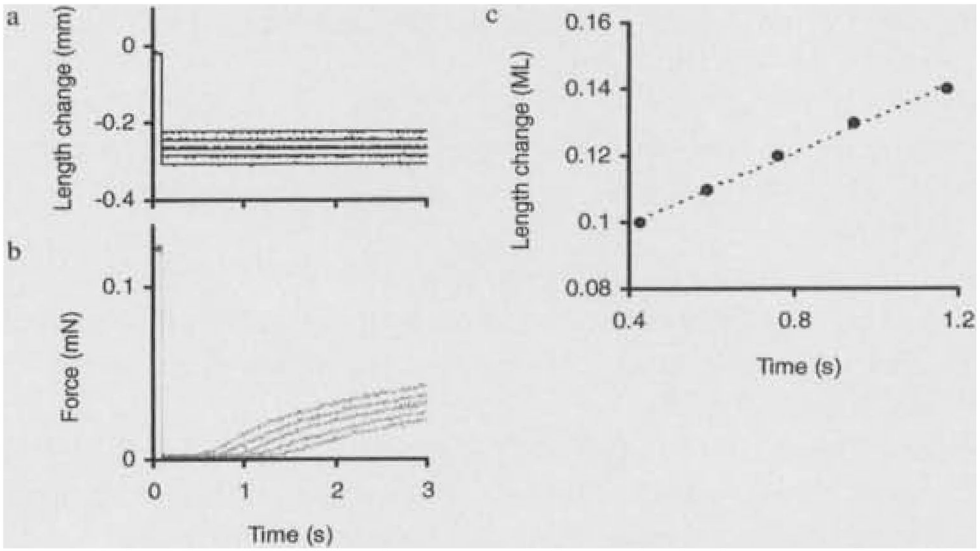

Figure 3.

Time-course plots show changes in muscle fiber length **(a)** and force **(b)** during a slack test performed on a human masseter fiber. **(c)** A length-time regression constructed from the estimated slack periods. Slope = V_0 in units of L/s, determined here as 0,054. A strong correlation to a linear regression ($R^2 > 0.99$) indicates that the fiber was in a viable condition for a value of V_0 to be approximated reliably. Statistical variability is tested for by assessing V_0 at the various muscle lengths, and reproducibility is tested for by the linear regression value.

Table

Myosin Content Correlates with V_0 for Single Fibers, in Limb but not Masseter Muscle

Anatomic Sampling Area	Fiber Type I Mean V_0 ± SD (n) Range	Fiber Type IIA Mean V_0 ± SD (n) Range	Fiber Type IIX Mean V_0 ± SD (n) Range	Heterogeneous Fibers Mean V_0 ± SD (n) Range
Limb muscles	0.460 ± 0.286 (18)	0.794 ± 0.225 (12)	2.22 (1)	0.726 ± 0.356 (9)
	0.125 – 1.141	0.555 – 1.307	-	0.281 – 1.520
Masseter muscle	0.590 ± 0.511 (26)	-	1.50 (1)	0.288 ± 0.219 (14)
	0.027 – 1.820	-	-	0.039 – 0.891