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Scientific Section

Skeletal Muscle Function and Fibre Types: the Relationship Between Occlusal Function and the Phenotype of Jaw-closing Muscles in Human

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Abstract. *Mammalian skeletal muscle cells are composed of repeated sarcomeric units containing thick and thin filaments of myosin and actin, respectively. Excitation of the myosin ATPase enzyme is possible only with presence of Mg-ATP and Ca^{2+}. Skeletal muscle fibres may be classified into several types according to the isoform of myosin they contain. Nine isoforms of myosin heavy chain are known to exist in mammalian skeletal muscle including type I, IIA, IIB, IIX, IIM, α, neonatal, embryonic, and extra-ocular. Healthy adult human limb skeletal muscle contains type I, IIA, IIB, and IIX myosin heavy chains. The jaw-closing muscles of most carnivores and primates have tissue-specific expression of the type IIM or 'type II masticatory' myosin heavy chain. Adult human jaw-closing muscles, however, do not contain IIM myosin. Rather, they express type I, IIA, IIX (as in human limb muscle), and myosins typically expressed in developing or cardiac muscle. The morphology of human jaw-closing muscle fibres is also unusual in that the type II fibres are of smaller diameter that type I fibres, except in cases of increased function and hypertrophy.*

This paper describes the relationship of fibre types and motor unit function to changes in human occlusion and masticatory activity.

Index words: Fibre Type, Motor Unit, Muscles of Mastication, Myosin, Physiology.

Refereed Scientific Paper

General Description of Cell Function

Skeletal Muscle Structure

Skeletal muscles consist of bundles of multinucleated cells, called muscle fibres, which lie side by side and run roughly parallel to the line of muscle action. Striated muscle fibres range from 5 to 100 μm in diameter and vary greatly in length, from 1 or 2 mm up to several centimetres, often spanning the entire length of the muscle. Along the centre of each fibre, occupying most of the intracellular space, are protein filaments called myofibrils. Consequently, nuclei and mitochondria are forced to peripheral locations in the cytoplasm (or sarcoplasm). Each myofibril comprises a continuous chain of contractile elements called sarcomeres. These are the basic units of muscle function, each capable of generating a vectoral force when activated. Sarcomeres are directionally aligned and work co-operatively, so that when muscle contracts tension develops along the axis of the myofibrils and, hence, along the muscle fibre itself. The hierarchy of this organization is illustrated diagrammatically in Figure 1 (for a general introduction to muscle structure and function see (Fawcett, 1994; Aidley, 1998).

More than a century ago, light microscopy revealed the distinctive banding pattern of striated muscle, an effect that comes from the alignment of sarcomeres in register across the myofibrils. Electron micrographs of fibres in longitudinal section show sarcomeres clearly demarcated by a dense region called the *Z line,* from which extends a light area consisting mostly of 'thin filaments' of the protein *actin*. These interdigitate with 'thick filaments' of the protein *myosin*, shown by a darker area called the *A band*. The lighter central portion of the A band is called the *H zone*. Cross-sections of the region between two A bands, the *I band*, profiles only of actin filaments, whereas that of the H zone profiles only myosin filaments. On close inspection, electron micrographs of sarcomeres reveal small projections, termed *cross bridges*, extending out from the myosin filaments. It is now well established that muscle contraction occurs as a result of interaction between cross bridges and actin filaments.

Substructure of Myofilaments

The actin filament, known as F-actin, looks like two strings of beads twisted into a two-stranded helix, each a polymer of globular monomeric molecules called G-actin (diameter 5·5 nm). Tropomyosin, a filamentous protein molecule, is wedged into the groves formed by the actin helix, and has

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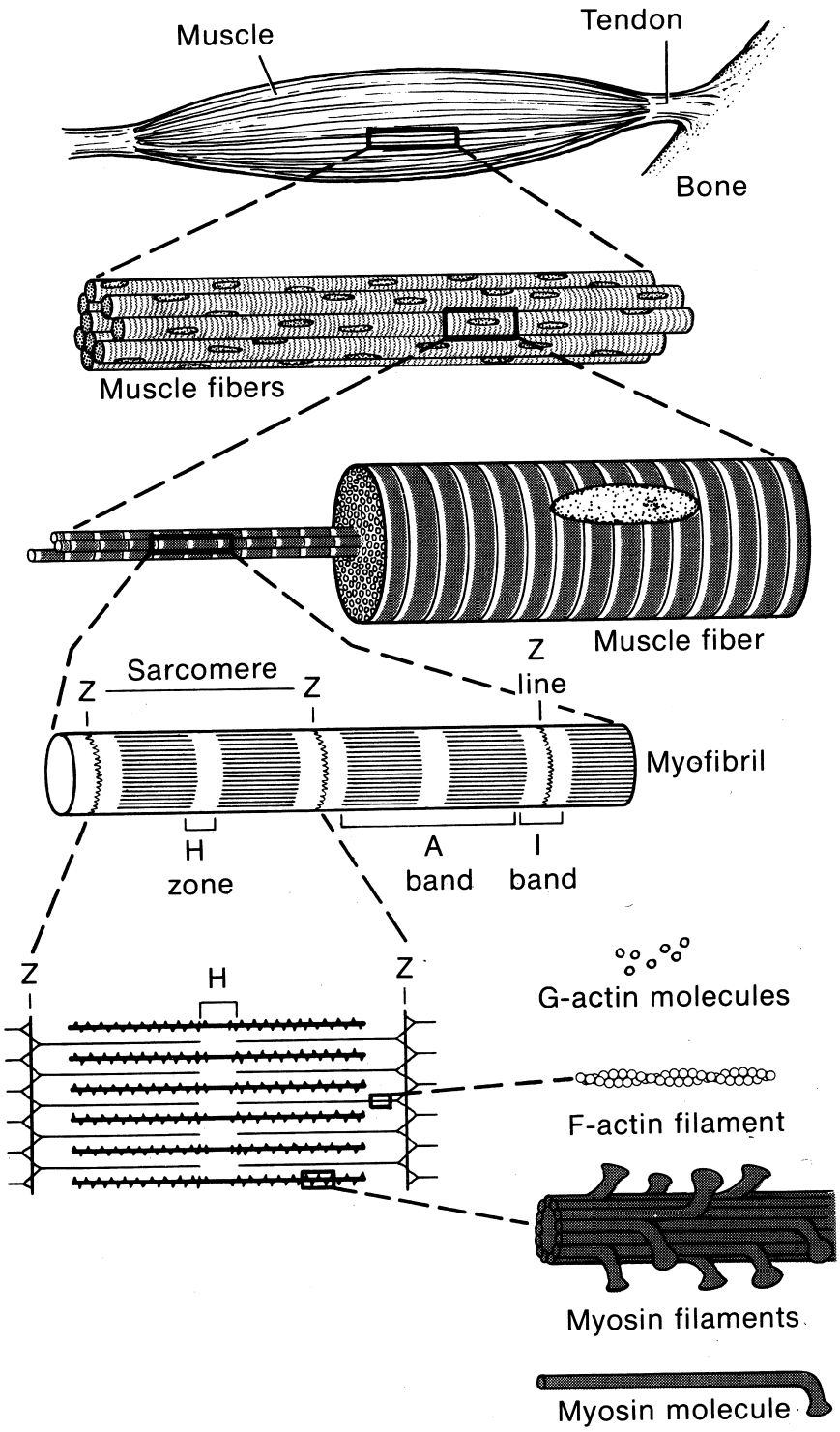


FIG. 1 Hierarchy of skeletal muscle organization (Bloom and Fawcett, 1968).

attached at intervals complexes of globular protein molecules, collectively called *troponin.* Monomeric myosin resembles a club with a slightly elongated head, approximately 150 nm long and 2 nm thick, whose 'shaft' is a twisted pair of peptide chains that can be divided into neck and tail sections. When treated with the proteolytic enzyme trypsin, myosin splits into two parts, *light meromyosin* (LMM) and *heavy meromyosin* (HMM). LMM constitutes most of the tail region, whilst HMM splits into subfragments after further enzymatic digestion: S1, the globular head, and S2, the neck. The head is a globular composite formed from ends of the main polypeptide plus several shorter polypeptides, the so-called myosin light chains. The head is of particular interest since it contains all the enzymatic and actin binding sites of the molecule. Light chain isoforms differ in muscle fibre types and appear to influence ATPase

activity of the myosin and shortening speed of the fibre. *In vitro*, myosin monomers spontaneously polymerize into thick filaments, in which the tail portions overlap and orientate toward the centre and the heads towards the ends. They are so arranged that myosin heads project out and spiral around the thick filament at repeating intervals of 14·3 nm.

Sliding Filament Theory

The micro-architecture of striated muscle is a superlative example of form predisposing function. During the stretching or contraction of a muscle, sarcomeres are observed to undergo relative changes in length. Based on such observations, H. E. Huxley and A. F. Huxley independently proposed the *sliding filament theory of muscle contraction* (Huxley & Hanson, 1954; Huxley & Niedergerke, 1954). According to this hypothesis, when a muscle contracts, the thick and thin filaments slide past each other. Thus, as actin filaments extend into the A band, the H band contracts and the Z lines move closer together. Thereafter, Huxley (1957) postulated the first precise model of the sliding filament theory in which each cross bridge acts as an independent tension generator that undergoes cycles of attachment, pulling, detachment, and reattachment when muscle cells are activated. For maintained contraction, cross bridge activity must be asynchronous, so that at any instant some myosin heads are attached to actin, while others are not. Two important assertions in his model were, first, the position of the active site on the myosin head oscillates by thermal agitation and, secondly, that the proximity between any pair of actin and myosin binding sites determines the probability of cross bridge formation or dissociation. Thus, during contraction as the distance between a site pair closes and the binding rate constant suddenly increases, making cross bridge formation likely. The bound complex is then pulled towards the centre of its displacement by elastic restoring forces in the myosin head, where the disassociation rate constant now predominates and the link breaks. The principle events presented in Huxley's cross bridge cycling model have stood the test of time, with theoretical refinements in detail of the mechanism (for a historical development see especially Huxley, 1969; Huxley & Simmons, 1971; Eisenberg & Greene, 1980; Geeves, 1991). Notably, the relationship between sarcomere length and the development of tension in activated muscle, that was predicted in the sliding filament model, was later verified experimentally (Gordon *et al.*, 1966). Assuming that a single actin-myosin cross bridge produces incremental force independently, tension should be proportional to the distance of filament overlap, since the number of available sites for cross bridge formation increases linearly with this distance. Figure 2 shows the relationship between tension and sarcomere length in an activated muscle fibre. Tension is seen to increase linearly between 3·65 and 2·25 μm, achieving a maximum typically between 1·85 and 2·25 μm when actin and myosin filaments overlap completely. If the fibre is stretched so that there is no overlap at all, tension becomes zero, whereas tension decreases steeply at maximal contractions, when myosin filaments impact and deform against the Z line. The model further predicts that during an isometric contraction (when the muscle is activated and develops tension without shortening) filament sliding occurs until elastic components are fully stretched, but then cross bridge activity persists because there is always some dissociation of actomyosin and links are constantly remade. This accounts for the consumption of ATP during isometric contractions. In isotonic contractions (where activated muscle is permitted to shorten), there is continuous movement of cross bridges past the point where dissociation occurs, so that on average the dissociation rate is higher. This allows faster cross bridge cycling and, therefore, a greater amount of energy is released. As shortening velocity increases the time window for cross bridge formation gets smaller, but because the binding rate of actin and myosin is finite the probability of cross bridges forming falls. Furthermore, since the dissociation rate also is finite, some cross bridges remain intact past their position of zero tension and are pulled by elastic forces in the 'wrong' direction, producing a *negative* force. As a result of these two effects, the faster a muscle shortens the less tension it develops.

Cross Bridge Function

One of the principal concerns in muscle research is the precise functioning of the cross bridges. The myosin head is known to have considerable flexibility due to elastic elements in the molecule (Burgess *et al.*, 1997). The current position is that contractile force arises from a large conformational change in the attached head of the myosin molecule, either in the binding angle made with actin (Huxley, 1969) or within the head itself (Rayment *et al.*, 1993). For cross bridge cycling to occur Mg-ATP and Ca^{2+} must be present. The myosin head is an ATPase, which is activated allosterically by the formation of the actomyosin complex. Interestingly, although the energy for each cycle comes from the splitting of ATP, kinetic studies have shown that the actomyosin complex rapidly dissociates when myosin binds ATP. Free myosin then hydrolyses ATP to form a stable myosin-products (ADP and Pi) complex, available to recombine with actin. Both kinetic studies of actin and myosin in free solution as well as fibre experiments have shown that transduction of the energy released by ATP hydrolysis into contractile force occurs during product release rather than the hydrolysis itself.

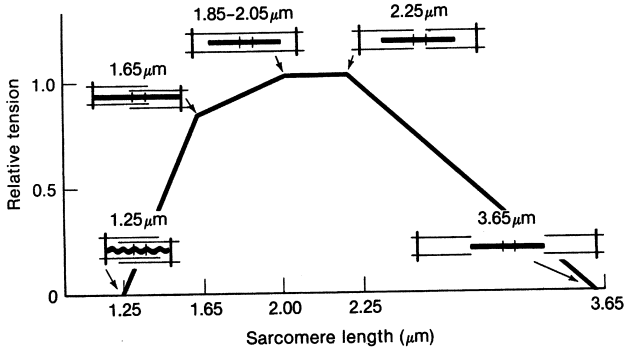


FIG. 2 Length-tension relationship of the sarcomere shown at critical points on the curve. *In-vivo* sarcomere length is generally constrained within the plateau region (Gordon *et al.*, 1966).

Calcium in Muscle Contraction

The important role of calcium ions in muscle activation was heralded by the work of Sidney Ringer and Dudley W. Buxton in the late nineteenth century. They found that preparations of frog heart cease to beat when calcium is absent from the bathing saline. The relationship between sarcoplasmic free calcium concentration ($[Ca^{2+}]_i$) and muscle contraction is now firmly established, and it is clear that calcium is active at extremely low levels. For example, in skinned skeletal muscle fibre preparations, where the sarcolemma has been stripped away to expose the naked myofibrils, the effect of $[Ca^{2+}]_i$ on contractile force can be measured directly. As shown in Figure 3A, tension rises sigmoidally from zero at a calcium concentration of about 10^{-8} to a maximum at about 5×10^{-6} (Hellam & Podolsky, 1969). The shape and position of the $[Ca^{2+}]_i$-force curve may vary slightly, reflecting differences in the range and degree of calcium sensitivity of the muscle fibre. This characteristic relationship between $[Ca^{2+}]_i$ and force closely resembles that between $[Ca^{2+}]_i$ and the rate of ATPase activity of homogenized myofibrils (Figure 3B). Calcium switches on the contractile machinery by binding with high affinity to four sites on the troponin complex, a trimeric assembly of protein subunits spaced at regular intervals along actin and tropomyosin filaments (Ebashi *et al.*, 1980). In the relaxed state, when $[Ca^{2+}]_i$ is very low, the arrangement of troponin and tropomyosin is such that they sterically impede the attachment of crossbridge heads to myosin binding sites on actin (see Figure 4). During muscle activation, as $[Ca^{2+}]_i$ increases it binds with the troponin C subunit, inducing a conformational change in troponin. In this configuration tropomyosin is pushed aside, allowing cross bridges to form.

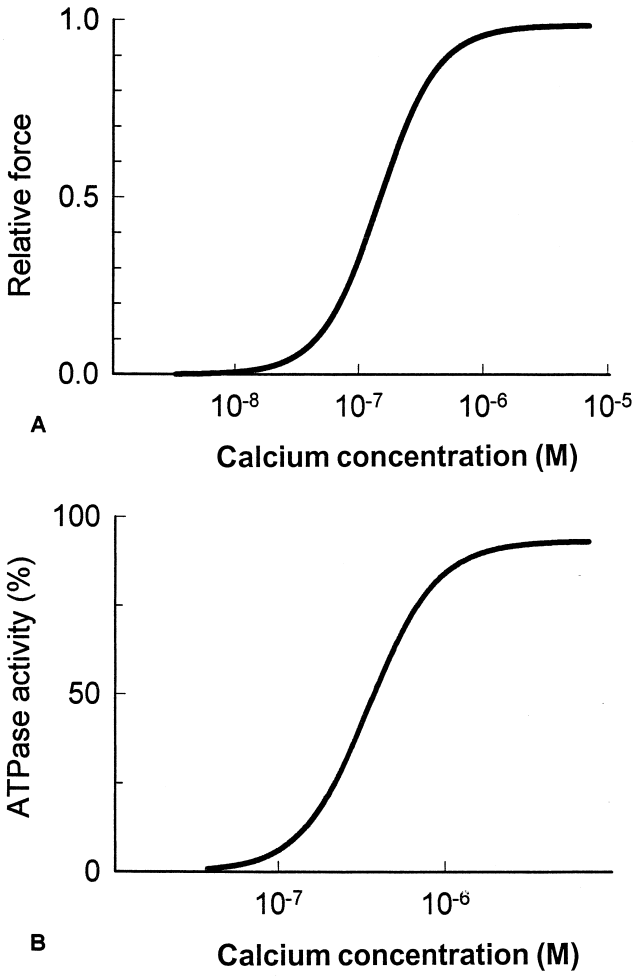


FIG. 3 (A) The relationship between calcium concentration and force recorded during a skinned fibre experiment (Hellam and Podolsky, 1969). (B) ATPase activity as a function of calcium concentration (Bendall, 1969).

Skeletal Muscle Fibre Types

The organization of connective tissue, the compartmentalization of fasicles and the geometric arrangement of origin and insertion to bone are important aspects of muscle organ function. Yet the principal determinant of movement and performance is controlled by actin-myosin interaction in the sarcomere. Skeletal muscle cell fibres can be systematically classified into types since typically in any given muscle cell, the majority of the sarcomeric thick filaments contain the same isoforms of myosin. Myosin is a large molecule composed of six amino acid chains. Two myosin heavy chains (MHC; molecular weight of each about 200,000 kDa) (Gazith *et al.*, 1970) and four myosin light chains (17–23 kDa; Perrie & Perry, 1970; Lowey & Risby, 1971). The myosin light chains may be divided into two groups, the alkali or essential chains and the regulatory light chains. The myosin heavy chain is both a structural protein and an enzyme. It enzymatically hydrolyses ATP and is therefore the key factor in determining the nature of excitation-contraction coupling and movement (Barany, 1967). The exact role of the myosin light chains in muscle contraction is still uncertain. In rat limb muscle the myosin light chains appear to modify the shortening speed of muscle fibres (Moss *et al.*, 1990; Bottinelli *et al.*, 1994), but in human skeletal muscle this effect has not been demonstrated (Larsson & Moss, 1993). There is general agreement, however, that the heavy chains determine the force–velocity characteristics of skeletal fibres, and in some specific muscles of mammalian species the light chains modify these characteristics. Given this, skeletal muscle fibres may be typed by describing the isoform of MHC they contain.

In mammalian skeletal muscle nine MHC isoforms have been identified. β/I, α, extra-ocular, neonatal, embryonic, IIA, IIB, IIX, and IIM. These proteins come from a large gene family, which at present is not completely described. All of the genes for the skeletal muscle MHC proteins have been characterized by cDNA and/or genomic cloning, and are found in two chromosomal clusters. (For an extensive review, see Weiss & Leinwand, 1996.) Protein and mRNA descriptions have also been made. (For an extensive review see Schiaffino & Reggiani, 1996.) The main isoforms in adult skeletal muscle are β/I, IIA, IIB and IIX. β and I are used to name the same slow contracting MHC with β designating its presence in heart muscle and I in skeletal muscle. The three 'type II' MHCs are fast contracting isoforms with average fibre diameter and shortening speed relative to each other as IIB>IIX>IIA. Embryonic and neonatal MHC isoforms are typically expressed during muscle development. IIM or 'type II masticatory' MHC has

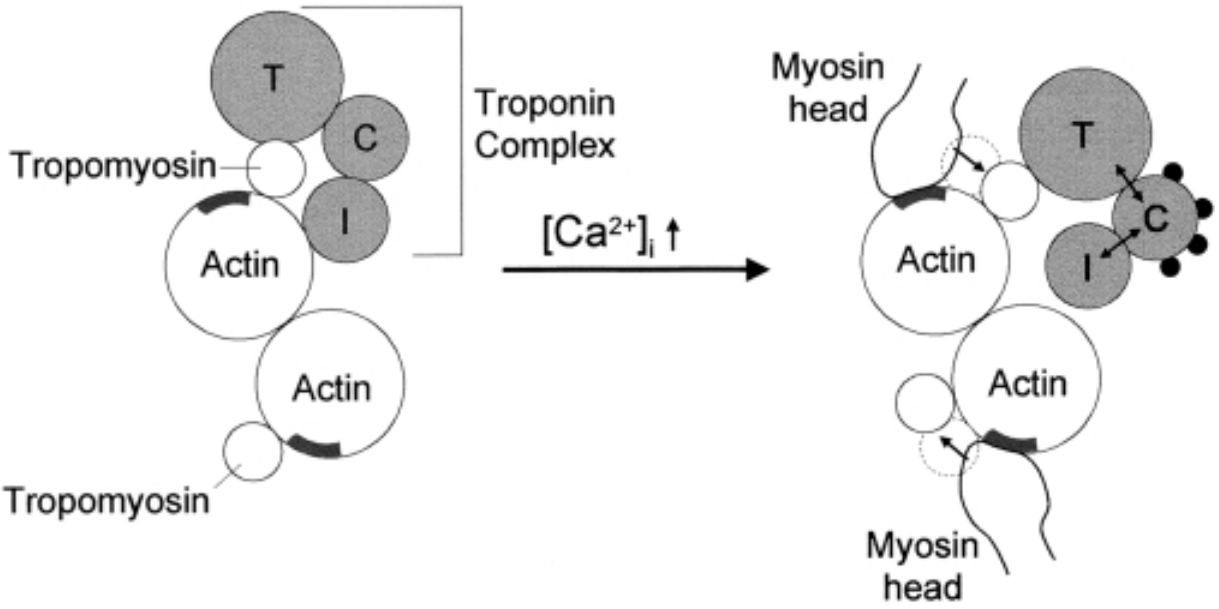


FIG. 4 The troponin complex, idealized in cross-section under resting conditions (left) and during activation (right) when $[Ca^{2+}]_i$ is high after (Ebashi *et al.*, 1980).

tissue-specific expression in the jaw-closing muscles of carnivores and primates (Rowlerson *et al.*, 1981, 1983) with its shortening speed described as 'super fast' relative to limb muscle myosin (Hoh *et al.*, 1991). Extraocular MHC has been identified in the extra-ocular (Sartore *et al.*, 1987) and laryngeal muscle (DelGaudio *et al.*, 1995). This isoform is almost always termed 'extra-ocular', since it was first identified in eye muscle, but in the otorhinolaryngology field another term has been used, 'type II-L' or type II—laryngeal, since it is the main MHC in the posterior cricoarytenoid muscle. The speed of shortening of these eye and laryngeal muscles is known to be faster than jaw-closing muscles, but this does not necessarily relate directly to the contraction speed of the extra-ocular myosin since (at least in extra-ocular muscle) embryonic, neonatal, α, and a slow-tonic MHC are also found (Lynch *et al.*, 1994). Finally, α is a fast-contracting MHC expressed in cardiac muscle. It also has rare tissue-specific expression in skeletal muscle where it is sometimes termed 'α-cardiac'. This isoform is of particular interest since it has been identified in both human and rabbit jaw-closing muscle (Bredman *et al.*, 1991; Sciote *et al.*, 1994). At present, no pleasing physiologic explanation is given as to why jaw-closing muscles of these two species need α-cardiac myosin for normal function. Although a fast contracting myosin in heart muscle, rabbit α-cardiac fibres in masseter have a shortening velocity slower than IIA fibres (Sciote & Kentish, 1996). Two likely explanations for this difference are: (1) there are differences in thick filament isoform arrangements in skeletal versus cardiac cells. Alternatively (2), there are small differences in protein sequence of the α-MHC isoform in cardiac versus skeletal muscle. Further molecular work is necessary to resolve these differences. So at present the reported comparable shortening speeds of adult skeletal myosins is extra-ocular > IIM > IIB > IIX > IIA > α-cardiac > I. There are many other characterized proteins and related genes present in the sarcomere, that contribute to the contractile processes. These include actin, tropomyosin, troponin T, troponin I, troponin C, C protein, H protein, M protein, capZ, titin, α-actinin, nebulin, and others, which have also been extensively reviewed (Schiaffino & Reggiani, 1996).

Given sequence conservation between myosins of different mammalian species and a large knowledge base regarding MHC isoform function, comparison of fibre type characteristics between different skeletal muscles in a large number of species has been conducted using a variety of techniques. These techniques include: myofibrillar ATPase activity assessed by histochemical staining or protein enzmye assays; antibody reactivity for specific myosin isoforms from immunohistochemical staining, ELISA assays and western blotting; histochemical staining to determine metabolic enzyme levels; SDS-PAGE and two-dimensional electrophoresis to identify both quantitative and qualitative protein differences; morphometric description of cell organelles by electron microscopy; *in situ* hybridization using nucleic acid probes; X-ray diffraction measurements; *in vitro* motility assays of actin and myosin; and physiologic description of force and velocity at the level of the single fibre, small fibre bundle, motor unit or whole muscle. In dentistry, whole muscle contractile properties of masticatory muscles have been extensively studied in human and animal models by two techniques, namely bite force measurements and EMG. Orthodontists have used these particular techniques to investigate craniofacial growth, stability of teeth or facial bones after clinical repositioning and neuromuscular responses to bite-modifying appliances.

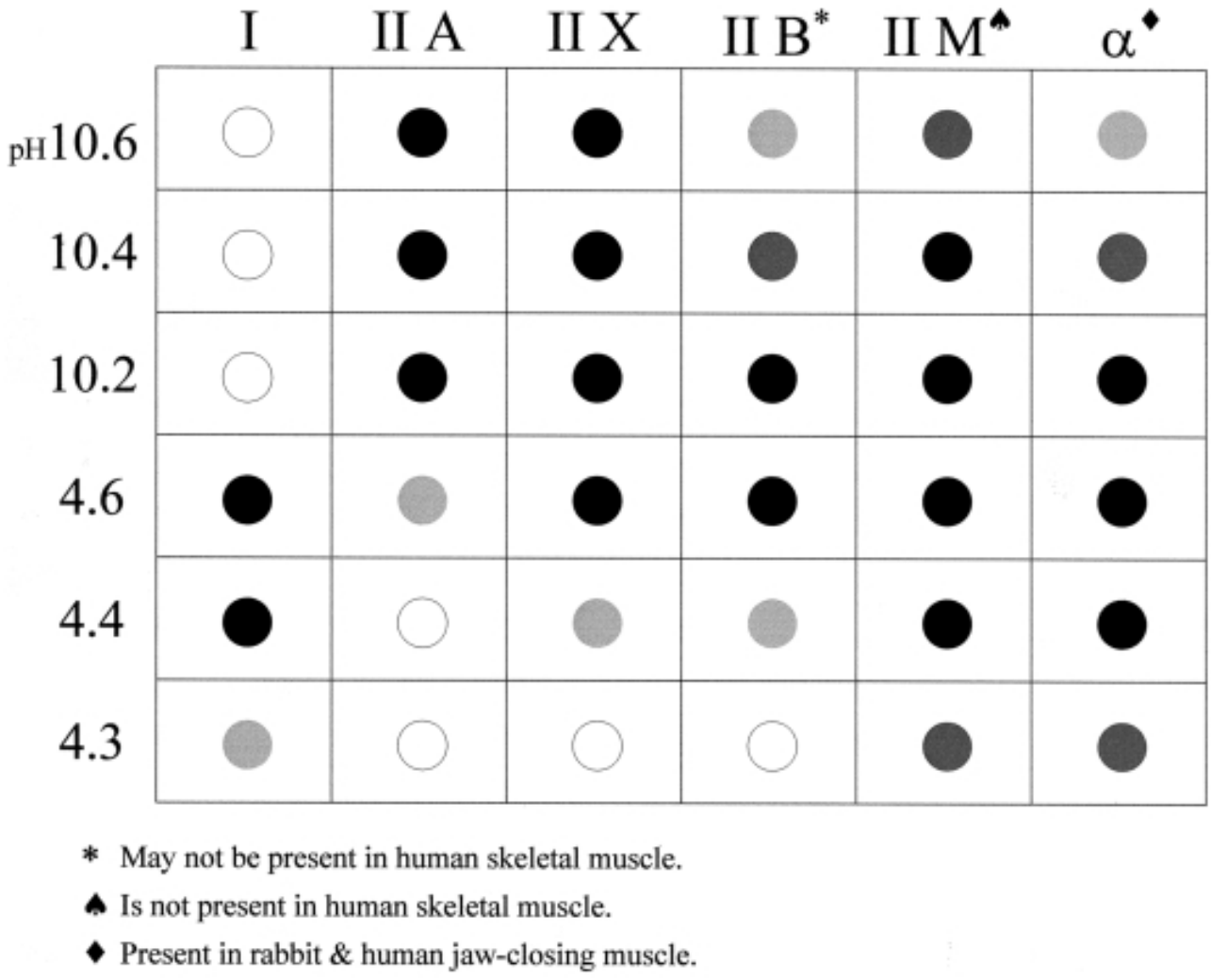
A further complication to characterizing skeletal muscle fibre types is that in many instances one or more of these techniques have been combined in attempts to give a more complete description of fibre types. Sometimes these combinations have had the intended effect, but the various methods of fibre typing are not always comparable. Since

the clinical interest in orthodontics is masticatory force and oral function, this review will summarize ATPase histochemical staining and immunohistochemical staining of muscle tissue sections comparing masticatory skeletal muscle to limb or somatic skeletal muscle.

Fibre Typing by ATPase Histochemistry

Barany (1967) demonstrated that if myosin is extracted from skeletal muscle and subsequently activated in the presence of actin, the amount of ATPase activity produced is directly proportional to the speed of shortening of the muscle from which the myosin was taken. Subsequent to this finding and as the nature of fast and slow isoforms of myosin became apparent, histochemists began developing assays to distinguish different fibre types. Since ATPase activity is ubiquitous in living organisms, myofibrillar ATPase stains which determine sarcomeric activity only were developed. There are a variety of myofibrillar ATPase stains, but in general they work by labelling inorganic phosphate precipitate when myosin hydrolyses ATP in the presence of Ca^{2+}. The stain is performed on frozen unfixed muscle sections since fixation destroys myosin enzyme activity. The Brooke & Kaiser method (1970) is in common usage today since it may distinguish fibre types by staining intensity (Table 1). When first developed this method distinguished the slow type I fibres from all of the fast fibres at pH 9·4, since the fast fibres had much higher ATPase activity. They later found that the ATPase activity of the different fast isoforms was pH specific such that pre-incubating muscle in acid or alkali buffers before histochemical staining activity could distinguish between type IIA or IIB fibres (Brooke & Kaiser (1970). Enzyme activity of II A fibres tends to be more stable in alkali pHs and IIB in acid pHs. The Brook and Kaiser method became widely utilized as a diagnostic tool for muscle pathology in human disease. In jaw-closing muscles Rowlerson (1981) first identified the IIM MHC by noticing that IIM fibres have ATPase activity over almost all pH ranges, and were therefore quite distinct from fibres of limb muscle. In general, this systematic classification can

TABLE 1 *Generalized myofibrillar ATPase histochemical profile for mammalian skeletal muscle fibre types (Brook & Kaiser method with modifications)*



be applied across all mammalian muscles, but some variability does exist between species. One particular example is mouse muscle where the acid and alkali reactivity of IIA and IIB fibres is not always consistent (Rowlerson, 1991).

Fibre Typing by Immunohistochemistry

After recognizing that differences in protein isoforms comprise the basis of histochemical classification, investigators developed fibre type-specific antibodies with reactivity for individual isoforms of MHC. The ability to electrophoretically separate isoforms of MHC assisted in the formation of these antibodies, but difficulty arose in specificity, especially in distinguishing between type II MHCs. Antibodies specific for type IIA and IIB were produced, and supported the earlier histochemical classification system. Subsequently, a third major type II MHC and fibre type was identified, the type IIX, and the histochemical classification system has been extended to include this fibre in some muscles (Gorza, 1990; Table 1). cDNA clones and mRNAs from all three type II isoforms have been characterized. *In situ* hybridization in tissue sections using riboprobes prepared from these cDNA clones has been compared to antibody staining, and at least in rat muscle, the hybridization matches antibody staining (DeNardi *et al.*, 1993). However, one presently unresolved issue in human skeletal muscle is the presence or absence of IIB MHC and the associated fibre type. Certainly from histochemical fibre typing, it has been concluded that the IIB fibre is a major component of fast muscle, but subsequent work has shown that the actual isoform is a homologue of IIX, not IIB (Sant'Ana *et al.*, 1997). Separate genes, mRNA and cDNA to human IIB and IIX have been identified, but to date only riboprobes from IIX cDNA have been found to hybridize to human skeletal muscle fibres. So, at present, the most likely conclusion is that earlier histochemical studies misclassified the IIX fibre as IIB. This and future discriminations of specific fibre types are likely to be answered only through the combination histochemical, immunological and molecular techniques. Much physiological information can be gathered about a muscle by a systematic classification of its fibres.

Fibre Type Composition of Masticatory Muscles

The anterior and posterior digastric are the main jaw opening muscles, and have been studied in man (Vignon *et al.*, 1980) and other small and large mammals (Rokx *et al.*, 1984; Rowlerson *et al.*, 1983). In most species studied the majority of fibres present are fast contracting type IIA and or IIB, with relatively few slow contracting type I fibres. Large herbivores tend to have more type I fibres with the cow having the most at about 60 per cent (Scapolo *et al.*, 1981). Human digastric muscles are reported to contain 29 per cent type I fibres and the remaining a mixture of type IIA and IIB (Eriksson *et al.*, 1982). Differences between the anterior and posterior bellies have been reported (for an extensive review see Rowlerson, 1990). The fibre types in digastric are similar to the types found in limb muscle, and in most species the digastric is a typical fast muscle with mixed fibre type populatons. The kinetics of jaw opening is not appreciably different between mammalian species.

Jaw closing kinetics, however, are quite variable between species and accordingly so are the fibre types. Animals with herbivorous diets such as cow, monkey, horse, and sheep contain only slow contracting type I fibres (Mascarello *et al.*, 1979). Small mammals like mouse, rat, and guinea-pig have up to 25 per cent type I fibres, with the remaining being mostly IIA and a small proportion of IIB (Suzuki, 1977; Hiraiwa *et al.*, 1978). Animals like cat, dog, ferret, and fox (often termed 'carnivores') may have from 0 to 50 per cent type I fibres with the remaining being the rapidly contracting type IIM (also termed 'super fast' fibres or type II masticatory). The distribution and percentages of type I fibres in these animals is highly variable between jaw-closing muscles and within compartments of any given muscle. Rowlerson (1983) separated primates into four groups when she characterized jaw-closing fibres. New World monkeys have from 1 to 20 per cent type I fibres with the remainder being IIM. Macaques varied greatly in type I fibres from 10 to 100 per cent with the remainder being IIM. Chimpanzee contained 30–60 per cent I, the remainder being IIM. Man contained 10 to 90 per cent type I and, significantly, *no* IIM fibres. The jaw-closing muscles of primates and carnivorous mammals are distinctive due to the IIM fibre type, but may still be separated functionally due to osteology. The primates have more acute gonial jaw angles and coronoid processes that allow temporalis muscle attachment at a level higher than the occlusal plane. The bones of the face and cranium in most primates are thick and dense, which allow for powerful bite force production. The carnivorous mammals tend to have more obtuse gonial jaw angles and coronoid processes that allow temporalis muscle attachment closer to the level of the occlusion which enables rapidity of jaw closure. Bones of the face and cranium in carnivorous mammals are not always thick and dense. The best example of a carnivorous mammal with relatively thin jaw bones is the tiny South American Opossum, Monodelphis Domestica (Sciote & Rowlerson, 1998).

The special case of man deserves careful description in relation changes in oral function through evolution. It is certain in prehistoric human populations that extreme wear of teeth was the rule. Young (1998)

> . . . cusps tend to play only a brief and transitory role in occlusal relationships. In many human groups the enamel is completely worn off from the occlusal surface of the first permanent molar by the time the second molar has erupted, and that of the second molar is largely gone by the time the third has erupted. Throughout maturity, then, 'normal' occlusal surface is a flat and featureless plane for the members of the population where heavy wear is the general rule. Even where wear is not so heavy and remnants of enamel stand above adjacent areas of work dentine, there is nothing remotely resembling the 'intercuspal position' that modern authors hold out as such an unquestioned ideal (Kraus *et al.*, 1969; Lauritzen, 1974; Thomson, 1975).

In colonial America, it is well known that those with anti-British sentiments often had unworn cusp tips, but were not likely to keep their teeth (most not from their sentiments,

but rather from the diet of the industrialized Western World). George Washington's dentures are commonly photographed, and the fact that Paul Revere made such prostheses is common knowledge to American grade school children. Perhaps the best anthropological description of evolutionary changes in the human dentition come from studies done at the University of Adelaide on Australian Aborigines from the 1920's till present time. Most Aboriginal people have transitioned from a primitive diet at the beginning of the this century to a modern Western diet at the end. Young (1998):

> From aboriginal dentitions, we have learned that functioning teeth wear in a 'natural manner rarely seen in civilized communities' (Barrett, 1958). Interactions between silaceous and phytolithic foods and teeth in motion produce wear patterns that reflect not only food abrasion, but also the habitual orientation and direction of tooth-to-tooth movements at each site in the mouth. Progressive attrition facets enamel, exposes dentin and changes tooth form. Thereby, it increases the efficiency of teeth as tools, enhancing shearing, crushing, and grinding on the occluding surfaces of opposing teeth. Proximal attrition between adjacent teeth preserves the intimate contact of working surfaces in an intact dental arch, an observation with orthodontic implications (Begg, 1954), for interproximal attrition results in a reduction in the perimeter of the dental arch (Campbell, 1925, 1938; Begg, 1954). As a result, the Australian aborigines, living under primitive conditions, rarely had malocclusions in the functional sense (Barrett, 1969). The evolutionary survival value of healthy teeth as tools to the Aboriginal was not lost on the dental anthropologist who observed that despite an entire life's exposure to abrasive food, the teeth were retained in a state of healty vigorous activity (Barrett, 1969). . . . Tooth wear progressively reduces cuspal heights and anterior overbites and permits a freer range of latero-medial and anteroposterior jaw movements than 'centric' occlusion would allow. Occlusal science was liberated from the restricitve myth that the unworn dentition of civilized man was 'ideal'. Jaws do not habitually close into a terminal position of 'balanced' bilateral maximum intercuspation, with no further movement, as simulated by the 'anatomical' articulator (Barrett, 1958), and edge to edge pre-canine occlusion were commonplace.

In contrast to Aboriginal diet earlier in this century, the contemporary Australian human culture seldom uses their teeth (Young 1998):

> . . . an amazing human culture with computer-age technology, biologically poorly adapted to working and sporting on its coastal surfscapes and arid sun-drenched playing fields. Its shell middens are recycled aluminum cans, labeled orthophosphoric, citric, and ascorbic acids. Its fresh-frozen fruits and vegetables are uncontaminated by a grain of sintered sand. Its beefsteak and barbecued prawn are tenderized more by marinade than by searing stone. And these diets require minimal mastication.

The same can be concluded about all people of the modern world. Miyamoto *et al.* (1996) recently studied amount and intensity of masseter activity in young adults by EMG. Almost all high amplitude bursts of activity were associated with chewing during meals. Throughout the entire day (24 hours), however, most bursts were of very low intensity and the total amount of time that teeth could possibly touch was 357 seconds in males and 419 in females. One may conclude that man progressed in evolution from a primate with cusp tips used for function, to a primate who rapidly wore cusp tips down to functioning planes with edge to edge incisal positions common, to modern man who very often lost most teeth due to oral diseases, to contemporary man who does not wear cusp tips (relative to his ancestors) and maintains most teeth due to the dentist's control of oral diseases. The direct causes that have led to the lack of occlusal wear in the contemporary population are still controversial. For certain the size of teeth continues to decrease (Brace & Nagai, 1982), but the persistence of malocclusion and lack of tooth wear may not be caused by genetic changes from natural selection. Brace (1977) proposes that such conditions are the result of relaxed selection pressure (phenotypes that are maintained since their presence or absence has no direct impact on survivability). Such phenotypes also termed 'proximate modification of phenotype' by some physical anthropologists are also related to changes in function. Muscles should reflect such changes in function, and therefore it is not surprising to find that human jaw-closing muscles often appear quite different from other human skeletal muscles or from jaw-closing muscles of other primates.

Human jaw-closing muscles are distinct from other primates in that do not contain the type II masticatory fibres. Human jaw-closing muscles are also distinct from limb muscles due to differences in fibre morphology and myosin isoform expression. ATPase histochemical staining of healthy adult limb muscle demonstrates that skeletal muscle is composed of a mosaic of type I and II fibres, with the type II fibres being of larger diameter (Figure 5). Most of the fibres are homogeneous for a specific myosin heavy chain isoform, although co-expression of myosin heavy chains within a single fibre is possible. Human jaw-closing muscles are composed of a relatively equal mixture of type I and II fibres, but the type II fibres are much smaller than the type I (Ringqvist, 1973; Eriksson, 1982; Thornell *et al.*, 1984). An additional complication is that human masseter may also have a variety of phenotypes that may be specific to individuals. Figure 6 represents ATPase histochemical staining of masseter biopsies of three different individuals. Biopsy A is from a healthy young adult female with no orthodontic problems. The fast type II (dark) fibres are small relative to the slow type I fibres. This is the most typical phenotype for human masseter (Sciote *et al.*, 1994). Biopsy B is from a patient who had a mandibular set-back operation as part of orthodontic treatment. Here, about half of the type II fibres are of diameter typical of human limb muscle. Biopsy C is of a healthy young adult female with no orthodontic problems. Two muscles were stained together; the masseter and the jaw-opener anterior digastric (bottom left of the panel) for comparative purposes. The anterior digastric has relatively normal sized type II fibres and masseter has no type II fibres. These biopsies were taken from the anterior superficial masseter since this area is reported to have the greatest variability in fibre type (Serratice *et al.*, 1976). So in any given patient it may not be

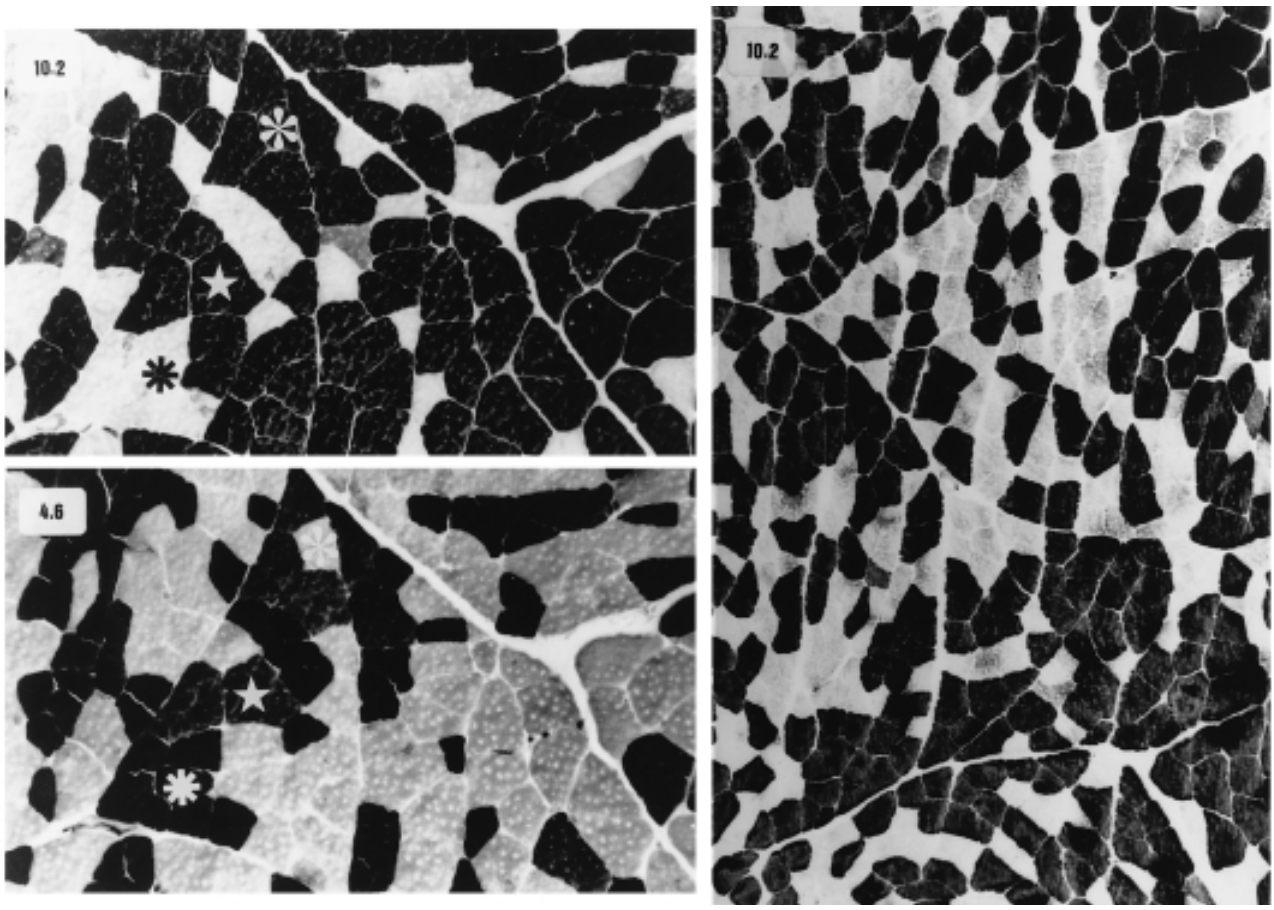


FIG. 5 Human rectus abdominus sections stained for myofibrillar ATPase activity after preincubation in buffers at pH 10·2 and 4·6. In the two serial sections at the left the white ✻ marks a type IIA fiber, the black ✻ a type I fiber and the white ✻ marks a type 'IIB' fiber (as defined histochemically). The low power view at the left shows the typical mosaic pattern of skeletal muscle.

possible to know what the actual percentage and type of fibres present in the jaw-closers.

Myosin isoform expression is also quite unusual for the jaw-closers. In addition to the typical myosin heavy chain isoforms found in healthy adult muscle, masseter at least, may also express neonatal, embryonic, and/or α-cardiac myosin heavy chains (Sciote *et al.*, 1994). Since myosin expression in many of the masseter fibres is heterogeneous for myosin heavy chain, it is very difficult to classify fibres into types with ATPase histochemistry alone. Given this variability and the recent information that IIB myosin heavy chain is not likely to be found in human skeletal muscle, future studies on fibre types in human masseter should include a combination of ATPase histochemistry, immuniohistochemistry, and *in situ* hybridization stains. Here, too, the amount of fibres with these non-adult skeletal muscle myosins varies between individuals. For example, in a biopsy study of 58 human masseters the percentage of α-cardiac fibres varied from 0 to 27 per cent, and neonatal fibres from 0 to 23 per cent (Sciote *et al.*, 1994). Individual variation in fibre types and percentages of fibre types in masseter may be proximate modifications of phenotype that do not have an impact on survivability, but orthodontists must consider if these modifications have an impact on success of clinical procedures. Specifically, does orthodontic relapse of teeth or surgical relapse of bone relate directly or indirectly to an individual patient's skeletal muscle phenotype?

One empirical example of change in muscle phenotype and function is a case report of an adult female with uncontrolled masseter spasm over a period of approximately 10 years. Due to chronic spasm the masseter was visibly hypertrophied. A biopsy from the anterior superficial area of masseter revealed fibres that looked quite normal when compared to limb muscle, but abnormal when compared to masseter! (Figure 7). Fibres were distributed in a mosaic pattern, with most of the type II fast fibres of larger average diameter than the type I fibres. An additional important finding was that there was no expression of the atypical neonatal, embryonic and α-cardiac myosins found in the 'normal' masseters. (This finding was produced by immunohistochemical staining not shown here, but also is seen by consistent staining intensity relative to pH pre-incubation for each fibre type shown in Figure 7).

It seems intuitive that the etiology of the fibre types in human masseter is directly related to dramatic changes in oral function. Other aetiologies have been suggested that may or may not be related to function: (1) jaw-closing muscles are branchial arch derivatives and develop from embryonic tissue distinct from body somites (Bredman *et al.*, 1991). (2) The pattern of nerve impulses influence

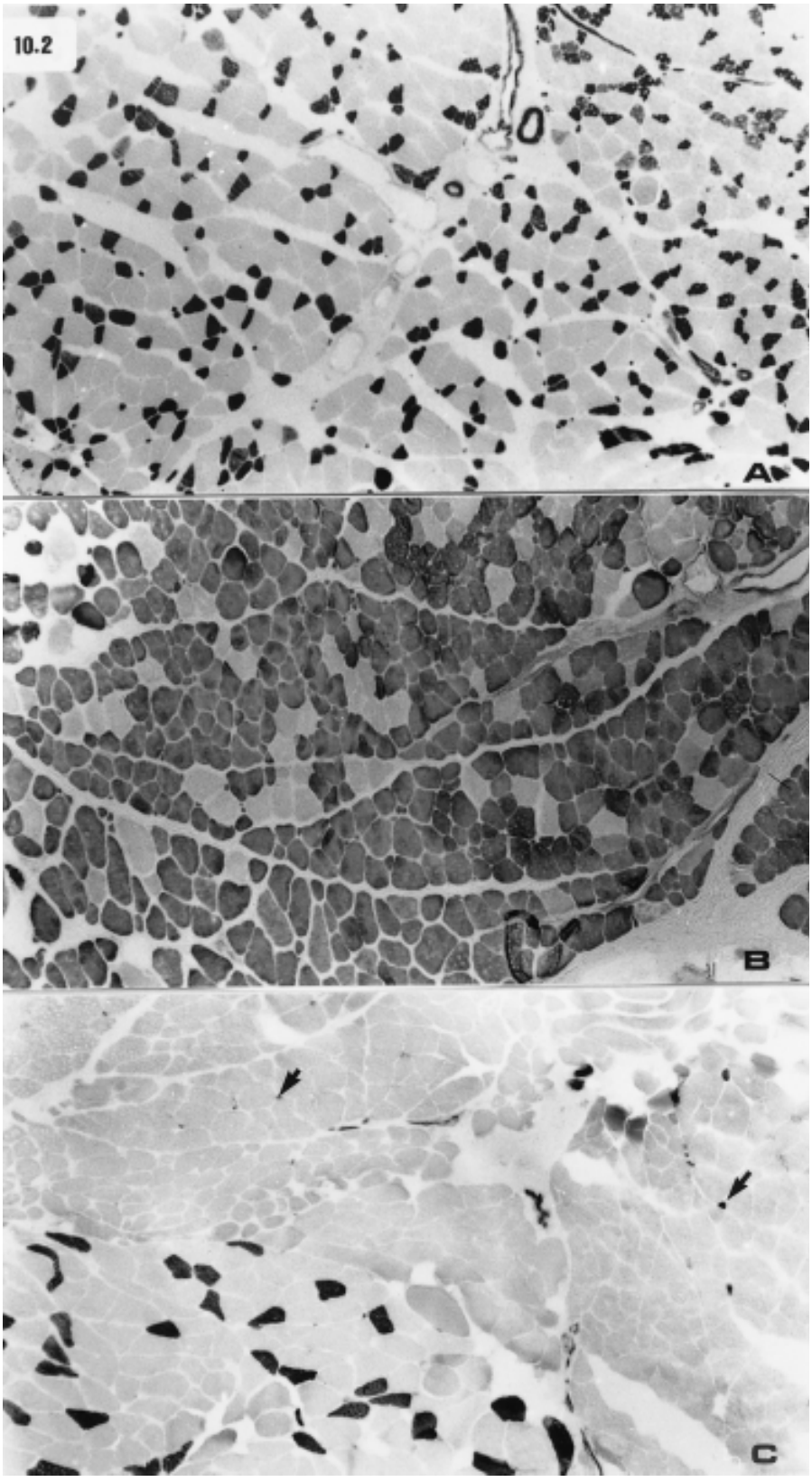


FIG. 6 Human masseter sections stained for myofibrillar ATPase activity after pre-incubation in buffer at pH 10·2 showing the variability in fibre type content between individuals. At this pH the 'type II' fibres are stained dark and the 'type I' fibres are stained pale. Panels A, B, and C are of different patients (see text). The lower left section of panel C is the human mylohyoid muscle.

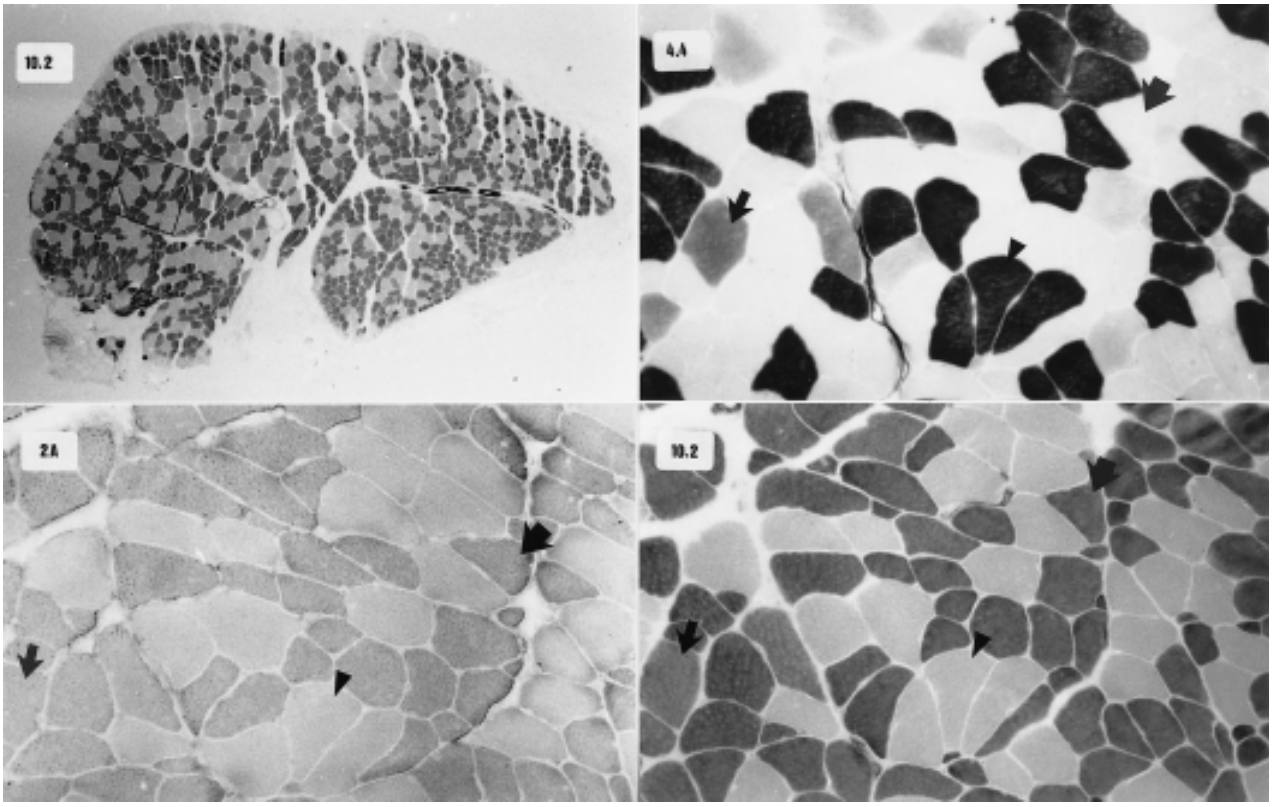


FIG. 7 Human masseter sections of a patient with masseter spasm and hypertrophy (see text) stained for myofibrillar ATPase activity after pre-incubation in buffers at pH 10·2 and 4·4 and for immunohistochemistry with IIA monoclonal antibody. Upper left panel is a low power view of the entire biopsy with the other three panels representing serial stained sections form the outlined square. Large arrowhead is a IIA fibre, small arrowhead is a fibre with heterogeneous myosin expression and the triangle is a type I fiber. Note that the type II fibres are comparable in diameter to similar fibres in rectus abdominus. Immunohistochemical staining was negative for neonatal, embryonic, α-cardiac, and IIM myosin antibodies.

myosin expression; and cranial nerves, rather than spinal nerves, innervate jaw-closing muscles (Soussi-Yanixostas *et al.*, 1990). (3) The period of development for jaw-closing muscle is later and longer, and somehow allows persistence of neonatal and embryonic myosin isoforms in adults (Soussi-Yanixostas *et al.*, 1990). (4) Developmental isoforms of myosin are transiently expressed from stretch-induced hypertorphy of muscle (Kennedy, *et al.*, 1986; Matthews *et al.*, 1990). (5) Finally, developmental isoforms of myosin are expressed at the tapered ends of skeletal fibres due to retention of neurotransmitter hydrolase AchE (Rosser *et al.*, 1995). Opposed to all these theories are experiments in which animal diets were modified to long-term intake of fine-grained foods. Most of these studies investigated changes in craniofacial growth, but in one study that looked at changes in masseter muscle phenotype in mouse, the change in diet was correlated to a decrease in the fibre diameter of fast twitch fibres (Maeda *et al.*, 1987).

Motor Units

Sherrington (1894) established that skeletal muscle fibres were organized into motor units, all of the fibres innervated by one α-motor neuron. The α-motor neuron connects the muscle to the central nervous system, and the motor unit acts as a functional unit in programmed movement and motor control. Sherrington also developed the size principle in motor unit recruitment. Recruitment is the excitation of an α-motor neuron which subsequently activates the motor unit, producing contraction. Motor units vary in size in two fashions, total number of fibres and cross-sectional diameter of the individual fibres. In performance of isometric tasks mammalian motor units are recruited in the order of small to large. This implies that as more force is generated by the task larger motor units are recruited. The sarcomere is the smallest functional unit in the fibre and the motor unit is the smallest functional unit in the muscle.

Later investigations connected the size principle to skeletal muscle fibre types by histochemical staining of tissue sections. This was first possible by the development of the glycogen depletion technique (Edström & Kugelberg, 1968). If a single α-motor neuron is repeatedly stimulated the fibres contained in its motor unit will eventually fatigue due to depletion of glycogen. Using a metabolic histochemical stain on sectioned muscle the fibres with depleted glycogen may be readily identified as belonging to a single motor unit. Serial sections may then be stained for myofibrillar ATPase activity or with antibody staining to identify myosin heavy chain isoform content to determine fibre type. Glycogen depletion animal studies upheld the size principle. Small motor units with small diameter type I fibres were slow-contracting and produced the least force. Larger units, containing IIA fibres, with faster contraction speeds were recruited secondly. The

largest and strongest units, containing IIB fibres were recruited last (Burke *et al*., 1971). All fibre types belonging to the same motor neuron were found to be histochemically similar (i.e. units were composed of all type I, IIA, or IIB fibres and never a mixture of different fibre types; Burke *et al.*, 1973). The same was true for human limb muscles in an interesting study conducted by (Garnett *et al.*, 1979). Even in rapid voluntary contractions in man, fast motor units cannot be preferentially recruited (Desmedt and Godaux, 1977).

Another important finding from the glycogen depletion technique was an understanding of the physical location of motor units in the muscle organ. Motor unit territory is variable in species and specific muscles since these differences are performance related. It is also the case in healthy muscle that fibres of many different motor units occupy the same area of muscle and are intermixed, giving a typical moasic pattern of differing fibre types with histochemical staining (Figure 5). Fiber type clumping, adjacent fibres of the same type and from the same motor unit is indicative of pathological conditions, such as denervation or re-innervation (Karpati & Engel, 1968). Such conditions occur, for example, from sprouting of a single motor neuron, which establishes endplates on regenerating fibres in the same area.

Motor unit characteristics are described in several ways that include: the size of the fibres in the unit; the number of fibres in the unit; the motor unit territory (area and position the unit occupies relative to the total muscle); the force produced by motor unit contraction; and finally the speed of shortening of the fibres in the motor unit. Muscles that require fine, precise motor control usually have many motor units with a small number of fibres in the unit. The best example is the human extra-ocular muscles used in positioning the eye. They are estimated to have 2970 motor units with 9 fibres in each unit (Feinstein *et al.*, 1955). The extra-ocular muscles also have a large representation in the motor cortex (Cushid, 1976). As an example of a relatively large human muscle which requires less precise movement the biceps brachii muscle is estimated to have 3552 motor units with 163 fibres in each unit (Christensen, 1959). The human temporalis is estimated to have 1331 motor units and 936 fibres in each unit and the masseter to have 1452 motor units and 640 fibres in each unit (Carlsöö, 1958). The masseter muscle does have a large representation in the motor cortex (Cushid, 1976) and is known to exhibit very fine movement in some of its functioning, so the size of its motor units were surprising. Since glycogen depletion experiments are not possible in living humans, investigators began applying bite force and electrophysiological measurements to human jaw-closing muscles to further describe motor units. Stalberg and Eriksson (1987) described some of the units in human masseter using these techniques and found most motor unit territory to be relatively small (from 0·6 to 4·5 mm. There were only a few motor units with very large territory in the range of 9·1–12·5 mm. The small motor units were confined to limited areas of masseter, but the few large motor units tended to span almost the whole muscle cross section. Large motor units spanning nearly the whole area of a limb muscle, to the best of our knowledge, have never been found in other studies (Buchthal and Schmall, 1980; Stalberg *et al.*, 1976). It is possible that the small motor units in masseter are used for fine motor functions and the very large motor units to stabilize the entire masseter muscle for balance or when maximal force is necessary. The electrophysiological study by Stalberg and Eriksson (1987), however, is in sharp contrast to the general description given by Carlsöö, (1958), who produced his results from an anatomic study of the innervation ratio. Carlsöö attempted to estimate the total number of α-motor nerves and the total number of skeletal fibres in the entire temporalis and masseter muscles. Stalberg and Eriksson's (1987) description of masseter motor units contained only those recruited to produce low bite forces and do not represent all of the units in the muscle. When similar EMG techniques were combined with magnetic resonance imaging of 162 motor units in human masseter, most of these units were found positioned between tendons and only a few (10 per cent) crossed tendons (Tonndorf and Hannam, 1994). This confirmed the idea that almost all masseter motor units are located in discrete compartments that may produce movement in a variety of directions, and some units cross tendons to stabilize compartments when necessary.

Animal experiments on motor unit territory and recruitment pattern do not necessarily provide answers for the way in which units work in human jaw-closing muscles given the very odd human phenotypes for masticatory skeletal muscle fibres. The only animal model which comes close to representing human jaw-closing muscle fibres is the rabbit, since its fibres express α-cardiac myosin heavy chain (Sciote and Kentish, 1996) and there is heterogeneous expression of myosin heavy chains in some individual fibres (Bredman *et al.*, 1991). In glycogen depletion experiments, which show the three-dimensional relationship of the total motor unit area to the total masseter muscle area for rabbit (Weijs *et al.*, 1993), the 11 motor units studied were restricted to small portions of masseter with total fibre number ranging from 40 to 424. The surprising finding in nearly half the motor unites investigated, however, was that not all fibres were of the same type. This observation directly contrasts Burke *et al.*'s (1973) finding in limb muscle that all motor units are homogeneous for fibre type. Those motor units found to be heterogeneous for fibre type contained a combination of fibre types, either α + IIA fibres or IIAB + IIA fibres. This data strongly suggests that in jaw-closing muscles of some mammals, fibres may be heterogeneous for myosin heavy chain isoforms and motor units may be heterogeneous for fibre type. However, the limited number of motor units examined in this study may not represent the whole muscle. Nevertheless, in general the α motor units had the smallest number of fibres and smallest territory, the IIA motor units had relatively much larger fibre number and territory, and the IIB units the largest fibre number and territory. Recruitment order of these unusual motor units was not described, but given anatomical description of unit size and myosin composition it is likely that rabbit masseter units are recruited in an orderly fashion from small to large and from slowest contracting to fastest contracting.

In human masseter, however, the recruitment of motor units has been investigated with electrophysiological techniques. The recruitment pattern is orderly for the size of the unit, but may not be orderly for the speed of shortening of the units. Yemm (1976, 1977) did find orderly recruitment for motor unit size and bite force, but found either no correlation between size and contraction speed, or a reverse

correlation between size and contraction speed of some units. Yemm suggested that for contraction speed it is quite possible that faster motor units are recruited *before* slow motor units. Goldberg and Derfler (1977) produced results similar to that of Yemm in that there was an orderly recruitment of masseter motor units for size, but no correlation between size and speed of contraction. Both studies confirm the well documented results of fibre typing studies done on muscle sections which clearly show that the fast contracting fibres in human masseter are much smaller than the slow contraction type I fibres. It is also not surprising that a reverse correlation in motor unit recruitment for speed of shortening cannot always be found since myosin expression in masseter fibres is often very heterogeneous. Recent studies have used different EMG techniques to investigate human masseter motor unit recruitment (Scutter and Turker, 1998), but very little additional information regarding recruitment order and speed of contraction have been obtained.

The size principle was first proposed for gradually increasing isometric contraction, but muscle contraction in living animals is not always isometric. Even in muscles that can be studied for isometric contraction, the same motor unit may have different recruitment thresholds for flexion versus extension (ter Harr Romeny *et al.*, 1982). Muscles that move bone in various directions do have consistently different motor unit recruitment based on the direction of force production. (Thomas *et al.*, 1978; Desmit, 1977; Schmidt and Thomas, 1981) Such observations have often been termed 'task specific behavior of motor units'. Ericksson *et al.*, (1984) have described such behaviour in human masseter and English (1985) confirmed that not all the motor units in a muscle are active during contraction. The idea that there are subpopulations of motor neurons that will respond differentially to directional movement is an exception to the size principal for both jaw-closing and limb muscles, but for reasons different than fibre type composition of the muscle. Task specific behaviour is observed in multifasiculated muscles that do perform a variety of patterned movements (Freund, 1983). Such muscles also have very sophisticated afferent input to their motor neuron pools that help modify the excitability of motor neurons controlling the motor units (Kanda *et al.*, 1977; Luescher *et al.*, 1979). In the case of human masseter this afferent information is produced principally by highly complex muscle spindle arrangements (Rowlerson, 1990) and peridontal afferents from the tooth ligaments (Linden, 1990).

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