Functional and molecular outcomes of the human masticatory muscles

FUNCTIONAL AND MOLECULAR OUTCOMES OF THE HUMAN MASTICATORY MUSCLES

Running title: Functional characteristics of the jaw muscles

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ABSTRACT

The masticatory muscles achieve a broad range of different activities such as chewing, sucking, swallowing and speech. In order to accomplish these duties, masticatory muscles have a unique and heterogeneous structure and fibre composition ~~of their fibers~~, enabling them to produce their strength and ~~speed of~~ contraction speed largely dependent on their motor units and myosin proteins that can change in response to genetic and environmental factors.

Human masticatory muscles express unique myosin isoforms, including a combination of thick fibers, expressing myosin light chains (MyLC) and myosin class I and II heavy chains (MyHC) -IIA, -IIX, α-cardiac, embryonic and neonatal and thin fibers, respectively.

In this review, we discuss the current knowledge regarding the importance of fiber-type diversity in masticatory muscles versus supra- and infrahyoid muscles, and versus limb and trunk muscles. We also highlight new information regarding the adaptive response and specific genetic variations of muscle fibers on the functional significance of the masticatory muscles, which influences craniofacial characteristics, malocclusions or asymmetry. These findings may offer future possibilities for the prevention of craniofacial growth disturbances.

AUTHOR CONTRIBUTIONS

All authors contributed to the development and successive revision of the drafts~~,~~ and reviewed the final version of the manuscript.

CONFLICT OF INTEREST

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INTRODUCTION

The stomatognathic apparatus achieves physiological activities, including mastication, deglutition, and speech; among these functions, mastication is the most ancestral function with a higher specialization (Horio and Kawamura, 1989). Mastication is defined as a rhythmic, highly co-ordinated neuromuscular function that involves, in each of its acts, all components of the stomatognathic apparatus, mainly executed by the masticatory muscles (Lewin, 1985). This mechanism, crucial for a correct trituration of food for digestion, is achieved by the co-ordination of the masticatory muscles and by a nervous control, which fundamentally allows an exertion of precise and strong occlusal forces (Horio and Kawamura, 1989).

To perform these functions, the masticatory muscles present a specific structural and heterogeneous fiber type composition, with fibers responsible for producing a wide range of contraction and force. The contraction speed of a fiber is related to the ATP production in the myofilament, which is composed of actin and myosin protein and can change in response to genetic and environmental factors (Schiaffino *et al*, 1994).

Several studies over the years have investigated the biomolecular processes that are responsible for the different functional types of muscle fibers and several classifications of the pathways have been documented to identify the functional properties underlying the phenotypes and plasticity of masticatory muscle fibers.

Based on the possibility of an interrelation between form and function, masticatory muscles have been investigated in individuals with different vertical craniofacial characteristics and malocclusions or asymmetry. Considering the importance of the ability of masticatory muscles to adapt their composition to various stimuli and conditions, the aim of this review is to broadly summarize how this functional significance influences craniofacial characteristics and particular adaptations of the muscles during pathological conditions, such as malocclusions or asymmetry.

MOLECULAR ASPECTS OF THE MASTICATORY MUSCLES

Masticatory muscles myosins

Human masticatory muscles are divided ~~branched~~ into elevator muscles (temporalis, medial pterygoid and masseter) and depressor muscles (digastric, lateral pterygoid, geniohyoid and mylohyoid) (Gans and Gaunt, 1991).

Masticatory muscles are skeletal muscles and are made up of individual cells known as muscle fibers. Muscle fibers contain myofibrils, which are the actual force generators. Myofibrils are composed of a series of sarcomeres, the functional units of muscle contraction. The sarcomere, the basic unit of the muscle, consists of thick filaments that are

largely composed of actin, myosin, troponin, and tropomyosin which, together with the regulatory proteins, troponin and tropomyosin, determine its functioning and contraction though ~~a~~ biomolecular synergy (Van Eijden and Turkawski, 2001).

The structure of the sarcomere is comparable between the different muscle categories of the human body; however, the sarcomere possesses different isoforms of contractile proteins (Fig. 1), which specifically determine the variations of muscle contraction for a specific group of muscles (such as the masticatory muscles). The mechanical properties of the muscle arise from the interaction of the filaments of myosin and actin (Schiaffino and Reggiani, 1994), which determine, the contraction speed through its cross-bridge structure~~, the speed of contraction~~ (Korfage *et al*, 2005a). However, the physiological activities of the masticatory muscles, are mostly dependent on their motor units, a single blend of motoneurons that branch into the muscle fibers with the neuromuscular junctions. The motor unit can be defined as the *functional contractile unit* of the muscle because it determines the size and the precision of the contraction force under various stimuli (Van Eijden and Turkawski, 2001).

Myosin isoforms

During the last few decades, several classifications of myosin isoforms have been developed. One of the first was the distinction of myosins due to their white or red color~~, white or red~~, and subsequently to their contraction speed ~~of contraction~~ as slow and fast fibers (Schiaffino *et al*, 1994). However, recently, an advance in fiber typing has been the classification of sarcomeric myosin.

The sarcomere is a complex structure composed of four light-chain (*MyLC*) myosin isoforms (molecular weight of 18-22 kilodaltons), divided into *regulatory* and *essential* MyLCs, and two heavy-chain (*MyHC*) myosin isoforms (molecular weight 190-225 kilodaltons) (Korfage *et al*, 2005a). In adult human muscles, three MyHC isoforms have been subsequently identified, which are correlated with the myosin ATPase based classification system. The slow twitch MyHC isoform I (S units) is present in type I fibers. The fast-twitch myosin isoform, MyHC-IIA (fatigue-resistant, FR, units) is present in -IIA fiber type ~~-IIA~~, and MyHC-IIB is present in -IIB fiber type ~~-IIB~~ (fast fatigable, FF, units). Both the myofibrillar ATPase activites and the MyHC based fiber composition are important for~~to~~ the contractile capacity of the muscle. For muscle contraction, *motor units* are recruited from the slower, *fatigue-resistant* fibers (I subtype) to the ~~faster~~ quicker *fast-fatigable* fibers (IIB subtype) (Sciote *et al*, 2003).

Moreover, with ~~a~~ newer monoclonal antibodies, it is possible to detect and further classify a third muscle fast fiber~~,~~ the "hybrid" type, characterized as expressing two or more mixed myosin isoforms ~~of myosin~~.

In fact, this *hybrid* MyHC fast type of myosin possesses a different composition compared to IIA and IIB fibers. These structural differences have prompted the classification of this myosin, using specific monoclonal antibodies, such as IIX sub-type (Schiaffino *et al*, 1988 and 1989). However, the identity of IIX MyHC fibers, though having been characterized by western blot analysis (LaFramboise *et al*, 1990), wasn't recognized as a specific myosin isoform due to a translational modification by other myosin isoforms. In fact, conclusive evidence was needed tohat showed a specific and different MyHC-IIX transcript of this isoform (DeNardi *et al*, 1993): DeNardi et al (1993) demonstrated that the motor unit constituted by MyHC-IIX presents similar properties (such as the relaxation time) of MyHC-IIA or -IIB ones. presented both half-relaxation and time properties comparable to units composed of MyHC-IIA and MyHC-IIB, with, hHowever, a fatigue resistance of MyHC-IIX is intermediate to that of the units MyHC-IIA and MyHC-IIB (Larsson *et al*, 1991).

In samples of animal muscle (rat), this hypothesis was confirmed because it was shown that type MyHC-IIX type fibers possess succinate dehydrogenase (SDH) staining (Larsson *et al*, 1991; Schiaffino *et al*, 1989), MyHC-IIX possesses and an intermediate contraction speed intermediate between fibers with a motor unit composed mainly of MyHC -IIA and -IIB isoforms (Bottinelli *et al*, 1991 and 1994). Immunohistochemical and biochemical analyses performed on these single muscle fibers showed that the functional properties of muscles are determined by their ATP activity (Bottinelli *et al*, 1994; Pette and Staron, 1990). The production of the ATP supply the energy for the contraction speed for all fibers, from the slowest (type I) to the fastest (IIB) (Bottinelli *et al*, 1996). IOn this way, a fundamental role is played by the hybrid fibers whichthat, depending on functional demands, can switch into a specific fiber form (slow or fast) optimizing the total enegy consumptiom of the muscle (Korfage *et al*, 2005b).

To start and sustain rhythmic movement during mastication, fiber types -I -II are liable for the majority of the production of force, especially in the absence or reduction of the number of teeth (Stal *et al*, 1994; Cannavale *et al*, 2013; Isola *et al*, 2017a). In the early phases, the first recruited fibers are the slow type (I), andthat are responsible for the greatest force production. Subsequently, when the force continues to increase, more fatigue- resistant (IIA) fibers are called up in addition to type I fibers; moreover, when great force is necessary, the fast- fatigable fibers (IIB) are recruited by the central pattern generator of the brain, responsible for the mastication pattern (Lewin, 1985; Piancino *et al*, 2017). The slow fibers (I) which are activated, that are first activated, first, show a high resistance to fatigue. However, when there is the necessity for extremely quick and great force, the *fast fatigable* fibers (IIB) are recruited. Through their anaerobic pathway (compared to type I), they can produce very great force, if only for a short time, and contribute to the precise movement of the mandible during mastication (Korfage *et al*, 2005 a and b). Additionally, muscle possesses a third type of fiber, the *fatigue-resistant* (IIA) type, that exhibits functional properties which

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intermediate between ~~the~~ types ~~,~~ I and -IIB. This sub-type of fiber permits a moderate amount of force to be produced for a prolonged period, because of their moderate anaerobic capability (Stål *et al*, 1994; Korfage *et al*, 2005 a and b).

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Myosin isoforms genes

Eleven sarcomeric genes that encode for MyHC were found in the genomes of mammals (Fig. 2, *A*), preserved in the evolution of vertebrates, ~~, and~~ each one coding for a specific isoform of MyHC (Berg *et al*, 2001). These MyHC isoforms ~~of MyHC~~, in ~~in~~ humans, are determined by a family of genes, with a clustering of two genes on chromosome 14 and six genes on chromosome 17, each one in two separate loci (Weiss *et al*, 1999).

In humans, genes that encode for the MyHC isoform are, respectively, MyHC -I, -IIA, -IIX, -IIB, -cardiac α, -extra-occular (normally present in the pharyngeal and extrinsic eye muscles), -fetal (called also -developmental or -neonatal) and -embryonic MyHC (Weiss and Leinwand, 1996). Human masticatory muscles can express all of these MyHC isoforms, as well as the -cardiac α isoform that is usually present in the heart (atrium), as well as also the -fetal isoform that is normally expressed in skeletal muscles during development (Butler-Browne *et al*, 1988; Monemi *et al*, 1996; Korfage *et al*, 2000). However, not every gene that encodes for MyHC isoforms is usually represented by translated protein isoforms. For example, the mRNA that encodes for the -IIB isoform in the human masseter muscle is abundantly expressed, although its cognate protein is detected in low concentrations. Moreover, three~~3~~ other isoforms, such ~~i e.,~~ as MyHC -M, -15 and -slow tonic, are exclusively found in some craniofacial and neck muscles, encoded by MYH16, MYH15 and MYH7b respectively (Horton *et al*, 2001).

The human masticatory muscles that specialize largely in jaw movement (Matarese *et al*, 2016, Cavuoti *et al*, 2015), i.e., the masseter, pterygoideus lateralis, and medialis, temporalis, mylohyoideus and digastricus, derive from the first branchial arch, presenting a common embryological origin (Schiaffino and Reggiani, 1994). However, their fiber arrangements and physiology are related to diet, craniofacial characteristics and eating and food habits, and are greatly variable between different species (Toniolo *et al*, 2008).

In many mammals and carnivores species (not human beings), distinct MyHC-M fibers encoded by a specific gene, ~~the~~ MYH16, have been detected in masticatory muscles (Rowlerson *et al*, 1983; Qin *et al*, 2002). The fibers are possessing this gene exhibit a contractility marked by a higher force and a minor lowering of the fast speed, which in turn is more useful for reaching a higher power level during chewing (Toniolo *et al*, 2008). However, Stedman *et al*, (2004) showed that in human beings there was an inactivation of the MYH16 gene due to a frameshifting mutation after evolution from chimpanzees and humans.

The inactivation of MYH16 in masticatory muscles in humans appears to be related to a strong reduction in the size of everyach single masticatory fiber and the whole volume of jaw muscles (Fig. 2, *B*). Stedman *et al* speculated that this frameshifting mutation startedappeared roughly 2.5 million years ago, when the transmigration of homo sapiens from Africa appeared, in association with the evolution of the human body, appeared (Walker and Leakey, 1993; Vekua *et al*, 2002; Stedman *et al*, 2004).

Evidence of ancient lineage divergence between chimpanzees and humans seems also seems to be related to the reduction of the size of masseters and temporalis due to a variation inof the morphology of the zygomatic arch and temporal fossa (Qin *et al*, 1994; Stedman *et al*, 2004). The reduction in the capacity of masticatory muscle contractions would have had a pleiotropic effect on the total craniofacial morphology in MYH16-null human ancestors. An intriguing theory suggests that the inactivation of MYH16 may be related to the possibility that this reduction in the volume of chewing muscles contributed as a stimulus for increased encephalization in Homo Sapiens (Wu *et al*, 2007; Yu *et al*, 2002; Stedman *et al*, 2004; Kang *et al*, 2010) (Fig. 2, *B*, a and d compared to g). In accordance, Stedman *et al* (2004) showed that the volume of the skeletal muscle fibres expressing the MYH16 gene transcript is proportional to the total amount of heavy chain myosin accumulating in the cell. Therefore, a frameshift mutation in MYH16 has resulted in an eightfold reduction in the size of the type II fibres in the human masticatory muscles as in comparison with the macaque monkey (Stedman *et al*, 2004). Moreover, studies of myostatin signalling demostrated that a genetic manipulation of muscle size has marked secondary effects on the anatomy of bony attachment sites (Hamrick *et al*, 2000). It was also likely demonstrated that diminished contractile force would translate into a reduction in stress across patent sutures, sites of dura-mater-patterned growth in the immature neurocranium (Warren *et al*, 2003).

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Functional significance of myosin composition of the masticatory muscles

There are some differences in the myosin composition between each masticatory muscle. The masticatory closer muscles possess a more composite architecture than the openers. They are multi-pennate and complexly layered, with many intramuscular aponeuroses, while the supra- and infrahyoid muscles show the opposite characteristics (Van Eijden *et al* 1997; Haviv *et al*, 2017). The fibers of jaw closing muscles are relatively short, while their attachment areas are relatively large. In addition, because of their broad attachment areas, the masticatory closer muscles can produce differential mechanical actions, such as chewing (Van Eijden *et al*, 1997). These functional characteristics allow the masticatory closers to produce broader force with a reduced speed compared to the supra- and infrahyoid muscles, and this is mainly due to their different myosin composition (Schiaffino and Reggiani, 1994; Bottinelli *et al*, 1996).

A comparison of the composition of fiber types between the elevator and depressor masticatory muscles demonstrated that the jaw closers present 75% type -I fibers (slow), while this percentage is reduced (by 40%) in the jaw-openers (Korfage *et al*, 2000) (Fig. 3, *A* and *B*). The jaw-closers exhibit 45% of ~~the~~ hybrid fibers, while the jaw-openers only contain 15%. Moreover, ~~al~~most of the hybrid fibers of the masticatory muscles express the -fetal (15%) and-cardiac α (25%) myosin isoforms, uniquely, among the human skeletal muscles (Korfage *et al*, 2000).

In order to explain these variances in the composition of the fiber types, many suggestions have been made. The jaw closer muscles possess a specific structure that allows them to produce greater force compared to the jaw openers that are specialized for mandibular~~the~~ movement ~~of the mandible~~ (Van Eijden *et al*, 1997). ~~.~~ This property~~is~~ is~~is~~ reflected by the composition of the fiber type because the masticatory closers present a slower contraction and a higher resistance while chewing food compared~~respect~~ to the jaw openers. For these reasons, the jaw closers contain a higher composition of type -I fibers while the jaw openers contain type -II fibers (fast, especially type -IIA). A higher percentage of slow fibers make the masticatory closing muscles slower muscles, which in turn enable them to perform more tonic and deliberate activities with a production of a continuous, gradual force compared to the masticatory opening muscles, more suitable for producing phasic, fast, and precise jaw movement (Van Eijden *et al*, 1997; Korfage *et al*, 2000).

Korfage *et al*, (2000) showed that the temporalis muscle presented fewer hybrid fibers and a high quantity of type -I and -IIA compared to the medial pterygoid and the masseter. This suggests that the temporalis muscle acts as slower muscle in comparison with other closing muscles and that it possesses more resistance due to the presence of a high composition of type -I fibers. The temporalis muscle is also~~presents also~~ a short length compared to the medial pterygoid and the masseter, with a shorter moment arm that reflects the possibility of producing ~~a~~ greater force during chewing or grinding (Van Eijden *et al*, 1997).

Moreover, ~~there~~ masticatory muscles also present~~s~~ ~~also~~ *intramuscular* differences in ~~the~~ fiber composition, and this is more evident in the closing muscles. The deep masseter contains a large~~great~~ amount of type -I fibers compared to the superficial masseter, while the posterior portion of the masseter possesses more type -IIB fibers compared to the anterior one (Eriksson, 1982; Korfage *et al*, 2000). Therefore, it appears that the anterior masseter fibers are more able to produce a ~~a~~ greater force which is helpful during chewing and~~,~~ especially during grinding (Monemi *et al*, 1996).

The above-described intramuscular differences appear to be associated with the morphology of the cranium and with the particular muscular actions during jaw movements (Blanksma et al, 1997). The temporalis attachment in the cranium makes the anterior portion more widely used than the posterior portion during jaw-closure (Van Eijden et al,

1997). Therefore, it appears that the anterior portion is the most efficient in motor tasks. This is in accordance with the results obtained by electromyographic studies that indicate that the anterior portion of the temporalis is more active than the posterior portion during chewing. This is true as well for the deep portion of the masseter, which is more active during chewing than the superficial one (Blanksma, 1995; Korfage and Van Eijden, 2003; Piancino *et al*, 2008).

Masticatory versus muscles of limb and trunk

Some notable differences were found between the masticatory and limb and trunk muscle of the human skeleton.

Compared to the muscle of the limb and trunk, the masticatory muscles possess a higher percentage of hybrid fibers (Korfage *et al*, 2005b), which express many myosin sub-types such as -cardiac α and -fetal isoforms. Moreover, compared to the muscles of limb and trunk, the masticatory muscles present more type -I, II and fetal MyHC isoforms. More specifically, in the masseter, type I fibers are more numerous ~~fewer~~ than in the muscles of the limb and trunk (Morris *et al*, 2001; Osterlund *et al*, 2011). These differences also include the expression and composition of the MyLC, i.e., compared to limb and trunk muscles, the masseter presents a higher expression of four different MyLC-1emb/atrial, -1f, -1s and -2s, that determines the tonic regulation of the fibers. Conversely, the muscles of limb and trunk express MyLC-2f -3f, which- is absent in the masseter (Soussi-Yanicostas *et al*, 1990; Stål *et al*, 1994; Ferlazzo *et al*, 2017).

Regarding the volume of- single fibers, the masticatory muscles present smaller fibers compared to the muscles of the limb and trunk (Korfage *et al*, 2005b). Compared to masticatory muscle, the limb and trunk muscles exhibit ~~the~~ type -II fibers which are larger in diameter compared to type -I (Polgar *et al*, 1973). This suggests that, in the masticatory muscles, the high presence of type -I fibers with a small cross-sectional area (CSA) ~~may be useful for the masticatory muscles and~~ could facilitate the greater exchange of nutrients and O_2 with the extracellular environment, increasing fiber resistance to fatigue, particularly in type -II fibers (English *et al*, 1998; Korfage *et al*, 2005b). Moreover, these differences in the volume of the fibers were related to a post- translational adaptation of type -I or due to the mutation of the MYH16 gene (Stedman *et al*, 2004).

Role of muscle specific Integrins

-Integrins are heterodimeric membrane proteins that are the interface between cells and between the cell and the extracellular matrix (ECM), and they also mediate cell-matrix adhesion (Hynes, 1992; Belkin *et al*, 1996). In muscle

fibers, these interactions are essential for muscular development, innervation, and pattern (Ervasti *et al*, 1990; Yoshida *et al*, 1994).

Each integrin is composed of α and β subunits, noncovalently linked. Among all subunits involved in the muscles, it is noted that the α7β1-integrin, located at myotendinous neuromuscular junctions, possesses an important role in muscle speed and contraction (Martin *et al*, 1996). In fact, suggested proof of the importance exerted in the maintenance of the skeletal muscle physiology by the α7β1-integrin was that the mutations of the α7 gene are associated with many congenital myopathies in humans (Hayashi *et al*, 1998).

The role of integrins was studied in the jaw muscle. Sinanan *et al*, (2008) in an in vitro study, showed that αv subsets of integrins (αvβ3 and αvβ5) mediate~~ing~~ cell-matrix and intercellular interactions, also regulate~~regulating also~~ cell adhesion and motility. Favaloro *et al*, (2009) analyzing biopsies of masseter muscle of -α~~alpha~~ and non-α~~alpha~~ chimpanzees showed that the α7A and β1A integrin isoforms play an important role in muscle regeneration, with the intriguing hypothesis that the MYH16 gene could negotiate the expression of integrins, determining in turn, muscle phenotype. Moreover, Cutroneo *et al* (2012), in a study on human biopsies of masseter muscles of subjects affected by unilateral posterior crossbite demonstrated that the amount of α7A and β1A integrins were significantly reduced in the crossbite side compared to the corresponding counterpart not affected by the crossbite malocclusion. These results obtained in humans lead to the intriguing hypothesis that the presence of a malocclusion may be influenced by the composition and arrangement of the muscle fibers.

THE HUMAN MASSETER MUSCLE

The masseter muscle is a composite masticatory multi-pennate closing muscle~~,~~ that grows in parallel with jaw-face skeletal growth and remodelling, and with the dental eruption. Masseter muscle morphology was ~~has been~~ previously studied in the different stages of human life; ~~throughout the human lifespan, i.e.,~~ prenatal (Barbet *et al*, 1992), postnatal (Bontemps *et al*, 2002), puberty (Vignon *et al*, 1980), adult (Korfage *et al*, 2000) and during old age (Monemi, 1999).

Structural aspects of the masseter muscle

The masseter is defined as a multipennate muscle with superficial, intermediate and deep fibers with two or usually three~~3~~ heads that originate from the zygomatic arch (Gans and Gaunt, 1991). The pennation provides more mechanical assets and a possibility of producing a higher contraction force, similar to the medial pterygoid.

The human masseter possesses insertions, caudally, on the posterior border of the mandibular ramus and the angle of the mandible. The muscle is composed of four groups of internal aponeuroses which arcthat are internal and associated sagittally, divided into septa along its length, from the mandibular border to the zygomatic arch (Lam *et al*, 1991). The pennation determines a variety of movement proportional to the vector of pennation (commonly with an angle of 20°) and the length of the muscle fibers (Raadsheer *et al*, 1994; Van Eijden *et al*, 1993).

The muscle fibers are divided into superficial, intermediate and deep portions.

The *superficial* fibers have an insertion area of about 2 mm on the mandibular angle with their main attachments on the edge of the mandibular angle (Gaudy *et al*, 2000). According to some authors, some fibers of the superficial portion of the masseter are in contiguity with the fibers of the medial pterygoid muscle. This would allow the muscle to have a "sling effect" which would help in the closing of the mandible (Lang, 1995; Gaudy *et al*, 2000); however, there is still no unanimous consensus on this. The superficial portion of the masseter muscle exerts pressure at a right angle to the posteriorly ascending occlusal plane of the molars.

The *intermediate* fibers of the masseter, shaped like a "fan" with a lower apex, originateing from the medial and central portion of the zygomatic arch. It terminates with insertions at the level of the outer face of the mandibular ramus, superiorly to the *superficial* fibers. It has been shown that with advancing age some fibers migrate to the deep muscle portion (Gaudy *et al*, 2000).

The *deep* portion of the masseter muscle, similar to the "fan shape" potion of the intermediate one, originates from the lower edge of the inferior third of the zygomatic arch with two portions, front and rear. These move vertically and down above the intermediate portion (Williams, 1995; Gaudy *et al*, 2000).

The contraction force of the *superficial* portion of this muscle results in elevation, contralateral movements, and mandibular protrusion. The *intermediate* and *deep* portions of the masseter allow the retrusive mandibular movements together with the action of the pterygoid muscles, which also favoursing contralateral and ipsilateral movements during elevation of the mandible (McMillan and Hannam, 1991).

The *volume* of the human masseter is about 140– 235 mm^3, smaller in females compared to male subjects. Thisese data waserе obtained by different techniques (3D ultrasound data, Bellington *et al*, 1999: for Computed Tomography -CT- data, Xu *et al*, 1994; for Magnetic Resonance Imaging -MRI- data, Boom *et al*, 2008), with a CSA of about 40-60 mm^2 for males and of 20-35 mm^2 for female subjects (Kitai *et al*, 2002). Usually, the volume of the masseter fibers is linked with human body characteristics such as height, (Raadscheer *et al*, 2004), body mass index (Satiroglu *et al*, 2005),

weight (Raadscheer *et al*, 2004; Raadscheer *et al*, 1996) and craniofacial characteristics, such as the facial type (Kitai *et al*, 2002; Isola *et al*, 2016 and 2017b; Matarese *et al*, 2017).

Moreover, the thickness of the masseter has been correlated, in males, with the height of the mandibular ramus (Kubota *et al*, 1998), the thickness of the alveolar process (Kubota *et al*, 1998) and the thickness of the mandibular symphysis (Kubota *et al*, 1998). In females, the thickness of the masseter was correlated with a maxillary intermolar width (Kiliaridis *et al* 2003), bizygomatic facial breadth (Raadsheer *et al*, 1996), and short vertical face height (Satiroglu *et al*, 2005; Raadsheer *et al*, 1996, Isola *et al*, 2015). Additionally, a negative correlation between the thickness and the volume of the masseter has been shown with some craniofacial characteristics such as the presence of a long facial type (Kiliaridis *et al*, 1991), anterior facial height (Raadsheer *et al*, 1996) and mandibular length (Raadsheer *et al*, 1996).

Masseter muscle myosin and functional significance

The functional significance of masseter hybrid fibers

The human masseter ~~participates~~ is involved in various tasks that require a multiplicity of forces and at different contraction speeds. In order to fulfil such tasks, it presents a large~~high~~ quantity of *hybrid* fibers which, as described above, have intermediate properties between MyHC -I and -II. Typically, the masseter muscle has a distribution of fiber types depending on different portions of the muscle. For example, at one extreme, there are more slow type (-I) and fatigue-resistant fibers, while at the other, there are more fast and fast fatigable fibers (type II), with the hybrid fibers are disseminated in the central portion of the muscle (Bottinelli et al, 1996). The high frequency of hybrid fibers suggests that uniquely among skeletal muscles this is related to a ~~the~~ specific functional demand to which the muscle responds (Kwa *et al*, 1995; Galler *et al*, 2002). It is precisely the presence of these hybrid fibers that provides a high functional plasticity of the muscle, which allows it to optimize contraction force by minimizing energy consumption, especially when compared to skeletal muscle which~~having~~ has only "pure" fibers (only type - I or type II) (Stienen et al, 1996).

Myosin characteristics in different craniofacial morphologies

The difference in craniofacial morphology was strongly associated with changes in the content and type of fibers in masticatory muscles, especially the masseter (Van Spronsen et al, 1992). It has been demonstrated by electromyographic studies that "long-face" hyperdivergent individuals possess a significantly lower bite force than

normal and hypodivergent individuals (Piancino et al, 2012). These clinical characteristics may be associated with a different composition of the muscle fibers in these subjects. In fact, it has been shown that long face subjects exhibit a higher proportion of fast fibers (type II) ~~than the normals~~ in some areas of the masseter than in the normal faces (Boyd et al, 1984). Moreover, ~~also~~ the vertical overlap of anterior teeth in centric occlusion was also associated with the fiber type composition of the masticatory muscles.

Rowlerson *et al*, (2005) showed that patients with an open bite presented with a higher proportion of type -I fibers (slow), while patients affected by deep bite presented with a higher proportion of fast fibers (type -II). There are several explanations as to why these factors influence such variations between subjects. The dynamic nature of fibers ~~between and~~ among individuals permits them to optimize their contraction speed properties associated with their muscular energy consumption. The typical, specific chewing pattern of these patients, ~~,~~which~~that~~ are associated with different forces and stretch, may have a strong effect on the expression of myosin in their fibers and may be correlated with the occlusal and craniofacial type.

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Other factors related to myosin fibers

Other relationships that influence myosin composition in the masseter muscle have also been noted. Some of the changes are related to hormone levels. For example, thyroid hormone concentration was shown to be useful for muscle fiber development and maturation. In rats, it was shown that hypothyroidism determineds a lag, especially in the masseter, in myosin maturation and transition to a specific isoform (d'Albis *et al*, 1990)~~.~~ and caused a~~n~~ upregulation of the -fetal isoform in the masseter muscle of the adult rat (Izumo *et al*, 1986).

Masticatory closing muscles (especially the masseter muscle) were also found to be sexually dimorphic in some animal experiments. The masseters of female rabbits were shown to present a lower proportion of ~~fibers~~ -II fibers compared to males (English *et al*, 1998). Similarly, in mice, the female masseter exhibited twice the proportion of ~~fibers~~ -IIB fibers compared to the male masseter, which, however, presented a higher percentage of type -IIA fibers (Eason *et al*, 2000a). In humans, Sciote *et al* (2013) it was demonstrated that the masseter muscle of males exhibits a higher proportion of both type -I and II fibers compared to females (Fig. 4, *A* and *B*).

The hardness of food consumed can also influence, in the long term, the phenotype of masticatory fibers. Many studies on animals have shown~~ed~~ that the daily hardness of food affected masseter muscle myosin composition. Furthermore, the type of daily diet also influenced the volume in their masticatory muscle fibers (Maeda *et al*, 1987, He *et al*, 2017). Additionally, previous reports have shown a reduction and degeneration of muscle fibers in murine masseter muscles

subjected to a daily diet of soft foods (Maeda *et al*, 1987, 1990). In accordance with these results, experiments in rats have shown a reduction of fiber~~s~~ volume (Kiliaridis *et al*, 1988; Miehe *et al*, 1999) and a significant increase in type -IIB fibers in the deep masseter fibers after consuming ~~a~~ soft food compared to those on a diet of~~with~~ hard food (Saito *et al*, 2002). Similar results regarding the fiber volume and composition have been demonstrated in rabbit masseter muscles following a 3-month diet of low consistency food compared to those fed with a high-consistency diet (Langenbach et al., 2003).

Masseter muscle fibers from the young to the elderly

During post-natal development, from suckling to chewing, the masticatory apparatus undergoes a fundamental conversion in function (Herring, 1985, Oghli *et al*, 2017). A decrease in muscle-fiber to muscle-length in different parts of the masseter muscle is observed during its development (Weijs *et al*, 1987; Herring and Wineski, 1986, Nam *et al*, 2017).

The human masseter muscle morphology at prenatal (28 foetal weeks) and postnatal (1.5 years) stages are~~is~~ characterised by the presence of distinct fiber populations of different sizes (Barbet *et al*, 1992, Tachibana *et al*, 2016). Large diameter fibers express MyHC-I exclusively or in association with MyHC-embryonic and MyHC-fetal isoforms. They give rise to adult type I fibers. Small diameter fibers express MyHC-embryonic, MyHC-fetal and MyHC-II isoforms and give rise to adult fiber types IIA, IIB, and IIC (Barbet *et al*, 1992). In puberty (10-13 years), the increase in type II fiber diameter is about half of that for type I fibers (Vignon *et al*, 1980; Bontemps *et al*, 2002).

In human~~s~~, the masseter exhibits differences in fiber~~s~~ arrangement from the young age to the elderly (Eriksson and Thornell, 1983; Monemi *et al*, 1998). Fibers of young and adult patients have been shown to possess a higher amount of slow fibers (type -I) (48-62%, respectively), with a lower proportion in elderly muscles (33%), whereas the proportion of type IIB fibers is greater in old age compared to young and adult age (young 19%, adult 27%, and elderly 37%, respectively). Other special features of the adult masseter muscle are the presence of MyHC isoform originally described in the heart, MyHC-α cardiac (Bredman *et al*, 1991; Pedrosa-Domellöf *et al*, 1992; Sciote *et al*, 1994).

The masseter muscle also shows, during ageing, important changes in the CSA of its fibers. In the young masseter, type I fiber diameter is smaller compared to those in the adult and the elderly, and type II A, and IIB fiber diameters are~~is~~ smaller compared to adult and elderly diameters (Eriksson, 1982; Bredman *et al*, 1991; Monemi *et al*, 1998).

Effects of orthognathic surgery in myosin changes of masseter fibers

Gedrange *et al*, (2006), in ten patients undergoing orthognatic surgery showed differences between the MyHC isoforms during different conditions. For example, in patients with retrognathism, the anterior region of masseter presented much more MyHC types I fibers compared to patients with prognathism. The results were the same for MyHC type IIX. Six months postoperatively, a significant decreasement in the volume of the masseter fibers was found in both groups of patients, with the highest reducdiminution found in type -I fibers of the anterior portion of the muscle in patients with retrognathism.

A previous reports,, both on a CTCT scan, showed that hemifacial macrosomia was associated with a low volume and development of the masseter and the other masticatory muscle fibers on the affected side compared to the non-affected side of the same patient (Huisinga-Fischer *et al*, 2001; Takashima *et al*, 2003), with a proportional increase of the jaw muscle hypoplasia with worsening morphological alterations of the mandible (Marsh *et al*, 1989; Kane *et al*, 1997). Raoul *et al* (2011), in patients with mandibular asymmetry undergoing orthognathic surgery, showed that in a condition of mandibular asymmetry there was an increase of type -II fibers in the latero-deviation side compared to the patients that had no asymmetry. By contrast, symmetric patients showedhad no significant differences in average fiber diameter between the two2 masseters.

Neuromuscular lack of coordination of the masseter muscle during asymmetrical malocclusion

The pattern of mandibular movement during chewing is influenced by skeletal or dental mal-relationships (Lewin, 1985; Woda *et al*, 2006; Piancino *et al*, 2006). We know that during asymmetrical malocclusion, involving the dental regions dedicated to mastication, posterior unilateral crossbite shows an asymmetrical chewing-pattern. This abnormal pattern determines an asymmetrical activation of both masseters (Piancino *et al*, 2009).

The unilateral posterior crossbite (UPS) has been defined as "An abnormal relationship of a posterior upper and lower teeth to the opposing teeth, in which normal buccolingual or labiolingual relationships are reversed" (AAO glossary 2012, 2017). If this malocclusion occurs early in the primary dentition, it will negatively influence the development of a proper oral motor control (Throckmorton, 2001).

Compared to subjects with physiological occlusion, children affected by UPS present a different chewing pattern in mastication on the crossbite side that results, eventually, in an unbalanced masticatory functioning between the both2 sides (Lewin 1985; Piancino *et al*, 2009). As previously described, Cutroneo et al (2012) showed that patients with UPS

had an imbalance of integrin expression in the masseter muscle between the both~~2~~ sides, with the side affected by UPS exhibiting a lower expression of different integrin isoforms, in accordance with electromyographic studies (Piancino *et al*, 2009 and 2012) (Fig. 5).

Moreover, the presence of an abnormal maxillo-mandibular anteroposterior relationship with the instable occlusal condition has all been associated with a different pattern of masticatory muscle activity. It was shown that, during maximal biting, patients with a class II malocclusion exhibit less electromyographic (EMG) activity in the masseter and temporal muscles compared to patients with normal occlusion (Van Eijden *et al*, 1993; Panchertz, 1980). Recently, in patients affected by class II malocclusions, positive effects on EMG chewing muscles activity has been shown by a functional orthopaedic therapy treatment using Sanders appliance (Di Palma *et al*, 2017) and, in patients with UPS, using a function generating bite (Piancino *et al*, 2006).

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CONCLUSIONS

Human evolution and some genetic influences on the development of malocclusion include inheritable effects on the heterogeneity and plasticity of muscle fibers in both masticatory muscles and jaw skeletal morphology. Moreover, the abundant presence of hybrid fibers in masticatory muscles demonstrates the important role played by the functional requirements of the stomatognathic apparatus that greatly influences their fiber composition. The high presence of hybrid fibers, associated with the "plasticity" of the masticatory muscles in order to optimize energy consumption ~~better~~ during contraction better, reflects the uniqueness of chewing muscles concerning all other skeletal muscles of the human body.

There is now a wealth of ancient human biomolecular information available to us, which is providing a complete view of human physiology and evolution. Maxillary and mandibular vertical and sagittal malocclusions are difficult to treat, in part because the underlying mechanisms which produce them are not well understood and may lead to relapse after treatment.

The results of the studies summarized in this review hopefully will open up a new scientific approach, which aims to profoundly investigate the physiology and the physiopathology of the stomatognathic apparatus in order to understand better the underlying factors that are the basis of masticatory disorders. Genetic and epigenetic studies offer an opportunity to identify new factors which will lead to the discovery of ~~the~~ specific molecular pathways involved in the ethiology and severity of chewing disorders, with the potential for improved diagnosis and treatments in the future.

FIGURE LEGENDS

Figure 1: SEM longitudinal section of a skeletal muscle sarcomere. In the upper part of the picture, multiple actin and myosin isoforms of the slow (green) and fast (red) fibers are shown (Luther, 2009).

Figure 2: *A*, Sarcomeric myosin gene expression in mammals and their corresponding protein pattern. §, present only in some species of mammals. *MYH7b expressed as protein only in extraocular muscles (Rossi *et al*, 2010). *B*, The evolution in the size and attachment of the temporalis muscle in the cranium from Macaca fascicularis (a–c), Gorilla gorilla (d–f) and Homo sapiens (g–i) (Stedman *et al*, 2004).

Figure 3: *A* and *B*, Percentage of MyHC isoforms in the different masticatory muscles (Korfage *et al*, 2000).

Figure 4: Comparison of mean fiber areas of masseter muscle fiber types in male and female subjects by vertical skeletal malocclusion groups. Data for mean fiber area values in μm^2 for normal vertical dimension (16 males and 42 females) (A) and deep-bite malocclusion categories (16 males and 15 females) (B). Bars represent one standard deviation of the mean, and the significance of the difference between males and females is indicated by the asterisks: *P < 0.05; ***P less than or equal to 0.0004 (Sciote *et al* 2013).

Figure 5: Immunohistochemical longitudinal section labelled for integrins of human masseter muscle of patient with UPS. *Control side*, not affected by UPS showed an increasing of α7A and β1A-integrin compared~~respect~~ to α7B and β1D isoform. Crossbite side, Expression of all of the integrin isoforms appears to be decreased compared to the integrins of the control side not affected by crossbite (Cutroneo *et al*, 2012).

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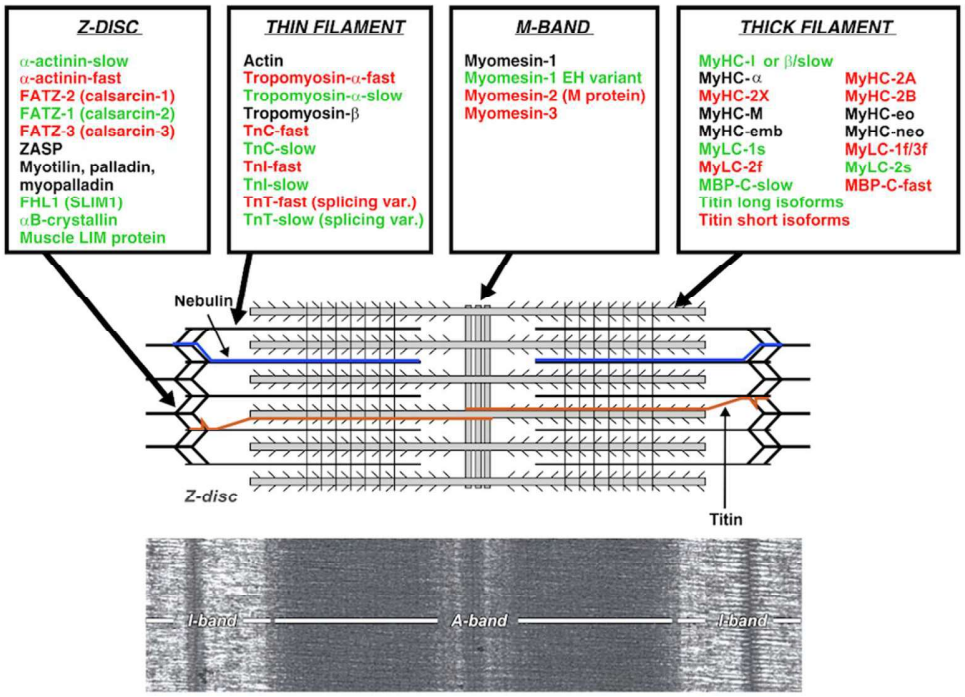

SEM longitudinal section of a skeletal muscle sarcomere. In the upper part of the picture, multiple actin and myosin isoforms of the slow (green) and fast (red) fibers are shown (Luther, 2009).

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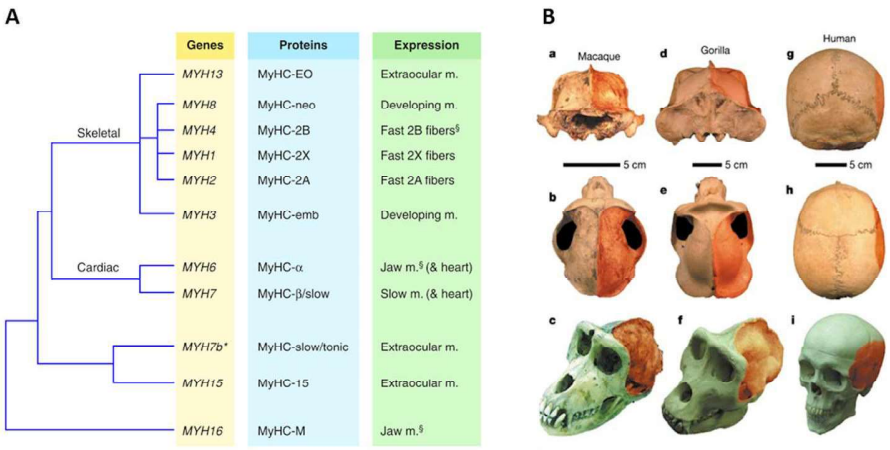

A, Sarcomeric myosin gene expression in mammals and their corresponding protein pattern. §, present only in some species of mammals. *MYH7b expressed as protein only in extraocular muscles (Rossi et al, 2010). B, The evolution in the size and attachment of the temporalis muscle in the cranium from Macaca fascicularis (a–c), Gorilla gorilla (d–f) and Homo sapiens (g–i) (Stedman et al, 2004).

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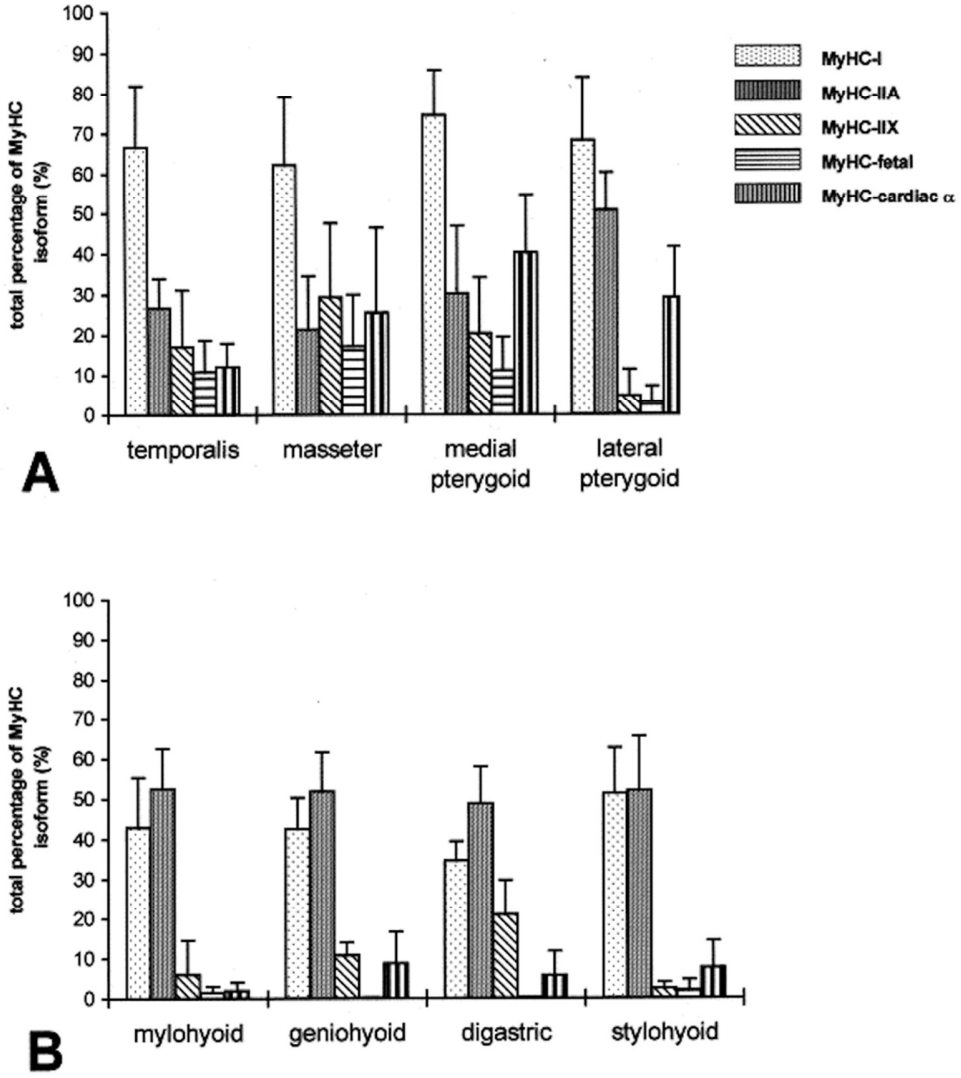

A and B, Percentage of MyHC isoforms in the different masticatory muscles (Korfage et al, 2000).

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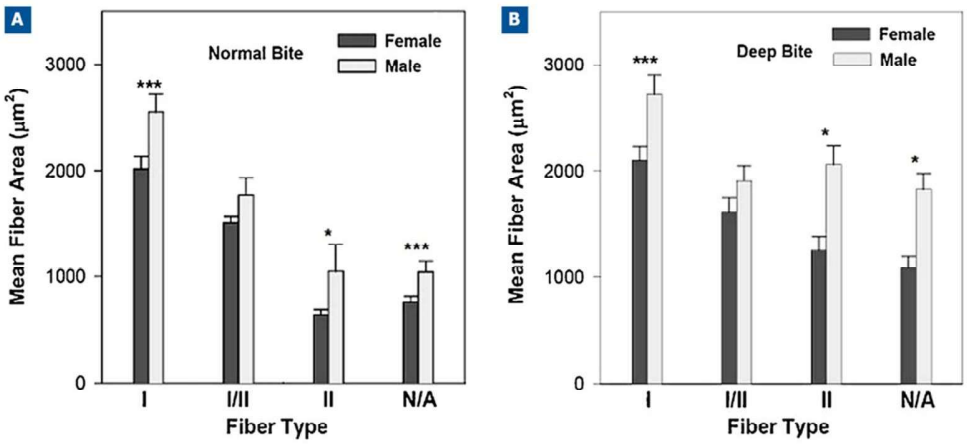

Comparison of mean fiber areas of masseter muscle fiber types in male and female subjects by vertical skeletal malocclusion groups. Data for mean fiber area values in µm2 for normal vertical dimension (16 males and 42 females) (A) and deep-bite malocclusion categories (16 males and 15 females) (B). Bars represent one standard deviation of the mean, and the significance of the difference between males and females is indicated by the asterisks: *P < 0.05; ***P less than or equal to 0.0004 (Sciote et al 2013).

102x46mm (300 x 300 DPI)