Myosin heavy chain composition of the human jaw muscles
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Introduction

The possible performance of the human jaw muscles is heavily dependent on the contractile properties of their fibres. Unfortunately, it is not feasible, or ethically acceptable, to measure these properties directly in vivo. However, they can be estimated post mortem by analysing the myosin heavy chain (MyHC) composition of the muscle fibres. These MyHCs are a determinant for the contractile properties of these muscle fibres. For instance, muscle fibres characterised with MyHC isoforms I, IIA, and IIX (the three main MyHC isoforms) are able to contract with increasing velocity (Reiser et al., 1985; Bottinelli et al., 1996). Consequently, a characterisation of the MyHC contents of the muscle fibres enables us to obtain a better insight into the possible performance of the human jaw muscles. Therefore, they have been analysed in this thesis.

It should be noticed that, except for MyHC, also other muscle specific proteins (e.g., sarcoplasmic reticulum Ca\textsuperscript{2+} ATPase (Jorgensen et al., 1979; Krenacs et al., 1989), troponin subunits (Galler et al., 1997b; Pette and Staron, 1997), \(\alpha\)-actinin (Schachat et al., 1985)) have fibre type specific isoforms which can modulate the contractile properties. Analysis of these proteins, however, falls beyond the scope of the present thesis.

The results in this thesis show that the MyHC contents of the jaw muscles is highly unusual. In addition to the MyHC-I, -IIA, and -IIX isoforms, they contain MyHCs which are typical for developing or cardiac muscle. Another remarkable feature is that...
many of the fibres are hybrid fibres, which co-express two or more different MyHC isoforms. The MyHC composition within these hybrid fibres appears to be very variable. The size of jaw muscle fibres is also unusual. The type II fibres have a smaller cross-sectional area than the type I fibres, in contrast to limb and trunk muscles where the reverse is true. Finally, large differences in fibre type composition and fibre cross-sectional areas have been observed between muscle groups, individual muscles, muscle regions, and individuals.

In the present chapter, the current knowledge of the variability in fibre type composition in the jaw muscles, particularly in human, is discussed in relation to its benefit and its origin.

**Why do the Jaw Muscles have Different Fibre Types?**

*Functional Significance of Fibre Type Diversity*

The human jaw muscles participate in a large variety of motor tasks, including biting and mastication of food of different textures and sizes, swallowing, talking, yawning, and singing. These activities require a diversity of forces which have to be maintained under various contraction velocities. To be able to perform the vast array of different tasks, the system has to contain many different muscles.

Generally, muscles are optimised to perform such tasks, however, they cannot be optimised for each task simultaneously. For example, a muscle that can contract at high speed cannot operate during a prolonged period, and vice versa. Therefore, if a muscle has to perform different tasks, it must contain a combination of fibres with different physiological properties. This combination may, for instance, be composed of at the one extreme the slowest, fatigue-resistant fibres and at the other extreme the fastest, fatigable fibres. The differences in fibre performance are the result of both qualitative modifications (e.g., myosin, troponin, and Ca$^{2+}$ pump isoforms with different kinetic rates) and quantitative modifications (e.g., densities of sarcoplasmatic reticulum and mitochondria) within the muscle fibres.

The observed variety of MyHC isoforms could reflect the requirements of the masticatory system regarding the contraction velocities of the various muscle portions. The unloaded shortening velocity ($V_{0}$), determined in human limb muscles by the slack-test technique (Edman, 1979), is about ten times lower in MyHC-I fibres
than in MyHC-IIx fibres: the V- in MyHC-IIB fibres, as expressed in some animals, is approximately 20% higher than the Vr in MyHC-IIx fibres (Bottinelli et al., 1994). MyHC-IIA fibres are intermediate between MyHC-I and -IIx fibres. When high speeds of contraction are required, these speeds can only be produced by the fibres that contain the fastest MyHCs. Furthermore, fast fibres are capable of producing more isometric force and more power (force times velocity) than slow fibres. Because of the force-velocity relationship, this difference will also be present at a similar speed of contraction.

An important advantage of the diversity in MyHCs is that these isoforms greatly help to optimise contractile function while minimising energy use. The energetic cost of contractions is dependent on two components, i.e., the ATP used by Ca\(^{2+}\) pumps and the ATP used by the cross bridges. In humans, fast fibres have an approximately 4 times higher ATP utilisation than slow fibres (Stienen et al., 1996). The tension cost, i.e., the ratio between expended energy and generated tension, is for type I fibres about three times lower than for type IIx fibres (Stienen et al., 1996). Slow fibres transfer energy more efficiently than fast fibres (He et al., 2000), although they are less powerful. Hence, fast fibres are less suitable of efficiently powering movements that occur at low frequencies. It seems, therefore, advantageous for the jaw muscles to use only fast muscle fibres when the occasion demands it and to use slow ones in less demanding situations. Furthermore, slow fibres are in general more fatigue resistant than fast fibres. Therefore, they are better capable of sustaining a motor task with a particular intensity than fast fibres.

The fact that not one muscle fibre type can perform all activities effectively, explains why the jaw muscles contain many fibre types. When a muscle is configured with one particular fibre type, thus is optimised to perform one type of activity, this will necessarily reduce its ability to perform another type of activity. To achieve a wider repertoire of movement velocities and to sustain different durations of activation, jaw muscles must use different fibre types. The more variation in fibre type composition of a muscle, the larger is its potential role in different motor tasks.

**Recruitment of Different Fibre Types**
The presence of a variety of different muscle fibres optimised to perform different tasks suggests a complexly organised nervous system where muscle fibres of different composition are connected to different motoneurons. Fortunately, such a
complex system is not necessary. Generally, a motoneuron is connected to a number of fibres of a similar type to form a so-called motor unit. Because the excitation threshold of a motor unit generally differs with its fibre types, only a relatively simple system is necessary.

In general, the recruitment of motor units follows the so-called size principle (Henneman et al., 1965; Hennig and Lomo, 1985). According to this principle they are recruited sequentially and in a strict, hierarchical order (and de-recruited in the reverse sequence). The smallest, and slow, fatigue-resistant motor units are recruited first. They produce small forces. With increasing force demands, the larger, faster, and more fatigable units join in. The largest, fastest, and most fatigable motor units are rarely used and recruited only during maximal effort for brief periods of time. It is this hierarchy of motor unit recruitment order that governs the neuronal control of different fibre types within the same muscle, or muscle portions (Pette, 1990; Pette and Staron, 2000). Important implications of the orderly recruitment of motor units are that the slower fibre types are more frequently used than the faster ones and that a finer modulation of force is possible at lower than at higher task intensities. This finer modulation is due to the fact that small motor units produce less force than the large motor units and that there are more small than large motor units (Kernell, 1992).

The applicability of the size principle has been established for limb muscles, but has also been reported for various jaw-closing muscles (human masseter and temporalis: Yemm, 1977; human masseter, Goldberg and Derfler, 1977; Desmedt and Godaux, 1979; Scutter and Türker, 1998; monkey temporalis: Clark et al., 1978; monkey masseter, temporalis and medial pterygoid: Lund et al., 1979). In general, the results of these studies show that, according to the size principle, the smallest motor units are recruited first, while the larger units are progressively recruited as force increases. The lowest force level at which the motor units are recruited, i.e., the recruitment threshold, appears not to be fixed but depends on the motor task. For example, the threshold varies with bite force direction (Hattori et al., 1991) and the duration of muscle contraction (Nordstrom and Miles, 1991). Furthermore, an increase in threshold has been associated with an increase in jaw gape and muscle length (Miles et al., 1986) and with a decrease in muscle contraction velocity (Desmedt, 1980). Many mechanisms responsible for this modulation of recruitment threshold have been proposed, including changes in excitability of the motoneuron pool, inhibition mediated by Golgi tendon organs, presynaptic inhibition, and
Renshaw inhibition (see for an overview: Van Eijden and Turkawski, 2001).

Muscle force is not only regulated by 'recruitment gradation' but also by 'rate gradation', i.e., by varying the rate of motor unit activation. Both strategies are used, although their relative contributions differ. In general, recruitment gradation is more applicable at low force levels and rate gradation at high force levels (Hennig and Lømo, 1987; Fournier and Sieck, 1988). This general principle may also be true for human jaw muscles. For example, at relatively low bite force levels (0-20% of the maximum bite force) between 50% (Goldberg and Derfler, 1977; Hannam and McMillan, 1994) and 87% (Scutter and Türker, 1998) of the motor units are recruited in the human masseter muscle.

**Related Recruitment of Jaw Muscle Fibres**

A question that still needs to be answered is how jaw muscle fibres with different properties are used during different tasks with widely varying demands. Similar to the situation in limb and trunk muscles (e.g., Gorassini *et al.*, 2000; Wakeling *et al.*, 2002), the different motor tasks in the jaw muscles are likely to be powered by different muscle fibre types. To produce low speed of the mandible or to produce sustained tension, for example to resist gravity, the slow, fatigue-resistant fibres can be expected to be responsible for the majority of force production. Most of the force produced during mastication can also be expected to be the result of the activity of slow fatigue-resistant fibres, with a contribution from the faster fatigue-resistant ones. As mastication speed or force increases, additional fast, fatigue-resistant fibres will be recruited, with a small contribution of the fastest, more fatigable fibres. High muscle speed and/or power are presumably required for tasks such as talking or biting, resulting in the fastest fibres becoming a more important contributor to the speed or power produced by the muscles.

This view about the task-related recruitment of different jaw muscle fibre types is, however, rather speculative and based on few experimental data (McMillan and Hannam, 1992). In order to understand the tremendous diversity of fibres and their recruitment, the properties of the jaw muscles should not be studied without examining the entire jaw system, because one cannot appreciate why, for instance, the distribution of fibre types is as it is without examining the other components (joints, muscle moments, masses, kinematics of movement, motor tasks) to which it is adapted. There are, however, a number of obstacles to integrating from jaw
muscle fibre type properties to mandibular movement and force production. Firstly, there are more than 20 muscles or muscle portions that contribute to mandibular movement and force production. Because of this redundancy the relative contribution of these muscles in performing a motor task is not a priori fixed (Van Eijden et al., 1990; Koolstra and Van Eijden, 2001). Therefore, it is difficult to appreciate and predict which particular muscle or muscle portion participates in a particular task and to appreciate the mandibular movement and force production from the fibre type composition of a single muscle. Secondly, thus far there is a lack of studies in which the proportion of each fibre population has been estimated within a muscle that is active at different tasks. As slow muscle fibres are intermingled with various types of fast muscle fibres, it is very difficult to experimentally identify which fibre types are active during particular motor activities. Electromyographic electrodes will pick up signals from fibres of all types, making it nearly impossible to discriminate which fibre types are powering a particular movement or force. The only robust technique that has been used in mammals is to record the activation directly from the motoneurons and to identify their fibre types at the conclusion of the experiment. This technique, which has been successfully done only in cats (Hoffer et al., 1987), is, however, extremely difficult.

Hybrid Fibres in the Jaw Muscles

One of the remarkable features of the jaw muscles is the abundance of hybrid fibres. These fibres have contraction properties which are intermediate between the MyHC isoforms they express (Larsson and Moss, 1993; Bottinelli et al., 1996; Widrick et al., 1996). Hybrid fibres that express both MyHC-I and -IIA, for instance, will thus be faster than pure MyHC-I fibres but slower than pure MyHC-IIA fibres. We also found that many hybrid fibres contain MyHC-cardiac α and MyHC-fetal isoforms that are normally not found in limb and trunk muscles. The contraction velocity of MyHC-cardiac α lies between the velocities of MyHC-I and MyHC-IIA fibres (Kwa et al., 1995b; Sciote and Kentish, 1996; Galler et al., 2002); the contraction velocity of MyHC-fetal is not yet determined unambiguously but seems to be slow (D'Antona et al., 2003).
Functional Significance of Hybrid Fibres
The occurrence of hybrid fibres in considerable numbers suggests that they match specific functional demands. Hybrid fibres could increase the capacity of muscles to produce a large variety of motor tasks, as they have contractile properties which lie between those of pure fibres. The greater the number of different hybrid fibres, the more a continuum in contractile properties arises which could contribute to a precise modulation of mandibular position and force. Whereas hybrid fibres represent a potentially unique design at the molecular motor level, motor unit recruitment schemes are also capable of providing smooth transitions throughout the contractile spectrum (see size principle). The large amount of hybrid fibres as seen in the jaw muscles (particularly in the jaw closers) raises two important issues with respect to the concept of motor units in human jaw muscles. Firstly, it is obvious that in these muscles the concept of one motoneuron, one MyHC isoform is not correct. Indeed, for motor units of the rabbit masseter muscle it has been demonstrated that a single motor unit can have muscle fibres with different MyHCs (Kwa et al., 1995b).
Secondly, the large number of different hybrid fibres and their variable MyHC contents (Chapter 7) suggests that the relatively simple classification of motor units into three distinct categories, namely S (slow, fatigue-resistant), FR (fast, fatigue-resistant), and FF (fast, fatigable) fibres (Barnard et al., 1971; Burke et al., 1971; Peter et al., 1972) fails to hold for jaw muscles. Our results indicate that human jaw muscle motor units display a continuous range of properties, as contractile speed and fatigability, which also has been demonstrated for the rabbit masseter (Turkawski et al., 1998; Kwa et al., 2003). Furthermore, also the mechanical properties of jaw motor units appear to be distributed as continua, without the clustering that suggests distinct types.

Why are there so many Hybrid Fibres in the Jaw Muscles?
Except for their functional significance, the presence of hybrid fibres could also be a reflection of the adaptive capacity of jaw muscle fibres. Muscle fibres have an inherent ability to alter their contractile properties so that their efficiency and economy of energy usage is optimised. They can change their phenotype in reaction to several influences (see below) by switching different isoform genes on or off. In mammals, the conversion of fibre types normally follows a strict order from MyHC-I → -IIA → -IX → -IIB or vice versa (Schiaffino and Reggiani, 1994). During these
transformations the fibres will change from a pure fibre type, that expresses only one MyHC isoform, into another pure fibre type, via hybrid fibre types that express the old as well as the new MyHC isoform. It might, therefore, be concluded that the jaw muscles are continuously switching one fibre type into another. Some papers question this strict order of MyHC transformation. For instance, under certain conditions, like space flight (Taimadge et al., 1996), or hindlimb suspension, alone (Stevens et al., 1996), or in combination with hyperthyroidism (Caiozzo et al., 1998), some hybrid fibres were found in limb muscles that express MyHC-I and -IIx but not MyHC-IIA. This particular hybrid fibre type is also found in the jaw-closing muscles (Chapters 4, 5 and 7). Thus, jaw-closing muscle fibres seem to divert from this strict order of MyHC transformations.

The MyHC expression within a fibre is not necessarily homogeneous. Muscle fibres contain many nuclei and, therefore, some of these nuclei might express a different MyHC isoform that is not expressed in other parts of the muscle fibre. This was noticed in fibres of denervated rat limb muscles (Schiaffino et al., 1988b) in which MyHC-fetal was regionally expressed in some fibres. Also, detailed investigation of longitudinally cut fibres in limb muscles from very old people, indicated that some fibres switch their fibre type along the length of the fibre or contain areas or nuclear domains in which the MyHC expression was different from other parts of the fibre (Andersen, 2003). This might explain the observation in Chapter 3, that the expression of MyHC-cardiac showed some mismatch between consecutive slides. Future experiments in which serial sections of jaw muscle fibres will be followed for a considerable length could confirm this. The functional implication of this particular diversity is not known.

A possible explanation for the many hybrid fibres in the jaw muscles has been related to a difference in innervation with respect to limb and trunk muscles, namely branchial versus spinal (Stal et al., 1987). However, this explanation is questionable, because we found that several muscles which are innervated by branchial nerves, like the digastric and the mylohyoid, contain only few hybrid fibres that express MyHC-fetal and/or -cardiac.

The many hybrid fibres in adult jaw muscles could also be regarded as a characteristic of development (Butler-Browne et al., 1988). During development, and also during regeneration, fibres can change their phenotype during which hybrid fibres arise. During these processes, the MyHC-fetal gene is temporarily.
upregulated. Still, this does not explain why these muscles continue to appear like a developing muscle.

We like to suggest another possible explanation. There is evidence that the membranes of normal muscle fibres are occasionally ruptured by stretch and/or stimulation (Petrof et al., 1993) which stimulates satellite cells to repair the damage. During the regeneration process, fibres transiently start to express MyHC-fetal and MyHC-I (Yang et al., 1997). The continued expression of these fibre types in adult jaw muscles might indicate a longer time for jaw muscles to heal. Indeed, the masseter is known to heal badly after an injury (Pavlath et al., 1998) which was related to the lower proportion of satellite fibres in this muscle. Furthermore, these satellite cells might be different from satellite cells found in limb and trunk muscles in respect to their genetic background, just as the muscle fibres are. This can be seen in the expression of transcription factors, like Pax-3, myf5, and MyoD, which are needed to express muscle specific proteins during development and during activation of satellite cells. For instance, a genetic difference is noticed after ablation of the Pax-3 transcription factor in mice which led to an absence in the formation of muscle fibres in the body but not in the jaw muscles (Tajbakhsh et al., 1997). Also, the timescale in which transcription factors occur is different. For instance, in chick somites the interval between the expression of MyoD after the onset of myf5 is only a few hours. In cranial muscles, however, this interval is 8-18 hours (Noden et al., 1999). Furthermore, the onset of MyHC expression is also delayed in the cranial muscles. Thus, it could be that the satellite cells of the jaw muscles and the jaw muscle fibres are also slower in the production of MyHC isoforms. This might result in a longer presence of hybrid fibres including those hybrid fibres that co-express MyHC-fetal.

We also found that many hybrid fibres in the jaw-closing muscles co-express MyHC-cardiac $\alpha$, which is in agreement with other studies (Bredman et al., 1991; Sciote et al., 1994). In the masseter of rabbit, this particular fibre type is often co-expressed with either MyHC-I or MyHC-IIA and it was thus thought that MyHC-cardiac $\alpha$ is an intermediate isoform between the slow MyHC-I and the fast MyHC-IIA isoforms (Hämäläinen and Pette, 1997; Peuker et al., 1998). Jaw muscle fibres can use this MyHC isoform as a ‘fine-tuning’ link protein between the slow type MyHC-I and the fast type MyHC-IIA isoforms. It is still not fully known why this particular MyHC isoform is upregulated in the jaw-closing muscles.
Differences in Fibre Type Composition between and within Jaw Muscles

In the present thesis, we have found many differences in the fibre type composition between and within the jaw muscles. In the next paragraphs we will discuss possible explanations for these differences.

**Adaptation of Muscle Fibres**

As mentioned earlier, muscle fibres have the capacity to adapt towards a new functional demand. The occurrence of adaptive changes has been related to a change in the amount of muscle activation and/or stretch. An increase of type I fibres has been reported after a long period of activation (Delp and Pette, 1994; Windisch et al., 1998) or electrical stimulation (Pette and Vrbová, 1992). Furthermore, a relationship has been demonstrated between the daily amount of activation of muscles (the so-called 'daily duty time'), and the proportion of type I fibres (Monster et al., 1978; Kemmell et al., 1998). In contrast, a muscle will convert slower fibres into faster fibres, during a period of reduced activity, for instance during bedrest, space flight (Edgerton et al., 1995), or when a muscle is immobilised in a shortened position. Repeated, rapid high-amplitude Ca**2+** transients in the sarcoplasm (Kubis et al., 2003), which occur during activation, have been suggested to play a role in the fast to slow fibre conversion. They will trigger the calcineurin signalling pathway that upregulates slow fibre-specific gene promoters (Chin et al., 1998).

However, activation is not the only influence. In experiments, in which a muscle was fixed in a stretched position by a plaster cast (Yang et al., 1997) there was still a conversion from fast to slow fibres; this conversion was even stronger when the muscle was also subjected to electrical stimulation. During stretch an increase of muscle IGF-I (Insulin Growth Factor-I) mRNA has been observed (Czerwinski et al., 1994; Goldspink et al., 1995). This particular IGF-I is a splice variant of the IGF-I gene found in the liver. The autocrine variant of IGF-I was cloned and named MGF (Mechano Growth Factor) (Yang et al., 1996; Goldspink, 1999). This protein increases myoblast proliferation required for hypertrophy or repair (Yang and Goldspink, 2002). MGF was not detectable in dystrophic mdx muscles even during stretch, and stretch combined with electrical stimulation. Therefore, it was thought that the dystrophin cytoskeletal complex might be involved in the
mechanotransduction mechanism. When this complex is defective, MGF and systemic growth factors are not produced resulting in the ensuing cell death and in a progressive loss of muscle mass. Also, when the cDNA of MGF is introduced into murine muscle fibres, an increase in mass by 20% is achieved within 2 weeks (Goldspink, 2003). The liver type of IGF-I is less potent.

Intermuscular Differences in Fibre Type Composition
The results of the present thesis show that the jaw-closing muscles (including the lateral pterygoid) contain more type I fibres and more hybrid fibres than the jaw-opening muscles and infrahyoid muscles (Chapter 2). The jaw-closing muscles seem thus more adapted to perform slow, tonic movements and to produce a smooth, gradable force. The jaw-opening muscles and infrahyoid muscles, on the other hand, seem to be more adapted to produce faster, phasic movements.

The differences in fibre type composition between the muscle groups suggest a difference in the daily amount of activity. The higher proportion of slow fibres in the jaw-closing muscles indicates that they could have a higher daily duty time than the jaw-openers, for instance, in maintaining the mandible in its resting postural position against gravity (Kitagawa et al., 2002). In extreme cases, like bruxism, the duration of activation might be longer than in normal individuals which might increase the formation of MyHC-I even further. It can thus be expected that these patients have a higher proportion of type I fibres. Thus far, no studies are available in which the daily activity of the muscle groups has been compared. At this moment, our department is studying the relationship between daily activity and fibre type composition using the jaw muscles of the rabbit as an experimental model (Langenbach et al., 2002, 2004). Preliminary results show that the digastric muscle of the rabbit has a higher daily duty time, and a larger proportion of MyHC type I fibres, than the masseter. Studies in which the activity of the jaw muscles are monitored for 24 hours could confirm this relationship in humans.

The differences in fibre type composition between jaw closers and jaw openers could also be related to the amount of stretch which could be larger in jaw-closing muscles than in jaw-opening muscles. The jaw-closing muscles consist of shorter fibres than the jaw-opening muscles and might, therefore, experience more stretch than the jaw-opening muscle fibres. This stretch would cause an upregulation of the MGF and thus a conversion from fast to slow type fibres. A possible difference
Chapter 8

of MGF upregulation between jaw-closing and jaw-opening muscles, however, is to our knowledge, not yet investigated.

Differences in activation and/or stretch might also explain the different fibre type compositions of muscles within a particular muscle group. Because of differences in 'neural drive' the muscles in a particular muscle group could differ in daily duty times, and because of differences in architecture and/or positions in the leverage system of the jaw, they can experience different amounts of stretch than another muscle from the same group.

**Heterogeneity in Fibre Type Composition within a Muscle**

We found a heterogeneity in fibre type proportion, particularly in the jaw-closing muscles. Heterogeneity in fibre type proportion within a muscle can be genetically determined. Deep muscle portions contain, in general, more type I fibres than superficial muscle portions (Johnson *et al.*, 1973). This is, for example, seen in the masseter (Chapter 3). Activation- and stretch-related fibre type adaptation might also contribute to the heterogeneity within a muscle. For instance, more type I fibres were found in the anterior muscle portions of the temporalis than in the posterior muscle portions (Chapter 4). The longer moment arm of the anterior muscle portions makes these portions more advantageous for jaw closing than the posterior muscle portions. Because of their vertical direction of pull, the anterior muscle portions can also contribute to more potential motor tasks than the posterior muscle portions. Thus, from a mechanical point of view it can be expected that the anterior portions are more activated than the posterior portions. Indeed, this was observed by measuring the EMG activity, as registered with fine-wire electrodes (Blanksma *et al.*, 1997). A similar situation is found in the anterior muscle portion of the masseter. This muscle portion has a longer moment arm than the posterior muscle portion. It seems thus more efficient to activate fibres in the anterior masseter to produce bite forces. Therefore, we speculate that the anterior portions of the temporalis and masseter are more frequently activated, which might lead to a higher proportion of MyHC type I fibres in these muscle portions.

In addition, architectural factors, such as the length of the muscle fibres and their position relative to the rotation axis of the jaw, might contribute to the amount of stretch-related fibre type adaptation. When the jaw is opened, e.g., during chewing, muscle portions with a longer moment arm, like the anterior portions of the temporalis
and masseter, have larger sarcomere excursions, and will thus be subjected to more stretch, than muscle portions with a shorter moment arm (Van Eijden and Raadsheer, 1992; Van Eijden et al., 1996). This might result in an upregulation of the MGF protein, which in turn will induce the fibres to express MyHC-I (Yang et al., 1997). Future experiments using antibodies which stain MGF could resolve whether there is indeed a relation between the regional expression of MGF and of MyHC type I within the jaw muscles.

Such heterogeneity in fibre type expression could not be observed in most of the jaw-opening muscles. In general, the cross-sectional areas of these muscles and their attachment sites are relatively small. This makes it unlikely that different muscle portions of a particular muscle can execute different mechanical functions. It can thus be concluded that the jaw-opening muscles are in general simpler in function than the jaw-closing muscles, both with respect to activation, architecture and fibre type composition.

Differences in Fibre Type Composition between Individuals

Large differences in the reported fibre type compositions of the various jaw muscles can be observed when the results of various studies are compared (Table 8.1). One of the major causes for these differences is a difference in methods used in the various studies to classify fibre types. In a number of studies the ATPase histochemistry method was used. By this method the hybrid fibres were classified as IIC or IM fibres depending on a difference in their staining pattern. Furthermore, ATPase histochemistry misses fibres expressing MyHC-fetal and MyHC-cardiac α. Also, some of the earlier studies did not, or could not, make a distinction between type IIA and type IIX fibres.

Despite consistent use of one of these techniques, the observed variability in fibre type composition in the same muscle of different individuals is large (Chapter 2 and 3). This is indicated by the large standard deviation values of the fibre type proportions. Except for genetic influences, there are several explanations for this variability. Systemic differences between individuals can lead to a different adaptation of muscle fibres. For instance, during ageing a number of systemic
### Chapter 8

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*Ringqvist, 1974

Vignon et al., 1980

Eriksson and Thornell, 1983

Korfage and Van Enden, 1999

Shaughnessy et al., 1969

Korfage and Van Eijden, 2000

Schiol et al., 1994

Stal et al., 1994

Monem et al., 1998

Korfage et al., 2000

Eriksson et al., 1981

Eriksson et al., 1982

Monem et al., 1999

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Table 8.1
Fibre type composition (mean %) in adult, human jaw muscles as found in literature. Fibres were classified by ATPase histochemistry, except for Korfage and Van Eijden (1999, 2000) and Korfage et al. (2000) in which immunohistochemistry was used, and Sciot et al. (1994) in which both methods were used. Note that ATPase classified IIB fibres contain MyHC-IIIX. The type IIB fibres in the study by Shaughnessy et al. (1989) are a combination of type IIB and IIC.

Changes occur which could relate to the observed differences in fibre type composition (see below). This is important because the muscle samples analysed in this thesis were taken from relatively old individuals. Furthermore, since the samples were taken from both sexes, it is also necessary to look how hormones can have an influence on fibre type composition.

Next to adaptation related to systemic influences there are also local, probably activation- and stretch-related influences, which might be responsible for the large interindividual variation in fibre type expression. These influences relate to, for instance, differences in food hardness, and the use of artificial prostheses. The latter is of particular interest since most of the individuals in this thesis were at least partially edentulous and wore a prosthesis. In the next few paragraphs, we will give some possible explanations, related to this adaptation in fibre type composition.
Ageing

Ageing leads to several complex changes which have a marked influence on skeletal muscles (reviewed by McComas, 1998; Basu et al., 2002). In limb and trunk muscles, it coincides with a reduction of muscle cross-sectional area, synthesis rate for MyHC (Balagopal et al., 1997), muscle strength (estimated at about 10% per decade starting at the age of 50 years (Larsson et al., 1979)), and shortening velocities of fibres (Larsson et al., 1997). The muscle fatigability increases, and the fibre type proportions and fibre cross-sectional areas change (Lexell, 1993). Furthermore, ageing coincides with a loss of motoneurons (Larsson, 1998). In limb and trunk muscles, reduction of muscle force is mainly the result of a loss of fibres, and to a lesser extent a reduction in fibre size, especially among the fast type II fibres. The proportion of type I fibres remains more or less the same while the proportion of type IIA fibres increases at the cost of the type IIX fibres (Monemi et al., 1999). It must be noticed, though, that the properties of the MyHC isoforms may change during ageing. It has been observed that many of the MyHC-I fibres in older subjects were either very slow, or very fast (Krivickas et al., 2001). There is also an increase in the population of hybrid fibres in elderly people (Klitgaard et al., 1990), especially of hybrid fibres co-expressing MyHC-I and -IIA (Andersen, 2003).

In jaw muscles, ageing coincides with a prolongation of muscle contraction time (Newton et al., 1993), a reduction of the reflex responses, in numbers and in amplitude, and an increased latency of oral reflexes (Smith et al., 1991; Kossioni and Karkazis, 1994). The cross-sectional area of the muscles is reduced with age (Newton et al., 1987), primarily due to a decrease of the cross-sectional area of the fibres (Monemi et al., 1998).

The fibre type composition of the jaw muscles changes also with age. In the masseter of elderly subjects, the proportion of pure type I fibres is decreased while the proportion of pure type II fibres is increased (Eriksson and Thornell, 1983; Monemi et al., 1999). As we used muscles from elderly subjects, this might imply that the percentage of type II fibres in the present study was relatively high, whereas the percentage of type I fibres was relative low. Furthermore, the proportion of hybrid fibres in the jaw-closing muscles of elderly subjects increases, particularly the proportion of fibres that co-express MyHC-fetal (Monemi et al., 1996). This might contribute to the relatively high proportion of MyHC-fetal positive hybrid fibres found in this thesis.
**Hormones**

Some of the changes during ageing are related to a change in the level of hormones. For instance, the levels of circulating anabolic hormones like thyroid hormone (Rubenstein et al., 1973), growth hormone, and insulin-like growth factor (IGF-I) are reduced with age (Proctor et al., 1998).

Thyroid hormone is important for the maturation of muscle fibres during development. Hypothyroidism in rat embryo (d’Albis et al., 1990) delays the MyHC isoform transition in all examined muscles, particularly in the sexually dimorphic muscles like the masseter (see next paragraph). Furthermore, it was noticed that hypothyroidism in adult rats caused an upregulation of MyHC-fetal in the masseter but not in other muscles (Izumo et al., 1986). In adult humans, the concentration of thyroid hormone decreases with age (Rubenstein et al., 1973) which might induce the reported increase of MyHC-fetal in the jaw-closing muscles of elderly individuals. Further investigations are required to verify this hypothesis.

As mentioned earlier, growth hormone is another hormone that might have an influence on the fibre type composition. The main mediator of its somatogenic action is insulin-like growth factor-I of which there are two different types (Goldspink, 2002), namely an insulin-like growth factor-I (IGF-I) which circulates in the blood and is produced by the liver, and a mechanogrowth factor (MGF) which is produced by the muscle fibres itself and acts only locally (see above). A gain in muscle weight of 20% was achieved after a period of 4 months when the liver type IGF-I was introduced into muscle fibres, by injecting the muscle with a recombinant adeno-associated virus directing overexpression of insulin-like growth factor I (IGF-I) in the muscle fibres (Barton-Davies et al., 1998).

Testosterone is a hormone that has a large influence on the jaw muscle fibres. Although castration of male adult rabbits has no significant effect on the fibre type proportion of the masseter, it has been demonstrated that when castration is performed when the rabbits are young adults the muscle maintained a similar fibre type distribution as in the females. This could be reversed when these rabbits had a brief exposure to testosterone (Reader et al., 2001; English and Schwartz, 2002). Jaw-closing muscles were found to be sexual dimorphic in the masseter of the rabbit (English et al., 1998, 1999; Eason et al., 2000b), rat (Ramamani et al., 1999), mouse (Eason et al., 2000a), and the temporalis of the guinea pig (Lyons et al., 1986). Male rabbit masseters have a significant larger proportion of fast fibres than female rabbit masseters (English et al., 1998). In mice, the male masseter has twice as many.
fibres containing MyHC-IIB as the female masseter, which in turn has twice as many fibres containing MyHC-IIA (Eason et al., 2000a). Furthermore, immunohistochemistry enabled the distinction of four different subtypes of MyHC-I, named I\(_1\), I\(_2\), I\(_3\), and I\(_4\) in rabbit (English et al., 1998; 1999; Eason et al., 2000b). This could be the result of post-translational modifications of the MyHC molecule (Pol-Rodriguez et al., 2001). The proportion of these individual subtypes differs between male and female rabbit. Subtypes of MyHC-I were also found to exist during development in limb and trunk muscles but were, in these muscles, converted into one single MyHC-I isoform (Hughes et al., 1993).

In humans, differences between male and female in the proportion of fibre types of the jaw muscles have been described in young adults. It was found that the male masseter had a larger number of type II fibres, while the female masseter had a larger number of type I and IM fibres (Tuxen et al., 1999).

**Food**

The main function of the jaw muscles is mastication. A recent study (Kemsley et al., 2003) showed that there is a large variation in the way people activate their jaw muscles during so-called ‘free-style’ chewing. The redundancy of muscles and muscle portions allows several ways in which they can be activated to produce a certain jaw movement (Koolstra, 2002). It can thus be expected that this difference in activation is reflected in the large individual variation in fibre type composition as observed in the present study.

The hardness of daily food can also have an influence on the phenotype of jaw muscle fibres. To our knowledge, no study is available which compared the influence of food hardness on the fibre type composition and fibre cross-sectional areas of jaw muscles in human. There are a number of studies in which the jaw muscles of animals were compared after they were put either on a hard or on a soft diet. It was demonstrated that long-term intake of an easily chewable, fine-grained diet results in degeneration of muscle fibres and muscle spindles in the masseter of mice (Maeda et al., 1987, 1990). Experiments with rats (Miehe et al., 1999; Saito et al., 2002) demonstrated that a soft diet, which requires less masticatory loading, facilitates a more MyHC-IIB-rich phenotype in the masseter muscle than a hard diet, which requires more loading. Type I fibre proportion, and fibre cross-sectional area in the deep masseter did not differ between rats that were put on a normal or a soft diet.
Although more type IIB, and less type IIA fibres were noticed in two regions of the deep masseter of rats that were kept on a soft diet. Such differences were not seen in the digastric muscle of these animals.

The cross-sectional area of the slow fibres decreased significantly in the masseter of rabbits that were put on a soft diet for three months compared to rabbits that were put on a diet of hard pellets (Langenbach et al., 2003). These animal groups did not show differences in fibre type composition. It must be noted that in this study only a distinction between type I and type II fibres, and fibres that expressed MyHC-cardiac α was made. Possible fibre type differences within the type II fibre group, therefore, could not be recognised.

A more rigorous approach was made in a study (Maxwell et al., 1980) in which all molars and incisive teeth were extracted in monkeys (Macaca mulatta). The investigators noticed, 4.5 years after the extraction, not only a significant decrease in the fibre cross-sectional area of the slow fibres in the masseter and temporalis muscles, but also a decrease in the proportion of slow fibres and an increase of fast, fatigable fibres in the posterior muscle part of the masseter. It is thus possible to see an adaptation of the fibre type a long time after the intervention.

**Adaptation Influences in Artificial Denture Wearers**

Most of the individuals in the present study had an upper and lower denture prosthesis. This could have an influence on the presented fibre type proportion and fibre cross-sectional areas. Generally, individuals with removable partial or complete dentures have a significant reduction in bite force and masticatory function (Fontijn-Tekamp et al., 2000). This might be reflected in the MyHC isoform proportion and the fibre cross-sectional areas of the jaw muscle fibres comparable to a diet on soft food. No studies have yet been performed that compared fibre type proportion and cross-sectional area of edentulous and dentulous humans. It can be expected that edentulous individuals have smaller muscle fibre cross-sectional areas and that the fibres become faster.

**Craniofacial Morphology**

The variation in facial forms is large among humans. Differences in skull shape are associated with differences in masticatory performance (Van Spronsen et al., 1992). For instance, individuals with an excessively large anterior face height, the so-called
long-face syndrome, produce a significantly smaller maximum molar bite force than those with a normal facial height (Proffit et al., 1983). This might be reflected by a difference in fibre type composition. According to one study (Boyd et al., 1984), long-face individuals have a higher percentage of fast fibres in some areas of the masseter, but this is refuted in another study (Shaughnessy et al., 1989). This discrepancy in study results can possibly be explained by the large inter-individual and intra-individual variation in fibre types of the jaw muscles (Chapter 3).

In animal studies, it is easier to investigate the relationship between craniofacial morphology and jaw muscle fibres, for instance, by surgically manipulating the position of the mandible. After increasing the vertical dimension of the jaw of guinea pigs, their masseter muscles became slower and had a decreased ATPase activity (Paik et al., 1993). Protrusion of the mandible in rats led to an adaptation of the lateral pterygoid and the superficial masseter (Easton et al., 1990); the contraction time increased in both muscles. The lateral pterygoid had a significantly greater area occupied by type I fibres. The superficial masseter, on the other hand, showed a significant increase in the area occupied by type IIA instead of type IIB fibres. It was concluded that both muscles became slower. A similar experiment in pigs led to an increase in the proportion of MyHC-I (and in mRNA for MyHC-I) in the anterior region of the masseter and in the posterior region of the temporalis (Gedrange et al., 2001). The fibres in these regions might experience more stretch.

Differences between Jaw Muscles, and Limb and Trunk Muscles with respect to the MyHC Isoforms

There are a number of differences between jaw muscles and limb and trunk muscles. Firstly, the jaw muscles contain many hybrid fibres, in contrast to limb and trunk muscles. Many of these fibres co-express MyHC-fetal and/or MyHC-cardiac α. Secondly, there is a difference in the fibre diameter between the two muscle groups. Type II fibres are larger than type I fibres in limb and trunk muscles, while in jaw muscles the opposite is true (Polgar et al., 1973).

Next to these differences, the question can be raised whether the MyHC-I isoform present in jaw muscles is the same as the one in limb and trunk muscles. For
instance, the type I fibres in the masseter are reported to be slower than the same fibre type in limb and trunk muscles (Morris et al., 2001). Furthermore, the variability in contractile properties among the type I muscle fibres is larger in the masseter than in limb and trunk muscles. This could mean that in the jaw muscles there are either more MyHC-I isoforms or that there is a post-translational adaptation of MyHC-I, for instance by glycation (Ramamurthy et al., 2001), deamination (Balagopal et al., 1997) or phosphorylation (English et al., 1998). It must be noticed that in rabbit and guinea pig, monoclonal antibodies could identify at least four different subtypes of MyHC-I (English et al., 1998). In the temporalis and pterygoid muscles (Chapters 4 and 5) some MyHC type I fibres had a different antibody staining pattern which also might suggest that there are more subtypes of MyHC-I. In the present study, this MyHC isoform was named MyHC-Ia. Whether there are subtypes of MyHC-I expressed in human jaw muscles, or whether MyHC-I is post-translationally adapted, is not yet clear.

Differences in contractile properties between fibres of the same type in jaw muscles and limb and trunk muscles might also be related to a difference in the expression of the light chain of the myosin protein (MyLC). In the human masseter, four different essential and regulatory MyLCs are expressed, namely MyLC-1s, MyLC-2s, MyLC-1f, and MyLC-1emb/atrial (Soussi-Yanicostas et al., 1990; Stal et al., 1994). The latter MyLC isoform is transiently expressed in fetal limb and trunk muscles. However, it remains present in jaw muscles. Furthermore, in limb and trunk muscles MyLC-2f and MyLC-3f are expressed, but they have not been found in the masseter.

In the rat, the large variability in contraction velocity of limb muscle fibres can mainly be accounted for by the essential MyLC composition of the fibres (Bottinelli et al., 1994; Bottinelli and Reggiani, 1995), in contrast to human limb muscles (Larsson and Moss, 1993). This might be caused by the fact that MyHC-IIB, which is found in rat but not in human muscles, is more sensitive to the regulatory MyLC isoform than other MyHC isoforms. However, the unusually slow MyHC type I fibres found in the human masseter might be related to the expression of MyLC-embryonic (Sciote et al., 2003). More study is needed to solve the question how jaw muscles differ from limb and trunk muscles in relation to these factors.

Fibres in the jaw muscles are smaller than fibres in limb and trunk muscles. Furthermore, type II fibres in the jaw muscles have a smaller cross-sectional area than type I fibres. While in limb and trunk muscle the reverse is true (Polgar et al.,
1973; Monemi et al., 1998). As smaller cross-sectional areas enable an increase of the exchange of \( \text{O}_2 \) and nutrients, the resistance to fatigue might thus be improved, especially in the fast fibres. Consequently, the smaller muscle fibres may be advantageous for the jaw muscles.

The origin of the difference in fibre diameters between jaw muscles and limb and trunk muscles could be related to the homeobox-containing gene Engrailed-2 (En-2). The presence of this gene has been demonstrated in the masseter, temporalis, and the medial and lateral pterygoid muscles of the mouse (Degenhardt and Sassoon, 2001; Degenhardt et al., 2002) and in some branchial arch muscles of the zebrafish (Hatta et al., 1990). The reporter of this gene is specifically expressed in myoblasts in the first branchial arch and is maintained in adult jaw-closing muscles only. However, in the anterior digastric and the tensor tympani, which are also derived from the first branchial arch, just as the jaw-closing muscles, this reporter gene was not detected. In transgenic mice, in which all muscle fibres expressed En-2, all fibres decreased in cross-section. An overexpression of this En-2 in fast muscles coincided with a decrease of the fast fibres and also with a shift in the metabolic properties of these fibres. The specific expression of En-2 in the jaw of normal, non-transgenic species can therefore play a role in specifying muscle-fibre characteristics that contribute to the physiologic properties of specific muscle groups (Degenhardt and Sassoon, 2001).

Differences in the Fibre Type Composition between the Jaw Muscles of Different Animals

The earlier paragraphs partially refer to studies in which animals are used, since the tests are either not feasible, or not acceptable, in humans. Comparing human skeletal muscles with those of animals can be difficult. In general, large animals have more fibres that express MyHC-I than small animals (Pellegrino et al., 2003). Adaptation of muscle fibres to a new demand can, therefore, be different among animals. For instance, an animal in which the masseter contains predominantly fibres that express MyHC-IIIX will, as a reaction to more activation and stretch, probably change its fibre types towards MyHC-IIIA but not to MyHC-I. An animal with a large proportion of MyHC-IIIA fibres might convert its fibres to MyHC-I. Although many
studies have investigated the fibre type transformation in several species it is, thus, difficult to compare the results of one animal with another.

The general rule for limb and trunk muscles that large animals have a higher proportion of slow fibres than small animals is also true for the jaw muscles. The masseter of the mouse, for instance, contains only fast fibres while a large animal like the cow contains only slow fibres. The cow, which is a ruminant, is thus well adapted for slow, long-term exercises. The masseter of cat, dog, and pig has a mixture of slow and fast fibres, although the fast fibre types predominate (Tuxen and Kirkeby, 1990). A special fibre type composition has been found in the masseter of the kangaroo. This species, which has more or less the same skull shape as sheep, and also eats the same type of food, has a masseter which predominantly expresses MyHC-cardiac $\alpha$ (Hoh et al., 2000). In contrast, the sheep's masseter predominantly consists of MyHC-I fibres. This has been contributed to a need for a higher velocity for grinding food because, in contrast to the sheep, the kangaroo does not ruminate.

In some animals, a special MyHC isoform that is named 'superfast' or MyHC-IIM has been identified. This MyHC isoform is found in the jaw-closing muscles of the cat, the American opossum (Rowlerson et al., 1981: Sciote et al., 1995), five species of Carnivora, six species of Primates, not including man (Rowlerson et al., 1983), and of the caiman and terrapin (Rowlerson, 1994). A more complete list with animals in which the jaw-closing muscles express MyHC-IIM is reviewed by Hoh (2002). The maximum contraction velocity of MyHC-IIM fibres is higher than that of MyHC-IIB fibres. The occurrence of MyHC-IIM in animals is associated with an aggressive bite which is required for predation and for defence (Rowlerson et al., 1983).

**Concluding Remarks**

The architectural and anatomical complex jaw muscles contain many fibre types which are normally not seen in large quantities in limb and trunk muscles. These fibre types differ in force production at various contraction velocities. The variation in fibre type enables the jaw muscles to produce a smooth gradable force and velocity. The dynamic nature of muscle fibres allows them to change their phenotype to optimise contractile function while minimising energy use. This explains the variability in fibre
type proportions found between individuals, muscles, and muscle portions. The amount of daily activation and stretch probably has a large influence on the expression of the MyHC isoforms in the muscle fibres. Next to these influences, there are other internal and external influences which also can affect the fibre type.

Internal and external influences on the muscle fibres can be different in jaw muscles than in limb and trunk muscles. However, the observed difference in MyHCs might also be related to a difference in genetic background. Although differences have been observed in the genes that are responsible for the development of jaw and other muscles, it is not yet known in which way these differences lead to the remarkable collection of fibre types that has been observed in the jaw muscles.