Myosin heavy chain composition of the human jaw muscles

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Jaw muscles are active during a large variety of motor tasks, like mastication, biting, talking, and swallowing. To execute these tasks, they have to control the position of the mandible and apply instantaneously changing forces to it. The jaw muscles can meet these different requirements by their complex architecture and heterogeneous fibre type composition, with fibres capable of producing a variety of forces and contraction speeds. The contraction speed of a fibre is largely dependent on the heavy chain of its myosin protein. The various isoforms of this myosin heavy chain (MyHC), and the way they are distributed over the different parts of the jaw muscles is the subject of this thesis.

**Functional Anatomy of the Jaw Muscles**

The human jaw muscles (Figs. 1.1, 1.2, 1.3, and 1.4) can be divided into two groups depending on their main function, namely the jaw closers and jaw openers. The jaw closers, which comprise the masseter, medial pterygoid, and temporalis, are the most complexly structured skeletal muscles in mammals (Hannam and McMillan, 1994). The masseter can anatomically be divided into a deep and a superficial part. The deep part originates from the whole length of the zygomatic arch and inserts onto the upper two-thirds of the lateral surface of the mandibular ramus. The superficial part originates from a strong tendon plate from the anterior two-thirds of the zygomatic arch and inserts onto the lower one-third of the ramus. The temporalis originates from the lateral side of the skull and its fibres attach to a thick tendon plate, which in turn inserts on the coronoid process and the inner side of the mandibular ramus. The
muscle is often, more or less arbitrarily, divided into an anterior and posterior part. The medial pterygoid is a heavily pennated, rectangular muscle. It originates from the pterygoid fossa and the medial surface of the lateral pterygoid plate and inserts on the lower part of the medial surface of the mandibular ramus.

The jaw openers comprise the lateral pterygoid, mylohyoid, geniohyoid, and digastric. The lateral pterygoid, which has an intermediate role in jaw opening and closing, has two heads. The superior head originates from the infratemporal surface of the sphenoid bone and the inferior head from the lateral surface of the lateral pterygoid plate. Both heads insert on the front of the mandibular neck and on the capsule of the temporomandibular joint. The digastric has an anterior and posterior belly which are united by an intermediate tendon. This tendon is held in place by a fibrous loop attached to the hyoid bone. The anterior belly attaches to the digastric fossa on the lower border of the mandible, close to the median plane. The posterior belly attaches to the mastoid notch of the temporal bone. The geniohyoid originates from the anterior surface of the body of the hyoid bone and inserts onto the inferior
The medial pterygoid (1) and the superior (2a) and inferior (2b) head of the lateral pterygoid.

mental spine. The mylohyoid forms a thin sheet that arises partially from the front of the body of the hyoid bone and from a median fibrous raphe which runs from the internal side of the mental symphysis of the mandible to the hyoid bone. This muscle inserts onto the mylohyoid line of the mandible. The digastric, mylohyoid, and geniohyoid do also act on the hyoid bone. Together with the stylohyoid these muscles are also known as the suprathyroid muscles, which are capable of elevating the hyoid bone during, for example, swallowing. In contrast, the so-called infrahyoid muscles, which comprise the sternohyoid, sternothyroid, thyrohyoid, and omohyoid, depress and stabilise the hyoid bone during functioning.

The jaw closers have a more complex architecture than the jaw openers. They are multi-pennate and complexly layered, with many intramuscular aponeuroses. Their fibres are relatively short, while their attachment areas are relatively broad. In contrast, the jaw openers have a parallel-fibred structure. They have less, but longer fibres than the jaw closers and their attachment areas are more circumspect.
Figure 1.3
Posterior view of the geniohyoid (1) and the mylohyoid (2).

Because of these different architectural designs the jaw closers have the capacity to produce larger forces than the jaw openers while, reversibly, the jaw openers have the capacity to produce a larger amount of shortening. In addition, because of their broad attachment areas, the jaw closers are capable of producing differential mechanical actions through a selective activation of different muscle parts (Van Eijden et al., 1995, 1996, 1997). Such a differential activation has indeed been demonstrated in a variety of species and for a large number of motor tasks (human: Blanksma et al., 1992, 1997; Van Eijden et al., 1993; rabbit: Weijs and Dantuma, 1981; Weijs et al., 1999; Langenbach et al., 2001; pig: Herring et al., 1979).

The selective activation of muscle parts is only possible if the motor unit territories in the muscles are small and restricted to specific areas. (A motor unit is the combination of a single motoneuron and all the muscle fibres it innervates.) Single motor units in the masseter have indeed been reported to occupy on average less than about 5% of the muscle’s cross-sectional area (human: McMillan and
Some of the jaw openers, namely the mylohyoid (1), and the anterior (2a) and posterior (2b) belly of the digastric. Also seen are the stylohyoid (3) and the infrahyoid muscles, namely the sternohyoid (4), the superior belly of the omohyoid (5), the thyrohyoid (6), and the sternothyroid (7).

Hannam, 1991; Tonndorf and Hannam, 1994; rabbit: Kwa et al., 1995a). Furthermore, both the territory occupied by its fibres and their average direction can be very different between different motor units from the same muscle. It has been demonstrated for rabbit masseter motor units that the positional and directional variation in motor unit forces is almost as large as the range of fibre positions and directions in the muscle (Turkawski et al., 1998). The restricted territories and possible variation in motor unit force provide an anatomical basis for accurate control of the combined muscle force vector. Except for this mechanical diversity, the functioning of the jaw muscles is highly dependent on the physiological properties of its motor units. These properties, like their force output, fatigability, and contraction speed, vary considerably (see for review: Van Eijden and Turkawski, 2001).
Motor Unit and Fibre Type Properties

A motor unit's ability to produce force is primarily dependent on its cross-sectional area, which depends on the number and cross-sectional areas of the constituent muscle fibres. The ability to resist fatigue is dependent on the metabolic properties of the fibres. Muscle fibres of fatigue-resistant motor units contain a substantial amount of aerobic end-oxidation enzymes. On the other hand, fibres of fatigable motor units are rich in glycolytic enzymes and low in enzymes of aerobic oxidative metabolism. The speed of motor unit contraction is largely dependent on the heavy chain of the myosin protein in the muscle fibres.

Myosin Heavy Chain Isoforms and Contraction Speed

Sarcomeric myosin is a complex hexameric structure and is composed of four light chain (MyLC) molecules (mol. wt. 16 - 20 kD), named essential MyLC and regulatory MyLC, and two heavy chain (MyHC) molecules (mol. wt. 200 - 220 kD) (Fig.1.5). The MyLC molecules play a part in the conversion of chemical energy into movement (Lowey et al., 1993), but these molecules are not considered in the present study. The MyHC subunit contains the ATPase activity which provides energy to generate force for muscle contraction. There are several isoforms of the MyHC molecule. These MyHC isoforms, which are encoded by a multigene family, are in humans clustered at two distinct locations, two genes on chromosome 14 and six on chromosome 17 (Weiss et al., 1999b). The MyHC isoform genes found in humans
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(Sartore et al., 1987; Butler-Browne et al., 1988; Smerdu et al., 1994; Stal et al., 1994; Weiss and Leinwand, 1996) include MyHC-cardiac α, MyHC-I (or -β), MyHC-IIA, MyHC-IIX, MyHC-IIB, and MyHC-extraocular which is expressed in the extrinsic eye and some pharyngeal muscles, and two developmental forms, namely MyHC-fetal (also named MyHC-neonatal, -perinatal or -developmental) and MyHC-embryonic. Although MyHC-cardiac α is normally expressed in the atrium of the heart, and MyHC-fetal in developing muscles, they can both be expressed in some mature jaw muscles (Butler-Browne et al., 1988; Bredman et al., 1991; Monemi et al., 1996). Not all MyHC genes are always translated into proteins. For example, mRNA encoding for MyHC-IIB is expressed abundantly in the human masseter but its protein was not found (Horton et al., 2001). The MyHC isoforms are highly conserved among mammals. Similar isoforms were found among human, rat, and mouse. Chromosome 14 and 17 are not the only locations where genetic information is located for the isoforms of human MyHC. Recently, three new locations have been found on chromosome 3, 7, and 20 that decode for three novel MyHC isoforms (Desjardins et al., 2002) which might be expressed under certain situations, although these situations are not yet known.

The different isoforms of MyHC are functionally unique and cannot be substituted for one another (Allen et al., 2000). The main difference between the MyHC isoforms is their rate of converting ATP into energy which determines the speed of actin-myosin detachment. There is a good correlation between the MyHC isoforms and the contraction velocity of fibres (Bottinelli et al., 1996). The contraction velocity increases successively from fibres that contain predominantly MyHC-I → MyHC-IIA → MyHC-IIX → MyHC-IIB. Using the slack-test technique (Edman, 1979), which determines the unloaded shortening velocity (Vₒ), it was shown that the Vₒ is consistently about ten times lower in MyHC-I fibres than in MyHC-IIX fibres; the Vₒ for MyHC-IIB is approximately 20% higher than the Vₒ for MyHC-IIX (Bottinelli et al., 1994). MyHC-IIA fibres are intermediate between MyHC-I and -IIIX fibres, and hybrid fibres (see below) are intermediate between the pure fibre types (Larsson and Moss, 1993; Bottinelli et al., 1996; Widrick et al., 1996). The contraction velocity of fibres co-expressing MyHC-cardiac α is said to lie between the velocities of MyHC-I and MyHC-IIA fibres (Kwa et al., 1995b; Sciote and Kentish, 1996); the contraction velocity of fibres expressing MyHC-fetal is not yet determined unambiguously but seems to be slow (D’Antona et al., 2003). Furthermore, it should be noticed that the consumption rate of ATP is higher in fibres expressing the fast MyHC isoforms, and
these fibres are thus more energy expensive than fibres expressing MyHC-I (He et al., 2000).

Muscle fibres do not always express just one MyHC isoform. Therefore, the fibres can be grouped as ‘pure’ fibre types, which express only one MyHC, and ‘hybrid’ fibre types, which express more than one MyHC isoform. These hybrid fibres are classified according to the MyHC isoforms they express. For example, fibres that co-express MyHC-I, MyHC-IIA, and MyHC-fetal are called MyHC-fetal+I+IIA fibres. Hybrid fibres are thought to be fibres that are in transition from one fibre type into another since they are frequently found in limb and trunk muscles of subjects during disuse or during extreme usage of the muscles (Klitgaard et al., 1990) or in fibres that are regenerating (Pette et al., 2002). However, they are also found in the jaw-closing muscles (rabbit: Kwa et al., 1995b; human: Stal et al., 1994; Monemi et al., 1999). Motor units that contain these hybrid fibres can have either one kind of hybrid fibre, or have hybrid fibres of which two MyHC isoforms are spread unevenly over the constituting fibres (Gates et al., 1991; Larsson and Moss, 1993). As was found in the masseter of the rabbit, these motor units have a continuous range of contraction speeds which match the continuous spectrum of MyHC contents of their hybrid fibres (Kwa et al., 1995b). This may imply that in jaw muscles a finer gradation of force and movement is possible than in limb and trunk muscles.

Muscle fibres are highly adaptive to environmental alterations, by changing one fibre type into another (Adams et al., 1993; Oishi et al., 1998). For instance, during resistance training in human the amount of MyHC-IIIX fibres decreased in favour of slower MyHC isoforms (Hather et al., 1991; Staron et al., 1994). Reversibly, disuse of the soleus muscle of the rat induced the conversion of MyHC-I fibres into MyHC-IIA fibres (Oishi et al., 1998). The conversion of fibre types normally follows a strict order from MyHC-I → -IIA → -IIIX → -IIB and vice versa (Schiaffino and Reggiani, 1994). Some muscle fibres in the rabbit co-expressed MyHC-cardiac α in combination with MyHC-I and -IIA (Peuker et al., 1998). It was, therefore, concluded that MyHC-cardiac α forms an intermediate step between MyHC-I and MyHC-IIA.

Some papers questioned this strict order of MyHC transformation. For instance, under certain conditions, like space flight (Talmadge et al., 1996), or hindlimb suspension, alone (Stevens et al., 1996), or in combination with hyperthyroidism (Caiczzo et al., 1998), hybrid fibres were found that express MyHC-I and -IIIX but not MyHC-IIIA. Thus, muscle fibres are not obligated to follow this strict order of MyHC transformations.
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Classification of Motor Units and Muscle Fibres

Differences in contraction velocity and fatigability made it possible to classify motor units physiologically into different types, namely S (slow contracting, fatigue-resistant), FR (fast contracting, fatigue-resistant), Fint (fast contracting, intermediate fatigable), and FF (fast contracting, fatigable) (Burke et al., 1971; Fournier and Sieck, 1988). The contractile speed is defined by the so-called twitch contraction time, i.e., the time necessary to build up force when a motor unit is elicited once. Slow and fast units have twitch contraction times of, respectively, more and less than 40 ms. Fatigue is usually defined as a decline in force during tetanic stimulation. If the tetanic force of a motor unit shows a relatively small or no decline, then the motor unit is called fatigue resistant. If there is a relatively large decline, then the motor unit is called fatigable.

Most information on the physiological properties of motor units comes from animal experiments by stimulating single motoneurons with an electrode into the motor nucleus. This kind of experiments cannot be performed in humans, because of the difficulty of accessing the motoneurons. Therefore, motor unit and fibre properties are, in general, extrapolated from histochemical staining of muscle fibres. The ability to resist fatigue is related to the amount of some metabolic enzymes, such as succinate dehydrogenase and citrate synthase. Enzyme histochemistry, therefore, makes it possible to identify and characterise individual slow and fast contracting muscle fibres based on these metabolic enzymes. Muscle fibres can then be classified into slow, oxidative (SO) fibres which are recruited for slow repetitive postural or chronic activity, and fast contracting fibres which are either oxidative and glycolitic (FOG), or only glycolitic (FG) (Barnard et al., 1971; Burke et al., 1971; Peter et al., 1972); these fibres are recruited for fast phasic contractions.

Based on the distinct instabilities of the myofibrillar ATPase activity of the muscle fibres in alkaline (Guth and Samaha, 1969) and acidic media (Brooke and Kaiser, 1970) fibres were initially classified into type I (slow) and type II (fast) fibres. Further refinement of the pH of the acidic media led to a subdivision of the type II fibres into a type IIA and IIB fibre. By using a double preincubation, each at a different pH, it was also possible to identify type IIX fibres (Sant'Ana Pereira et al., 1995a). Some muscle fibres could not easily be classified by ATPase histochemistry into one of the aforementioned types, leading to a further classification into subgroups, like IIC and IM (Ringqvist, 1971, 1973; Rowlerson et al., 1981; Eriksson et al., 1982). These fibres were found in muscles that were regenerating or in
muscles that were subjected to training, but they were also found in large numbers in the jaw-closing muscles.

In the last few decades a technique has been developed for the identification of proteins that could serve as a marker for the classification of muscle fibres. Hybridoma cell lines could raise antibodies against the diverse MyHC isoforms. These isoforms of MyHC can be considered as molecular markers of a complete pattern of protein expression. This pattern is the molecular equivalent of the fibre type as determined by physiology (Bottinelli et al., 1999). A different approach is the separation of the muscle proteins on high glycerol containing gels (Laemmli, 1970: Talmadge and Roy, 1993). With this technique, also known as SDS-PAGE, it is possible to separate the different MyHC isoforms. Laser densitometry makes it further possible to calculate the concentrations of the different isoforms in whole muscles or muscle portions, and in single hybrid fibres (Giulian et al., 1983).

Classification based on the MyHC isoform expression, as detected by immunohistochemistry, is largely similar to the classification based on ATPase histochemistry. Thus, ATPase classified type I fibres express MyHC-I, and type IIA fibres express MyHC-IIA. However, some problems were encountered in using the ATPase technique. Firstly, some fibres of the rat, which were classified as type IIB, had a contraction velocity that was lower than what was normally found for type IIB fibres (Schiavino et al., 1989). It is now well established that ATPase classified type IIB fibres comprise two different MyHC isoforms. In mammals, a subdivision was made into MyHC-IIB and MyHC-IIX fibre types (Schiavino et al., 1989; DeNardi et al., 1993). Since this MyHC-IIX isoform is homologous with the human ATPase classified type IIB fibres, it was thus more appropriate to denote these type IIB fibres as MyHC-IIX fibre types (Santana Pereira and Moorman, 1994). Secondly, with ATPase histochemistry it is not possible to classify the whole spectrum of hybrid fibres that might occur in muscles. For example, the MyHC contents of fibres in jaw muscles which were classified by ATPase histochemistry as type IM or IIC fibres is not always unambiguous. Immunohistochemistry showed that it could be stained with antibodies against MyHC-I, MyHC-IIA, and MyHC-I+IIIX, and in many cases also with an antibody against MyHC-cardiac (Bredman et al., 1992; Monem et al., 1999). Finally, with ATPase histochemistry it is not possible to distinguish fibres that express MyHC-fetal and/or MyHC-cardiac. These MyHC isoforms are abundantly expressed in the muscle fibres of the jaw muscles (Butler-Browne et al., 1988; Stal et al., 1994). It is, thus, clear that ATPase histochemistry has its limitations in
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distinguishing the various fibre types. Therefore, in the present study immunohistochemistry using monoclonal antibodies against MyHC isoforms was used to classify the fibres of the human jaw muscles.

Distribution of Fibre Types in Human Jaw Muscles

Information from Literature

Several studies are available that investigated various aspects of fibre type composition and/or fibre cross-sectional area of the human jaw muscles (Johnson et al., 1973; Ringqvist, 1973, 1974; Serratrice et al., 1976; Vignon et al., 1980; Eriksson et al., 1981, 1982; Ringqvist et al., 1982; Eriksson and Thornell, 1983; Thornell et al., 1984; Shaughnessy et al., 1989; Sciote et al., 1994; Stal et al., 1994). These studies showed a number of differences between jaw muscles, and limb and trunk muscles. A prominent feature of the jaw muscles is that they have more IM or IIC, or hybrid fibres than limb and trunk muscles and that the diameter of type II fibres is smaller than that of type I fibres, while the reverse is true in limb and trunk muscles. Furthermore, these studies pointed to a heterogeneous fibre type distribution in the jaw muscles. For instance, the posterior superficial part of the masseter contains less type I fibres than the other muscle parts (Eriksson and Thornell, 1983), and the anterior belly of the digastric contains more ATPase classified type II B fibres than the posterior belly (Eriksson et al., 1982). The studies also observed a large interindividual variation in fibre type composition.

These studies, however, have a number of limitations. Firstly, almost all studies used ATPase histochemistry to classify the muscle fibres. As mentioned earlier, this technique is not capable to detect, for example, the various MyHC isoforms and hybrid fibre types. Secondly, most studies used only one biopsy, or a small number of biopsies, while their location was not always well defined. Thirdly, the samples from different muscles were not always from the same person. Since the variability in fibre type distribution within muscles and between individuals might be large, comparison of different muscles or muscle parts is hampered. To decrease the amount of variability it is thus necessary to take samples from well-defined regions of muscles from the same individual. Fourthly, the available literature gives an incomplete picture of the fibre type distribution of both the jaw closers and openers.
Most information is available on the masseter. The other jaw-closing and -opening muscles were but scarcely investigated. Thus far, no study compared the intra- or intermuscular fibre type distribution of all human jaw muscles. Moreover, the fibre type composition of the human infrahyoid muscles was never examined. Finally, no studies are available that measured the contents of different MyHC isoforms within single hybrid fibres. This is probably caused by the fact that the small jaw muscle fibres are difficult to isolate. Still, it is important to investigate the MyHC isoform proportion in hybrid fibres because a variation in the proportion of a particular MyHC isoform could explain the continuum of contractile properties which exists within the jaw muscles (Kwa et al., 1995b; Turkawski and Van Eijden, 2001).

Factors that might be responsible for Differences in Fibre Type Composition

The large number of anatomical and functional differences among and within the jaw-closing and -opening muscles suggests that different muscles and muscle portions are specialised for certain functions. Electromyographic studies have, indeed, demonstrated a task-dependent differential activation of muscle groups (jaw closers versus jaw openers), muscles, and muscle portions (McNamara, 1973; Möller, 1974; Vitti and Basmajian, 1977; Mahan et al., 1983; Belser and Hannam, 1986; Blanksma and Van Eijden, 1990, 1995; Van Eijden et al., 1990, 1993).

As pointed out, a prominent feature of muscle fibres is their ability to alter their phenotype to a certain function. It is thus likely that the specialisation for a particular function is also reflected by differences in fibre type composition and that the MyHC isoform composition of a muscle group, muscle, or muscle portion is related to, for example, the amount of its activity. It is generally known that muscles that are more tonically activated, like the soleus, have more fibres that express MyHC-I than muscles that are more phasically activated, like the extensor digitorum longus. In cat (Kernell et al., 1998) and in human limb muscles (Monster et al., 1978), it was noticed that muscles with larger daily duty times contained more type I fibres. Reversibly, in muscles that ceased to be activated, for instance after a spinal cord injury or hindlimb suspension, MyHC-I was downregulated while fast MyHC isoforms were upregulated (Talmadge, 2000). Thus, it can be expected that there are more type I fibres in muscles, or muscle portions, that are more active. With respect to the jaw muscles, it might be hypothesised that the jaw-closing muscles have more MyHC-I fibres than the jaw-opening and infrahyoid muscles because they are more tonically active to
elevate the jaw against gravity (Stalberg and Eriksson, 1987). Similarly, intramuscular differences in fibre type composition may be explained. For example, a heterogeneous activation pattern has been demonstrated in the temporalis muscle during various bite force tasks. A change in bite force direction showed an alteration in activity which was generally the smallest in the anteriormost region and the largest in the posteriormost region of the muscle (Blanksma and Van Eijden, 1990, 1995). This suggests a gradual increase of MyHC-I fibres across the muscle which goes from posterior to anterior.

Differences in fibre type composition between muscle groups, muscles, or muscle portions, might also be related to differences in their positions and orientations within the leverage system of the jaw. Muscle portions that are closer to the rotation axis of the mandible undergo less excursions and should produce more force to generate a particular force at the teeth than muscle portions that are further away from this axis. This might possibly affect the fibre type composition, but how this is reflected is still not clear. A well-known fact is that deep muscle portions generally contain more type I fibres than superficial muscle portions. Whether this is related to differences in activation and/or to mechanical factors is also not clear.

Except for these functional aspects, other factors that might affect fibre type composition should be considered. Firstly, there is a difference in nerve supply of muscles, namely by branchial or spinal nerves (Butler-Browne et al., 1988; Stal et al., 1994). This might have a possible influence on the difference in fibre type composition between jaw and limb muscles. The jaw-closing muscles, and also some jaw-opening muscles, are derived from the first branchial arch and are innervated by a branchial nerve. Other jaw-opening muscles are derived from the second branchial arch or from a somite and are innervated by either a branchial or a spinal nerve. Secondly, there might be a difference in the genetic pathway for expressing muscle-specific proteins during embryogenesis. For instance, Pax-3 plays a regulatory role in somitic mesoderm but not in the nonsomatic cranial muscles (Tajbaksh et al., 1997). Thirdly, differences in the chronological or spatial activation of some transcription factors have been reported. The expression of several muscle-specific transcription factors peaks later in the masseter than in other muscles (Noden et al., 1999; Yamane et al., 2000). Finally, the regulation of the myosin gene family is under control of a complex set of processes including activity, hormonal, and metabolic factors. For instance, activity and/or stretch induce the upregulation of an autocrine, local growth factor, also called mechano growth factor (MGF) (Goldspink, 2002).
which causes addition of new sarcomeres, upregulation of protein synthesis, and changes in gene transcription. These factors can all have an impact on the expression of MyHC isoforms.

Aims

In the present study, the fibre type composition of the human jaw muscles was determined. Detailed knowledge of the fibre type composition combined with a thorough discussion of the concomitant functional and anatomical differences improves our insight into the significance of jaw muscle function and heterogeneity. In Chapter 2, we compared the jaw closers and the supra- and infrahyoid muscle groups and hypothesised that, because of their different functions, the jaw-closing muscles contain more slow type MyHCs and have larger fibre cross-sectional areas than the supra- and infrahyoid muscles. We expected these larger areas because the jaw-closing muscles have to produce more force during functioning. Among and within the jaw muscles a large number of anatomical and functional differences has been reported. Different muscle groups, muscles, and muscle portions can be specialised for certain functions and these differences might be reflected by differences in fibre type composition. Therefore, in Chapter 3, differences between and within the jaw muscles (closers and openers) were compared. Thus, we took all the jaw closers, supra- and infrahyoid muscles, sectioned them in a cryomicrotome and classified the fibres in several anteroposterior and mediolateral regions with antibodies against MyHC.

An example of a human jaw-closing muscle that is heterogeneous in function is the temporalis. By investigating this muscle in detail, thus by selecting many muscle regions across the muscle from anterior to posterior, it is possible to compare the fibre type distribution of a large number of muscle regions. We hypothesised, in Chapter 4, that the more anteriorly positioned muscle regions contain more MyHC-I fibres than the more posteriorly positioned regions.

Similarly, we compared in detail the fibre type composition of the medial pterygoid and the lateral pterygoid. These muscles differ in function and architecture but not in their innervation and genetic background. In addition, the two heads of the lateral pterygoid have different functions. The inferior head is supposed to be
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concentrically active during opening of the jaw, while the superior head is supposed to be eccentrically active during closing of the jaw. Whether this has any influence on the fibre type composition was investigated in Chapter 5.

To describe the fibre type composition of various jaw muscles we determined the relative number or the cross-sectional area of a particular fibre type using antibodies against MyHC isoforms as markers. Alternatively, the MyHC isoforms can be separated by gel electrophoresis and the integrated density of their bands can be calculated. The advantage of this technique is that the MyHC contents can be determined in muscles, even if the muscles are difficult to cut in a microtome. It remains to be investigated whether both methods give similar results in the jaw muscles. In Chapter 6, we compared the methods on biopsies from the digastric muscle.

By using either ATPase histochemistry or immunohistochemistry, it is not possible to measure the amount of particular MyHC isoforms in hybrid fibres. A more suitable method is to perform gel electrophoresis on single muscle fibres. Therefore, in Chapter 7, the MyHC contents of single fibres of human jaw muscles were determined to see whether, and how, the proportions of individual MyHC isoforms vary within the hybrid fibres.

Finally, in Chapter 8 of this thesis, we have discussed the various findings of the present study as well as those from literature in relation to the adaptive properties of jaw muscle fibres to environmental stimuli or perturbations, like ageing, hormones, stretching and activation.