Extra- and intrafusal muscle fibre type compositions of the human masseter at young age
In perspective of growth and functional maturation of the jaw-face motor system

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Cover image: Muscle cross-section from young masseter stained by monoclonal antibody MHCn against myosin heavy chain-fetal, showing extrafusal muscle fibres, and intrafusal muscle fibres within a muscle spindle. Note above, muscle spindle containing numerous intrafusal fibres. Variability in staining intensity reflect variable physiological properties of fibres.
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Gud ge mig sinnesro att acceptera det jag inte kan förändra,
med att förändra det jag kan
och förstå att inse skillnaden.

Till min familj
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ABSTRACT

Muscles control body posture and movement by extrafusal and intrafusal (muscle spindle) fibres. The purpose of this thesis was to provide insight into the muscular basis for human jaw function at young age. Extrafusal and intrafusal fibres in the young masseter, and for comparison young biceps, were examined for composition of fibre types and myosin heavy chain (MyHC) isoforms by means of morphological, enzyme-histochemical, biochemical and immuno-histochemical techniques. For evaluation of plasticity during life span the data for young muscles were compared with previous reported data for adult and elderly muscles.

The results showed significant differences in extrafusal fibre types and MyHC expression between young masseter and young biceps and between young masseter and masseter in adults and elderly. Compared with young biceps, young masseter was more intricate in composition of extrafusal MyHC expression. Muscle spindles were larger and more frequent in the masseter than in the biceps. Masseter and biceps muscle spindles showed fundamental similarities but also marked differences in MyHC expression.

The results suggest that the young masseter is specialized in fibre types already at young age and shows a unique fibre type growth pattern. Whereas masseter extrafusal fibres display marked plasticity in fibre types and MyHC isoforms during life span muscle spindles/intrafusal fibres are morphologically mature already at young age and precede extrafusal fibres in growth and maturation. Results showed similarities in intrafusal MyHC expression between young masseter and biceps, but also differences implying muscle specific proprioceptive control. Differences in fibre types and MyHC expression between young masseter and young biceps extrafusal fibres are proposed to reflect diverse evolutionary and developmental origins and accord with the masseter and biceps being separate allototypes of muscle.

Keywords
Jaw, limb, human, muscle, morphology, fibre type, myosin heavy chain, muscle spindle
ABBREVIATIONS AND DEFINITIONS

Adult masseter  Masseter muscle samples from subjects mean age 28 years
Biceps  Short, inner, head of the biceps brachii muscle
Childhood  Age 3-7 years
Deep  Deep region of the masseter
Development  Changing from an immature to a mature stage
EF  Extrafusal fibres
Elderly masseter  Masseter muscle samples from subjects mean age 74 years
GE  Gel electrophoresis
Growth  Change in size with age
IF  Intrafusal fibres
IHC  Immuno-histochemistry
mAb  Monoclonal antibody
mATPase  Myosin adenosine triphosphatase
Maturation  The process of becoming mature
Muscle allotype  Muscles which are developmentally, structurally and functionally distinct (e.g. limb/trunk, jaw, extra ocular)
MYH  Sarcomeric myosin heavy chain gene
MyHC  Myosin heavy chain
MyHC-I  MyHC- slow twitch, MyHC-β cardiac
MyHC-α c  MyHC-α cardiac
MyHC-emb  MyHC-embryonic
MyHC-fet  MyHC-fetal
MyHC-sto  MyHC-slow tonic
MS  muscle spindles
PAP  Peroxidase-antiperoxidase
PBS  Phosphate buffer saline
Sup ant  Superficial masseter, anterior region
Sup post  Superficial masseter, posterior region
Young masseter  Masseter muscle samples from subjects aged 3-7 years, mean age 5 years
SVENSK SAMMANFATTNING


Avhandlingens syfte var att undersöka muskelfibertypsammansättningen i käkmuskulatur och som jämförelse armmuskulatur vid tidig ålder. Muskelprover från masseter och biceps muskulatur undersöktes med avseende på muskelfibertyper och kontrakta proteiner (myosin heavy chain MyHC) i extrafusala- och intrafusala muskelfibrar. För att undersöka plasticitet, det vill säga förändringsmönster för muskelfibertyper under livscykeln, jämfördes resultaten med tidigare publicerade data för vuxna och äldre individer. Ett flertal tekniker användes för muskelfiberanalys; morfologiska, enzym- och immun-histokemiska samt biokemiska metoder.


Sammanfattningensvis tyder resultaten på att masseter muskeln redan i tidig ålder är specialiserad vad gäller fibertyper samt uppvisar ett unikt tillväxtmönster. Medan extrafusala fibrar uppvisar åldersrelaterade förändringar i sammansättning av muskelfibertyper och kontrakta proteiner är muskelspoler med dess intrafusala fibrar morfologiskt mogna redan i tidig ålder. Muskelspoler tillväxer och mognar före det extrafusala muskelfibersystemet, vilket tyder på behov av reflexstyrd kontroll av käkrespektive armrörelser. Resultaten tyder på att masseter muskeln utvecklingsmässigt, strukturellt och funktionellt är skild från biceps.
This thesis is based on the following papers, which will be referred to in the text by their roman numerals:

I. Differences in fibre type composition between human masseter and biceps muscles in young and adults reveal unique masseter fibre type growth pattern
   Österlund C, Thornell L-E and Eriksson P-O
   The Anatomical Record, 2011: 294;1158-1169.

II. Muscle spindle composition and distribution in human young masseter and biceps brachii muscles reveal early growth and maturation
    Österlund C, Liu J-X, Thornell L-E and Eriksson P-O
    The Anatomical Record, 2011: 294;683-693.

III. Remarkable heterogeneity in myosin heavy chain composition of the human young masseter compared with young biceps brachii
    Österlund C, Lindström M, Thornell L-E and Eriksson P-O
    Submitted, 2011

IV. Intrafusal myosin heavy chain expression of human masseter and biceps muscles at young age shows fundamental similarities but also marked differences
    Österlund C, Liu J-X, Thornell L-E and Eriksson P-O
    Manuscript

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BACKGROUND

The muscles control body movements by virtue of two muscle fibre systems, the extrafusal fibres and the intrafusal fibres. These systems have quite different functional roles. The extrafusal fibres build up the muscle mass, generate force and execute movements. The intrafusal fibres are part of the muscle spindles - sensory receptors for muscle control and movements. This thesis investigates the structural basis for jaw sensory-motor control at young age. Specifically, the masseter muscle in childhood was examined for compositions of fibre types and contractile proteins, myosin heavy chain (MyHC) isoforms, of the extrafusal and intrafusal fibres, i.e. the basis for power, velocity and endurance of muscle contraction, and the finely tuned adjustments of tension and length of muscle. Determination of composition and distribution of muscle fibre types and MyHC isoforms in a given muscle is critical for a better understanding of its physiological properties. In this context, there is a gap of in-depth knowledge for the human young masseter muscle. To elucidate the possible influence of differences in genetic and embryological origins, innervation and functional context on extra- and intrafusal fibre types and expression of MyHC isoforms, a spinal nerve innervated muscle, the young biceps brachii, was included in the study. Finally, the masseter and biceps muscles were evaluated for age-related plasticity of fibre types and MyHC expression during the life span by comparing the results with previously reported data for adults (Eriksson, 1982; Stål, 1994; Liu, 2004) and elderly (Monemi, 1999).

INTRODUCTION

Muscles and muscle fibres

Skeletal muscles
Humans have about 640 skeletal muscles (Grounds and Shavlakadze, 2011) of which numerous jaw, face, tongue and neck muscles are activated in natural jaw function, i.e integrative jaw-neck motor control. Skeletal muscles are made up of individual cells known as muscle fibres. Muscle fibres contain myofibrils, the actual force generators. The myofibrils are composed of series of sarcomeres, the functional units of muscle contraction. The sarcomere consists of thick filaments that are mainly composed of myosin and thin filaments composed of actin, troponin and tropomyosin. Interaction between the filaments is the basis for muscle contraction (Fig. 1).
Figure 1: Schematic illustration of components of skeletal muscle. Whole muscle and a bundle of muscle fibres. Muscle fibres are composed of myofibrils, which contain myofilaments. The myofibrils have distinct repeating microanatomical units termed sarcomeres, which represent the basic contractile units of the muscle. The sarcomere is composed of thick and thin filaments, myosin and actin, respectively. Together with regulatory proteins, troponin and tropomyosin, chemical and physical interactions between the actin and myosin cause the sarcomere length to shorten. Figure published with kind permission from Per Stål, Umeå University.
Muscles in human adults
Each human muscle is unique in fibre type- and myosin compositions. The muscles in the jaw-face region are further specially designed in comparison with spinal nerve innervated muscles such as leg, arm, hand and neck muscles (Fig. 2). Cranial nerve innervated muscles, e.g. extra ocular, jaw, face and tongue muscles, differ in composition of fibre types and contractile proteins. Therefore, by mapping the fibre type and MyHC composition of muscles, a better understanding of its physiological properties for sensory-motor control can be achieved.

Figure 2: Schematic illustration of muscles in human adults in relation to the cortical representation. Note that the jaw, face, tongue and hand regions have large representation in the cortex, which means that more brainpower is dedicated to controlling these body parts. Figure published with kind permission from Per Stål, Umeå University.
**Muscle fibre types**

Skeletal muscle fibres can be characterized qualitatively by enzyme-histochemical and immuno-histochemical (IHC) techniques. Enzyme-histochemistry has been generally used to classify muscle fibres on the basis of reactions at alkaline and acid pH. The fibres are classified into type I fibres and type II fibres with subtypes IIA, IIB and IIC (Brooke and Kaiser, 1970). Human adult skeletal type I fibres have weak mATPase staining at alkaline pH and strong staining at acid pH. They have low glycolytic activity and high oxidative capacity and are resistant to fatigue and belong to slow twitch motor units that have relatively long contraction time. Type IIA fibres have strong mATPase staining at alkaline pH and weak staining at acid pH, belong to fast twitch motor units that contract fast and are relatively resistant to fatigue. Type IIB fibres have strong mATPase staining at alkaline pH and weak staining at pH 4.3, have high glycolytic activity and low oxidative capacity, are fatigable and belong to fast twitch motor units. Type IIC fibres show strong mATPase staining at alkaline pH and intermediate staining at acid pH (Dubowitz, 1985, 2007). Type mATPase-intermediate fibre type, type IM, is a typical fibre type in human jaw muscles, but normally not present in limb and trunk muscles. (Ringqvist, 1973).

An advancement in fibre typing was the generation of monoclonal antibodies (mAbs) to different MyHC isoforms. (Schiaffino, 2010). By IHC technique the mAbs reveal MyHC composition of individual fibres. In adult human muscles, three MyHC isoforms have been identified, which are correlated to the myosin ATPase based classification system. The slow twitch MyHC isoform I/β-cardiac is present in type I fibres. The fast-twitch myosins, MyHC-IIa is present in type IIA fibres, and MyHC-IIx is present in type IIB fibres. The unique expression of any one of these MyHC isoforms in a single muscle fibre is responsible for the distinct fibre types observed in skeletal muscles after staining for myofibrillar ATPase following acid or alkaline preincubations (Termin et al., 1989). Type IIC fibres which in general are rare, appear during development and in pathological conditions can contain a mixture of slow and fast isoforms (Pette and Staron, 2000; Dubowitz, 2007) and isoforms related to muscle development or muscle fibre regeneration i.e. MyHC-embryonic and MyHC-fetal isoforms (Schantz and Dhoot, 1987; Monemi et al., 1999a; Pette and Staron, 2000; Schiaffino, 2010). Both the myofibrillar ATPase activities and the MyHC based fibre types composition of a muscle can be used to predict the contractile properties of the muscle. The mAbs make it possible to detect fibres expressing a single MyHC isoform ("pure" fibres) or two or more mixed isoforms ("hybrid" fibres).
Electrophoretic separation of MyHC isoforms by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE GE) procedure (Bar and Pette, 1988) allows quantification of separate isoforms.

What decides the characteristic properties of a muscle?
Muscles differ in contraction speed, force generation, fatigability and endurance. Numerous different properties decide the characteristics of muscle fibres and whole muscles such as the content of various sarcomeric proteins, enzyme activity, capillarisation, fibre cross-sectional area, muscle architecture, and influence of genetic programs, motoneurones and different hormones.

Sarcomeric myosin

A sarcomere is the basic contractile unit of a muscle (Fig. 1). Sarcomeric myosin, the most abundant protein in skeletal muscle, is a conventional class II myosin (Pette and Staron, 1990; Schiaffino and Reggiani, 1994; Bottinelli and Reggiani, 2000).

The myosin molecule is an important motor protein in the sarcomere. It powers muscle contraction and determines the contractile speed and thus belongs to the foundation of muscle structure and function. It is a complex of two heavy chains and four light chains that exist in multiple isoforms. An isoform is a particular protein with slight variations in amino acid composition (Schiaffino and Reggiani, 1994). In muscle cells, MyHC isoforms contain both the ATPase and the actin-binding site- determinants of speed and force of contraction (Pette and Staron, 1990; Bottinelli et al., 1991). Slow MyHC isoforms predominate in slow-muscles, fast MyHC isoforms in fast-muscles. The MyHC composition is regarded as the best marker of the functional heterogeneity among muscle fibres (Larsson and Moss, 1993).

Genome
A multigene family, formed by gene duplications approximately 75-110 million years ago encodes the MyHC isoforms (Fig.3). The gene organization is highly conserved through evolution in terms of genomic organization and the order of the MyHC genes (MYH) on the chromosomes (Schiaffino and Reggiani, 1996; Weiss et al., 1999; Berg et al., 2001). Eleven MYH genes have been detected in the mammalian genome and expressed in striated muscles in a tissue-specific and developmental stage-specific manner (Schiaffino and
Muscle plasticity

The adaptive potential of muscle tissue is an important evolutionary achievement because it improves the ability of survival. Both jaw and limb muscles are highly adaptive to physiological demands. This adaptive responsiveness has been termed "plasticity of muscle" (Pette and Staron, 2001). Muscle plasticity is the ability of a muscle cell to alter either the quantity of protein and/or the type of protein (i.e., isoform), in response to any stimulus (use or inactivity) (Baldwin and Haddad, 2002). A given protein can be replaced by another protein, which is better suited for a specific physiological or pathological state. The need for new motor skills as the child grows and develops will place extra demands on the muscle plasticity during these formative years.
Muscle development

Embryology
Muscles of different anatomical origins also have different embryological origins. Skeletal muscle cells derive from the paraxial mesoderm. Jaw muscles (masseter, temporalis, the lateral and medial pterygoids and the anterior digastric) originate from the head paraxial mesoderm (somitomeres) and migrate into the first branchial arch, whereas muscles of facial expression and the posterior digastric migrate into the second branchial arch (Noden, 1983; Noden and Francis-West, 2006). Tongue, cervical and limb muscles are derived from the somitic mesoderm.

Myogenesis
Muscle fibres are multinucleated cells formed as a result of myogenesis. During myogenesis mononucleated muscle precursor cells (myoblasts) proliferate and then differentiate and fuse to form thin multinucleated muscle cells (myotubes). Primary myotubes form around 12 weeks of gestation for the masseter muscle and around 8-10 weeks for the biceps (Barbet et al., 1991). A second generation of myotubes forms around the primary fibres. The muscle spindles and the extrafusal fibres arise from different myogenic lineages. There are special myotubes during development that are destined to be muscle spindles (Pedrosa and Thornell, 1990; Soukup et al., 1995). However, the precise origin of a spindle precursor is unknown. Skeletal muscles develop initially without innervation; myoblasts proliferate and differentiate and fuse to form primary myotubes. Motor nerves establish contact to the myotubes accompanied by sensory contacts, which trigger divergent differentiation of primary myotubes into extrafusal fibres and intrafusal fibres, respectively (Zelená, 1994). The differentiation into extrafusal slow-twitch fibres are motor nerve dependent, whereas fast-twitch fibres can differentiate in the absence of nerves. The differentiation into intrafusal fibres is dependent of sensory innervation to develop MyHC-slow tonic. The myotubes mature and grow in width (hypertrophy) and in length into multinucleated muscle cells or fibres. Failure of nerve contact or inactivity results in decrease in muscle mass (atrophy).

The muscle fibres stabilise in length as skeletal bone growth ceases. An average adult human extrafusal muscle fibre reaches about 30-60µm in diameter and 20-30 mm in length (range about 2-600 mm) (Grounds and Shavlakadze, 2011), whereas intrafusal fibres diameter are about 10-20µm and 3-4 mm length (Eriksson and Thornell, 1990). Some myoblasts persist between the sarcolemma (muscle cell membrane) and the overlying
basement membrane. These cells are called satellite cells (Mauro, 1961) and are responsible for generation of new myoblasts for muscle regeneration in muscle repair, and providing nuclei to growing muscle fibres.

**Jaw functions**

In natural jaw function, the jaw muscles interact with other muscle groups in order to achieve specific tasks. The jaw and neck muscles act as an integrative sensory-motor system during jaw actions by simultaneous recruitment of jaw and neck muscles and concomitant movements in the temporomandibular, the atlanto-occipital and the cervical spine joints. Natural jaw function is by definition “integrated jaw-neck function” (Eriksson et al., 1998; Eriksson et al., 2000). The involved muscles act as synergies, i.e. coordinated activations of groups of muscles for a specific task or function. The central nervous system selects the appropriate muscle activity patterns to achieve a behavioural goal (d’Avella et al., 2003). Jaw functions like mouth opening, suckling, swallowing, gaping and breathing are based on innate reflexes necessary for surviving. During maturation, eating behaviour (mastication and swallowing) and communication (speech and mimic) activate by learning. Additional jaw muscle functions are positioning the mandible, posture control, and mediation and execution of jaw reflexes (jaw opening and closing reflexes).

Jaw muscles and limb muscles perform different types of functions and thus have evolved different fibre types to match these specific functional demands. Jaw and limb muscles, respectively, are said to belong to distinct higher order classes called allotypes, developed from different lineages of myoblasts, committed to form a specific subset of myofibrillar proteins (Hoh, 1993; Hoh, 2002). Satellite cells in jaw muscles are pre-programmed to express masticatory myosin even when re-innervated by a limb fast nerve (Hoh and Hughes, 1988).

**Jaw-face growth**

Combination of genetic and epigenetic (regulated gene expression by response to the environment) is responsible for the adult jaw-face morphology. Development of the jaw-face region takes place early in foetal life, but is underdimensioned in favor of development and growth of the cranium (Fig. 4). The muscles growth and attachment are closely associated
with the jaw-facial skeleton morphology, which undergoes growth and remodelling throughout childhood. The muscles must readjust their insertion and site of origin. The mandible and the maxilla grow and remodel in a posterior-superior manner leading to jaw displacement in forward and downward directions. As a consequence, the lower face (below the eyes) becomes longer and larger to accommodate the expansion of nasal region and the eruption of permanent teeth. The cranium, instead, grows much less than the lower part of the face after the first year of life, because most of the cranium growth, takes place in the neonatal and infancy period. At childhood (the age of 3-7 years), the cranium has already reached about 80-90 % of its final size (Mann, 1984). The lower face grows (condylar grow) between 20 to 60 % of the total size, and reaches its full size at approximately 14 years of age (Buschang et al., 1999). A pronounced shift in motor behaviour occurs during teeth eruption when mastication succeeds suckling and develops and improves throughout early childhood (Green et al., 1997). The jaw muscle capacity varies between growing individuals as measured by bite force (Kiliaridis et al., 1993) and muscle thickness (Raadsheer et al., 1996). At the age of 6 years maximum bite force is about 50 % (85 Newton) that of the maximum bite force at the age of 18-20 years (176 Newton) (Braun et al., 1996). It can be assumed that changes in skeletal morphology and transitions in functions during growth and maturation from childhood to adulthood will be reflected in the muscle morphology.

Figure 4: Schematic illustration of the relative growth of the skull (blue) and lower face (beige) of neonate and adulthood skulls. The figure is redrawn from Dal Martello and Maloney (Dal Martello and Maloney, 2006). At birth the skull has reached about 60-65% of its final size and in childhood nearly full size. The size of the face (below the eyes) at birth is around one eight of the total size of the head, whereas in adult, it is half of it.
**Masseter muscle**

The masseter muscle, which is the strongest jaw closing muscle, is essential in eating and communication behaviours. The muscle grows in parallel with the jaw-face skeletal growth and remodelling and with teeth eruption. The masseter muscle morphology is previously studied in prenatal (Barbet et al., 1992), postnatal (Vignon et al., 1980; Soussi-Yanicostas et al., 1990; Barbet et al., 1992; Bontemps et al., 2002), puberty (Vignon et al., 1980; Soussi-Yanicostas et al., 1990; Barbet et al., 1992; Bontemps et al., 2002), adult (Ringqvist, 1973, 1974; Eriksson, 1982; Stål, 1994; Korfage et al., 2000) and in elderly (Monemi, 1999) ages.

**Architecture**

The human masseter muscle comprises of two main muscle portions, a superficial portion with vertically-posteriorly directed fibres and a deep portion with vertically oriented fibres (Ebert, 1938/39; Schumacher, 1961) (Fig. 5). The muscle portions are divided by internal tendinous or connective tissue septa, which run in parallel alternatively from the zygomatic arch and the mandible. Between these tendon layers, numerous short muscle fibres are arranged in a multipennate form. This intricate anatomy suggests that the masseter is organized into functionally separate compartments (Schumacher, 1961; Widmer et al., 2007), activated as synergic groups.

![Diagram of the masseter muscle](image.png)

**Figure 5:** Schematic illustration of the masseter muscle portions; masseter superficialis anterior (sup ant), masseter superficial posterior (sup post) and masseter deep (deep).
Innervation
Sensory and motor branches of the trigeminal nerve innervate the jaw muscles. The trigeminal motor nucleus lies in the mid pons level in the brainstem and the component of the trigeminal sensory system that relay information from muscle spindles is located in the mesencephalic trigeminal nucleus at the mid brain level of the brainstem.

The motor unit
The central nervous system does not operate "in muscles", i.e. a muscle is never activated as a whole, but instead activates individual motor-units. Thus, the motor unit is the basic unit of motor activity. It consists of a single α-motor neuron and the set of muscle fibres innervated by this neuron (first described by Sherrington, 1929). A given muscle fibre belongs to only one motor unit. When a motor unit is activated, all of its fibres are activated and produce a force. Muscles that require precision and fine movement control usually have many motor units with a small number of fibres in each unit. The masseter motor units contain fewer muscle fibres than those in limb muscles (Stålberg et al., 1986). There is an orderly recruitment of motor units. Small, slow contracting, fatigue resistant motor units, produce small forces. With increasing force demands, large, fast-contracting fatigable motor units join in. Slower fibre types/motor units are more frequently used than faster ones. When more force is needed, the larger motor units are recruited. Control of muscles, including jaw muscle function, relies on selective recruitment of motor units.

The masseter muscle from prenatal age to puberty
The human masseter muscle morphology at prenatal (28 foetal weeks) and postnatal (1.5 years) stages is characterised by presence of two distinct fibre populations of different fibre size (Barbet et al., 1992). Large diameter fibres express MyHC-I exclusively or in association with MyHC-embryonic and MyHC-fetal isoforms. They give rise to adult type I fibres. Small diameter fibres express MyHC-embryonic, MyHC-fetal and MyHC-II isoforms and give rise to adult fibre types IIA, IIB and IIC (Soussi-Yanicostas et al., 1990; Barbet et al., 1992; Bontemps et al., 2002). In puberty (10-13 years), the type II fibre diameter is about half of that of type I fibres (Vignon et al., 1980).

The adult and elderly human masseter muscle
The adult human masseter muscle, like other jaw closing muscles, is distinct from limb muscles due to smaller fibre diameter and differences in enzyme-histochemical fibre types and MyHC isoform expression. In addition to type I fibres and small type II fibres with subtypes IIA, IAB, IIB and IIC, the
masseter typically contains fibres which are intermediate to type I and type II in mATPase staining and diameter, termed mATPase-IM fibres, IM (Ringqvist, 1973; Eriksson, 1982; Eriksson and Thornell, 1983).

The adult human masseter shows regional differences in fibre type composition (Eriksson, 1982; Eriksson and Thornell, 1983; Stål, 1994a; Monemi et al., 1998; Korfage et al., 2000; Korfage et al., 2005a). The deep masseter portion contains the highest proportion of type I fibres and in the superficial masseter portion, the posterior part contains more type IIB fibres than the anterior part (Eriksson, 1982; Stål et al., 1994a; Korfage et al., 2000). The elderly masseter contains a larger proportion of type IIB fibres, type IIA fibres and a larger amount of fibres expressing developmental MyHC (Monemi et al., 1996).

The regional specialization and heterogeneity in composition and distribution of fibre types, suggest that the human masseter is genetically destined to develop, grow and mature into not one muscle, but an entity of functionally different muscle portions acting in a task-related synergy (Eriksson and Thornell, 1983). Moreover, aging is associated with significant phenotypic alterations in muscle structure (Monemi et al., 1998).

GE of adult and elderly masseter muscle extract shows content of MyHC-I, MyHC-IIa, MyHC-IIx and MyHC-fetal (Butler-Browne et al., 1988; Monemi, 1999a). IHC studies have demonstrated that in contrast to type I fibres containing MyHC-I and type II fibres containing MyHC-II, the type IM fibres express both MyHC-I and MyHC-II isoforms (Eriksson, 1982; Thornell et al., 1984). Later studies confirmed and extended this finding of a heterogeneous MyHC composition of the adult masseter fibres, and presented evidence of proteins characteristic of developing muscles, MyHC-neonatal/fetal and MyHC-embryonic isoforms (Butler-Browne et al., 1988; Soussi-Yanicostas et al., 1990). Other special features of the adult masseter 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; Stål et al., 1994a), and that the type IM fibres typically contain mixtures of two or more MyHC isoforms (Stål et al., 1994a; Monemi et al., 1999a; Korfage et al., 2005b). Interestingly, a transition in masseter contractile proteins during aging opposite to that of limb muscle, has been identified by relatively more MyHC-II and MyHC-fetal isoforms and more fibres with a mixture of MyHC isoforms in the masseter of elderly (Monemi et al., 1999a).
Other specialized muscles

Diversity in MyHC expression is also seen in extra ocular (Kjellgren et al., 2003) and laryngeal (Hoh, 2005) muscles, and mirrors evolutionary development into allotypes which are genetically different from that of limb and trunk muscles (Hoh, 1993; Hoh, 2002). Thus, some cranial muscles have emerged with multiple MYH genes and MyHC isoforms reflecting a considerable phylogenetic plasticity in relation to functional demands (Hoh, 2002). The craniofacial muscles are evolutionary, morphologically and molecularly distinct from trunk muscles, and the craniofacial muscles myogenic gene network probably arises independently of the trunk muscle regulatory program (Sambasivan et al., 2011).

Biceps brachii muscle

The biceps brachii muscle, which in this thesis is included for comparison, is of different embryonic origin, innervation, of simpler architecture and involved in more gross movements than the masseter muscle (Fig. 6). The human biceps brachii muscle fibre type composition has been described in all age groups including prenatal, postnatal, childhood, adulthood and in elderly (Brooke and Engel, 1969; Colling-Saltin, 1978).

Figure 6: Schematic illustration of the biceps brachii muscle.

In limb and trunk muscles the type I and type II fibres are of about the same diameter and homogeneously distributed (Dubowitz, 2007). The adult biceps muscle is made up of types I, IIA and IIB fibres of about equal proportions and diameters evenly distributed over the muscle cross-section (Eriksson, 1982; Stål, 1994a), and type IIC fibres which in limb and trunk muscles appear only during development and in pathological conditions (Pette and Staron, 2000; Dubowitz, 2007).
Muscle spindles

Muscle spindle structure
Muscle spindles are muscle mecanoreceptors sensitive to muscle length and change in muscle length. Because they respond to stimuli from within the muscle they belong to so called proprioceptors, which provide information about position of body parts in postures and movements to the central nervous system. They consist of a bundle of small muscle fibres, intrafusal fibres, located in parallel with extrafusal fibres, partly surrounded by a fusiform fluid-filled connective tissue capsule. They are unique by receiving both a motor and a sensory innervation. Due to regional differences in morphology the muscle spindle has been separated into the A-region-constituting its central equatorial, and the B-region- bilateral polar regions reaching to the capsule end, and the C-region- extracapsular, where some intrafusal fibres extend beyond the capsule to fuse with extrafusal fibre tissue (Barker and Banks, 1994) (Fig. 7).

Intrafusal fibre types
Mammalian intrafusal fibres differentiate into three fibre types. Two “bag” fibres develop from primary and secondary myotubes, and “chain” fibres differentiate from secondary myotubes (Kucera and Walro, 1989, 1990; Pedrosa-Domellöf et al., 1991; Pedrosa-Domellöf and Thornell, 1994). Figure 8 shows masseter muscle spindle with bag and chain fibres.
Nuclear bag, (dynamic bag1) and nuclear bag2, (static bag2), with bag of nuclei, and nuclear chain (static chain) fibres, with a single row of nuclei, have been classified on basis of morphology, myosin ATPase activity (Ovalle and Smith, 1972) and physiological properties (Matthews, 1971, 1981; Banks, 1994). In addition, an acid stable bag, fibre (AS-bag1) has been distinguished in human masseter muscle spindles (Eriksson and Thornell, 1985, 1990; Eriksson et al., 1994).

**Innervation**

Intratusal fibres both send afferent innervation to the central nervous system and receive efferent innervation from the central nervous system. Initially the dual innervation is established with the primary myotubes (Zelená, 1994). Two types of afferents, the primary (Ia) and the secondary (II), and in addition two types of efferents, dynamic and static $\gamma$- motoneurons innervate the intratusal fibres. Also $\beta$-motoneurons, connect to muscle spindles (Boyd and Smith, 1984; Bradley, 1995), as well as to extratusal fibres. Afferent nerves contact the central equatorial muscle spindle region. The afferents have responsible for supplying the central nervous system with information on muscle length and velocity changes of length. The efferent $\gamma$- nerve fibres contact the polar ends. By $\gamma$-nerve fibres length and velocity changes causes intratusal muscle fibres to regulate the muscle length. The muscle spindle afference from the masseter muscle is relayed to the mesencephalic trigeminal nucleus in the brainstem. The fact that more than two-thirds of the myelinated nerve fibres in a muscle nerve innervate the
muscle spindles (Boyd and Smith, 1984) implies strong functional impact of the very small intrafusal muscle fibre population (Fig. 9).

Figure 9: Cross-sections from masseter muscle spindles in childhood sample 6 years (left) and in adulthood sample 58 years (right). Note the peripheral nerves (arrows) in near relation to the muscle spindles. Bar 50 µm.

MyHC isoforms
In human muscles, intrafusal bag fibres express MyHC-slow tonic, MyHC-I (slow-twitch), MyHC-embryonic and MyHC-fetal and MyHC-a cardiac isoforms. Chain fibres express MyHC-embryonic, MyHC- fetal and MyHC-II (fast-twitch).

Muscle spindle density
Muscle spindle density varies significantly between different muscles (Boyd and Smith, 1984) and can be regarded as an indicator of functional differences. High muscle spindle density characterizes muscles initiating fine movements (e.g. lumbrical muscles and extra ocular muscles) (Bruenech and Ruskell, 2001; Boyd-Clark et al., 2002; Soukup et al., 2003) or maintaining posture (e.g. neck muscles) (Boyd-Clark et al., 2002; Liu et al., 2003), whereas low spindle density is characteristic of muscles initiating gross movements (e.g. biceps brachii).

Muscle spindle functions
Muscle spindles play multifunctional roles contributing in proprioception, postural and movement control, motor learning and in plasticity of motor behaviors (Windhorst, 2007, 2008), in predicting future kinematic states by acting as forward sensory model (Dimitriou and Edin, 2010), and as
suggested, in the genesis and spread of chronic muscle pain (Johansson et al., 1999; Blair et al., 2003; Johansson et al., 2003).

Muscle spindle activity in the masseter muscle regulates jaw position and movements during chewing and speech (Smith et al., 1991; Scutter and Turker, 2001). Excitation of muscle spindle afferent fibres monosynaptically activates jaw-closing a-motoneuron in the trigeminal motor nucleus, causing jaw-closing or jaw stretch reflex. In childhood, the proprioceptive information from the masseter muscle spindles is highly important for developing fine movement and postural control of the jaw for eating and speech behaviours and for posturing the mandible during brisk walking/running (Smith et al., 1991; Miles et al., 2004; Miles, 2007). While children are developing jaw motor skills, the reflex development occurs coincidently (Smith et al., 1991). The masseter muscle spindles activity regulates the jaw motor pattern to smooth and relatively stable jaw positions and movements with impact from the proprioceptive reflex (Jaaskelainen, 1993; Scutter and Turker, 2001; Miles et al., 2004; Miles, 2007) and information from other peripheral receptors (periodontal receptors, golgi-tendon organs).

**Adult masseter and biceps muscle spindles**

The human adult masseter muscle (Voss, 1971) compared with the biceps muscle shows higher spindle density and larger and more complex spindles, especially in the deep masseter portion. The spindles are located closely together in clusters, sharing the same capsule and described as compound in arrangement. Similar to the adult biceps (Liu et al., 2002), the muscle spindles in the adult masseter (Eriksson and Thornell, 1985, 1987, 1990; Eriksson et al., 1995) show large variations in fibre type composition with heterogeneous intrafusal fibre type composition. In the adult masseter (Eriksson et al., 1994) and biceps (Liu et al., 2002), bag, fibres mainly express MyHCs- slow-tonic and I; AS-bag, fibres MyHCs- slow-tonic, fetal and embryonic; bag, fibres MyHCs- slow-tonic, I and fetal, and chain fibres MyHC- II, fetal and embryonic.
Myofascial pain

Myofascial pain in the jaw system is characterized by pain at rest and function, tiredness/stiffness, weakness and tenderness of jaw muscles. Diagnosis is based on history and clinical examination (Dworkin and LeResche, 1992; Okeson, 2008). Examination may reveals limited mouth opening. Women report pain more frequently, severely and of longer duration than men. The reason for this difference is unclear and in what way pain modulation is influenced by sex hormones have gained increasing interest (Dao and LeResche, 2000; LeResche, 2000; Ernberg, 2004; Shinal and Fillingim, 2007).

The mechanisms behind muscle pain are not fully understood. Both peripheral and central mechanisms are involved. Nociceptive afferent, Aδ- and C fibres mediate pain. In the masseter, the nociceptive afferent fibres from the trigeminal nerve have their cellbodies in the trigeminal ganglion and terminate in the subnucleus caudalis in the brain stem. The nociceptive activity from muscle tissue is sensitive to stimuli that can modulate the pain transmission. One molecule in the pain transmission is serotonin (5-HT). Studies suggest that serotonin in the central nervous system can reduce pain, whereas serotonin in blood contributes to local progression of muscle pain. Serotonin in the human masseter has been studied by means of microdialysis technique (Ernberg et al., 1999) and intramuscular injection of granisetron, a selective 5-HT receptor antagonist, into the masseter muscle has been found to reduce myofascial pain (Christidis et al., 2007).

Myofascial pain in the jaw-face can be paralleled by pain in the neck, shoulders and back, and also by generally spread pain. Recent findings show that pain and dysfunction in trigeminally, as well as spinally, innervated areas mutually predict the onset of new symptoms in the other region, indicating common pathophysiological mechanisms and individual vulnerability (Marklund et al., 2010).

In the national guidelines issued by the National Board of Health and Welfare in Sweden, myofascial pain can have a major impact on oral health including impaired ability to eat, chew and speak and influence the individual’s mental and social well-being. Effort to identify, assess and reduce myofascial pain are therefore of importance.
Purpose of the thesis

The general aim of this thesis was to increase the knowledge about the structure and function of the human jaw system at young age. Information on the structural basis for human masseter function at young age, in terms of composition and distribution of extrafusal muscle fibre types and contractile proteins/MyHC isoforms, is currently lacking. Likewise, there is no knowledge for the human young masseter on the morphology and distribution of muscle spindles and the expression of MyHC isoforms in the intrafusal fibre population. Such knowledge would present a more profound understanding of natural masseter function and therefore jaw motor control, as well as assessment of dysfunction at young age. Furthermore, data from this age group are essential for evaluation of age-related plasticity of the human masseter extra- and intrafusal fibres during the life span, knowledge that would mirror adaptation of motor and sensory control during growth and aging.
AIMS & HYPOTHESES

Aims

This thesis is part of a larger project focused on increasing the knowledge on the structural basis for sensori-motor control of human jaw function

- The specific aim was to examine the extra- and intrafusal muscle fibres of the human young masseter muscle for composition and distribution of fibre types and for expression of MyHC isoforms.

- For comparison and to elucidate the influence on muscle growth of genetic and embryologic origins, innervation and functional context, the young biceps brachii muscle was included in the study.

- Possible age related changes from childhood to adulthood and old age, i.e. muscle plasticity during life span, was evaluated by comparing the results with previously reported data on the human masseter and biceps muscle in adults and elderly.

Hypotheses

Changes in jaw-face skeletal morphology and jaw function from childhood to adulthood are paralleled by alterations of fibre types and MyHC expression
- in masseter extrafusal fibres and
- in masseter intrafusal fibres (muscle spindles)

The masseter muscle is divergent from limb muscle in growth pattern.
Questions to be answered for the young masseter and biceps brachii muscles

1. What is the extrafusal muscle fibre type composition in childhood?
2. Are there any intra-muscular regional differences in distribution and size of fibre types in the masseter muscle for this age group?
3. What is the MyHC composition in the extrafusal muscle fibres?
4. What are the morphological characteristics of muscle spindles and the composition of intrafusal fibre types and MyHC isoforms in childhood?
5. What similarities and differences are there between the young masseter and biceps muscles in their extra- and intrafusal muscle fibre populations?
6. What similarities and differences are there between the young masseter and biceps muscles vs. muscles in adults and elderly in their extra- and intrafusal muscle fibre populations?
### MATERIALS & METHODS

#### Materials

**Muscle samples**
Subjects and muscle samples included in this thesis are shown in table 1.

Table 1: Subjects and muscle samples. M= Morphological analysis, EHC= enzyme-histochemistry, IHC= immuno-histochemistry, GE= SDS-PAGE gel electrophoresis, WB= Western blot technique, f= female subject, m= male subject.

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Masseter sup ant</th>
<th>Masseter sup post</th>
<th>Masseter deep</th>
<th>Biceps</th>
<th>Paper</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 years</td>
<td>f</td>
<td>M EHC IHC</td>
<td>M EHC IHC</td>
<td>M EHC IHC</td>
<td>M EHC IHC GE</td>
<td>I, II, III, IV</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(biceps)</td>
</tr>
<tr>
<td>3 years</td>
<td>m</td>
<td>M EHC IHC GE</td>
<td>M EHC IHC GE</td>
<td>M EHC IHC</td>
<td>M EHC IHC GE</td>
<td>I, II, III, IV</td>
</tr>
<tr>
<td>4 years</td>
<td>m</td>
<td>M EHC IHC GE WB</td>
<td>M EHC IHC GE</td>
<td>M EHC IHC</td>
<td>M EHC IHC GE</td>
<td>I, II, III, IV</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(biceps)</td>
</tr>
<tr>
<td>6 years</td>
<td>m</td>
<td>M EHC IHC GE</td>
<td>M EHC IHC GE</td>
<td>M EHC IHC</td>
<td>M EHC IHC GE</td>
<td>I, II, III</td>
</tr>
<tr>
<td>7 years</td>
<td>f</td>
<td>M EHC IHC GE</td>
<td>M EHC IHC GE</td>
<td>M EHC IHC</td>
<td>M EHC IHC GE</td>
<td>I, II, III</td>
</tr>
<tr>
<td>7 years</td>
<td>f</td>
<td>M EHC IHC GE WB</td>
<td>M EHC IHC GE</td>
<td>M EHC IHC</td>
<td>M EHC IHC GE</td>
<td>I, II, III, IV</td>
</tr>
<tr>
<td>22 weeks</td>
<td></td>
<td>WB</td>
<td></td>
<td></td>
<td>WB</td>
<td>III</td>
</tr>
<tr>
<td>3 months</td>
<td>f</td>
<td>WB</td>
<td>WB</td>
<td>WB</td>
<td>WB</td>
<td>III</td>
</tr>
</tbody>
</table>
The muscle samples were obtained one to three days post mortem, a delay which does not hamper reliable fibre typing (Eriksson et al., 1980). The muscle samples were rapidly frozen in propane chilled with liquid nitrogen and stored at -80°C until analysis.

**Ethic approval**
All human muscle tissue samples in this thesis were collected before 1990 in accordance with the National Board of Health and Welfare in Sweden, regulation 1975:190, concerning the exploitation of biological material for research purposes (Socialstyrelsens föreskriften 1975:190 rörande tillvaratagande av biologiskt material för forskningsändamål)

**Methods**

**Morphology**
Serial muscle cross-sections, stained for enzyme-histochemistry and IHC were analyzed using a light microscope (Leica DMR) connected with an image analyses system (Leica QWin), and then photographed (Leica DC 220) at x2.5, x10 and x40 magnification. Two masseter samples from each of the anterior (sup ant) and posterior (sup post) superficial portions and the deep (deep) portion were examined. Each sample was explored in six to eight randomly selected fields (Fig. 10). On average 1500 individual fibres/subject were examined in the masseter. In the biceps, from six subjects, one muscle sample was analysed in seven to eight random fields and on average 650 individual fibres/subject. Classification of fibre types was performed at x40 magnification. The diameter of each fibre was calculated by measuring the circumference of each fibre directly on the computer screen.
Figure 10: Sampling for identification of individual extrafusal fibres. In the light microscope, overview at x2.5 magnification magnification, random fields were marked (left). Each field was photographed at a higher magnification, x40 (middle) for serial cross-sections with three enzyme-histochemical stainings and ten mAbs stainings (middle). Classified fibres were marked with its circumference on the computer screen at x40 magnification (right).

Muscle spindles
Distribution and composition of muscles spindles including muscle spindle density, single and compound spindles, muscle spindle capsule diameters, relative frequency of intrafusal fibre types, intrafusal fibre type diameters, number of intrafusal fibres per muscle spindle and MyHC expression were examined. For morphological analysis the serial cross-sections of the muscle spindles were devided into localisation of muscle spindle regions. Muscle spindle region-A included, the central part of the spindle capsule, the equator and the juxta-equatorial part. This part contains "paraxial fluid space". Muscle spindle region-B was from the end of the fluid space to the capsular end. Muscle spindle region-AB was defined as the region in between A and B. Muscle spindle region-C was extracapsular.

Examination of fibre types and MyHC isoforms content and distribution
Enzyme-histochemistry and IHC methods were used for classification of muscle fibre types. Quantification of MyHC isoforms was performed by gel electrophoresis (GE). These three methods enabled comparison with previously reported data from our laboratory. For detailed description of fibre typing of extra- and intrafusal fibres see papers I and II, and for evaluating MyHC content and distribution of extra- and intrafusal fibres with IHC see papers III and IV. Details concerning quantification of MyHC isoforms from whole muscle extracts determined by GE separation are described in paper III. Primary antibodies for IHC staining are listed in (Table 2).
Table 2: MyHC monoclonal antibodies (mAbs) used for immuno-histochemistry and the corresponding MYH genes, short name and references.

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>MyHC</th>
<th>Genes$^a$</th>
<th>Short names</th>
<th>References</th>
</tr>
</thead>
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<tr>
<td>MyHC mAb</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALD19$^a$</td>
<td>MyHC-slow tonic</td>
<td>MYH14</td>
<td>MyHC-sto</td>
<td>(Sawchak et al., 1985)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Pedrosa et al., 1989)</td>
</tr>
<tr>
<td>A4840$^b$</td>
<td>First developmental MyHC-I</td>
<td>MYH7</td>
<td>MyHC-1/β cardiac</td>
<td>(Cho et al., 1993; Hughes et al., 1993)</td>
</tr>
<tr>
<td>A4951$^b$</td>
<td>Second developmental MyHC-I</td>
<td>MYH7</td>
<td>MyHC-1/β cardiac</td>
<td>(Cho et al., 1993; Hughes et al., 1993)</td>
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<tr>
<td>N2261$^b$</td>
<td>Third developmental MyHC-I, MyHC-IIa, MyHC-fetal/perinatal, MyHC-extracocular, MyHC-α cardiac</td>
<td>MYH7 MYH2 MYH8 MYH13 MYH6</td>
<td>MyHC-I+IIa</td>
<td>(Hughes et al., 1993; Liu et al., 2002)</td>
</tr>
<tr>
<td>A474$^b$</td>
<td>MyHC-IIa</td>
<td>MYH2</td>
<td>MyHC-IIa</td>
<td>(Hughes et al., 1993; Liu et al., 2002)</td>
</tr>
<tr>
<td>SC71$^c$</td>
<td>MyHC-IIa</td>
<td>MYH2</td>
<td>MyHC-IIa</td>
<td>(Schiaffino et al., 1989; Liu et al., 2002)</td>
</tr>
<tr>
<td>BF35$^c$</td>
<td>MyHC-I, MyHC-IIa, MyHC-embryonic, MyHC-fetal/perinatal, MyHC-extracocular, MyHC-α cardiac, MyHC-slow tonic</td>
<td>MYH7 MYH2 MYH3 MYH8 MYH13 MYH6 MYH14</td>
<td>MyHC-“all except IIx”</td>
<td>(Schiaffino et al., 1989; Liu et al., 2002)</td>
</tr>
<tr>
<td>NCL-MHCn$^d$</td>
<td>MyHC-fetal/perinatal</td>
<td>MYH8</td>
<td>MyHC-fet</td>
<td>(Ecob-Prince et al., 1989; Weiss et al., 1999)</td>
</tr>
<tr>
<td>F1652$^a$</td>
<td>MyHC-embryonic</td>
<td>MYH3</td>
<td>MyH-emb</td>
<td>(Silberstein et al., 1986)</td>
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<tr>
<td>F88$^e$</td>
<td>MyHC-α cardiac</td>
<td>MYH6</td>
<td>MyHC-α c</td>
<td>(Léger, 1985)</td>
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<tr>
<td><strong>Laminin mAb</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5H2$^e$</td>
<td>Laminin-α2 chain</td>
<td>LAMA2 Ln α2</td>
<td></td>
<td>(Sewry et al., 1998)</td>
</tr>
</tbody>
</table>

$^a$ Official gene nomenclature according to OMIM (http://www.ncbi.nlm.nih.gov/omim/)
$^b$ Gift from Donald A. Fischman (Cornell University; New York, NY)
$^c$ Purchased from Developmental Studies Hybridoma Bank (Department of Biological Sciences, University of Iowa; Iowa City, IA, USA)
$^d$ Gift from Prof. S. Schiaffino (University of Padova; Padova, Italy)
$^e$ Purchased from Novocastra Laboratories (Newcastle upon Tyne, UK)
$^f$ Gift from Dr. Jean J. Léger (Institut de la Sante et de la Recherche Meicale, Unite 249; Montpellier, France)
Western blot analysis
The presence of MyHC-fetal was determined using Western blot analysis and the mAb NCL-MHCn (Novocastra Laboratories Ltd).

Relative cross-sectional area (RCA)
To evaluate the relative muscle mass of each fibre type, the relative cross-sectional area (RCA) was calculated by the formula: total cross-sectional area of each fibre type/total cross-sectional area of all fibres x100.

Coefficient of variation (CV)
In addition to mean fibre diameter, the variability in fibre type diameter was assessed with the coefficient of variation (CV) by the formula: (Standard deviation x1000/Mean fibre diameter). In normal limb and trunk muscles the CV is less than 250 and any sample with CV greater than this indicate abnormal appearance (Dubowitz, 1985, 2007).

Statistics
Means and standard deviations (SD) were used for descriptive statistics. The precision in the estimation of mean was calculated by the formula: SD / √n, expressed by the standard error of the mean (SE). As the number of subjects was low, the study population could not be assumed to be normal distributed and consequently non-parametric statistics were applied. Statistical calculations were performed in computer programs (InStat 3.0 and Prism 5.0 for Macintosh). The null hypothesis was rejected at the 0.05 level of significance.

Wilcoxon signed rank test, a paired non-parametric test, was used to check if there were any significant differences in relative frequency of fibre types and fibre type diameter between fibre types, masseter portions and the masseter and biceps muscles (I).

Paired t-tests, were used to test if there were any significant differences in muscle spindle density, muscle spindle capsule diameter, intrafusal fibres per muscle spindle, relative frequency of intrafusal fibre types, and intrafusal fibre diameters between masseter portions, masseter and biceps brachii (II). Paired t-tests were used to test if there were any significant differences in relative content of MyHC isoforms between masseter regions and between masseter and biceps muscles (III).
Mann-Whitney test, an unpaired non-parametric test, was used to calculate whether two independent groups had equally large values. Mann-Whitney tests were used to test for any significant differences between the different age groups, young vs. adult and young vs. elderly (I, II).

The relationship between mean values from GE and IHC analyses was evaluated by Pearson correlation coefficient ($r$).
RESULTS

Extrafusal muscle fibre type composition (Paper I)

Fibre types
In the young masseter types I, IM, IIC, IIAB, IIB, and rarely type IIA fibres were distinguished. The masseter muscle fibres were generally rounded in shape. The young masseter muscle showed marked intra-muscular variability in fibre type composition (Fig. 11) as well as inter-individual variation.

Figure 11: Muscle cross-section from young masseter sample, stained with mAb A474 (MyHC-IIa). Note intra-muscular variability. Bar 300µm

In the young biceps types I, IIA, IIAB, IIB, and rarely types IM and IIC fibres were found. The biceps brachii fibre types were more angular and evenly distributed over the muscle cross-section.

Relative frequency of muscle fibre types
In the young masseter the mean values of fibre type proportions were, type I 47.7 %, IM 21.2 %, IIC 7.3 %, IIA 0.1 %, IIAB 4.8 % and IIB 18.8 %. The young masseter showed regional differences in fibre type proportions. The type I fibre proportion was larger in the deep region, 53.9 %, than in the sup ant, 45 %, and sup post, 43.8 %, regions (p= 0.03, both comparisons). The type IM fibre proportion was smaller in the deep region, 14.9 %, than in the sup ant, 28.4 %, and sup post, 21.4 %, regions (p=0.03 and 0.05, respectively).
In the young biceps the mean values of fibre type proportions were for types I 54.5%, IM 0.2% IIC 0%, IIA 24.7%, IIAB 18.8% and IIB 1.8%. The type I fibre proportion was larger than those of the types IIA and IIB fibres (p=0.03 for both comparisons). The types IIA and IIAB proportions were larger than that of type IIB (p=0.03 for both comparisons).

**Muscle fibre type diameters**
In the young masseter, the order of mean values of fibre diameters were, types I 25.4µm >IM 20.8µm >IIC 16.2µm >IIB 14.6µm >IIAB 14µm. The type II fibre diameter (mean fibre diameter for types IIA, IIAB and IIB) was significantly smaller than type I (p=0.02).

In the young biceps, the order of mean values of fibre diameters were type I 26.1µm >IIB 24.6µm >IIA / IIAB 22.5µm.

**Variability in fibre type diameter (CV value)**
The CV values for young masseter and biceps brachii muscles were (mean, SD) 179 (20) and 140 (17), respectively.

**Relative cross-sectional area (RCA) of fibre types**
Percentage of RCA formed by different fibre types in different masseter portions and in the biceps at young age, showed that the type I fibre RCA values in all young masseter regions were about the same as for the corresponding value for young biceps brachii.

**MyHC content and distribution of extrafusal muscle fibres (Paper III)**

**MyHC content**
In the young masseter the results from GE showed an apparent inter-individual variability in density of the different MyHC bands. Three bands were identified in the gels loaded with 2.0µl muscle extract as MyHC-I, MyHC-IIa and MyHC-IIx, and an additional faint band above the MyHC-I corresponding to MyHC-α cardiac (Liu et al., 2002). The MyHC-I band, with the highest mobility, was the most prominent. It was larger in the sup ant masseter than in the sup post masseter (p=0.032). No band representing
MyHC-fetal was detected but this could be expected since this developmental MyHC isoform is known to be present in the masseter of young adults and elderly (Monemi et al., 1996). Therefore, the GE analysis was extended to include gels loaded with 3.0µl muscle extract to allow low content isoforms to appear. The 3.0µl gels revealed more details for the low mobility bands with three low mobility bands including a band inbetween the MyHC-IIa and the MyHC-IIx bands. This band corresponded to MyHC-fetal (Liu et al., 2002). Also, a faint band was seen with lower mobility than that of the MyHC-IIx, corresponding to MyHC-embryonic (Liu et al., 2002).

In the young biceps the results showed an apparent inter-individual variability in density of the different MyHC bands. Three bands were identified in the gels loaded with 2.0 µl and 3.0 µl muscle extract as MyHC-I, MyHC-IIa and MyHC-IIx (Liu et al., 2002). The MyHC-I band was the most prominent.

To confirm the presence of MyHC-fetal a Western blot analysis using the mAb MHCn was performed. In the fetal samples, the result showed one band approximately at the level of MyHC-IIa. Notably, two bands were detected in the masseter samples aged 3 months and 4 and 7 years. One band appeared at the level of the band in the fetal samples. This band was also seen in the elderly masseter. The other, “extra”, band was located in between the MyHC-IIa and MyHC-IIx bands. This “extra” band was also seen in the adult masseter (sup ant). No reaction of bound anti-MHCn was observed in the young and adult biceps specimens.

MyHC distribution
In the young masseter analysis with IHC showed a marked variability in staining with all mAbs and of all enzyme-histochemical fibre types. A MyHC isoform not previously described, tentatively termed IIx’, was detected in the masseter. Based on pooled data for all subjects in the masseter, 44 % of the fibres contained one MyHC isoform, either MyHCs- I, IIa, IIx or IIx’. The rest of the fibres, 56%, showed mixtures of two to four isoforms in twenty-two combinations, fifteen when less frequent combinations, <1 %, were excluded. MyHC-fetal and MyHC-α cardiac isoforms always occurred in combinations with other isoforms. Matching the mATPase fibre types with MyHC isoforms revealed a systematic continuum of the MyHC isoforms. Analysis of masseter regional differences for slow and fast MyHCs showed a larger proportion of fibres containing fast MyHC in the sup ant and sup post masseter than in the deep masseter (p= 0.026 and p=0.012, respectively). The proportion of MyHC-IIx was larger in the sup post than in the deep masseter (p=0.039).
In the young biceps, the staining pattern with mAbs was simpler than in young masseter with no stained fibres with mAbs MHCn, F88, F1652 and ALD19. Based on pooled data 99% of the fibres contained only one isoform.

**Correlation between GE and IHC analysis**

In the young masseter, there was a strong correlation between the GE data and the IHC data for MyHC-I (r=0.895, p=<0.0001), moderate correlation for MyHC-IIx (r=0.540, p=0.021) and no correlation for MyHC-IIa (r=0.184 p > 0.05).

**Comparison of extrafusal muscle fibres of young masseter with young biceps**

Young masseter and biceps were similar in that type I fibres outnumbered other fibre types and were of the same diameter. The results showed significant differences in composition and distribution of fibre types between young masseter and young biceps. The type IIB fibre proportions in the sup post portion were larger than in the biceps (p=0.03). Young masseter differed from biceps by smaller mean fibre diameter for type II fibre diameters (i.e. types IIA, IIB and IIB) in the masseter than in the biceps (p=0.03). Both muscles showed a predominant content of MyHC-I. Differences were seen between the young masseter and biceps with higher MyHC-IIa content in the biceps (p=0.017). A fundamental difference was no expression of MyHC-fetal and MyHC-α cardiac isoforms in the biceps muscle. Young masseter extrafusal fibres showed a much more intricate MyHC distribution including unique MyHC isoforms compared with biceps fibres. Heterogeneous IHC staining, and fibres co-expressing MyHC isoforms were less pronounced in the biceps.

**Comparison of extrafusal muscle fibres of young masseter with adults and elderly**

**Fibre type proportion**

The young masseter differed in age-related changes in composition and distribution of fibre types compared with adults (Eriksson, 1982; Eriksson and Thornell, 1983) and elderly (Monemi et al., 1998). Young and adult masseter muscles showed an overwhelming proportion of type I fibres (48% and 62%, respectively), with a lower proportion in elderly muscles (33%),
whereas the proportion of type IIB fibres was larger with age (young 19%, adult 27%, and elderly 37%, respectively). The type IM proportion was largest in young masseter (young 21%, adult 6%, and elderly 13%, respectively) (Fig. 12). The different age groups of the masseter showed regional differences in relative frequency of fibre types but in different ways. The young masseter, compared with data from the adult masseter (Eriksson, 1982; Eriksson and Thornell, 1983), showed larger type IM proportion in the sup ant and sup post portions (p=0.04, p=0.002, respectively), smaller type IIB proportion in the sup post (p=0.002) and smaller type I fibre population in the deep portion (p=0.03). The young masseter, compared with data from elderly masseter (Monemi et al., 1998a), showed larger type I proportion in sup ant and sup post portions (p=0.009, p=0.002, respectively), larger type IM proportions in sup ant and sup post portions (p=0.015, p=0.018, respectively), smaller type IIA proportion in sup ant, sup post and deep masseter portions (p=0.009, p=0.012, p=0.005, respectively), smaller type IIAB proportion in sup post portion (p=0.005) and smaller type IIB proportions in sup ant and sup post portions (p=0.015, p=0.005, respectively).

**Figure 12:** Relative frequency (%) of fibre types in young masseter compared with data from adult (Eriksson, 1982; Eriksson and Thornell, 1983) and elderly (Monemi et al., 1998) masseter muscles. Mean and SD.

**Fibre type diameter**
In the young masseter, type I fibre diameter was smaller compared with adult and elderly (p=0.002, p=0.001, respectively), type IM fibre diameter was smaller compared with adult and elderly (p=0.002, p=0.001,
respectively), type IIC fibre diameter was smaller compared with adult and elderly (p=0.002, p=0.014, respectively), type IIAB fibre diameter was smaller compared with elderly (p=0.045), and type IIB fibre diameter was smaller compared with adult (p=0.005) (Eriksson, 1982; Eriksson and Thornell, 1983; Monemi et al., 1998). Comparison of mean fibre diameter for young masseter fibres with adult and elderly showed 1.8 times larger diameter in the adult masseter and 1.4 times larger in the elderly masseter (Eriksson, 1982; Eriksson and Thornell, 1983; Monemi et al., 1998). No age-related difference was found for masseter type II fibre diameter (Fig.13).

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<th>Fibre type</th>
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<td>IM</td>
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<td>IIC</td>
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<td>IIA</td>
<td>39.1</td>
<td>27</td>
<td>22.5</td>
<td>74.6</td>
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Figure 13: Illustration of fibre type diameter in young, adult (Eriksson, 1982; Eriksson and Thornell, 1983) and elderly (Monemi et al., 1998) masseter and biceps muscles. Every fibre type is labelled for its absolute diameter.
Relative cross-sectional area (RCA)

Percentage of overall muscle fibre cross-sectional area formed by different fibre types of masseter regions and biceps in young, adult (Eriksson, 1982; Eriksson and Thornell, 1983) and elderly (Monemi et al., 1998) muscles (Fig. 14).

![Diagram showing RCA (%) of different fibre types in different masseter regions and in biceps of young, adult (Eriksson, 1982; Eriksson and Thornell, 1983) and elderly (Monemi et al., 1998) muscles. Note the predominance of type I fibre muscle mass.]

Figure 14. RCA (%) of different fibre types in different masseter regions and in biceps of young, adult (Eriksson, 1982; Eriksson and Thornell, 1983) and elderly (Monemi et al., 1998) muscles. Note the predominance of type I fibre muscle mass.
**MyHC content**

Comparison MyHC content between young masseter and adult and elderly muscles showed smaller proportion of MyHC-I with age accompanied by larger proportions of MyHC-IIX, MyHC-IIX (Fig. 15). The MyHC-fetal content was largest in elderly masseter, especially in the sup ant region (Monemi et al., 1999a). The deep masseter showed no major age-related differences in MyHC content.

![Graph](image)

Figure 15: Relative content (%) of MyHC-I, MyHC-IIX, MyHC-IIX and MyHC-fetal in young, adult and elderly masseter (Monemi et al., 1999a). Mean and SD.

**MyHC distribution**

IHC showed higher proportion of MyHC-I, MyHC-fetal and MyHC-α cardiac in young masseter vs. smaller proportion in adult and elderly (Stål et al., 1994a; Monemi et al., 1999a). Young masseter individual fibres showed four single and fifteen mixed "MyHC based fibre types" and in total twenty-six when occasional combinations were included (<1%). Adult masseter, individual fibres show eight "MyHC based fibre types", three single and five mixed and nine when occasional combinations included (<1%) (Stål et al., 1994a). Elderly masseter individual fibres show eleven "MyHC based fibre types", three single and eight mixed, and sixteen when occasional combinations included (<1%) (Monemi et al., 1999a) (Fig. 16).
Figure 16: Proportion (%) of single (shaded) and combinations of MyCH isoforms in extrafusal fibres in the young masseter and biceps muscles and for comparison adult (Stål et al., 1994a) and elderly (Monemi et al., 1999a). Note apparent differences between masseter and biceps.
Comparison of extrafusal muscle fibres of young biceps with adults and elderly

The young biceps differed in age-related changes of relative frequency of fibre types between young age, adulthood and elderly with larger type IIB proportion in elderly (p=0.004) (Monemi et al., 1998) (Fig. 17).

Figure 17: Relative frequency (%) of fibre types in young biceps compared with adult (Eriksson, 1982; Eriksson and Thornell, 1983) and elderly (Monemi et al., 1998) biceps. Mean and SD.

The mean muscle fibre diameter was 3.0 times larger in the adult biceps than in young biceps and 2.2 times larger in elderly biceps (Eriksson, 1982; Eriksson and Thornell, 1983; Monemi et al., 1998) (Fig. 13).

RCA (%) of different fibre types showed a predominant area of type I fibres in young biceps with a smaller area in adult and elderly biceps in favour of larger type II fibres area (Eriksson, 1982; Eriksson and Thornell, 1983; Monemi et al., 1998) (Fig. 14).
In the biceps GE showed the content of MyHC-I largest in young and elderly biceps and smallest in adult accompanied by large amounts of MyHC-IIa and MyHC-IIx in adult biceps (Monemi, 1999) (Fig. 18).

![Biceps](image)

Figure 18: Relative content (%) of MyHC-I, MyHC-IIa, MyHC-IIx and MyHC-fetal in young, adult and elderly biceps (Monemi et al., 1999a). Mean and SD.

Detected MyHC isoforms with IHC showed for the biceps no major age-related differences in proportions for MyHC-I and MyHC-IIa. The proportion of MyHC-IIx isoform was larger in elderly (Stål, 1994a; Monemi, 1999a). Young biceps fibres showed four "MyHC based fibre types", two single and two mixed and six when occasional combinations were included (<1 %). Adult biceps individual fibres showed four "MyHC based fibre types", three single and one mixed and five when occasional combinations were included (<1 %). Elderly biceps individual fibres showed five "MyHC based fibre types", three single and two mixed (Fig. 16).
**Muscle spindles (Paper II)**

*Muscle spindle density*
In the young masseter the muscle spindle density, MS/cm², was higher (mean, SD) in the deep 43 (16) than in the sup ant 27 (11) and sup post 4 (6) masseter portions, and higher in the sup ant than in the sup post. In the young biceps the density was 20 (13), which was lower than that of the deep masseter (Fig. 19).

![Figure 19: Muscle spindle density (MS/cm²) in the anterior (Sup ant) and posterior (Sup post) superficial and deep (Deep) masseter and biceps muscles. Mean and SD.](image)

*Compound muscle spindles*
In the young masseter compound muscle spindles were most frequent in the deep portion, 24 %, compared with the sup ant, 10 %, and sup post, 0 % portions. In the young biceps 4 % of the spindles were compound.

*Capsule diameter*
For both the masseter and biceps, the diameter of muscle spindle regions- A and AB were significantly larger than that of region-B. The diameter of region-A was significantly larger than that of region-AB. The mean capsule diameter (µm, mean SD) of pooled data for single muscle spindles was 96 (37) in the masseter and 76 (28) in the biceps. There were no significant differences in capsule diameter between masseter portions or between masseter and biceps (Fig. 20).
Intrafusal fibre type composition (Paper II)

Intrafusal fibre types
In the young masseter the number of intrafusal fibres per muscle spindle was on average 8.3 (min-max 1-23) in single and 18.8 (min-max 7-38) in compound muscle spindles. Of the examined spindles, 90 % contained bag₁, 15 % AS-bag₁, 76 % bag₂, and 96 % chain fibres. The average composition of intrafusal fibre types in single muscle spindles was 2 bag₁, 1 bag₂ and 4 chain fibres. Single muscle spindles contained 67 combinations of intrafusal fibre types, whereas compound muscle spindles contained 22 combinations. A unique allotment of number of intrafusal fibres was presented in 70 % of the muscle spindles.

In young biceps there were on average 7.4 (min-max 3-19) intrafusal fibres in single muscle spindle and 22 (min-max 22-23) in compound muscle spindles. Of the examined spindles, 96 % contained bag₁, 45 % AS-bag₁, 28 % bag₂ and 100 % chain fibres. The average composition of intrafusal fibre types in single muscle spindles was 2 bag₁ and 4 chain fibres. Single muscle spindles contained 39 combinations of intrafusal fibre types, whereas compound muscle spindles contained 2 combinations. A unique allotment of number of intrafusal fibres was present in 79 % of the muscle spindles.
**Intrafusal fibre type diameter**

The intrafusal fibre type diameter in young masseter and biceps muscles are seen in (Fig. 21). The intrafusal fibre diameter did not differ between the masseter muscle portions, but did between the masseter and biceps (p=0.045).

![Intrafusal fibre diameter](image)

Figure 21: Intrafusal fibre type diameter in young masseter and biceps muscles. In the masseter, bag₂ fibres were significantly larger than both bag₁ and chain fibres. Also in the biceps, bag₁, AS-bag₁ and bag₂ fibres were significantly larger than chain fibres. Masseter bag₁ fibres were significantly larger than those of the biceps (p=0.049). The mean intrafusal fibre diameter for pooled data of bag₁, AS-bag₁, bag₂ and chain fibres (µm, mean SD) was significantly larger in the masseter, 13.5 (2), than in the biceps, 10.0 (1) (p =0.045).

**MyHC composition of intrafusal fibres (Paper IV)**

The mAb staining pattern of the young biceps intrafusal fibres was similar to that of the masseter for mAbs ALD19, N2261 and F1652. For the other mAbs some differences were seen. Details are presented in paper IV. In both muscles eight MyHC isoforms- slow tonic, I, IIa, IIx’, IIx, α-cardiac, fetal and embryonic were detected.

In the young masseter bag₁ fibres contained predominantly MyHC isoforms slow-tonic, α-cardiac and I and substantial proportions of IIa, IIx’, IIx and fetal; AS-bag₁ contained predominantly slow-tonic, α-cardiac, I, IIa and substantial proportions of fetal, IIx’ and embryonic; bag₂ contained predominantly slow-tonic, I, α-cardiac, fetal, IIa and substantial proportions of IIx’; chain contained predominantly IIa and fetal and substantial proportions of embryonic, α-cardiac and I.
Comparison between the three muscle spindle regions for differences in MyHC content showed for the masseter bag, fibres smaller proportion of MyHC-I in the A+AB region, and for masseter bag, and bag$_2$ fibres larger proportion of MyHC-fetal in the A+AB region than in the B region. There were 56 combinations of MyHC isoforms, 24 when less frequent combinations, <1%, were excluded (Fig. 22). The most common MyHC combination for bag, and AS-bag, fibres was slow tonic + I + IIa + α-cardiac, for bag$_2$, fibres was slow tonic + I + IIa + fetal + cardiac and for chain fibres was IIa + fetal.

In the young biceps bag, fibres contained predominantly MyHC isoforms slow-tonic and I and substantial proportions of IIx, IIa and α-cardiac, IIx and embryonic; AS-bag, contained predominantly slow-tonic, I and IIx’ and substantial proportions of IIa and fetal embryonic; bag$_2$ contained predominantly slow-tonic, I, IIa, fetal and α-cardiac and substantial proportions of embryonic; chain contained predominantly fetal, IIa and I and substantial proportions of embryonic and α-cardiac. In young biceps bag, fibres showed larger proportion of MyHC-IIx’ in the A+AB region, and bag$_2$ fibres showed larger proportion of MyHC-fetal in the A+AB region than in the B region. There were 38 combinations of MyHC isoforms, 26 when less frequent combinations, <1%, were excluded (Fig. 22). The most common MyHC combinations for bag, fibres was slow tonic + I + IIx’, for AS-bag, fibres was slow tonic + I + IIa, for bag$_2$, fibres was slow tonic + I + IIa + fetal and for chain fibres was IIa + fetal.

Figure 22: Proportion (%) of single (shaded) and combinations of MyHC isoforms in intrafusal fibres in the young masseter (left) and young biceps (right) muscles. The pie diagrams show for the young masseter 24 MyHC isoform combinations represented with ≥1%, and for the young biceps 26. Note co-expression (combinations) of MyHC isoforms in generally all fibres.
Comparison of muscle spindles and intrafusal fibres of young masseter with young biceps

The muscle spindle density in the young deep masseter portion was higher than in the biceps (p=0.044). There were no significant differences in capsule diameter between young masseter and biceps muscle spindles. The masseter contained significantly more bag\textsubscript{2} fibres, 16 \% versus 4 \% (p=0.008) and less chain fibres, 52 \% versus 64 \% (p=0.024), than the biceps. Masseter bag\textsubscript{1} fibres were significantly larger in diameter than those of the biceps (p=0.049). The mean intrafusal fibre diameter for pooled data of bag\textsubscript{1}, AS-bag\textsubscript{1}, bag\textsubscript{2} and chain fibres (µm, mean SD) was significantly larger in the masseter, 13.5 (2), than in the biceps, 10.0 (1) (p=0.045).

There were notable similarities and marked differences in MyHC distribution of intrafusal fibres for young masseter and biceps. Similarities- Eight MyHC isoforms. Mixtures of two to six isoforms in all intrafusal fibres. Numerous of combinations of MyHC isoforms. Bag\textsubscript{1}, AS-bag\textsubscript{1} and bag\textsubscript{2} fibres showed MyHC-slow tonic, and bag\textsubscript{2} also MyHC-I. Chain fibres showed MyHC-II\textalpha and MyHC-fetal. Some combinations were seen in both muscles. In chain fibres, the two most frequent MyHC isoform combinations were seen in both muscles. Differences- In the young masseter, 98 \% of bag fibres showed MyHC-\alpha cardiac vs. 30 \% in the young biceps, all AS-Bag, showed MyHC-\alpha cardiac vs. 10 \% in the biceps, 35 \% of bag\textsubscript{2} fibres showed MyHC-IIx vs. none in the biceps, 17 \% of chain fibres showed MyHC-I vs. 61 \% in the biceps. The most frequent MyHC isoform combinations in bag, and bag\textsubscript{2} fibres differed between the muscles.

Comparison of muscle spindles and intrafusal fibres of young masseter and biceps with adults

Comparison of young masseter muscle spindles with muscle spindles in adult muscles showed that the muscle spindle density was significantly higher (p=0.005) in young masseter, 25, than in adult (Eriksson and Thornell, 1987) masseter, 7. In both young and adult masseter the deep portion showed the majority of spindles (young 61 \% and adult 74 \%), and compound muscle spindles occured in a significant amount. Young masseter did not differ from the adult (Eriksson and Thornell, 1990) in number of intrafusal fibres of single spindles (young 8.3, adult 7.3), capsule diameter of single spindles (young 96 µm, adult 98 µm) or intrafusal fibre diameter (young 13.5 µm, adult 16 µm). The ratio between the mean bag fibre
diameter (pooled data for bag₁, AS-bag, and bag₂ fibres, n=439) and the mean diameter of extrafusal type I fibres (Österlund et al., 2011a) was 0.8 (16.5 µm/21.7 µm) for the young masseter versus 0.4 (18.1 µm/43.9 µm) for the adult (Eriksson and Thornell, 1990).

Comparison of muscle spindles and intrafusal fibres of young biceps with muscle spindles in adult (Liu et al., 2002) muscles showed a similarity in enzyme-histochemical staining pattern of intrafusal fibre types, mean number of intrafusal fibres per muscle spindle heterogeneity in combinations of intrafusal fibre types and lack of bag₂ fibres.

MyHC-I was identified in chain fibres in both the masseter and biceps, although in diverse proportions, about one-fifth in masseter and two-thirds in young biceps. These fibres were in contrast to reports of lack of MyHC-I in chain fibres in both the masseter (Eriksson et al., 1994) and biceps (Liu et al., 2002) of adults. Likewise, whereas we could detect MyHC-α cardiac in about one-fifth of the chain fibres of both the young masseter and biceps, chain fibres in the adult biceps lack this isoform (Liu et al., 2002).
DISCUSSION

The main findings in this thesis were, firstly, that the young masseter differed profoundly from the young biceps muscle in composition and distribution of extrafusal fibre types and MyHC isoforms, and in age-related plasticity from childhood to adulthood. This suggests that the human masseter is specialized in fibre types already at young age and has a unique pattern of growth of fibre types and contractile proteins. Secondly, findings of fundamental similarities in intrafusal MyHC expression between young masseter and biceps, but also marked differences implying muscle specific proprioceptive control. Findings of similarities in mATPase fibre types and MyHC expression of intrafusal fibres between young masseter and biceps muscle spindles and spindles in adult muscles suggest early maturation of muscle spindles and that muscle spindles/intrafusal fibres precede extrafusal fibres in growth and maturation. This in turn implies early need and capacity of reflex, proprioceptive control in learning, performing and improving jaw skills and arm behaviour. Thus, the hypothesis of masseter extrafusal muscle plasticity during life span in parallel with changes in jaw-face skeletal morphology and jaw function, and the hypothesis that the masseter is divergent from limb muscle in growth pattern could be verified. However, the hypothesis of changes in masseter intrafusal (muscle spindle) fibre types and MyHC expression from childhood to adulthood was rejected.

Extrafusal fibre types (Papers I and III)

Extrafusal fibres in perspective of evolution and development
To explain the findings of fundamental differences between the masseter and biceps in extrafusal fibre types and MyHC expression, and in muscular plasticity during life span, they can be viewed in perspective of evolution of the MyHC genes and current knowledge on the diversity in evolution and development between the craniofacial and the limb and trunk muscles.

Firstly, in the last decade, all the genes in the human genome has been characterized and an evolutionary tree has been created, which shows that in human muscles eleven genes are linked to the expression of MyHC isoforms and have over the years evolved for specific purposes. Hoh has studied in detail the evolution of jaw closing muscles and limb and trunk muscles in vertebrates (Hoh, 2002). He refers the phenomenon of phylogenetic muscle plasticity to adaptive changes in muscle properties in response to changes in functional load during phylogeny. The functional properties of jaw closing
muscles across species are complex and varied. Jaw muscles show a much greater phylogenetic plasticity than limb and trunk muscles. In terms of myosin expression it fits with the extensive repertoire of myosin expression in jaw closers across species. In contrast, limb muscles show a minimal degree of phylogenetic plasticity and manage to optimize contractile functions by means of a limited number of MyHC isoforms.

Secondly, recent studies have provided evidence that the skeletal muscles of the head that control mastication, facial expression, and eye movements are evolutionary, morphologically and molecularly distinct from those of the trunk and limbs (Sambasivan et al., 2011). In evolutionary terms the head of vertebrates is thought to be a novel structure and some muscles; extra ocular, jaw and facial muscles are also a vertebrate novelty. They all derive from the cranial mesoderm, an embryonic tissue that is also unique to vertebrates. It has been suggested that the head muscles have arisen independently of trunk muscles, which means that the cranial mesoderm derived progenitors are evolutionary distinct from the somatic muscle progenitor pool (Sambasivan et al., 2011). Furthermore, it is now known that different genetic regulatory cascades operate within the individual craniofacial muscle groups, and that there is a remarkable relationship with cardiomyogenesis (Sambasivan et al., 2011).

Finally, Hoh introduced the term allotype for muscles, which were developmentally, structurally and functionally distinct. The first group of muscles to be an allotype separated from limb and trunk muscles was the jaw muscles (Hoh, 2002). The results in this thesis concord with the masseter and biceps being different allotypes of muscles (Hoh, 1993; Hoh, 2002), and with the divergence between jaw and limb muscles in growth patterns and regulatory proteins (Pavlath et al., 1998; Rios and Marcelle, 2009). The findings are in line with results from previous studies on human jaw opening and jaw closing (Eriksson, 1982; Eriksson and Thornell, 1983; Stål, 1994; Monemi, 1999) facial (Stål et al., 1990), tongue (Stål et al., 2003; Granberg et al., 2010) and extra ocular muscles (Kjellgren et al., 2003) as well as small and large limb muscles (Eriksson, 1982; Stål et al., 1987) showing that each muscle has its special fibre type composition.

Thus, the present results of key differences in structure and growth pattern between the human masseter and biceps most probably reflect diversity in evolutionary and developmental origins for jaw and limb muscles.
Fibre type composition

The findings in the young masseter of specialized composition and distribution of fibre types already at three years of age suggests early diversified and fine-tuned internal motor control for specific tasks. The deep masseter with vertically oriented fibres and slow twitch motor units (Stålberg et al., 1986; Stålberg and Eriksson, 1987) and a predominance of type I fibres and slow MyHC and high muscle spindle density, probably is of special importance for anti-gravity function in jaw positioning. More type IM fibres composed of slow and fast MyHCs in mixture in the anterior superficial masseter, and more type IIB fibres and fast MyHCs and few spindles in the posterior portion near the fulcrum imply regional specialization of the superficial masseter in force development and endurance during biting, chewing, swallowing and speech. The internal diversity tells that the superficial masseter is genetically destined to develop, grow, and mature into not one muscle, but an entity of different muscle portions acting in a task-related synergy. Moreover, marked inter-individual heterogeneity in MyHC expression of the young masseter, compared with the young biceps, reflects individual characteristics in functional properties.

The young masseter and young biceps muscles were similar in that type I fibres, with slow MyHC, outnumbered other fibre types and were of similar diameter. This suggests that at young age both muscles are composed mainly of slow-twitch, fatigue resistant motor units and have high oxidative capacity. However, differences in composition for the other fibre types reflect basic diversity in morphology and function.

Significant age-related muscular plasticity from childhood to adulthood was revealed when comparing the young masseter with data from the adult (Eriksson, 1982; Eriksson and Thornell, 1983) and elderly (Monemi et al., 1998) masseter. Thus, young masseter contains more type IM and less type IIB fibres in the superficial portion, and less type I fibres in the deep portion than the adult (Eriksson, 1982; Eriksson and Thornell, 1983) masseter. The young masseter has more types I and IM proportions and less types IIA and IIB proportion in superficial portion than elderly masseter (Monemi et al., 1998).

Fibre type growth pattern

Smaller type II fibre diameter in the young masseter than in young biceps reflects diverse fibre type growth patterns. Generally, small diameter fibres in the masseter together with multipennate muscle architecture (Ebert, 1938/39; Schumacher, 1961; Gaspard, 1987) should allow a relatively high number of motor units. In fact, there is evidence from electrophysiological
studies of fewer muscle fibres per motor unit in the masseter than in large limb muscles but about the same number as in the small first dorsal interosseus muscle (FDI) (Stålberg et al., 1986). The FDI, like the masseter, has a large cortical representation and is used in finely graded movements. The average muscle fibre diameter of the FDI is about the same as in other limb muscles (Polgar et al., 1973) and thus much larger than that of the masseter. Therefore, given that the FDI is of equal volume as the masseter, or rather smaller, the masseter should contain a higher number of motor units than the FDI. High number of motor units, in turn, implies a wide span of continuous contractile properties in the masseter as suggested by EMG studies (Yemm, 1977).

Our comparison between young and adult (Eriksson, 1982; Eriksson and Thornell, 1983) and elderly (Monemi et al., 1998) masseter and biceps muscles showed significant differences between their fibre type growth patterns. The difference in fibre type growth pattern between the masseter and biceps reflects differences in development and maturation of fibres, optimally designed to meet special force and speed demands in a cost and energy effective manner. The small type II fibres can be the result of both genetic program and phenotypic adaptation.

*Extrafusal MyHC expression*

The young masseter differed from the young biceps in MyHC expression of the extrafusal fibres, the masseter being more complex by containing six isomyosins including exclusive MyHCs- fetal and a-cardiac, in addition to slow and three fast MyHC isoforms. Typically, the young masseter displayed a more variable mAbs-staining pattern, and a major proportion of fibres composed of mixtures of different MyHC isoforms resulting in a higher number of “MyHC-fibre types”. Notably, there was a continuum of single and combinations of MyHC isoforms systematically related to enzyme-histochemical fibre types promoting correlation between mATPase fibre types and MyHC isoform expression. The heterogeneous MyHC composition in the young masseter may reflect an immature composition of MyHCs at young age, which during growth and maturation, in parallel with craniofacial changes, teeth eruption and improvement of jaw functions, will evolve and adapt to functional demands.

The result provides evidence that the young masseter muscle is unique in MyHC expression, including unconventional MyHC isoforms as well as hitherto unrecognized spliced isoforms of MyHC-fetal and MyHC-IIx. Differences in MyHC expression vs. young biceps are proposed to reflect diverse evolutionary and developmental origins and accord with the masseter and biceps being separate allotypes of muscle. Comparison with
MyHC expression in adults and elderly revealed differences between masseter and biceps in MyHC plasticity, and therefore alterations in contractile properties, during life span.

*Slow- and fast MyHC expression*

For slow MyHC in the young masseter, there was generally good agreement between the IHC and GE results (73% and 86%, respectively) and the proportion of enzyme-histochemical fibres expressing slow MyHC (83% types I, IM and IIC). Similar congruence was not seen for the fast MyHCs. IHC stained 45% of the young masseter fibres for fast MyHC in accordance with the proportion of enzyme-histochemical fibre types expressing fast MyHC (55% types II and IM). However, fast MyHC content in the GE was only 13%. This seemingly disparity in results of the two methods can be explained. Firstly, the mAbs against MyHCs used have very high sensitivity. Thus individual fibres, which contain relatively small amounts of fast isomyosins, as reflected in the gels, will nevertheless appear as stained fibres by IHC. Secondly, in young masseter, the type II fibre diameter is smaller than the type I type diameter (Österlund et al., 2011b), which means that a given proportion of fibres detected with mAbs will correspond to a relatively smaller muscle mass quantified by GE.

The findings for fast MyHC distribution may be due to high mAb sensitivity. With respect to specificity of the used mAbs, we were aware that Western blots of the used mAbs have revealed that some have broader reactivity pattern than originally published or proposed by manufacturer. This holds for the mAbs SC71, A474 and N2261. The first two are reported to identify MyHC-IIa, but show affinity, albeit weaker, also for MyHC-IIx (Soukup & Thornell, unpublished). The mAb N2261 showing very strong affinity for MyHC-IIa, also detects MyHC-I and MyHC-extra ocular, whereas the mAb BF35 reacts with MyHCs I, α-cardiac, IIa, slow-tonic and extra ocular (Liu et al., 2002). This taken into account when classifying MyHC staining in relation to ATPase fibre typing, nevertheless we had to introduce a fibre type MyHC-IIx’ as the staining pattern of these fibres did not accord with presumptive staining patterns of used mAbs. The mAb BF35 negative fibres are supposed to reflect fibres expressing MyHC-IIx, being the only MyHC which they do not detect. In the present study however, a proportion of the mAb BF35 unstained fibres were also unstained for the mAb N2261, indicating lack of MyHC-IIa. On the other hand, the fibres stained with the mAbs SC71 and A474, which preferentially detect MyHC-IIa according to the manufacturer and researchers introducing them (Schiaffino et al., 1989; Hughes et al., 1993; Liu et al., 2002). However, these antibodies also have been shown to have affinity for MyHC-IIx, albeit clearly weaker than for
MyHC-IIa. Thus, unfortunately these mAbs do not reliably separate fibres containing MyHCs-IIa and IIx (Smerdu and Soukup, 2008). Until further information is available, we tentatively interpret the MyHC-IIx’ fibre to reflect the occurrence of a spliced isoform of MyHC-IIx. Interestingly, the young biceps lacked this isoform, which may reflect some disparity in molecular structure between cranial and limb fast MyHC isoforms. This may be related to divergence between the masseter and biceps in evolutionary and developmental origins (Sambasivan et al., 2011).

Actually, existences of spliced MyHC isoforms are not unique. Indeed isoforms of MyHC-I have been presented in human muscle related to development (Hughes et al., 1993). However, using the same mAbs, we did not observe distinct evidence for spliced variant of MyHC-I in childhood.

**MyHC-fetal and MyHC-α cardiac expression**

A fundamental difference between young masseter and young biceps was the presence of MyHC-fetal and MyHC-α cardiac isoforms in the masseter, but not in the biceps. The results of the two methods with respect to amount and occurrence of MyHC-fetal and MyHC-α cardiac in the young masseter also seemed to be at variance. By IHC, both isoforms were detected in about half of the young masseter fibre population, dispersed over the complete fibre type population though preferentially expressed in IM and IIC fibres. By GE, they were detectible in the overloaded gels but faintly visible in the regular mini-gels. Like the result for fast MyHC content, the finding for fetal and α-cardiac isoforms may be due to high mAb sensitivity. The result confirms that these isoforms, which in limb muscles are expressed only during muscle development, are constituent contractile proteins in the human masseter muscle during the life span (Butler-Browne et al., 1988; Soussi-Yanicostas et al., 1990; Bredman et al., 1991; Barbet et al., 1992; Pedrosa-Domellöf et al., 1992; Eriksson et al., 1994; Ściote et al., 1994; Stål et al., 1994a; Monemi et al., 1996; Monemi et al., 1999a; Korfage et al., 2000; Bontemps et al., 2002).

Our result of two bands stained for MyHC-fetal with Western blot technique was unexpected but might be of considerable interest in terms of MyHC expression and phylogenetic regulation. One band corresponded in mobility with a band in the fetal sample known to reflect the MyHC-fetal isoform (Butler-Browne et al., 1988). This band co-migrated with the MyHC-IIa band in our gels. The other band had slower mobility and co-migrated with the MyHC-IIx band. We could exclude that the extra band was related to MyHC-embryonic, both on the basis of known mobility of MyHC-embryonic in our gel system (Kjellgren et al., 2003), and the fact that the mAb F1652, specific
for human MyHC-embryonic (Karsch-Mizrachi et al., 1989), stained our fetal control samples but left young masseter samples unstained. Further studies are needed to ascertain the existence of the two fetal isoforms and their functional significance, but our results indicate the existence of a so far unrecognized spliced variant of MyHC-fetal in human skeletal muscles. The special MyHC expression in the masseter reflects evolutionary aspects on the jaw-closing muscles. The significance of small proportions of fetal and α-cardiac contractile proteins in the masseter and for jaw function during growth and maturation has not clearly been revealed. Recent molecular studies by mRNA techniques indicate adaptive changes in MyHC-fetal expression in the human muscle in response to altered biomechanics from dental and surgical treatments (Harzer et al., 2010; Oukhai et al., 2010). Experimental results in rat cardiac myocytes indicate that even small amounts of MyHC-α cardiac significantly augment myocyte power output (Herron and McDonald, 2002). An age-related decrease of this isoform is suggested when the present result in young masseter is compared with data from adult and elderly masseter.

**Muscle spindles and intrafusal fibre types (Papers II and IV)**

The results revealed both differences and similarities between the young masseter and the young biceps in muscle spindle morphology. The masseter showed higher spindle density, larger number of intrafusal fibres per spindle, larger average fibre diameter and more compound muscle spindles than young biceps. The masseter spindles contained more bag, fibres and less chain fibres than the biceps spindles. The young masseter and young biceps were similar in their strikingly heterogeneous allotment of types and number of intrafusal fibres. Therefore, the “average” composition of intrafusal fibre types should not be taken as “the typical” muscle spindle appearance. Rather, most spindles were unique in the combination of intrafusal fibre types. This variability in morphology between spindles and muscles suggest highly differentiated proprioceptive abilities allocated in governing mandibular and arm positions and movements. Furthermore, young masseter and biceps muscle spindles expressed a broad repertoire of MyHC isoforms in a complex pattern and still use genes that appeared early in evolution. The findings of fundamental similarities in intrafusal MyHC expression between young masseter and biceps, but also marked differences implying muscle specific proprioceptive control, for jaw- as well as arm motor skills.
**Muscle spindle density**

The present finding of almost three times higher muscle spindle density in the deep than in the superficial masseter, accords with findings in the adult masseter (Eriksson and Thornell, 1987). From experience that smaller muscles have higher muscle spindle densities than larger muscles it was suggested that higher density is appropriate for small muscles involved in fine adjustments (Barker and Banks, 1994; Banks, 2006). Comparison of human small and large muscles acting in parallel demonstrated that smaller muscles contained about 3.7 times the spindle density of larger muscles. This difference was related to greater relative excursions of the smaller muscles, which therefore were appropriate for length sensors (Peck et al., 1984). Length changes of spindles depend on features such as extrafusal fibre length, degree of pinnation and moment-arm length (Gans and Bock, 1965; McClearn, 1985). The masseter is multipennate with relatively short muscle fibres. The deep masseter, with vertically oriented fibres and a predominance of type I fibres/slow motor units (Stålberg and Eriksson, 1987), seems to be appropriate to house length sensors involved in fine position control of the mandible. Studies indicate that this regional specialization is early evolved. The number of spindles supplying the jaw muscles seems to become larger from the lower primates towards man. This increasing jaw muscle spindle density accords with increasing complexity of jaw skills along the evolutionary line of the primates (Kubota and Masegi, 1977), indicating impact on mandibular movement control in chewing and speech, a function evolved only in man.

**Intramuscular fibre type composition**

Bag₁ fibres in young biceps muscle spindles were relatively scarce. There was a lack of bag₂ fibres in the young masseter and biceps muscle spindles. Spindles lacking bag₂ fibres have previously been reported for the human adult biceps (Liu et al., 2002) and deep neck (Liu et al., 2003) muscles. Bag₁ fibres are known to play a major role in the production of the velocity-sensitive (dynamic) response in the primary endings whereas bag₂ fibres mediate the length sensitivity (static) along with chain fibres (Boyd, 1980; Matthews, 1981; Barker and Banks, 1994). Furthermore, five of the young masseter spindles lacked chain fibres, but of these, all contained bag₁ and four contained bag₂ fibres, suggesting ability for both dynamic and static sensitivity. Lack of bag₂ and chain fibres would again reflect diversity in physiological qualities between spindles and muscles, preferentially in static length sensitivity properties. One could speculate that the proportions of MyHCs- slow-tonic, I and α-cardiac in bag₁ is correlated with “dynamic” properties, and that MyHCs- Ila and fetal in bag₂ and chain fibres are significant myosin isoforms for “static” actions.
In line with classification of the “intermediate” AS-bag, fibre on basis of its mATPase staining profile (Eriksson and Thornell, 1985, 1987, 1990; Eriksson et al., 1994), IHC placed this fibre type in between bag₁ and bag₂ fibres by showing MyHCS- I, α-cardiac and IIa, isoforms present in both bag₁ and bag₂. Its physiological characteristics and role in proprioceptive control remains to be examined.

Only one intrafusal fibre type combination overlapped all three masseter portions, and few combinations overlapped the young masseter and biceps. Also different subjects were unique in fibre type composition of muscle spindles, with hardly any overlapping combinations between subjects. Although noted in a limited number of subjects, this variability in morphology between spindles, between muscle portions and between muscles and subjects suggest highly differentiated proprioceptive abilities allocated in governing jaw and arm positions and movements. The finding of a resemblance in muscle spindle morphology between young and adult (Eriksson and Thornell, 1987) masseter and between young and adult (Liu et al., 2002) biceps, suggest that muscle spindles in these muscles are morphologically mature already at young age and precede the extrafusal fibres in growth and maturation.

**Intrafusal MyHC expression**

Precise determination of muscle fibre types and their composition of contractile proteins in a given muscle are critical for in-depth knowledge of muscle diversity and better understanding of physiological properties and pathophysiological behaviour. The MyHC isoforms detected in the intrafusal fibres are coded by separate genes. The fast skeletal gene cluster codes for MyHC-IIa (MYH2), MyHC-IIx (MYH1), MYH3 and MYH8 genes code for the developmental MyHC-embryonic and MyHC-fetal isoforms. Two cardiac myosin genes MYH6 and MYH7 code for MyHC-α cardiac and MyHC-β cardiac/slow-twitch. Furthermore, unlike extrafusal fibres the intrafusal fibres contained MyHC-slow tonic, the evolutionary second most ancient MyHC isoform, coded by MYH14 (Rossi et al., 2010), after the most ancient masticatory IIM isoform, coded by MYH16 (Hoh, 2002; Stedman et al., 2004). MyHC-slow tonic was first recognized in presumptive muscle spindle fibres in human 10-11 weeks of gestation old foetuses (Thornell et al., 1988; Pedrosa-Domellöf and Thornell, 1994). Thus, the broad repertoire of MyHC isoforms in the intrafusal fibres still uses genes appeared early in evolution.

Interestingly, the number of muscle spindles in the jaw muscles seems to increase in the evolutionary series from lower primates towards man (Kubota and Masegi, 1975, 1977), with the highest spindle density in the jaw-
closing muscles. The intricate MyHC expression seen in human muscle spindles probably reflects the higher complexity of their sensory and motor innervation (Kucera, 1986). The sensory and motor innervations are well known factors influencing the expression of MyHC isoforms in intrafusal fibres (Pedrosa et al., 1989; Soukup et al., 1995; Walro and Kucera, 1999).

**Clinical implications**

This thesis will contribute to a better understanding of growth, maturation and aging of human skeletal muscle fibres and muscle spindles with special focus on the jaw sensory-motor system. The findings that the masseter has a complicated structure with intra-muscular variability, regional differentiation and fibre type diameter differences that change and adapt over the life span, will be of value in examination by means of, e.g. EMG, muscle biopsy and microdialysis techniques.

**Training**

In response to load, muscle fibres can alter in MyHC isoforms and cross-sectional area, which determine their contraction velocity and maximum force generation. Reduced muscular activity induces transition towards faster more fatigable fibre types and a decrease in fibre cross-sectional area, whereas increased muscular activity elicits transition towards slower more fatigue-resistant fibre types and enlargement of fibre cross-sectional area (c.f Grunheid et al., 2009; Vreeke et al., 2011). Soft food diet reduces the capacity of the masseter muscle in animals (Kiliaridis et al., 1988) with decrease in cross-sectional area of fibres co-expressing MyHCs- I and a-cardiac (Vreeke et al., 2011). The finding that jaw muscles can adapt structurally to reduced or increased load has implications for selection of rehabilitation program.

**Muscle pain**

Pain influences jaw muscle proprioception and deteriorate motor performance. The effect of muscle pain on motor control is, however, not fully understood. Experimentally induced jaw muscle pain causes transient decrease in the maximum voluntary contraction resulting in lower bite force (Svensson et al., 1998a; Svensson et al., 1998b; Svensson et al., 2004). Moreover, muscle pain causes a change in coordination and decrease in movement amplitude and velocity (Lund, 1991; Stohler, 1999; Svensson and Graven-Nielsen, 2001). It has been shown experimentally that pain will via the Y-muscle spindle system, increase the sensitivity of the muscle spindles,
resulting in increased reflex-mediated muscle stiffness (Johansson et al., 1999). Likewise, incorrect information from the jaw muscle spindle system may cause a sensory mismatch, with disturbed proprioceptive information, which may cause disturbance of the postural and movement system of the jaw (Ro and Capra, 2001; Capra et al., 2007).

Finally, it is intriguing to speculate that the evolutionary and developmental relations between the heart and jaw muscle as reflected by MyHC-α cardiac expression also in jaw muscle may have some bearing for the clinical experience that jaw-face pain can sometimes be referred heart pain (Kreiner et al., 2007).

Disease
Muscle diseases like hereditary myosin myopathies, are caused by mutation in skeletal MyHC genes, which may affect MYH8 (codes for MyHC-fetal), and can give clinical signs as jaw trismus and problems with mastication. Two another diseases affects MYH3 (codes for MyHC-embryonic) or MYH7 (codes for MyHC-I), which can give rise to facial anomalies and jaw muscle weakness (Oldfors, 2007).

Future perspective

From structure to function- Study of integrative jaw-neck motor control in childhood.
Previous findings of coordinated mandibular and head-neck movements during natural jaw-opening closing tasks suggest a functional linkage between the jaw and the neck sensori-motor systems. Studies also indicate that this functional connection between the jaw and neck is innate (Zafar et al., 2000; Häggman-Henrikson and Eriksson, 2004; Eriksson et al., 2007). Studies of comorbidity of jaw and neck myofascial pain have tried to explain amplification and spread of muscle pain, by pain modulating mechanisms via central sensitization (Arendt-Nielsen and Henriksson, 2007). Furthermore, there is experimental evidence of intersegmental reflex connections between the masseter and neck muscles (Hellström et al., 2000; Hellström et al., 2002). To evaluate the functional maturation of the jaw-neck motor coupling in childhood, a pilot study examined concomitant mandibular and head-neck movements during jaw opening-closing motor activities in 5-year-old children (Fig. 23). The result demonstrated that the movement pattern in children was irregular and variable compared with the well-coordinated movement patterns seen in adults. This suggests an
immature reflex motor programming of jaw actions in children, although as demonstrated in the present thesis, the muscle spindle system may be morphologically well differentiated and matured.

Figure 23: Movement patterns of concomitant mandibular (blue) and head-neck (red) movements during maximal jaw-opening-closing movements in a child aged 5-years (left panel) and young adult 23-years (right panel). Note irregular and variable mandibular and head-neck movements for the child (left panel) and regular and well-coordinated movements for the adult (right panel). (Zafar, Österlund, Eriksson, pilot data).
**SUMMARY**

*Extrafusal fibre types*

**Young masseter**
- Young masseter contained fibre types I, IM, IIC, IIAB, IIB and scarce IIA. Type I fibres predominated. The inter-individual variation was marked.
- The deep portion showed more type I, and less type IM than the superficial portion.
- Type II fibres were smaller in diameter than type I, and smaller than biceps type II.
- Compared with adult masseter, young masseter showed larger proportion of type IM and smaller proportion of type IIB in the superficial portion, and smaller proportion of type I fibres in the deep portion.
- Compared with elderly masseter, young masseter showed larger proportions of types I and IM, and smaller proportions of types IIA, IIAB and IIB in the superficial portion.
- The fibre diameters of types I, IM and IIC diameters were smaller in the young masseter than in the adult and elderly masseter. The type IIB diameter was smaller than in adults and type IIAB diameter smaller than in elderly.
- The average fibre diameter was 1.8 times larger in adult and 1.4 times larger in elderly masseter.

**Young biceps**
- Young bicep contained types I, IIA, IIAB and few IIB.
- Compared with adult and elderly biceps, young biceps showed smaller proportion of type IIB.
- The average fibre diameter was 3.0 times larger in adult and 2.2 times larger in elderly masseter.

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**Extrajusal fibre MyHC expression**

Young masseter

- GE identified MyHCs- I, Iia, IIX and small proportions of fetal and α-cardiac isoforms.

- MyHC-I predominated with larger content in the sup ant than in the sup post masseter.

- Two MyHC-fetal bands were identified.

- Young masseter showed six isomyosins, MyHCs- I, Iia, IIX, fetal and α-cardiac.

- The content of MyHC-IIa and IIX was larger in the superficial than in the deep masseter.

- The majority of fibres, 56%, co-expressed two to four MyHC isoforms, i.e. were mixed in MyHC composition.

- Compared with adult and elderly masseter, young masseter showed more MyHC-I, less MyHCs- Iia, IIX and fetal isoforms and more mixed fibres.

Young biceps

- GE and IHC showed MyHCs- I, Iia and IIX. MyHC-I predominated.

- Only occasional fibres contained mixtures of two MyHC isoforms.

- Young biceps showed more MyHC-I and less fast isoforms MyHCs- Iia, IIX and fetal than the adult and elderly biceps.

**Muscle spindles**

- Muscle spindle density was higher in the deep masseter than in the superficial and in the biceps, and higher in the sup ant than in the sup post masseter.

- Compound muscle spindles were frequent in the deep masseter, and few in the biceps.

- Muscle spindle diameter was larger in the A and AB-regions than in the B-region.
- Muscle spindles were very heterogenous in allotment of types and number of intrafusal fibres.

- Intrafusal fibre number was larger in the deep masseter than in the biceps.

- Young masseter muscle spindles contained more bag₂ fibres and less chain fibres than the biceps spindles.

- The mean intrafusal fibre diameter was larger in the young masseter than in the young biceps.

- Bag₂ fibres were larger in diameter than bag₁ and chain fibres.

- No major differences in spindle morphology were found in comparison with spindles in adult masseter and biceps, respectively.

**Intrafusal fibre MyHC expression**

Similarities between young masseter and young biceps in intrafusal MyHC expression

- Eight MyHCs- slow-tonic, I, IIa, IIx’, IIx, fetal embryonic and α-cardiac.

- Co-expression, mixtures of two to six isoforms in all intrafusal fibres.

- Numerous of combinations of MyHC isoforms. Bag₁, AS-bag₁ and bag₂ fibres showed MyHC-slow tonic, and bag₁ also MyHC-I. Chain fibres showed MyHCs- IIa and fetal.

- In chain fibres, the two most frequent MyHC isoform combinations were seen in both muscles.

Differences between young masseter and young biceps in intrafusal MyHC expression

- In the young masseter, 98% of bag₁ fibres showed MyHC-α cardiac vs. 30% in the young biceps, all AS-Bag₁ fibres showed MyHC-α cardiac vs. 10% in the biceps, 35% of bag₂ fibres showed MyHC-IIx’ vs. none in the biceps, 17% of chain fibres showed MyHC-I vs. 61% in the biceps.

- The most frequent MyHC isoform combinations in bag₁ and bag₂ fibres differed between the muscles.
CONCLUSIONS

Extrafusal fibres

- The results suggest that the human masseter is specialized in composition of muscle fibre types already at young age and shows a unique fibre type growth pattern, including relatively small type II fibres.

- The findings of unconventional MyHC isoforms as well as hitherto unrecognized spliced isoforms of MyHC-fetal and MyHC-IIx provide evidence that the young masseter muscle has a unique MyHC composition.

- The finding of differences in fibre types and MyHC expression between young masseter and the masseter in adults and elderly suggests marked plasticity of extrafusal contractile proteins during growth and aging.

- Differences between masseter and biceps in composition and distribution of fibre types and MyHC isoforms, and in muscular plasticity during life span are probably related to divergent evolutionary and developmental origins, resulting in fibres optimally designed to meet special force and speed demands in a cost and energy effective manner. The differences accord with the masseter and biceps being separate allotypes of muscle.

Muscle spindles/Intrafusal fibres

- The MyHC-IIx’ isoform demonstrated in masseter and biceps intrafusal fibres as well as in masseter extrafusal fibres has not previously been reported in human intra- or extrafusal fibres.

- Findings of fundamental similarities in intrafusal MyHC expression between young masseter and biceps, but also marked differences implying muscle specific proprioceptive control, probably related to diverse evolutionary and developmental origins.

- Findings of similarities in mATPase fibre types and MyHC expression of intrafusal fibres between young masseter and biceps muscle spindles and spindles in adult muscles suggest early maturation of muscle spindles. This in turn implies early need and
capacity of reflex, proprioceptive control in learning, performing and improving jaw skills and arm behaviour.

**General conclusion**
- This thesis will contribute to a better understanding of growth, maturation and aging of human skeletal muscle fibres and muscle spindles, with special focus on the jaw sensory-motor system. The results will also be of scientific and clinical value in examination by means of e.g. EMG, muscle biopsy and microdialysis techniques.

**Verified hypotheses**
- The hypothesis of masseter extrafusal muscle plasticity during life span in parallel with changes in jaw-face skeletal morphology and jaw function.
- The hypothesis that the masseter is divergent from limb muscle in growth pattern.

**Rejected hypothesis**
- The hypothesis of changes in masseter intrafusal (muscle spindle) fibre types and MyHC expression from childhood to adulthood was rejected.
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Min familj

Min familj är skogens träd, starka med årsringar många

Deftande blommor växer bland mossa och knotig gren i den rikaste mull

Här finns fåglar som kvittrar, stigar som letar sig till drömmar och mål att fånga

Bland brokiga stammar drar vinden ostyrig och varm och viskar tillsfull

Min familj är samling och verklighet i all sin härighet


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