MUSCLE TONE IMBALANCE IN HUMAN UPPER EXTREMITY

An experimental study of muscle adaptation to altered tension

Fredrik Einarsson

Department of Orthopaedics
Göteborg, Sweden
2008

UNIVERSITY OF GOTHENBURG

ISBN 978-91-628-7610-4

Till Gerd och Lars

Muscle Tone Imbalance in Human Upper Extremity

An experimental study of muscle adaptation to altered tension

Fredrik Einarsson

Departments of Orthopaedics and Hand Surgery, Institute of Clinical Sciences
at the Sahlgrenska Academy, University of Gothenburg

Aim:

The aim of this thesis was to improve outcome after tendon transfer and rotator cuff surgery by investigating the impact on response to passive mechanical testing and change in structural characteristics associated with longstanding changes in the tension of skeletal muscle in the human upper extremities *in vivo*.

Patients and methods:

The investigational method was *in vitro* assessment of human upper extremity muscles. Muscle biopsies were harvested from both healthy controls and patients with conditions representing different types of change in tension in the muscle-tendon unit; upper extremity muscles from patients with spastic contractures, subscapularis muscles from patients with residual internal rotation contractures of the shoulder following obstetric brachial plexus injury and supraspinatus muscles from patients with full thickness rotator cuff tears with retraction.

Mechanical testing was performed by passively stretching single muscle fibres and muscle bundles, using the laser diffraction technique to measure changes in sarcomere length parallel to registrations of tension.

Morphology was assessed using light microscopy and standard staining techniques, including immuno-assay.

Results:

I. The mechanical testing and fibre size of muscle biopsies from human upper extremities are unaffected by freeze storage at -20°C for up to four weeks.

II. Stress relaxation after passive stretching follows a predictable regression pattern related, in amplitude and duration, to the measured sarcomere length after stretching. The time taken to reach a relatively stable tension plateau following the stretching of single fibres is two minutes or more in clinically relevant sarcomere lengths.

III. The mechanical quality of the extracellular matrix in muscle bundles from patients with spastic contractures is compromised, resulting in impaired mechanical performance even though the actual muscle fibres are stiffer than normal controls. This indicates that compensatory mechanisms take place in both muscle and extracellular matrix.

IV. The relative increase in the stiffness of the subscapularis muscle measured between single fibres and bundles is greater than that of normal controls, indicating a compensatory mechanism related to the extracellular matrix in children with obstetric brachial plexus palsy.

V. The subscapularis muscle from children with residual internal rotation contracture following obstetric brachial plexus injury showed essentially normal muscle histology. This indicates that the longstanding loss of amplitude of the muscle secondary to the denervation of antagonist muscles is the most probable cause of internal rotation, at least in children without severe deformation of the glenohumeral joint. A direct injury to soft tissue at delivery and subsequent fibrosis might be a contributory factor.

VI. The supraspinatus muscle from rotator cuff tears with a longstanding, significant retraction responds normally to passive mechanical testing in comparison to the healthy ipsilateral deltoid muscle, indicating that the overall stiffness in retracted rotator cuff tears is not primarily related to the mechanical or morphological deterioration of the muscle tissue. A reduction in absolute muscle volume through the loss of serially coupled sarcomeres is a possible explanation, although no evidence of the presence of this mechanism in humans has previously been demonstrated.

Conclusions:

To improve the outcome at tendon transfer surgery, assessments of the tension in the muscle-tendon unit to be transferred at surgery should preferably be made with a minimum of two minutes' delay following stretching.

Human upper extremity muscles are sensitive to changes in tension over time in different aspects.

The spastic condition with deranged neural regulation, including irregular changes in tension, appears to have a profound impact on muscle fibres, as well as on extracellular matrix mechanics.

The longstanding reduction in tension, as exemplified by the subscapularis muscle of shoulders with a persistent internal rotation following brachial plexus injury and the supraspinatus in retracted rotator cuff tears, appears to have only mild effects on muscle mechanics, but compensatory changes in the extracellular matrix can be detected, affecting the mechanical performance in the muscle-tendon unit as a whole. A possible feedback system sensitive to mechanical stimuli may involve both the direct mechanical interaction of intra- to extracellular proteins and indirect communication through the up- and down-regulation of the production of structural proteins.

Key words:

Muscle biopsy, freeze storage, upper extremity, shoulder, human, muscle, sarcomere, stress-strain relationship, stress relaxation, stiffness, elasticity, mechanical testing, morphology, spasticity, obstetric brachial plexus palsy, rotator cuff tear, tendon transfer

LIST OF PAPERS

This thesis is based on the following papers

I. Passive mechanical features of single fibres from human muscle biopsies - effects of storage
Einarsson F, Runesson E, Fridén J
Journal of Orthopaedic Surgery and Research, 2008; 3:22

II. Stress relaxation of human upper extremity muscles: implications for tensioning at tendon transfer surgery
Einarsson F, Runesson E, Fridén J
Manuscript

III. Inferior mechanical properties of spastic muscle bundles due to hypertrophic but compromised extracellular matrix material
Lieber R, Runesson E, Einarsson F, Fridén J
Muscle and Nerve, 2003, 28; 11:464-471

IV. Subscapularis muscle mechanics in children with obstetric brachial plexus palsy
Einarsson F, Hultgren T, Ljung B-O, Runesson E, Fridén J
Journal of Hand Surgery (European Volume) 2008; 33:507-512

V. Structural characteristic of the subscapularis muscle in children with medial rotation contracture of the shoulder after obstetric brachial plexus injury
Hultgren T, Einarsson F, Runesson E, Hemlin C, Fridén J, Ljung B-O
Manuscript

VI. The supraspinatus muscle in retracted rotator cuff tears responds normally to passive mechanical testing: a pilot study
Einarsson F, Runesson E, Karlsson J, Fridén J
Manuscript

SUMMARY OF PAPERS

Paper I

Question: Does the freeze storage of muscle biopsies affect the results of mechanical testing?
Method: Human biopsies (n=5) from the upper extremity extremities were harvested and divided into two specimens; one for immediate (fresh) analysis and the other kept in a -20°C freezer for four weeks and then analysed. Both specimens were prepared for mechanical testing and for histological analysis
Evaluation method: Single fibres were tested for passive mechanical properties using the laser diffraction technique and analysed in a light microscope for defined morphological parameters.
Results: There were no significant differences at mechanical testing or at histological evaluation between samples that were analysed fresh or following storage.

Conclusion: Freeze storage of muscle specimens retains mechanical properties.

Paper II

Question: At what time following passive stretching of human muscle single fibres does the fall in tension level out to a more stable plateau?
Method: Human biopsies (n=12) from the upper extremities were harvested and prepared for single fibre passive mechanical testing.
Evaluation method: Single fibres were exposed to stretching and subsequent stress relaxation, during which tension decay was recorded at set time intervals. Sarcomere length was registered after each stretch. Multivariate analysis of regression curves was performed.
Results: The time to complete stress relaxation is directly related to sarcomere length after the stretch. A more stable tension plateau could be detected after two minutes following passive stretching of single fibres in clinically relevant sarcomere lengths.

Conclusion: Assessments of tension in the muscle-tendon unit should preferably be made after a minimum of two minutes' delay at tendon transfer surgery.

Paper III

Question: What is the impact on the passive mechanical performance of spasticity on single fibres and muscle bundles?
Method: Human biopsies from the upper extremities were harvested from patients with spasticity (n=9) and from healthy controls (n=21). Specimens were prepared for single fibre and muscle bundle passive mechanical testing and histological evaluation.
Evaluation method: Single fibres and muscle bundles were tested for passive mechanical properties using the laser diffraction technique. Indirect calculations with regard to the contribution of extracellular matrix were made. General tissue morphology was assessed and morphometry including fibre per bundle count, measurement of fibre and bundle size and staining for fast and slow myosin heavy chain was performed.
Results: Single fibres from patients with spasticity are shorter in slack sarcomere length, but stiffer than single fibres from healthy controls, whereas bundles from healthy controls are stiffer. The fraction of extracellular matrix in bundles from patients with spastic muscle contractures is much more abundant, however significantly more compliant than the extracellular matrix from healthy controls.

Conclusion: Profound mechanical changes (decreased stiffness) can be detected in the extracellular matrix of upper extremity muscles from patients with spasticity, affecting the whole muscle mechanical performance.

Paper IV

Question: Does the long-standing loss of amplitude in a muscle affect muscle mechanics?
Method: Human biopsies (n=9) from the subscapularis muscle from children with residual internal contracture of the shoulder following obstetric brachial plexus palsy (OBPP) were harvested and prepared for single fibre and muscle bundle mechanical testing. These specimens were compared with healthy controls (n=7).
Evaluation method: Single fibres and muscle bundles were tested for passive mechanical properties using the laser diffraction technique. Calculations of the contribution to mechanical stiffness from the extracellular matrix were made.
Results: Single fibres from patients with OBPP had a wider range of linear deformation when passively stretched. The relative increase between single fibres and bundles was significantly higher for OBPP patients compared with healthy controls.

Conclusion: A mild compensatory increase in stiffness is seen in the extracellular matrix of the subscapularis muscle from patients with persistent internal rotation of the shoulder after obstetric brachial plexus injury.

Paper V

Question: Does the longstanding loss of amplitude in muscle affect muscle morphology?
Method: Human biopsies (n=13) from the subscapularis muscle from children with residual internal rotation contracture of the shoulder following obstetric brachial plexus palsy were harvested.
Evaluation method: Muscle bundles were prepared for histological evaluation in light microscope using standard staining techniques and immunoassay for myosin heavy chain types.
Results: The majority of the patients showed essentially normal histology, whereas one patient with an extremely traumatic delivery and severe shoulder deformity showed a marked increase in the extracellular fraction, indicating fibrosis.

Conclusion: The morphology of the subscapularis muscle from patients with OBPP is essentially normal, indicating that the progressive shortening of the muscle due to weak, denervated antagonists is the main cause of rotation contracture, although the effects of the direct soft tissue trauma may be added in severe injuries.

Paper VI

Question*:* Does the longstanding loss of amplitude, as in the supraspinatus muscle in retracted rotator cuff tears, affect muscle mechanics and morphology?
Method*:* Biopsies (n=7) from the supraspinatus muscle from seven patients with a retracted rotator cuff tear were harvested. Samples (n=7) from the deltoid from the patients' same side were used as controls.
Evaluation method: Single fibres and muscle bundles were tested for passive mechanical properties using the laser diffraction technique. Muscle bundles were prepared for light-microscopic histological evaluation, regarding collagen and fat content, using standard staining techniques.
Results: Single fibres and muscle bundles responded normally to passive mechanical testing. Morphology was judged as normal.

Conclusion*:* The supraspinatus muscle in retracted rotator cuff tears is mechanically normal. This observation challenges the belief that muscle undergoes degeneration and "fatty infiltration".

CONTENTS

ABBREVIATIONS

ATP	**A**denosine **T**ri **P**hosphate
CT	**C**omputed **T**omography
CP	**C**erebral **P**alsy
ECM	**E**xtra **C**ellular **M**atrix
H&E	**H**aematoxylin and **E**osin
IF	**I**mmuno **F**luorescence
Ig	**I**mmuno**g**lobulin
LASER	**L**ight **A**mplification by **S**timulated **E**mission of **R**adiation
LM	**L**ight **M**icroscopy
MHC	**M**yosin **H**eavy **C**hain
MRI	**M**agnetic **R**esonance **I**maging
MTU	**M**usculo **T**endinous **U**nit (a muscle and its tendon)
OBPP	**O**bstetric **B**rachial **P**lexus **P**alsy
PCSA	**P**hysiologic **C**ross **S**ectional **A**rea
ROM	**R**ange **O**f **M**otion
RCT	**R**otator **C**uff **T**ear
SD	**S**tandard **D**eviation
US	**U**ltra **S**onography

DEFINITIONS

Compliance	the inverted value of stiffness of a body (how *easily* a body is deformed)
Elasticity	the resistance to deformation of a physical body (a muscle fibre for example)
Extracellular matrix	tissue in between and surrounding muscle fibres and other tissue
Fibre **Fiber**	Br English Am English
Force (F)	$F = m \bullet a$, expressed in Newton (N) where m is mass (kg) and a is acceleration $\Delta v / \Delta t$ $v = l / t$
Grating equation	$d \theta_m = m \lambda$, where d is the spacing of the grating θ is the angle of the light beam m is an integer (the +/- order of the light maxima) and λ is the wavelength of the emitted light
Hook's law;	$F(r) = - k \bullet x$, where F(r) is the restoring force of elasticity = negative value of the elasticity in a physical body, k is the spring coefficient or stiffness, and x is the distance of deformation of the body

Hook's law gives;	$F(r) = - k \bullet x$, where $F(r)$ is the restoring force of elasticity = negative value of the elasticity in a physical body, k is the spring coefficient or stiffness and x is the distance of deformation of the body
PCSA	physiologic cross sectional area = $\frac{\text{muscle mass (g)} \bullet \cos \theta}{\rho\ (\text{g/cm}^3) \bullet \text{fibre length (cm)}}$
Pressure (P)	$P = F / A$ where A = area expressed in Pascal (Pa) or N / m^2
Stiffness (K)	material-specific elasticity (at a given point of length of the object), usually expressed as N/m
Strain	deformation per length unit of the material
Stress	force per area unit of a material
Upper extremity	the arm, including the hand and shoulder
Young's modulus (E)	modulus of elasticity, a measure of the stiffness of a materials (within given limits of length of the object, the linear portion of the stress-strain curve), Δ stress (Pa)/ Δ strain, usually expressed in Pa

PREFACE

Can we really *know* anything?

The accumulation of what we call knowledge can be made in different ways. This thesis accepts the scientific tradition which postulates that, if we can repeat an experiment several times with similar results each time, the underlying phenomenon, responsible for the result, is true. According to this tradition, it is not possible, in the true scientific meaning of the word, to *prove* anything in science, except from philosophy and mathematics. Within other disciplines, such as biology, we can only provide *evidence* for facts and calculate with different degrees of possibility, always encountering uncertainty.

This thesis provides evidence of the adaptation of human upper extremity skeletal muscle as a response to changes in tension in the musculo-tendinous unit. Changes in tension have been exemplified by three conditions; spasticity, obstetric brachial plexus palsy and full-thickness tear of the rotator cuff.

What we “know” today will be revised tomorrow.

INTRODUCTION

MUSCLE MECHANICS

Human skeletal muscle

Muscle function is the foundation of movement, whether it is breathing, chewing, running or pressing the buttons of the remote control. Human muscles are characterised as smooth or striated. Smooth muscles are engaged in bowel motion and bladder control, among other functions, and are only under indirect voluntary control. There are two types of striated muscle; heart muscle designed for rhythmic contraction and skeletal muscle enabling voluntary controlled motion.

Basic features

To suit different muscles to different tasks, variations in a few basic features of the muscle are seen; the length and cross-sectional area, pennation (direction of muscle fibres in relation to the axis of force generation), number of muscle bellies (uni-, bi- or tricipity) and fast or slow twitch of the individual fibres. The variation in and different combinations of these features produce muscular performance ranging from static low torque to dynamic high torque and from fine-tuned to gross motor activities.

Macroarchitecture

Common to all skeletal muscles is a bony origin, a muscle belly (the actual mus), a musculo-tendinous junction and a tendinous insertion to bone. Muscle tissue consists of bundles of fibres which in turn consist of numbers of myofibrils (Fig 1).

The smallest functional muscle unit; the sarcomere

The myofibrils are subunits in the muscle cell (fibre) and are composed by serially coupled units; sarcomeres. The sarcomeres are the contractile units of the muscle and are composed by regularly arranged proteins. The repeated arrangement of proteins are visualised as a light and dark striation pattern of the muscle fibres (Fig 2).

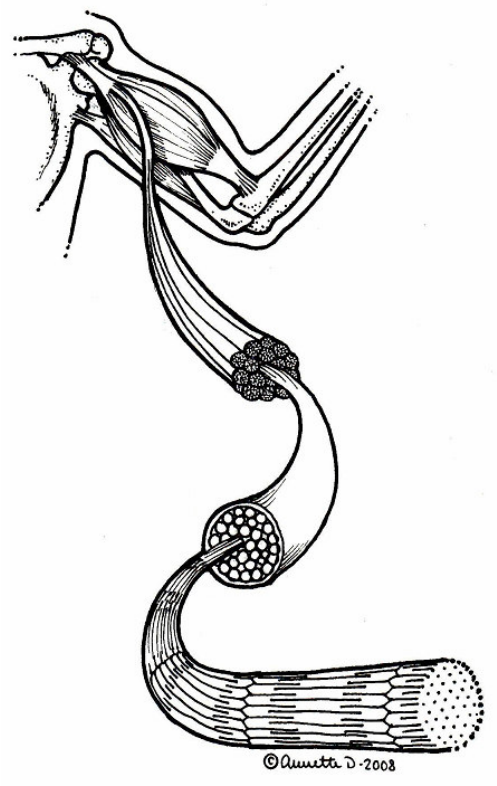

Figure 1. Muscle: tendinous origin, muscle belly, muscle fascicle, fibre and fibrils.

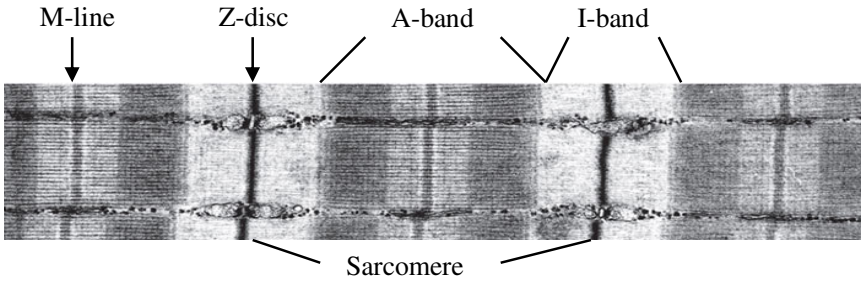

Figure 2. Ultrastructure of the sarcomere: the regularly arranged thin and thick filaments make up for defined parts of the sarcomere (Z-disc, I-band, A-band and M-line)

The extracellular matrix

The muscle cell is surrounded by extracellular matrix (ECM), which is the extracellular part of tissue and includes the interstitial matrix and the basement membrane. Interstitial matrix is composed of an interlocking mesh of fibrous proteins and glycosaminoglycans (GAGs) produced by resident cells (fibroblasts) and secreted into the ECM. Basement membranes are sheet-like depositions of ECM on which various epithelial cells rest (Järvinen et al. 2002).

Cell to ECM adhesion is regulated by specific cell surface cellular adhesion molecules, known as integrins that bind cells to ECM structures, such as fibronectin, collagen and laminin, and also to integrin proteins on the surface of other cells. Attachment to the extracellular domain initiates intracellular signalling pathways, as well as association with the cellular cytoskeleton via a set of adapter molecules such as actin (Gao et al. 2006). The ECM plays an important role in cell-to-cell communication (Kjaer 2004).

Mobile proteins

The muscle cell is mainly composed of three types of regularly arranged proteins; actin, myosin and titin (Figs 3 and 4). At neural depolarisation the subsequent release of calcium ions induces the metabolism of adenosine triphosphate (ATP), which structurally deforms cross-bridges between myosin and actin, leading to a shortening of the muscle fibre; actin filaments sliding in between the thick myosin filaments (sliding filament theory).

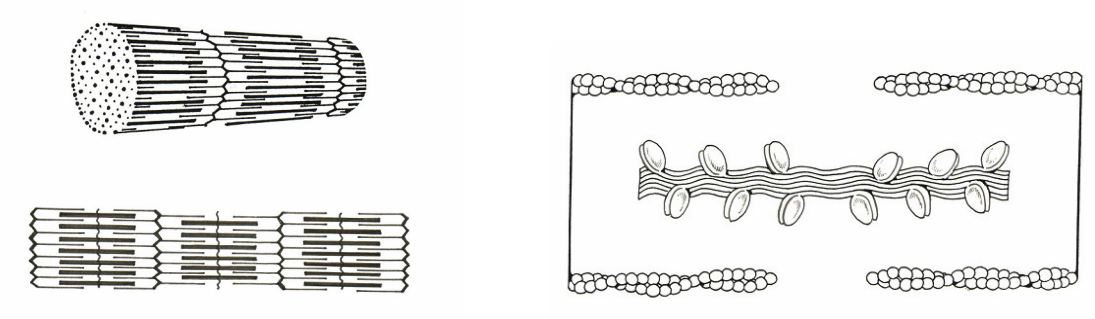

Figure 3 A and B. To the left **(A)**: schematic drawing of repeated units (sarcomeres), overlap of thick and thin filaments (myosin and actin) and to the right **(B):** schematic drawing of the myosin and actin ultra-structure. (© A Dahlström)

Fibre types

Several types of muscle fibres can be identified. The classification schedule differs, depending on the method of fibre identification that is used. The most common method for muscle fibre typing is based on the expression of different proteins on the myosin molecule (on its heavy chain). Mouse antibodies against at least seven different proteins related to the myosin heavy chain are available. Usually human muscle fibres are classified as type I ("slow twitch fibres") and type II ("fast twitch fibres"). The type II fibres are sub-divided into type II-A and II-B. Furthermore there is an intermediate type named type II-X (neonatal or developmental). Very simplified type I fibres have slow oxidative (aerobic or oxygen-

dependent) capacity and high "endurance", whereas type II fibres have fast glycolytic (anaerobic) or oxidative capacity and lower endurance (Lieber 2002; Harridge 2007).

The *sliding filament theory* was proposed in the early 1950s and explains the variation in force generation by muscle length. The validity of this hypothesis was tested by Julian, Gordon and Huxley, among others, who performed pivotal experiments with single muscle fibres from the frog that they stimulated to contraction at various fibre lengths (Gordon 1966; Gordon et al. 1966) (Fig 5).

Titin; a gigantic molecular bungee cord

Titin was not, however, discovered until the 1970s. The protein was first seen as slender filaments in electron microscopy when sarcomeres were stretched beyond the actin-myosin overlap (gap filaments).

Titin is the largest protein encoded in the human genome; a single molecule spans over half the sarcomere (Fig 4), and it is the third most abundant muscle protein after myosin and actin. A typical titin molecule has a length of approximately 1 μm and a molecular weight of up to 4 MDa (Granzier and Labeit 2007).

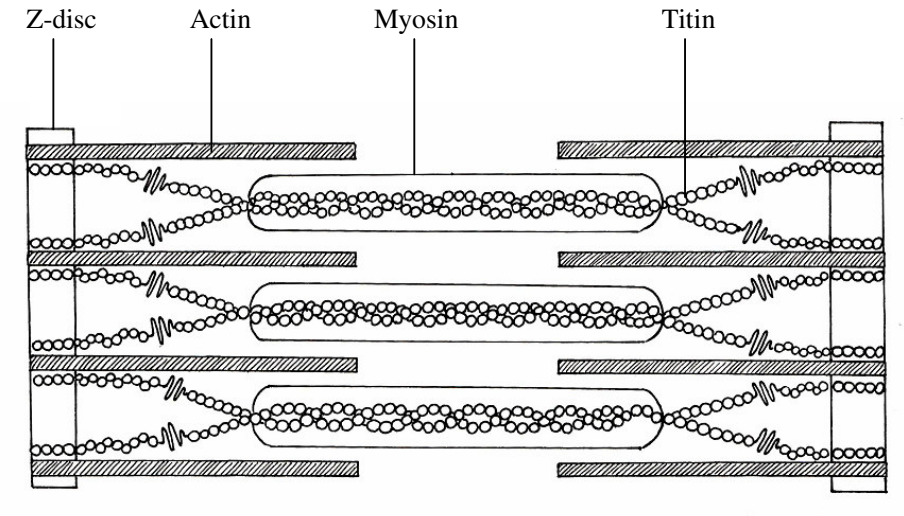

Figure 4. Schematic drawing of the sarcomere; titin in relation to the Z-disc, thick and thin filaments. Titin spans half the length of the sarcomere. (© A Dahlström)

Titin consists of repeated modules of mainly two types; immunoglobulin-like and fibronectin type III. The two end parts of the molecule bind to the Z-disc and the thick filaments (myosin) respectively. The "functional" units are located primarily in two different regions

within the molecule; the so-called PEVK domain (rich in the amino acids proline (P), glutamate (E), valine (V) and lysine (K)) and a few individual Ig domains in the I-band region (Fig 4). Both these regions are flexible and unfold upon stretching, dampening the stretching force acting on the muscle cell, thereby protecting muscle fibres from damage due to overstretching (Linke et al. 1998; Li et al. 2002; Linke et al. 2002). Furthermore, it appears that titin acts as a restoring spring in cells that have been shortened below muscle slack length (Preetha et al. 2005).

The expression of different isoforms of titin produces variations in muscle cell stiffness. Heart muscle, which is relatively stiffer than skeletal muscle, expresses different isoforms compared with skeletal muscle (Granzier and Irving 1995).

Active tension

The major determinant of the potential for force production is the physiological cross-sectional area, which is closely related to muscle volume (Maughan and Nimmo 1984). In a number of experiments in the 1890s, the Swedish physiologist Magnus Blix demonstrated that the active contraction force of a muscle varies with the length of the muscle cell (Blix 1894).

These experiments provided heart physiologists with part of the explanation of the Frank Starling mechanism and were later explored by Gordon et al, as previously mentioned (Gordon 1966), who demonstrated the relationship between sarcomere length and active tension (contraction force) (Fig 5).

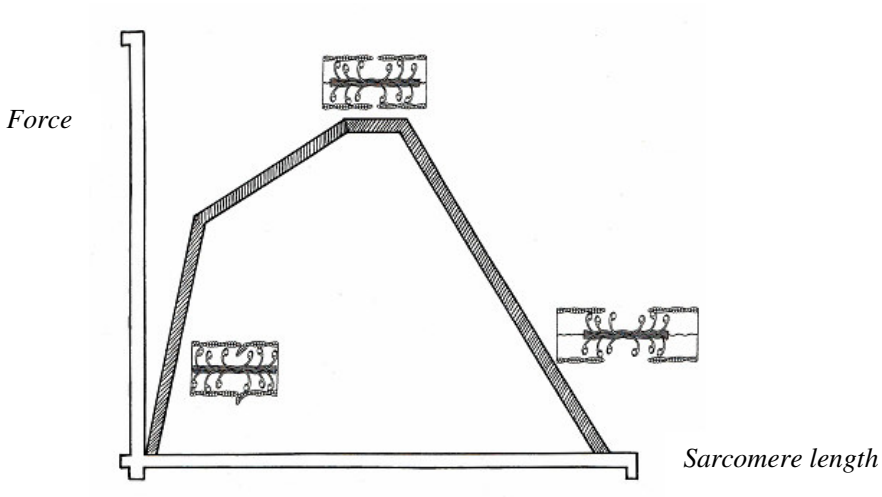

Figure 5. Force-length relationships. Optimum force production occurs when myosin-actin overlap is maximal. (© A Dahlström)

Passive tension

The ability of a muscle to lengthen is important, as it allows for the maximum length of both activated and non-activated muscles and maximum length is essential for full range of motion in a joint and for optimal force production (Gajdosik 2001). The lengthening of a muscle is restricted by the total tension in the muscle; i.e. the sum of active (contractile) and passive tension.

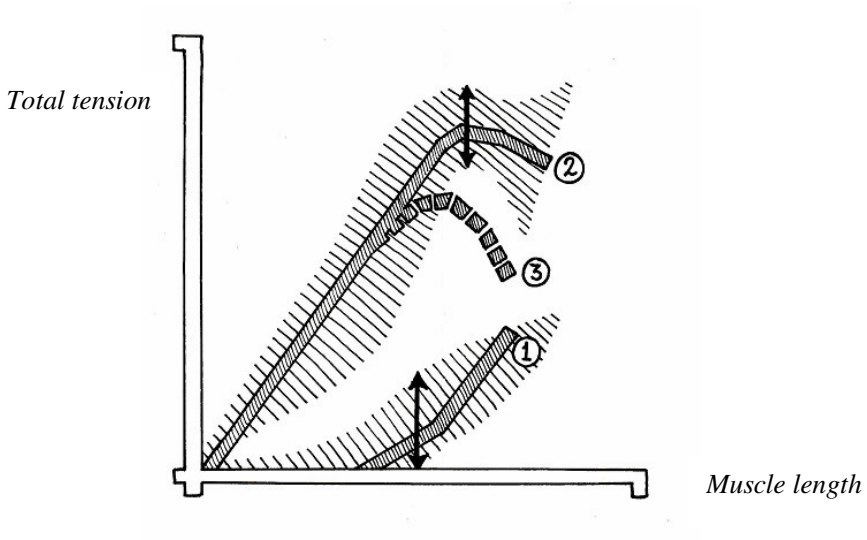

Figure 6. The total tension in a muscle (2) is the sum of the active force production (3) and the passive tension (1). (© A Dahlström)

In experiments with frog semitendinosus muscle; Magid and Law showed that the major determinant of passive tension in muscle is related to muscle fibres, not predominantly the connective tissue (Magid and Law 1985). In a review, Gajdosik refers to experiments on rats, humans and cats when concluding that resistance during passive muscle lengthening is mainly influenced by the amount of muscle tissue, reflecting the amount of titin, and to some extent also extracellular connective tissues, with the perimysium as the major extracellular contributor to resistance (Gajdosik 2001). Variability in slack sarcomere length is predominantly a reflection of the expression of different titin isoforms (Labeit and Kolmerer 1995).

What do the results of experiments exposing muscle tissue to passive stretching tell us about active muscle function?

A prerequisite for force production is filament overlap. Filament overlap is directly proportional to sarcomere length, which in turn relates to whole muscle length and is adjusted by passive restraints. The latter is strongly supported by data testing heart muscle showing that the sarcomere length is predominantly regulated by titin (Fukuda et al. 2001). Passive restraints are thus acting as a restoring forces both at (over-) stretch and at joint angles where the whole muscle is theoretically allowed to shorten below "resting length" (to "slack length").

The results of passive mechanical testing cannot be directly translated into measures of active function. However, the function of passive restraints is one of several prerequisites for active function and the results of passive mechanical testing indicate in a general way (changes in) the expression of muscular proteins, directly affecting active function (Huijing and Jaspers 2005).

TENDON TRANSFER IN ORTHOPAEDIC SURGERY

Tendon transfers are used to improve or restore limb function, both in primarily neurological conditions and in muscle-tendon ruptures with secondary functional deficit (Ejeskär and Dahlöf 1988; Gerber 1992; Warner 2001)

In hand surgery, tendon transfers are commonly used to improve the hand function in patients with peripheral or central nerve injuries (spinal cord lesions, cerebral palsy and stroke). A healthy muscle is usually transferred to compensate for a deficient muscle on the opposite side.

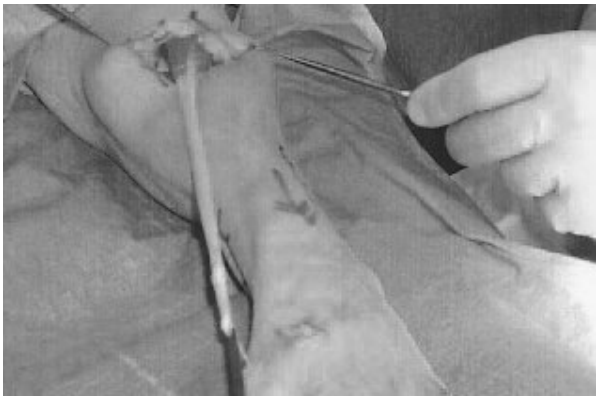

Figure 7. Tendon transfer in the forearm.

In shoulder surgery, several transfers have been described specifically for massive irreparable rotator cuff tears (Gerber 1992; Warner 2001; Degreef et al. 2005; Gerber et al. 2006; Gerber et al. 2007). The most commonly used transfer is the latissimus dorsi transfer indicated for predominantly posterior-superior rotator cuff defects, i.e. rupture of both the supraspinatus and infraspinatus. It has also been suggested for use as an addition to prosthesis surgery, e.g. the restoration of external rotation, which is not restored by a reversed shoulder prosthesis alone, in rotator cuff-deficient patients (Gerber et al. 2007). Pectoralis major transfer is used for anterior-superior defects, i.e. combined subscapularis and supraspinatus tears (Galatz et al. 2003).

Even if no actual transfer is made during the direct repair of the rotator cuff, the underlying soft tissue problems are similar to those of tendon transfers and the direct repair of large/massive rotator cuff tears can be viewed as a special case of tendon transfer.

Several factors, including donor muscle cross-sectional area and fibre length (Fridén et al. 2001; Fridén et al. 2004), perioperative muscular release, tensioning of muscle-tendon to be

transferred and how aggressive the postoperative rehabilitation programme is, all affect the clinical outcome (Fridén and Lieber 2002; Fridén and Lieber 2002).

There is confusion about definitions with regard to various muscle length terms. Both functional and operational definitions exist in parallel. Optimal length refers to the length at which myofilament overlap is optimal for maximum force generation. Resting length is variable and cannot be clearly defined more precisely than the length when the muscle is without active tension. Muscle lengths in relation to a given joint angle are usually known as *in situ* or *in vivo* length (Fridén and Lieber 1998).

Optimally, the chosen donor muscle has mechanical parameters corresponding to the deficient muscle, resulting in full functional recovery. The functional loss following transfer of the healthy muscle should preferably be minimal and without any harvest site morbidity. Failures caused by overstretching the transferred muscle-tendon unit and poor tendon-to-bone healing may result in reduced joint range of motion, weakness and imbalance in the affected joint.

The impact of peroperative tensioning of the transferred tendon has been assessed in several studies (Fridén et al. 2001; Lieber and Fridén 2001; Fridén and Lieber 2002; Fridén and Lieber 2002; Lieber and Fridén 2004). Studies provide support for a reduction in the serial number of sarcomeres when positioning (and immobilising) a tendon and its muscle at high tension (Fridén et al. 2000). The risk of producing a tenodesis rather than a dynamically functioning muscle-tendon unit at tendon transfer has been highlighted in studies of tendon transfer in the forearm (Fridén and Lieber 2002).

There is a lack of scientific-based guidelines as how to determine the degree of muscle-tendon unit release and tensioning during surgery to optimize the functional result (Green et al. 2005). Intra-operative sarcomere length measurements have been suggested to verify optimal tensioning (Lieber and Fridén 2004), however, this technique has never reached common clinical use. Even though assessment of passive tension in the muscle-tendon unit is poor as predictor of optimal muscle length (Lieber and Fridén 2004), it is the most frequently used technique in tendon transfer.

CONDITIONS STUDIED

SPASTICITY

The upper motor neuron syndrome is a collective term that refers to different types of motor behaviour in patients who have lesions of the descending corticospinal system. Lesions involving the upper motor neuron, its pathways and connections can occur at the level of the cortex, internal capsule, brain stem, or spinal cord (Mayer and Esquenazi 2003). Examples of disorders in this category are spasticity, stroke, traumatic spinal cord injury and cerebral palsy. Spasticity is a motor disorder characterised by a velocity-dependent increase in tonic stretch reflexes ("muscle tone") with exaggerated tendon jerks, resulting from the hyperexcitability of the stretch reflex (Lance 1980).

The unintentional contractions are not optimal and result in a functional weaknesses (Rose and McGill 1998). In the upper motor neuron syndrome, the flexors have more influence than the extensors over the wrist joint which sets the hand in a typical flexed position (Pontén et al. 2005). In the long term this imbalance may lead to bony deformation of the joint.

Although basic scientific descriptions of spasticity are being formulated, clinical confusion still exists (Ivanhoe and Reistetter 2004). The primary emphasis of research on spasticity has been to characterise the static and dynamic properties of the nervous system. Rose et al. (Rose et al. 1994) showed that prolonged dynamic electromyographic activity in lower limb muscles was related to a drift towards muscle fibre type I in children with cerebral palsy and concluded that the increased fibre size variability may be associated with a progression of changes secondary to prolonged stimulation. The structural and functional properties of skeletal muscle in spasticity have received far less attention. Reports suggesting muscle abnormalities can be found in the literature, but they are based on indirect calculations of joint dynamics or static measurement of biopsy properties.

OBSTETRIC BRACHIAL PLEXUS PALSY

Obstetric brachial plexus palsy (OBPP) comprises symptoms related to the stretching, rupture or avulsion of roots from the brachial plexus (C5-Th1) acquired at birth. This injury is a peripheral nerve injury, and not an upper motor neuron syndrome. The frequency is reported to range from 0.17 to 1.56 per 1,000 live births (Bager 1997; Mollberg et al. 2005). Internal (or medial) rotation contracture of the shoulder is the most common and the most severe secondary deformity in obstetric brachial plexus palsy. It occurs frequently in C5-6 and C5-6-7 lesions (Narakas groups 1-2) (Narakas 1987; Birch 2000) primarily denervating the external rotators of the shoulder; the infraspinatus muscle (and the teres minor muscle).

In a large number of children the contracture is complicated by deformity of the shoulder joint itself including deformation of the glenoid, the humeral head, the coracoid process and the acromion (Sever 1925; Pearl and Edgerton 1998; Waters et al. 1998; Kambhampati et al. 2006). In some patients this leads to a dorsal subluxation or dislocation of the humeral head (Birch and Chen 1996). The medial rotation contracture not only limits the mobility of the shoulder, but also affects the whole upper limb. It sets the arm in an unfavourable position and limits the use of the hand. The cause of the deformity is not well understood.

There are two main explanatory models for residual impairment, of the internal rotational contracture of the shoulder following obstetric brachial plexus injury. Gilbert among others has suggested that the contracture is the result of muscular imbalance, caused by the nerve injury. In a partial plexus lesion, the external rotators innervated by C5 and C6 become paralysed, whereas the internal rotators retain their innervations through C7 and C8. This creates a situation in which the subscapularis muscle, acting as an internal rotator, has no antagonists (Gilbert 1993); (Kon et al. 2004).

The other main theory favours contracture as an effect of direct tissue injury caused by the birth trauma resulting in fibrosis and the loss of elasticity in the internal rotators (Zancolli and Zancolli 2000). Birch and Chen reported that biopsy specimens from the subscapularis muscle in five children with rotation contracture and obstetric plexus palsy showed changes “consistent with post-ischemic fibrosis” (Birch and Chen 1996). In a series of 183 children operated on due to posterior subluxation/dislocation Khambhapati et al. reported that fibrosis-like changes were observed peroperatively in 42 cases (Kambhampati et al. 2006). This report was not supported by histological evaluation of biopsy specimens, merely by ocular and

manual assessment at surgery. Furthermore, 101 of these children had a subluxation and 82 a chronic dorsal dislocation of the humeral head, indicating severe impact of the shoulder joint in all these children.

There is still a lack of complete knowledge relating to the cause of internal rotation contracture of the shoulder in children following obstetric brachial plexus injury.

ROTATOR CUFF TEAR

The rotator cuff is the common name of the group of muscles and their tendons (the subscapularis, supraspinatus, infraspinatus and teres minor respectively) enclosing the capsule of the glenohumeral joint. They are mainly dynamic stabilisers of the humeral head to the glenoid in the glenohumeral joint.

The subscapularis is the largest muscle in terms of volume and acts as an internal rotator. The infraspinatus and the teres minor act as external rotators and are thus antagonists to the subscapularis muscle. The supraspinatus is mainly an abductor, but it is also a depressor of the humeral head.

A rotator cuff tear is defined as a structural injury to the rotator cuff comprising extremely heterogenic injuries ranging from partial tears to massive full-thickness tears. It is almost exclusively an injury to the tendon, without any association with nerve injury or direct injury to the muscle component. A tear is complete when the full depth of the tendon is injured; otherwise, the tear is partial. Total tears are further defined in size by medial *retraction* and width.

The supraspinatus is involved in almost all tears. It is injured in up to nine out of ten cases, most commonly with the infraspinatus, and isolated in half the cases (Cofield et al. 2001; Goutallier et al. 2003). The clinical entity of supraspinatus tears was described and discussed by Codman already back in 1911 (Codman 1911). Isolated subscapularis tendon ruptures are rare (3-10%) (Cofield et al. 2001; Goutallier et al. 2003).

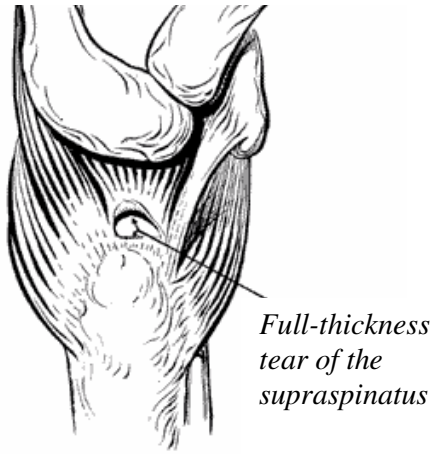

Figure 8. Lateral view of a right shoulder: rotator cuff, full-thickness tear of the supraspinatus tendon.

The prevalence of rotator cuff tears is closely related to age, indicating a strong relationship with degenerative changes. Sher et al. reported a prevalence of 4% under the age of 60, and 28% at ages above 60 years (Sher et al. 1995). Milgrom et al. reported a prevalence of 50% at ages above 70 years (Milgrom et al. 1995). In a study by Yamaguchi et al. (Yamaguchi et al. 2006), 588 patients with unilateral shoulder pain were evaluated by ultrasonography with regard to the presence and size of rotator cuff tears in both shoulders. Two hundred and twelve patients had an intact rotator cuff in both shoulders, 199 had a unilateral rotator cuff tear (either partial or full-thickness) and 177 had a bilateral tear (either partial or full thickness). The average age was 49 years for patients with no rotator cuff tear, 59 years for those with a unilateral tear and 68 years for those with a bilateral tear. Calculations indicated a 50% likelihood of a bilateral tear after the age of 66 years. The average size of a symptomatic tear was 30% greater than that of an asymptomatic tear. Patients who presented with a full-thickness symptomatic tear had a 36% prevalence of a full-thickness tear on the contralateral side (Yamaguchi et al. 2006).

Symptoms associated with a rotator cuff tear are pain, typically with the arm at and above shoulder level, ache at night disturbing sleep, reduced range of motion and reduced strength. Depending on the injury pattern, the shoulder is weak in abduction/external rotation or abduction/internal rotation (posterior-superior and anterior-superior lesions respectively).

Inflammatory parameters are shown to be increased in patients with rotator cuff tears (Blaine et al. 2005). The symptoms probably relate to the underlying inflammatory reaction, but there are obviously rotator cuff tears, which are asymptomatic, as shown by Yamaguchi et al. (Yamaguchi et al. 2006).

It is possible that both "symmetrical" tears, leaving the balance between the anterior (i.e. subscapularis) and posterior (i.e. infraspinatus and teres minor) force couples intact, or a tear that leaves the rotator cable intact are less likely to cause biomechanical derangement and subsequent adherent symptoms (Burkhart et al. 1993).

The majority of ruptures appear to have a non-traumatic cause, whereas a more defined trauma can usually be identified in tears in younger patients. Causal factors are divided into extrinsic and intrinsic to the rotator cuff (Löhr and Uhthoff 2007; Nho et al. 2008). In 1972, Neer presented a surgical method to relieve symptoms related to impingement syndrome. He emphasised the importance of mechanical restraints, i.e. the humeral head and coraco-acromial arch, as a cause of wear to soft subacromial tissue (Neer 1972). According to Neer; subacromial changes develop in a continuum from acute bursitis to partial and total tears of the rotator cuff (Neer 1983). In a recent report, Norlin and Adolfsson concluded that good long-term results following arthroscopic decompression without direct repair of small, full-thickness rotator cuff tears may support the theory of mechanical impingement as a cause of rotator cuff tears (Norlin and Adolfsson 2008).

As opposed to mechanical impingement, some authors emphasise muscular imbalance in the glenohumeral joint or weakness and use the term "functional" impingement (Irlenbusch and Gansen 2003). Most probably, the cause of impingement syndrome, as a pre-stage to rotator cuff tear, is multifactorial (Neviaser and Neviaser 1990; Nho et al. 2008).

There is still no complete understanding of the pathophysiology and natural history following a full-thickness tear and retraction of the rotator cuff (Zingg et al. 2007). Safran et al. measured a mean retraction of 34 mm twelve weeks after creating infraspinatus tears in dogs (Safran et al. 2005). Gerber et al. measured a total, mean retraction in infraspinatus tears created in sheep of 4.5 cm of which 3 cm occurred within one hour (Gerber et al. 2004). In the same study, it was shown that the structural basis for the so-called fatty infiltration is found in interfascicular and interfibrillar fat deposition (Gerber et al. 2004). Furthermore, in a study using a sheep model in which Meyer et al. showed that the mean muscle fibre length had shortened from 32 to 16 mm following a created infraspinatus tear, the muscle fibres were found to be essentially normal in microscope with an unchanged fibre diameter and

myofibrillar structure, while interstitial fat and fibrous tissue had increased from 3.9% to 46% of the muscle volume (Meyer et al. 2004).

In 1994, Goutallier et al. presented a five-stage grading scale for “fatty infiltration” in the torn rotator cuff on CT, based on observations from 63 patients (Goutallier et al. 1994). Preoperative CT was compared with CT 1.5 years postoperatively, with a correlation of the postoperative results to infraspinatus “degeneration”. The authors used terms like “fatty infiltration” and spoke about “degeneration” of the rotator cuff. They concluded that … “it seems preferable to operate on wide tears before irreversible muscular damage takes place”.

This paradigm comprising muscular damage or degeneration and fatty infiltration has been widely accepted among shoulder surgeons. Fuchs et al. modified the Goutallier fatty degeneration index for MRI from a five- to a three-stage scale (Fuchs et al. 1999). In a several studies, the results have been coupled to CT or MRI staging of fat content/“fatty infiltration” (Goutallier et al. 2003; Fuchs et al. 2006; Shen et al. 2008).

However, limited direct mechanical and structural data relating to muscles in retracted rotator cuff tears in humans are available. The underlying biology that relates to the poor outcome of repair of rotator cuff tears presenting with a high relative fat content on preoperative CT or MRI has still not been well explained.

AIMS OF THE STUDY

Does freeze storage of muscle biopsies affect their mechanical properties?

At what time, following passive stretching of human upper extremity muscles, can stress relaxation be observed?

What is the impact of spasticity on the passive mechanical performance of single fibres and muscle bundles?

Does the long standing loss of amplitude in human upper extremity muscle affect muscle mechanics and structure?

How does the lack of muscle tension variation affect the functional outcome of upper extremity tendon transfer surgery and rotator cuff repair?

Are full-thickness rotator cuff tears with retraction associated with impaired performance in passive mechanical testing of the supraspinatus muscle?

PATIENTS

Table 1. Overview of conditions studied, patients and controls included, methods used and statistical analysis made in the different studies. PMT = passive mechanical testing.

Study	CONDITION	PATIENTS (n/muscle)	CONTROLS (n/muscle)	METHOD	STATISTICAL ANALYSIS
I.	-	-	5 / upper extremity	PMT, morphology	t-test, Mann-Whitney U
II.	-	-	6/ upper extremity	PMT	(regression analysis)
III.	Spasticity	9/ upper extremity	21/ upper exremity	PMT, morphology	ANOVA, post hoc t-test
IV.	Brachial plexus palsy	9/ subscapularis	7/ upper extremity	PMT	Two sided t-test for unpaired observations
V.	Brachial plexus palsy	13/ subscapularis	-	Morphology	(regression analysis)
VI.	Rotator cuff tear	7/ supraspinatus	7/deltoid	PMT, morphology	Wilcoxon sign rank test

Study I

Open biopsies from upper extremity muscles were harvested from five patients undergoing surgery due to a fracture or removal of an internal fixation device. No neuromuscular or rheumatic disorder was found in these patients and the muscles were judged as healthy. Their age ranged from 24 to 68 years; four men and one woman. Biopsies were divided into two pieces of which one acted as a control (tested fresh) and one was treated (freeze storage). One biopsy was harvested from each of the following muscles; the deltoid, flexor pollicis brevis, extensor pollicis longus, extensor carpi radialis longus and brachioradialis.

Study II

Two biopsies from upper extremity muscles were harvested in each of six healthy patients of whom one was a woman and five were men with a median age of 35 (15-68) years. Biopsies were prepared for single fibre passive mechanical testing. There were no controls in this study.

Study III

Muscle biopsies were harvested from nine patients with spasticity caused by cerebral palsy; their mean age was 9.3 (3.1) years. Biopsies from 21 patients, with a mean age of 27.5 (5.4) years, undergoing surgery for non-neuromuscular disorders such as fracture repair, joint fusion and tendon repair, acted as controls. For spastic bundles, the following muscles were tested; biceps brachialis (n=3), extensor carpi radialis longus (n=1), flexor carpi ulnaris (n=2),

pronator teres (n=2) and subscapularis (n=1) muscles. For control bundles, the following muscles were tested: adductor pollicis longus (n=9), brachioradialis (n=1), extensor carpi radialis longus (n=3), flexor digitorum superficialis III (n=5) and flexor digitorum superficialis V (n= 3).

Study IV

Nine children with a median age of 6 (1–16) years undergoing open shoulder surgery for obstetric brachial plexus palsy were included in the study. Of the nine children, six were girls and three boys. All the children had a contracture of the shoulder characterised by the partial or total loss of passive and active external rotation, ranging from mild (45° of passive external rotation) to severe (<10° of passive external rotation). Seven of the nine children had a partial C5-C6 lesion; two of the nine children had a partial C5-C6-C7 lesion. None of the children had clinical signs of rupture or avulsion and none had undergone previous surgery. Seven patients with a median age of 24 (range 15–33) years who were unaffected by neuromuscular disorders contributed "control biopsies" from six uninjured upper limb muscles, representing the adductor pollicis brevis, adductor pollicis longus, brachioradialis, extensor carpi radialis longus, flexor carpi ulnaris and flexor digitorum superficialis.

Study V

Biopsy specimens were harvested from the subscapularis muscle in thirteen children who underwent corrective surgery for rotation contracture of the shoulder, caused by obstetric brachial plexus lesions. The median age at operation was 74 (11-186) months. All the patients had obstetric brachial plexus lesions involving the two or three upper spinal nerves, C5, C6 ± C7, thereby primarily affecting the shoulder and the elbow, but not so much the hand. In all patients, the nerve lesion recovered spontaneously, without any nerve repair. There were no controls in this study.

Study VI

Seven patients with a symptomatic rotator cuff tear for at least three months were included in the study. Five were women and two were men, with a median age of 65 (51-78) years at the time of surgery. Five patients underwent an open repair; two underwent an arthroscopic subacromial decompression. Muscle biopsies were harvested from the supraspinatus and the ipsilateral deltoid in all patients during rotator cuff surgery treating tears with a retraction of at least two cm, measured on preoperative MRI and verified during surgery. The median "tear age" was 12 (5-24) months and the median tear size was 15 (4-30) cm^2.

METHODS

The methods used in this study are well-established methods that can render clear answers to defined questions.

MUSCLE BIOPSY

The open biopsy technique

An open biopsy technique is usually preferred in order to obtain a specimen of sufficient size and quality for thorough evaluation (Dubowitz and Sewry 2007). In all the studies, except for the last one, biopsies were harvested with an open technique using careful dissection with scissors, forceps and a knife. The size of the open biopsies was approximately 5x5x10 mm.

Vacuum needle biopsy

In study VI, the aim was to investigate the supraspinatus muscle from rotator cuff ruptures with retraction, however, obtaining a muscle biopsy from the supraspinatus muscle with sufficient quality for mechanical testing from patients with a rotator cuff tear with an extensive retraction has previously not been described (Irlenbusch and Gansen 2003), without an additional incision.

Biopsies of excellent quality were successfully harvested using a vacuum-assisted biopsy needle. Vacora™ (Bard, New Jersey, USA) is a biopsy needle designed for, and clinically used to, obtaining breast biopsies in tumour diagnostics. The device consists of two pieces; a working handle ("driver") and the actual biopsy probe. The probe used in this study (product no. VB10140) was 1400 mm long, with an outer diameter of 3.6 mm and an inner diameter of 3.4 mm, corresponding to Gauge 10. The semi-circular opening near the tip of the probe enables specimens of up to 19 mm in length to be harvested. With a muscular weight of about 34 grams (Ward et al. 2006) and a density of less than 1 g/mm^3, a sample from the supraspinatus muscle constitutes less than 0.5% of the total volume of the supraspinatus muscle. The technical specification from the manufacturer estimates the weight of a breast biopsy at 170 mg, which corresponds to 0.5% of the mean weight of a supraspinatus muscle (Ward et al. 2006).

Figure 9. Vacora™ biopsy needle and driver. The probe is connected to a syringe via a short tube. Both the syringe and the proximal part of the probe are placed inside the handle.

The re-chargeable handle builds up under-pressure via the syringe. A circular sleeve retracts and the biopsy slot is exposed sucking soft tissue into the opening. The mechanism automatically cuts the soft tissue as the sharp sleeve rotates back in place closing the biopsy slot. The cut is slightly transverse to the direction of the fibres leaving the middle part of the sample intact. To harvest a biopsy from the supraspinatus muscle the needle is directed from laterally in the direction of the muscle fibres.

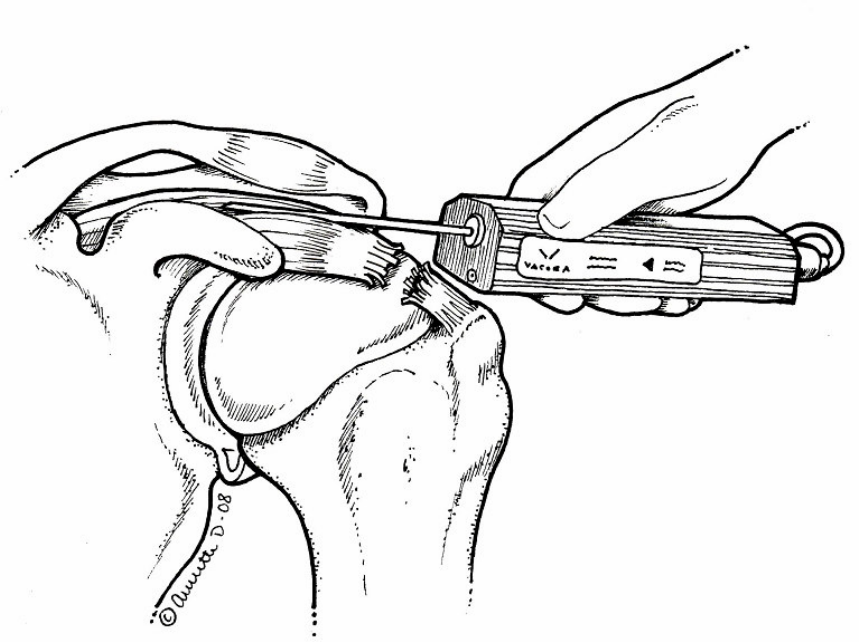

Figure 10. Harvesting of a supraspinatus muscle sample in the subacromial space. (© A Dahlström)

This can be done during an arthroscopic procedure (subacromial decompression or rotator cuff repair) or mini-open cuff repair. At arthroscopy, it is possible to visualise the fibre direction. In an open procedure, the fibre direction has to be estimated in relation to the long axis of the ruptured tendon. Macroscopic inspection of the biopsies was judged to be close to perfect. Mechanical testing of single fibres and muscle bundles, together with morphological evaluations of cryosections of biopsies, showed muscle cells with preserved integrity and a lack of structural damage due to the biopsy procedure (Einarsson et al. 2008).

LASER DIFFRACTION TECHNIQUE

The laser diffraction technique for sarcomere length measurement, as a part of passive mechanical testing, was used in all the studies except Study V.

The light emitted by a laser device is precise, monochromic (without variations in wavelength) and coherent (electromagnetic waves are in phase) in contrast to "ordinary" light that spreads light in various wavelengths, phases and directions. Skeletal muscle is striated as a result of the arrangement of sarcomeres with myosin, actin and Z-discs in repetitions. A slit, or several slits, is known to spread (diffract) a ray of light. The diffraction pattern as a result of light passing through a grating is seen as the light point maxima.

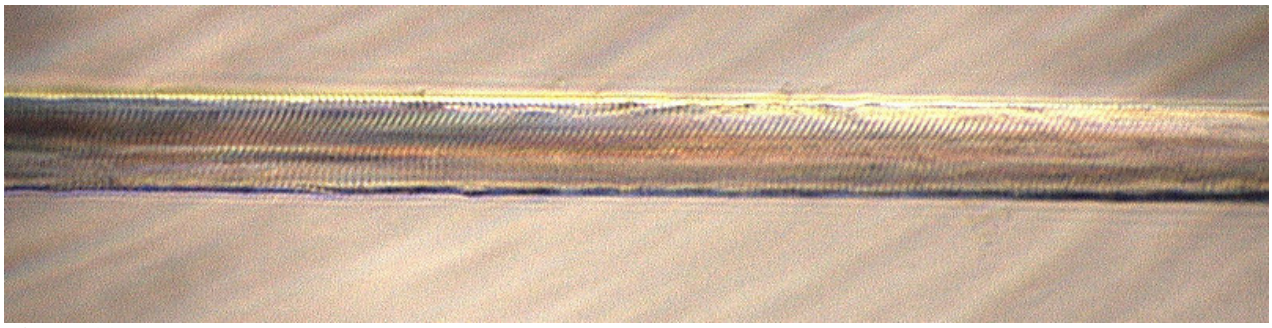

Figure 11. Single muscle fibre with clearly visible striation pattern in microscope.

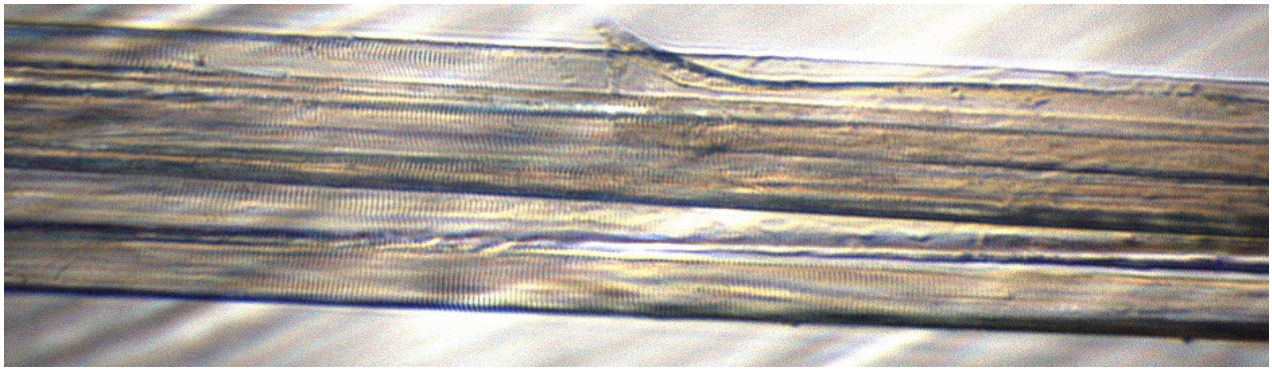

Figure 12. Stretched muscle bundle in microscope.

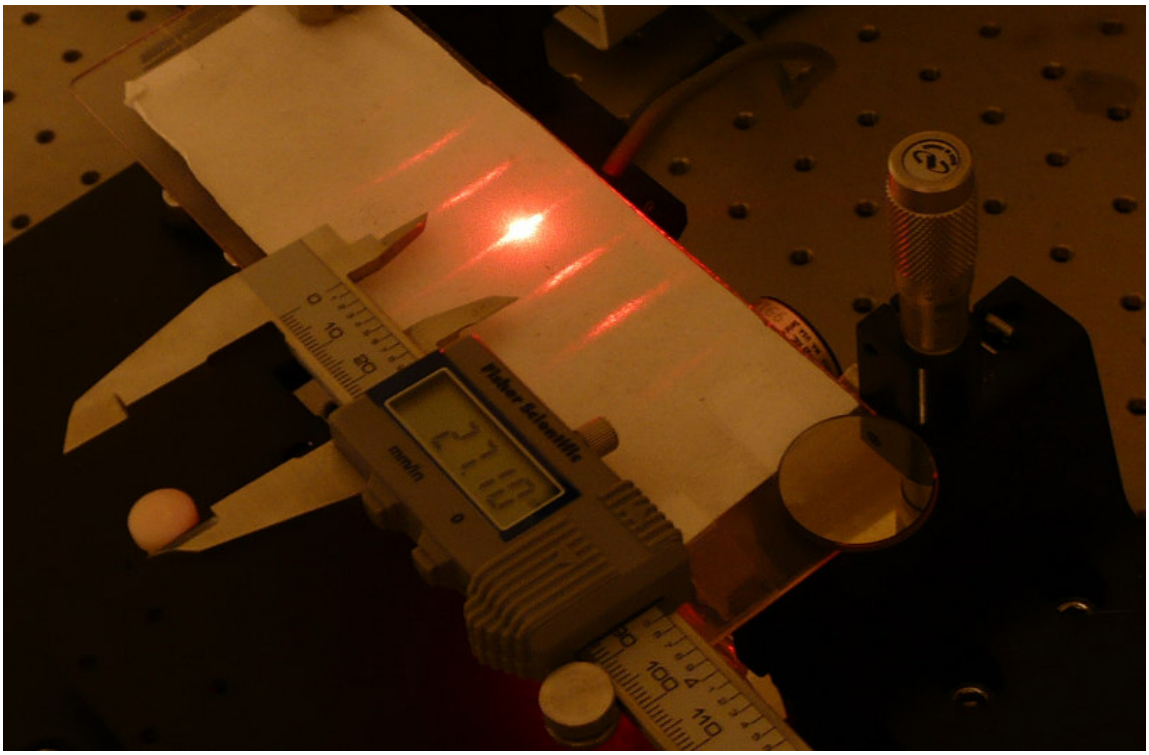

Figure 13. Diffraction pattern of a laser beam through a single fibre.
Measurement of the distance of the 1'st to 1'st diffraction order with a digital calliper.

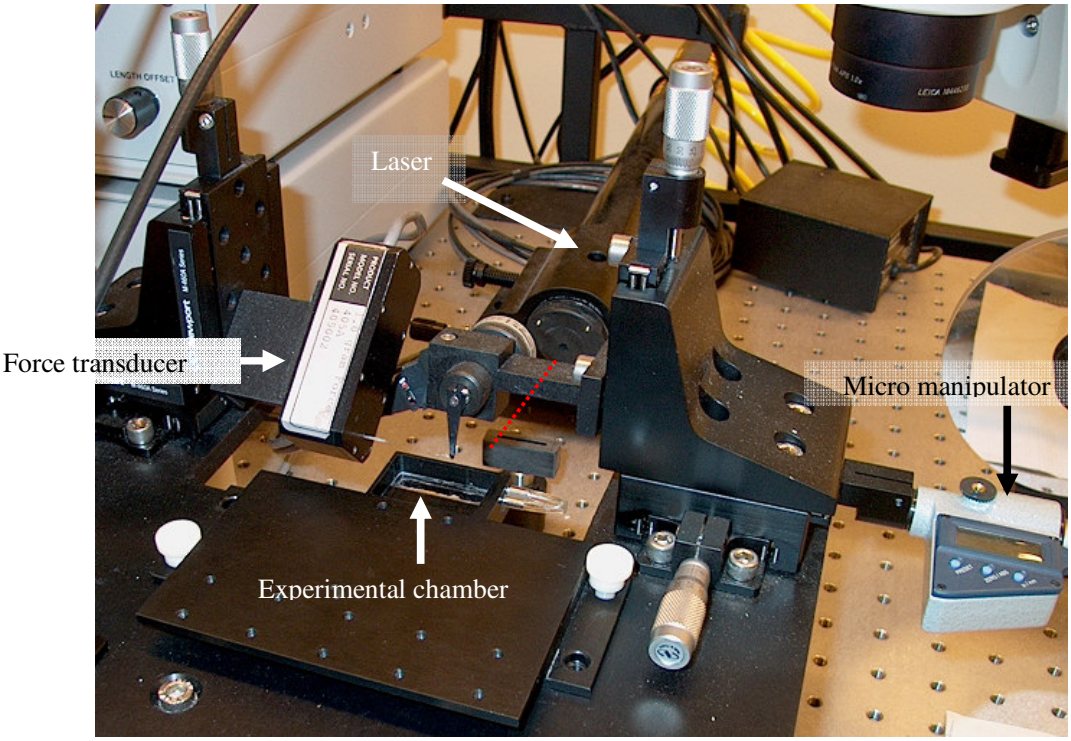

Figure 14. Experimental set up for passive mechanical testing at the Lundberg Laboratory for Orthopaedic Research.

If the wavelength of the emitted light and the distance between the different light point's maxima are known, the spacing of the grating can be calculated according to the formula:

$$d \sin\theta_m = m\lambda.$$

where

d is the spacing of the grating

θ is the angle of the light beam

m is an integer (the +/- order of the light maxima) and

λ is the wavelength of the emitted light

As a result, the sarcomere length can be calculated by measuring the distance between the light point maxima of diffraction from a laser with light with a defined and known wavelength (Baskin et al. 1979; Yeh et al. 1980; Lieber et al. 1984).

PASSIVE MECHANICAL TESTING

In Studies I-IV and VI, passive mechanical testing was performed.
In Studies I and II, single fibres were tested, whereas both fibres and bundles were tested in Studies III, IV and VI. To test passive mechanics, single fibres and bundles were dissected under an epi-illumination microscope (Leica $MZ9_5$, Heerbrugg, Switzerland) with 40 x magnification.

The fibre/bundle was then transferred to the experimental set up which consisted of a transilluminable chamber, filled with relaxing solution, and two titan-wires. One wire transmitted force to a force transducer/voltmeter (Model 405A, Aurora Scientific Inc, Ontario, Canada, sensitivity 10V/g) and the other was connected to a micromanipulator (Mitutoyo, Tokyo, Japan).

Slack sarcomere length was measured with the fibre floating free in the chamber. The fibre/bundle was then tied to the metal threads with 10-0 sutures. To prevent slippage bundles were secured by a second pair of sutures. The diameter and length of the fibre/fibre bundle was calculated from a digital photo image (Model DC 300, Leica, Heerbrugg, Switzerland) mounted on a microscope at the laser set up

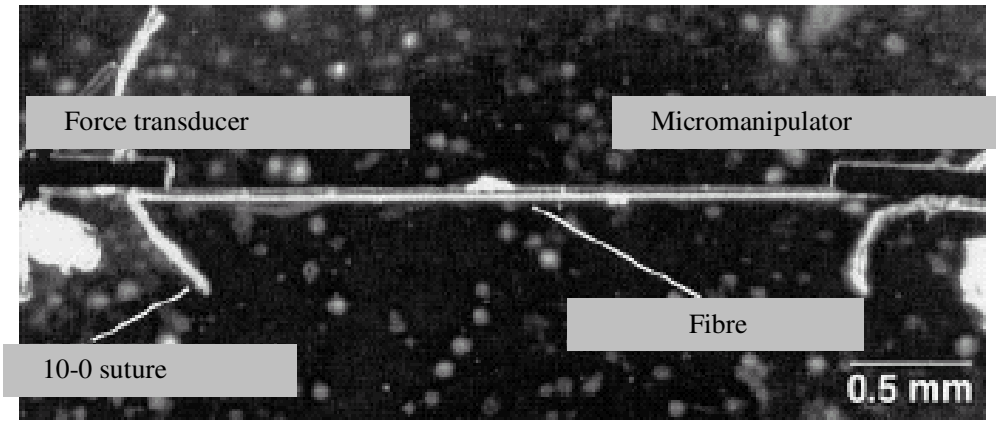

Figure 15. Single muscle fibre, in the experimental chamber, mounted to two titan-threads by 10-0 suture, in microscope.

Studies I and IV

Samples were lengthened in 250-µm increments in velocity-independent steps until the fibres were stretched a total of 4.0 mm, after which subsequent lengthening progressed in 500-µm increments. Stress relaxation was permitted for one minute. Recordings of tension in the fibre/bundle were made at stretching and at one minute. Two or three measurements of light interference point distance of 0-1st, 0-2nd, 1st-1st or 2nd-2nd order were made once following each stretch in order to calculate sarcomere lengths. Samples were stretched until mechanical failure occurred, resulting in the loss of tension. Calibration of the laser diffraction measurements using grating with known spacing was made at the end of mechanical experiments.

Study II

A protocol identical to the one described above was used, except for the time of stress relaxation, which was allowed until tension decay was insignificant (up to 12 minutes), defined as a variation between tension recordings of less than 10% on condition that the absolute value of change in tension was less than 10 mV.

Studies III and VI

A protocol identical to the one described above was used, except for the time of stress relaxation that was permitted for two minutes. Tension was registered at both one and two minutes in Study VI and at two minutes in Study III.

STRUCTURAL EVALUATION

In Studies I, III, V and VI, structural analyses were performed. In Study I, only standard staining with haematoxylin and eosin (H&E) was used, whereas H&E staining was supplemented by immunohistochemical procedures in Studies III, IV, V and VI. All morphological and immunohistocemical analyses, regardless of staining technique, were carried out using a light microscope (Nikon E600, Tokyo, Japan).

Morphology

For all the morphology analyses, the OCT-embedded muscle biopsies were cut in a cryostat (Microm HM 500, Walldorf, Germany) in 10 μm thick sections. Samples used for routine histology were prefixed for five minutes in Histofix (Histolab, Göteborg, Sweden) and subsequently stained. In Studies I and III-VI, haematoxylin and eosin were used. In Study VI, morphological analysis was supplemented by staining with Sirius red for the distribution and content of collagen and Sudan black for the distribution and content of fat. Muscle cross sections were measured for single fibre diameter according to Dubowitz (Dubowitz and Sewry CA 1985). Areas in the section were chosen with the emphasis on finding a polygonal or circular shape in the cut fibres and avoiding areas with semicircular or longitudinal cuts.

Immunohistochemical procedures

To examine the distribution of myosin heavy chain (MHC) proteins, mouse monoclonal antibodies were used against human MHC slow, MHC fast and MHC neonatal and MHC developmental (Novocastra Lab. Ltd., Newcastle, United Kingdom). Non-fixed sections were subjected to the primary antibody for one hour, washed in buffer and then subjected to the secondary antibody for one hour. The visualisation of antibodies was obtained using DAB (diaminobenzidine, Sigma Ald, St. Louise, USA).

Study I

At least 150 fibres were measured and counted on each slide. Overall morphology was based on the homogeneity of cells, the presence of inflammatory cells and the position and density of nuclei. Atypical findings were recorded. Fibre occupancy was calculated as a quota of fibre area per total measured area including extracellular matrix.

Study II

No structural analysis was performed in Study II.

Study III

Samples/bundles were labelled with primary antibody to the slow- and fast myosin heavy chain respectively.

Study IV

Analysis of single fibres after mechanical tests

To determine the MHC composition in single fibres, the samples were run on a Hoefer SE 600 gel electrophoresis system (Amersham Pharmacia Biotech, Buckinghamshire, UK). The gels consisted of a 4% stacking part and a 6% resolving part. The gels were run overnight at constant current, whereas silver staining (Bio-Rad, Hercules, CA, USA) was performed to visualise the form of MHC expressed in the fibre. The gels were then dried, pictured and classed as type I, type II-A, type II-X or hybrid (a mixture of MHC I and II types). As a size control, a prestained, broad-range protein ladder (Bio-Rad, Hercules, CA, USA) was used and a sample biopsy of the whole muscle served as a control for all possible MHC types.

Analysis of fibre bundles after mechanical testing

Transverse serial sections were cut (8–10 μm) on a cryostat (Microm, Walldorf, Germany). Sections were stained with H&E and with mouse monoclonal antibodies against human MHC slow and MHC fast (Novocastra Lab. Ltd., Newcastle, UK).

Study V

Antibodies against the cytoskeletal protein desmin, as well as the basal lamina-associated protein laminin (DAKO, Glostrup, Denmark), were used to demonstrate the integrity of intermyofibrillar cytoskeletal network and fibre boundaries respectively. In each biopsy, three to four randomly selected areas were analysed for fibre diameter, fibre area, central nuclei, angular fibres and necrotic fibres. Mean values were calculated for each subject. The percentage of extracellular matrix as an indication of fibrosis was calculated by subtracting the area occupied by muscle fibres from the total area of the region of interest.

Study VI

A qualitative analysis of samples stained with haematoxylin eosin was made noting fibre size variability, the rough amount of extracellular matrix and the presence of intracellular nuclei, fibrosis and necrosis. Fibres per bundle were counted. The quantification and location of collagen content was performed using sections stained by Sirius red. Sections stained with

Sudan black were assessed for the amount and distribution of fat. Fibres per bundle were counted and fibres were characterised as fast or slow by myosin heavy chain typing.

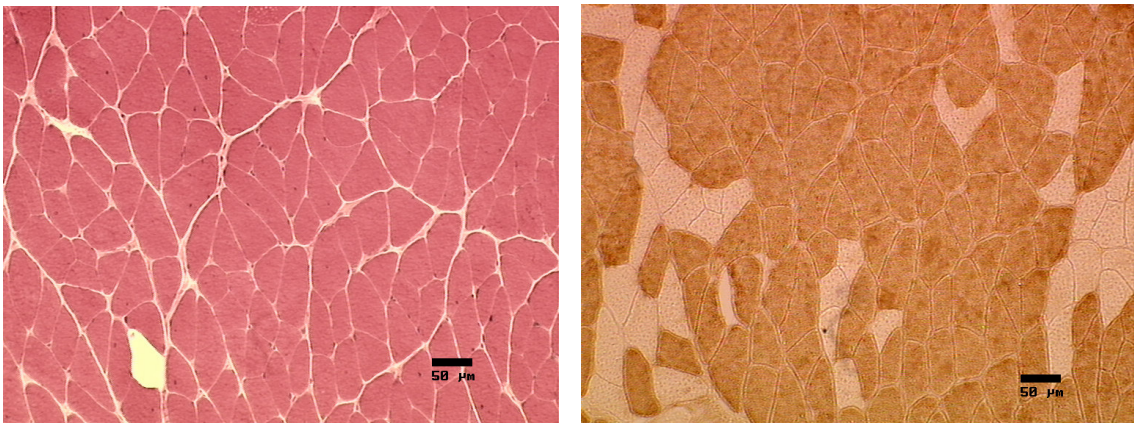

Figure 16 A and B. Representative cryosections;
on the left hand **(A)**: staining with haematoxylin and eosin,
on the right hand **(B)**: staining for slow isoforms of myosin heavy chains.
From study V; subscapularis muscle.

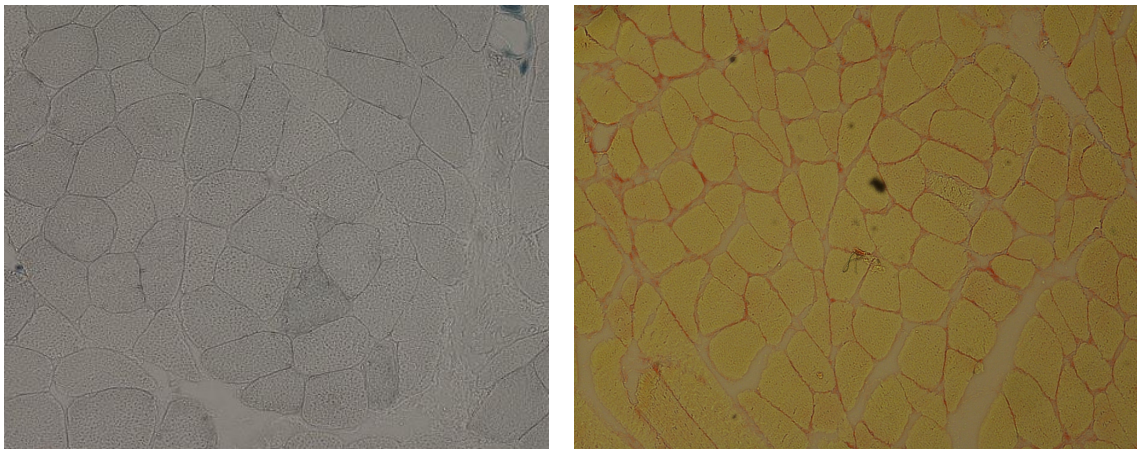

Figure 17 A and B. Representative cryosections; on the left hand **(A)** staining with Sudan black (for fat), on the right hand **(B)** staining with Sirius red (for ECM). From study VI; deltoid muscle.

STATISTICS

Study I

A two-sided Student's t-test for paired observations was used to detect differences in mean fibre size between the different preparations of the same biopsy. The Mann-Whitney U-test was used to test for differences in the mean of fibre occupancy (part of a section occupied by fibres exclusive of extracellular matrix).

Study III

The data were grouped by specimen type (fibre vs. bundle) and tissue type (normal vs. spastic) and analysed by two-way analysis of variance (ANOVA). The data were screened for normality and skew to justify the use of parametric tests. Post-hoc multiple t-tests were used to make specific comparisons between bundles and cells of a given type and between matching specimens of different types. The multiple t-tests were therefore corrected for these four comparisons, which were chosen a priori. The mathematical partitioning of the tangent modulus and ultimate tensile stress was based on the assumption that muscle cells and the ECM acted mechanically in parallel. Bundle area vs. sarcomere length data were fitted to a second-order polynomial and coefficients of determination were used to determine fit quality.

Study IV

An analysis of the muscle deformation curve was conducted by comparing the relationship between bundle area and sarcomere length at each point of elongation. The data were grouped by experiment type (fibre vs. bundle) and tissue type (normal vs. contracture) and analysed using a two-sided Student's t-test for unpaired observations.

Study VI

For tests of the location of the median of the modulus of elasticity, for comparisons of the difference and ratio of the tangent modulus between single fibres and bundles, for comparisons of the mean collagen content, for comparisons of the means of myosin heavy chain types and for comparisons of mean fibre diameter between samples, a two-tailed Wilcoxon's signed rank test was used. The observed confidence intervals for the range of tangent modulus values and fibre diameter were established using a one-sample t-test in SPSS 16.0 (Chicago, Il, USA).

Studies II and V

No statistical evaluations were made in Studies II and V, but regression analysis was made with regard to time to stress relaxation (Study II) and the relationship between fibre size and age (Study V) respectively.

ETHICS

All the studies included in this thesis were approved by the local Human Ethics Committee at the University of Gothenburg (approval no S166-1 and 681-07) and at Karolinska Institutet, Stockholm (approval no 214/99).

RESULTS

Results below are presented as measured value and (SEM).

Studies I and II can be viewed as tests of methodology in healthy muscle; the results are not related to longstanding changes in tension.

Study I

It appears that the results of the passive mechanical testing and structural analysis of muscle single fibres and bundles respectively are not obscured by freeze storage.

Mechanical property comparisons

Comparisons of stress-strain curves demonstrated a substantial variability between patients and muscles, but essentially identical responses between the different treatments of the biopsy samples for each individual. The predominant shapes of the stress-strain graphs were exponential or sigmoidal. The mean ratio for the tangent modulus between stored and fresh samples was 1.12 (0.05) with a variation coefficient of 12%.

Structural property comparisons

No structural derangements were seen in the stored samples. All the slides used for measurements demonstrated tightly packed and usually polygonally shaped muscle fibres with normal staining characteristics. The muscle fibres were organised into well-defined fascicles. Extracellular space was sparse. There were no significant differences in fibre diameter between fresh and stored samples. Nor was there any significant difference in fibre occupancy between fresh and stored samples, 94.5 (0.8) vs. 91.4 (2.7)%.

Study II

The aim of this study was to establish a time point at which tension decay progresses slowly, based on the stress relaxation pattern of single fibres from human upper extremity muscles. After detailed assessment of several stress vs. time plots, the deflection point between the steep and shallow part of the stress relaxation curve was estimated to correspond to a stress relaxation of approximately 75% of the total stress amplitude. The time taken to reach a relatively stable tension-plateau following the passive stretching of single fibres was registered from only a few seconds, in short sarcomere lengths, up to several minutes in

supra-physiological sarcomere lengths. The relationship between time to stress relaxation and sarcomere length was interpreted as a direct proportionality. A detailed analysis of the stress relaxation of each fibre in a range at a supposed sarcomere length at fixation at tendon transfer in the forearm, i.e. 3.78 (0.52) (Fridén and Lieber 1998), revealed that the median time to a reduction of 75% in stress was two (range 2-3) minutes. The mean time for a stress relaxation of 75% at a relative sarcomere length of 1.2 and 1.8 μm was 82 (range 74-84) and 175 (range 156-184) seconds respectively.

Study III

Spasticity appears to have a profound impact on both mechanical response and structural characteristics. Single fibre mechanics revealed a shorter slack sarcomere length and increased stiffness, whereas calculations of the contribution of the extracellular matrix to mechanical performance showed significantly impaired passive mechanical performance of the extracellular matrix in bundles from patients with spastic contractions, compared with controls.

General bundle characteristics

In overall terms, bundles obtained from spastic muscles were inferior in quality compared with normal muscle. Spastic bundles were more mechanically fragile and the length of the spastic bundles that were tested was significantly shorter than the length of normal bundles ($p<0.05$). Diffraction patterns from spastic bundles were more diffuse and of a lower intensity compared with those obtained from normal muscle, although sarcomere lengths were easily calculated. Spastic bundles were characterised by a relatively large amount of extracellular material. Only approximately 40% of the spastic bundle was occupied by muscle fibres, compared with 95% of the normal bundle.

Bundle mechanical properties.

In contrast to the mechanical results obtained from single cells of normal muscle in which the sarcomere length-stress relationships were predominantly linear (Fridén and Lieber 2003), all sarcomere length-stress records from fibre bundles were non-linear. The average sarcomere length range over which the bundle tangent modulus is reported in the current study, 1.22 (0.11) μm, representing an average minimum sarcomere length of 2.79 (0.13) μm, to an average maximum sarcomere length of 4.02 (0.18) μm, was significantly shorter ($p<0.0001$) compared with the range previously reported for the modulus of single cells wherein linear stress-strain curves were usually observed 2.27 (0.19) μm. The slack sarcomere length was

significantly shorter in fibre bundles of normal muscle compared with single cells, whereas the slack sarcomere length was significantly longer in fibre bundles of spastic muscle compared with single cells using a simple t-test ($p<0.001$). Two-way ANOVA revealed a significant difference between spastic and normal tissue types for slack sarcomere length ($p<0.005$). For both normal and spastic muscle, the tangent modulus was significantly larger in bundles compared with single fibres ($p<0.0001$), but the difference was more pronounced for normal muscle than spastic muscle; the spastic muscle bundle modulus was only twice the single-fibre modulus, whereas the normal muscle bundle modulus was more than 16 times larger than the modulus of a normal single muscle cell.

Study IV

The passive mechanical testing of the subscapularis muscle from children with persistent internal rotation contracture of the shoulder following obstetric brachial plexus palsy (OBPP) and older controls with samples from a range of upper extremity muscles did not reveal any significant differences when compared within the same type of preparation (i.e. subscapularis single fibres vs. control single fibres and subscapularis bundles vs. controls bundles respectively). However, an analysis of the change in the tangent modulus measured from single fibres to bundles in the two groups (i.e. subscapularis vs. controls) revealed a greater relative increase in the subscapularis group.

The slack SL was significantly ($p<0.01$) shorter in single fibres from OBPP patients than normal controls, viz. 2.01 (0.03) versus 2.26 (0.08) mm. This difference was not found between fibre bundles. The tangent modulus for both single fibres and fibre bundles demonstrated higher means for OBPP patients, compared with normal controls, but no significant difference was seen. The relative increase in stiffness between single fibres and fibre bundles was 6.7 and 5.3 times for OBPP samples and controls respectively. This constitutes a 26% larger increase in stiffness for the OBPP samples compared with normal controls. The tangent modulus for fibre bundles corrected for the number of fibres in the bundle was calculated without any difference between OBPP patients and controls, viz. 8.0 (3.1) versus 6.7 (2.3) kPa.

Biopsies from OBPP patients consisted of only subscapularis muscle, whereas control biopsies were harvested from several different muscles. As a result, the co-ordinates from controls were more scattered. In spite of this, the deformational curves fitted well between samples. The area of single muscle fibres from OBPP patients was significantly smaller than the area of normal single cells, viz. 4 703 (502) versus 7 350 (690) mm^2 ($p<0.01$). The area per fibre in fibre bundles was also significantly smaller in fibre bundles from OBPP patients,

compared with normal controls, viz. 2916 (343) versus 6677 (996) mm^2 ($p<0.01$). Fibre bundles from OBPP patients consisted of more fibres than normal muscle bundles, viz. n=29 (4.6) versus 18.2 (2.4), but without any differences in the cross-sectional area between samples. There were no significant differences in the MHC composition between the single-fibre samples for subscapularis samples and normal controls, viz. 16/27 type I; 9/27 type II and 2/27 hybrid fibres versus 11/18, 4/18 and 3/18.

Study V

In twelve of thirteen subscapularis muscle samples from children with persistent internal rotation contracture of the shoulder following obstetric brachial plexus injury, relative homogeneity was revealed with regard to morphology. However, one patient, with an extremely complicated delivery, identified as an outlier that had a dislocated humeral head on preoperative CT, was noted with marked muscular changes; increased fibre size variability, an increase in extracellular fraction (21%) and a drift in fibre type composition towards type II dominance compared to the other twelve patients (66 vs. 25 %).

CT scans

CT scans from seven patients revealed a subluxation of the humeral head in the glenohumeral joint in three patients and a dislocation in one of the patients.

Histopathology

Muscle morphology was considered normal in twelve of thirteen patients. One of the patients (the outlier) exhibited a marked change in muscle morphology. Biopsy sections from this outlier showed an abnormal mean fibre diameter relative to age and the mean fibre occupation was 79%, indicating excessive fibrous tissue. No difference in the occurrence of angular and necrotic fibres was noted, however. Angular and necrotic fibres were measured with a mean fraction of 1.3 (0.5) and 1.0 (0.3)% respectively. The twelve patients had a mean value of 9.3 (3.9)% of fibres with central nuclei. Five patients had central nuclei levels of less than 3%, four patients had levels between 3-10% and three patients had levels of more than 10%. A positive regression ($r^2=0.76$) was found between age and fibre diameter, indicating a significant ($p<0.001$) linear relationship.

Fibre type distribution

In twelve subjects, we found a predominance of MHC type I fibres with a mean of 75%. The immunolabelling of desmin and laminin was considered normal. The distribution of MHC isoforms in the outlier showed a marked difference compared with the other twelve patients with a type II fibre proportion of 66% (vs. 25% compared with the other twelve children). Fibre type grouping was also detected.

Study VI

The passive mechanical testing of supraspinatus muscle samples from retracted rotator cuff tears revealed a normal response in comparison with the testing of muscle samples from patients' ipsilateral deltoid.

Mechanical testing

Stress-strain curves within individuals displayed obvious similarities in shape, with a trend towards greater compliance by the supraspinatus muscles. However, in terms of the modulus of elasticity, there were no differences in the location of single fibres or bundles; viz. 67 (13) vs. 56 (14) and 102 (23) vs. 94 (20) MPa. There was no difference (p=0.40) in the relative increase in the modulus of elasticity from single fibres to bundles when the deltoid and supraspinatus was compared. The observed confidence interval (95%; 2.5-97.5%) for deltoid single fibres was 38-97, while it was 44-160 MPa for bundles. The corresponding figures for the supraspinatus were 21-97 for single fibres and 46-142 MPa for bundles.

Morphology

There was no difference in the mean fraction of collagen in the histological sections between muscle types as measured by Sirius red; 1.55 (0.15) vs. 1.39 (0.13) % (p=0.75) (deltoid and supraspinatus respectively). The ratio of slow to fast fibres (fast/slow), as defined by the count of myosin heavy chain staining in histological bundle preparations, did not differ between samples; viz. deltoid 1.61 (0.48) vs. supraspinatus 1.64 (0.35), (p=0.57). The mean fibre diameter was measured as 76.6 (5.9) and 64.5 (6.6) μm for deltoid and supraspinatus respectively; this is not a significant difference (p=0.176) between samples at the chosen level of confidence (95%). The observed confidence intervals (lower 2.5% to upper 97.5%) were 63-91 and 48-81 μm for the deltoid and supraspinatus muscles respectively. The qualitative evaluation was judged without any significant difference in fibre size variability, the occurrence of necrotic fibres, the presence of fibrosis and the content and distribution of fat and extracellular matrix.

DISCUSSION

This study provides evidence of the adaptation of muscle tissue to change in tension over time thereby confirming current knowledge (Huijing and Jaspers 2005; Harridge 2007; Magnusson et al. 2008). However, there is no, one single, generally applicable response pattern that can be identified as a summary of Studies III-VI. All the studies in this thesis tested human muscle tissue and all the results relate to human conditions. The effects of change in muscular tone were evaluated in three different conditions. No reports on the mechanical testing of the subscapularis muscle in children following obstetric brachial plexus injury or in the supraspinatus from retracted rotator cuff tears could be found in the literature. In this respect, the data presented in this study are unique. Methods are used consistently throughout this study; morphology, including the typing of fibres with regard to myosin heavy chains, and passive mechanical testing. The combination of testing single fibres and muscle bundles at passive mechanical testing made it possible to calculate the contribution of passive restraint from the extracellular matrix, something that is otherwise difficult to assess directly.

Tendon transfer is a technique that is used to restore function in neurological and musculo-tendinous injuries. The goal is to achieve dynamic function in the transferred muscle-tendon unit, but a more static tenodesis is sometimes achieved. It is a challenge to objectify the optimal tensioning of a transferred muscle-tendon unit at surgery. It is reasonable to assume that if a surgeon wants to perform iterated assessments of the tension in muscle-tendon units following stretching at tendon transfer surgery, with comparable levels of tension between cases, the “feel” of tension is more easily reproduced at the tension plateau after, and not during, the initial steep tension decay. Study II showed that a “safe” time to make this assessment is two minutes after stretching.

The structural basis for the inferior mechanical properties of the spastic ECM is not known. Study III confirms previous findings of fibre size variability (Rose et al. 1994). Morphologically, the spastic ECM appeared disorganised, “loose”, and hypercellular. This may represent a dynamic reorganisation of the spastic ECM in response to changes in muscle fibre mechanical properties, or the changes in muscle fibre mechanical properties may be an attempt by the muscle cells to compensate for the deranged ECM. The exponential shape of the stress strain curves was interpreted as being due to either interaction between fibres or to the added presence of the ECM.

Persisting symptoms following obstetric brachial plexus injury range from mild internal rotation of the shoulder to severe bony deformation and chronic dislocation of the glenohumeral joint. This is explained by weakness in the partially denervated external rotators of the shoulder and/or capsular fibrosis, as a result of brachial plexus injury or a direct soft-tissue trauma respectively. Close to normal passive mechanics and structural analysis of the subscapularis muscle from children with persistent internal rotation of the shoulder after obstetric brachial plexus injury were interpreted as supporting neural injury affecting the external rotators as the predominant cause, at least in the majority of children with mild residual symptoms. To date, Birch has dealt with more than 600 children with cases of posterior dislocation. According to him (personal communication), it appears clear that severe fibrosis of the subscapularis muscle is not uncommon and he has come to the view that the internal contracture is largely caused by haematoma or by tearing during a difficult delivery. An analysis of the results related to the outlier patient in Study V supports a possible mechanism of this kind, although no evaluation of the capsule tissue was made to confirm this.

With regard to structural changes in the muscular component of the rotator cuff following rotator cuff tears with retraction, there is a common belief that the muscle degenerates and undergoes fatty infiltration (Goutallier et al. 1994). Another *possible* explanation is a reduction in the serial number of sarcomeres as a result of the fall in muscle tension that follows a rotator cuff tear. Even though there is no evidence of this regulatory mechanism in humans, it is known and reported in animals (Goldspink et al. 1974; Gajdosik 2001).
A rotator cuff tear may therefore be followed by an *absolute* reduction in muscle volume. This may be interpreted as a *relative* loss of muscle volume and an adjacent relative increase in the amount of fat ("fatty infiltration").

The direct repair of a rotator cuff tear may result in either a functional repair or a tenodesis, caused by overstretching a short muscle. Study VI shows a normal response to passive mechanical testing of the supraspinatus muscle from rotator cuff tears with retraction. Supplementary qualitative structural analysis supports the appearance of normal supraspinatus muscle tissue. This is in contrast to previous descriptions of "fatty infiltration" and degenerative changes in the muscle component, based on findings on CT and MRI, following rotator cuff tear and retraction (Goutallier et al. 1994; Fuchs et al. 1999). The amount of fat increases with age, in both the supraspinatus and deltoid muscles (Ashry et al. 2007). It *may* therefore be a mistake to assume that the muscle degenerates, which is partly supported by previous reports (Gerber et al. 2004; Shen et al. 2008).

SUMMARY

The results in this thesis reveal that the extracellular matrix is an important functional structure of muscle tissue. No general rule for the adaptation of muscle in response to long standing changes in tension can be derived.

The pathophysiology of spasticity is complex. Study III adds to the existing knowledge, showing profound changes, predominantly in the mechanical performance of the extracellular matrix. The conclusion is that the “feel” of passive tension when stretching a muscle-tendon complex in spastic muscles is predominantly generated by muscle fibres and to a lesser extent by the extracellular matrix. This information should be kept in mind during surgical corrections of spastic contractures.

The normal mechanical appearance, supported by essentially normal semi-qualitative histological evaluation, in Study VI supports the hypothesis that a reduction in the number of serially coupled sarcomeres actually occurs in rotator cuff muscles following tears. This statement needs to be confirmed in further studies. However, if this is true, it has implications for surgical repair, especially of massive, chronic tears.

Biopsies for histological analysis can be harvested using the fine needle biopsy technique, but, when harvesting muscle in order to perform mechanical testing, the muscle structure needs to be perfectly intact. During the work with Study VI, there was a need for a technique that made it possible safely to harvest supraspinatus muscle, through the surgical incision, while preserving the muscle structure. A vacuum-assisted biopsy needle technique was tested and was found to deliver close to perfect muscle biopsies. This technique provides a useful tool in further research with regard to structural and mechanical changes in the muscle component of rotator cuff tears.

CONCLUSIONS

Freeze storage of muscle specimens retains mechanical properties

Assessment of tension in the muscle-tendon unit should preferably be made after a minimum delay of two minutes during tendon transfer surgery

Spasticity causes profound mechanical changes in the extracellular matrix of upper extremity muscles that affects the entire mechanical performance of the muscle.

In most children, the cause of internal rotation contraction in the shoulder following an obstetric brachial plexus injury relates in most children to a muscular weakness due to neural injury, although the direct soft tissue injury may be an additional important factor in children with an extremely complicated delivery.

The supraspinatus muscle from retracted rotator cuff tears has potential for full recovery. Restoring retracted tears may need staged surgery or alternative surgical solutions, in order to avoid overstretching a shortened muscle.

CLINICAL RELEVANCE

TENDON TRANSFER

Assessments of tension in the muscle-tendon unit that are made after a delay of two minutes following passive stretching will leave the muscle with a significant reduction in stress, as measured in single fibres. The progressing decay is slow, allowing for a high correlation at inter-case comparison.

SPASTICITY

The “feel” of passive tension when stretching of a muscle-tendon complex in patients with spastic contractures is predominantly generated by muscle fibres and to a lesser extent by the extracellular matrix compared with normal muscle tissue, and this should be kept in mind during surgery.

OBSTETRIC BRACHIAL PLEXUS INJURY

It *may* be necessary to differentiate (surgical) treatment with regard to how pronounced the soft tissue injury is.

ROTATOR CUFF TEARS

Mechanically and structurally normal supraspinatus muscles increase the opportunity to restore function. Surgery to restore mechanical integrity and function in a shoulder with a retracted rotator cuff tear should preferably allow for the gradually stretching of the muscle over time to avoid overstretching.

FUTURE PERSPECTIVES

It was not within the scope of this study to investigate pathways regulating the expression of proteins (Lum and Beachy 2004; Day and Yang 2008), even though future studies in this area might add substantial information to promote our understanding of the pathophysiology of clinical symptoms like those studied in this thesis. However, the kind of investigations presented in this thesis are necessary to link advanced basic research to clinical problems (Huijing and Jaspers 2005).

For further research on the structural and functional results of the torn rotator cuff, the use of pre- and postoperative MRI, pre- and postoperative functional testing (scoring) and whole muscle testing during surgery could be added in order to produce a complete investigation coupling structural and mechanical data to clinical outcome. The polymeras chain reaction (PCR) technique could be used in order to quantify the expression of mRNA by different iso-forms of titin. Radiostereometric analysis (RSA) could possibly be used in order to evaluate changes in glenoscapular motion prior to and following direct repair, latissimus dorsi transfer or surgical restoration of function with a reversed shoulder prosthesis. No such data are as yet available.

Restoring function in massive chronic rotator cuff tears is a challenge. A combination of new/alternative surgical techniques and "biological engineering" might be successful. In a recent report, the augmentation of tendon-to-bone repair, by addition of growth factors and bone morphogenetic proteins, was investigate in a sheep model (Kovacevic and Rodeo 2008). Optimally, a surgical repair technique would achieve the gradual stretching of the muscle, possibly involving a (bio-degradable) material that bridges the defect of tendon to bone and increases in stiffness as time elapses over a long period of time (months) and, if possible soaked with muscle-specific, growth factors that are slowly released.

Mekaniska och strukturella egenskaper i human övre extremitetsmuskulatur: evidens för anpassning som svar på förändrad spänning

Fredrik Einarsson
Göteborg 2008

Målet med denna avhandling var att förbättra resultaten vid så kallad sentransfereringskirurgi och vid reparation av skada i axelns muskel-senmanschett, så kallad rotatorkuffruptur.

Metoden var att undersöka mekaniska och strukturella egenskaper i human övre extremitetsuskulatur (axel, arm och hand) med förändrad spänning under en längre tid.

Den huvudsakliga tekniken var analys av muskelprov (biopsier) med avseende på mekaniska och strukturella egenskaper. Muskelprov från patienter som genomgick operation av övre extremiteten togs till alla delstudier. Dessa biopsier har till de olika delstudierna hämtats dels från friska kontroller, dels från patienter med spasticitet, nervrotskada efter förlossning (brachialplexusskada) respektive skada i axelns muskel-senmanschett (rotatorkuffruptur).

Genomgående har muskelprovernas passiva mekaniska egenskaper testats (elasticitet) i både enskilda muskelfibrer och muskelbuntar. Vävnadsförändringar i muskelbuntar har undersökts avseende både kvantitativa och kvalitativa egenskaper.

Spänningsminskning i enskilda muskelfibrer planar ut till en relativt stabil nivå först efter cirka två minuter i normalfysiologiska längder av de mista kontraktila enheterna i muskeln (sarkomererna), vilket har betydelse för så kallad sentransfereringskirurgi där sena med muskel flyttas till en ny position för att uppnå ökad funktion. I samband med sådana ingrepp sträcker kirurgen på muskeln och bör sedan antagligen vänta två minuter innan spänningen i muskeln värderas innan senan fixeras definitivt.

Hos patienter med spastisk kontraktur kunde de mest uttalade förändringarna noteras med en ökad mängd bindväv av försämrad mekanisk kvalitet som viktigaste resultat.

Hos barn med inåtrotationskontraktur i axeln efter förlossningsrelaterad skada av armens nervplexa (brachialplexusskada), visade resultat att subscapularis-muskeln har väsentligen normala mekaniska och strukturella egenskaper. Inåtrotationskontrakturen kan, hos dessa barn med milda restsymtom, förklaras av svaga utåtrotation-muskler som en följd av nervskadan, medan barn med mer komplicerad förlossning sannolikt har ytterligare pålagring i form av en direkt mjukdelsskada mot muskel och ledkapsel i samband med förlossning.

Hos patienter med rotatorkuff ruptur tolkades fynden från mekanisk testning av supraspinatus-muskeln som normala, vilket dels talar för att det finns potential att återställa den förlorade funktionen, men även att tidigare uppfattning om att muskeln genomgår en degeneration efter sådan skada ifrågasätts. I samband med reparation av rotatorkuffruptur bör hänsyn tas så att muskeln inte översträcks med ett försämrat resultat som följd.

Denna avhandling visar att muskel som står med ändrad spänning förändrar sig både avseende egenskaper i muskelfibrer och i omkringliggande bindväv. Dessa resultat bekräftar tidigare beskrivning av förekomsten av en reglerad (molekylär) kommunikation mellan muskel och bindväv, där bindväven har en betydelsefull roll.

ACKNOWLEDGEMENTS

The work of this thesis was carried out at the Department of Orthopaedics, Sahlgrenska University Hospital (Ortopeden Östra and the department of Hand Surgery, Sahlgrenska) and at the Lundberg Laboratory for Orthopaedic Research at the Sahlgrenska Academy, University of Gothenburg. I wish to express my sincere gratitude to everyone who has made this thesis possible.

Jan Fridén, my tutor, professor of Hand Surgery at the Department of Orthopaedics, Sahlgrenska University Hospital, for accepting me as a PhD-student, for patience, encouragement, skilled scientific guidance and for help towards independence. Some hard work, a few fatigue periods and an intense finish; just like a good marathon ought to be?

Jón Karlsson, my co-tutor, professor and head of the Department of Orthopaedics, Sahlgrenska University Hospital, for providing excellent conditions to complete this thesis and for extremely efficient guidance in practical, clinical and scientific matters. Stórmenni lætur aðra vaxa og ná árangri.

Eva Runesson, PhD, at the Lundberg Laboratory for Orthopaedic Research, for introducing me to practical laboratory work, for substantial help with mechanical testing and morphological analysis, for valuable discussions and essential encouragement. Keep up the good work!

Tomas Hultgren, MD, co-author in Study IV and main author of Study V, for teaching me about the clinical problems relating to obstetric brachial plexus injuries and for smooth collaboration.

Tommy Hansson, professor at the Department of Orthopaedics, Sahlgrenska University Hospital, for providing the opportunity to perform this thesis and for valuable criticism.

Björn Rydevik, professor at the Department of Orthopaedics and head of the Section for Anaesthesiology, Biomaterials and Orthopaedics, Sahlgrenska Academy, for providing the opportunity to perform this thesis.

The staff, at the orthopaedic surgical theatre, out patient clinic and patient wards at Ortopeden Östra**;** I always felt welcome, thank you!

During the work with Study VI I had good help at Ortopeden Östra, thank you **Lotta Falkendal**, for brilliant, smooth help handling muscle specimens, **Ulrica Rosenquist,** for coordinating and re-coordinating theatre lists, **Erna Koskel**, for help organising numerous outpatient clinics and **staff at the surgical theatre** for endurance pulling through a few long lists.

Things were sorted out, thank you **Linda Johansson**, for kind and endless assistance in all the practical matters in the preparation of this dissertation and **Maria Knall**, research coordinator, for swift and bright help.

Manuscripts and frame story were spiced and revised, thank you **Anette Dahlström**, for illustrations**, Ninni Sernert**, for lay-out guidance and **Jeanette Kliger**, for excellent linguistic revision.

Katarina Helander, **Jonas Isberg and Ann-Charlott Söderpalm**, colleagues at, what used to be, Ortopeden Östra and fellow PhD-students, for many rewarding discussions. Your good company made the travel even more joyful.

Olof Lundin, MD, PhD, Perago Ortopedklinik, for introducing me to rotator cuff surgery, for mutual understanding of the need to hit a long, straight driver and for encouragement.

Björn Naes, MD, my first mentor, for introducing me to orthopaedics, for believing in me and for being a role model, still.

Tobias Kjellberg, for encouraging me to pursue (orthopaedic) surgery and for friendship.

Erik, my brother, for your unconditional support in life, **Lars**, my late father, for your love and **Gerd**, my mother**,** for life, for your wits, caring, patience and for being a role model.

Kristin, my wife, for your love, efficient life-coaching and for letting me be part of your life and **Karl Filip** and **Lovisa**, our children, merely for existing.

REFERENCES

Ashry R, Schweitzer ME, Cunningham P, Cohen J, Babb J, Cantos A. Muscle atrophy as a consequence of rotator cuff tears: should we compare the muscles of the rotator cuff with those of the deltoid? Skeletal Radiol 2007;36:841-5.

Bager B. Perinatally acquired brachial plexus palsy - a persisting challenge. Acta Paediatr 1997;86:1214-9.

Baskin RJ, Roos KP, Yeh Y. Light diffraction study of single skeletal muscle fibres. Biophys J 1979;28:45-64.

Birch R (2000). Obstetric Brachial Plexus Palsy. In: *The Growing Hand*. Gupta A, Kay S, Scheker LR. London, Mosby**:** 461-474.

Birch R, Chen L. The medial rotation contracture of the shoulder in obstetric brachial plexus palsy. Journal of Bone Joint Surgery 1996; 78-B 68.

Blaine TA, Kim YS, Voloshin I, Chen D, Murakami K, Chang SS, Winchester R, Lee FY, O'Keefe R J, Bigliani LU. The molecular pathophysiology of subacromial bursitis in rotator cuff disease. J Shoulder Elbow Surg 2005;14:84S-89S.

Blix M. Die Länge und die Spannung des Muskels. Skandinavische Archive für Physiologie 1894.

Burkhart SS, Esch JC, Jolson RS. The rotator crescent and rotator cable: an anatomic description of the shoulder's "suspension bridge". Arthroscopy 1993;9:611-6.

Codman EA. Rupture of the supraspiantus tendon. Boston Medical and Surgical Journal 1911;CLXIV:708-710.

Cofield RH, Parvizi J, Hoffmeyer PJ, Lanzer WL, Ilstrup DM, Rowland CM. Surgical repair of chronic rotator cuff tears. A prospective long-term study. J Bone Joint Surg 2001;83-A:71-7.

Degreef I, Debeer P, Van Herck B, Van Den Eeden E, Peers K, De Smet L. Treatment of irreparable rotator cuff tears by latissimus dorsi muscle transfer. Acta Orthop Belg 2005;71:667-71.

Dubowitz V, Sewry CA (2007). *Muscle biopsy A practical approach*. Amsterdam, Saunders Elsevier.

Dubowitz V, Sewry CA FR, Sewry CA. (1985). *Muscle biopsy - A practical approach*. London, Baillière Tindall.

Einarsson F, Runesson E, Fridén J. The supraspinatus muscle in retracted rotator cuff tears responds normally to passive mechanical testing: a pilot study. Manuscript 2008

Fridén J, Albrecht D, Lieber RL. Biomechanical analysis of the brachioradialis as a donor in tendon transfer. Clin Orthop 2001:152-61.

Fridén J, Lieber RL. Evidence for muscle attachment at relatively long lengths in tendon transfer surgery. J Hand Surg [Am] 1998;23:105-10.

Fridén J, Lieber RL. Mechanical considerations in the design of surgical reconstructive procedures. J Biomech 2002;35:1039-45.

Fridén J, Lieber RL. Tendon transfer surgery: clinical implications of experimental studies. Clin Orthop 2002:S163-70.

Fridén J, Lieber RL. Spastic muscle cells are shorter and stiffer than normal cells. Muscle Nerve 2003;27:157-64.

Fridén J, Lovering RM, Lieber RL. Fiber length variability within the flexor carpi ulnaris and flexor carpi radialis muscles: implications for surgical tendon transfer. J Hand Surg [Am] 2004;29:909-14.

Fridén J, Pontén E, Lieber RL. Effect of muscle tension during tendon transfer on sarcomerogenesis in a rabbit model. J Hand Surg [Am] 2000;25:138-43.

Fuchs B, Gilbart MK, Hodler J, Gerber C. Clinical and structural results of open repair of an isolated one-tendon tear of the rotator cuff. J Bone Joint Surg 2006;88-A:309-16.

Fuchs B, Weishaupt D, Zanetti M, Hodler J, Gerber C. Fatty degeneration of the muscles of the rotator cuff: assessment by computed tomography versus magnetic resonance imaging. J Shoulder Elbow Surg 1999;8:599-605.

Fukuda N, Sasaki D, Ishiwata S, Kurihara S. Length dependence of tension generation in rat skinned cardiac muscle: role of titin in the Frank-Starling mechanism of the heart. Circulation 2001;104:1639-45.

Gajdosik RL. Passive extensibility of skeletal muscle: review of the literature with clinical implications. Clin Biomech 2001;16:87-101.

Galatz LM, Connor PM, Calfee RP, Hsu JC, Yamaguchi K. Pectoralis major transfer for anterior-superior subluxation in massive rotator cuff insufficiency. J Shoulder Elbow Surg 2003;12:1-5.

Gao M, Sotomayor M, Villa E, Lee EH, Schulten K. Molecular mechanisms of cellular mechanics. Phys Chem Chem Phys 2006;8:3692-706.

Gerber C. Latissimus dorsi transfer for the treatment of irreparable tears of the rotator cuff. Clin Orthop Relat Res 1992:152-60.

Gerber C, Maquieira G, Espinosa N. Latissimus dorsi transfer for the treatment of irreparable rotator cuff tears. J Bone Joint Surg 2006;88-A:113-20.

Gerber C, Meyer DC, Schneeberger AG, Hoppeler H, von Rechenberg B. Effect of tendon release and delayed repair on the structure of the muscles of the rotator cuff: an experimental study in sheep. J Bone Joint Surg 2004;86-A:1973-82.

Gerber C, Pennington SD, Lingenfelter EJ, Sukthankar A. Reverse Delta-III total shoulder replacement combined with latissimus dorsi transfer. A preliminary report. J Bone Joint Surg 2007;89-A:940-7.

Gilbert A (1993). Obstetric Brachial Plexus Palsy. In: *The Hand*. Tubiana R. Philadelphia, WB Saunders Co. 4**:** 575-601.

Goldspink G, Tabary C, Tabary JC, Tardieu C, Tardieu G. Effect of denervation on the adaptation of sarcomere number and muscle extensibility to the functional length of the muscle. J Physiol 1974;236:733-42.

Gordon AM, Huxley AF, Julian FJ. Tension development in highly stretched vertebrate muscle fibres. J Physiol 1966;184:143-69.

Gordon AM, Huxley AF, Julian FJ. The variation in isometric tension with sarcomere length in vertebrate muscle fibers. J. Physiol. 1966;184:170-192.

Goutallier D, Postel JM, Bernageau J, Lavau L, Voisin MC. Fatty muscle degeneration in cuff ruptures. Pre- and postoperative evaluation by CT scan. Clin Orthop Relat Res 1994:78-83.

Goutallier D, Postel JM, Gleyze P, Leguilloux P, Van Driessche S. Influence of cuff muscle fatty degeneration on anatomic and functional outcomes after simple suture of full-thickness tears. J Shoulder Elbow Surg 2003;12:550-4.

Granzier H, Irving T. Passive Tension in Cardiac Muscle: Contribution of Collagen, Titin, Microtubules and Intermediate Filaments. Biophysical Journal 1995;68:1027-1044.

Granzier H, Labeit S. Structure-function relations of the giant elastic protein titin in striated and smooth muscle cells. Muscle Nerve 2007;36:740-55.

Green DP, Hotchkiss RN, Pederson WC, Wolfe SW (2005). *Green's Operative Hand Surgery*. Philadelphia, Elsevier.

Harridge SD. Plasticity of human skeletal muscle: gene expression to in vivo function. Exp Physiol 2007;92:783-97.

Huijing PA, Jaspers RT. Adaptation of muscle size and myofascial force transmission: a review and some new experimental results. Scand J Med Sci Sports 2005;15:349-80.

Irlenbusch U, Gansen HK. Muscle biopsy investigations on neuromuscular insufficiency of the rotator cuff: A contribution to the functional impingement of the shoulder joint. J Shoulder Elbow Surg 2003;12:422-6.

Ivanhoe CB, Reistetter TA. Spasticity: the misunderstood part of the upper motor neuron syndrome. Am J Phys Med Rehabil 2004;83:S3-9.

Järvinen TA, Jozsa L, Kannus P, Järvinen TL, Järvinen M. Organization and distribution of intramuscular connective tissue in normal and immobilized skeletal muscles. An immunohistochemical, polarization and scanning electron microscopic study. J Muscle Res Cell Motil 2002;23:245-54.

Kambhampati SB, Birch R, Cobiella C, Chen L. Posterior subluxation and dislocation of the shoulder in obstetric brachial plexus palsy. J Bone Joint Surg 2006;88-B:213-9.

Kjaer M. Role of extracellular matrix in adaptation of tendon and skeletal muscle to mechanical loading. Physiol Rev 2004;84:649-98.

Kon DS, Darakjian AB, Pearl ML, Kosco AE. Glenohumeral deformity in children with internal rotation contractures secondary to brachial plexus birth palsy: intraoperative arthrographic classification. Radiology 2004;231:791-5.

Kovacevic D, Rodeo SA. Biological augmentation of rotator cuff tendon repair. Clin Orthop Relat Res 2008;466:622-33.

Labeit S, Kolmerer B. Titins: giant proteins in charge of muscle ultrastructure and elasticity. Science 1995;270:293-6.

Lance JW. The control of muscle tone, reflexes, and movement: Robert Wartenberg Lecture. Neurology 1980;30:1303-13.

Li H, Linke WA, Oberhauser AF, Carrion-Vazquez M, Kerkvliet JG, Lu H, Marszalek PE, Fernandez JM. Reverse engineering of the giant muscle protein titin. Nature 2002;418:998-1002.

Lieber RL (2002). *Skeletal muscle structure, function and plasticity*. Lippincott Williams & Wilkins.

Lieber RL, Fridén J. Clinical significance of skeletal muscle architecture. Clin Orthop 2001:140-51.

Lieber RL, Fridén J. Implications of muscle design on surgical reconstruction of upper extremities. Clin Orthop 2004:267-79.

Lieber RL, Yeh Y, Baskin RJ. Sarcomere length determination using laser diffraction. Effect of beam and fiber diameter. Biophys J 1984;45:1007-16.

Linke WA, Kulke M, Li H, Fujita-Becker S, Neagoe C, Manstein DJ, Gautel M, Fernandez JM. PEVK domain of titin: an entropic spring with actin-binding properties. J Struct Biol 2002;137:194-205.

Linke WA, Stockmeier MR, Ivemeyer M, Hosser H, Mundel P. Characterizing titin's I-band Ig domain region as an entropic spring. J Cell Sci 1998;111:1567-74.

Löhr JF, Uhthoff HK. [Epidemiology and pathophysiology of rotator cuff tears]. Orthopade 2007;36:788-95.

Magid A, Law DJ. Myofibrils bear most of the resting tension in frog skeletal muscle. Science 1985;230:1280-2.

Maughan RJ, Nimmo MA. The influence of variations in muscle fibre composition on muscle strength and cross-sectional area in untrained males. J Physiol 1984;351:299-311.

Mayer NH, Esquenazi A. Muscle overactivity and movement dysfunction in the upper motoneuron syndrome. Phys Med Rehabil Clin N Am 2003;14:855-83, vii-viii.

Meyer DC, Hoppeler H, von Rechenberg B, Gerber C. A pathomechanical concept explains muscle loss and fatty muscular changes following surgical tendon release. J Orthop Res 2004;22:1004-7.

Milgrom C, Schaffler M, Gilbert S, van Holsbeeck M. Rotator-cuff changes in asymptomatic adults. The effect of age, hand dominance and gender. J Bone Joint Surg 1995;77-B:296-8.

Mollberg M, Hagberg H, Bager B, Lilja H, Ladfors L. High birthweight and shoulder dystocia: the strongest risk factors for obstetrical brachial plexus palsy in a Swedish population-based study. Acta Obstet Gynecol Scand 2005;84:654-9.

Narakas AO. [Injuries of the brachial plexus and neighboring peripheral nerves in vertebral fractures and other trauma of the cervical spine]. Orthopade 1987;16:81-6.
Neer CS, 2nd. Anterior acromioplasty for the chronic impingement syndrome in the shoulder: a preliminary report. J Bone Joint Surg 1972;54-A:41-50.
Neer CS, 2nd. Impingement lesions. Clin Orthop Relat Res 1983:70-7.
Neviaser RJ, Neviaser TJ. Observations on impingement. Clin Orthop Relat Res 1990:60-3.
Nho SJ, Yadav H, Shindle MK, Macgillivray JD. Rotator cuff degeneration: etiology and pathogenesis. Am J Sports Med 2008;36:987-93.
Norlin R, Adolfsson L. Small full-thickness tears do well ten to thirteen years after arthroscopic subacromial decompression. J Shoulder Elbow Surg 2008;17:12S-16S.
Pontén E, Fridén J, Thornell LE, Lieber RL. Spastic wrist flexors are more severely affected than wrist extensors in children with cerebral palsy. Dev Med Child Neurol 2005;47:384-9.
Preetha N, Yiming W, Helmes M, Norio F, Siegfried L, Granzier H. Restoring force development by titin/connectin and assessment of Ig domain unfolding. J Muscle Res Cell Motil 2005;26:307-17.
Rose J, Haskell WL, Gamble JG, Hamilton RL, Brown DA, Rinsky L. Muscle pathology and clinical measures of disability in children with cerebral palsy. J Orthop Res 1994;12:758-68.
Rose J, McGill KC. The motor unit in cerebral palsy. Dev Med Child Neurol 1998;40:270-7.
Safran O, Derwin KA, Powell K, Iannotti JP. Changes in rotator cuff muscle volume, fat content, and passive mechanics after chronic detachment in a canine model. J Bone Joint Surg 2005;87-A:2662-70.
Shen PH, Lien SB, Shen HC, Lee CH, Wu SS, Lin LC. Long-term functional outcomes after repair of rotator cuff tears correlated with atrophy of the supraspinatus muscles on magnetic resonance images. J Shoulder Elbow Surg 2008;17:1S-7S.
Sher JS, Uribe JW, Posada A, Murphy BJ, Zlatkin MB. Abnormal findings on magnetic resonance images of asymptomatic shoulders. J Bone Joint Surg 1995;77-A:10-5.
Ward SR, Hentzen ER, Smallwood LH, Eastlack RK, Burns KA, Fithian DC, Fridén J, Lieber RL. Rotator cuff muscle architecture: implications for glenohumeral stability. Clin Orthop Relat Res 2006;448:157-63.
Warner JJ. Management of massive irreparable rotator cuff tears: the role of tendon transfer. Instr Course Lect 2001;50:63-71.
Yamaguchi K, Ditsios K, Middleton WD, Hildebolt CF, Galatz LM, Teefey SA. The demographic and morphological features of rotator cuff disease. A comparison of asymptomatic and symptomatic shoulders. J Bone Joint Surg 2006;88-A:1699-704.
Yeh Y, Baskin RJ, Lieber RL, Roos KP. Theory of light diffraction by single skeletal muscle fibers. Biophys J 1980;29:509-22.
Zancolli A, Zancolli R (2000). Reconstructive surgery in brachial plexus sequelae. In: *The Growing Hand*. Gupta A, Kay S, Scheker L. London, Mosby**:** 805-823.
Zingg PO, Jost B, Sukthankar A, Buhler M, Pfirrmann CW, Gerber C. Clinical and structural outcomes of nonoperative management of massive rotator cuff tears. J Bone Joint Surg 2007;89-A:1928-34.