EFFECT OF NERVE INJURY ON MUSCLE FATIGUE AND SENSORY-MOTOR PERFORMANCE OF THE HAND

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Presentations and publications

Abstarcts

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2- Ebied AM, Kemp GJ, Frostick SP. The influence of local anaesthesia on the sensory-motor performance of the hand.

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Publications

1- Ebied AM, Kemp GJ, Frostick SP: The role of cutaneous sensation in motor function of the hand. *Journal of Orthopaedic Research* 22:862-866, 2004

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Fatigability of reinnervated first dorsal interosseous muscle following ulnar nerve palsy.

Abbreviations and Definitions

Abbreviations

AP	Action Potential
$[Ca^{2+}]_i$	Intracellular Calcium
$[Ca^{2+}]_o$	Extracelluar Calcium
$[K^+]_0$	Extracellular Potassium
$[K^+]_i$	Intracellular Potassium
$[K^+]_o$	Extra cellular Potassium
1stDI	First Dorsal Interosseous
2PD	Two Point Discrimination
^{31}P MRS	Phosphorus Magnetic Resonance Spectroscopy
A/D	Analogue Digital Convertor
Acetyl CoA	Acetyl coenzyme A
ACh	Acetyl Choline
AChRs	Acetyl Choline Receptors
ADP	Adenosine Di-Phosphate
AE	Abbreviation of a volunteer's name
Ag	Silver
AM	Abbreviation of a volunteer's name
Amp	Amplitude
ARJ	Average Rectified Jerk
ARV	Average Rectified Value
ATP	Adenosine Triphosphate
ATP_f	Free ATP
BM	Abbreviation of a volunteer's name
Ca^{2+}	Calcium
CAR	Central Activation Ratio
CI	Confidence Interval
Cl	Chloride
CMRR	Common Mode Rejection Ratio

CNS	Central Nervous System
CO_2	Carbon Di-Oxide
Con	Concentric
CPK	Creatine Phosphokinase
CSA	Cross Sectional Area
CT	Twitch Contraction Time
CV	Co-efficient of Variation
CV	Conduction Velocity
DHP	Dihydropyridine
dP/dt	Rate of Force Development
DS	Abbreviation of a volunteer's name
E	Energy
Ecc	Eccentric
ECC	Excitation Contraction Coupling
EMG	Electromyography
EPP	End Plate Potential
FCU	Flexor Carpi Ulnaris
FFT	Fast Fatigue Transform
FG	Fast Glycolytic
FI	Fatigue Intermediate
fig	Figure
FOG	Fast Oxidative Glycolytic
FR	Fatigue Resistant
g	Gram
H^+	Hydrogen
H_2O	Water
HCO_3^-	Bicarbonate
HPAD	Homosynaptic Post-Activation Depression
Hz	Hertz
iEMG	Integrated EMG
$InsP^3$	Inositol Triphosphate
IT	Interpolation Twitch

J	Jerk
K^+	Potassium
K_{ATP} channel	ATP-dependent K^+ channel
kg	Kilogram
kJ	Kilo-Joule
LDH	Lactate Dehydrogenase
LTN	Long Thoracic Nerve
LTNP	Long Thoracic Nerve Plasy
MDF	Median Frequency
MEP	Motor End Plate
Mg^{2+}	Magnesium
MHC	Myosin Heavy Chain
min	Minute
mM	Mille-mole
MN	Motoneuron
MNF	Mean Frequency
mRNA	Messenger ribonucleic acid
MRS	Magnetic Resonance Spectroscopy
ms	millisecond
MU	Motor Unit
MUAP	Motor Unit Action Potential
mV	Mille-volt
MVC	Maximum Voluntary contraction
N	Neuton
Na^+	Sodium
NARJ	Normalised Average Rectified Jerk
NMR	Nuclear Magnetic Resonance
PAD	Primary Afferent Depolarization
PCr	Phosphocreatine
P_i	Inorganic Phosphate
PIPJ	Proximal Interphalangeal Joint
PO_4^{3-}	Phosphorus Oxide

QA	Quickly Adapting
RMS	Root Mean Square
s	Second
S	Slow
SA	Slowly Adapting
SEM	Standard Error of the Mean
SNR	Signal/Noise Ratio
SO	Slow Oxidative
SO_4^{2-}	Sulpher Oxide
SR	Sarcoplasmic Reticulum
STD	Standard Deviation
T	Time
TT	Transverse Tubule
V_{max}	Maximum Shortening Velocity

Definitions

Electromyographic (EMG) signals represent the electrical activity associated with a contracting muscle and considered as the algebraic summation of the different action potentials detected within the field of the recording electrodes.

The Motor Unit: is the single smallest controllable muscular unit and consists of a single α-motor neuron, the neuromuscular junction, and the muscle fibres it innervates.

Motor Unit Action Potentials (MUAP): consists of the spatio-temporal summation of the individual muscle fibre action potentials.

Impedance: is the degree of resistance met by an electric current travelling through a material. All forms of material including biological tissues impose impedance to the transmission of electrical current.

An electrode is a device or unit through which an electrical current enters or leaves an electrolyte; i.e., the electrode is the site of connection between the body and the collection system.

Noise is any unwanted signal detected alongside the wanted signal and can result from distant sources such as power cords, polarization potentials from the metal electrolyte junction or neighbouring muscles.

Gain is the amount of amplification applied to the signal with the use of amplifiers.

Bandwidth is the range of frequencies within which amplifiers and filters would be working. Signals within this bandwidth pass through with minimal diminution, while those outside are suppressed or eliminated.

Common Mode Rejection Ratio (CMRR) is a measure of the ability of a differential amplifier to eliminate the common mode signal.

Common Mode Signals are those detected by both electrodes, usually originating from distant sources such as power supplies and distant muscles, and considered of no interest or as noise.

Jerk (J) is defined as the rate of change of acceleration, the third derivative of distance x with respect to time t ($J = d^3x/dt^3$).

Integrated Jerk ($= \int |J| dt$) averages the jerk over the whole movement segment.

Detour is a parameter used to measure the magnitude of deviation, from straight line, that the path of a line can take to connect two points. Detour is a measure of indirectness and equals (= [trajectory length]/[distance from start to finish] – 1).

Work is defined as the product of force times distance i.e. Work = Force x Distance. Work is expressed in kilopond meters or joules.

Power is the term used to describe how much work is accomplished per unit of time. Power is expressed in watt (W) and is defined as 6.12 $Kpm^{\bullet} Min^{-1}$.

Power =Work/Time

Abstract

Denervation has a deleterious effect on muscle function. There is a controvery regarding the underlying causes of the deficient function observed in reinnervated muscles. The first part in this thesis examines some aspects of the integrated relation between muscles and nerves and possible effects of denervation on muscle fatigue. The second part investigates the relation between cutaneous and muscle sensation and the overall hand sensory-motor performance. Protocols were designed to examine fatigability of the serratus anterior muscle using both isometric and dynamic muscle exercises; and myoelectric fatigue was examined using both surface and fine wire electrodes. EMG was acquired using MP100 machine (BIOPAC Systems, Inc., Santa Barbara, California), and dynamic exercises were performed on a KIN-COM machine (Chattanoga, Inc., Oxfordshire, UK). EMG median frequency and amplitude were calculated. Repeatability studies were conducted on eigth healthy volunteers with mean age of 29-35 years and revealed superiority of surface electrodes and isometric mode of muscle contractions in investigating fatigability of this unique muscle. Five patients with reinnervated serratus anterior muscles following long thoracic nerve palsy were studied with the established methods of testing. Median frequency analysis showed no increase in fatigability of reinnervated serratus anterior compared to control group, however, amplitude changes were different suggesting disturbed motor unit recruitment in reinnervated muscles.

Fatigability of reinnervated first dorsal interosseous muscles following ulnar nerve palsy was investigated using an intermittent and a sustained isometroic contraction protocols. Two groups, the first consisted of four healthy volunteers and the second of seven patients with reinnervated first dorsal interosseous following decompression of ulnar nerve were examined. Analysis of control data did not show a difference in fatigability or strength between dominant and non-dominant hands. In the patients' group, myoelectric and mechanical fatigue charateristics of the reinnervated hands were not different from control. However, reinnervated muscles were found to be weaker. In these experiments intermittent contractions were found to induce higher degree of fatigue compared to the sustained when the mode and contraction levels were standardised.

In the second part of the thesis experiments were designed to examine the effect of cutaneous anaesthesia induced by median nerve block (similar to a severe case of carpal tunnel syndrome) on subjects' ability to perform two tasks: the first was to maintain a submaximal level of force and the second was a fine handwriting task. The second task represented a functional form of evaluation to the sensory-motor performance of the hand. Cutaneous anaesthesia did not alter the subjects' ability on maintaining a submaximal level of force, but resulted in a reduction of their smoothness in performing fine hand manipulations.

For the first time, fatigability of the serratus anterior muscle was investigated and protocols were developed that would allow investigating fatigability of this important muscle. No difference in fatigability was found between reinnervated and normal control muscles, however, there evidence was found for weakness of reinnervated first dorsal interosseous compared to control.

An objective method of evaluation of hand function was introduced and used to record the effect of cutaneous anaesthesia on hand performance. This would open the way for similar methods of electronic assessment and rehabilitation of the hand.

Chapter I: GENERAL REVIEW OF LITERATURE

1.1 Electrical and cellular mechanisms associated with muscle contraction

1.1.1 Muscle resting and action potentials

The resting membrane potential is the result of a difference in electrical potential that exists between the inside of a cell and its fluid environment. In an inactive mammalian muscle the inside of the cell is around 85 mV negative relative to the outside (224).

The resting membrane potential is the result of the difference in ion concentration on the two sides of the membrane. Inside the muscle fibre the most common cation is K^+. The anions are supplied by PO_4^{3-}, SO_4^{2-} and HCO_3^- together with amino acids, polypeptides and proteins. The interstitial fluid outside the cell has a composition similar to plasma with Na^+ and Cl- being the dominant cation and anion respectively in addition to smaller amounts of K^+, Ca^{2+}, Mg^{2+}, HCO_3^- and PO_4^{3-} (224).

The difference in the composition of the intracellular and extracellular fluid is due to two main reasons. The first is that a large proportion of the organic anions in the interior of the fibre is part of the cell structure and therefore cannot migrate to the outside. Secondly, the resting membrane is semi-permeable allowing K^+ and Cl- to transfer freely while impeding the passage of Na^+. Because K^+ (high intracellular concentration) is moving against its concentration gradient and does not fully balance the anions present within the cell, the slight excess of anions intracellularly is responsible for the internal negativity of the resting membrane potential (32).

The membranes of muscle and nerve have the ability to develop transient changes in potential, which can be transmitted from one point to another. The propagated change of membrane potential is the Action Potential (AP) or impulse (224).

The action potential magnitude and propagation have been explained by ionic changes. It has been shown that as the membrane potential is reduced there is an initial flow of Na^+ ions into the cell and this was followed by a flow of K^+ in the

opposite (outward) direction. It is obvious that the membrane permeability to Na^+ changes during the action potential. Under resting condition the membrane is largely impermeable to Na^+. However, during the action potential Na^+ permeability not only increases but the increase becomes self-regenerative. As the membrane becomes more permeable to Na^+ these ions will diffuse into the fibre down their concentration gradient and under the internal negativity of the cell. The entry of Na^+ ions will depolarise the membrane because being positively charged the ions make the inside of the cell less negative. Further depolarisation causes the membrane to become even more permeable to Na^+ and the cycle is repeated (224).

The functional significance of the AP is that once an impulse has been initiated at a site, excitatory changes automatically take place in neighbouring regions of the membrane and cause the impulse to travel the length of the fibre. The travelling of the AP is dependent on the difference of potential between the region of the membrane at which the AP is momentarily located and the more distant sites that are still in the resting state. At the crest of the AP the interior of the fibre is about 30mV positive with respect to the outside while other parts still exhibit the resting potential of 85mV negative to the outside. Because of this difference in potential current will travel between the two regions of the fibre (346).

At the end of an action potential the muscle fibre will have gained Na^+ and lost some K^+. The quantities are so small that a motor nerve axon or muscle fibre can conduct several thousand action potentials without difficulty. During and following periods of muscle activity the changes in Na^+ and K^+ composition of muscle fibres are corrected by means of the Na^+-K^+ pump. The pump actively expels Na^+ outside the cell and attracts K^+ to the inside (346).

1.1.2 Neuromuscular transmission

The neuromuscular junction is the site where excitation spreads from the motor axon to the muscle fibre. In this excitation sequence the impulse invades the motor nerve terminal and allows Ca^{2+} ions to enter and to release Acetyl Choline (ACh) from the synaptic vesicles. The ACh diffuses across the synaptic cleft and combines with receptors in the muscle fibre membrane. This combination alters the permeability of the membrane causing it to depolarise and fire an impulse (3).

The role of the ACh is to act as a chemical intermediary in the transfer of excitation from nerve to muscle. During this process ACh serves to amplify the small impulse current in the motor nerve terminal into one that is sufficiently large to trigger an impulse in the muscle fibre (3).

Ca^{2+} ions have been found to be essential to the process of neuromuscular transmission. When the impulse invades the axon terminal it depolarises the membrane through opening of Na^{+} channels. This depolarisation then activates Ca^{2+} channels allowing Ca^{2+} ions to enter the axon terminal down their concentration gradient. This inward Ca^{2+} current starts slower and lasts longer than the Na^{+} current. It has been found that an impulse invading the axon terminal causes a 10-fold rise in Ca^{2+} concentration in the region of axoplasm adjacent to the plasmalemma known as the active zone. It is in the immediate vicinity of the Ca^{2+} channels that ACh is discharged into the synaptic cleft (203).

The process of Ca^{2+} mediated release of ACh is responsible for most of the synaptic delay that happens between the excitation in the nerve and its subsequent appearance in the muscle fibre. In a mammalian junction the delay in excitation transimission at the neuromuscular junction is about 0.2 ms (18).

When ACh is released from the vesicles into the synaptic cleft it instantly interacts with the ACh Receptors (AChRs). Once ACh attaches to its respective receptors the channel in the centre of the receptor opens to allow K^{+} and Na^{+} ions to flow through. These ions travel in opposite directions moving down their respective concentration

gradients. Therefore, K^+ moves outside and Na^+ inside the cell. The membrane potential tends to fall and this depolarisation is known as the end-plate potential (EPP). When the EPP is fully developed it changes the membrane potential from –85 mV to –15 mV. The rising EPP is overtaken by an action potential that arises in the membrane adjacent to the AChRs and is due to the opening of voltage gated Na^+ channels. The voltage-gated channels are particularly concentrated in the end-plate region and secondary synaptic clefts. Action potential then spreads from the neuromuscular junction area to both ends of the muscle fibre (224).

1.1.3 The sliding mechanism theory for muscle contraction

Within a muscle fibre each myofibril is composed of many myofilaments. These myofilaments are of two types myosin or thick filaments and actin or thin filaments. Each myosin filament is surrounded by a hexagonal array of the actin filaments. Actin and myosin filaments overlap each other in a special arrangement (118) fig..

The sliding mechanism for muscle contraction has been established. Actin filaments on both sides of the myosin propel towards each other and slide along the intervening myosin filament while myosin filaments remain stable and do not alter their shape. This process of sliding is controlled by the formation of cross bridges that arise from the myosin filaments, which momentarily attach themselves to the actin filaments and propel them into the new position (118).

It was proposed that each cross bridge would act as an independent force generator and that the force developed in a contraction would depend on the number of simultaneous interactions between cross bridges and the actin filaments. Stretching a muscle within its physiological limit was found to alter cross bridge formation and attachment angle to the actin filaments in a way that would increase the generated force in a subsequent isometric contraction (32).

Two types of proteins are attached to the actin filaments tropomyosin and troponin. These proteins play an important role in the contraction process. Troponin is a complex of three polypeptides of which troponin I exhibits an inhibitory effect to the

muscle contraction by preventing interaction between troponin and myosin. During muscle contraction this inhibitory effect is overcome by a rise in cytosolic Ca^{2+}. Changes in troponin molecules lifts the tropomyosin molecule away from the actin filament. This movement of tropomyosin exposes sites on the actin filament for interaction with myosin heads allowing for the muscle contraction process to proceed (32).

Ca^{2+} ions play an important role in muscle contraction. A rise in Ca^{2+} concentration in the vicinity of actin and myosin filaments signals the initiation of contraction. Ca^{2+} acts as a messenger between the action potential and the contractile apparatus. The process in which depolarisation of the transverse tubule (T-tubules) and release of Ca^{2+} from Sarcoplasmic Reticulum (SR) take place is called excitation-contraction coupling (100).

T-tubules are situated at regular intervals along the muscle fibre and run inward toward its centre. T-tubules meet each other and form a net-work or a continuous structure. The main function of the t-tubules is transmission of signals to the interior of the muscles fibres. Action potentials recorded superficially at the surface plasmalemma of a muscle fibre are transferred along the t-tubular system to activate central myofibrils (346).

Electrical signal travelling down the t-tubules uses two types of Ca^{2+} channels in order to release Ca^{2+} from the SR. The first type is termed DHP because it is blocked by dihydropyridine. This type of channel is found in high concentration in the membrane of the T-tubules and act as a voltage sensor that transmits a signal to the second type of Ca^{2+} channel found on the surface of the SR (100).

For muscle contraction to terminate, Ca^{2+} ions are pumped back from the cytosol into the SR. As this pump moves Ca^{2+} against a large concentration gradient it requires ATP to provide the necessary energy. For each cycle of this pump two Ca^{2+} ions are transported into the SR in return for two K^{+} ions. This unequal exchange of positive charges causes the cytosol around the SR to become increasingly negative (32, 100).

1.1.4 Muscle fibre types

It is generally agreed that human skeletal muscles are composed of three main fibre types. These fibre types are type I or slow fibres and type II or fast fibres. Type II fast fibres are further classified into type IIa and IIx. This classification is based on histochemical characteristics or the specific isoform of myosin ATPase enzyme found in different fibres. Functional differences between these myosin isoforms explain why fast fibres shorten more rapidly than slow fibres. For example, the myosin isoform found in fast fibres has a high ATPase activity and this promotes a rapid breakdown of ATP and provides the needed energy for a high speed of muscle shortening. In contrast, the myosin isoform found in slow fibres has a low ATPase activity and therefore shortens at a slower rate compared to fast fibres (35).

Type I or slow fibres, also called slow oxidative (SO), contain a large number of oxidative enzymes, high mitochondrial volume and are surrounded by more capillaries. In addition, type I fibres have high myoglobin content. All these characteristics provide these fibres with large capacity for aerobic metabolism and high resistance to fatigue. In terms of contractile properties type I fibres have slower maximum shortening velocity (V_{max}) and produce lower specific tension compared to type II (35, 282).

Two subtypes of fast fibres exist in humans: type IIx and type IIa. Type IIx fibres are called fast glycolytic (FG) have a relatively small number of mitochondria, a limited capacity for aerobic metabolism and are less resistant to fatigue than SO fibres. Type IIx are rich in glycolytic enzymes, which provides them with large anaerobic capacity. The myosin ATPase activity in type IIB is higher than other fibre types resulting in the highest V_{max} of all fibre types. Type IIB, however, are less efficient than other fibre types and this is mainly because of the high energy expenditure per unit of work performed (282).

The second type of fast fibres type IIa or fast oxidative glycolytic (FOG) have biochemical and fatigue characteristics that are between type SO and FG. Therefore, although they have higher V_{max} compared to type SO their V_{max} is lower than FG.

This fibre type is highly adaptable and with endurance training they can increase their oxidative capacity to levels equal with type SO (282).

It is important to note that traditional classifications for muscle fibre types have used type IIB nomenclature to describe the fast fatigue human muscle fibre type. Ennion et al (1995) used a method of single muscle fibre analysis to investigate the presence of RNA transcripts for various isoforms of the myosin heavy chain (MHC) gene in histochemically, immunohistochemically and electrophoretically characterized individual muscle fibres from adult human vastus lateralis muscle. It was shown that the human equivalent to the rat type IIX MHC gene was expressed. This observation was taken to suggest that the previously classified IIB muscles fibres in human muscle express a MHC isoform equivalent to the rat IIX, not the IIB, and would therefore be more accurately classified as IIX fibres(95). Type IIB fibres are the fastest muscle fibre type in small mammals (32).

1.1.5 Motor units

A motor unit is a term that describes a motoneuron and the muscle fibres it supplies . A muscle is composed of many motor units and the muscle's activity is determined by the combined activities of individual units (247).

Muscle fibres within the same motor units have been found to share a common pattern and follow the characteristics of their innervating motoneurones (338). Therefore, within the same motor units muscle fibres were found to be comparable in their ability to develop tension and generate force in addition to similarities in their contraction time, glycogen storage and resistance to fatigue.

Burke et al have defined the criteria of three groups of motor units according to their fatigue index, which is the ratio of the unfused tetanic force after 2 minutes of repetitive stimulation to the force of the first tetanic contraction of the test (38). The three groups are fast fatigable (fatigue index < 25%), intermediate (fatigue index between 25% to 75%) and fatigue resistant (fatigue index > 75%). Gillespie has further adopted this classification and added a fourth group using the twitch contraction time (CT) to differentiate between the slow and fatigue resistant types.

Accordingly four types of motor units were defined: fast fatigue (FF) (fatigue index <25%; CT < 30ms), fatigue intermediate (FI) (fatigue index between 25% to 75%; CT <30 ms), fatigue resistant (FR) (fatigue index > 75%; CT< 30ms) and slow (S) (fatigue index >75%; CT> 30ms) (123).

The innervation ratio of motor units i.e. the number of muscle fibres per motoneuron varies widely from few fibres in extraocular muscles to thousands of fibres in large lower limb muscles. The innervation ratio of a motor unit has been found to correlate closely to its tension producing capability (247).
It has been demonstrated that the fibres of a motor unit are distributed within a localised area within the muscle, and that the size of this territory is specific to the muscle and the size of the motor unit. Bodine et al studied the motor units of the leg muscles in 11 cats. For the tibialis anterior (TA) muscles, the territory of individual motor units were found to occupy between 8 and 24% of the total muscles' cross sections. In general slow (SO) motor units were found to occupy smaller territory compared to the fast units. For the soleus muscle, known to have a homogenous high percentage of SO motor units, the territories of slow motor units were found to occupy higher percentage of the muscles' cross sectional area ranging between 41 and 76% (27).

Fast motor units appear to occupy a larger territory across the muscle surface area compared to the slow ones. Larsson et al reported the sizes of five MU in the medial head of cat medial gastrocnemius to range between 7% and 32% of the muscles' cross section. The mean of two SO MUs was 15% while that for the FF MUs was 23%. In the rat medial gastrocnemius the sizes of the MU territories of FF, FR and SO MUs were found to be 18%, 11% and 8% of the muscle's volume respectively (195).

In compartmentalised muscles i.e. muscles that have separate anatomical compartments each innervated by a separate primary nerve branch and show mechanical partitioning, the fibres that belong to a certain MU are distributed within the boundaries of separate compartments. MUs also appear to occupy only a portion of that compartment. This finding has functional implication and would be consistent with the concept of differential activation of individual compartments during movements conducted by highly compartmentalised muscles. If this occurs parts of

the muscle will be passive but come into activity and contribute to mechanical function of the muscle at certain sequence of different parts of the movement (247).

1.1.6 Energy consumption for muscle contraction

Muscle fibres obtain their immediate energy by splitting Adenosine Tri-Phosphate (ATP). When ATP is split it gives Adenosine Di-Phosphate (ADP), inorganic phosphate (P_i) and Energy (E). ATP $\Longleftrightarrow$ ADP $^+$ P_i $^+$ E (282).

ATP consumption is necessary for every step in the process of muscle contraction. For example, ATP is required for the detachment of the myosin cross-bridges from the actin filaments; hence allows these bridges to move to new positions on the filaments. ATP is also needed for two active ionic pumps; the first is the Na^+-K^+ pump that maintains the muscle in an excitable state during muscle activity, and the second returns Ca^{2+} to the SR at the end of excitation period. Additionally, ATP is required to build up phosphocreatine (PCr) stores in the resting fibres, for phosphorylation of enzymes by protein kinases and for conversion to cyclic adenosine monophosphate (cAMP) at the fibre plasmalemma (146).

Muscle fibres differ from other cells in having a substantial energy reserve in the form of PCr. As ATP is expended it is instantly replenished from PCr by the action of creatine kinase. Although this pathway for ATP production is extremely useful for exercises of short durations and moderate or high intensity the amount of energy produced through it is relatively minor. As the muscle continues to contract and energy provided by the PCr becomes inadequate, muscle fibres become totally dependent on oxidation of fat and glucose. The latter is largely derived from glycogen. The enzyme pathway that consumes fat and glucose converts both fuels to acetyl coenzyme A (acetyl CoA) and then break the latter down to CO_2 and H_2O under aerobic conditions (146).

At the end of the process of glucose breakdown (glycolysis) for each molecule of glucose there is a gain of two ATP molecules and the end product is pyruvic acid. Once pyruvic acid is produced in the cytosol it is taken up in the mitochondria and

oxidized to CO_2 and H_2O. The oxidation of pyruvic acid begins with its decarboxylation and combination with coenzyme A (CoA) to create the intermediary metabolite acetyl CoA that combines with oxaloacetic acid to make citric acid, a six-carbon molecule. Through a series of reactions two of the carbon atoms are oxidised and removed as CO_2, resulting in the eventual formation of oxaloacetic acid. This metabolic cycle, the citric acid cycle, results in the formation of 12 molecules of ATP for each molecule of acetyl CoA. As two molecules of acetyl CoA are produced from each glucose molecule; the citric acid cycle creates 24 ATP molecules. The glycolytic pathway (glucose to pyruvate) adds another 12 molecules of ATP, making a total of 36 ATP molecules for each glucose molecule consumed (146).

In prolonged exercises all muscle glycogen will be consumed and continued effort will then largely depend on lipid metabolism. Fat available to muscle as fatty acids or triglyceride (three fatty acid molecules linked to glycerol). Fatty acids are broken down through a sequence of metabolic steps that eventually result in formation of acetyl CoA and hydrogen ions as by-products. Hydrogen ions are taken into the respiratory chain to produce ATP. Meanwhile acetyl CoA enters the citric acid cycle to produce further ATP (224).

1.1.7 muscle receptors

For the muscles to work effectively the motoneurons must be able not only to commence discharging but also to adjust its rate of discharge depending on the nature of the task performed and the loads applied to the moving part (220). This adjustment of motoneuron firing rate depends on the continuous flow of information to the CNS from receptors in the muscles, joints and the skin. Muscle receptors will be described in this section while skin and joint receptors will be dealt with in future parts of this thesis.

Four types of muscle receptors have been defined. These are the muscle spindles, Golgi tendon organs, Paciniform corpuscles and the free nerve endings (219).

1.1.7.1 Muscle spindle

A muscle spindle is complex type of receptor that varies in diameter (widest part) from 80-250 μm. Its length is usually several mm but can be up to 10mm. The number of the spindles within muscles is quite variable with highest density of spindles found in small muscles of the hand and the neck muscles. This is most probably related to the ability of hand and neck muscles to perform small and accurate movements (219).

A muscle spindle has a characteristic fusiform shape due to the presence of a connective tissue capsule that contains fluid in its middle. In the longitudinal axis of the spindle the central and peripheral parts are referred to as equatorial and polar parts respectively (219).

Two types of muscle fibres are present in the spindle the intrafusal fibres that are present inside the spindle and the extrafusal fibres that surround it. The *intrafusal* fibres are classified into two types. The *nuclear bag* fibres that contain an extraordinary number of nuclei in its middle. The second type of *intrafusal* fibres is the *nuclear chain* in which the nuclei are more evenly distributed along the length of the muscle fibre. The *bag fibres* are less numerous comparable to the *nuclear chain* type and have been classified according to their myosin ATPase histochemical staining into bag_1 *and* bag_2. (219)

Intrafusal muscle spindle fibres are supplied by γ-motor (fusimotor) axons, which can be identified in the ventral roots of the spinal cord by their small diameter. Two types of connections between the γ-motor axons and intrafusal fibres have been described. The first is the plate ending that resembles an extrafusal fibre neuromuscular connection and usually attaches to a nuclear bag fibre. The second type is the trail ending that is usually seen with nuclear chain type of fibres. Occasionally spindles have α-motor axon that is termed β-axon and form a plate ending to a nuclear bag fibre (219).

Muscle spindles receive two types of afferent nerve supply *Group Ia axon*, and *Group II axon*. Each muscle spindle receives a large *Group Ia axon* which terminates around the equatorial part of the intrafusal fibres forming the *primary ending*. *Group II axons*

terminate on the nuclear chain and bag_2 fibres to constitute the *secondary ending* (219).

Intrafusal muscle fibres were found to respond differently to excitation through their axons. Contractions of the bag fibres and in particular the bag_1 were found to be slower and weaker comparable to the chain fibres. As the intrafusal fibres receive their innervations on the polar ends away from the central nuclear part, it is the central part that is stretched by the contracting ends. Contraction of intrafusal fibres was found to increase their sensitivities to stretch (219, Brooks, 2003 #646).

When a muscle spindle is exposed to stretch, Ia axons fire a high frequency burst of impulses while stretching takes place, termed the dynamic response. This original response is followed by a steady discharge of impulses when the muscle spindle is held in its new position termed the static response. Meanwhile, type II axons have only a static response. It is important to note that the Ia axons have a much lower threshold to stretch compared to the Group II axons. It has been suggested that the difference in response between the two types of axons is mainly a reflection of the fact that Group Ia axons supply both nuclear bag and chain fibres, while Group II axons are only limited to the nuclear chain fibre type (220, 221).

Impulses from Group Ia and II were found to modulate the motoneuron discharge rate through spinal reflexes. It has been shown that the Ia discharge rate has an excitatory effect on the homonymous (supplying the same muscle) motoneuron. This connection provides a means for rapid adjustment of muscle length following any stretch or change in the applied load. Group II axons resemble Ia axons in providing an excitatory effect on the homonymous motoneuron though the connection is usually through more than one interneuron i.e. disynaptic (220, 221).

Muscle spindles initiate the muscle tone and tendon reflexes. By tendon reflex it is meant that a sharp tap on a tendon stretches the muscle belly and the spindles contained within it evoking an impulse volley in the Ia axons. These axons in turn evoke a brisk reflex contraction of the muscle. The electrical equivalent of the tendon reflex is the H-reflex. In the H-reflex the impulse volley in the Ia axons is set up by an electrical stimulus to the motor nerve, rather than by a mechanical perturbation (116).

1.1.7.2 Golgi tendons and free nerve endings

The tendon organs are encapsulated receptors found at the musculotendinous junction. A Golgi tendon organ is typically 0.5-1.0 mm long and 0.1-0.2 mm in diameter. Sensory impulses are transmitted from the tendon organ to the spinal cord through a relatively large diameter nerve axon termed Ib axon (224).

Tendon organs respond to muscle contraction by discharging impulses that have an inhibitory effect on the motoneuron of the same muscle and excitatory to the motoneurons of the antagonist muscle. These reflex connections contribute to adjustments of the muscle force (116).

The last type of muscle receptor and the most abundant is the free nerve endings. The free nerve endings transfer their impulses to the spinal cord through the smallest myelinated nerve fibres (Group III afferents) and the nonmyelinated fibres (Group IV afferents) (1).
Group III and IV afferents are sensitive to mechanical changes within the muscle such as contraction, pressure and stretching. Some of these nerve endings are specifically sensitive to chemical changes like K^+ ion or lactic acid concentration. The nociceptive endings are a specific type of the nerve endings sensitive to the release of neuropeptides like bradykinin and arachidonic acid and therefore respond to stimuli that cause tissue damage (1).

1.1.8 Muscle fibre plasticity and training effect

An interesting phenomenon about muscle fibres is their ability to switch from one MHC category to another, termed the muscle plasticity. The change in muscle fibre myosin isoform can be induced in response to changes in motoneuron-specific impulse patterns, neuromuscular activity, and mechanical loading. Depending on the type, intensity, and duration of changes in any of these factors, muscle fibres adjust their phenotype to meet the altered functional demands. Fibre-type transitions resulting from multiple qualitative and quantitative changes in gene expression occur sequentially in a regular order within a spectrum of pure and hybrid fibre types (272).

Muscle fibres response to training depends on its intensity, duration and number of repetitions. Endurance training (low intensity, multiple repetitions) like marathon running and cycling is believed to facilitate the increase in slow twitch/SO fibre type within muscles (282). Green et al 1979 studied the influence of an extensive history of participation in high intensity activity on muscle fibre type, fibre size, and metabolic profile. Biopsy samples from the vastus lateralis muscle were obtained from ice hockey players prior to and following the season and compared with control subjects. No significant differences were found in the percentage (49.6 vs. 43.8%) or the size of the SO fibres between the study and control groups, nor was there any significant alteration following the season of play in these variables. For the fast fibre subgroups, a reduction in the FG (12.2 vs. 3.9%) and an increase in FOG (38.0 to 45.2%) fibre populations occurred over the season. It was suggested that transitions in muscle fibre types were limited to the fast fibre subtypes and thus consist of fast to less fast transitions (131).

Luginbuhl et al (1984) studied the effect of intense interval running on the fibre type composition of soleus (S), plantaris (P), deep vastus lateralis (DVL), and superficial vastus lateralis (SVL) of the rats. The muscles were assessed by histochemical ATPase analysis for distribution of fibre type and biochemically for citrate synthase activity (an aerobic marker). Training has induced a significant increase in citrate synthase activity in all muscles. The distribution of fibre types within the S (85% slow-twitch) and SVL (100% fast-twitch) remained unchanged with training.

However, significant increases in the percentage of type I (slow-twitch) fibres in both the P and DVL were observed with concomitant decreases in the type II (fast-twitch) population. Additionally, training induced significant changes in the fast-twitch subtype populations of the DVL with conversion from type IIB to IIA. These findings provided evidence that exercise-induced fibre type transformations occur both within the fast-twitch population and between fast-twitch and slow-twitch fibres in certain muscles of the rat following a high intensity interval-training program (204).

It is important, however, to note that the MHC changes are species specific. Jaschinski et al studied the influence of forced contractile activity induced by chronic low frequency stimulation (CLFS) of rat extensor digitorum longus muscle on mRNAs specific to four myosin heavy chain isoforms [MHCIIB, MHCIIx, MHCIIa, and MHCI]. Marked changes were noted in MHCII subtypes. The MHCIIB mRNA reached ~50% its normal level in 3-day stimulated muscles and its amount continued to decay, reaching an almost 35-fold lower level in 42-day. Similarly, MHCIIx mRNA level decayed with long stimulation periods and by 42 days it was reduced by 4.5-fold. In contrast, MHCIIa mRNA started to increase after 7 days, and after 42 days its level was 3.4-fold elevated over control. MHCI exhibited only small changes and was 1.8-fold elevated in muscles exposed to CLFS for 42 days. The first significant increase was noted in 60-day stimulated muscles when it amounted to ~10%. In 100-day stimulated muscles, MHCI amounted to $12.0 \pm 4.0\%$, corresponding to an approximately twofold increase over control (159).

In contrast to Jaschinski's findings, Leeuw et al (1993) demonstrated that MHCIIx, which is the predominant fast myosin isoform in rabbit tibialis anterior, was exchanged for MHCIIa and the latter was replaced by the slow MHCI following a protocol of repetitive electric stimulation of the tibialis anterior muscle. It has been proposed that in rabbits full conversion of MHCIIB to MHCIIa then finally to MHCI was possible. These findings supported the proposal that the training effect on the fibre type is dependent on the muscle (whether it is predominantly slow of fast twitch fibres) as well as on the animal species (197).

In addition to the alteration of muscle fibre myosin isoforms, training also results in a multitude of metabolic and structural changes within the muscle. It is well known that endurance training, employing an appropriate duration per day, frequency per week, and submaximal intensity per exercise bout, can produce an increase in mitochondrial content. This increase is usually ranging from 50 to 100% within about six weeks (323).

The marked improvement in endurance performance that results from mitochondrial biogenesis is a consequence of changes in muscle metabolism during exercise. During acute muscle contraction, the concentration of free ADP (ADP_f) rises. This increase drives the creatine phosphokinase (CPK) equilibrium reaction toward the formation of ATP and creatine. ADP_f is also a substrate and allosteric activator in the glycolytic pathway, and it controls active mitochondrial respiration. As endurance training increases the mitochondrial content of skeletal muscle without large effects on CPK or glycolytic enzymes, it has been assumed that with endurance training a greater part of the energy requirement of a given work effort would be derived from aerobic metabolism. This was referred to as a greater sensitivity of mitochondrial respiration to ADP_f since a lower increase in the concentration of the metabolite is required to attain the same level of oxygen consumption. The reduced increase in ADP_f would attenuate glycolysis and the formation of lactic acid, and consequently sparing of PC (147).

A higher mitochondrial content as a result of chronic exercise improves lactate oxidation, resulting in lower rates of release from muscle. In addition, the higher the mitochondrial content per gram of muscle, the lower the rate of respiration required per mitochondrion for any given workload. Therefore, the increased mitochondrial content brought about by endurance training not only leads to a reduced lactate production but also enhances the disposal of lactate (147).

The response of muscle fibres to strength training (high-resistance training) is different to that mentioned in endurance training. Strength training is associated with muscle enlargement (increase in cross sectional area). This increase in muscle size has been attributed to muscle fibre hypertrophy (increase muscle fibre size) and hyperplasia (increase in number). Fast twitch (type II) fibres develop slightly more

specific tension (i.e. force/cross-sectional area) than type I fibres. Hence, strength training causes enlargement of both type II and I fibres but with preferential enlargement of the former compared to the latter (206).

In contrast to the influence of training, muscle unloading is known to produce atrophy. This unloading can be in form of simple disuse, reduced weight-bearing activity, immobilization, bed rest or an extended stay in weightlessness (19, 88).

Animal studies revealed changes in muscle fibres MHC isoforms in response to muscle atrophy that follow hind limb suspension. This change in MHC isoforms is associated with change in the contractile velocity that shifts type I fibres from a low to higher contractile speed to approach that of type II fibres (315).

In humans the situation is different. Berg et al examined the effect of six-weeks of bed rest on the quadriceps muscle of seven healthy men. This period of bed rest was found to reduce the quadriceps maximum isometric torque by 24 ± 10%, and the maximum concentric torque by 29 ± 12%. Using enzyme histochemical analyses with ATPase staining the types of the muscle fibres were determined and cross sections were measured. Clinical and MRI methods were also used to determine the whole muscles' cross sectional areas (CSA). After bed rest the mean fibre CSA and diameter were reduced by 17.6 ± 13.6 and 7.8 ± 9.0% respectively. Type I fibres' CSA and diameter decreased by 18.2 ± 13.9 and 10.3 ± 7.9%, respectively. Type IIA and IIB fibres showed no change. However, the relative CSA occupied by type I fibres and the MHC composition have not changed. The authors concluded that muscle atrophy as a result of bed rest did not change the fibre type composition. Moreover, it was suggested that the reduction in muscle and fibre CSA could not account for the reduction observed in maximum torque, hence a reduction in central neural drive was proposed as at least partially responsible for that decline (19) .

It has been argued that although transformation of muscle fibre protein could not be observed in humans this does not necessarily signify that fibre-type transformation is not in progression during a six-weeks bed rest period. Human muscle fibres are characterized by the specific MHC isoforms. Three distinct types have been described

MHCIβ / slow, type IIa and IIx. In addition to these fibres there are few hybrid forms of fibres co-expressing various amounts of two MHC isoforms that can be recognized (4). Anderson et al 1999 studied the effects of a 37-day period of bed rest on MHC expression on both mRNA and protein level in human skeletal muscle fibres using in situ hybridization, immunocytochemistry, and ATPase histochemistry. No significant change was observed in fibre-type distribution as determined by ATPase histochemistry and immunocytochemistry. Using the in situ hybridization technique it was noted that there was a reduction in the proportion of fibres positive for mRNA for Iβ or IIa. In contrast there was an increase in the number of fibres simultaneously positive for IIa and IIx, as well as for fibres solely positive for IIx. Another group of fibres was recognised and found to test positive for type I fibre at protein level. At the mRNA level these fibres were simultaneously positive for MHC isoforms for (Iβ, IIa and IIx), or positive for (Iβ and IIx) (4).

It has been suggested that muscle fibres can be of two categories. The first category represents the stable or steady state fibre phenotypes that test positive for a certain protein and its corresponding MHC isoform. The second category represents fibres in a transitional state during which a mismatch between the MHC expression at the mRNA and protein levels exist. For example some fibres may test positive for MHC type I at protein level while at the mRNA level a positive test can be found for Iβ and IIa simultaneously (159).

It was concluded that this mismatch between protein and mRNA expression of MHC was a reflection of an incomplete transitional process. Longer periods would be necessary for the changes seen at the level of MHC transcripts to be observed at the isoprotein level and for the newly formed isoproteins to be incorporated into the myofibrils (159).

These findings were supported by the results of recently conducted experiments by Trappe et al 2004. Six human subjects were tested after 86 days of bed rest. The results confirmed an increase in the number of hybrid MHC isoform fibres within the vastus lateralis muscle from 13% before to 49% after. Three forms of the hybrid fibres were observed I/IIa, IIa/IIx and I/IIa/IIx. The increase in hybrid fibres number was associated with a significant reduction in type I fibres. Type II fibres are known to have higher contractile velocity and higher ability to develop force compared to

type I. The influence of muscle fibre transformation was seen as an increase in the overall contractile velocity of atrophied muscles. However, muscle atrophy is accompanied by a decline in the muscle force generating capacity as a result of the reduction in muscle CSA and neural drive (331).

1.2- Fatigue and its mechanisms

Muscle fatigue has been defined as the failure to maintain force output leading to a reduced performance (6). Edwards (1981) introduced a more precise definition, which is the failure to maintain the required or expected power output (90). This definition recognizes that the ability to sustain a given work capacity without decrement requires the maintenance of both force and velocity. Any factor that reduces the rate of force development (dP/dt) would contribute to fatigue by decreasing the percent of peak force obtained in the first few seconds following muscle activation. Hence, the velocity and power achieved would also be compromised (106).

It has to be emphasised however that maximal force generating capacity of muscles starts to decline once exercise commences so that fatigue begins almost at the onset of the exercise and develops progressively before the muscle fails to perform the required task. Another definition of fatigue has therefore been introduced, which is the loss of the capacity for developing force and/or velocity of a muscle resulting from muscle activity under load that is reversible by rest (346). Finally, fatigue refers not only to a physiological or pathological state in which muscles perform below their expected maximum, but to a symptom reported by subjects in whom there may be no obvious defect in muscle performance (346).

Muscle fatigue is a complex phenomenon its aetiologies are still to be clearly established and various new factors are being discovered. Bigland-Ritchie (1984) has defined the major potential sites of fatigue as 1- excitatory input to higher motor centres, 2- excitatory drive to lower motor neurons, 3- motor neuron excitability, 4- neuromuscular transmission, 5- sarcolemma excitability, 6- excitation-contraction coupling, 7- contractile mechanism and metabolic energy supply and metabolic accumulation. Considerable controversy exists to the role of each of these sites particularly the relative importance of central and neuromuscular transmission (step 1-4) versus peripheral (steps 5-8) mechanisms in the aetiology of muscle fatigue (23).

In investigating fatigue two terms are commonly used to express the method of muscle activation, "voluntary activation" that refers to notional level of drive to

muscle fibres and motoneurons. The second term is the "maximum evocable force" which is the force produced when the muscle is fully activated by volition or appropriate electrical stimulation and is the formal term for true maximal force (346).

The time course of fatigue and possibly the underlying mechanisms depend to a large extent on the experimental protocol. In general, there are two regimes for induction of fatigue continuous high frequency stimulation and repeated tetani (346).

Fatigue resulting from continuous high-frequency stimulation is called high frequency fatigue. This type of fatigue develops rapidly with a tension-decline half time of 5-30 sec. Recovery after high frequency fatigue is also rapid and has an initial component requiring only few seconds to be complete. The rate of decline of force was found to be slower or less steep during maximum voluntary contraction compared to the continuous high frequency stimulation. Though continuous high frequency stimulation does not occur during normal activity, the resulting type of fatigue is a useful model to study fatigue mechanisms in particular the ionic changes (346).

Fatigue that results from repeated tetanic stimulation (low frequency fatigue) develops at a much slower rate than the high-frequency fatigue. The rate of force decline depends on several factors like the stimulation scheme, the motor unit and fibre type investigated. Similarly, the recovery period after low-frequency fatigue is slow and consists of multiple phases during which force-generating ability is reduced (336, 346).

1.2.1 - Central fatigue

It is not possible to specify all the sites within the CNS that contribute to voluntary activation of muscle, central fatigue and supraspinal fatigue. The traditional meaning of central fatigue mechanisms includes a chain that originates from high levels within the CNS via descending paths to the motoneurons and then via motor axons to the neuromuscular junction. A number of arguments has been presented to support the contribution from each of these sites to the fatigue process (116).

1.2.1.1 Is voluntary activation sub-maximal in maximal efforts?

If in maximal voluntary efforts the CNS fails to generate maximal evocable force then a reserve exists that could be used when necessary. There are two possibilities, the first is that voluntary activation is maximal at the start of exercise i.e. the limit to the performance develops through peripheral mechanisms of fatigue. The alternative possibility is that voluntary activation is submaximal and therefore, central mechanisms of fatigue present at least partial contribution to the motor performance or the development of the fatigue process (116).

Merton (1954) tried to answer the question of whether a voluntary force is limited by the capacity of the nervous centres and conducting pathways to deliver motor impulses to the muscle fibres or by the intrinsic contractile properties of the fibres themselves. The author compared the force generated by maximal voluntary adduction of the thumb to the adduction force generated through artificial tetanic stimulation of the ulnar nerve at the wrist joint, superimposed twitches were added while the muscles were contracting at different percentages of their MVC. In the same experiment superimposed twitches were delivered during fatiguing contraction to discover whether adding an artificial stimulatory impulse could reverse the decline in force. The effect of tetanic twitch was also recorded under ischaemic conditions. Merton reported that maximum voluntary activation of the muscle generated equal tension compared to the tetanic stimuli. A linear correlation was found between the increment in force following superimposed stimuli and the original level of muscle contraction. The lower the percent of MVC the larger the increment of force that

happened with the interpolated tetanic stimuli. At any point on the decline in force slope, superimposed tetanic stimuli failed to increase tension. When fatigue was induced under ischaemic conditions muscles failed to develop tension when tetani were applied during the recovery period (236). Three conclusions were drawn from this study. The first was that during a maximum effort the muscle fibres whose motor nerve was stimulated were already contracting maximally i.e. volition has successfully activated muscle fibres to its maximum contraction capacity. Secondly, the relation between voluntary force and the size of interpolated twitch indicated that absolute maximum force could be predicted by linear extrapolation. Finally, if fatigue was a phenomenon of the central nervous system it seemed most improbable that recovery should be delayed by occluding the circulation to the arm (236).

It has been argued that the experiments conducted by Merton which ruled out the presence of central factors in fatigue lacked essential details about the instructions given to subjects. For example presence of visual feedback about the level of force, verbal encouragement during the MVC and time allowed between the repetitions of the tests, which influence the level of force generated during fatigue experiments (117).

Other experiments with the use of twitch interpolation technique have presented different results from those reported by Merton. Grimby et al examined the relative role of central and peripheral mechanisms of fatigue in voluntarily maintained maximum contraction of the extensor hallucis longus muscle. Superimposed tetanic stimulations of 50Hz were delivered through surface electrodes to the common peroneal nerve. Voluntarily maintained dorsiflexion torques were found to decline more rapidly compared to those contractions supported by the superimposed tetanic stimuli. These findings suggested that the reduced neural drive had contributed to the drop of force and the development of fatigue (134).

The effect of training on muscle strength has been investigated to support the influence of central factors on muscle performance and fatigue. McDonagh et al studied the effect of daily MVC training sessions for five weeks on the elbow flexors of four men compared to a control group. Following these training sessions the supramaximal stimulated isometric twitch force and voluntary isometric contractions were measured. Training was found to increase the MVC by 20% while electrically evoked forces remained unchanged (225).

In another experiment Narici et al reported the results of training sessions performed over 60 days and detraining over 40 days. Trained and non-trained legs of four males who performed maximal isokinetic knee extension exercises were assessed. The MVC of knee extension, integrated EMG (iEMG) and cross sectional area (CSA) were assessed. For the trained legs there was an increase in CSA by 8.5%, iEMG by 42.4% and in MVC by 20.8%. No change in CSA of the untrained legs was found. However, there was an increase in iEMG by 24% and MVC by 8.7%. The authors proposed that the increase CSA (only 8.5%) could only account for 40% of the increase in MVC. Increased neural drive has been suggested as contributing to 60% of the increase in MVC. This view was supported by the remarkable increase in iEMG (255).

Yue and Cole evaluated the effect of four weeks of imagined training on the MVC of the hand abductor digiti minimi. This muscle was used because it is rarely used in large sustained efforts. Voluntary strength increased by 22% in those undertaking imagined training and 30% in those training with real contractions. This study provided unequivocal evidence that voluntary efforts of the tested muscle did not produce maximal evocable force before training (358).

The conclusion that can be drawn from the above studies is that if the increase in voluntary strength that happened with training exceeded what could be attributed to the local changes in the muscle, then some improvement must have occurred in the CNS. This CNS effect can be through learning or altered patterns of muscle and motor unit recruitment (255, 358).

1.2.1.2 Motor unit recruitment and "muscle wisdom"

Motor units have been found to follow a certain order of recruitment during voluntary muscle contractions (247). Henneman (1981) established the motor unit size principle stating that during voluntary muscle contractions motor units with slow conduction velocity, producing low force levels are recruited first then followed by those with fast conduction velocity that produce larger forces. This principle links motoneuron (MN) properties like small size and low axonal conduction velocity with muscle fibre properties, for example small twitch force, long contraction time, slow fibre conduction velocity and low fatigability (247).

Sokoloff et al examined the hypothesis that MNs with slow conduction velocity (CV) are recruited before MNs with higher CV. The authors studied MN recruitment in two muscle pairs, the lateral gastrocnemius (LG) and medial gastrocnemius (MG) muscles, and the MG and posterior biceps femoris (PBF) muscles in decerebrate cats. During stretch stimulation recruitment order was predominantly from low to high CV for 29/34 pairs of motor axons or 85% of the sample. Mechanical stimulation of the heel skin allowed evaluation of the recruitment order across the LG and MG during an additional natural stimulus. In all trials of these MN pairs (27/27), the MN with the slowest CV was recruited first. The authors concluded that the MN recruitment order according to the size principle is still applicable to MNs within the same muscle (310).

Motor commands generated in the CNS of mammals are ultimately translated into skeletal muscle force through two processes, by varying the number of MU participating in a contraction and modulating the rate at which APs drive active MUs (rate coding). The concept of MU substitution has been introduced and postulated to offset the effects of fatigue. This concept describes a process where higher threshold MUs are recruited to replace lower-threshold MUs that have stopped firing. A related concept used synonymously is motor unit rotation. By this it is meant that MUs alternate their activity in a cyclical fashion. Hence, substitution of one MU by another would be followed by back-substitution of the original unit (57). Westgaard and De Luca (1999) conducted a study on the human trapezius muscle during sustained low-level isometric contraction, a manipulation task with mental concentration and

copying a text on a word processor. The authors provided support for MU substitution during prolonged contractions. It was speculated from this study and others that MU substitution protects MUs from excessive fatigue in sustained contractions (347).

Marsden et al (1979) have made a key observation in regard to the MU behaviour. During a sustained MVC the firing rate of a single MU in the first dorsal interosseous muscle was found to decline from 60-80 Hz at the start of the contraction to ~20Hz after 30 seconds (215). This reduction in MU discharge rate that accompanies the fall of force during isometric voluntary contraction has been the subject of interest by many researchers.

Jones et al 1979 conducted their experiments on the adductor pollicis muscle of the hand. The loss of force during isometric MVC was compared with that due to 60 s of maximal ulnar nerve stimulation at different frequencies. Stimulation of the ulnar nerve by 80Hz stimuli was necessary to produce forces that match the MVC. With continuous stimulation at 80 Hz, force decreased to about 80% of its initial value in the first 12 s, then more rapidly to reach less than 20% by 60 s. A continuous tetanus at 80 Hz was interrupted at intervals by stimulation at 20 Hz. After 12 s of stimulation at 80 Hz the force had declined considerably whereas that at 20 Hz was well maintained. Thus stimulation at 20 Hz gave substantially more force than the high-frequency stimulation i.e. high frequency stimulation actually reduced force generation in the fatigued muscle (169). Jones and Bigland-Ritchie (1986) in a subsequent study provided some evidence that the mechanism underlying high-frequency stimulation impairment of muscle excitation was related to alteration in extacellular ion concentration particularly the reduction in extracellular Na^+(25).

Sustained MVC are normally associated with a reduction in the MUs' firing rate. Usually MUs' discharge rate are reduced from 30 impulses s^{-1} to about 15 impulses s^{-1} during sustained MVC for 60 sec. This reduction in MU discharge rate is associated with slowing in contractile speed, which should promote a shift in the force-frequency curve towards lower frequencies, thus allowing for maximum muscle or MU force to be achieved with lower activation rates (23).

The above-mentioned findings led to the proposal, referred to as muscle wisdom, that the decrease in motor neuron output observed during MVC is an adjustment of the

CNS that optimises rather than undermines force output during fatigue (216). It was suggested that once MUs are recruited at a high firing rate near maximal force it can be maintained with frequencies well below those initially required for the contraction to start. It has been shown that asynchronous stimulation of several groups of MUs produced higher forces than if they were all discharging synchronously. Therefore, a low firing rate in sustained voluntary efforts is not on its own sufficient to indicate that a muscle is not producing its optimal force. The critical factor in fatigue with voluntary efforts is whether the firing rate falls too much so that sub-maximal force is produced (116).

The hypothesis of muscle wisdom has recently been revisited by Fuglevand and Keen (2003). The authors repeated Jones' experiment on the adductor pollicis muscle using lower rates of stimulation. In contrast to Jones' high frequency rate of stimulation with 80-100 Hz stimuli, Fuglevand and Keen started their experiment with 30 Hz stimuli that were maintained for 60 s in one experiment and reduced to 15 Hz over 60 s in another. This pattern of decrease in stimulus frequency corresponds to the natural change in MU firing rates recorded during sustained MVC of the adductor pollicis. The authors found that during continuous stimulation at 30Hz force declined by 20% over the course of 60 s. In the same test the amplitude of the evoked EMG responses (M wave) exhibited slight potentiation over the first 20 sec, then gradually declined through the remainder of the fatigue test. In the second test when stimulus rate was reduced progressively from 30 to 15 Hz, the force output declined by about 40%, which is double the force loss that occurred when the stimulus rate was maintained at 30 Hz. A sustained MVC was found to decrease by 40% in 60 s similar to the second test when the stimulus rate was reduced from 30 to 15 Hz. The results from this test did not support the muscle wisdom theory that attributed fatigue process solely to muscular mechanisms. The authors suggested that reduction in neural drive accounts at least partially to the development of fatigue (113).

1.2.1.3 Contribution to muscle fatigue at spinal level

The exact role of muscle spindles to the development of fatigue is not well defined. Several arguments suggest that muscle spindle inputs can facilitate human motoneurons during isometric contractions and fatigue. First, when the small fibres and fusimotor axons were blocked the firing rate of motor units decreased and became more irregular (29). Second, Bongiovanni et al (1990) examined the effect of high frequency muscle vibration on weak or moderate voluntary contraction in fatigued muscles and the outcome was assessed by measuring the motor unit firing rates from the ankle dorsiflexors. Vibration was found to provide a facilitatory effect that allowed the maintenance of motor unit firing in the presence of fatigue. It was concluded that there is a facilitatory effect conveyed through the discharge of type Ia afferents that tends to decline with the occurrence of fatigue; vibration was able to compensate for this afferent input and facilitate the motor drive (30)

Finally, Griffin et al investigated the effect of two protocols of fatigue on the motor unit discharge rate. The fatigue protocols were dynamic and isometric following a similar scale for force production and the only difference was in regard to the arm movement. Motor units discharge rate from the lateral head of triceps muscles of 12 subjects were studied. During the isometric task there was a significant drop in motor unit discharge rate from 13.0±2.7 Hz (mean ± STD) to a minimum of 9.2±1.3 Hz. In contrast, during the dynamic task the firing rate was constant with discharge rate at the minimum point (11.0±1.4 Hz), and at the end (12.2±2.0 Hz) were not significantly different from the initial firing rate of 13.0±2.7 Hz. This study confirmed that the motor unit discharge rate tended to decline when the movement was substituted for isometric force pulses. The maintenance of motor unit discharge rate during dynamic fatiguing contractions was attributed to higher muscle spindle activation and blood flow during dynamic than during isometric fatiguing contractions (133).

Decreased excitation from Ia afferents, due to decreased firing frequencies of muscle spindles, has been suggested to be one mechanism that could cause central fatigue. It is also possible that the size of the excitatory postsynaptic potential induced by each Ia

afferent action potential decreases during fatigue. The strength of the excitation achieved by a same-sized compound Ia afferent action potential on its homonymous motoneuron pool can be measured by means of the Hoffmann reflex (H reflex). A decrease in the H reflex can be attributed to decreased motoneuron excitability and/or to increased presynaptic inhibition of Ia afferents. Presynaptic inhibition of Ia afferents could arise either from primary afferent depolarization (PAD) which lasts for up to 500 ms, or from homosynaptic postactivation depression (HPAD) , with a duration of up to 15 s. Whereas the PAD interneurons are controlled by both afferent and descending circuits, HPAD is believed to be caused by an intrinsic mechanism within the Ia terminal itself and only occurs when the muscle spindle is, or has recently been, discharging. It is possible that reduced facilitation from Ia afferents via presynaptic inhibition could be influential enough to weaken the net excitatory input to the motoneuron pool and thereby contribute to an impaired ability to fully activate a muscle (133).

Nordlund et al 2004 recently investigated the peripheral and central contributions to fatigue during isometric intermittent maximal voluntary ankle plantar flexions. The authors demonstrated a reduction in the H reflex when measured after the onset of a fatiguing protocol. This reduction was attributed to the presynaptic mechanisms. It was difficult to deduce from this study whether the decrease in presynaptic inhibition was due to decreased PAD-mediated inhibition or decreased HPAD. The distinction between the two presynaptic mechanisms is important for the interpretation of whether excitation from muscle spindles increases or decreases during fatiguing exercises. Whereas decreased HPAD would be caused by decreased muscle spindle firing, decreased PAD-mediated inhibition with sustained muscle spindle firing would imply an increased excitation from the Ia afferents onto the motoneuron pool (258).

Another possible mechanism for the reduction in the H reflex is a decrease in motoneuron excitability due to increased inhibition from group III and IV afferents.

Group III and IV muscle afferents innervate free nerve endings distributed widely throughout a muscle. These afferents are either silent or maintain low background discharge rate (<1 Hz) and respond to mechanical, biochemical and thermal changes

within the muscle. The discharge of these afferents in response to the various changes is dependent on the type, intensity, and duration of contraction as well as muscle perfusion (115).

Kaufman et al 1983 examined the effect of static contractions as well as capsaicin (100-200 micrograms) and bradykinin (25 micrograms) on the activity of the groups III and IV afferents of the gastrocnemius muscles in anaesthetized cats. No relationship was found between those fibres stimulated by contraction and those stimulated by chemicals. The authors suggested that although both groups III and IV muscle afferents were evoked by static exercise, group III fibres were likely to be stimulated by the mechanical effects of muscular contraction, whereas at least some group IV fibres were likely to be stimulated by the metabolic products of muscular contraction (186).

Andreani et al investigated the response of groups III and IV afferents to dynamic contractions induced by electrical stimulation of the mesencephalic locomotor region in anaesthetised cats. The set of dynamic contractions was of a low level that although has increased the O_2 consumption by 2.5 times from the resting state, did not cause a significant change in the pH or CO_2 levels. For the group III afferents there was a significant increase in discharge rate that commenced within two seconds of the onset of contraction. 68% of the recorded action potentials were discharged in response to the contraction phase of the protocol, and 12 out of 18 of the afferents that responded to the contraction were discharging synchronously. All group III afferents responded to nonnoxious probing of the triceps surae muscles. Six of the 24 group III afferents responded weakly to stretching the calcaneal tendon.

For group IV afferents, eight of the 10 afferents were stimulated by dynamic exercise. Of the eight afferents stimulated by exercise, only two discharged synchronously with the contraction phase of the dynamic cycle. Like the group III afferents that responded to exercise, the group IV afferents responded to exercise within 2 s of its onset. This increase in activity was maintained throughout the entire period of exercise. After the exercise period ended, the afferent discharge rates returned rapidly to their resting levels. During exercise, 44 % of the action potentials discharged by the group IV afferents occurred during the contraction phase of the dynamic contraction cycle. Each of the ten group IV afferents responded to noxious probing of the muscles; they

did not, however, respond to nonnoxious probing. Similarly, none of the ten responded to mild tendon stretch.

Andreani and her colleagues concluded that the sensitivity of group IV afferents to mechanical stimuli did not appear to be high. They speculated that these unmyelinated afferents responded to metabolic changes that happened within the muscle but did not change the pH. In contrast group III muscle afferents were sensitive to mechanical distortion of their receptive fields indicated by their response to tendon stretch and nonnoxious probing of their receptive fields. Moreover, group III afferents responded briskly at the onset of tetanic contractions. The sensitivity to mechanical distortion possessed by group III afferents probably explains the synchronous discharge with the contraction phase of the dynamic cycle (1).

It has been proposed that group III and IV afferents initiate a reflex modulation of motoneurons firing rates during muscle fatigue (273). Pettorossi et al have studied the influence of blocking these fibres (using Capsaicin a selective neurotoxin that eliminates some muscle afferents that belong to group III and IV afferents) on motor unit excitability and the monosynaptic reflex during fatigue in spinalized rats. Muscle fatigue was induced by a series of increasing tetanic electrical stimuli (85 Hz, 600 ms) delivered to the lateral gastrocnemius (LG) muscle nerve. Potentials from the spinal cord LG motor pool and from the ventral root were recorded in response to afferent stimulation. The compound action potentials of the nerve supplying the LG muscle before and 2 h after injection of different doses of capsaicin into the LG muscle were compared. The size of the potential wave associated with group III fibres, corresponding showed a reduction of about 20 %, and that associated with group VI a reduction of about 80 %. The effect of repetitive muscle stimulation on the size of the pre- and postsynaptic waves was remarkably changed after capsaicin treatment. In capsaicin-treated rats, the potentials did not show inhibition in response to 12-, 30- and 60-train sequences and remained enhanced at the same level reached after 3- and 6-train sequences, so there was no correlation between the muscle fatigue level and potential size. On the other hand, the control groups showed a significant progressive decrease of the potential size after the 12-train sequence (273).

The authors suggested that capsaicin-sensitive fibres are responsible for the reflex inhibition induced by muscle fatigue. Since high doses of capsaicin injected into the

LG muscle block most of group IV and some group III muscle afferents, the disappearance of the reflex reduction could be attributed to the lack of inhibitory input from these fibres. The capsaicin-sensitive fibres may influence the monosynaptic reflex by postsynaptic inhibition of the motoneurons, or by presynaptic inhibition of the afferents impinging upon the motoneurons (273).The presynaptic effect may be mediated by interneurons or may be due to a direct influence of capsaicin-sensitive afferents on the large Ia spindle afferents, since anatomical evidence of a direct synaptic contact between these small fibres and large afferent axons has been discovered (116).

1.2.1.4 Attributes to fatigue at supraspinal sites

Transcranial magnetic or electrical stimulation over the primary motor cortex produces excitatory EMG responses in most muscles at short latency called motor evoked potentials (MEPs). The size of the MEPs depends on two main factors, first, is the excitability of the underlying motor cortex, and secondly, is the strength of the mono- and oligo-synaptic corticofugal connections with motoneurons. Other factors are related to the excitability of the motoneuron and the properties of the muscle fibre AP (116).

Transcranial magnetic stimulation also generates separate inhibitory effects at a cortical level that can result in a silence in the EMG after the MEPs have been evoked during a voluntary effort. Stimuli at levels below those that evoke the MEPs can reduce ongoing EMG suggesting involvement of inhibitory cortical circuits. As the stimulus intensity increases the silent period lengthens (116).

In a similar fashion to the technique of twitch interpolation, in which a motor nerve is stimulated during a voluntary fatigue task to note any increment in force, trascranial magnetic and electric stimuli were used. Taylor et al used transcranial magnetic stimulation superimposed during a fatigue task of the biceps muscle. During three min MVCs, voluntary activation of the biceps fell to an average of 90.7% from an average of > 99%. When the transcranial magnetic stimulus was applied an increment in force (relative to the voluntary force) was found. The magnitude of the increment in force was initially small (1%). However, after two mins of sustained MVCs this increment increased to 9.8%. The authors suggested that motoneuronal output became

suboptimal during the contraction, i.e. central fatigue developed and accounted for a significant loss of maximal voluntary force (321).

Todd et al examined the relation between twitch interpolation through nerve stimulation, transcranial magnetic stimulation and the degree of force increment at rest and fatigue. The biceps and triceps muscles in eight subjects were examined during brief maximal and submaximal contractions. Without fatigue, motor cortical stimulation evoked larger superimposed twitches than motor nerve stimulation. The amplitude of the superimposed twitch decreased with an increase in voluntary force when evoked by either motor cortical or motor nerve stimulation. With motor cortical stimulation, a perfect linear correlation ($r^2 = 1$) was found between the superimposed twitch and the background voluntary force for contraction strengths between 50% and 100 % MVC. Similar correlation was found between the magnitude of superimposed twitch and the level of force (MVC %) when transcranial motor stimulation was applied during fatiguing contraction.

Is the change in the motor cortical behaviour a direct cause for central fatigue? this question remains for the most part unanswered (116).

To summarise the ideas presented under the central fatigue heading it would be emphasised that voluntary activation of muscle is commonly not maximal in measurements of isometric strength. This deficiency varies with the subject, task and muscle group. It is therefore recommended that on designing an experiment using MVC, attention should be paid to the following details, 1- Fatigue protocols with voluntary activation should be accompanied by some instructions and practice. 2- Visual feedback should be given during the exercise. 3- Verbal encouragement should be given. 4- Subjects should be allowed to reject a test if they considered it to be submaximal (116).

With fatiguing tasks requiring maximal effort central fatigue may add substantially to the decline in performance. Thus central fatigue is at least partially attributed to failure of supraspinal drive to motoneurons. This is usually termed supraspinal fatigue that acts to protect the muscle from further peripheral fatigue at the expense of true maximum performance.

During isometric contraction the CNS uses a strategy whereby the firing rate of motor units is declined. This reduction in discharge rate takes advantage of the change in force-frequency relationship that shifts to the left during fatiguing contraction (113).

During fatigue, intramuscular receptors (spindles and tendon organs) provide a feedback about the decline in muscle performance and change in muscle length. At a spinal level this produces both excitatory and inhibitory influences on the motoneuron pool that could contribute to the decline in motor unit firing rate observed during maximal isometric contraction (133). Additionally the input from small-diameter muscle afferents particularly group IV reduces voluntary drive through a supraspinal action and not via direct postsynaptic inhibition of motoneurons. The action of group III and IV muscle afferents is complex and exerted at multiple points in the pathway responsible for force production (273).

1.2.2- Peripheral mechanisms of fatigue

Peripherally conducted steps that contribute towards the generation of muscle contraction include impulse conduction in the motor axons and their terminals, neuromuscular transmission, conduction of impulses in the muscle fibres, excitation-contraction coupling, and the contractile process itself.

1.2.2.1 Sarcolemma resting potential

The resting membrane potential of skeletal muscle is largely a K^+ dependent potential, thus any change in K^+ conductance or concentration gradient across the sarcolemma will affect the resting potential. It is known that muscle fatigue is associated with loss of intracellular potassium $[K^+]_i$ and a gain in Cl, Na^+ and water. The extent of ion and water shift is greater in fast compared with slow twitch muscles (303).
It has been suggested that alteration in sarcolemma function induces muscle fatigue by preventing cell activation. It is thought that K^+ efflux and inhibition of the Na^+-K^+ pump causes cell depolarisation, reduced action potential amplitude and possible complete inactivation (306). Edwards (1981) proposed that cell depolarisation would provide a safety mechanism to protect the cell against ATP depletion and Ca^{2+} accumulation as inactivation at the first step of the ECC rather than latter during the contraction process would prevent activation of subsequent ATP-utilizing steps and increase in the intracellular Ca^{2+} (90).

Juel using microelectrodes to measure Na^+ and K,$^+$ observed a higher K^+ level in the fast mouse extensor digitorum longus (EDL) of 182 mM compared to 168mM in the slow soleus muscle. This difference in K^+ level was correlated with a difference in the resting potential between the two muscles as the EDL muscle showed a more negative resting potential compared to the soleus (175). Juel found the cellular K^+ loss to exceed Na^+ gain during fatigue induced by electrical stimulation. After one minute of repetitive stimulation (400-ms, 40 Hz with one impulse/s) the resting potential was found to depolarise by 12 mV in the slow soleus compared to 18 mV in the fast EDL muscle. The author also observed an increase in extracellular potassium $[K^+]_0$ and suggested that the fall in resting potential is likely to be due to the fall in $[K^+]_I$ and increase in $[K^+]_0$ (175).

It has been suggested that the resting potential depolarisation observed in fatigued muscle cells is the result of combined effects of reduced $[K^+]_I$, elevated $[K^+]_0$ and increased K^+ conductance. The increased K^+ conductance could result from activation of ATP-dependent and /or Ca^{2+} -dependent K^+ channel. Davies et al studied the ATP-dependent K^+ channel (K_{ATP} channel) in frog skeletal muscle. At intracellular pH (pH_i) 7.2 the K_{ATP} channel showed little activity at one mM and was inactive at three mM ATP. However, at pH_i of 6.3 the K_{ATP} channel activity was detectable at one and three mM ATP concentrations. Therefore it was suggested that K^+ efflux increases as the pH_i falls during exercise. This would contribute to the depolarisation of the resting potential and reduced action potential amplitude with possible depolarisation block of the sarcolemma or T tubular action potential (61).

If the membrane hypothesis is the main factor in the development of muscle fatigue this would mean that the sarcolemma Na^+-K^+ pump is unable to maintain the ionic gradient for K^+ and Na^+, which is essential for maintenance of the resting potential and cell excitability. It was suggested that Na^+-K^+ pump might be limited by insufficient ATP. However, the essential ATP level for maintaining the Na^+-K^+ pump is around 0.5mM, and during contractile activity cell ATP rarely falls below 3 mM (303).

Medbo and Sejerstd (1990) showed that both the rise in plasma concentration during maximal exercise in human and its decline during recovery followed an exponential course with regard to time. Post-exercise the plasma K^+ content fell below the pre-exercise level by 0.5 mM, which suggests that the pump sensitivity was stimulated by the exercise. It is clear from these results that fatigue resulting from cell depolarisation would rapidly reverse within few minutes post-exercise. Hence, the membrane theory cannot explain the slowly recovering phase of muscle fatigue (229).

1.2.2.2 Sarcolemma action potential

The question of whether muscle fatigue can be attributed to disturbances in membrane excitability (either sarcolemma or t tubules) has been an interesting subject for years. Merton (1954) demonstrated that a maximal voluntary effort developed the same tension in the human adductor pollicis muscle as tetanic stimulation elicited through the ulnar nerve and this relation was maintained as fatigue developed. During extreme fatigue the sarcolemma action potential did not diminish in amplitude leading Merton to conclude that fatigue was not caused by failure of neuromuscular junction or sarcolemma but rather by events within the muscle cell (236). Edwards and Lippold found that with submaximal level of isometric muscle contraction there was an increase in surface recorded action potential amplitude. This finding was explained by the recruitment of additional motor units to maintain the force of fatiguing muscles (91).

Kernjevic and Miledi observed a reduced sarcolemma action potential frequency with fatigue in rat skeletal muscle and luettgau et al (1987) found action potentials to drop out without effect on mechanical force when frog muscles were stimulated at frequencies above 40Hz [for review (106)]. An explanation for these findings was provided by Jones et al (1981). During fatiguing muscle contractions EMG and action potential usually show an initial and early increase due to the recruitment of additional motor units. When the muscle starts to be fatigued EMG amplitude and AP fall, however, the optimal stimulating frequency for force development decreases as muscle fatigue develops (168).

Muscle fatigue is therefore associated with changes in the sarcolemma action potential characterised by a reduced amplitude, prolonged duration and increased amplitude of the early after-potential. The question that remained unanswered is whether these changes are a cause or a result of the muscle fatigue process. If the AP amplitude is enough it would fail to initiate or at least reduce the T-tubular charge movement, which would inhibit SR Ca channel opening and Ca release (291). Standing against the sarcolemma AP theory as the main cause for fatigue are the results from Metzegner and Fitts who showed that muscle's peak force generating

capacity was significantly more depressed after high compared with low frequency stimulation yet the reduction in AP amplitude was similar in both regimes of stimulation (237). Moreover, in the post-exercise period AP amplitude recovers faster than force, hence the AP fatigue theory cannot explain the delayed recovery of force in the post-exercise period (237).

1.2.2.3 T tubular system

It has been recognised that a primary role of the t-tubular system is to allow the sarcolemma action potential to propagate into the core of the fibre. A large percentage of the surface area of the T tubular system is in close proximity to the terminal cisternae of the SR. This junction between the T-tubular system and the SR has been found to be highly developed in fast compared with slow twitch fibres (269).

The coupling mechanisms responsible for the propagation of the AP into the T-tubule and subsequent release of Ca^{+} from the SR have been studied. It is believed that the T-tubular action potential is sensed by an intramembranous T tubular protein (dihydropyridine (DHP) receptor), which during activation undergoes a voltage driven conformational change (T tubular charge movement) that triggers Ca^{2+} release from adjacent SR Ca^{2+} channels (100).

Activity induced changes in the ionic status of the T tubular lumen and intracellular fluid compartments could contribute to the development of muscular fatigue by directly reducing the extent of T tubular charge movement. While an elevated T tubular Ca^{2+} might mediate fatigue by blocking conduction of the AP into the axial core of the fibre, low Ca^{2+} could directly reduce intramembranous T tubular charge movement leading to reduced Ca^{2+} release and force production (276).

Howell and Snowdowne (1981) observed a linear fall in peak tension as extracellular Ca^{2+} was increased from 1 to 20 mM. This treatment had no effect on the surface action potential amplitude but the conduction velocity was reduced. It has been suggested that an activity induced increase in T tubular Ca^{2+} might slow the T tubular AP conduction velocity to the point of conduction block producing incomplete activation of the axial core of the fibre (150). Howell et al observed myofibrillar

waviness in the axial core of the fibres activated in 10 mM Ca^{2+}, and attributed this to T tubular AP conduction block (149).

Consistent with the theory of T tubular conduction block, Westerblad et al found high frequency stimulation to produce a spatial gradient of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) with higher concentrations near the edges of the fibre (345). The low Ca^{2+} observed towards the centre of the fibres was attributed to the AP conduction block in the T tubule network. For the T tubule Ca^{2+} to contribute significantly to the force decline during contractile activity it has to reach 15 mM. However, T tubular Ca^{2+} was measured by Bianchi and Narayan, and found to reach 28 mM following 120 muscular twitches.

It has been argued that a modest increase in T tubular Ca^{2+} (increase of 5-10 mM) would serve to stabilise the DHP receptor and thus preserve charge movement and reduce the likelihood of fatigue. Luettgau demonstrated that the resting potential was depolarised and the peak K^+ contracture tension reduced in low extracellular Ca^{2+} ($[Ca^{2+}]_o$) of 0.2 mM. Further reduction of the $[Ca^{2+}]_o$ to zero resulted in complete inhibition of the AP and peak K^+ induced contracture. The author has also observed the relation between $[Ca^{2+}]_o$ and the potential threshold. Low $[Ca^{2+}]_o$ shifted the activation threshold to the left with weak contractions recorded at 10-20 mM $[K^+]_o$ or resting potential of –60 mV. Increasing the $[Ca^{2+}]_o$ from the control value of 1.8 to 5 mM increased the threshold potential from –35 to –18 mV (203). It has therefore been suggested that if T tubular depolarisation develops with fatigue it would be more likely to cause inactivation if the T tubular Ca^{2+} was reduced (203).

1.2.2.4 T tubule-sarcoplasmic reticulum junction and Ca^{2+} release from the terminal cisternae.

The exact mechanism of transduction across the TT-SR junction has not been fully established. The morphological relationship between the SR Ca^{2+} release channel (ryanodine receptor) and the intramembranous TT charge sensor (DHP receptor) supports the hypothesis that the main mechanism involves mechanical or allosteric coupling. Two additional mechanisms the Ca^{2+} -induced Ca^{2+} release and activation by inositol triphosphate ($InsP_3$) are thought to play roles in regulating Ca^{2+} release in skeletal muscles (100).

During fatigue exercises the amplitude of Ca^{2+} transient has been shown to be reduced (345). Research has shown that depletion of Ca^{2+} from the SR is unlikely to be fully responsible about the initiation of fatigue [for review (106)]. Eberstein and Sandow demonstrated that caffeine, a compound that stimulates Ca^{2+} release from the SR, reverses the tension loss of fatigued muscles. If fatigue were caused by depletion of SR Ca^{2+}, caffeine would have failed to produce significant tension [for review (106)]. Gyorke (1993) noted no decline in the T tubular charge movement despite a significant reduction in the amplitude of Ca^{2+} transient during fatigue simulations of single frog muscle fibres. The author concluded that the reduced Ca^{2+} transient was caused by a direct inhibition of the SR Ca^{2+} release channel and not to disturbance in T tubular action potential or the DHP charge sensors (136).

It has been proposed that the rate and extent of Ca^{2+} release from the SR could decline during contractile activity if either the coupling step between the TT and SR membrane, or the release process itself were inhibited (106). Volpe et al suggested that the linking step could involve the second messenger $InsP_3$. Myoplastic $InsP_3$ has been shown to increase rapidly with stimulation and depletion of $InsP_3$ could facilitate the onset of fatigue (337).

Although the evidence reviewed above suggested that the releasable stores of SR Ca^{2+} are unlikely to be depleted with the onset of fatigue, a redistribution of Ca^{2+} from the SR release site to the Ca^{2+} - binding protein parvalbumin and the SR pump must

occur. This redistribution would decrease the driving force for Ca^{2+} release, producing a reduced rate of release (106).

Allen et al studied the Ca^{2+} transient in in *Xenopus laevis* fibres, and Westerblad et al evaluated mouse muscles. In both studies the authors found that in muscle fatigue the amplitude of Ca^{2+} transient initially increased as force declined after which both force and Ca^{2+} transient declined. It was apparent that the resting $[Ca^{2+}]_i$ rose continuously throughout the stimulation period. The authors concluded from their studies that fatigue was initially caused by metabolic factors (such as increased H^+ or P_i) acting via direct inhibition of the cross bridges. They attributed the early rise in tetanic $[Ca^{2+}]_i$ to a saturation of the myoplasmic Ca^{2+} buffers rather than to an increased Ca^{2+} release from the SR. As stimulation continued, a reduced Ca^{2+} release became quantitatively more important in the fatigue process. The observation that 10 mM caffeine had little effect early in fatigue but greatly increased both the Ca^{2+} and force late in fatigue supports their contention that the depression of Ca^{2+} release and its contribution to fatigue develops after the initiation of the fatigue process by Ca^{2+} - independent factors acting directly at the cross bridges (2).

1.2.2.5 Lactic acid, intracellular pH and fatigue

The major source of acid production in skeletal muscle is the anaerobic production of lactic acid from glucose and glycogen. At physiological pH the acid produced primarily dissociates into lactate and H^+ (298).

A relationship between lactic acid production and muscle activity has long been recognised. At workloads above 50-60% of the maximal aerobic capacity blood lactate concentration increases and generally averages 10-20 mM following a short duration of maximal voluntary exercises. Maximal muscle lactate is ~10 mM higher than blood lactate and significant increases can occur within 10 s. of the initiation of supramaximal exercises (298). Karlsson and Saltin found high muscle lactates to be consistently associated with exhaustion following repeated one min bouts of maximal bicycle exercise (185). The same authors studied metabolic changes following three different workloads leading to exhaustion at 2, 6 and 16 min of bicycle exercise. Muscle lactate averaged 16.1 mM at the two highest loads but only 12.0 mM at the

lowest load. They concluded that fatigue at the two highest loads might have been caused by the high lactate, while fatigue at the lowest load was clearly dependent on other factors. Increase in muscle and blood lactate levels have been observed in studies that involved isometric and dynamic exercises (184).

It is known that fast muscles (FG & FOG) and fast single motor units fatigue more rapidly and to a greater extent than slow muscles (SO) and slow motor units. After both static and dynamic voluntary contractions to exhaustion in humans, the fast type FG and FOG fibres were found to have higher lactate (25-27 mM) than the slow type SO fibres (15.8 mM). A high level of correlation ($r = 0.86$) was observed between fatigability and the percentage of fast muscle fibre content (328). In skeletal muscles stimulated *in vitro* the fast EDL fatigued faster and showed higher lactates and lower pH than the slow-twitch soleus muscle. In both muscles the lactate increase and pH decrease were highly correlated with the decline in tension (332).

The relation between muscle bicarbonate level and functional performance of the muscle has been studied. In high bicarbonate (25 meq/1), the fatigue-induced prolongation of relaxation following a tetanus was found to be less and recovery of force was faster than muscles incubated in low bicarbonate (1meq/1). The faster recover of force in the high bicarbonate was associated with a faster lactate efflux. Despite the faster lactate efflux in high bicarbonate the decline in force during stimulation was not affected by the external bicarbonate concentration (211). It has been suggested that the effect of reduced extracellular bicarbonate on the degree of fatigue depends on the duration and intensity of the exercise. With high intensity short duration exercise a reduced extracellular buffer was found to have no effect, however, an increased fatigability was observed in heavy endurance exercise in humans and during prolonged in situ stimulation of the rat hindquarter (312).

The pH_i of frog and mammalian skeletal muscles is ~7.0. With high intensity exercise the pH_i falls to values as low as 6.2 (184). The question is whether the change in pH_i is the causative factor in fatigue? Skinned fibre studies have shown acidosis to depress tension in skeletal as well as cardiac muscles (97). Reduction of pH from 7.0 to 6.2 not only showed a decline in maximum tension in presence of optimal Ca^{2+} levels but also increased the free Ca^{2+} required for initiation of contraction i.e.

increased the Ca^{2+} level activation threshold. The force- Ca^{2+} curve was found to be shifted to the right such that higher free Ca^{2+} was required to reach a given tension (97). When muscle pH was reduced from 7.0 to 6.2 fast twitch vastus lateralis muscle was found to have a greater decline in force compared to the slow twitch soleus muscle (238). The fact that reduced pH has resulted in a reduction in muscle tension in the presence of optimal levels of Ca^{2+} is an indication that this effect is not a simple interference of high H^+ level with Ca^{2+} binding to troponin (97, 238).
It is currently thought that protons directly inhibit force by reducing the cross-bridge transition from the low- to the high-force state. The direct effect of H^+ on the cross-bridge could involve a reduction in the number of cross bridges and/or a reduction in the force produced per cross bridge (106).

Renaud et al (1987) studied the effect of reduced pH_i with high CO_2 on the peak force and muscle fibre tension in the mouse soleus and EDL muscles. Reduction in pH_i resulted in a decline of peak force by 24 and 30% in the two muscles respectively. Stiffness was only reduced by 9 and 14% respectively. These results suggest that the primary effect of low pH_i is to reduce the force developed per cross-bridge (288). In agreement with these results Edman and lou conducted their studies on living single fibres during the development of muscle fatigue to 75% of the initial force. The authors observed only 9% reduction in fibre stiffness. In non-fatigued fibres stiffness reached its peak before force and after fatigue this difference was magnified as the rate of rise of force ($^+dP/dt$) was markedly depressed while the rate of stiffness development remained unchanged. The result implied that fatigue altered the rate of transition from cross bridge attachment state (reflected by stiffness) to the high force state of cross bridge (89).

In addition to the decline in force, fatigue has been known to result in a reduction in the rate of force development (dP/dt). The decline in dP/dt can be partially explained by the decrease in the number of active cross bridges acting in parallel caused by incomplete activation (inhibition of ECC) or direct inhibition of the cross bridge. If the peak $^+dP/dt$ in an intact muscle is limited by the cross bridge transition rate from the weakly bound low force state to the strongly bound high force state, then the rate for cross bridge binding may be reduced in the fatigued cell. Regardless of what

limits the rate of force development the available evidence suggests that its decline with fatigue is at least partially caused by the development of low pH_i (106).

1.2.2.6 Inorganic phosphate (P_i) and muscle fatigue

With contractile activity P_i increases with the decrease in PCr and both show a significant correlation with the development of fatigue. The handling of P_i appears to depend primarily on a mitochondrial membrane carrier protein for the transport of the P_i into the mitochondria. The carrier appears to be specific for the divalent anion $(HPO_4)^{2-}$ form. After fatigue both P_i and PCr recover with similar time course, which is generally related to the recovery in force. However, the relation between P_i and force is not always linear (155).

Weiner et al observed the P_i to increase more rapidly than the decline in the peak force of MVC. After two min of contraction the level of P_i plateaued while the MVC continued to fall (344). Dawson observed a high correlation between the decline in force and the increase in diproteinated $(H_2PO_4)^-$ form of P_i in frog skeletal muscle during fatigue(62). These results support the hypothesis that the increase in P_i contributes to the development of fatigue.

Controversy exists in regard to the active form of P_i that could have a role in the aetiology of fatigue. When the total P_i and $(H_2PO_4)^-$ forms were plotted against force until the occurrence of fatigue a simple inverse relation was observed between $(H_2PO_4)^-$ level and force. Zero force was found to be obtained at $(H_2PO_4)^-$ concentration of ~20 mmol/kg wet weight. It has been suggested that diproteinated $(H_2PO_4)^-$ form of P_i is a causative factor in fatigue development (350).

Millar and Homsher (1990) found the decline in force to be linearly related to the logarithm of the average P_i concentration. The authors found the slope of the relative tension versus log $[P_i]$ to be the same at pH of 7 and 6.2 (240). It has been argued that if tension depends on the logarithm of P_i concentration then the question cannot be answered by determining the relationship between P_i (total or diproteinated form) and force as both forms will increase by the same factor as fatigue develops (106).

High P_i has been shown to shift the force-Ca^{2+} relationship to the right i.e. higher Ca^{2+} level would be needed for muscle activation. This has functional implications, as the amplitude of Ca^{2+} transient is known to be reduced in fatigued muscle cells. A P_i – induced decrease in strongly bound cross bridges would reduce thin filament activation resulting in accentuation of the decline in tension particularly at sub-optimal Ca^{2+} level. This effect would explain the right shift in the force-Ca^{2+} curve and reduced peak force associated with high P_i levels (106).

1.2.2.7 High-energy phosphates and muscle fatigue

ATP provides an immediate source of energy for force generation by the myosin cross-bridges. Adenosine 5'-triphosphate is also needed for the Na^+-K^+ pump function, which is essential for the maintenance of a normal sarcolemma and T-tubular action potential. Moreover, ATP is a substrate of the SR ATPase and hence is required for the Ca^{2+} reuptake by the SR. Therefore; adequate tissue levels of ATP must be maintained if fatigue is to be avoided (282).

High intensity exercises are associated with a rapid decline in PCr, reaching 5-10% of its pre-exercise level within 30 s from the onset. In contrast ATP shows a modest decline and rarely falls below 60-70% of the pre-exercise content (314). The reduction in PCr occurs in all muscles (fast and slow) but the decrease is larger in the fast-twitch compared to the slow-twitch muscles. This difference is likely to be a reflection of the three to fourfold higher ATP utilization rate in fast compared to slow skeletal muscles (314). During high speeds of locomotion the slow fibres may become functionally unloaded and/or develop low tension due to slow activation and low dP/dt, either of which could lower the energy cost. The fibre type difference was demonstrated in a study by Ivy et al who studied single slow and fast twitch fibres isolated from the vastus lateralis muscle of humans at exhaustion. The authors found greater fall in the PCr content in the fast (FG) fibres compared to the slow (SO) ones (158).

The changes in Cr and P_i with contractile activity show an inverse correlation with PCr. This correlation would be expected as PCr participates in the creatine kinase (CK) reaction ($PCr + ADP + H^+ \longrightarrow Cr + ATP$). This reaction is driven by the cell

utilization of ATP ($ATP + H_2O \longrightarrow ADP + P_i + H^+ + Energy$). Another reaction important in the maintenance of cell ATP during intense activity is the adenylate kinase reaction ($2ADP \longrightarrow ATP + AMP$). With high intensity exercise, increased H^+ and ADP concentration serve to stimulate the CK and adenylate kinase reactions. These reactions maintain high ATP and low ADP (282).

ATP concentration in skeletal muscle rarely drops below 60-70% of the pre-exercise level even in cases of extensive fatigue. Nassar-Gentina et al demonetrated that fatigued semitendinosus muscles contained 70% of their pre-exercise ATP level. When the fatigued muscles were stimulated by caffeine the muscles generated considerable extra-tension and the remaining ATP was reduced by 50%. These results indicated that fatigue was not caused by the lack of ATP availability (256).

Sahlin et al suggested that ATP could limit performance without depletion due to a decrease in the free energy of ATP hydrolysis. After maximal bicycle exercise in humans the free energy of ATP hydrolysis decreased from 54 to 50 Kilo-Joule (kJ) Sahlin, 1990 #600}. However, Dawson et al found the fall in free energy to be small and had no correlation to the fall in force (62).

The important question in the study of muscle fatigue is whether or not the concentration of cellular ATP declines to a critical level that would compromise the force-generating capacity and/or the cycle rate of the cross bridge? The evidence from the above mentioned and other studies is that it does not. Fatigue is likely to be produced by other factors that reduce the ATP utilization rate before availability of ATP becomes a limiting factor (106).

1.2.2.7.1 Cell phosphocreatine can it be a limiting factor?

The relationship between force and PCr during and following contractile activity appears to be dependent on the type of exercise (dynamic or isometric) and the relative intensity of the work. The usual pattern for PCr is to decline considerably faster than the decline in force (282).

Following contractile activity PCr recovers in two phases. The initial phase is fast and has a half time of 20-30 sec, which is then followed by a slower phase requiring 20 minutes or more before full recovery. The second or slow phase of recovery was found to be slower following isometric compared with dynamic exercise (282).

The second slow phase of PCr recovery following contractile activity was found to have a high inverse correlation with lactate. It was suggested that the increased H^+ concentraction altered the equilibrium state of the CK reaction and thus slowed the PCr recovery. This could explain the slowed recovery following isometric exercises, an exercise regime that is likely to produce high muscle lactate and low pH compared with dynamic exercises (282).

Thompson and Fitts (1992) noted that PCr of single frog semitendinosus fibres show a high correlation with force during recovery from fatigue. A close inverse correlation between the recovery of PCr and H^+ concentration during recovery from fatigue was found. The authors suggested that the correlation between PCr and force could be attributed to the fatigue inducing effects of H^+. Although pH_i can account for the force changes that happen during the slow phase of PCr recovery, the initial rapid phase seems to be independent of pH_i (327).

In summary, PCr can only limit performance if its depletion reduces ATP re-synthesis markedly to a level that would inhibit force production. The results from the above studies suggest this to be unlikely(327). With high intensity exercises PCr declines faster than force while ATP remains relatively adequate even at exhaustion (282). The correlation between PCr and force during the post-fatigue recovery period is likely not causative but rather related to the effect of high H^+ concentration which inhibits force and PCr re-synthesis (327).

1.2.2.8 Muscle glycogen and fatigue

The rate of muscle glycogen utilization is dependent on the type and intensity of the work. The magnitude of muscle glycogen utilization was found to increase from 0.3 to 3.4 glucose units.kg^{-1}.min^{-1} as the relative work load increased from 25% to 100% of the subjects' maximal oxygen uptake (Vo_{2max}) (336). At work intensities <60%

Vo_{2max} , muscle glycogen remains high as free fatty acids (FFA) provide the primary substrate for energy requirements. Therefore, fatigue resulting from prolonged work at low exercise intensities cannot be attributed to muscle glycogen depletion (336).

Although muscle glycogen is rapidly metabolised at work loads > 90% Vo_{2max} , fatigue develops rapidly within minutes while muscle glycogen remains high. At work loads between 65 and 85% Vo_{2max} muscle fatigue is highly correlated with muscle glycogen depletion (336). Bergstrom and Hultman found the highest glycogen utilization rate to occur during the initial phase of exercise. Glucose administration was found to have no influence on the rate of glycogen depletion. The authors proposed that muscle glycogen is not only the primary energy source during moderate to heavy exercises but that muscle glycogen is an obligatory substrate and therefore its depletion was likely to cause fatigue (20).

Research has evaluated the question of whether there is a correlation between the muscle fibres' MHC, oxidative capacity and the rate of depletion of glycogen, which presumably contribute to the development of fatigue. Gollnick et al noted that during prolonged exercises of moderate intensity in humans the SO fibres were depleted first. At later phases of the exercise the fast twitch fibres were also depleted of glycogen (128). There was however, no attempt to differentiate between type FOG and FG fibres in these tests. Animal experiments showed that glycogen depletion occurred in the SO and FOG fibres at all exercise intensities while in the FG fibres, glycogen stores were not altered except at the final stages of prolonged exercise or at high exercise speeds (201). Astrand et al employed intense exercises of the arms and legs to produce fatigue within five minutes but glycogen was depleted by < 50% (7).

It was suggested that glycogen depletion could only contribute to fatigue during prolonged endurance exercises performed at 65-90% of the subject's Vo_{2max} or in repeated high-intensity exercise bouts (7).

1.3- Nerve injuries

Nerve injuries can occur as a result of a variety of agents including mechanical, chemical, thermal and ischaemic. Early classifications of nerve injuries have provided several categories that represented the mechanism as well as the common features of the injured nerve like compression, contusion, stretch and laceration or division. These terms were neither specific enough to define the degree of injury nor carried a prognostic value. Seddon has described a three category classification system of nerve injuries that involved neurapraxia by which it is meant block of conduction without structural damage; axonotmesis that reflects damage to the axons while the continuity of the epineurium is preserved; and finally neurotmesis to express complete nerve section (302) (table 1.1).

Sunderland has provided a histopathological classification system that defined five grades of nerve injuries reflecting the degree of damage to various nerve structures that progressively increases from grade I to V (317) (table 1.1).

Grade I nerve injury in Sunderland's classification corresponds to Seddon's neurapraxia and represents a transient block of nerve function that can extend from few days to few months before recovery. Histologically the axons are intact and there is no Wallerian degeneration. Motor function was found to be affected first and recovered last in these injuries; therefore atrophy of the muscle can happen whilst awaiting recovery. In grade II injury, axons are disrupted and Wallerian degeneration occurs in the distal part of the nerve. However, because the endoneurial tubes remain intact in these cases, axons re-grow to innervate their original end organs. The interval from onset of injury to recovery depends to a great extent on the level of injury. In more proximal injuries the nerve takes longer to travel to its end organs as opposed to distal nerve lesions (205, 317).

In grade III injuries there is intrafascicular damage with disruption of the nerve axons and endoneurial tubes. Distal axonal and Wallerian degeneration in addition to possible proximal axonal disintegration can all occur. Though the axons can regenerate within the fascicles, possibilities for intrafascicular bleeding, oedema and fibrosis are present that can hamper the establishment of axon regeneration and

consequently the final outcome. This type of lesion is seen with traction as well as compression within a closed compartment with resultant axon degeneration and replacement by fibrous tissue (205, 317).

Grade IV nerve injuries are characterised by complete disruption and disorganisation of the fascicles. Although the continuity of the nerve trunk is preserved, a nerve segment will be filled with ruptured fascicles, scar tissue, Schwann cells and regenerating axons covered with intact epineurium and can enlarge to form a neuroma. Loss of continuity of the nerve trunk occurs in grade V injuries.

Following nerve transection variable amounts of scar tissue forms between the nerve ends and regenerating nerve ends become impeded in it. The chances of nerve regeneration without surgical repair are very slim (205, 317). Grade IV and V nerve injuries carry the worse prognosis because of the possibility of mixing of nerve fibres, retrograde degeneration, loss of some neurones and considerable degree of scar formation that can imbed regenerating axons (196, 205, 317).

Table 1.1. Classifications of nerve injuries

Type		Functional Disorder	Anatomical/ physiological basis	Prognosis
Physiological Conduction block (a)*		Local conduction block	Intraneural circulatory arrest. Metabolic ionic block with no nerve fibre pathology.	Immediately reversible
Physiological Conduction block (b)*		Local conduction block	Intraneural oedema. Metabolic block with little or no nerve fibre pathology. Increased intraneural fluid pressure.	Reversible within days or weeks.
Seddon	Sunderland			
Neurapraxia	Grade I	Local conduction block. Motor function and proprioception mainly affected. Some sensation and sympathetic function may be preserved.	Local myelin damage, primarily thick, myelinated fibres. Axonal continuity preserved. No wallerian degeneration.	Reversible within weeks to months.

Axonotmesis	Grade II	Loss of nerve conduction at level of injury and within distal nerve segment.	Loss of axonal continuity, wallerian degeneration. Endoneurial tubes preserved.	Recovery requires axonal regeneration. In this injury correct orientation of growing fibre is likely since endoneurial tubes are preserved and correct targets will be reinnervated.
	Grade III	Loss of nerve conduction at level of injury and with distal nerve segment.	Loss of continuity of the axons and endoneurial tubes. Perineurium intact.	Endoneurial pathways disrupted and disoriented, bleeding and oedema lead to scarring. Axonal misdirection. Poor prognosis. Surgery may be required.

	Grade IV	Loss of nerve conduction at level of injury and within distal nerve segment.	Loss of continuity of the axons, endoneurial tubes and perineurium. Epineurium intact.	Rupture and total disorganisation of guiding elements of the nerve trunk. Intraneural scar formation. Axonal misdirection. Poor prognosis. Surgery required.
Neurotmesis	Grade V	Loss of nerve conduction at level of injury and within distal nerve segment.	Transection or rupture of entire nerve trunk.	Recovery requires surgical adaptation and co-aptation of nerve ends. Prognosis dependent on the nature of the injury as well as local and general factors.

Table1.1 shows Seddon and Sunderland classifications of nerve injuries. Grade a & b metabolic conduction block are shown at top according to Lundborg's explanations of these degrees of nerve damage and possible prognosis (205).

1.3.1- Pathophysiology of nerve compression

Nerve compression injuries can occur as acute or chronic lesions. The severity of the injury and the functional recovery after these lesions are dictated by the magnitude and the duration of the pressure exerted on the nerve. The functional outcome and period of recovery following these injuries varies to a considerable degree reflecting the wide spectrum in the pathological processes that can be associated with these injuries (205).

Impaired nerve function in compression lesions has been attributed to vascular and mechanical effects (296). When a nerve is compressed externally the intraneural blood vessels can be obstructed. With an external pressure of 20-30 mmHg retardation of venous flow was observed in rabbit tibial nerves; when this pressure was increased to 80mmHg complete disruption of the blood flow was noticed (262).

The carpal tunnel is a common site for nerve compression in humans where the median nerve is entrapped underneath the deep transverse ligament at the level of the wrist joint. Gelberman (1981) reported a tissue pressure of 32mmHg around the median nerve in a group of patients with carpal tunnel syndrome compared to 2.5 mmHg in a control group of healthy volunteers (120).

External pressure that leads to complete cessation of blood flow can damage the endothelial cells of the intraneural microvessels resulting in increased permeability to water, ions and proteins. Accumulation of these materials can increase the intraneural pressure and reduce the blood flow (119).

The effect of increased local pressure around the sciatic nerve of rats and its influence on the endoneurial fluid pressure was investigated. It has been reported that when the nerve was subjected to a pressure of 80mmHg for four hours, a four-fold increase in endoneurial fluid pressure occurred. This increase in endoneurial fluid pressure was reported for up to 24 hours after relieving the pressure, denoting that no drainage of endoneurial fluid had taken place (253, 281).

Nerve compression was found to interfere with both the slow and rapid axonal transport systems. External pressures as low as 30mmHg for two hours were found to

partially block the slow axonal transport proteins. When the pressure was increased to 50mmHg complete block of fast and slow transport systems was observed (59, 60). This block was reversible within days in most cases.

Compression of nerves leads to mechanical deformation as nerve tissues are redistributed into the non-compressed areas. Such deformation leads to stretching of the nerve components as they get redistributed from areas of high to low pressure. Therefore, a compression lesion may create shearing forces particularly at the edge of the compressed zone (205).

The effect of compression on different nerve structures varies to a considerable degree. Large myelinated and superficially located nerve fibres are more susceptible to the compression effects when compared to the small unmyelinated ones (295). This has been explained by the reduced amount of perineurium that surrounds large and superficially located nerve fibres and protects them against external pressure (295). It was also found that in large fibres there is normally a narrowing of the axon at the nodes of Ranvier, which can form an obstacle to the movements of the axoplasm with resultant displacement of the axolemma and the nodes attached to it. These findings would explain the clinical observation of sparing the sensory function in the early stages of a nerve compression syndrome compared to the motor one, as motor fibres are usually large and superficially located (317).

1.3.2- Ulnar nerve compression

1.3.2.1- Aetiology of ulnar nerve compression

The common sites for ulnar nerve entrapment are around the elbow. One of the common sites is at the arcade of Struthers, proximal to the elbow joint, which is formed by the deep investing fascia of the forearm and the superficial fibres of the medial head of triceps (205).

Osborne (1970) confirmed the reduction of the cubital tunnel volume with flexion of the elbow. The same author also defined a tight fascial band that connects the 2 heads of the Flexor Carpi Ulnaris (FCU) as a cause for nerve compression (264). The ulnar nerve can also be compressed at the level of the wrist joint.

1.3.2.2- Outcome of ulnar nerve compression

Although the effects of nerve compression in its early stages with predominantly vascular phenomena are likely to be reversible, structural changes that result from chronic compression are not expected to recover quickly or even completely (205). Chronic compressions can result in various degrees of nerve injuries ranging from grade I to IV in Sunderland's classification with variable outcome depending on the magnitude of pressure as well as the period of entrapment (317).

Patients with ulnar nerve entrapment have been classified according to their presenting symptoms into three categories: the first category includes those with intermittent paraesthesia (tingling) and hyperaesthesia (painful paraesthesia) in the ulnar nerve distribution. The second includes those with persistent hypoaesthesia (reduced sensory power) and varying degrees of weakness or atrophy of intrinsic hand muscles. The final group includes those with marked atrophy and weakness of their intrinsic hand muscle. Recovery was reported to be usually good for the first category, variable and takes longer periods for the second and worse in the last category where incomplete recovery with residual hand function deficiency is usually expected (278, 342).

1.3.3- The long thoracic nerve

The Long Thoracic Nerve (LTN) arises directly from the anterior branches of C5,C6 and C7 spinal nerve roots and supplies the Serratus Anterior (SA) muscle (351) .

1.3.3.1- Long thoracic nerve palsy (LTNP)

Various possible mechanisms have been described in association with LTNP. Since the early description by Horwtiz and Tocantins (1938) it was recognised that trauma in the form of distraction of the shoulder from the neck by either a caudad thrust on the shoulder or contralateral angulation of the head away from the shoulder (217) puts the nerve under tension and potentially causes a stretching injury. The trauma theory by distraction has been further supported in other reports (132, 335).

Along its course the LTN can be exposed to compression at many sites (78). In an early cadaveric study, scalenus spasm or injury has been suggested as a possible cause for nerve injury. More recently, Hester et al on a dissection of six fresh cadavers described how a tight fascial band of tissue extends just superior to the middle scalene muscle insertion on the first rib, and acted as an additional digitation proximal to the serratus anterior muscle. With progressive manual abduction and external rotation, the long thoracic nerve was stretched across the fascial band (141). In another cadaveric dissection of 40 fresh cadavers the LTN was found to turn posteriorly with an angle of 30° as it comes out of the axillary sheath at the level of the 2nd. rib. Elevation of the arm was found to move the axillary sheath superiorly and accentuate the nerve angulation and stretching (87).

Compression of the LTN can happen along the chest wall where it is crossed by a leash of vessels from the thoraco-dorsal artery and vein. Irritation of the nerve with scar formation has been reported to cause nerve entrapment at this point. Surgical release of the nerve achieves good recovery in resistant cases that did not improve with non-operative measures (212).

Patients with long thoracic nerve palsy present with pain, weakness and limitation of shoulder elevation. Scapulothoracic movements are usually deficient with scapular winging (prominence of the vertebral border of the scapula), medial translation of the scapula and rotation of the inferior angle of the scapula toward the midline (83, 259, 329).

Non-operative treatment in form of physiotherapy is primarily directed at improving coordination of periscapular muscles and strengthening the serratus anterior and is usually the first line of treatment. Good results have been reported to occur in the majority of patients, with reduction in pain and improvement of the shoulder range of motion (ROM) (83, 349). Friedenberg et al have presented the results of a non-operatively treated series of 50 patients with LTNP, with a minimum follow up of 48 months. The outcome was reported as either good or poor depending on both subjective patients' feeling of their strength and pain and also on clinical assessment of shoulder ROM and muscle strength. Fifty six percent of these patients were reported to have good results, and 20% were poor. The rest of the patients (24%) had equivocal scores that did not qualify them to be classified under either good or poor results groups (108).

Though good results could be achieved in the majority of patients there was a substantial number of patients (46 % in the above study) who had residual deficiency of serratus anterior function. It has been our observation as well as others (107) that, recovery of the reinnervated serratus anterior is usually incomplete with residual winging, easy fatigue of the shoulder and lack of the ability to perform strenuous activity or maintain arm position above the head for lengthy periods. This deficiency is usually more notable in patients who perform heavy manual jobs.

As in other reinnervated muscles this deficiency in not well understood. Weakness and easy fatigability have been theorized as possible causes for deficient function of reinnervated muscles. To differentiate between fatigue and weakness of the serratus anterior muscle it would be necessary to measure its force. Mechanical measurement of the human's serratus anterior muscle force generating capacity is not possible, as its function cannot be isolated. An alternative method of measuring muscle fatigability is the EMG analysis or the myoelectric manifestation of fatigue. Methods

for assessment of serratus anterior function and the use of EMG in assessing fatigability will be explained in the next sections.

1.4- Denervation process and muscle structure and function

1.4.1- Denervation - reinnervation induced changes on muscle fibres and motor units

Denervation has been shown to induce various alterations on the characteristics of the affected muscle at the fibre, motor unit and connective tissue levels (79, 355). These alterations can be broadly attributed to mechanisms associated with muscle disuse (unloading), lack of neural supply or the influence of the re-innervating motor neuron (8).

Unloading of muscles was discussed under (1.1.8 Muscle fibre plasticity and training effect). Unloading has been shown to influence muscle fibre characteristics with tendency for the muscle fibres MHC to transform into more fast contracting and fatigable category. For example type IIa fibres were found to transform to type IIx or IIB (315). The slow contracting MHC type I fibres have been found to be more resistant to this transformation process especially at the protein level. However, at the MHC isoform mRNA level this transformation was found to take place (159).

Denervated muscles from different species (cats, rats and humans) have been examined to delineate the influence of this process on the muscle fibres and motor units histological and contractile properties. Gillespie et al studied the influence of denervation-reinnervation on the lateral gastrocnemius (LG) and soleus (S) muscles in rats. The muscles were reinnervated with their common nerve branch that normally supplies the fast twitch LG and the slow twitch S. Using myosin ATPase staining it was found that reinnervation has induced a significant effect on the fibre type distribution of the LG. The fibre type proportions in the control LG muscles were found to be FG 42%, FOG 48% and SO 10%. In the reinnervated muscles these proportions were changed to FG 35%, FOG 37% and SO 28%. The control S muscles were entirely oxidative, with a small proportion of FOG (20%) among 80% of SO fibres. reinnervated soleus was found to contain an average of 30% FOG fibres and 70% SO fibres (123).

A comparison between the histochemical fibre type and motor units' contractile properties was made. For the LG, the control muscles contained 10% SO fibres and

9% slow motor unit. When the SO fibres were increased to 28% in the reinnervated muscles a corresponding increase of the slow motor units to 30% was observed. In contrast, the FOG fibres of the soleus muscle have slightly increased from 20% to 30% but was accompanied by an unmatched increase in the muscles' fast motor units from 20% to 70%. The changes introduced in the proportion of motor units after reinnervation were attributed to the lack of specificity in reinnervating muscle fibres i.e. slow MUs were not necessarily reinnervated by a slow motor neuron. Instead, the slow MUs were re-innervated by fast motor neurons. Therefore a mismatch between the characteristics of the motor neuron and the reinnervated muscle fibres must have happened. For the SO fibres reinnervated by fast motoneurons a change in the muscle fibres' contractile velocities to meet that of the motoneuron was observed. However, the transformation process did not involve the muscle fibre protein (MHC) (123).

For the fast muscle fibres that were reinnervated by slow motor neuron the change was noted at the contraction velocity and the MHC levels. Therefore, the increase in slow motor unit number that was observed in the LG was accompanied by a corresponding increase in the SO fibres. This dissociation between the histochemical (fibre type) and physiological (motor unit contractile properties) in the soleus muscles (80% SO fibres) was attributed to the lack of plasticity of this muscle. Though the reinnervating motor nerve has introduced a significant change at the level of motor unit contraction velocity a corresponding change at the level of muscle fibre myosin isoprotein could not be achieved (123).

It has been proposed that postural muscles with high proportion of SO fibres have a higher threshold for denervation associated changes compared to the fast muscle that contain mainly FG and FOG fibres (122). This statement was challenged by Sheilds et al (1995) who studied acute and chronically paralysed soleus muscles in human subjects. Ten subjects with chronic > a year and three with acute < six weeks complete spinal cord injury were recruited. The denervated soleus muscles were stimulated by surface electrodes applied over the course of the tibial nerve, and ankle plantar flexion torque was measured. Histochemical analysis for denervated soleus muscle fibre type was also performed. Calculation of the fatigue index (FI) revealed increased fatigability of the chronically paralysed muscles as six subjects had a fatigue index of < 0.25, two had FI of 0.30 and another two were < 0.50. it was clear

from these fatigue indices that none of the tested chronically denervated muscles acted as a predominantly type I oxidative muscle. The short-term denervation in acutely paralysed muscles did not induce increased fatigability. The mean FI of acutely paralysed muscles was 0.88.

The biopsy specimen from a chronically paralysed patient revealed that the soleus muscle was almost totally comprised of type II fibres. It was also concluded that large proportion of these fibres were type IIB. Many conclusions have been drawn from this study. Most importantly it has been shown that even slow muscles can show denervation-induced changes in their fatigue characteristics. This effect was found to be time dependent, the longer the denervation period the more likely that the process of muscle fibre transformation to be completed (305).

A well recognised problem associated with nerve injury is the lack of specificity of the growing axons towards their end targets. When a nerve is repaired after complete transection some axons will successfully pass the injury site. These re-grown axons do not necessarily pass through their original endoneurial tubes and may re-innervate foreign end organs. As there is a reduction in the number of the axons few but not all the motor units are likely to be reinnervated (317).

An interesting phenomena about nerve injuries is the ability of surviving nerve axons in partially denervated or reinnervated muscles to sprout. Sprouting means collateral re-innervation where healthy axons form sprouts that extend across to neighbouring denervated muscle fibres and form new neuromuscular junctions. Sprouting can arise from the nodes of Ranvier or the motor end plate itself (224). Following denervation with complete or incomplete nerve section, surviving or regenerating motor axons were found to be able to re-establish the motor end plates though they are not specific or have preferential re-innervation pattern (130).

Rafuse and Gordon 1998 studied the influence of nerve section followed by repair, nerve crush and partial denervation on the characteristics of the MUs in the medial gastrocnemius (MG) muscles in cats. The muscles were examined after a mean of six months from the original denervation surgery and compared to normal controls. Muscles that were exposed to partial denervation and reinnervated muscles after crush

injuries contained more motor units with fatigue indices between the FR and FF i.e. fatigue intermediate (FI) than control. These muscles have also contained MUs that were not possible to classify because of the difference between contractile properties and fatigue indices. MUs were classified according to the criteria of Gillespie et al with the FR motor units normally having the longest twitch contraction time (> 30 ms) and produce the lowest force when compared to similar MUs. The unclassifiable MU had mixed properties that although they were FR they had a twitch contraction time of < 30 ms. Following complete nerve section and repair the number of unclassified MU was even higher. These unclassified MUs were thought to be the result of the reinnervation of a MU by a foreign motor neuron that resulted in a mismatch between the characteristics of the two. These changes were attributed to the lack of specificity between the characteristics of the denervated MUs and the reinnervating motor axons. Motor axons that succeed in establishing connection with denervated MU usually sprout to establish new connections with the surrounding denervated motor units. Therefore, it is common for denervated MUs to receive foreign motor neurons that carry different fatigue and contractile characteristics to their fibres (283).

Recently, Lehnert et al examined the histochemical properties of reinnervated extensor digitorum longus muscles in rats. The authors defined 4 types of muscle fibres based on their myofibrillar ATPase and succinate dehydrogenase (SDH) activities. In addition to the 3 known types (I, IIa and IIB) of muscle fibres a fourth group was described and termed "succinate dehydrogenase intermediate (SDH) Int." (198). Early in the reinnervation process, marked reduction of type IIa & IIB muscle fibres was noted and accompanied by an increase in the proportion of SDH Int. fibres. Although this preferential change in muscle fibre type became less significant after 180 days post-surgery, there continued to be increased proportions of slow (type I) and SDH Int. fibres. The presence of SDH Int. fibre type was considered proof for destabilization and reorganisation of motor unit properties and was correlated with the time course of recovery following both end-to-end repair and nerve graft (198).

Normally there is a correlation between the force generating capacity of different MUs and their contraction and fatigue characteristics (247). FF motor units are known to have the highest level of force generating capacity and the highest innervation ratio

(largest in size); conversely, slow MUs have the lowest force and innervation ratio (smallest in size). Hence, the normal order of MU force is S< FR< FI = FF (247). In the Rafuse and Gordon study this order was reversed in reinnervated muscles when the number of reinnervating motor axons was less than 25% and 50% of normal, following nerve to nerve and nerve to muscle repair respectively. It was suggested that on re-innervation when only a small number of motor neurons is available, slow motoneurons have higher potential for sprouting with a resultant increase in their innervation ratio and the ability to develop high level of force (284).

The denervation period has been shown in various studies to influence the outcome when reinnervation takes place (8). Finkelstein et al examined the medial gastrocnemius muscles of the rat following different periods of denervation with time from nerve cut to re-suture ranging from 0 to 56 days. Muscles that had immediate repair of their nerve were found to have 25% reduction of their muscle mass at eight weeks from injury. Longer periods of denervation were associated with a further reduction in muscle mass. The SO fibres were found to atrophy to a greater extent than did the FG and FOG fibres. With denervation up to seven days SO fibres were atrophied by 25-30% while the FOG and FG fibres showed only a 10% reduction in cross section. Longer periods of denervation were associated with greater reduction of fibres' cross sectional area. 21-56 days of denervation resulted in atrophy of the SO fibres by 65-80% and of the FG and FOG by 25-50%. Despite this disproportional reduction of the different muscle fibres the fatigue index of denervated muscles (0.92 ± 0.45) was not different from control (0.58 ± 0.28) (103).

Maximum tetanic force was found to have an inverse relation to the period of denervation. Hence, muscles with longer periods of denervation had less maximum tetanic force compared to others with shorter periods of denervation. Muscles with immediate repair of their nerves recovered only 50% of their maximum tetanic tension after 8 weeks of recovery.

The findings from this study confirmed the reduction in muscle mass and force and their correlation to the denervation period. Their was no difference in the fatigability of reinnervated muscle and this would be consistent with their histochemical findings as denervated muscles were found to have a higher proportion of the SO fibres compared to control. It is important to note that in this study the treatment groups were treated as one group with self and cross-reinnervated muscles' results summed

together. In the cross-reinnervated muscles the nerve for the soleus that predominantly comprised of slow motoneurons was used. This would result in a formation of motor units that are primarily fatigue resistant, hence this finding of no difference in fatigability between reinnervated and control muscles (103).

Denervation induced changes in muscle afferents and different muscle receptors are likely to influence fatigue characteristics of reinnervated muscles (330). Unloading of the muscle that reduces the afferent activity is associated with transformation or shift of muscle fibres from slow towards fast twitch (9, 39).

Following nerve repair muscle spindles were found to have an abnormal firing pattern in response to sustained stretch, and this affected over 50% of the afferents for several months (10, 11). A similar abnormal behaviour was noted for many Golgi tendon organs that had a reduced firing rate in response to sustained tetanic contractions (301). In a study of rat tibialis anterior muscles following peroneal nerve division and immediate repair, type III and IV afferents were found to be reduced in number up to seven months after surgery. Even those afferents that recovered and regained their activation by extracellular chemicals (KCl and lactate) were not possible to activate by prolonged contraction resulting in fatigue (66). It was proposed that the lack of afferent response to changes in metabolite levels during muscle contraction can lead to loss of the protective (muscle wisdom) inhibitory reflex and delays the occurrence of fatigue (66).

Brunetti et al examined the mechanical and histochemical changes induced on the lateral gastrocnemius muscle of the rat by deprivation from group III and IV afferents. The function of these groups of muscle afferents was eliminated by the use of capsaicin (neurolytic agent). Maximal twitch tension was reduced by 13% in deafferented muscles compared to control. Fatigue resistance of deafferented muscles was higher than control as denoted by the increase in their fatigue index by 45%. Deafferentation was also found to alter the fibre type composition of the affected muscles with an increase in the percentage of type I and IIa and a reduction in type IIB fibres. These effects were explained by two hypotheses, first was the elimination of nociceptive and ergoceptive (type III and IV) fibre functions with a resultant increase in fast motoneuron discharge leading to slow fatigue resistant muscle fibres.

The second was the elimination of type III and IV proprioceptive effect on the monoreflex that regulate motoneuron discharge leading to delayed appearance of fatigue. (34).

1.4.2- Biochemical changes in dennervated muscles

Nerve section was found to affect the enzymatic profile of dennervated muscles. Hearn (1959) observed a reduction in activity of succinate dehydrogenase, cytochrome oxidase and aldolase in all fibre types following nerve section (139). Reduction in glycolysis and oxidative phosphorylation were also noted in denervated muscles and found to be similar to the changes observed in dystrophic muscles (109). Hogan et al demonstrated that alteration in enzymatic activity was due to changes in the enzyme protein population and not due to altered muscle kinetics. Examining dennervated gastrocnemius muscle showed that fast contracting fibres has lost alpha-glycerophosphate dehydrogenase, aldolase, pyruvate kinase and muscle type lactate dehydrogenase (LDH), whereas slow fibres from the soleus muscles lost isocitrate dehydrogenase, malate dehydrogenase and heart type LDH (143).

In vitro analysis of skeletal muscles after denervation showed alterations of the muscle energetics that may be partially attributed to disuse atrophy. Disuse atrophy of rat hind limbs was found to decrease the concentration of cytochrome oxidase and monoamine oxidase and the specific activity of these enzymes. The decrease in enzyme activity observed with disuse atrophy may be the reverse of the training effect. In other words the oxidative capacity of the muscles decreases because of inactivity and hence a lower need for energy supply (review (109)).

Magnetic resonance spectroscopy (MRS) has provided a valuable tool for studying the biomechanical changes in denervated muscles. Frostick et al have examined the rabbit hind limb denervated muscles using 31 phosphorus MRS (^{31}P MRS) and reported significant alterations of the high and low energy phosphate metabolites (110). Human reinnervated muscles were studied by the same authors who observed a condition of metabolic myopathy in the muscle cells with changes in phosphocreatine, adenosine triphosphate and inorganic phosphate (111).

Frostick (1987) examined denervated animal and human muscles using ^{31}P MRS and observed a decrease in PCr/ATP ratio and an increase in P_i/ATP ratio in all denervated muscles. On reinnervation to a clinically assessed MRC grade V these ratios tended to return to normal.

In the animal model of dividing the sciatic nerve in rabbits, a gradual fall in the PCr/ATP and Pi/PCr ratios was observed in addition to increased Pi/ATP ratio. The difference between denervated and control group was found to be significant early post surgery and suggested an early onset of abnormalities in the high-energy phosphate metabolism. The author attributed these findings to the state of interdependence between muscles and their supplying nerves, hence the rapid alteration in the metabolic state of muscles observed early after denervation (109).

Sodium channel density in the sarcolemma is normally set to meet the contractile requirements of muscle fibres and thus contribute to the phenotypic features of myofibres (242). In skeletal muscle, a high density of sodium channels in the sarcolemma allows fast-twitch fibres to fire at higher frequencies in order to allow rapid and fast contraction, whereas slow-twitch muscle fibres have a lower density of sodium channel that suits their slow motor neuron input. This low density of sodium channels in slow-twitch muscle fibres may reduce the propensity to fatigue during long periods of contractions (242). Activation of sodium channels by artificial agents (veratridine and aconitine) was found to reduce muscle endurance and to slow the initial rate of force recovery in rat soleus muscle. This effect has been attributed to an increase in sodium influx exceeding the capacity of Na^+-K^+ ATPase to remove Na^+ from the sarcolemma (138).

Desaphy et al (2001) investigated the influence of one, two and three weeks of unloading on the hind limb muscles of the rats. After one week of unloading the soleus muscle sarcolemma was found to have a higher density of sodium current compared to control. This difference has gradually increased from one to three weeks. The authors suggested that this increase in sodium channel density if not compensated for by a simultaneous increase in Na^+-K^+ pump activity can influence the fatigue characteristics of unloaded muscles (75).

The change in sodium channel density was found to be influenced by the activity of the innervating motor neuron. Milton et al (1995) examined the effect of reinnervating a slow twitch muscle using a motor axon that normally supplies a fast twitch muscle in the mouse hind limb. This experiment resulted in an increase in the Na^+ channel density of the reinnervated slow twitch muscle to almost twice its normal value. The change in sodium channel density was suggested as one of the reasons for impaired

motor deficiency and possible increased fatigability in immobilized and reinnervated muscles (241).

It is clear from the above review that denervation can alter the muscle fibre distribution and contractile properties through various mechanisms. Some of these mechanisms have been shown to increase muscle fatigability with a shift from slow fatigue resistant to fast fatigue fibre type (39, 9) while others can induce the opposite change (66, 103). The final outcome of muscle function is likely to be governed by the original functional properties of the muscle, the degree of nerve injury, the period of denervation, the extent of reinnervation and motor neuron sprouting.

1.5- Serratus anterior muscle

1.5.1 Serratus anterior muscle anatomy and function

The serratus anterior muscle is a broad, flat muscle formed by multiple digitations arising from the upper eight or nine ribs in the midaxillary line and inserted into the ventral surface of the scapula along its vertebral aspect (217). Three functional components of the serratus anterior have been identified, a superior cylindrical mass that attaches to the superiomedial border of the scapula and anchors the arm during its overhead rotation. A long thin wide intermediate band that connects the third, fourth and fifth ribs with vertebral border of the scapula and draws the scapula forward. The lower five slips from the sixth through the tenth ribs that converge on the lower angle of the scapula to rotate the inferior angle of the scapula upward and laterally across the chest wall (132).

The scapula represents the stable base for the humeral head and alters its position in response to glenohumeral movements. This characteristic scapular movement augments and facilitates mobility of the whole shoulder complex and is known as scapulothoracic rhythm (340). The scapula has three plans of motion; elevation, depression, protraction (lateral and forward motion along the thorax), retraction, upward and downward rotations (83) fig.1.1.

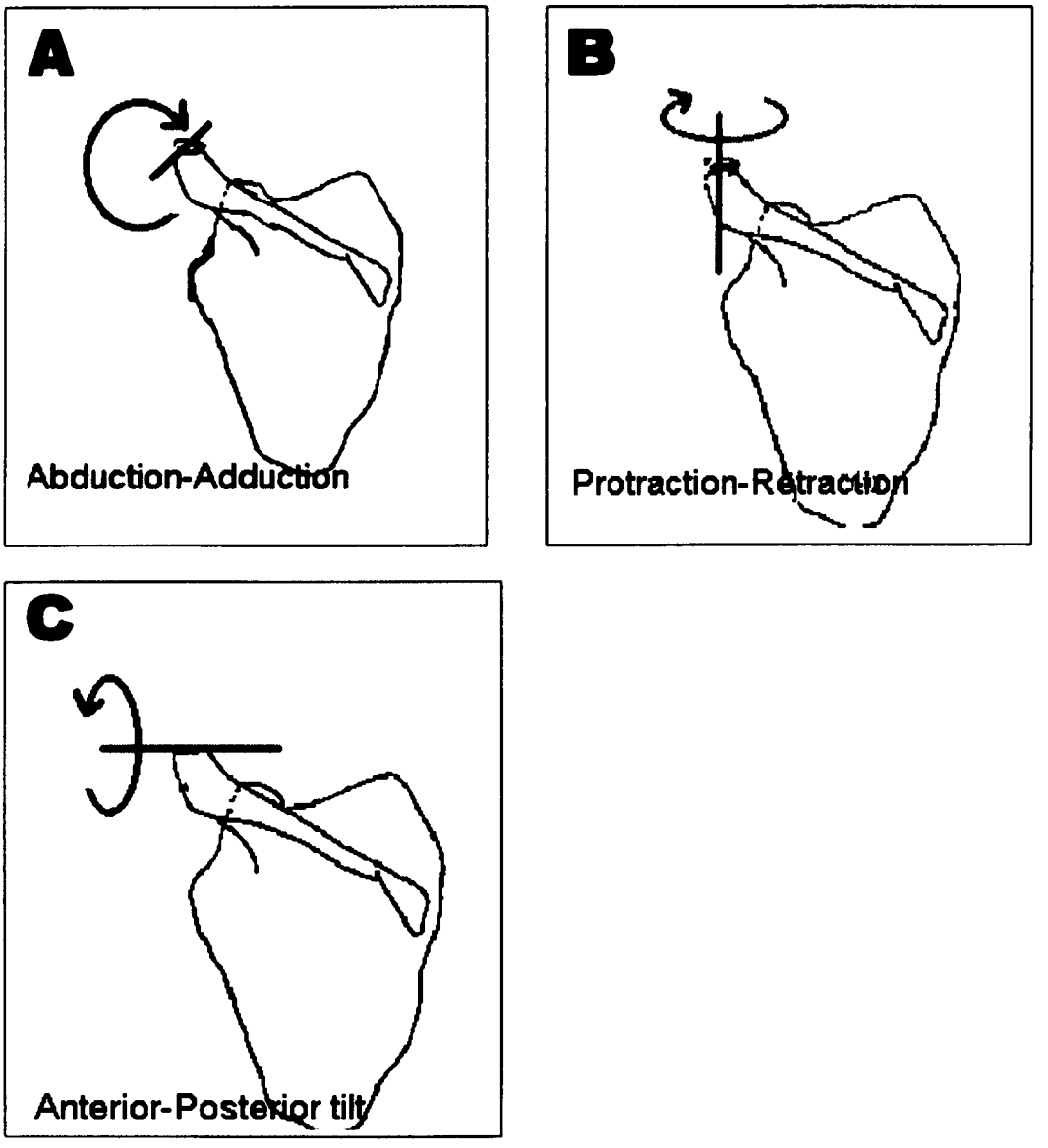


Figure 1.1 shows the different directions of movement of the scapula. (A) shows the abduction-adduction movement on the plan of the scapula around an antero-posterior axis. (B) shows the protraction-retraction movement or lateral and medial rotation around a vertical axis. (C) shows the antero-posterior tilting around an axis parallel to the spine of the scapula.

Multiple muscle groups are involved in scapular motion. Particularly the trapezius and serratus anterior are important for scapular elevation and upward rotation and the surrounding muscles cannot compensate for the loss of their function (266). The serratus anterior action allows the inferior angle of the scapula to swing more laterally and forward than does the superior angle, causing the glenoid cavity to tilt upwards (266) fig.1.2.

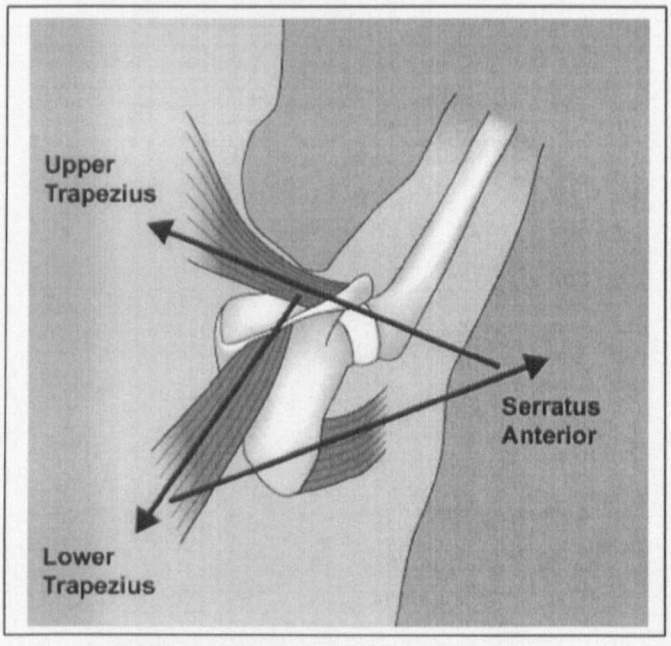


Figure 1.2 shows the direction of action of the serratus anterior muscle during forward elevation that allow forward pulling of the inferior angle of the scapula and upward rotation of the glenoid cavity. The action is aided by the action of the upper and lower parts of the trapezius muscle. After D Stoller, S Copeland, L Bigliani, R Emery, A Amis, A Chippindale, in Interactive Shoulder CD 2000, Primal Pictures, London.

Testing serratus anterior function is usually performed by one of two methods: with the patient standing, the arm is forward flexed to about 90°, using the palms of the hand the patient is asked to push against a vertical surface. In this position the serratus anterior function is to try and pull the thorax backwards as the arms are trying to push the body back fig 1.3. The position of the scapula is observed during this test. The same mechanism of serratus anterior loading applies to a push-up test. The second

method of testing the serratus anterior is whilst the patient is standing or sitting with the arm in a forward flexed position of 120°-130°. The examiner applies pressure on the dorsal surface of the arm between the shoulder and elbow joints in the direction of arm extension while using the other hand to feel for movements of the inferior angle of the scapula fig. 1.4. Serratus anterior weakness can be observed as difficulty to achieve or maintain this degree of arm forward flexion or by winging of the scapula. The second test emphasises the upward rotation action of the serratus anterior and usually used to test the serratus anterior function in doubtful cases or to test its strength (266).

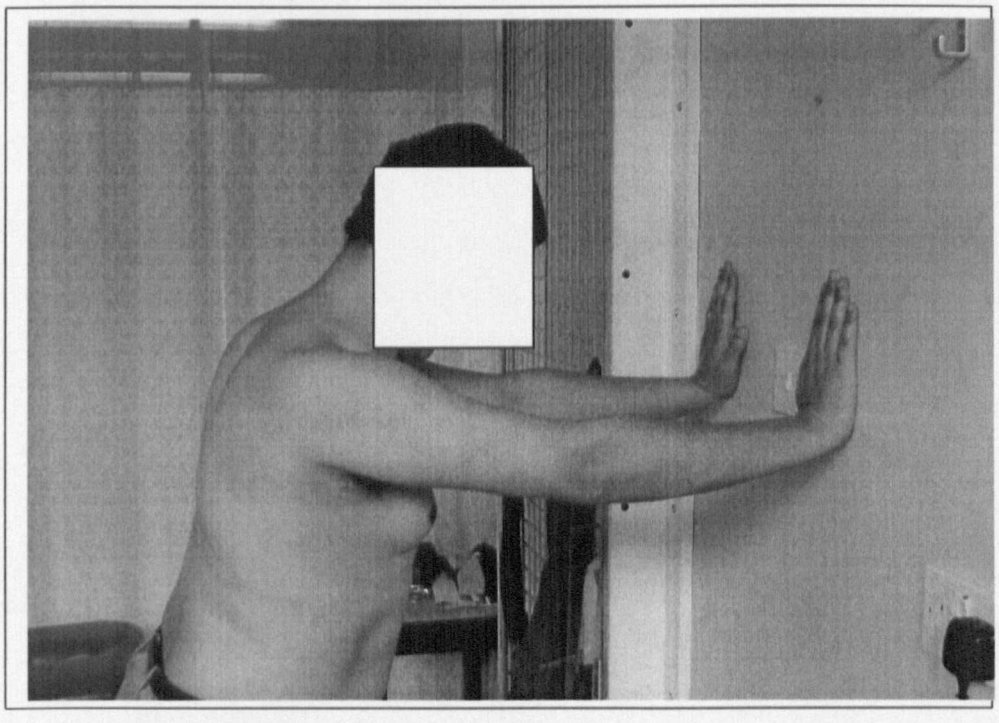


Fig. 1.3 testing the serratus anterior weakness by asking the subject to push forward against fixed resistance while the arm is in 90° forward flexion. Serratus anterior function is stabilising the scapula that is being pushed back by the reactive forces of body weight.

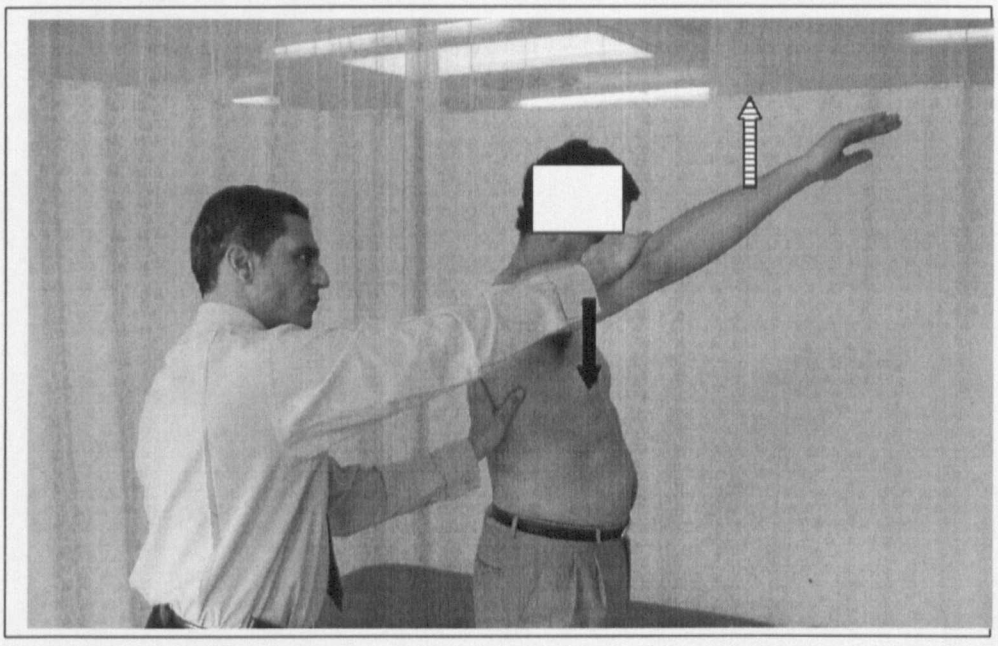


Fig. 1.4 with the subjects arm in 120° of forward flexion, one of the examiners hands is pushing the subject's arm downwards, ⬇

while the subject is trying to elevate his arm upward ⇧. The examiners left hand is feeling the inferior angle of scapula for any tipping or retraction

1.5.2- Testing of scapular position and movements

Quantitative assessment of the scapular stabilizers and scapular position is performed by one of 2 clinical tests: the first test is the lateral slide test described by Kibler (1991), and the second test is the DeVita's test (1990).
De-Vita's test compares the position of the scapula between each side in one position. With the subject standing, the distance between the tip of the 3rd thoracic vertebral spinous process and the inferior angle of the acromion is measured using a piece of string (80).

In Kibler's lateral slide test the position of the scapula is compared between the injured and non-injured sides in relation to a fixed point in the spine at three different positions that subject the serratus anterior to varying degrees of loads. The first position is with the arms relaxed by the sides, the infero-medial border of the scapula is defined and marked. The distance between this point and the nearest spinous process is then measured. The same spinous process will be taken as a reference for the other two measurements. The second position is with the hands at the hips with the fingers pointing forward and the thumb towards the back; and the shoulder assuming a position of 10° extension. The distance from the previously defined spinous process and the inferomedial border of the scapula is measured. In the third position the arm is placed in 90° forward flexion, and the shoulder is fully internally rotated (188). The measurements are repeated again in the last position between the same landmarks in its new position. Reliability tests of this test have confirmed good intra-tester and inter-test reliability (318).

The authors have accepted a side to side difference of up to 1.5 cm in the distance measured from the inferior angle of the scapula to the reference spinous process and considered it as normal (188). Using EMG evaluation showed almost no muscle activity in the first position, low level of activity in the serratus anterior and lower fibres of the trapezius in the second position, and around 40% of maximum level of activity in the serratus anterior, trapezius and rhomboids (188).

1.5.3- isometric and dynamic testing of the serratus anterior muscle

Muscle contractions can be isometric or dynamic (isokinetic). When a muscle contracts in an isometric mode this means that there is an increased tension as force is generated but the length of the muscle remains constant. Conversely, during the dynamic mode of contraction force generation is accompanied by a change in the muscle length. The change in muscle length during dynamic contraction can be in the form of shortening as in concentric (Con) contraction. If the change in length is in form of lengthening the contraction mode is called eccentric (Ecc).

Con type of muscle contraction can occur with or without an external resistance to the muscle's action. If an external resistance is present it is smaller in magnitude compared to the muscle's generated force resulting in shortening of the muscle during contraction. For the Ecc mode of contraction there is an external load that exceeds in magnitude the generated force or torque by the contracting muscle and acting in an opposite direction. The externally applied load is greater in magnitude to the muscle's generated force or torque. Hence, the muscle increases in length as it contracts.

Experiments with dynamic contractions are usually performed with the use of an isokinetic machine that controls the externally applied load, the ROM and the velocity of movements.

For the serratus anterior a Con mode of dynamic contraction would be achieved during forward elevation of the arm. During this movement the serratus anterior action is to rotate the scapula laterally and upward as well as protracting the medial border of the scapula i.e. the origin and insertion of the serratus anterior are getting closer to each other. In contrast, an Ecc mode of contraction would be achieved when the arm is forced from an upward elevated position by an extension force. The return of the arm to the side of the body will be accompanied by medial and inward rotation of the scapula and retraction. The contracting serratus anterior will allow for controlled return of the scapula into its normal position. Hence, there is lengthening of the muscle during contraction.

In this thesis isometric and dynamic modes of muscle contractions have been used in different experiments to induce fatigue of the serratus anterior muscle. Isometric contractions have been used for the first dorsal interosseous muscle fatigue protocols. Fatigue patterns and changes observed with different types of contractions will be discussed in the relevant sections throughout the thesis.

1.6- EMG and myoelectric manifestation of fatigue

In the first section of this chapter the ionic basis of the resting membrane potential has been explained. The resting membrane potential is around 85 micro-volts. When a stimulus arrives from the motor axon to the end plate it initiates the opening of the Na^+ channels close to the ACh receptors and secondary synaptic clefts at the MEP region. The intracellular flow of Na^+ and movement of K^+ to the extracellular space results in membrane depolarisation. This depolarisation rapidly travels from the excited towards the resting parts of the membrane that responds in a similar way by opening the Na^+ channels allowing propagation of the AP (224).

The amplitude of an individual AP varies depending on muscle fibre diameter, distance between active fibre and recording site and electrode properties. The sum of action potentials generated by different muscle fibres that constitute a motor unit presents the Motor Unit Action Potential (MUAP) (224).

Within a contracting muscle there are multiple motor units that are usually discharging at different rates. Some of these motor units may have alternate periods of activity while synchronised action can be found in other occasions. An Electromyography (EMG) signal is a spatial and temporal interference pattern of the electrical activity arising from the activated motor units located within the receptive field of the recording electrodes (45). The shape of the EMG interference pattern is therefore influenced by the number of recruited MUs, their size, shape and architecture, firing rates, duration of firing, time of recovery, and degree of synchronization in recruitment (254).

1.6.1- EMG recording

For digital recording of EMG the following key components would be required: electrodes, amplifiers, filters, analog-to-digital (A/D) converters and a PC.

Electrodes are usually classified according to whether they are invasive like needle and wire electrodes or non-invasive being attached to the skin surface (surface electrodes) (189).

Surface electrodes are non-invasive, easy to apply and can provide valuable information even in inexperienced hands. Therefore, they are more commonly used in humans. They are attached to the skin using adhesive strips or collars. Silver (Ag) electrodes are electrically more stable when covered by a layer of silver chloride (AgCl). Therefore, Ag-AgCl surface electrodes are widely used for EMG recording (189).

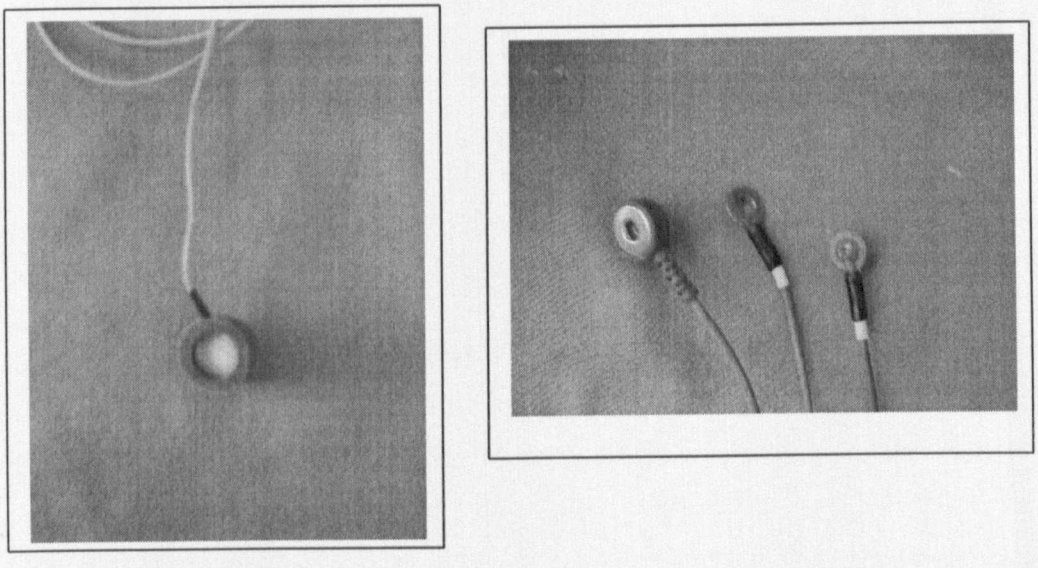


Figure 1.5 shows two types of Ag-AgCl surface electrodes of different diameters that have been used in this thesis for EMG recording. The concavity of these electrodes is normally filled with conductive gel to improve contact and conductivity of EMG signals.

Surface electrodes can be used in both monopolar and bipolar configurations. Monopolar electrodes detect the electrical activity from the underlying muscle in respect to a reference electrode placed in an unrelated or electrically quiet area

(ground electrode). Monopolar electrodes are more prone to pick up any and possibly unwanted electrical signals from the surrounding muscles (333).

The EMG electrical signals are liable to meet resistance while passing through any material including the biological tissue. They are also liable to acquire noise from different sources like motion artefacts and alternating power line interference. Therefore, attention to the details of EMG acquisition is essential if meaningful data are to be acquired (45).

A common problem met in using surface electrodes is the impedance at the skin-electrode interface. Impedance here is meant to express the resistance met by the EMG electrical signals during their propagation. The magnitude of this impedance depends on the site of electrode application in relation to the active part of the muscle. It is also influenced by the thickness of underlying adipose layer separating skin from muscle and time since application. Sweat that accumulates between the skin and an electrode that stays in place for sometime improves conductivity and reduces the magnitude of impedance (114).

To minimise impedance the dead superficial epidermal and oily layers of skin have to be removed from the area of skin-electrode interface (14). This has been found to reduce the impedance by a factor of 10 (189).

Needle electrodes have various designs including monopolar, concentric monopolar, bipolar and multipolar. The needles are usually insulated apart from the tip that remains as a detection area. All needle electrodes have high impedance due to their small detection area (14). They are particularly suitable for studying individual motor units and can be repositioned to detect other motor units. As they are invasive, they cause discomfort and have a localised recording area they are not suitable for biomechanical studies that are intended to characterise a group of muscle activity. They are rarely used in children or those who fear needles (14).

Fine wire electrodes were first introduced by Basmajian and Stecko (1962) (16). The original design consists of two fine wires 25 μm in diameter, fully insulated apart from their ends and inserted using 27 gauge hypodermic needles. Fine wires are usually made of silver, platinum or nickel chromium alloys and insulated apart from

their tips by nylon, polyurethane or Teflon. These electrodes were found to have obvious advantages over the needle electrodes. As they are very thin in diameter they are relatively less painful once the needle is withdrawn and less likely to break or cause bleeding. The wire electrodes are staggered and hooked at the ends and this allows them to engage in the muscle fibres and reduces migration. Minor degrees of movement 100 μm can place the electrodes in a completely different field of muscle fibres. The diameter of a muscle fibre in an adult's large muscle is roughly 50μm. It was, therefore, recommended that the studied muscle should be allowed to contract few times prior to the start of actual testing to allow these wires to settle and engage themselves in the muscle fibres (14).

1.6.1.1 *Noise in EMG and how can it be reduced or eliminated?*

The basis for function of an EMG acquiring electrode whether it is surface or indwelling (wire and needle) is to form a layer of charge at the interface between the metal electrode and an electrolyte solution. The presence of a charge gradient at the electrode-electrolyte interface produces a potential termed the half-cell potential. This potential is dependent on the electrode material. In EMG recording, all recording electrodes should be made of the same material to minimise the half-cell potential difference (45).

Coating the electrodes with a layer of AgCl stabilizes the electrode-electrolyte interface of Ag electrodes. That is why Ag-AgCl electrodes are widely used for EMG surface recording (5).

In surface EMG recording the electrode-skin interface has reactive impedance. This impedance is dependent on electrode size, the signal frequency and the current density at the electrode (5). Godin et al demonstrated that electrode impedance was reduced with the increase in electrode diameter and increase in the signal frequency (127).

High electrode-skin impedance can result in a reduction of the signal amplitude, waveform distortion, and power line interference in the recorded EMG. This problem can be minimised if the following precautions are taken. Careful preparation of the skin with the use of alcohol or lipid solvents like ether in addition to rubbing the skin with conductive paste or gel reduce the electrode-skin impedance. Paste-filled

electrodes generally exhibit lower impedance than dry electrodes. Finally, the use of a signal amplifier with high input impedance that is at least 100 times greater the highest expected electrode impedance i.e. an input impedance of 100 MΩ or greater (45).

For surface electrodes, motion can result in disturbance of the electrode charge layer and deformation of skin under the electrodes. Relative movement between the electrode and underlying skin causes disturbance of the electrode charge layer that can be the source of noise. Noise is any unwanted signal detected together with the desired signals. Having a conductive gel or paste at the electrode skin junction reduces this adverse effect of motion and damps the mechanical disturbance (319).

The other type of motion noise is the result of a potential difference that exists across layers of skin. The value of this potential changes with deformation or stretching of the skin. Reducing skin impedance, by abrading the superficial epidermal layer, lowers the degree of noise that can arise from skin deformation (319).

The power density of motion noise is usually below 10 Hz but can be as high as 20 Hz. High pass filters are usually set to cut off signals below 10 Hz, and this usually eliminates most of motion artefact noise without interfering with the myoelectric signals (53).

Cables that connect the recording electrodes to the amplifiers can be a source of noise. When cables are subjected to a surrounding electric or magnetic field, current is generated and can be mixed with the acquired signals. Friction and deformation of the cables can also generate static charges that propagate through the recording system. Reducing cables length and movements helps in reducing this type of noise (343).

Another source of noise arises from alternating current power line interference. Electromagnetic fields exist around power lines and electric equipment. Presence of such fields can result in power line interference signal in the recorded EMG (343). Using bipolar recording electrodes with differential amplifiers can eliminate or reduce this type of noise (see section on common mode rejection ratio and differential amplifiers).

1.6.1.2 Selection of the site for electrode application

One of the problems that have been encountered in EMG recording is cross talk. By cross talk it is meant the unintended recording of EMG signals arising from muscles in the neighbourhood of the muscle under investigation. It has been suggested that the magnitude of this problem is usually higher with surface than indwelling electrodes (311).

In choosing the position of a surface electrode consideration has to be given to the possibility of cross talk, motion artefacts, innervation zone and direction of muscle fibres (353).

The impedance of muscle tissue is both frequency and direction dependent. As muscles are non-homogeneous structures, fibres are arranged to form fluid filled channels that possess lower impedance than their surrounding connective tissue. Hence, recording parallel to the fibres will encounter lower resistance than if it is done perpendicular to the fibres direction (77). It is also advisable to place the electrodes in the region mid-way between the centre of the innervation zone and the distal tendon (353).

EMG signals acquired with a bipolar configuration are less liable to be contaminated by non-desirable signals from surrounding muscles. Having two detection surfaces a bipolar configuration detects two potentials from the muscle of interest with respect to a reference (ground) electrode. These two potentials are then transferred to a differential amplifier that eliminates the common mode signals (identical signals detected simultaneously by both electrodes) and keeping only the relevant signals that arose from the intended muscle. So, a bipolar configuration acts as a bandpass filter with a bandwidth inversely proportional to the space between the two recording electrodes (45).

Surface electrodes are intended to record all electric activities arising from different motor units of the studied muscle that can be detected within their recording field. Selectivity is dependent on the size of the recording area (area of contact with underlying skin) and the space between the recording electrodes. The most selective electrodes are those with the smallest detection surface and the shortest inter-electrode

distance (114). In general, surface electrodes are less selective i.e. record signals from more motor units, compared to the needle and wire electrodes.

1.6.1.3 Sampling rate and resolution

The amplified and filtered EMG signal is a continuous signal in regard to time or analogue. To allow handling of this signal by a digital computer it has to be converted into a discrete-in regard to-time or a digital signal. Once converted to a digital signal, the signal takes the form of a sequence of numbers (193).

Most recent EMG recording machines bypass the analogue recording and EMG is sampled directly for storage and off line processing through an analogue-to-digital (A/D) converter. Resolution of an A/D converter is determined by the number of bits (binary integers) per sample i.e. the number of bits defines the number of discrete levels available for the signal to be decomposed into. A 16-bit A/D converter divides the input voltage range into 63,536 discrete levels while a 12-bit A/D converter into 4096. A 16-bit A/D converter is preferred as it allows sampling of signals with wide range of amplitudes, thus signals are given the discrete level closest to their actual level. A 16-bit A/D converter has been used for EMG acquisition in the experiments presented in this thesis (179).

The sampling frequency determines the maximum frequency in the signal to be digitized that can be adequately represented. The sampling or Nyquist theorem states that if a signal contains frequencies that range from 0 to F_N (Nyquist frequency) then the minimum sampling frequency that can be used is $2F_N$ (193). It is believed that on sampling a band limited signal, a sampling frequency above double of the highest frequency content is necessary to be able to reconstruct that waveform. Choosing a sampling frequency double the highest frequency of the signal allows enough information to recreate the waveform with accuracy (157).

Nilsson et al (1993) explained the setting of this sampling frequency by a phenomenon called aliasing. If the frequency content of a signal is above half the sampling frequency misinterpretation or aliasing occurs (257). The authors gave an example to this phenomenon by what is seen in television in cowboy films when cartwheels appear to run backward or more slowly than the cart. In this case, the

signal frequency is the rotation of the cartwheels, and the sampling rate is the presentation rate of the television image (30Hz for example). If the cartwheels are rotating at 15 rotations/second (Hz) or more the resulting image will distort the true rotation of the wheels (257).

Gitter and Stovlov (1995) claimed that the Nyquist rate is too slow and sampling rates should be three to ten times the maximal frequency content in the signal (125). Nilsson et al (1993) reported altered onset timing of evoked muscle surface EMG signals when sampled below four times the highest signal frequency (257). However, Ives et al (2003) suggested that over sampling (using high sampling frequency above the Nyquist rate) does not add any significant advantage to the amplitude and timing analysis of surface EMG (157).

Sampling rates for surface and wire EMG recording have been defined according to the known highest EMG frequency acquired with both types of electrodes (see table next page). Sampling frequency of 2000/ sec. has been used for the experiments presented in this thesis.

1.6.1.4 Amplifiers

The magnitude of amplification applied to the recorded signal with the use of amplifiers is called the gain. Amplifiers can reduce the effect of the noise when a differential gain is applied to the signal, which improves overall noise rejection by raising the signal strength above the noise floor found in subsequent electronics (45).

With bipolar recording electrodes the EMG is differentially amplified. Signals that have been identically recorded by the two electrodes are called the common mode signals. These are typically the noise that has been superimposed on the real EMG signal during their travel in the system. These common mode signals are eliminated on amplification. Therefore, differential amplifiers play an important role in reducing the noise assuming that the recorded noise signals are similarly recorded by the two electrodes (45, 333).

Assuming that the gain is G, the desired signal is m and the undesired signal or noise is n. With monopolar amplifiers the desired signal and noise are similarly amplified

i.e. monopolar: amplified signal = G* ($m^{+}n$). With bipolar recording the desired signals recorded by both electrodes will be amplified while the noise (identically recorded by both electrodes is eliminated i.e. bipolar amplified signal = G*(($m_1^{+}n$)-($m^{2+}n$)) = G*(m_1-m_2). (from Basmajian and Deluca 1986)

The quality of the amplified signal is measured by the signal to noise ratio, the higher the ratio the greater the noise reduction. While the ability of the amplifier to reject the common mode signals is termed the common mode rejection ratio (CMRR), CMRR = differential gain/common mode gain, the higher the CMRR the better the cancellation of common mode signals (353).

CMRR should be greater than 10,000 (80dB). This means that the difference in voltage between the recording and reference electrodes should be amplified 10,000 times more than the common voltage at both electrodes. Incomplete eradication of noise in bipolar recording can then arise from one of two sources; either a low CMRR or that noise signals are not identical at both ends (257).

As amplifiers work within a range (bandwidth) of frequencies they act like filters with gain. The function of an amplifier then is to remove the signals outside its working bandwidth. Hence, the frequency setting of the amplifier should be high enough to eliminate low frequency signals (< 20 Hz) that usually result from movement artefacts. The lower limit for the amplifier bandwidth should also be low enough so real signals would be amplified. The lower limit for amplifier setting is usually 10-20 Hz. Signals of lower frequencies are usually unstable and unpredictable. The upper limit of the bandwidth depends on the method of recording. The maximum frequency recorded by surface electrodes and wire electrodes are usually around 400 Hz and 650 Hz respectively. The upper limit for amplifier frequency setting is usually 500 Hz for surface and 1000 Hz for wire electrodes (257).

1.6.1.5 Digital filters

A digital filter is a computer program or algorithm that can remove unwanted frequency components from a signal. Filters have been classified according to the range of frequencies they work on into high-pass, low-pass, band-pass and notch filters (193).

The setting of a particular filter determines its cut-off limit. Certain filters are used to eliminate particular types of noise. For example, high-pass and low-pass filters are useful in removing low and high frequency noise respectively. Therefore, high and low-pass filters are used to eliminate noise that arises from disturbances produced by the electronics of the amplifiers, electrode impedance noise, movement artifact, and magnetic noise (257, 333).

When considering filter setting, the frequency content of the EMG signals should be defined. For surface electrodes the frequency of the desired EMG signals usually lie in the range between 10 Hz to 340 Hz. Therefore filters and amplifiers are usually set at a cut-off limit of 10 Hz to 500 Hz i.e. frequencies above 500Hz and below 10Hz are eliminated. Signals below 10 Hz and above 500 Hz are usually unstable and considered as undesired noise. Wire electrodes are able to record a wider range of frequencies, hence filters are usually set at 10 Hz to 1000 Hz. Nilsson et al have provided a guide to the range of frequencies that can usually be recorded with different types of electrodes and accordingly determined the cut-off frequency limit for the filters and amplifiers used with them (table below) (257).

Electrode type	High frequency limit	Peak frequency	Cut-off for filters and amplifiers	Minimum sampling frequency
Concentric needle EMG	600Hz	115-225 Hz	2-10,000 Hz	20 KHz
Surface EMG	340Hz	75-102 Hz	6-500 Hz	1 KHz
Wire EMG	650Hz		2-1000 Hz	2 KHz

(257)

1.6.2- Analysis of EMG signals

EMG amplitude is usually reported as an Average Rectified Value (ARV), or Root Mean Square (RMS). Rectification is a process by which the negative deflections of the signals are eliminated and being either removed in half wave rectifiers or converted into positive deflections in full wave rectifiers. Therefore, full wave rectifiers takes the absolute value of the signal and retains all the signal's energy (15).

The rectified value can then be smoothed. Smoothing acts like a low-pass filter to eliminate the high frequency signals. The resulting value is called the Mean Absolute Value (MAV). Alternative to the process of rectifying the EMG signal is to calculate the root mean square (RMS). Calculation of the RMS effectively rectifies the signals. Both methods of calculation, the ARV and RMS, were found to be very similar with very little difference between them (137).

EMG amplitude is usually used in biomechanical and biofeedback studies to indicate the pattern of muscle activity or presence of muscle contraction. It can also be used to predict the level of exerted force, though the relation is not always linear. To compare between different subjects or the level of activity in different muscle, a normalisation process would be necessary (137).

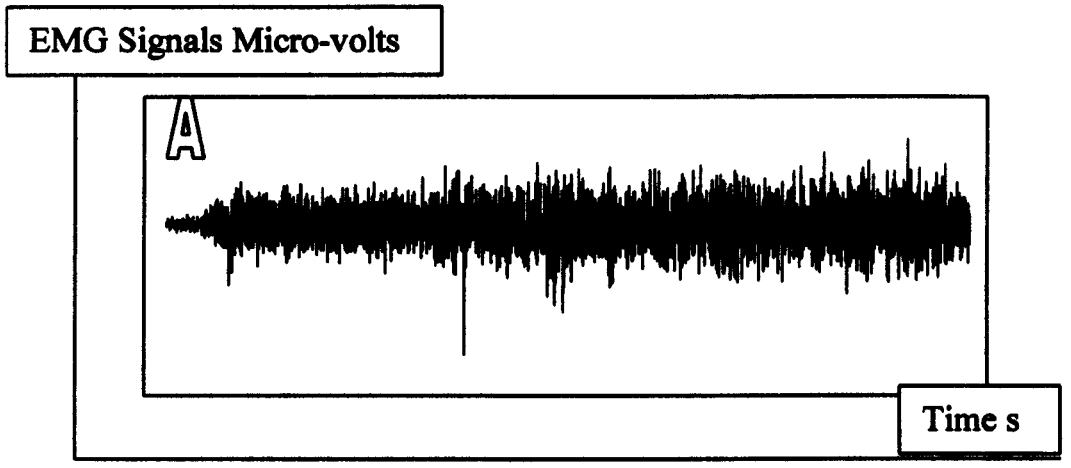
Normalisation is performed by averaging the measurable AMV or RMS in respect to either the maximum measurable value or to the mean of the cycle of movement. In some studies averaging is performed to the recorded EMG signals during a maximum voluntary contraction (MVC) (249).

1.6.2.1 Fourier spectral analysis

Fourier analysis is the representation of a periodic function as a Fourier series or a sum of trigonometric functions, which are sins and cosines. The Fourier transform is a mathematical method to analyse a periodic function into a sum of a large number of cosine and sine waves with frequencies of f, 2f, 3f and so on, where f is the lowest frequency in the function analysed and 2f, 3f and so forth are harmonics. The input of

the Fourier transform is the periodic function or signal to be analysed. The output is the amplitude of each sine and cosine wave in the series (104, 193).

By convention both cosine and sine waves of a given frequency are often lumped together into one quantity describing the amount of that frequency present in the signal. This quantity is called the spectral intensity or power and a plot of this as a function of frequency is the power spectrum (104) fig 1.6



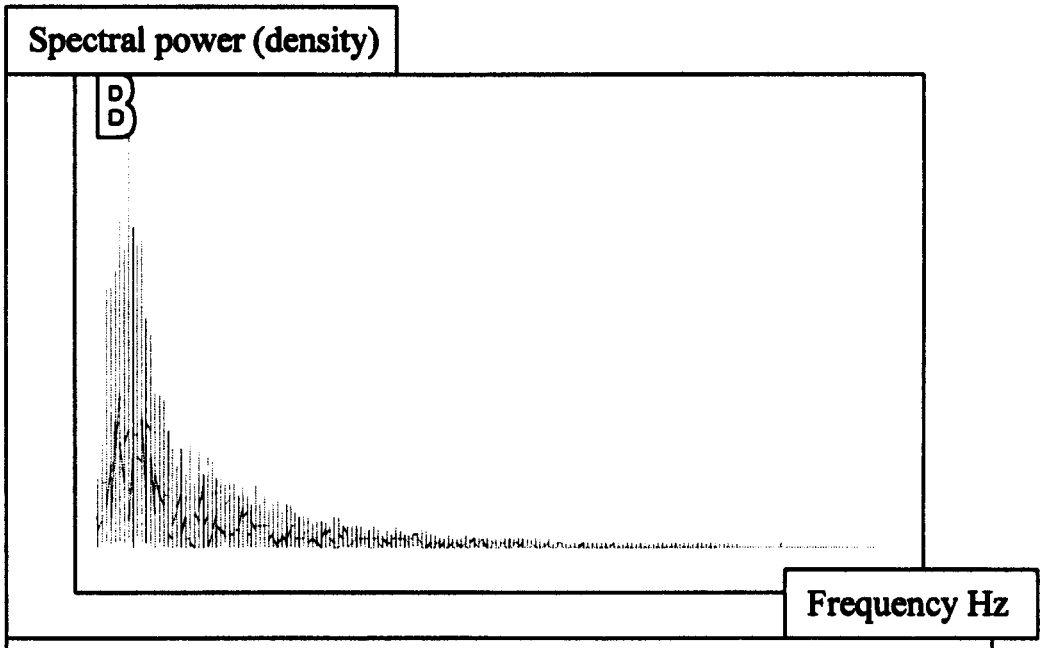


Figure 1.6 A shows the raw data of EMG signals plotted as a function of time.
B. shows the power spectrum of the EMG plotted as a function of frequency.

Two parameters are commonly calculated from the power spectrum, which are the median frequency (MDF) and the mean power spectrum (MNF). The MDF is the middle most frequency value, which has equal number of frequencies above and below it, while the MNF is the average of the amplitude spectrum (15).

1.6.3 EMG as a measure of fatigue

Central to the process of muscle contraction and fatigue is the order, rate and pattern of discharge of motor units. Motor units were found to follow a certain order of recruitment with the slow fatigue resistant motor units being recruited earlier than the fast fatigable units. It was suggested that this order of recruitment would allow the higher threshold motor units to substitute for the other units that have stopped firing (270). Motor units may be alternating their function in a cyclical fashion; with those motor units that have stopped firing being re-recruited to take the place of recently recruited ones (299).

The motor unit discharge rate was found also to be modulated during fatiguing contractions. During MVC, motor unit discharge rates were found to drop by around 50% after 60 seconds and accompanied by a fall in force. Bigland-Ritchie et al recorded the discharge rates of individual motor units from human adductor pollicis during MVC. The authors recorded a progressive decline in the range and mean rate of motor unit discharge; with those units that had highest initial discharge rates changing their rate most rapidly (21). In a similar experiment Jones et al have studied the effect of high stimulus rates on the force production in the adductor pollicis muscle and could demonstrate that, although high rates of stimulus was needed at the beginning of the contractions; maintaining this high stimulus led to a rapid fall in force (169).

Reduction in motor unit discharge rates was not considered as the direct cause of decline in force but rather as a mechanism to maintain force level that would fail if higher rates of discharge were to continue and to optimise the force output from motor units that have slowed down their contractile speed (21, 169, 216). This phenomenon was termed the muscle wisdom.

Modulation or change in motoneurons' firing rate during fatiguing contractions was viewed by many researchers as a mechanism of adaptation to the fatigue process already happening. Jones et al used high stimulus rate of 100 Hz that was maintained throughout the stimulus period of the adductor pollicis muscle and found this rate of stimulation to be associated with a drop in force. In contrast, when the stimulation rate was reduced after 20 seconds of the start of contraction the force level was

maintained. Therefore, it was suggested that the reduction in firing rate of motoneurons is a protective mechanism that avoids muscle damage if a high rate of motoneuron discharge was to be maintained (168).

Other researchers considered the drop in motoneuron discharge rate as indirect evidence for central fatigue (116). Fuglevand et al argued that the stimulation rate used in Jones' study was non-physiological and exceptionally high. The authors studied fatigability of the adductor pollicis muscle using a stimulus rate of 30 Hz that was maintained in one experiment and reduced to 15 Hz in another. When the stimulus rate was maintained at 30 Hz the force level was better maintained in comparison to the decline in force that was observed when the rate of stimulation was reduced from 30 Hz to 15 Hz. It was proposed that reduction in motoneuron discharge rate is at least partially responsible for the decline in force during a sustained MVC (113).

1.6.3.1 EMG amplitude

Changes in the potentials associated with muscle fatigue have been recorded since the 1940s. Seyffarth documented a reduction in potentials in association with intermittent contractions of the forearm muscles. A few years later, Loofbourrow (1948) and Lindqvist (1959) documented their observations of reductions in motor unit potentials with progressive fatigue [for review (14)].

Controversy exists in relation to the relative changes of muscle EMG amplitude that accompany the fatigue phenomenon. This controversy, is largely arising from the inconsistency in the terms and definitions used when EMG amplitude has been reported in the past, this is in addition to the differences in experimental settings between voluntary and electrically elicited and maximum or submaximal levels of contraction. In many occasions the MUAP and muscle fibres AP have been considered as representative of the EMG amplitude (77). Though muscle fibres' AP and MUAP contribute to the surface EMG, and can in part be responsible for some of the changes observed under fatigue inducing contractions these terms cannot be used interchangeably.

Using surface electrodes, Eason (1960) recorded an increase in the integrated EMG from the flexor digitorum superficialis during sustained submaximal voluntary contraction. The author attributed his findings to the recruitment of additional motor units to compensate for the fatigued ones [review (15)].

Moritani et al (1986) have studied the EMG fatigue characteristics of the biceps brachii muscle using surface and wire electrodes during both MVC and 50% MVC exercises. The authors recorded a significant decline in both EMG amplitude Root Mean Square (RMS) and mean power frequency (MPF) together with a reduction in motor unit frequency during sustained MVC. In contrast, during 50% MVC although MPF was found to fall, the EMG amplitude RMS and motor unit firing frequency increased (248).

Yamada et al (2002) studied the electromyographic characteristics of fatigue in the vastus lateralis muscles of 14 male subjects. The fatigue test was conducted during isometric contractions of 60% and 20% MVC. The MPF of voluntary EMG obtained from vastus lateralis muscles was found to consistently decline throughout the test period. Conversely, EMG amplitude presented as the average rectified value increased. Similar findings with reduction of MDF and increase in surface EMG amplitude was reported by Masuda et al (1999) when fatigue of the vastus lateralis muscle was induced with the use of 50% MVC (356).

Changes in EMG variables (power spectrum and amplitude) have been studied during workload field activities. Hägg et al recorded surface EMG from the left and right trapezius muscles of a surgeon performing an operation that continued for 50 min. The authors recorded an increase in EMG amplitude presented as average rectified values over 10 s. windows. This increase in amplitude was accompanied by a decline in MNF and MDF of the surface EMG (137).

It is clear from the above studies that submaximal fatiguing contractions are associated with an increase in surface EMG amplitude. This increase can be attributed to the increase in motor unit firing frequency, synchronisation of motor unit firing rate (357), or to the order of motor unit recruitment. Motor units of large size, high level of force production and consequently high magnitude of AP are usually recruited later after the small units with low force level have already started the contraction (357).

Interestingly, a simultaneous increase in RMS of surface EMG and decrease of EMG amplitude recorded by needle electrodes was seen and classified as a paradox (363). This finding has been explained on the basis of changes in the intracellular action potential profile and the difference in the distance between the electrodes and the active muscle fibre (76, 77). Additionally, wire and needle electrodes have a limited field of recording that is usually confined to a limited number of fibres or single MU in contrast to the surface electrodes that have a wider recording field reflecting the activity of more than one MU. Thus, differences between the localised changes of a muscle fibre AP or MUAP recorded by a needle electrode and the net surface EMG can at least partially explain the amplitude differences recorded by Zijdewind.

The response of surface EMG amplitude to fatiguing voluntary and electrically elicited maximum contractions is different from its response to submaximal contractions. Hultman et al (1983) investigated the influence of continuous electrical stimulation of the quadriceps muscle in 17 healthy subjects using 20 Hz stimuli with a voltage range between 30-120 V for 75 s. surface EMG amplitude recorded from the quadriceps muscle was found to be reduced in parallel with the force reduction (152).

Pincivero et al (2000) examined the vastus lateralis, vastus medialis and rectus femoris of 30 healthy subjects using three sets of 5 s. MVC. The authors reported a decline in surface EMG amplitude that was more apparent in the third set of contraction compared to the first (275).

In a recent study, Mullany et al (2002) examined the EMG activity of knee extensor agonists and a knee extensor antagonist muscles during fatiguing isometric extensions across a range of force levels. Five female subjects performed isometric knee extensions at 25%, 50%, 75% and 100% of their maximal voluntary contraction MVC with the knee flexed to 75°. Surface EMG was recorded with bipolar electrodes from the agonist muscles (vastus lateralis, vastus medialis, rectus femoris) and antagonist (biceps femoris). For all muscles the EMG amplitude RMS increased with respect to time during the fatiguing contraction task in response to submaximal contractions (25%, 50% and 75% MVC). In contrast, EMG amplitude RMS was found to decline with time during the 100% MVC. The difference between the increase in EMG amplitude with 25% MVC task and the decline in amplitude associated with 100% MVC was statistically significant (251).

The findings from these studies indicate that changes in EMG amplitude in response to fatigue are dependent on the level of force exerted. It is generally believed that the pattern of muscle activation and motor unit recruitment in a short duration forceful contraction is different from low force level, long duration endurance activity (104). For example, during sprint running fast fatigue strong motor units are recruited first resulting in production of high level of force that lasts for a relatively short period, conversely long distance runners rely on activation of slow, fatigue resistance motor units (282). Similarly in maximum voluntary contractions it is likely that all motor units are activated with the start of the contraction. Development of fatigue is associated with a reduction in motoneuron firing rate that results in a reduction in the motor units firing rate and would be observed as a reduction in EMG amplitude (104).

1.6.3.2 Changes in EMG power spectrum with fatigue

During a sustained voluntary or electrically elicited contraction the surface myoelectric signals become progressively slower. During muscle contraction, this slowing is very evident and it appears to be a combination of scaling (stretching in time and in amplitude) and a change of shape of the EMG signals or the M-wave (233).

If the real signals are referred to by $x(t)$ and $x(kt)$, with power spectral densities $P(f)=|X(f)|^2$, and $P_k(f)=P(f/k)/k^2$. The mean frequency (MNF) f_{mean} and the median frequency (MDF) f_{med} of the power spectral density $P(f)$ would be defined as:

$$f_{mean} = \int_0^\infty f \cdot P(f)\, df / \int_0^\infty P(f)\, df$$

$$f_{med} = \int_0^{fmed} P(f)\, df = \int_{fmed}^\infty P(f)\, df$$

The relationship between the MNF (or MDF) of $x(t)$ and that of $x(kt)$ is:

$$f_{mean,k} = kf_{mean}\ f_{med,k} = kf_{med}$$

From (233).

These formule can be applied to either voluntary or electrically elicited signals. The formule indicate that in the case of signal scaling with no other signal modification, MNF and MDF change by the same percentage during the period of contraction. However, during a sustained contraction, it is not uncommon for MNF and MDF not to change by the same percentage, indicating a change of the spectral shape (234).

One of the fatigue theories described by Jones et al (1981) is the membrane theory. In this theory it was proposed that the fatigue associated increase in extracellular K^+ results in depolarisation of the sarcolemma and T tubules, a decrease in AP spike height and slowing of the muscle fibre membrane conduction velocity (CV). This reduction in CV slows propagation of the AP, which may eventually fail to propagate along the surface of the muscle and thus result in a loss of force (168).

Lindstrom and Magnusson (1970) correlated between the compression of power spectrum (and therefore the decrement of MNF and MDF) and the reduction of muscle fibre membrane CV during a sustained contraction (202). It was originally thought that this relation is linear i.e. if CV decreases by a percentage, MNF and MDF also decrease by the same percentage (202). However, in practice, this is rarely the case indicating that factors, other than CV affect MNF and MDF. Brody et al conducted their investigations on the relation between pH changes, CV and MDF. They concluded that the relation between CV and MDF is situational and dependent on other biochemical changes in the milieu of the contracting muscle fibres; and changes in MDF are influenced by factors other than the pH and CV (31). These factors so far have not been clarified fully (77).

Researchers have agreed on the value of spectral analysis of EMG signals as a predictor and indicator of muscle fatigue during isometric contractions (17, 22, 43, 64, 113, 153, 194, 230, 232, 234, 316).
As increase of extracellular K^+ is a key factor in the rate of reduction of the conduction velocity and consequently the MDF. EMG power spectrum analysis has been used to compare different fatigue protocols, which are associated with different rates of K^+ accumulation. Similarly, muscles with different proportions of fibre types that vary in their conduction velocity and fatigability can be characterised with the use of EMG power spectrum parameters (297, 316).

Merletti et al (2002) studied the difference in fatigability of the biceps muscles between two groups of patients, the first was a group of eight elderly men with age range between 67-86 years and the second was of ten young patients with age range 23-34 years. The fatigue protocol was in the form of six voluntary contractions lasting 30 s each: two at 20% MVC, two at 40% MVC, and two at 60% MVC, in random order. Elderly subjects showed a significantly smaller slope of MNF and CV at 40% and 60% MVC with respect to young subjects. The young subjects showed a significant increase of MNF and CV slope when torque level increased from 20% MVC to 40% MVC and from 40% MVC to 60% MVC, whereas the elderly subjects showed a significant increase of these slopes only between 40% and 60% MVC. The authors concluded that the myoelectric manifestations of muscle fatigue were higher in young than in elderly subjects in the biceps brachii muscle. These findings were

attributed to the change in MU firing rate and fibre transformation towards slow fatigue resistant fibre type with ageing (230).

Surface EMG spectral analysis was also used to characterise fatigability of tibialis anterior muscles in patients with hereditary sensory-motor neuropathy and myotonic dystrophy in comparison to healthy controls. Patients with myotonic dystrophy were shown to have increased fatigability represented by the larger slope of reduction in their MDF compared to controls (200).

Merletti et al have studied the repeatability of surface EMG variables recorded from the human vastus medialis and tibialis anterior muscles during electrically evoked fatiguing contractions. The authors found the initial values of MNF and MDF to be most repeatable across trials and experiments on the same subject. Of the fatigue indices, the normalised initial slope and area ratio had a high level of repeatability. The authors, however, cautioned against inconsistency in choosing electrodes' positions on repeating the tests (234). Rainoldi et al have studied repeatability of surface EMG variables using different levels of isometric voluntary contraction of the biceps brachii muscle. The authors reported a better repeatability of the CV compared to the MDF and MNF in this muscle; the normalised rate of decrease in MNF had higher repeatability than that of the CV (286). Finally, repeatability studies on the quadriceps muscle showed a good agreement between tests and subjects on the initial values of MNF and EMG amplitude (285).

1.6.4 EMG in different types of muscle contractions

Most fatigability studies that employed EMG spectral analysis as a method of fatigue evaluation have used an isometric mode of muscle contraction. During dynamic contractions the muscle force or torque and the joint angle (position and velocity) vary. Hence, there are associated changes in the number of active MUs with possible different fibre types and firing rates. Moreover, dynamic contractions are associated with changes in the geometric relation between the active muscle fibres and the recording surface electrode, the geometric relation to the innervation zone and tendon; and the muscle fibre lengths. Previous research has confirmed alteration of the received single fibre AP and MUAP during dynamic contractions that was attributed to the change in the relation between the active muscle fibres and the recording electrodes (99).

EMG amplitude and spectral parameters have been reported in a number of studies using dynamic mode of muscle contractions(43, 194). It has been proposed that during dynamic contractions the signals are assumed to be quasi-stationary, i.e. stationary during short time intervals of 0.5–2 s. Under this assumption spectral analysis based on the Fourier Transform may be applied. Controlling the velocity and level of force exerted during the dynamic contractions with the use of isokinetic machines, and analysing similar parts of the movement cycles provide additional consistency to the recorded data (104).

Christensen et al (1995) compared the EMG amplitude and spectral variables during non-fatiguing static and low level ($10°s^{-1}$) velocity dynamic exercises of the biceps brachii. In the dynamic exercise the elbow was moved from a position of 80° flexion to 100° flexion and returned to the starting position in a concentric – eccentric mode. In the static part of the experiment the elbow was kept at 90° flexion while a two kg weight was held in the hand against gravity. The two kg weight corresponded to 9.8% of the MVC. The mean RMS of the EMG amplitude varied between 10-12% of the maximum EMG during both static and dynamic parts of the test indicating low level of muscle activity. Calculated EMG parameters amplitude RMS and power spectrum MNF and MDF showed no difference between static and dynamic exercises. The authors concluded that low velocity, low force level dynamic contractions that

produce equal force and durations during the concentric and eccentric phases of the exercise are comparable in their EMG changes (43). These results were in parallel with Muro's findings who found no difference between the EMG power MNF during isometric and dynamic exercises performed at a similar contraction level of 20% MVC when the angular velocity of the dynamic movement was 0.17 rad. s^{-1} (252).

In another study during-the-day repeatability of the EMG variables and peak torque from the 3 components of the quadriceps muscle were tested during dynamic exercises. Both peak torque and EMG amplitude RMS had high degree of repeatability. Reproducibility of the MNF of the EMG power spectrum for all muscles was good when the mean of the contraction was used (194). The same research group conducted another study to validate the EMG variables as indicators for fatigue using maximum isokinetic knee extension exercises. High correlation was found between peak torque and MNF. A positive correlation between the EMG amplitude RMS and knee extension peak torque was found in the majority of subjects. The authors suggested that EMG power spectrum MNF has good validity criteria with respect to biomechanical fatigue during dynamic maximum contractions (121).

It can be seen that dynamic protocols have been approached with caution. Specially designed protocols are needed to avoid errors that can arise from changes in muscle length, force or electrode position when EMG power spectrum analysis is used in defining muscle fatigue. Recently, suggestions have been made to use the instantaneous median frequency in the EMG analysis of dynamic exercise fatigue protocols. The theoretical advantage of this method relies on the ability to analyse non-stationary signals as it follows the Cohen class of time-frequency transform. This type of transform is particularly suitable for the analysis of EMG signals recorded during dynamic contractions because it constitutes the class of bilinear time-frequency transforms which are invariant to time and frequency shifts. This property is important to correctly represent the time evolution of the frequency content of the surface EMG as any time delay or frequency shift applied to the signal results in an equivalent delay or shift in the time frequency representation (293). A limited number of publications used this method and it remains to be seen whether it will be possible in the future to use dynamic protocols of exercise more regularly in fatigue studies.

1.7- Sensation and contribution to motor performance

1.7.1- Sensory receptors types and modes of function

Glabrous "non-hairy" skin has been found to have the highest level of innervation density and the highest percent of sensory receptors compared to other body parts (322). Afferent fibres have been defined to be mainly of group A and C fibres. Type A fibres have been correlated with touch, cold, pricking pain and tickle sensations; while type C are correlated with warming, burning pain and itch. The ratio of type A to type C fibres in peripheral nerves varies from 1:1 ratio in hand and facial nerves to 1:5 in proximal body nerves (164).

Sensory receptors have been classified on a structural and functional basis. On a structural basis 2 types were defined; encapsulated and non-capsulated free nerve endings. Encapsulated receptors are the Meissner corpuscle, Pacinian corpuscle and Merckel cell-neurite complex. The non-capsulated receptors are free nerve endings, like the Ruffini nerve endings, and many more poorly defined endings of type A-delta and C fibres that provide cold and warmth, nociceptive, itch and tickle sensations (164).

Functionally, receptors have been classified according to their sensitivity and adaptability to stimuli. Quickly adapting (QA) receptors were found to produce signals that rapidly decline to zero or to a base line value in response to a constant stimulus; while the slowly adapting (SA) receptors will continue to discharge throughout the stimulus period. QA receptors will re-start discharging again if the stimulus changes its character or intensity while the SA receptors will modify their discharge frequency or rate (165). Examples of the QA receptors are the Meissner's and Pacinian corpuscles and mechanoreceptor free nerve endings.

Slowly adapting receptors have been classified into SA type I that have no resting phase discharge like the Merckel cell neurite complex and SA type II that maintain a slow regular rate of discharge in the resting state like the Ruffini's nerve endings (165, 167).

In the muscles the sensory receptors are the muscle spindles, Golgi tendon organs and the small group III and IV free nerve endings (221). Muscle spindles have also been found to modulate their discharge pattern by modulating the tension of its intrafusal fibres through the small gamma efferent function. A property that would allow this receptor to respond to the change of muscle tension at different muscle lengths (219).

The second type of receptors located within musculotendineous junctions is the Golgi tendon organ; which is a SAII receptor providing a continuous feedback about the state of tendon tension (220).

Joint capsules have been found to be rich in Ruffini's, SAII type of receptors. These receptors were classified according to their positions and rate of response to different joint angles. As they seem to have different rates of discharge in response to various degrees of flexion and extension (322). Two other types of QA receptors have been found in joint capsules: the pacinian and paciniform corpuscles with similar morphological and functional characteristics to those of the cutaneous receptors (322).

1.7.2- Dennervation and recovery of sensory receptors

The degree of central nervous control over reinnervated muscles is usually disappointing (263). Sensory axons represent more than half the fibres that constitute a muscle's nerve supply and their disruption is likely to influence the muscle's proprioceptive function. Banks et al have investigated the effect of nerve transection and direct repair as well as nerve graft on the recovery of sensory receptors of cats' hindlimb muscles. After 50 weeks of nerve repair functionally identified muscle spindles and tendon organ were reduced to 25% and 45% respectively. Nerve graft was found to increase the magnitude of deficiency in muscle sensory recovery (11). Collins et al have examined the ability of different afferent types to re-establish connection with muscle receptors after nerve section of medial gastrocnemius muscle nerve in cats, and found a dramatic reduction in the number of afferents that were able to establish connection with muscle spindles and tendon organs. There was also a reduction in the conduction velocity for those afferents that survived even after 9

months of recovery (52). To determine the effect of cross-reinnervation on muscle sensory recovery Ip et al reported the effect of re-innervating the soleus muscle using the extensor digitorum longus nerve. Following 449 days of recovery the number of muscle spindles that established normal innervation was 3%; abnormal innervation was present in 54% and no nerve endings were found in 43%. Similarly in 80 recognised tendon organs 38% had normal innervation, 29% abnormal innervation and 33% without visible innervation. The authors concluded that both self and cross-reinnervated muscles had a severe disruption of reinnervation patterns that can account for the functional abnormalities (156).

Kanamaru et al (1993) examined human biceps muscles reinnervated with intercostal nerves following brachial plexus injury. Out of 14 patients examined, tapping of the reinnervated muscle produced a somatosensory evoked potential in only 4 indicating the return of the local reflexes in small percentage of cross reinnervated muscles. (182).

The innervation pattern can be restored to some extent if the nerve injury was less severe as in cases of nerve crush assuming that reinnervation happens within a short period; but even though residual deficiencies have been recorded (239, 300). Hyde et al have detected failure or rapid decline in the firing rate of regenerated muscle spindle afferents to a held phase of muscle stretch after 140 days of recovering from nerve crush injury. This finding was attributed to an increase in the muscle spindle threshold to stretch stimulation (154). In another experiment the monosynaptic H reflex and its effect on walking was assessed in adult rats following various degrees of nerve injury. The value of the reflex remained high in all groups except the group of nerve crush in which normal values were detected at the end of three months recovery period (334).

Denervation has also been found to affect cutaneous receptors both histologically and functionally. Histological changes were found in the dermal plexus, free nerve terminals and encapsulated receptors. Both dermal plexus and nerve terminals persist as empty trains of Schwann cells surrounded by a fine endoneurial sheath, but there is some evidence to suggest that they can be used by regrowing nerve axons for up to a year following nerve injury (317). Dellon et al have reported degeneration of nerve

terminals and lamellar complex in different types of skin receptors (69, 71). However denervated complex receptors like the Meissner's corpuscles, Pacinian corpuscles and Merckel cell neurite can survive for up to 1 year and maintained the possibility of recovery if they receive a growing nerve terminal (181, 354). The question is whether poor sensory recovery following long periods of dennervation is the result of end organ failure or lack of regenerating axons to establish a functioning connection. There is some evidence to suggest that the availability of regenerating nerve axons is the main determinant to the final outcome. A correlation has been drawn between the severity of nerve injury and the final outcome (317).

Studies with the use of microneurography have allowed recording from single receptors during recovery from nerve injuries in humans. It has been reported that during the early stages of recovery from nerve injury recovering afferents can have multiple receptive fields supplying both slow and rapidly adapting receptors; this is in addition to decline in the discharge rate from slowly adapting fibres after a short period of stimulation (208). These effects seemed to be transitional and reinnervated rapidly adapting and slowly adapting type II receptors were recorded to recover normal threshold, discharge characteristics and receptive fields. Only the slowly adapting type I receptors continued to have smaller receptive fields and slow response compared to normal (207).

The degree of nerve injury and length of the period between injury and repair has been found to have the biggest influence on the fate of sensory function after nerve injury (33). Following nerve crush, axons were found to be guided by the old Schwann tubes in the distal nerve stumps to reinnervate their old receptors, while in nerve section mixing of the axons and cross re-innervation of receptors is more likely and this can explain the better sensory function after recovering from the less traumatic nerve crush (148).

1.7.3- Proprioception and co-ordination of the hand movement

Proprioception generally refers to the sensory modality that defines the sense of position, movement and force (220). Controversy still exists as to which of the sensory feedback sources (skin, muscle and joint) provides the most contribution to proprioception; particularly at the hand level (172).

Previous research has demonstrated a primary role for the muscle receptors in providing movement and position senses of large body joints like the hip and knee (48, 220, 221). However, this fact was questioned for the hand, which has been considered unique and does not represent the rest of the body (46, 47). Clark et al tested the possible contribution from articular receptors on the ability of humans to detect movement of the distal interphalangeal joint in the middle finger. The authors found a modest but significant reduction in the ability of subjects to detect movement. However, because this effect was modest and had been detected under a condition of reduced sensory input from the muscles, they concluded that articular contribution to the movement sense of small hand joints is minimal (47). This finding was in agreement with Burke et al's results with the use of microneurographic technique to record single afferents discharge arising from cutaneous, muscle spindles and joint receptors (37).

Moberg (1983) conducted his experiments on a number of patients while performing surgical release of the median nerve under local anaesthetic. He had the chance to stretch the long tendons of the fingers during these operations and record the patients' response. The author reported that increasing the muscle length did not contribute to the ability of patients to detect finger movement. Increasing muscle length to the extreme that leads to skin displacement in the forearm resulted in a vague subjective feeling by patients that their fingers were moving in the direction of muscle lengthening. His conclusion was that muscle stretching does not contribute to the sense of interphalangeal joints movement (246). These observations and others (36, 102), have focused more attention on the importance of the cutaneous receptors to the proprioceptive modality in the hand.

Microneurographic studies have confirmed the value of the mechanoreceptors in the dorsal aspect of the hand on providing detailed kinematic information about finger

movements. Experiments by Collin et al found that electrical stimuli applied to skin on the dorsal aspect of the finger or skin stretch to produce illusory feelings of finger movement (50). In the same experiments muscle vibration produced the same effect in a higher proportion of subjects. Further expansion of this experiment has shown evidence for integration between the information provided by the cutaneous and muscle receptors (51). The role of cutaneous sensation according to this experiment was not just facilitatory as had previously been suggested (223), but rather represented an integral part in the interpretation of the data provided to the CNS. Whether sensory input from one finger can influence or have a facilitatory role to the signals arising from other fingers in the same hand has been denied by recently conducted experiments (287).

The illusory feeling of finger movement with muscle vibration has revived the traditional belief that muscle signals are a primary source for proprioceptive input that has been supported by various experiments but was denied by Moberg's observations.

The controversy about the relative importance of the three types of receptors (skin, muscle and joint) to hand proprioception has not been resolved as each of these seems to have an important role (287). More importantly, most of the experiments that have been undertaken to elucidate the pattern or threshold of activation of various types of receptors are arguably non-physiological (151). As these types of stimuli are not normally encountered in real life; and perhaps of more interest is the influence that these sensory inputs would have on the normal function of the hand and its ability to perform various tasks.

1.7.3.1 Evaluation of hand sensation and reflection on its function

Sensations from the glabrous skin of the hand are essential for refined motor manipulations. It is common for patients who suffer from lack of sensation in their hands to present for consultation primarily because of a motor deficiency (163, 166, 245, 246). Research has also clarified the important role of sensory integrity at the tip of the fingers to adjust gripping and holding forces (166).

Sensory function of the hand has usually been defined by measuring the threshold of detecting various stimuli that represent the sensory modalities of touch, heat and cold, two point discrimination (2PD) and vibration (172). Tactile sensation represents an important part of the hand sensory evaluation. Many tests have been advocated to measure this sensory modality; of particular importance are light touch, Semmes-Weinstein monofilament test and the two-point discrimination. Light touch can usually be assessed with the use of hairs or cotton buds and can be tested as either static or moving. Moving touch test is believed to stimulate the rapidly adapting receptors while the static test relies on the slowly adapting ones (317).

The Semmes-Weinstein monofilament test is designed to test the subject's ability to detect a specific amount of pressure delivered to the skin through a series of nylon filaments. These graduated nylon filaments are calibrated in terms of the logarithm of 10 times the force required to buckle the filaments (199). The filaments are applied to the skin in a descending order with thicker filaments applied first, until the patient cannot feel the stimulus. The filament has to be stable over the skin surface and amount of pressure placed at the filament is just enough to make it buckle (277). Test-retest reliability of this test was found to be high, but can easily be compromised if slippage of the filament tip on the skin surface occurs as this leads to a lower buckling stress (199). Equally important to pressure sensitivity is the point localisation. Ability to localise the stimulus site is usually impaired in patients with nerve injury (267). Nerve injury and repair results in a change in the input signals arriving to the somatosensory cortex from different parts of the hand due to the misdirected nerve fibres (209). Therefore, a disorganised cortical map can impair the tactile localisation (177).

One of the important and most studied tests for sensory evaluation and innervation density is 2PD. In this test a moderate pressure is applied on the skin using either one or two blunt points of a calibrated disk (or paper clip), and the patient is asked to decide whether he/she has felt one or two points. The distance between the two points is initially set at a distance that can be easily detected, for example 15 mm, then gradually reduced until the patient cannot differentiate between being touched at one or two points. In 1992 the Pressure-specifying Sensory Device was introduced to allow computerised measurement of two-point discrimination in regard to the distance between the two points and the pressure threshold at which these two points were perceived (73). Dellon 1979 has advocated the use of moving 2PD as a means of assessing the rapidly adapting mechanoreceptors (68). The test involves moving a paper clip along the skin surface from proximal to distal direction. The threshold for detecting moving 2PD was found to be lower than the static test suggesting that it may be a more sensitive test for measuring sensory status of the hand (68).

The correlation between these neurological tests and overall hand performance following nerve injuries or compression has been questioned, as improvement in some or all of these separate tests does not necessary mean good sensori-motor performance of the hand (317). Therefore, efforts have been made to present more objective ways of hand evaluation and to correlate between sensory modalities and hand performance. In the 1930's the Minnesota Rate of Manipulation test was presented and measured the time necessary for a patient to place 5 subsets of wooden disks on a board. This early attempt was followed by the Purdue Pegboard Test in the 1940's to time the skill of assembling a pin, a collar and 2 washers and place them into holes in a board (70). Jebsen and colleagues then presented another test in which the time for using different writing and eating utensils was measured (160). Recently, another version of functional hand testing was presented from the Mayo clinic under the name of Mayo Dexterity Test; in which different sizes of squares, cylinders, metal pins and collars are screwed into place (72). In all these tests unrestricted visual feedback remained a constant factor.

In 1958 Moberg presented the pickup test. In this test commonly used objects like coins, safety pins, washers etc are collected from the surface of a table, identified without visual feedback then dropped in a box. The test was intended to measure

stereognostic sense (ability to identify objects by touch) as well as timing the skill of picking up the objects and dropping them in a box (244).

Trials have been made to correlate between the various sensory tests and the functional performance of the hand represented by one of the available functional sensory motor tests. Moberg has correlated between the object identification part of the pick up test and the return of sudomotor function represented by the ninhydrin sweat test (244). Novak et al studying patients following median nerve injury found a strong correlation between static and moving 2PD and hand function measured by the pick up test (260).

Despite these great efforts of developing functional and more objective hand assessment tests that allow incorporation of both sensory and motor components in performing the tasks, it remains of great concern that these tests rely on timing tasks but lack the ability to measure how these tasks are being performed and how movements are being executed. Slow, clumsy and jerky performance of denervated hands is easily recognised when compared to the smooth effortless manipulations of the normal hand but have hardly been measured objectively (171). What is needed, is to develop a method for quantifying the overall sensory motor performance in terms of how the movements are performed, manipulations combined and movements executed.

Chapter II: Fatigability of the serratus anterior

2.1- Introduction

The movement and stability of the scapula, and thus of the glenohumeral joint and the upper limb, depend mainly on the scapulothoracic muscles (12, 83). The role of trapezius and serratus anterior in scapular elevation and upward rotation cannot be replaced by other muscles (12, 266). The effect of serratus anterior dysfunction is dramatically seen in the scapular winging of long thoracic nerve palsy (349). Serratus anterior is a broad, flat muscle formed by multiple digitations arising from the upper 8-9 ribs in the midaxillary line and attaching to the vertebral aspect of the ventral surface of the scapula. Three functional components have been identified: a superior cylindrical mass that attaches to the superiomedial border of the scapula and anchors the arm during overhead rotation; a long wide intermediate band that connects the 3^{rd}-5^{th} ribs with the vertebral border of the scapula and draws the scapula forward; and the lower five slips from the 6^{th}-10^{th} ribs that converge on the lower angle of the scapula to rotate its inferior angle upward and laterally (83).

Scapular winging, though not frequently reported in the literature, is not uncommon, and is potentially disabling. Physiotherapy programs that concentrate on periscapular muscle co-ordination and rehabilitation may produce satisfactory recovery (132), otherwise operative intervention is usually warranted (341). Many patients, despite partial recovery, continue to suffer residual winging and fatigue especially with overhead and endurance activities fig. 2.1. However, there have been no measurements of the fatigue properties of this anatomically unique muscle.

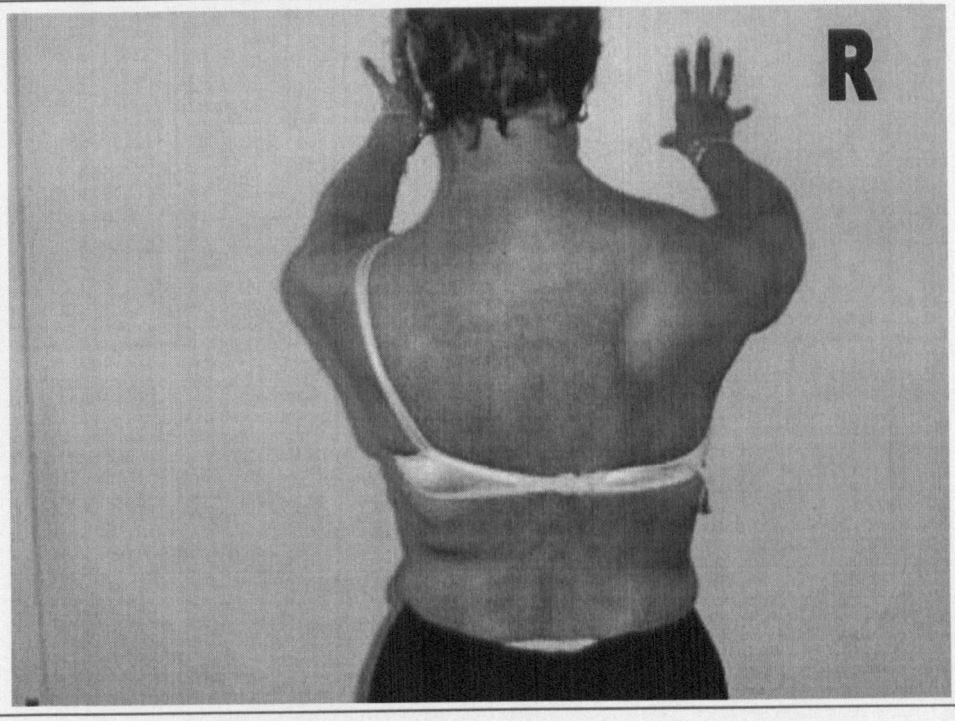


Figure 2.1 shows winging of the scapula on the right side on attempt for forward elevation of the arm, in a patient with long thoracic nerve palsy.

2.1.1- Is the deficiency observed in reinnervated serratus anterior muscle function a reflection of increased fatigability?

As presented in the review section, the denervation-reinnervation process is associated with various changes in the muscle's fibre MHC isoforms, mRNA and muscle metabolism. It is common for denervated muscles to have an increase in the percentage of type II (FG and FOG) and a reduction in the type I fibres. This is in addition to an increase in Na^+ channel density and alteration of glycogen metabolism that were observed with disuse atrophy. Studying paralysed human soleus muscle, Shields et al, showed increased fatigability of chronically paralysed muscles (>1 year) compared to the acutely paralysed ones (4-6 weeks). The authors used electrical impulses delivered through surface electrodes applied over the tibial nerve to stimulate the denervated soleus muscles. Flexion torque at the ankle joint was measured using an adjustable force transducer. Despite the increased fatigability of the denervated muscles the measured torques generated in the chronically paralysed soleus muscles were similar to the voluntary plantar flexion torques reported for non-paralysed individuals (305). It has been suggested that chronically paralysed muscles have increased fatigability compared to normal.

For the denervated muscles to have an increase in fatigability fibre type must have been changed from the slow fatigue resistant towards the fast fatigue. This fibre type conversion was found in many cases to be incomplete (122). Even if a change in muscle characters occurs, it usually converts the SO type of fibres to FOG but does not progress to a full conversion from SO to FG muscle fibre type (56). It has been suggested that the altered interaction between nerve and muscle following denervation influences the isomyosin expression in muscle fibres leading to faster contractile properties but less effect on oxidative capacity and fatigability (161). Animal experiments showed increased potential of slow MUs to sprout and increase their size compared to the fast ones, which in turn would enhance reinnervated muscle's ability to resist fatigue (92).

In voluntary contraction of muscles the order of recruitment of motor units starts with weak fatigue resistant motor units followed by the stronger, fast contracting fatigable

units (57, 74). Reversal of motor unit recruitment order has been proposed as an explanation for the increased fatigability of paralysed muscles in animal experiments (98). However, recent investigation of human partially and totally paralysed muscles revealed a scatter in the order of recruitment with some weak units found to have high electrical threshold concluding that factors other than the reversed activation order contribute to the increased fatigability observed in paralysed muscles (326).

Fatigability of reinnervated muscles in humans has hardly been investigated. It is not uncommon for patients recovering from nerve injury to complain from tiredness or easy fatigability. Whether these symptoms are real reflections of increased fatigability of reinnervated muscles is not clear. In this chapter fatigability of reinnervated serratus anterior muscles will be tested and results will be discussed.

2.1.2- Myoelectric fatigue (EMG as a tool in investigating fatigue)

Classically fatigue has been investigated by measuring the maximum voluntary or electrically elicited force or torque generated by a certain muscle or group of muscles (22). However, this may not always be possible and researchers have been developing other methods to indirectly investigate and measure fatigue.

Electromyography (EMG) has been introduced as a valuable tool to investigate fatigue for clinical and research purposes (104, 231-234, 285). Concentration has been focused on two main variables of the EMG signals and their correlation with muscle fatigue, EMG amplitude and spectral analysis.

Amplitude can be characterised by measuring either the root mean square or the average rectified value of the signals (232). Special care should be practiced whenever amplitude results are interpreted as the test setting can dramatically influence the results. Testing muscle fatigue in a submaximal mode of contraction results in an initial increase in EMG amplitude (22, 285, 286), which can be followed later by a decline in EMG amplitude along with the fall in force production (363). The increase in EMG amplitude has been attributed to the recruitment of additional motor units to compensate for decline in force, increased firing rate/frequency or synchronisation of motor unit recruitment (357).

Some researchers argued that amplitude rise is a manifestation of enhanced electrogenic Na-K pumping with increased intracellular action potential (142). Others denied the possibility of increased action potential in individual fibres and suggested a better synchronisation of potentials from individual fibres as a reason for the amplitude rise (152).

Simultaneous recording of EMG using surface and needle electrodes may show a paradox of increase in amplitude recorded by the surface and decline recorded by the needle of a single fibre (135); this has been explained by the length of depolarisation zone influenced by the fibre-electrode distance and was seen in other experiments using two surface electrodes at different distances from the active muscle (135).

Dimitrova and Dimitrov attributed the rise or fall in the motor unit potential and M-wave amplitude to the change in intracellular action potential (IAP) profile and the difference in position of the recording electrodes in relation to the active fibres (76, 77).

Dimitrova et al proposed that the MUAP can be represented as a linear summation of temporal and spatially propagating single fibre action potentials (SFAP). SFAPs have phases that reflect propagation of depolarised zones from the end plates to the fibre ends as well as the excitation arising from the end plate. Hence, the formation of an electric field and its magnitude is a spatial character, and as the size of the individual SFAP is determined by the IAP whose effect is distance dependent; so are the MUP and amplitude. Therefore, differences in the position of an electrode in relation to the active muscle fibre influences the data recorded by different electrodes (77).

The second parameter that has been used to investigate fatigue is spectral analysis. Muscle fatigue is known to be associated with ATP and phosphocreatine depletion, reduction of pH at the sarcolemma and increased $[K^+]_o$ that produce progressive decrease in the action potential CV (31, 176, 297). Of the spectral variables, the time dependent decline in median and mean power frequency during muscle contraction have received considerable attention. Many studies (64, 230, 232)have demonstrated the value of these parameters in defining myoelectric fatigue even before the actual

decline in force occurs. Repeatability studies have supported the use of these two variables in different settings (231, 285).

2.1.3- Static and dynamic protocols of inducing muscle fatigue

Most of the experiments that have used EMG for evaluating myoelectric fatigue were performed in a static mode using ismoteric contraction of the examined muscles. Some researchers suggested using dynamic modes of muscle testing instead of isometric, as the latter does not always reflect workload conditions (194). Meanwhile, dynamic exercises can introduce various sources of error in the recorded EMG signals due to the change in the position of the electrodes in relation to the active muscle, skin movements and change in the muscle length (280).

To avoid sources of error expected with the use of dynamic modes of exercises, some investigators suggested the use of isokinetic machines. The advantage of using an isokinetic machine is to standardise the experiments' protocol in terms of velocity of movement, the range of movement, and the minimum torque accepted to move the machinery lever arm(104).

Christensen et al have compared the EMG characteristics of static and low velocity dynamic non-fatigue muscle exercises. No difference could be found in the EMG amplitude or spectral variables "mean and median frequency" between the two exercise protocols. There was also no difference between the concentric and eccentric parts of the dynamic exercises. Their conclusion was that slow dynamic contractions at low force level do not adversely affect the results of power spectrum analysis (43). In another study, good reproducibility was reported for surface EMG variables and peak torque during dynamic contractions (194). The authors have also compared the various myoelectric fatigue parameters between the dynamic and static fatiguing muscle exercises and showed a similar significant fall in median frequency and rise in amplitude. Although isometric contractions resulted in a significant fall in muscle fibre conduction velocity, dynamic exercises caused only a small reduction (218).

Bonato et al have suggested processing the EMG signals over very short periods to acquire the instantaneous median and mean frequencies to avoid the influence of the change in muscle fibre length on the recorded signals (28).

Of the dynamic modes of muscle testing concentric (Con) contractions were found to induce greater fatigue and a different pattern of decline in force when compared to eccentric (Ecc) exercises (268). Movements with low angular velocity of 10 - 20°s^{-1} have been shown to have good repeatability of EMG amplitude, MNF and MDF between different subjects (43). It is not clear, however, what would be the repeatability of EMG variables with moderate velocity (90° s^{-1}) fatigue inducing dynamic exercises. The experiments presented in this chapter investigated this subject applying similar methods of recording and analysis to the dynamic and static protocols of exercises. Caution and attention to the differences in the mechanisms of inducing fatigue in different experiments were practiced while interpreting the results.

2.1.4- Surface and intramuscular wire electrodes in fatigue studies

Surface electrodes have been commonly used in fatigability studies but only a small number of experiments have employed wire electrodes. Wire electrodes may be more specific particularly in studying deep and small muscles. However, the reproducibility of wire electrode results has been a matter of contention and was found to be poorer compared to surface electrodes (173, 192). Previous studies of the serratus anterior have employed both types of electrodes, particularly for determining the patterns of muscle activity (126, 162, 261).

2.2- Aims of the experiments:

The aims of these experiments were, first to determine the most reproducible protocol for examining myoelectric fatigue in the serratus anterior muscle. Second to compare control and pathological data from a group of patients with reinnervated serratus anterior following long thoracic nerve palsy.

2.3- Hypothesis tested:

Following the establishment of the most reproducible protocol (type of exercises and electrodes) it was hypothesised that reinnervated serratus anterior muscles are more fatigable. Therefore, experiments have been conducted to compare EMG fatigue parameters between patients and control groups.

2.4- Subjects and methods

These experiments were approved by the local ethics committee and all volunteers and patients signed a consent form.

EMG studies were carried out on 8 male healthy volunteers aged 29-35 years (the right side was tested in seven and the left in one), of whom four were studied twice to define repeatability. Repeatability tests were performed one to three weeks apart. Surface and wire electrodes were simultaneously employed in this control group.

Five patients aged 22-59 years with scapular winging following long thoracic nerve palsy were studied using surface electrodes. Four of these patients had had surgical treatment by decompression of the long thoracic nerve $\geq$ 12 months before study (212) after failure to improve with $\geq$ 12 months of physiotherapy and rehabilitation to the periscapular muscles. The remaining patient was treated solely by physiotherapy. All patients had achieved good recovery in terms of scapular winging (side-to-side difference < 1 cm using Kibler's test (188)) but continued to complain of fatigue and a sense of scapular instability following lengthy overhead or strenuous activities.

2.4.1- Muscle-testing protocols

Two isometric (static) and one isokinetic (dynamic) exercise tasks were performed on every subject. For the isometric tasks: the first task **(Isometric I protocol)** consisted of 60 s upward pushing at 120° of shoulder forward flexion, using a KIN-COM machine (Chattanoga, Inc., Oxfordshire, UK), fig. 2.2. With the subject sitting, a cuff, linked to a force-recording cell and connected to a lever, was wrapped round the arm 10 cm distal to the tip of the acromion. The arm was positioned in 120° forward flexion from neutral. Subjects were asked to push upward, using their maximum force. Maximum voluntary force was determined as the best of three 3 s upward pushes separated by 5 s relaxation. Subjects were then instructed to reproduce this force for 60s, given visual feedback about their force level through the screen (fig. 2.3) in addition to verbal encouragement to produce the maximum possible force.

The second task **(Isometric II protocol)** consisted of 60s maximum forward pushing against a fixed resistance, a wall, with the arm in 90° forward elevation (the classic

test for examining the serratus anterior clinically and demonstrating scapular winging) fig. 2.4.

In the third **(dynamic)** task the patient sat with a stabilisation belt across his chest and his arm connected, through a cuff and force recording cell, to the mobile lever of the KIN-COM machine working in isokinetic mode. The cuff was positioned 10 cm from the tip of the acromion. The centre of rotation of the lever was adjusted to be at the level of the humeral head. After taking the weight of the arm to compensate for gravity the machine was set on continuous isokinetic (concentric Con -eccentric Ecc) mode for shoulder flexion-extension between 0°-120°, at a moderate velocity of 90°/s. The subject pushed upward in both flexion and extension for 90 seconds fig. 2.5. During the Con phase of movement the direction of movement and pushing by the subject were the same (positive work). In contrast, during the Ecc phase the direction of movement was opposite to the direction of pushing by the subject (negative work). The minimum force necessary to start moving the lever was set at 1 kg.

As the recorded torque measured not the force generated by the serratus anterior muscle, but rather the indirect effects of the overall loading of the scapular stabilisers, it was used only to give feedback about the level of performance. Throughout the experiments subjects were allowed to reject or repeat a trial if they felt that they had not produced their maximum effort. The set up of these voluntary protocols followed the guide lines suggested by Gandevia 2001 and discussed in an earlier section (1.2.1).

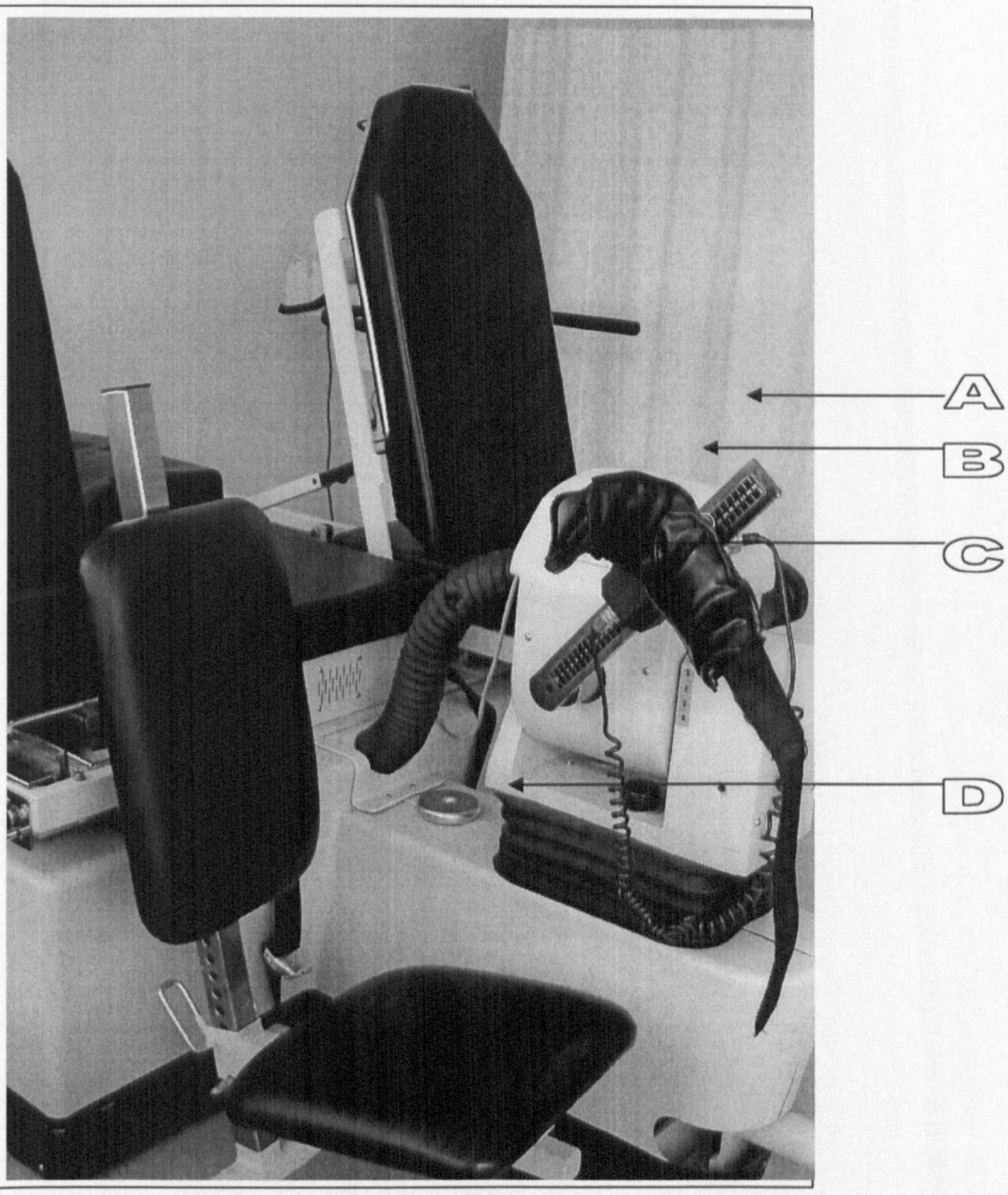


Figure 2.2 Kin-Kom isokinetic machine (A) Is the lever arm connected to the body of the machine. (B) Cuff connected to a force detector cell and used for connection to the subject's arm. (C) Centre for rotation of the lever arm, its height has been adjusted to meet the level of the humeral head. (D) Chair for seating subjects during the exercise protocol.

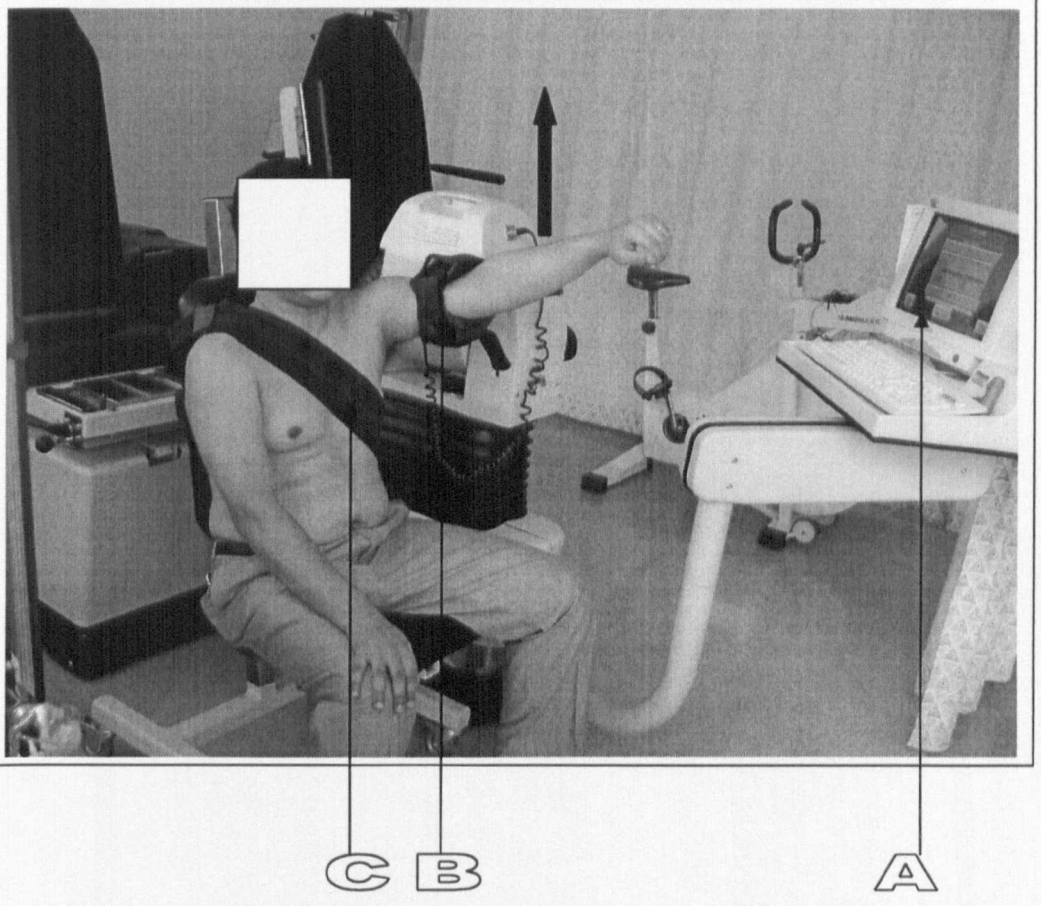


Figure 2.3 showing the general set up for **Isometric I protocol** loading of the serratus anterior with the arm in 120° forward flexion and the upward directed arrow ⬆ defines the upward direction for pushing. (A) Screen providing visual feedback about the generated torque. (B) Cuff, attached to the force-recording cell, wrapped around the subject's arm. (C) A subject is stabilised to the chair by the use of belt to minimise contribution of body movements to the generated torque.

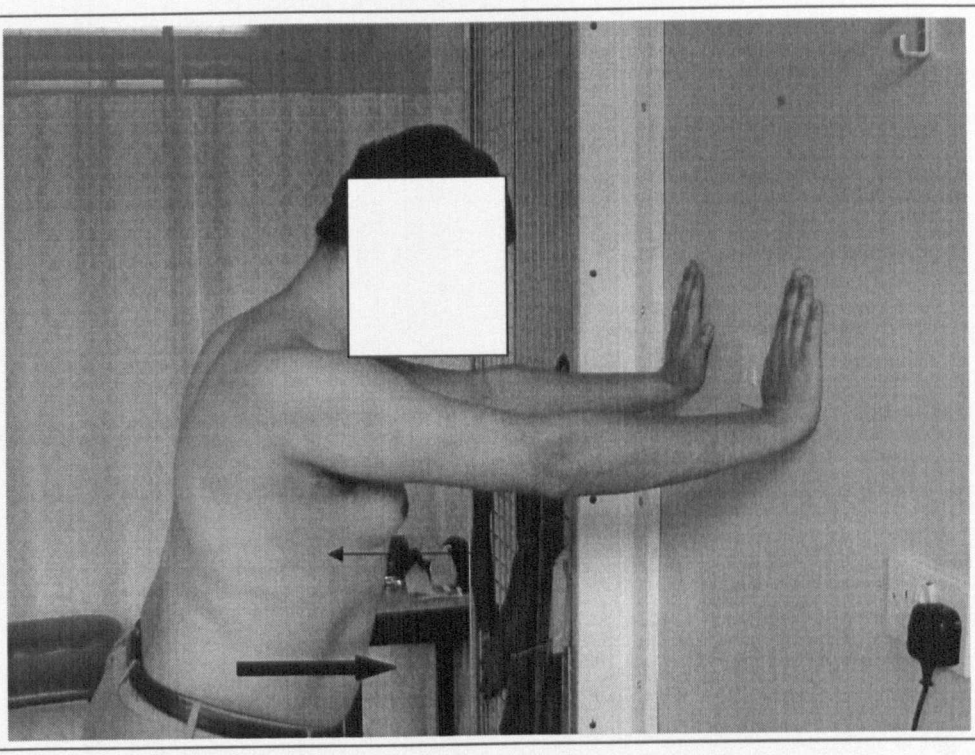


Figure 2.4 shows the position for loading the serratus anterior during the **Isometric II protocol** with the arm in 90° forward flexion, the subject is pushing forward against fixed resistance (a wall). In this position reaction forces to the body weight are directed backwards ← through the arm into the scapula, while serratus anterior action is to maintain stability of the scapula by pulling the inferior angle forward. →

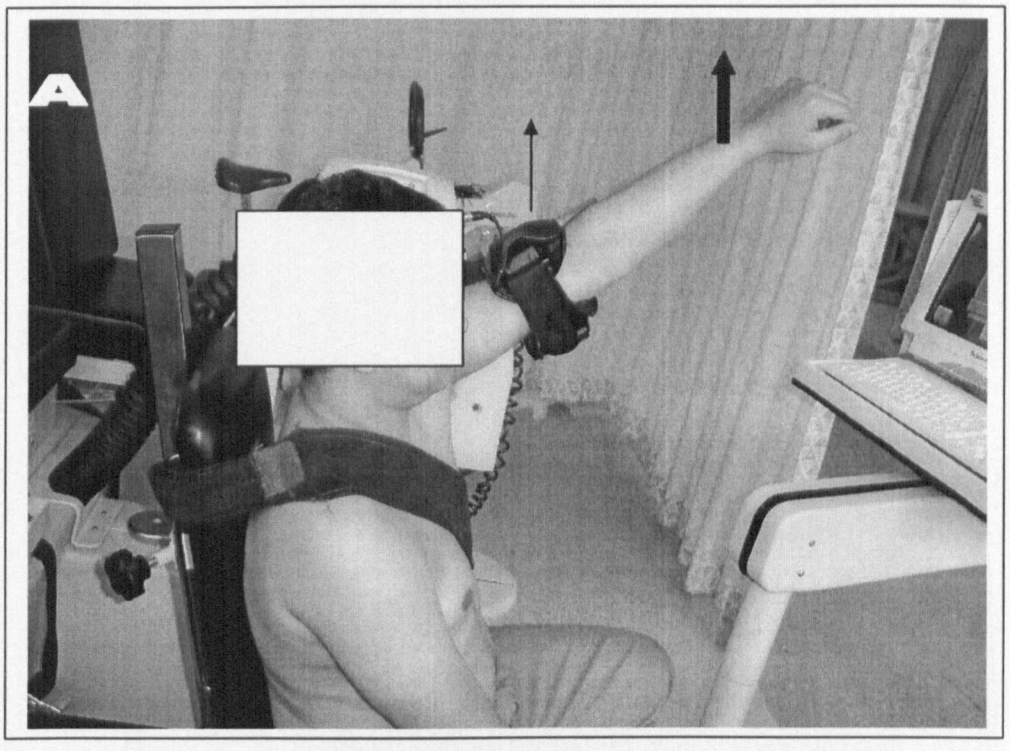


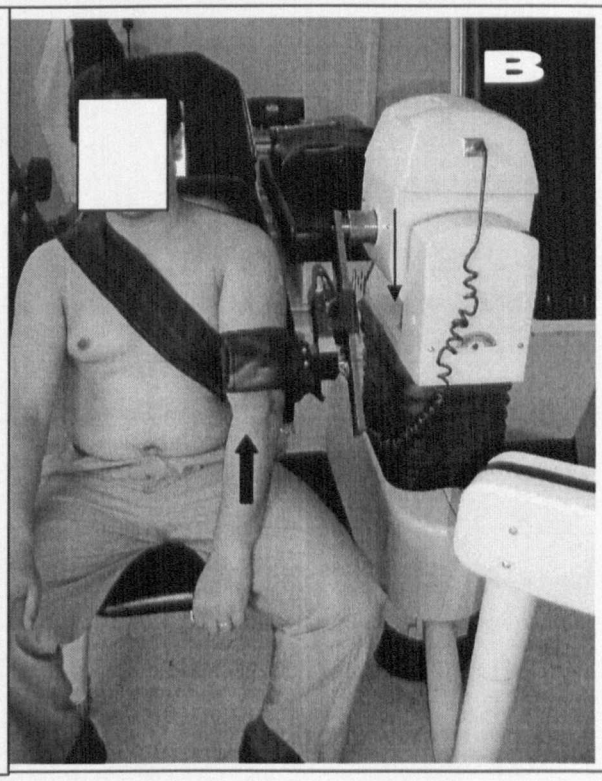
Figure 2.5 shows the **Dynamic protocol** of serratus anterior loading.

↑ **Concentric Contraction**

↓ **Eccentric Contraction**

⬆ Show the direction of pushing by the subject.

(A) Concentric phase, as direction of movement and force are the same. (B) Eccentric phase, as direction of force is against the direction of movement.



2.4.2- EMG acquisition and analysis

EMG data were acquired simultaneously using a set of two surface electrodes and two double fine wire electrodes. Surface electrodes were applied over the serratus anterior muscle as described by Basmajian (13). With the arm raised, two electrodes were aligned along the mid-axillary line at a level above the inferior angle of scapula fig.2.6.

One wire electrode was inserted into the serratus anterior as described by Delagi (67): with the subject prone and the arm dangling over the edge of a bed, the wire was inserted just lateral to inferior angle of scapula fig. 2.7. A second wire electrode was inserted in the serratus anterior as described by Goodgold (129): with the subject prone, the vertebral border of the scapula was identified and lifted manually to create a space between it and the chest wall. The wire was inserted between the vertebral border of the scapula and chest wall, with its tip lying close to the thoracic surface of the inferior angle of scapula fig. 8.

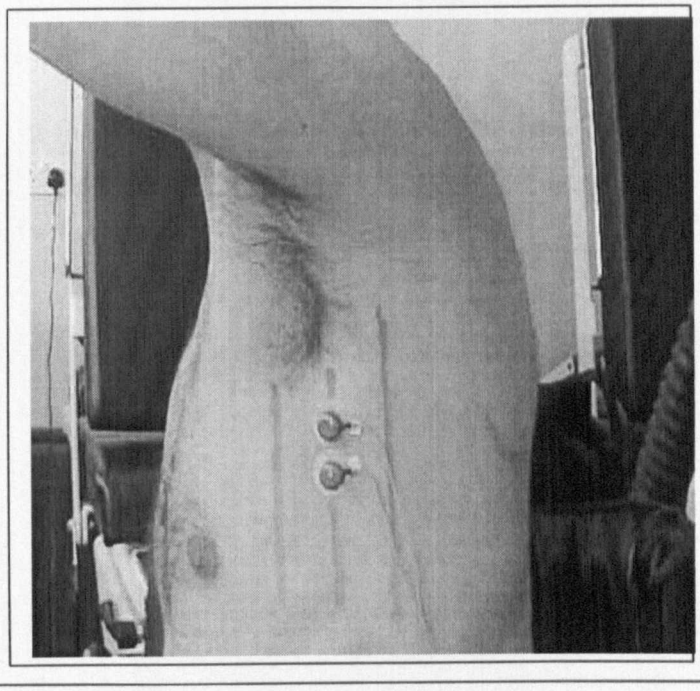


Figure 2.6 shows the position of two surface electrodes applied according to Basmajian's description along the midaxillary line and just above the level of the inferior angle of the scapula.

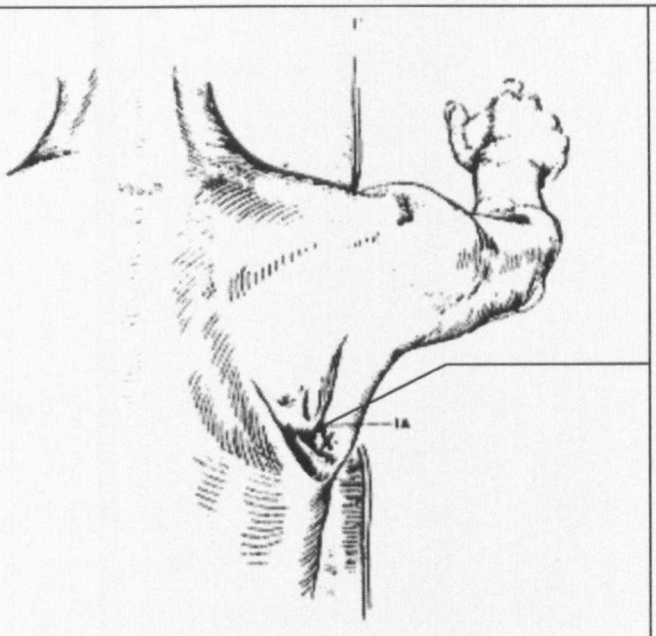


Figure 2.7 shows the position of the subject prone in a coach and the arrow shows the direction for wire insertion lateral to the inferior angle of the scapula into the aponeurosis of serratus anterior muscle.

After Delagi et al (1975) in *Anatomic guide for the electromyographer.* New York, Springfield & Illinois, 1975. (67)

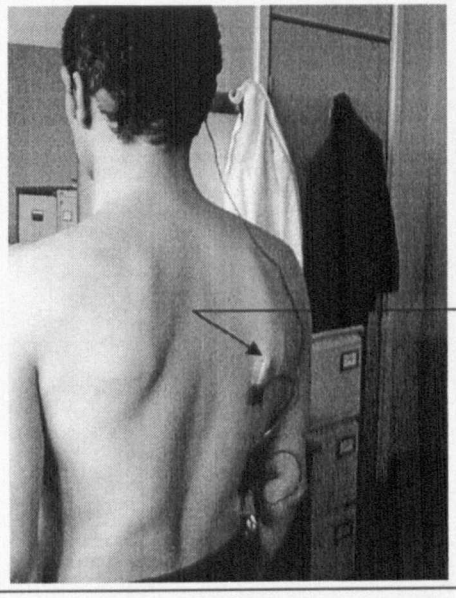


Figure 2.8 shows the position of fine wires applied according to the technique of Goodgold medial to the inferior angle of the scapula. The arrow gives the direction for wire insertion into the serratus anterior aponeurosis.

A note was taken of the sites of electrode insertion in reference to bony landmarks (spinous processes and inferior angle of the scapula), and an attempt was made in the second test to re-insert the electrodes in the same sites. The disposable 50μm fine wire electrodes, based on the design of Basmajian and Stecko (16), are supplied ready-loaded in a 25 Ga. hypodermic needle (Chalgren Enterprises, Inc, Gilroy California). They are of nickel-chromium alloy, insulated with nylon and sterilised by electron beam: 2 mm of metal are exposed at the tips, the hooked ends being staggered.

Surface electrodes were Ag-AgCl with an 8 mm contact area (BIOPAC Systems). The concavity was filled with conductive gel (Gel 100, BIOPAC Systems, Inc., Santa Barbara, California). Disposable Ag-AgCl electrodes with 10 mm contact areas were used over the tip of the olecranon as a ground. The electrodes were connected to three channels of an MP 100 EMG machine (BIOPAC Systems, Inc., Santa Barbara, California). Differential input amplifiers were used with a common mode rejection ratio of 100 dB minimum, input impedance 10 Ω, bandwidth 10-4000 Hz and gain 2000. Data were acquired with a Biopac system MP 100 data acquisition unit (BIOPAC systems, Inc. Santa Barbara, CA) with A/D resolution of 16 bits. Signals were sampled at 2500 Hz, and bandpass filtered at 10-1000 Hz for fine wires and 10-500 Hz for surface electrodes.

Bipolar electrodes were selected to eliminate the common mode signals (noise) (45, 333). A sampling rate of 2000 was chosen according to the Nyquist theory (193) as it is more than double the highest signal frequency that can be recorded by surface and wire electrodes (157, 257).

To insure that electrodes were applied correctly and were recording EMG signals from the serratus anterior muscle, manual loading of the subjects' serratus anterior was performed after completion of the set up and before starting the real test. If one of the EMG channels was found to have a high level of noise, or was recording out of phase in comparison to the mechanical loading of the muscle, alteration of the electrodes' positions was made until the problems were eliminated.

2.4.3- Analysis of EMG signals

Data were analysed using Acqknowledge software (BIOPAC Systems, Inc. Santa Barbara, CA), dividing each 60 s task into 20 intervals (data assigned to the midpoint). Median frequency was obtained through FFT. Median frequency was found to drop dramatically and usually shows unstable figures during the first 5s. Therefore, the first 5s of the data were omitted from the analysis (104).

Amplitude was calculated as the Average Rectified Value (ARV). Smoothing was found to improve the signal/noise ratio (45). Signals were therefore, smoothed (2000 sample/window), using AcqKnowledge 3.5 software. Smoothing is a transformation that computes the moving average of a series of data points and replaces each value with the mean value of the moving average "window". Smoothing therefore has a similar effect to that of a low pass filter with the advantage of being faster than a digital filter. The mean value smoothing formula is:

$$f_{output}(n) = \sum_{k=n-(m/2)}^{k=n[(m-1)/2]} f_{input}(k)/m$$

where m is the number of points in the window and n is the sample number.

(AcqKnowledge 3.5, soft ware guide, BIOPAC Systems, Inc. Santa Barbara, CA, http:// www.biopac.com)

Signals were then rectified (400 sample/window). Rectifying the data is a form of integration to provide an overall mean. This process can be performed by either calculation of the RMS or the ARV. Both methods of calculation were found to provide similar results with very little difference between them (137). Following the integration process, rectified data were normalised. Normalisation was performed by calculating the mean amplitude of every individual channel through the exercise period, then dividing the whole channel signals by this mean. Therefore, the relative amplitude was presented as a percentage of this mean. This process of normalisation has previously been evaluated in our department (249).

2.4.4- Statistical analysis

Statistical power calculation for the sample size necessary to demonstrate a significant difference between groups was not possible as the size of clinically significant difference and spread of data around the mean were not known.

Collected data were transferred to excel spreadsheets (Microsoft word 2000) for calculation of rates of change between start and end points of the different curves (slopes) using *linest* function in the spread sheet (Excel, Microsoft Word 2000). *Linest* is a mathematical method to calculate a straight line that best fits the data, and returns an array that describes the line. The equation for the line is $y = mx + b$, where the dependent y value is a function of the independent x value. m is the regression coefficient and b is a constant. The constant b value is a logical value specifying whether to force the constant b to equal zero (26).

The slope is the vertical distance divided by the horizontal distance between any two points on the line, which is the rate of change along the regression line. The equation for calculation of slope is as follows:

$$b = n \sum xy - (\sum x)(\sum y) / n \sum x^2 - (\sum x)^2$$

Standard Error (SE) of slope was calculated by calculating the linear regression array that provides values for the slope, slope SE, intercept and intercept SE (26).

To examine whether a certain slope is significantly different from zero a t value was calculated using the equation $t = \alpha - 1/SE(\alpha)$, when α is the slope and $SE(\alpha)$ is the standard error of slope. After calculating the t value, a t-distribution test was used to find a P value for each slope (26).

Slope values were expressed relative to the starting values: for frequency this was done by linear regression; for amplitude, where the time course was markedly non-linear, a two-point slope calculation was performed between t =3-9 s and t= 42-60 s. These time-points have been chosen arbitrarily to provide the best possible robustness and precision.

Between-study variability was calculated for each subject's pair of separate measurements, expressed as a coefficient of variation (CV = SD/mean %), and the

root mean square value taken for all subjects. Between-person CV was calculated using the means of the paired data for all subjects, correcting for the duplicate measurements by subtracting from the observed between-person CV^2 half the between-study CV^2.

As the collected data were numerical and continuous, parametric methods for statistical analysis were used. The student's *T-test* is used to determine whether two samples are likely to have come from the same two underlying populations that have the same mean (26). Differences were compared using the paired or unpaired t-test. A paired t-test was used to compare repeated measurements from the same patient between two trials, while unpaired t-test was used to compare between control and patients (26). The alpha error was set at 5% with $P < 0.05$ taken as statistically significant.

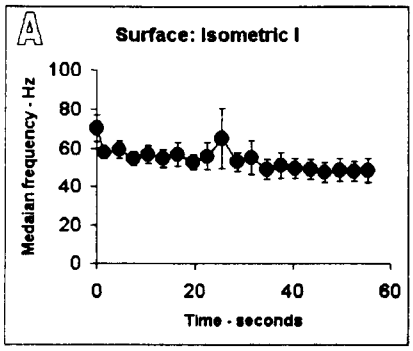
To provide an insight about the range of data around the mean the 95% confidence interval was calculated for statistically significant results. The confidence level was calculated with the use of significance level (1 - alpha), standard deviation and sample size (26).

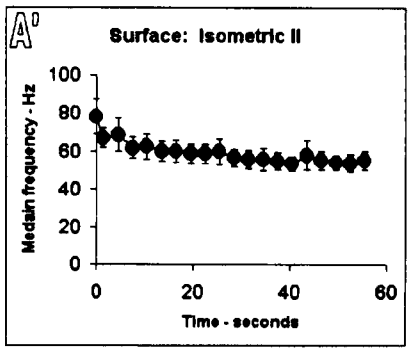
2.5- Results

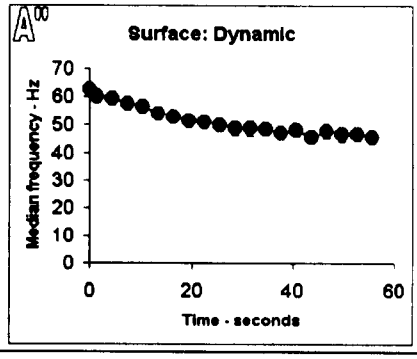
2.5.1- Repeatability results

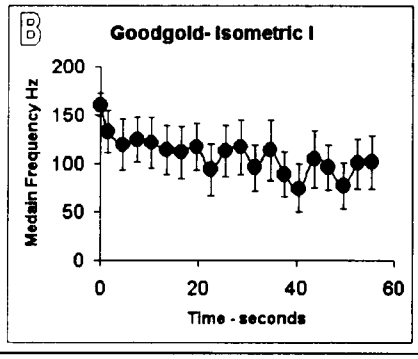
The main general observations in these studies were in relation to the changes in MDF and amplitude. The MDF showed gradual progressive decline throughout the course of the fatigue protocols. The decline of MDF was observed with surface and wire electrodes methods for EMG recording. No difference was noted between the rate of decline in MDF associated with isometric and dynamic fatigue protocols of exercises. Fig. 2.11 shows clearly the sharp fall in MDF during the first few seconds of exercise (even before the occurrence of mechanical fatigue), followed by a slower rate of fall during the rest of exercise. It was evident that surface electrodes gave less variability between subjects than fine wires, both around the linear regression line in an individual subject and within the same testing session. Both wire electrode insertion methods (Delagi and Goodgold, see methods for details) gave qualitatively similar results, in regard to the degree of variability within and between subjects.

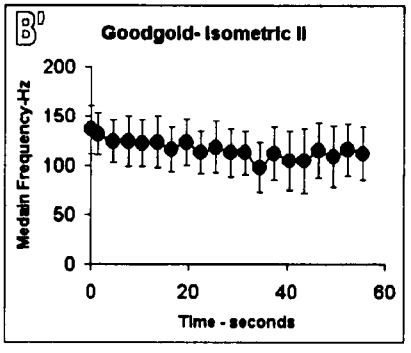
Calculation of the EMG amplitude showed gradual increase in the amplitude values. In general, amplitude figures were more variable than the MDF data, however, surface electrodes again gave less variability compared to the wire acquired results.

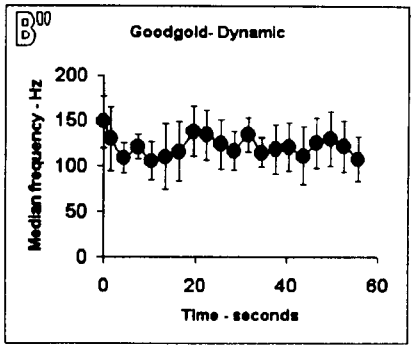


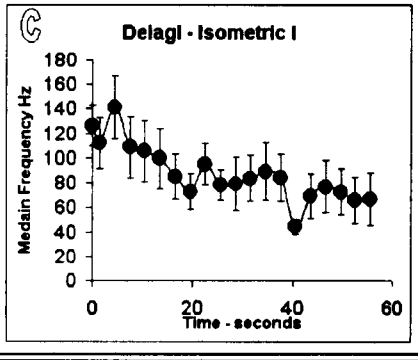


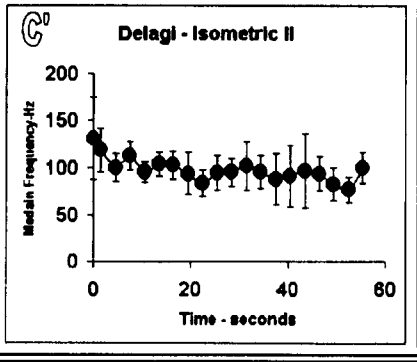












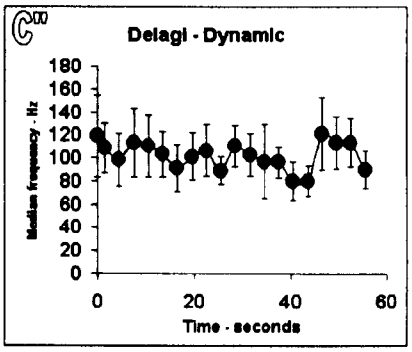


Figure 2.11 Median Frequency presented as mean and SEM (error bars).

The figure presents the mean data from both trials. The upper most panel of figures (A,A',A'') presents the data recorded with surface electrodes. The middle figures (B,B',B'') are data recorded with fine wires using Goodgold technique, while the lowermost figures (C,C',C'') present data recorded with wires according to Delagi technique. It is clear from this figure that surface electrode data were much less variable (smaller error bars) compared to the wire electrode methods. See text for detailed interpretation of the findings.

The next step was to compare the mean slopes in the two repeated studies in each person. Considering the general shape of the time course, it was decided to calculate by linear regression a slope over time from 5s to 50s. The choice of these intervals was to some extent arbitrary, being a trade-off between accuracy of instantaneous slope measurement, and the robustness of the result.

The measurement of slope was performed on the spreadsheet data as a function of the statistical methods in the Microsoft Excel 2000 programme and as explained in the statistical section earlier.

An important point that arose from calculating these slopes was that the initial slope (first 5 s) shows dramatic reduction in the MDF. This finding is in agreement with other studies that found the rate of change of MDF to be much higher during the first 5 s of voluntary and electrically elicited contractions compared to the rest of the exercise period (232). The rapid decline in MDF during the first 5s from the start of the exercises was unlikely to reflect real mechanical fatigue status of the muscles. Therefore, the initial slopes (first 5 s) were omitted from the analysis.

Having calculated the mean slopes for individual subjects in the different trials, these means were compared. It was obvious that surface electrodes produced more reproducible results compared to the wire electrode recording methods Table 2.1.

The use of surface electrodes gave better agreement between studies for both MDF and amplitude compared to the wire recording methods. Concerning MDF slope the overall between study CV for surface electrodes was 22%; comparatively the CV for Delagi and Goodgold wire electrode methods were very poor 224% and 194% Table 2.1.

Table 2.2 shows the results of the amplitude slopes CV between studies and subjects by the different protocols and recording methods.

Median Frequency Variability Between-study CV						Between subjects in the same trial CV
	Surface					
	AE	BM	AM	DS	RMS	
Isometric I	47%	5%	9%	24%	27%	32%
Isometric II	1%	31%	29%	7%	22%	50%
Dynamic	11%	0%	21%	24%	17%	14%
RMS (Isometric I & II)	33%	22%	22%	18%	24%	42%
RMS (all)	28%	18%	21%	20%	22%	35%
	Delagi					
	AE	BM	AM	DS	RMS	
Isometric I	10%	73%	16%	93%	60%	24%
Isometric II	6%	56%	11%	93%	54%	35%
Dynamic	738%	58%	156%	64%	380%	38%
RMS (Isometric I & II)	9%	65%	14%	93%	57%	30%
RMS (all)	426%	63%	91%	84%	224%	33%
	Goodgold					
	AE	BM	AM	DS	RMS	
Isometric I	4%	2%	84%		49%	28%
Isometric II	78%	101%	220%	16%	127%	295%
Dynamic	492%	174%	98%	255%	294%	89%
RMS (Isometric I & II)	55%	71%	166%	16%	95%	209%
RMS (all)	288%	116%	147%	180%	194%	179%
	Mean wire					
	AE	BM	AM	DS	RMS	
Isometric I	8%	52%	61%	93%	61%	36%
Isometric II	55%	81%	156%	66%	98%	53%
Dynamic	627%	129%	130%	186%	340%	173%
RMS (Isometric I & II)	39%	68%	118%	81%	82%	45%
RMS (all)	364%	93%	122%	126%	207%	107%

Table 2.1 presents the results of the between study and between subject Coefficient of Variation CV using different methods of recording and different exercise protocols. The abbreviation AE, BM, AM and DS are the initials of the healthy subjects who were recruited to this repeatability study; RMS = Root Mean Square. The mean wire column presents the results of the combined wires results.

EMG Amplitude Variability

	Between-study CV					Between subjects in the same trial CV
	Surface					
	AE	BM	AM	DS	RMS	
Isometric I	56%	4%	24%	45%	38%	37%
Isometric II	22%	18%	1%	14%	16%	29%
Dynamic	53%	8%	6%	16%	28%	79%
RMS (Isometric I & II)	42%	13%	17%	33%	29%	33%
RMS (all)	46%	12%	14%	28%	29%	53%
	Delagi					
	AE	BM	AM	DS	RMS	
Isometric I	92%	92%	121%	14%	89%	47%
Isometric II	19%	8%	461%	145%	242%	99%
Dynamic	2593%	216%	273%	-152%	1310%	391%
RMS (Isometric I & II)	67%	66%	337%	103%	182%	78%
RMS (all)	1498%	136%	317%	121%	771%	236%
	Goodgold					
	AE	BM	AM	DS	RMS	
Isometric I	109%	13%	66%		71%	46%
Isometric II	112%	16%	222%	51%	127%	2583%
Dynamic	241%	110%	5054%	-48%	2531%	223%
RMS (Isometric I & II)	111%	14%	161%	51%	101%	1827%
RMS (all)	166%	64%	2921%	49%	1463%	1497%
	Mean wire					
	AE	BM	AM	DS	RMS	
Isometric I	101%	66%	94%	14%	77%	46%
Isometric II	80%	12%	361%	109%	193%	76%
Dynamic	1841%	172%	3579%	112%	2016%	303%
RMS (Isometric I & II)	91%	47%	264%	78%	147%	62%
RMS (all)	1066%	106%	2078%	91%	1170%	182%

Table 2.2 shows the EMG amplitude variability between studies and persons at different protocols and recording methods using Coefficient of Variation CV. Better agreement between studies and persons can be seen with the surface electrodes compared to either or both wire methods combined.

The abbreviations are the same as in table 2.1

Having established that surface electrodes were producing less variable data compared to wire methods the results for the different exercise protocols were compared. The three exercise protocols showed qualitatively similar results in terms of MDF slopes. Using a t-test, the mean MDF slopes between Isometric I and Isometric II protocols were compared. No significant difference (P= 0.9) was found between the slopes of the isometric protocols. Similarly, the comparison bewteen the combined data from Isometric I and II protocols and the dynamic protocol showed no difference (P= 0.88).

A similar comparison was made between the mean amplitude slopes using surface electrodes during the three fatigue inducing protocols. No significant difference was found between the isometric protocols (P= 0.7); but a significant difference was found between the combined isometric versus the dynamic protocol (P =0.001) with static exercises producing greater increase in amplitude Fig 2.12.

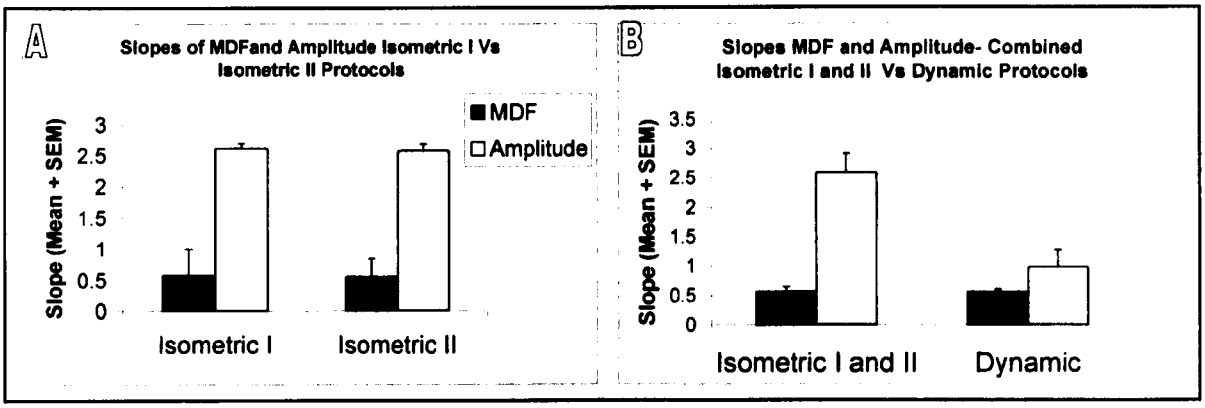


Fig 2.12 (A) compares the slope of MDF and amplitude between the Isometric I and II protocols using surface electrodes. Data are presented as mean + SEM (error bars) no significant difference was found between the compared slopes using t-test with P<0.5. (B) Compares the slope of MDF and amplitude between the combined isometric I & II results and the dynamic protocols. No significant difference was found between the MDF slopes, however, the amplitude slopes were significantly different (P= 0.01) with the isometric exercises producing higher magnitude of rise in amplitude. The Key in A applies to the panel B.

2.5.2- Comparison of isometric versus isokinetic exercise protocols.

For the purpose of comparison, and as isometric exercise protocols were found to produce similar slopes for MDF and amplitude their data were combined into a single mean. Similarly, data from both wire electrode insertion techniques were combined.

2.5.2.1- Isometric exercise protocols

Figure 2.13 summarises mean data on the relative changes of MDF and amplitude during the isometric protocols of exercises. With the surface electrodes, changes in amplitude were larger but more variable, especially after about 30s. The rate of fall in MDF was similar with surface and both wire electrodes: the mean rate of fall was 0.6±0.1% initial value per second (% s^{-1}) with both electrodes. The rate of rise of amplitude with both fine wire electrodes was significantly smaller than that recorded by surface electrodes (1.3±0.2% s^{-1} vs 2.6±0.3% s^{-1}, P=0.02), and more variable. With the wire electrodes, median frequency measurements were less variable than amplitude, but still more variable than with surface electrodes.

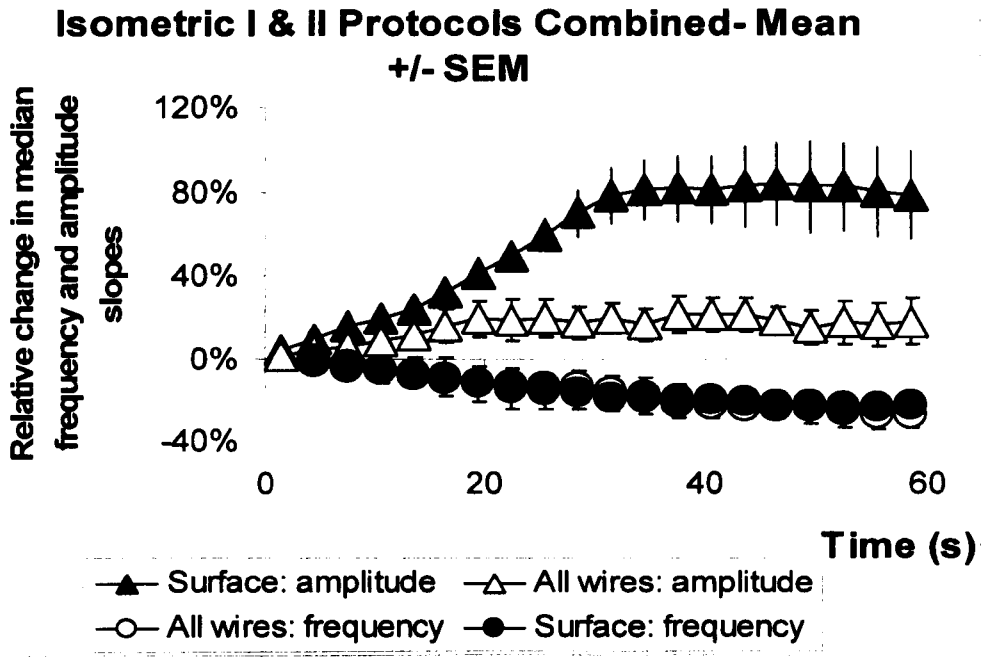


Figure 2.13 shows the mean results of relative amplitude and MDF acquired with surface electrodes ± SEM (error bars). Amplitude results are presented relative to the mean (ARV) of the whole period of exercise, while MDF is related to the starting value. See text for description of the changes.

2.5.2.2- Dynamic exercises & surface electrodes

The reduction in MDF during dynamic exercises recorded with surface electrode was comparable to that observed with the isometric exercises. However, EMG amplitude was found to be less marked in comparison to the rise in amplitude seen with isometric exercises fig. 2.14. This difference in amplitude slope between Isometric and dynamic exercises with the use of surface electrodes was significant (P= 0.001) fig 2.14. The amplitude slope (mean ± 95% CI) was 3-1.8 for the isometric protocol and 1.6-0.3 for the dynamic fig. 2.15.

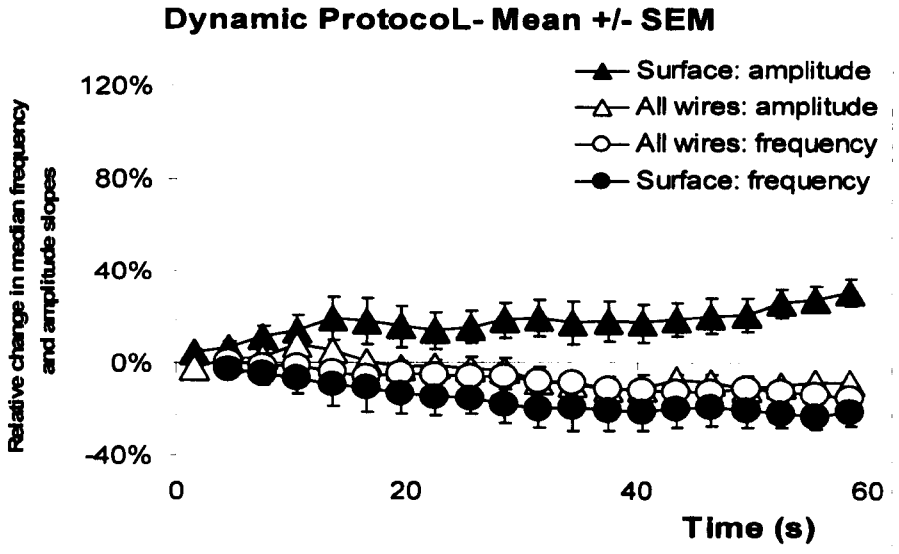


Figure 2.14 shows the mean data of relative MDF and amplitude acquired with surface and wire electrodes during dynamic exercises.

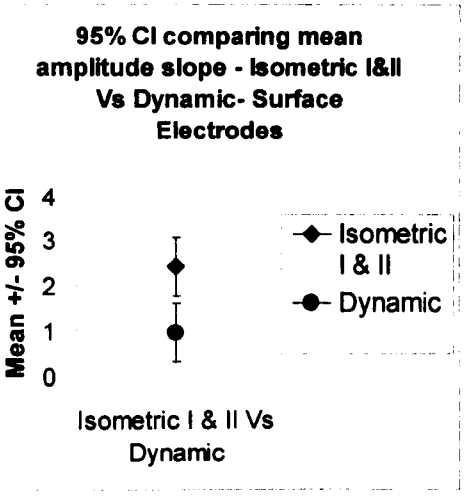


Figure 2.15 presents the Mean ± 95% CI for the EMG amplitude during Isometric I & II and Dynamic protocols of exercises.

2.5.2.3- Dynamic exercises & wire electrodes

Wire acquired data during dynamic exercises were different from those of the isometric exercises. Dynamic exercises produced a decline in amplitude in contrast to the rise seen with isomeric exercises and this difference was significant (P=0.02). The 95% CI was (1.4 to 0.4 for isometric and 0.3 to 0.1 for dynamic)

The MDF decline slope was also significantly different when wire electrodes acquired results were compared between isometric and isokinetic exercises (P=0.03). Figure 2.14 shows the mean relative MDF and amplitude data acquired with the use of surface and wire electrodes during dynamic exercises.

2.5.3- Comparison of patients versus control data (surface electrodes)

Having established superiority of surface electrodes in studying fatigability of the serratus anterior muscle. Five patients with reinnervated serratus anterior muscles following long thoracic nerve palsy were studied.

Patients' MDF and amplitude slopes were significantly different from zero during the Isometric protocols of testing. MDF and amplitude slopes during dynamic exercises were not significantly different from zero Table 2.3.

Patients' full slope for median frequency & amplitude						
Isometric	Slope	SE Slope	Intercept	SE Intercept	T value	P value
Amplitude	0.398	0.112086	93.516916	4.370141672	3.55045	0.0018
Median Frequency	-0.2145	0.0476357	75.10937	1.857275186	-4.5039	0.0002
Dynamic						
Amplitude	-0.016	0.041138	100.02407	1.603936164	-0.3885	0.7014
Median Frequency	-0.1743	0.0305126	84.517736	1.189659436	-5.7132	1E-05

Table 2.3 presents detailed linear regression array analysis of the patients' MDF and amplitude slopes during isometric and dynamic protocols. The P value defines whether the slope is significantly different from zero. MDF and amplitude slopes during isometric exercises were different while dynamic protocol findings were not.

During isometric protocols patients had a similar decline in their MDF throughout the testing period, not significantly different (0.6±0.1 % s^{-1}) from controls. However, the amplitude slope was significantly different (0.9±0.4 % s^{-1}, P=0.01) from that of control with a delayed but less marked increase followed by a rapid decline Fig 2.16A. 95% CI for control was 3 - 1.8 and for patients 1.6 - 0.2 (Mean ± 95% CI).

During the dynamic exercises patients had a less marked and delayed decline in their MDF slopes and a minimal change in their amplitude.

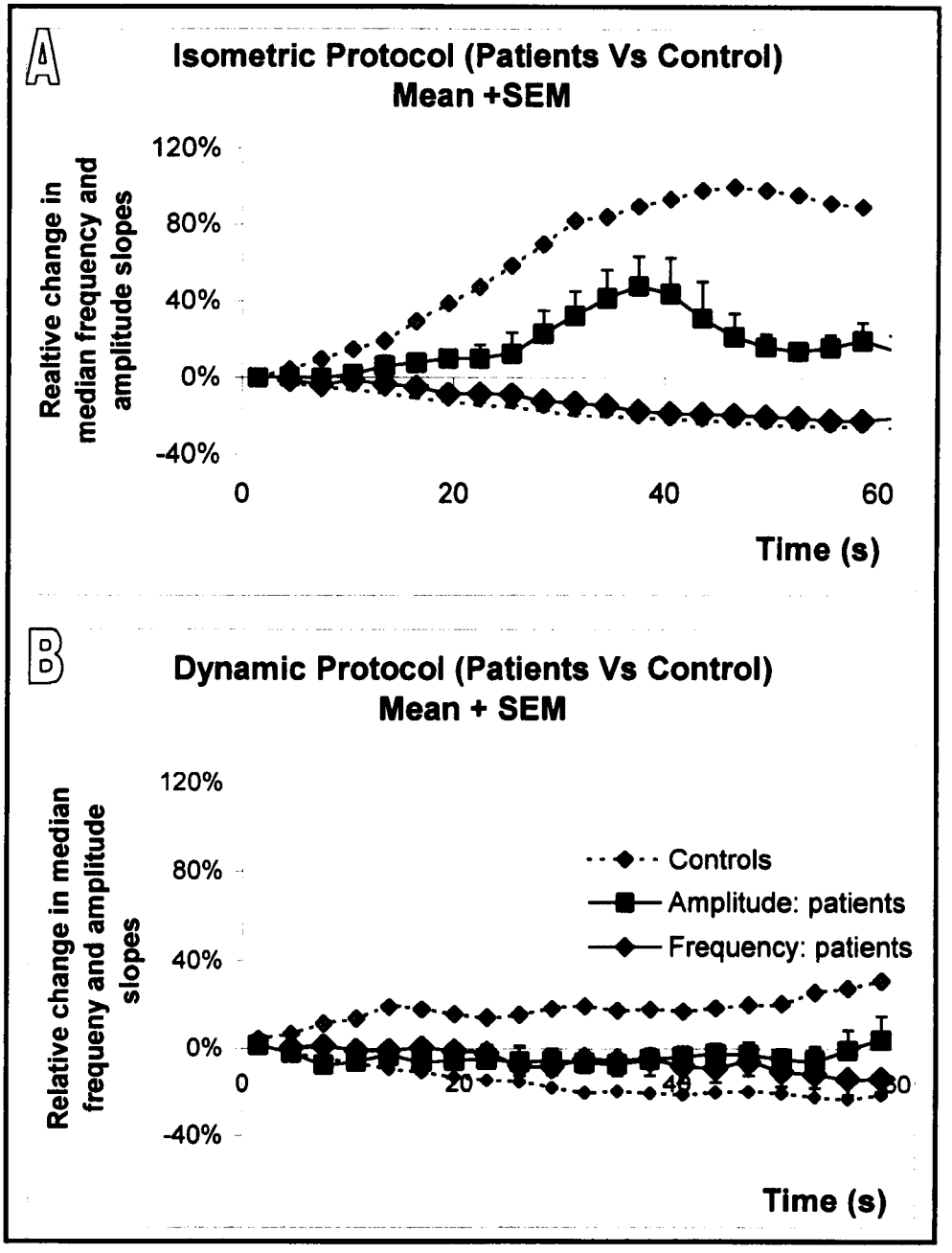


Fig 2.16 presents comparison between patients and control data during both isometric and dynamic exercise protocols using surface electrodes. Data are presented as Mean ± SEM (error bars). Amplitude is presented as a relative value % compared to the mean amplitude (ARV) of the whole exercise period, while MDF changes are related to its starting value. See text for details of the changes. The key in (B) applies to both subfigures.

Figure 2.17 shows the cumulative MDF and amplitude slopes as combined means. Isometric studies resulted in a lower MDF and amplitude slopes in patients compared to controls. The difference in amplitude slope was significant whilst in frequency was not. Dynamic exercise results were significantly different between patients and controls at both amplitude and MDF mean slopes.

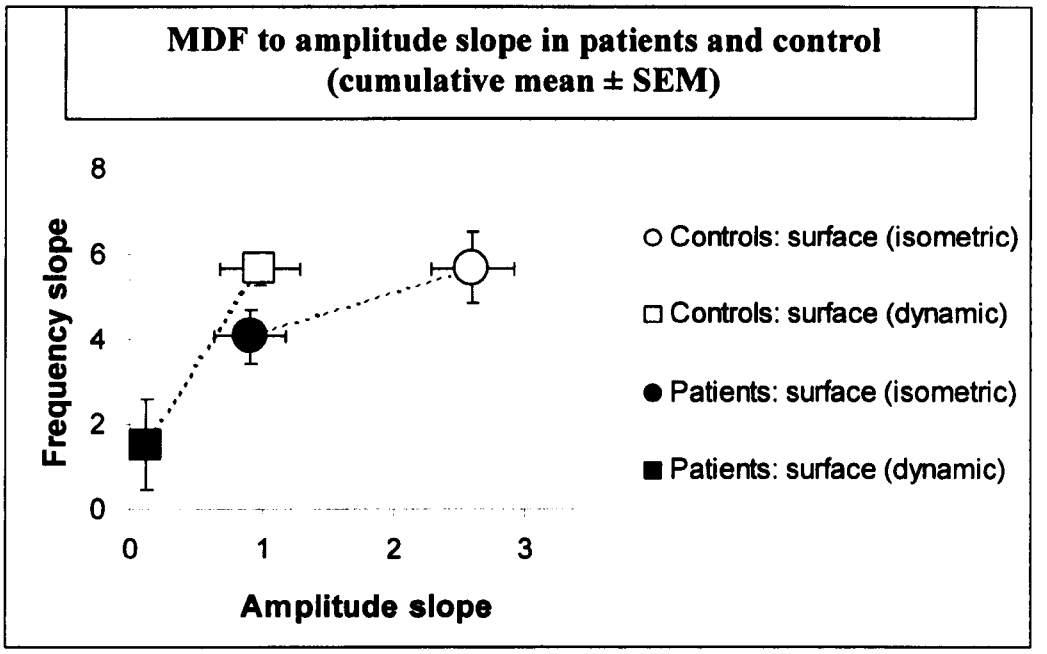


Figure 2.17 shows cumulative results for both patients and controls during both isometric and dynamic exercises. Data presented as mean ± SEM (error bars) Comparative exercise protocols results are connected by (.....) to facilitate comparison.

2.6- Discussion

In this chapter experiments have been designed to define the most repeatable method of studying myoelectric fatigue of the serratus anterior and the most suitable protocol of inducing fatigue in this muscle. This is in addition to comparing fatigability of reinnervated serratus anterior following long thoracic nerve palsy, to control data. Therefore in addition to the general discussion about the findings from these experiments attempts will be made to answer these questions:

1- Are surface electrodes better compared to wires in studying fatigability of the serratus anterior muscle?
2- What is the most suitable way of loading serratus anterior muscle and inducing fatigue?
3- Can dynamic exercises produce reproducible results, and what are the main differences between them and the isometric protocols?
4- Do reinnervated serratus anterior muscles show manifestations of easy fatigability compared to control?

2.6.1 Control and repeatability results

This chapter presents the first report of fatigue measurements of the serratus anterior, a muscle that combines an essential role in the movements of the upper limb with an anatomy that makes force measurements in isolation impossible. In general, surface electrodes are used to study superficial muscles, while wires are reserved for small, deep muscles (such as the rotator cuff muscles (249)) or when localised fibre or motor unit action potentials are being characterised. The results of this experiment agree with previous studies of other muscles (173, 178) that showed better reproducibility with surface electrodes than with inserted wires. Komi and Buskirk reported the repeatability of integrated EMG (iEMG) recorded with wire and surface electrodes from the biceps brachii muscles during isometric and dynamic fatigue protocols. Subjects were examined on three different days and at different levels of contractions. The overall reliability coefficients for iEMG with surface and wire electrodes were 0.88 and 0.62 respectively (192).

One of the factors that contribute to poor reproducibility with wire electrodes is the change in position of the wires in relation to the active motor units (173). The hooked

wires used in these experiments are designed to engage in the muscle fibres and reduce wire movements. Surface electrodes, however, are liable to similar errors due to skin movements in relation to the underlying muscle.

A second factor that would influence the level of repeatability of EMG data recorded by either wire or surface electrodes is the relation between the recording electrode and the active muscle bulk. Serratus anterior is composed of many digitations that combine before inserting on the anteromedial surface of the inferior angle of the scapula. The muscle is functionally divided into upper and lower parts, with different levels of activity in various tasks and arm positions (341). The territory of an MU within a highly compartmentalised muscle like the serratus anterior is confined to one compartment, and does not extend beyond its fascial boundaries (247). Both the Delagi and Goodgold techniques insert wires into the musculo-tendinous part of the muscle, which may not always represent the muscle's net level of activity. Fine wires are likely to reflect the activity that takes place in a single or small number of motor units. Surface electrodes were inserted within an ellipse that measures 4 cm in its widest part, giving a more representative measure of muscle activity.

The third factor could be the heterogeneous pattern of intramuscular distribution of muscle fibre types (muscle fibre regionalization) (187). It is, therefore, likely for surface electrodes (larger pick up area) to give more representative data about the muscle's myoelectric changes with fatigue compared to wire electrodes that have a more selective field in recording.
Other reported reasons for the poor reproducibility of wire electrodes are pain, intramuscular bleeding and inhomogeneous structure of the musculature (174). In these experiments once the needles were removed the fine wires caused minimal discomfort. Fine wires with 50 μm diameter are unlikely to cause serious disruption of the muscle's internal structures or bleeding. However, these factors can at least theoretically influence the repeatability of fine wire results.

The difficulty in re-inserting wires exactly into the same place can explain poor reproducibility between test days. However, this applies to both wire and surface electrodes.

A well-known disadvantage of surface electrodes is cross-talk (noise from other muscles or cardiorespiratory activities). De Luca et al (1998) applied surface electrical stimulation at 20 pulses per second to the tibialis anterior muscle and were able to record signals from the peroneus brevis and soleus muscles. The authors concluded that as much as 16% of EMG activity from the tibialis anterior could be recorded elsewhere in the leg (63). Koh et al were able to record 11% to 16% of EMG activity arising from the quadriceps femoris (following femoral nerve stimulation) in the medial and lateral hamstring muscle groups (190). However, these studies had the disadvantage of superficial stimulation through the skin. Superficially applied signals are likely to stimulate skin receptors, which is associated with various sensori-motor reflex arcs. These reflex arcs can initiate activity in different muscles of the studied limb (311).

Solomonow et al conducted a study on six adult cats' legs. All leg muscles were denervated except the nerve to medial gastrocnemius muscles. Supra-maximal stimuli were initially delivered to the medial gastrocnemius, which were followed by maximal and sub-maximal stimuli. Surface and wire electrodes were applied to the tibialis anterior and lateral and medial gastrocnemius muscles for EMG recording. With surface electrodes the authors recorded 5% of absolute mean EMG values as cross-talk in the neighbour (non-stimulated) muscles. This level of cross-talk was 2.5% with wire electrode recording. Cross talk was found to increase to 16% when muscles were covered with substantial amount of subcutaneous fat. The authors concluded that cross-talk at such low level is unlikely to influence the interpretation of clinical studies (311).

In this study the serratus anterior is the main muscle that controls protraction of the scapula and acts mainly on anterior and lateral rotation of the inferior angle of the scapula. Other muscles involved in controlling scapular rotation (trapezius and rhomboids) are located away from the surface electrode recording area and have different phases of activity during shoulder movements compared to the serratus anterior. The latissmus dorsi muscle is close to the recording area. However, this muscle's function is extension of the forward-elevated arm. As the isometric and dynamic protocols of exercises were designed to produce forward flexion it is unlikely that the latissmus dorsi could have produced a significant level of activity during these exercise protocols.

If cross-talk was to interfere significantly with the recorded signals in these experiments it would have produced a greater degree of variability in the surface electrode acquired data compared to the wires, which is not the case in these experiments. Hence, cross-talk remains at least a theoretical source of error in our experiments but is unlikely to have produced significant bias to the results.

In these experiments two isometric protocols of exercises were used to induce fatigue of the serratus anterior muscle. The basic mechanical way of loading the serratus anterior in both protocols is essentially the same with the serratus anterior used to stabilise the scapula against an anteriorly directed displacing force delivered through the arm. The isometric protocols produced similar results and a good level of agreement between studies and subjects (see table 2.1 & 2.2) that could be clearly seen. The slope of MDF and amplitude (resulting from both protocols) were very similar when surface electrodes were used fig 2.13 & 2.14.

Repeatability of dynamic exercises using wire electrodes and in recording EMG amplitude was particularly poor (tables 2.1& 2.2). However, there was a good level of reproducibility of median frequency results during dynamic exercises using surface electrodes.

There is paucity in the number of studies that have used myoelectric fatigue measurements e.g. spectral analysis and EMG amplitude during dynamic exercises. Christensen et al compared the EMG variables during static and dynamic fatiguing contractions and could not find any difference. However, in their study only low angular velocity (7° to 10° s^{-1}) of elbow flexion-extension and low force (~ 10% of maximum voluntary force) was used (43). Dynamic contractions performed at low force levels and low velocity may have a similar recruitment pattern to the static contractions (308). In another study by Larsson et al, who used dynamic exercises of moderate velocity (90°/s^{-1}) and moved the knee between 15° to 90° of flexion, good reproducibility of mean frequency and amplitude RMS was found with intraclass correlation of (0.83-0.98). However, their study tested the during the day reproducibility without changing the position of the electrodes (194).

The change in muscle fibre length and level of force exerted during dynamic contractions can cause differences in the calculated EMG spectral variables at different stages of the movement and when compared to isometric contractions (280). With surface recording of EMG during dynamic contractions skin movements over different parts of the active muscle can introduce a source of inconsistency and bias to the results.

Dynamic protocols of exercises presented in this chapter produced a low CV% between studies' and person's results with the use of surface electrodes. Reproducibility of the spectral shift or decline in MDF using surface electrodes was comparable to the isometric exercises. However, reproducibility with wires was generally poor with both exercise protocols. In the dynamic protocols, moderate velocity (90°/ s^{-1} isokinetic Con/Ecc mode of testing) was used and produced a good level of reproducibility with the use of surface electrodes fig 2.11. Merletti et al recommended the use of an isokinetic machine when dynamic exercises are used to induce fatigue, as they contribute to the consistency in recording and analysing the cycles of movement (233). The results from this study agreed with Christensen and Larsson's results in reporting good level of repeatability of EMG variables with dynamic exercises despite the difference in angular velocities used in the different experiments.

Though dynamic modes of testing provide more physiological methods of muscle loading, further investigation of other muscles is needed before recommending this protocol for general use with myoelectric fatigue studies. Therefore, the isometric mode of exercises was used in the second part of the experiment that involved patients' evaluation.

The gradual decline in MDF throughout the fatiguing exercises observed in these experiments agreed with previous studies confirming the spectral shift referred to as the myoelectric manifestation of fatigue (213, 231, 232, 234, 235, 243, 297). This decline in spectral variables during fatiguing contractions has been correlated with the reduction in muscle fibre conduction velocity and metabolic and pH changes during muscle contraction (31, 64).

Ebenbichler et al investigated the EMG fatigue patterns associated with isometric extension exercises of the knee joint using 30%, 50% and maximum voluntary contractions performed on 18 healthy subjects. The EMG MDF values at 3 consecutive MVC fatiguing exercises during the same session was reported as mean ± STD. For the vastus medialis (VM) the MDF values were 99.1± 29 Hz, 86.2 ± 22 Hz and 94.4 ± 29 Hz. Comparable values were also reported for the vastus lateralis with MDF values of 95.1 ± 28, 105.2 ± 29 and 99.3 ± 26 (84). Felici et al investigated the repeatability across days of the mean initial values of MDF from 6 normal subjects in 5 consecutive days after 80% eccentric contraction of the biceps muscles. The authors reported STD values equal to 4.5% of the mean (101). These degrees of spread of data around the mean compare favourably with the MDF data recorded in this study. The initial MDF values recorded in this study during two different trials of isometric contractions performed on two different occasions were 60.9 ± 7.5 Hz and 56.7 ± 6.7 Hz (mean ± STD).

Merletti and Roy (1996) have used the rate of change of MDF as a measure of muscle fatigability (234). The slopes of EMG mean and median frequency during fatigue inducing exercises have subsequently been used in various experiments to characterise muscle fatigue (101, 230, 231, 285). In this study considerable variability was noticed between the absolute EMG figures of different subjects. However, the absolute slopes correlated significantly with the starting values, so that the relative slope used here was the appropriate quantity, and shows only modest variability.

The decline in MDF during fatiguing exercises has been observed in similar studies. Merletti et al (1990) studied the myoelectric manifestation of fatigue associated with voluntary and electrically elicited contractions of the tibialis anterior muscle. With 80% MVC sustained over 20 s the MDF was found to decline from 100 Hz to 75 Hz (232). Merletti et al (1996) studied fatigability of the tibialis anterior muscle at different levels of contractions ranging from 50% to 80% MVC that were sustained for durations of 170 s to 90 s respectively. At the end of these fatiguing contractions the normalised MDF has declined to 62 % and 38% of its initial value following the 50% and 80% MVC fatigue protocols respectively (234).

Considering the experiments presented in this chapter, isometric contractions resulted in a reduction of the MDF from 80 Hz at the beginning of the contractions to 60 Hz after 60 s. With dynamic contractions the initial value of the MDF was lower, 60 Hz and by the end of 60 s fatigue protocol the MDF had declined to 40 Hz.

Although there was no significant difference of the MDF slope between static and dynamic exercises using surface electrodes, the amplitude slopes differed significantly. The smaller rise in amplitude during dynamic exercises could be explained by the fact that dynamic (isokinetic) exercises induce less fatigue and delayed reduction in force generating capacity of muscles compared to isometric exercises (268). However, if dynamic exercises had induced less fatigue this would have resulted in a smaller degree of decline in the MDF, which was not the case.

The more logical explanation for the difference in EMG amplitude change between the dynamic and isometric protocols of exercise would be a difference in the MU recruitment. Dynamic exercises have been found to rely primarily on recruiting additional MUs to compensate for the decline in force as fatigue develops. In contrast, isometric exercises tend to increase the MU discharge rate then recruit additional MUs (309). In a fatigue protocol with maximum voluntary contraction most MUs are usually recruited at the beginning of the test leaving little room for recruiting additional units. Hence, dynamic exercises would have a lower rate of increase in EMG amplitude compared to the isometric.

A contradiction between the amplitude slope recorded by the surface and wire electrodes during same exercises was clearly seen during dynamic exercises where a rise recorded by surface electrodes and a simultaneous fall recorded by the wires. Zijdewind et al have attributed these paradoxical changes to either a decrease in the total motoneuronal activity that arrive to the muscle (localised central fatigue) (363) or to local factors that involve electrophysiological muscle fibre properties. The same authors in a more recent study confirmed this contradiction between the EMG amplitude recorded with surface and wire electrodes and proposed the possibility of heterogeneous pattern of intramuscular distribution of different categories of muscle fibres "muscle fibre regionalisation" (359). Dimitrov el al suggested that a difference in the distance between the active fibres and electrode (length of depolarisation zone)

leads to similar changes. Gydikov et al, using two surface electrodes at different distances from an active motor unit, recorded an increase in EMG amplitude in the far electrode and decrease in the electrode that was placed closer (135). Dimitrova and Dimitrov have theorised that EMG amplitude changes are reflections of intracellular action potential profile and the further away the recording electrode from the active fibre, the more likelihood for the recorded amplitude to increase (77).

MUs are known to discharge in a cyclical fashion i.e. motor units recruited at the beginning of fatiguing contractions are known to reduce their discharge rate (21). Meanwhile, other MUs are recruited to replace those who stopped firing (270). As wire electrode recording fields are usually limited to an MU, wire electrode recorded signals would present a MUAP, which is known to decline with continued contraction. In contrast, surface EMG would be able to record signals arising from additional motor units recruited at different stages of the contraction cycle. Large motor units, with high threshold and high level of electric activity have been shown to be recruited later after the contraction had already been started (357). The contribution of these large MUs to the EMG activity would account for the augmentation of EMG amplitude recorded with surface electrodes in isometric and dynamic experiments presented in this chapter.

An interesting observation that emerged from this study was in regard to the EMG amplitude. EMG amplitude has been shown to have different responses to different levels of fatiguing muscle contractions, with 100% MVC there is usually a decline in the amplitude. Conversely, submaximal contractions (< 90% MVC) were found to be associated with an increase in the EMG amplitude (152, 275). In these experiments subjects were asked to produce their maximum forward flexion force of the arm and it was assumed that this would produce the MVC of the serratus anterior. However, the augmentation of the EMG amplitude suggests that the serratus anterior muscles were sub-maximally loaded. Mullany (2002) observed an increase in EMG amplitude recorded from the knee extensor muscles with submaximal voluntary isometric contractions at 25%, 50% and 75% MVC, which was statistically different from the decline in EMG amplitude observed with 100% MVC (251). There is no other method to ascertain whether the serratus anterior was sub-maximally loaded, as isolation of the serratus anterior muscle mechanical force is not technically possible.

2.6.2 Comparison of patients and control results

Having confirmed that surface electrodes were superior to the wire in recording myoelectric fatigue of the serratus anterior muscle and the pattern of MDF and amplitude slope; five patients with a history of long thoracic nerve palsy that was treated either surgically (four patients) or non-operatively (one patient) were studied. The main symptom in this group of patients was the residual sense of easy fatigue and inability to maintain the position of the arm above the shoulder level, despite the improvement in the scapular winging that was recorded clinically using Kibler's test.

In this experiment increased fatigability of reinnervated serratus anterior muscles in patients with reinnervated serratus anterior muscles could not be demonestrated. The results confirmed a similar slope for the EMG median frequency compared to normal. Meanwhile, the amplitude slope was reduced by about half. This is unlikely to be explained by differences in the muscle loading (impossible to assess, because of the possible compensation by other muscles). Decline in MDF with fatiguing muscle contractions has been correlated with the slowing of CV at the muscle fibre membrane and increased $[K]_o$. Lindeman et al (1999) reported a steeper MDF slope (-0.37) of the surface EMG recorded from the vastus medialis muscle in patients with congenital myotonic dystrophy compared to healthy subjects (-0.06) (200). Merletti et al (2002) have shown a significant difference between the myoelectric manifestation of fatigue recorded from the biceps muscles between a group of elderly subjects compared to a young group of healthy volunteers. Young subjects had a steeper slope of the MDF compared to the elderly subjects indicating higher fatigue resistance in the older group muscles. This difference was attributed to the change in muscle fibre type with higher percentage of SO fibre type in the older group muscles compared to the young (230). The normal slope of MDF observed in the EMG of reinnervated serratus anterior muscles in this study suggests that muscle fibre type and MU conduction velocity remained unchanged.

Although disuse and denervation have been shown to induce muscle fibre changes at the level of the mRNA of the MHC (159), these changes were found to be usually incomplete and limited i.e. it is not possible for the denervation process to fully convert SO type I to FG type IIb or IIx fibres (315). Denervated muscle fibres were found to change their contractile properties from slow to fast contracting but to retain

their fatigue resistance properties (123). Moreover, it is important to note that denervation induced conversion of muscle fibre type is dependent on the severity of nerve injury. Nerve crush injury was found to produce milder changes in the characteristics of reinnervated muscles compared to complete nerve division (103). Serratus anterior paralysis as a result of LTNP is likely to be either a result of traction (87, 265) or compression (141, 212) injuries that represent a milder grade of nerve damage with no or limited disruption of the endoneurial tubes (317). Therefore, they are less likely to induce a mismatch between the reinnervated MUs and the growing motoneuron that can induce significant changes to the muscle's fatigue properties.

In a group of 10 patients 12 years post microsurgical nerve repair of brachial plexus injury, Chammas et al have confirmed significant weakness of partially re-innervated biceps muscles when compared with the healthy side. However, there was no difference in the fatigue or endurance of the partially re-innervated muscle when they exerted the same percentage of their own maximum voluntary force (41).

The lower amplitude slope observed in the patients group can be explained either by the inability of the reinnervated muscles to increase their rate of discharge or to recruit higher threshold large motor units and/or the lack of strong fast contracting motor units.

The less marked increase in EMG amplitude of reinnervated serratus anterior muscles can be explained by the reduction in their central drive. Central drive to MUs of wasted and weak muscles has been shown to be reduced by up to 40% and has been suggested as a reason for the reduction in EMG amplitude (180). This reduction in motoneuron discharge rate can be the result of a central command to protect these deficient muscles from overexertion and early fatigue or due to local reflexes controlled at the spinal level. Feedbacks from muscle spindles through Ia afferent fibres contribute to the maintenance of motoneuron discharge rate (29). Reduction in the number of functioning afferents associated with denervation (11, 52) can therefore account for the reduction in motoneuron discharge rate.

Another explanation that can account for the smaller increase in EMG amplitude is the lack of large, strong fast contracting motor units in reinnervated serratus anterior muscles. Rafuse et al showed that slow motor axons have a higher potential to sprout

following denervation compared to the fast ones (283). This would increase the number of slow MUs and be reflected in the EMG as a reduction of the amplitude.

Finally, it is known that reduction in muscle cross-sectional area (as in case of denervation) is accompanied by increase in non-contractile structures such as fat and connective tissue (289). The increase in the amount of non-contractile structures in reinnervated serratus anterior can account for some of the observed reduction in amplitude changes in the patients' group.

The subtle changes in amplitude points to a deficiency in the muscles' ability to develop high levels of force. For technical reasons it is not possible to comment on the serratus anterior force generating ability.

2.7- Conclusion

For the first time protocols for studying fatigability of the serratus anterior using isometric and dynamic modes of muscle testing have been developed. Isometric testing of the serratus anterior in two arm positions, Isometric I & II protocols, were not different in their ability to induce fatigue. Although subjects were instructed to produce their maximum voluntary contractions with arm movements in the different protocols of testing, the increase in EMG amplitude recorded from the serratus anterior suggests that these muscles were loaded sub-maximally.

Dynamic exercises with moderate angular velocity 60° s-1 and MVC were used in the third protocol. EMG power spectrum analysis and amplitude (ARV) were found to be reproducible when analysis was performed over intervals of 3 s. Although, these results support the use of dynamic exercises in myoelectric fatigue studies caution and further experiments are necessary before this method of investigation can be routinely used.

Surface electrodes were found to produce more reproducible results compared to fine wires and would be recommended for investigating myoelectric fatigue of the serratus anterior.

Finally, the findings from this chapter did not prove the hypothesis that reinnervated serratus anterior muscles would be more fatigable compared to normal. This conclusion is based on the EMG findings of a muscle; where it is not possible to correlate these findings to the mechanical properties. However, the less marked increase in EMG amplitude in patients compared to normal suggests an abnormality in the reinnervated serratus anterior MUs distribution and/or discharge rates.

Chapter III: Recovery of reinnervated first dorsal interosseous muscle

3.1- Introduction

In an earlier section of this thesis (Chapter II), the results of an experiment on fatigability of the reinnervated serratus anterior muscles were presented. The results from the experiments on the serratus anterior could not prove a difference in the fatigability of the reinnervated muscles compared to control based on the analysis of the EMG power spectrum. In the case of the serratus anterior muscle, mechanical fatigue could not be measured due to particular difficulties related to the anatomy of the muscle and impracticality in separating its action. Therefore, investigating the fatigue phenomenon following nerve injury in another muscle for which both mechanical and myoelectric manifestation of fatigue can be analysed was sought.

The first dorsal interosseous (1^{st} DI) is one of the commonly studied human muscles in regard to fatigue. As this muscle is superficial, easily located and can be stimulated either voluntarily or by ulnar nerve stimulation (359).
The main function of the first dorsal interosseous muscle is abduction of the index finger. In conjunction with its anatagonist (the first palmar interosseous muscle) they contribute to flexion of the 2^{nd} MP joint. In histological studies of human muscles, 1^{st}.DI was found to have a homogenous structure with almost even distribution of both type I and IIa and IIb muscle fibres. Type I muscle fibres were found to represent about 57% of the fibre population in this muscle. This is in contrast to other hand muscles that were found to have a higher proportion of the fatigue resistant slowly contracting type I (44). Despite this difference in muscle fibre composition the 1^{st}. DI was found to have a similar fatigue characteristic changes in torque and EMG amplitude when compared to the adductor pollicis; a muscle known to have a higher proportion (80%) of type I muscle fibres. This finding was recorded with electrically elicited contraction using ulnar nerve stimulation. Conversely, in voluntary induced submaximal contractions, 1^{st} DI was found to have a briefer endurance when compared to the adductor pollicis (362, 363). In this context if mechanical fatigue can be defined as the time dependent decrease in the ability of the muscle to maintain a specified force or joint torque within a given window centred around the target level, endurance would refer to the time during which the muscle contractile force remained within the target window (234).

In voluntary induced fatigue studies using submaximal contractions, decline in force is usually paralleled by augmentation of the surface recorded EMG amplitude. A finding that suggested a compensatory increase in the number of activated motor units , increase in motor unit rate of discharge or increased motoneuronal drive to substitute for the progressively reduced force (359). Conversely, maximum voluntary contractions are associated with reduction in surface EMG that was attributed to the decline in MU discharge rates seen with 100% MVC activities (22).

3.1.1 The response of muscles to different fatigue protocols

Fatigue is a complex, multifactorial phenomenon and has been associated with impairments at a number of sites, ranging from central activation to myofilament interaction (as reviewed in section 1.2). Fatigue was found to be task specific, in that for a given task one particular site or mechanism may be more or less responsible for the decline in a muscle force generating ability (294). Task variables that can be manipulated by the investigator and influence the prevailing mechanism of fatigue include the level of subject motivation, the neural strategy (pattern of muscle activation and motor command), the intensity and duration of activity, the speed of contraction and the extent to which the activity is continuously sustained (294).

Fatigue that results from employing submaximal level of contractions has been linked to mechanisms that develop solely within the muscle itself. Bigland-Ritchei et al (1986) investigated the fatigue characteristics that developed in the quadriceps and soleus muscles during isometric 50% MVC performed by intermittent periods of contraction over 6 s followed by 4 s of relaxation. These 50% MVC fatigue task were interrupted by asking the subjects to exert their MVC or delivering supra-maximal electric stimuli to the relaxed muscles. With the development of fatigue and the drop of the MVC to 50% of the initial value, electrical stimulations failed to achieve any increment in force indicating that fatigue was not a result of reduction in central drive (24). During these experiments there was no significant change in muscle lactate, pH, PC and glycogen depletion was minimal. The authors attributed the development of fatigue to failure of ECC (24).

Because muscle contraction increases the demand for ATP by an order of magnitude and fatigue is a consequence of muscle contraction, the metabolic cost of contraction is believed to be a primary factor in fatigue (294). Despite the lack of consensus regarding the relationship of specific metabolic by-products to fatigue and the mechanisms by which they act in vivo, several studies have demonstrated that voluntary exercise and stimulation protocols that produce the greatest metabolic changes also produce the greatest fatigue (42, 279), supporting the theory that fatigue is related to metabolic cost, although factors other than metabolites contribute to the reduction in force.

Number of studies have demonstrated that during intermittent, isometric, electrical stimulation with trains of pulses rather than twitches, shorter duration contractions produce greater fatigue than longer contractions when force, total contraction time and number of stimulation pulses were controlled (144, 313). Fatigue protocols using short-duration contractions produced greater ATP turnover as well as greater fatigue (144, 313).

Duchateau et al (1985) investigated the effect of sustained and intermittent contractions on mechanical failure during muscle fatigue in the human adductor pollicis electrically stimulated at 30 Hz delivered to its motor nerve. Contractions resulted from sustained stimulation for 60 s were compared to three protocols of 60 one-second contractions, which were separated by 2 s, 1 s and 0.5 s intervals of relaxations in three different intermittent protocols. The sustained protocol of muscle contraction resulted in a reduction of force by 60% of its initial value. The intermittent protocols differed in their effect but the intermittent protocol with 0.5 s intervals of relaxations between the one-second 60 contractions resulted in a 65% reduction of force from its initial value.

Hogan et al (1998) investigated the effect of two intermittent stimulation protocols on the development of fatigue and metabolic changes in the dogs' gastrocnemius muscles. The two stimulation protocols were similar in their total contraction period as well as the ratio of the contraction to relaxation intervals with the first being 0.25 s of stimulation and 0.75 s relaxation and the second comprised of 1 s of contraction and 3 s relaxation. O_2 supply was maintained at a similar level during both stimulation

protocols. The authors found the intermittent protocols with short durations of contractions to result in a higher rate of fatigue development with drop of force to 70% of its initial value compared to 91% with the long contraction protocols. Calculated intracellular [H^+] was also significantly higher during short- duration contractions compared with the long. The O_2 cost for the developed force was significantly greater (39%), during the contractions of short duration. The calculated ATP utilization rate was 32% greater and the ratio of ATP utilization to developed force was 70% greater during the short-duration contractions compared with the long (144).

The factors involved in the development of fatigue in high intensity short duration contractions are clearly different from those eliciting fatigue in submaximal prolonged contractions. With high intensity sustained contractions there is recruitment of all fibre types, high contraction frequency and high degree of anaerobic metabolism (282). The dependence on anaerobic metabolism results in an increase in intracellular H^+ and P_i, which are known to reduce peak force (81).

Research has shown that central drive plays an important role on the ability to maintain a voluntary level of force. McComas et al have shown that 10 out of 17 males and 4 out of 11 females were not able to maximally activate their plantar flexor muscles (224).

One of the relations that received considerable attention in the fatigue phenomenon is the force-fatigability relationship, the greater the force exerted during a task the more rapidly the muscle fatigues. Classic endurance tests with sustained isometric muscle contractions revealed a hyperbolic relation between force and endurance limit (i.e. time to a certain fatigue task) (96). Clark et al examined the effect of sustained isometric contraction on surface (EMG) and force signals derived from the jaw closing muscles. Subjects produced and sustained 25%, 50%, 75% and 100% of their isometric MVC. A consistent inverse relation was found between the endurance limit (time to a certain fatigue task) and the magnitude of force (49).

A feature of the force-fatigue relationship is its dependence on the absolute force exerted during the task. McKenzie and Gandevia examined this relation in a group of subjects who performed intermittent isometric contractions of their elbow flexors. These contractions were performed at two different muscle lengths; the optimal length and a shorter length. MVC at the shorter muscle length was found to be reduced by 25%. Fatigability was defined as the magnitude of decline in peak force after 18 repetitions of 100% MVC. Fatigability of the elbow flexor muscles was found to be greater at the optimal muscle length, peak force reduced by 61%, compared to the shorter muscle length for which the peak force has dropped by 55% (226). A similar finding was observed by Fitch and McComas when they examined fatigability of the tibialis anterior muscle using MVC and percutaneous electric stimuli at optimum and shortened lengths. Significantly greater reductions in twitch and tetanic torques were found after the fatiguing procedure had been conducted at the optimum muscle length rather than with the muscle in a shortened position (105).

It is not clear whether this force-fatigability relationship would be found in reinnervated muscles. Experiments on the serratus anterior muscle in the previous section showed no increase in fatigability. The peak force or MVC of the serratus anterior could not be measured. The work presented on this chapter attempted to examine the relation between fatigability and peak force on reinnervated first dorsal interosseous muscles.

3.1.2- Anatomy of the first dorsal interosseous, mechanical testing and EMG recording

The bulk of the first dorsal interossseus muscle is located on the first web space between the 1st and 2nd metacarpal bones. The muscle has 2 belies attached proximally to the radial side of the 2nd and ulnar aspect of the 1st metacarpal bones Fig 3.1. The distal attachment of the muscle is into the radial side of the proximal phalanx of the index finger and the extensor expansion at the base of the index. The function of this muscle is abduction of the 2nd metacarpophalangeal joint through its attachment to the radial side of the proximal phalanx, but also it plays a role in flexion of the 2nd.MPJ and extension of the PIPJ through its insertion into the extensor mechanism(351).

Because the 1st DI muscle is superficially located and easily accessible, surface electrodes have been regularly used to study its myoelectric manifestation of fatigue (320). One or two surface electrodes can be used, though the use of 2 electrodes is usually favourable to allow the common mode rejection of superimposed noise on the real EMG signals (257). Loading of the muscle is achieved through abduction of the 2nd MPJ in either the flexed or extended positions.

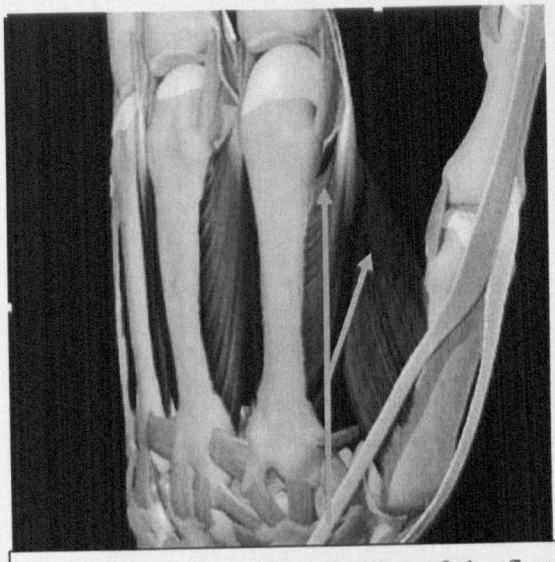


Figure 3.1 shows the 2 bellies of the first dorsal interosseous muscle originating from the ulnar side of the 1st and the radial side of the 2nd metacarpals. Courtesy of Professor Mc Grouther and Dr O'Higgins, Interactive Hand Anatomy CD 1998, Primal Pictures, London.

3.1.3 Functional outcome following ulnar nerve decompression

The 1st. DI muscle is innervated by the deep branch of the ulnar nerve. Therefore, it is not uncommon for patients with ulnar nerve compression whether at the elbow or wrist levels to suffer from denervation of this muscle. Denervation of the 1st DI results in a reduction of the index's abduction force that would be reflected on the hand function as a deficiency in the patient's pinch grip.

In addition to the 1st DI, ulnar nerve palsy can lead to denervation of other small muscle in the hand like the adductor pollicis and lumbrical muscles. At sensory level ulnar nerve palsy results in altered sensation in the palmar aspect of the little and ulnar half of the ring fingers.

The outcome of ulnar nerve release has been correlated with the severity of nerve compression and the length of the denervation period (278, 342). Patients have been classified into three categories. The first category includes those with predominately sensory symptoms for whom full recovery would be expected after nerve decompression. The second category comprises of those with mild muscle wasting in whom motor recovery takes longer time and may be incomplete. The final group includes patients with moderate to severe motor deficiency and muscle wasting. Recovery in this final group is usually incomplete (342).

This clinically based classification is supported by other animal studies (8). Finklestein et al examined the medial gastrocnemius muscles of the rats following different periods of denervation with time from nerve cut to re-suture ranging from 0 to 56 days. Muscles that had immediate repair of their nerve were found to have 25% reduction of their muscle mass at eight weeks from injury. Longer periods of denervation were associated with higher degrees of reduction in muscle mass (103).

Clinical assessment of motor function of the hand is usually based on the medical research council (MRC) grading of muscle strength. This system classifies muscles into six grades from 0 to V with grade 0 being the worst and grade V the best (see table 3.1) (171).

MRC grade	Description of muscle function
Grade 0	Complete paralysis
Grade I	Flickers of muscle contraction but no movement
Grade II	Muscle is able to move the nearby joint with elimination of gravity
Grade III	Muscle is able to move the nearby joint against gravity
Grade IV	Muscle is able to move the nearby joint against gravity and some external resistance
Grade V	Normal muscle strength

Assessment of sensory function usually includes the pain and touch modalities in addition to other special tests that measure the innervation density (see section 1.7.3.1 Evaluation of hand sensation and reflection on its function). Of particular importance is the 2PD. The value of the 2PD has been found to be lowest at the tip of the fingers where it usually measures 3-5 mm. Higher values are normally found in the proximal parts of the hand and less sensitive parts of the body (171).

3.2- Objective of the experiment:

The objectives of these experiments were multiple folds. First, to investigate fatigability of reinnervated/partially denervated first dorsal interosseous muscle and examine whether these muscles have different fatigue characteristics when compared to control. Second, to correlate between mechanical and myoelectric manifestations of fatigue of the first dorsal interosseous muscle in a group of patients who had ulnar nerve palsy and compare them to controls.

3.2.1- Hypothesis tested

The hypothesis was that reinnervated first dorsal interosseous muscles would demonstrate more fatigability compared to control hands.

3.3- Material and methods

The study was approved by the local ethics committee and all patients and volunteers have given consent to participate in this study.

3.3.1- Subjects

Two groups of subjects were recruited to this study. The first group consisted of 4 healthy volunteers, three males and a female with average age of 31 years (range 24-37 years). All subjects were right hand dominant and both hands were studied. None of these volunteers had a history of neurological diseases and clinical assessment showed normal function.

Seven patients (four males and three females) with average age of 58 years (range 31-71 years) were also recruited to this study. Five were right and two were left hand dominant. The right side was the affected side in six and the left in one patient. All patients had decompression of their ulnar nerves at the elbow level following the diagnosis of cubital tunnel syndrome. The average time between the operation and performing this experiment was 20 months (range 6-40 months). Affected (reinnervated) and unaffected hands were examined in the same testing session.
The exclusion criteria included those who had bilateral compression of the ulnar nerve and those who had mild involvement of the ulnar nerve with predominantly sensory symptoms. Patients who suffered from systemic disease that would affect their nerve function like diabetes mellitus were also excluded.

All patients reported some improvement of their sensory and motor function following the ulnar nerve decompression, however, four of these patients had residual feeling of hypoasthesia in the ulnar nerve distribution of the affected side. Using 2PD for sensory testing showed a slight non-significant increase on the affected sides (average 5.5mm) to the non-affected sides (average 4 mm). Three of the patients had a clinically noticeable degree of wasting of the 1st DI. Using MRC grading for assessment of the first dorsal interosseous motor function the average grade for the reinnervated hands was IV, while the average grade for the unaffected hands was V.

3.3.2- First dorsal interosseous fatigue protocols

While the subject was seated, the elbow was flexed and the wrist was slightly extended to allow comfortable resting and support of the hand in a table fig 3.2.

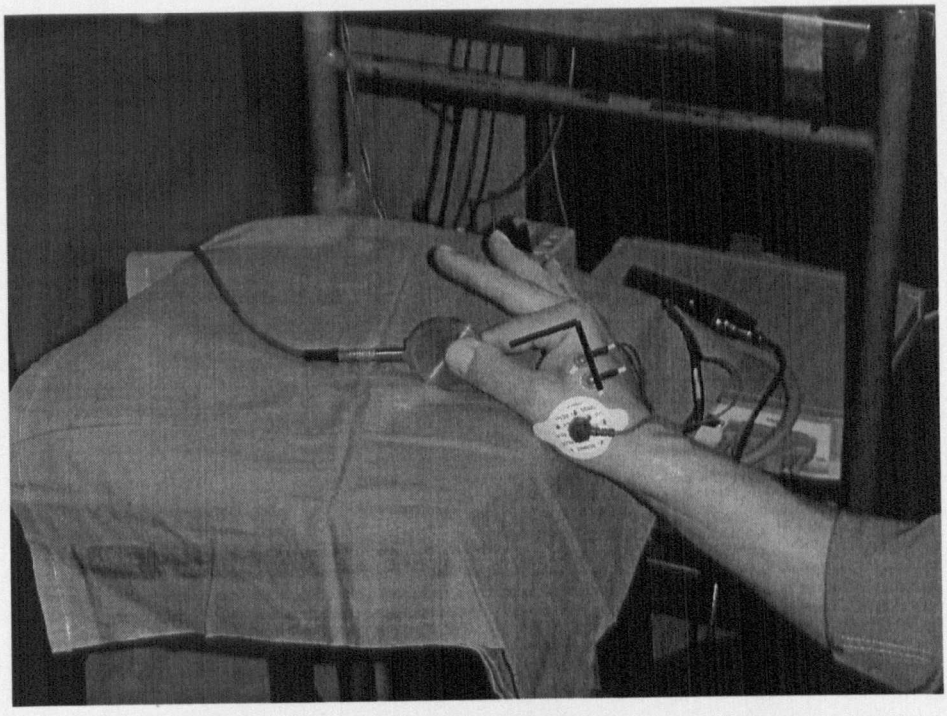


Figure 3.2 shows the set up and position of the hand during the fatigue exercise. The index finger is hold in 90° of flexion at the MPJ and 5°-10° of abduction. The electrodes are positioned along a line perpendicular to the axis of the proximal phalanx and along the fibres of the first dorsal interosseous.

The index finger of the subject was positioned in about 10° of abduction and 90° of flexion while the thumb was positioned in about 30° abduction to oppose the index finger and allow holding a force-recording disc in a key pinch manoeuvre Fig 3.2. A force-recording disc (Biometrics, E link evaluation system, "V800S", Biometrics, UK) with its force-recording surface facing the radial side of the index finger was used to assess the key pinch grip strength. The ulnar three digits were kept away from the index finger to avoid their contribution to the abductor force of the index finger. The force disc was connected to one of the channels in an MP100 machine with a

visual feedback through a PC screen about the level of force generated when the force disc was squeezed.

Subjects were asked to push the force-recording disc using abduction of the index finger against a fixed thumb. At the beginning of the test subjects were asked to generate their maximum pinch force for brief periods of 3 seconds, and this was repeated 3 times with 5 s relaxations in between. The strongest grip force recorded was considered the subject's own maximum voluntary contraction of the first dorsal interosseous "MVC".

Two fatigue protocols were designed, the first consisted of sustained 100% MVC for 120 s. The second consisted of intermittent 100% MVC for periods of 9 s separated by 3 s intervals of relaxation Fig 3.3. The fatigue protocols were continued until the subject was unable to create 50% of his MVC.

Intermittent protocols of fatigue are known to reduce the influence of central factors in the development of fatigue (96). This protocol has also been designed to examine the difference between intermittent and continuous exercises in terms of their ability to induce fatigue.

Attention was paid to the position of the index finger and the test was terminated if the index finger lost its abducted position, or if the subject moved his middle finger trying to support an index finger unable to maintain the abducted position.

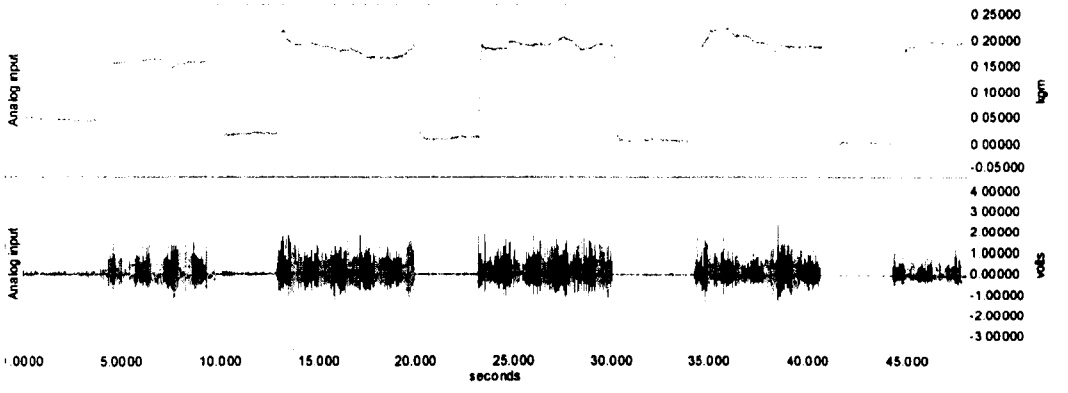


Figure 3.3 shows a sample of the raw data acquired during the intermittent exercise protocol. The top channel is the force channel while EMG was simultaneously acquired on the second channel.

3.3.3- EMG recording

The surface anatomy of the first dorsal interosseous was defined by asking subjects to abduct their index finger against resistance. Surface electrodes were placed along the axis of the muscle. As shown in figure 3.2 while the 2nd. MPJ was flexed to 90° a line was drawn along the radial side of the proximal phalanx of the index finger then a perpendicular line was dropped from the end of this line towards the base of the thumb. Two 5mm diameter Ag-AgCl surface electrodes were placed along the muscle with inter-electrode distance of 5 mm. A third ground electrode was placed over the dorsal surface of the thumb Fig 3.2; an electrically quite area with no muscles directly underneath the skin.

Prior to the attachment of these electrodes the skin surface was shaved and repeatedly cleaned with alcohol. Using sterile blunt ended needles the superficial epidermal layer of the skin underneath the electrodes was scratched to reduce the skin impedance (45). The electrodes were attached to the skin using double-sided adhesive plastic rings and the concavity of the electrodes were filled with conductive gel (Gel 100, BIOPAC Systems, Inc., Santa Barbara, California).

Surface and ground electrodes were connected to an MP100 Biopac EMG acquisition machine (BIOPAC Systems, Inc., Santa Barbara, California) with A/D resolution of 16 bits. Differential input amplifiers were used with a common mode rejection ratio of 100 dB minimum, input impedance 10 Ω, bandwidth 10-4000 Hz and gain 2000. Signals were sampled at 2500 Hz, and bandpass filtered at 10-500 Hz. These set up criteria were based on the general rules for surface EMG recording explained on the previous sections 1.6.1 EMG recording and 2.4.2 EMG acquisition and analysis.

The force was acquired using a force-detection cell (Biometrics, E link evaluation system, "V800S", Biometrics, UK) connected to a second channel of the MP100 machine. At the beginning of each test the force channel was zeroed to eliminate any noise that may alter the absolute force recording. Subjects were allowed a visual

feedback to their force level through the PC screen and were encouraged throughout the test to achieve the required force level.

3.3.4- Calibration of the force recording cell

As the force recording disc connected to the MP100 machine provides force reading in volts it was necessary to calibrate the force recording channel to provide readings in g. Gradually increasing weights starting from 20 g up to 1500 grams were used to calibrate the force cell.

In an excel spreadsheet the recorded volts were plotted against the corresponding weights used, and the best fitting line was inserted using linest function (Excel, Microsoft Office 2000) using the equation *(y = mx + b)*, where the dependent *y* value is a function of the independent *x* value, *m* is the regression coefficient and *b* is a constant fig 3.4. Having calibrated the force recording cell (Biometrics, E link evaluation system, "V800S", Biometrics, UK) the recorded data were converted into Newton (N) where one Newton on the surface of the earth is equal to 101.972 grams (290).

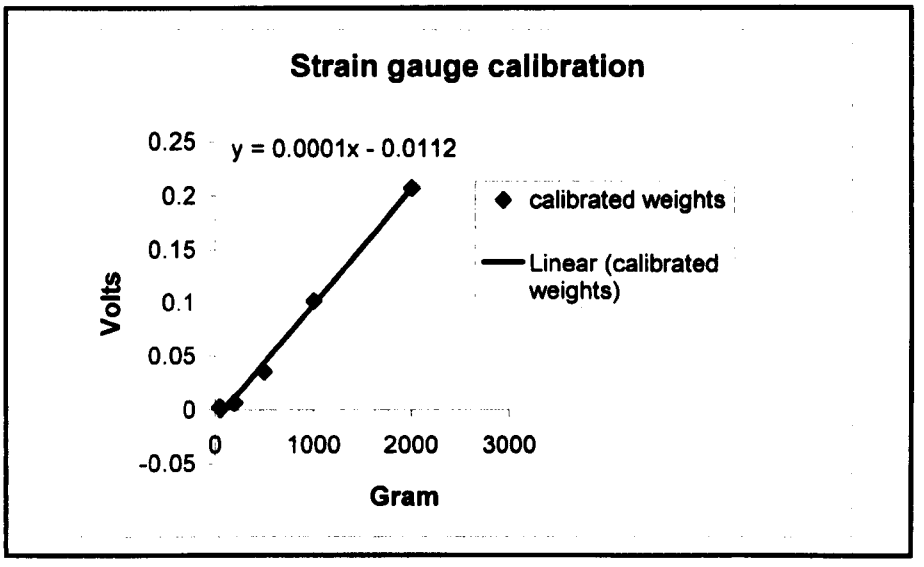


Figure 3.4 shows the process of calibration of the force recording disk. Recorded voltages recorded on the MP 100 machine were plotted against corresponding applied weights. The line that best fits the data was found using the least squares method.

3.3.5- Data analysis

EMG analysis: using Acknowledge-3.5 (BIOPAC Systems, Inc., Santa Barbara, California) the recorded signals were bandpass filtered at 10-500Hz. The signals were then divided into intervals each of 3 seconds duration. The signals on each of these intervals were fast Fourier transformed and the median frequency was calculated. The EMG amplitude was also calculated by smoothing the signals (2000 signals/window) then rectified (400 signals/window). The smoothed rectified signals of the test period was then normalised to the mean EMG amplitude value of the patients own EMG signals of their MVC contraction performed at the beginning of the test. Therefore a relative value or % of the maximum voluntary contraction EMG amplitude was defined for comparison purpose.

Force analysis: the recorded key-pinch grip torque over the exercise period was similarly divided into equal intervals of 3 seconds. The recorded torque was normalised to the mean of 3 seconds MVC at the beginning of exercises to provide relative force or subject's % of his own MVC. Subjects' MVC was converted from volts to kg using the calibration equation to allow comparison of the absolute force. Collected data were then transferred to a spreadsheet for statistical analysis.

3.3.6- Statistical analysis

Data were transferred to excel spreadsheets (Microsoft word 2000) for calculation of rates of change or slopes, expressed relative to the starting values: this was done by linear regression for EMG parameters including median frequency and amplitude as well as for changes in relative force. The linear regression analysis was calculated using *linest* function on the spreadsheet (Excel, Microsoft Word 2000) (see section 2.2.4 for details of calculation of slopes and formulae).

Differences between slopes and MVC were compared using the paired t-test when two sides of the same subject were compared or when different experiments (two set of data) were repeated by the same hand. This selection was justified by the

recommendation given in Bland's book on medical statistics (26) and has been widely used on similar experiments that compared fatigability and force of the two hands in the same subject (304, 320, 360, 361). In comparing the difference between absolute force generated by reinnervated and unaffected hands the non-parametric Wilcoxon's test (54) was added to ensure validity of the results.

The Wilcoxon's test is a non-parametric method for the comparison of a pair of samples whose component data have differences, and makes no assumption about the Normality (Gaussian distribution) of the sample population. The two-sided test uses the null hypothesis that the median of the differences is zero (54). A confidence interval was constructed for the difference between the population medians. A significant level of 95% with $P<0.05$ was taken as statistically significant.

95% CI was calculated for the significant results as previously described (section 2.2.4).

To calculate the endurance (time to a certain fatigue task) of tested muscles the time to decay of force to 75% MVC was calculated and compared between reinnervated and unaffected hands.

To find whether there was a correlation between endurance and the MVC of different hands, Pearson correlation coefficient test was used. The test returns *r* value, which represent the strength of the linear relation between the two variables. The *r* value for the regression line is calculated by the following equation:

$r = n(\sum XY) - (\sum X)(\sum Y) / \sqrt{[n\sum X^2 - (\sum X)^2][n\sum Y^2 - (\sum Y^2)]}$. To produce a P value r^2 was calculated as well as the degrees of freedom (26).

3.4- Results

3.4.1- Control group:

Decline of force was noticed throughout the fatiguing exercise with reduction from 100% MVC to about 20% at the end of the continuous exercise protocol of 120 s fig. 3.5. The slope of force decline or the rate of development of fatigue was not significantly different between dominant and non-dominant hands during the continuous or intermittent fatiguing exercises P = 0.1 and P = 0.4 respectively.

As a general observation for the different fatigue protocols the decline in force was accompanied by a similar decline in EMG power spectrum parameters. MDF has been found to drop gradually and in parallel with force Fig 3.5 & 3.6. The slope of MDF was not different between dominant and non-dominant hands in either of the fatigue protocols with P = 0.7 for the continuous and P = 0.2 for the intermittent fig 3.5 & 3.6.

EMG amplitude was also found to decline with the occurrence of fatigue. In the continuous protocol the dominant hand EMG amplitude slope was – 0.27 ± 0.1 s^{-1}, and the non-dominant hand was – 0.76 ± 0.2 s^{-1} (P = 0.1). With the intermittent protocol comparison of the EMG amplitude between dominant – 0.6 ± 0.6 s^{-1} and the non-dominant hands – 1.1 ± 0.06 s^{-1} showed no difference (P = 0.4).

Though, no difference in fatigability could be demonstrated between dominant and non-dominant hands, fatigue protocols (continuous Vs intermittent) were found to elicit different responses. In the dominant hand the continuous protocol (sustained 100 % MVC) was found to produce lower rate of decline in force with a slope of – 0.55 ± 0.06 compared to the intermittent protocols that produced a much steeper slope of – 1.9 ± 0.2. This difference in force slope was significant (P = 0.006) fig 3.7A. Similar comparison was made in the non-dominant hand. However, the continuous exercise protocol was found to produce less fatigue compared to the intermittent but the difference fell just short of being statistically significant (P = 0.09) fig 3.7A. Data from dominant and non-dominant hands were combined and the continuous was compared against the intermittent protocol. The mean slope of decline in force with the continuous protocol was – 0.65 s^{-1}, while the intermittent protocol produced a

much steeper decline in force with a mean slope of – 1.5 s^{-1}. This difference was statistically significant with a P = 0.005 fig 3.7B.

In agreement with the higher rate of fatigue development with intermittent compared to the continuous protocols, EMG power spectrum MDF was found to be different between the two types of exercises. The slopes of MDF with continuous exercises were – 0.3 ± 0.1 s^{-1} (dominant), - 0.25 ± 0.1 s^{-1} (non-dominant). These slopes were different from those recorded with intermittent protocols of – 2.2 ± 0.2 s^{-1} (dominant) and – 1.2 ± 0.3 s^{-1} (non-dominant). When these slopes were compared using paired t-test the results were significantly different in dominant and non-dominant hands with P = 0.003 and P = 0.01 respectively. These results confirmed a higher rate of development of fatigue with intermittent compared to the continuous protocols of exercises.

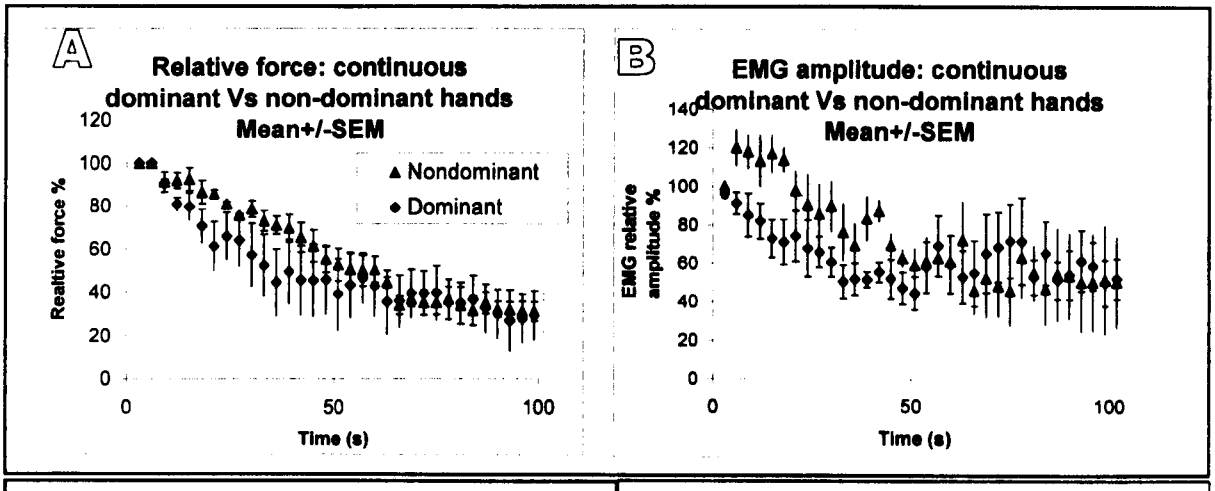
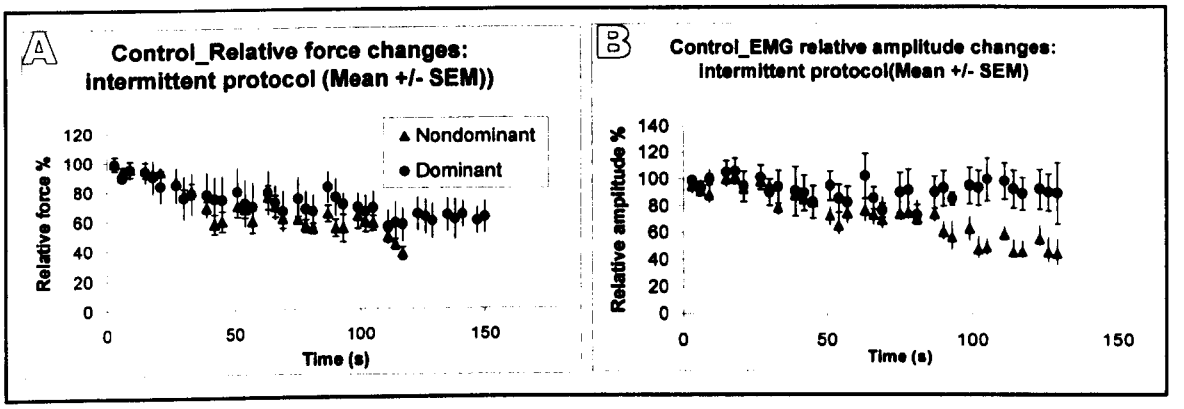


Figure 3.5 shows the results of continuous 100% MVC fatigue exercise in the control group. Data from dominant and non-dominant hands are compared. No significant difference was found. The key in subfigure A applies to the rest of the graph.



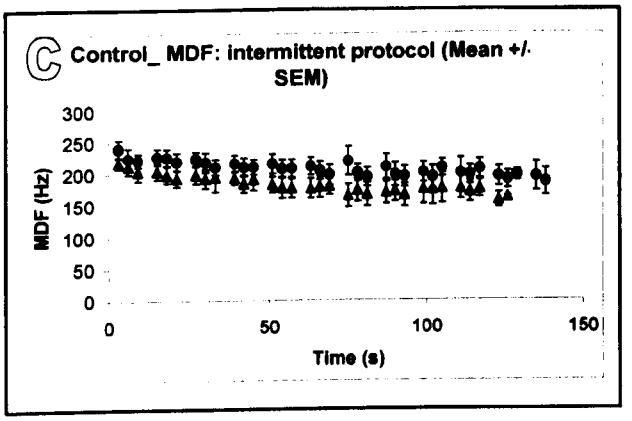


Figure 3.6 presents the data acquired from the intermittent fatigue protocol. The data are presented as (mean ± SEM). Data from dominant and non-dominant hands compared. No significant difference was found (see text for details). The key in (A) applies to the rest of

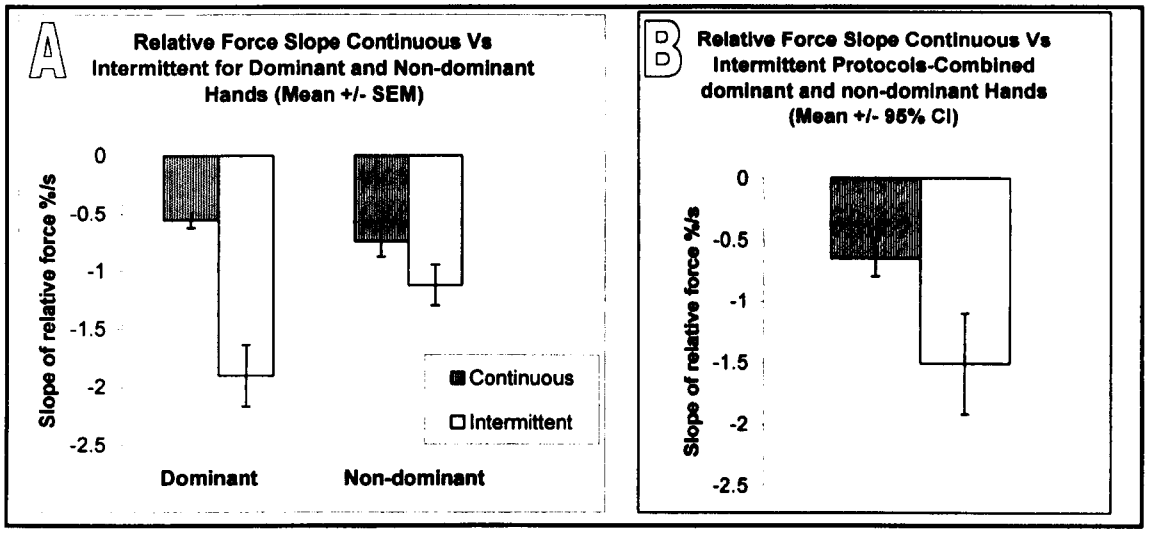


Figure 3.7 A. shows the relative force slope in dominant and non-dominant hands during the continuous and intermittent protocols of exercises. Significant difference was found between the two protocols in dominant hands but not in the non-dominant.

3.7 B. The relative force slopes (data from dominant and non-dominant hands combined) of continuous Vs intermittent protocols. The results were significantly different (P = 0.005).

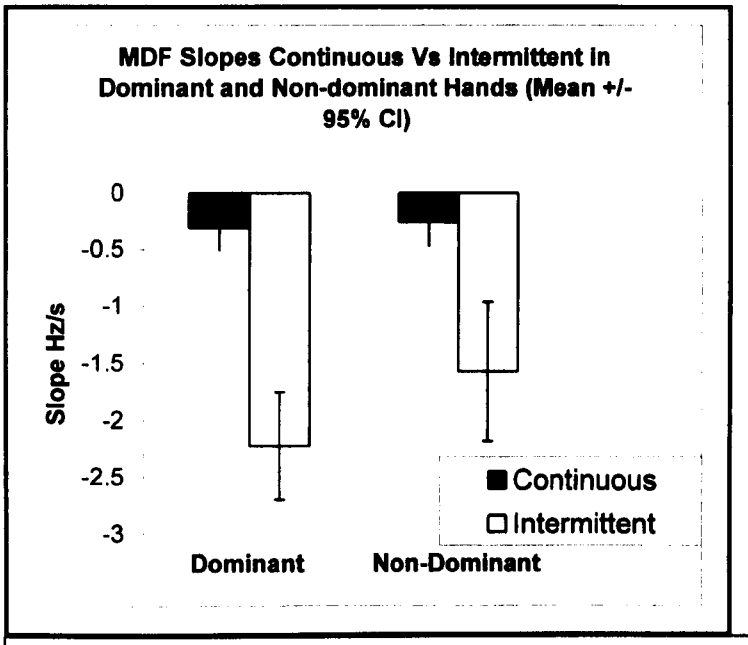


Figure 3.8 presents the MDF slopes in continuous Vs intermittent protocols, data from dominant and non-dominant hands. Significant difference was found between the two protocols. Data are presented as mean ± 95% confidence intervals.

Comparison of strength in dominant and non-dominant hands:

The maximum force that could be exerted by the subjects when they maintained their pinch grip for 3 s was compared. The mean MVC for the dominant hands was 21.58 N, not significantly different from the non-dominant hand with a mean MVC of 23.32 N fig 3.9 and attached table.

Control: Absolute force

	MVC		n
	Mean	SE	
Nondominant	23.32	0.121	4
Dominant	21.58	0.143	4
P	0.383		

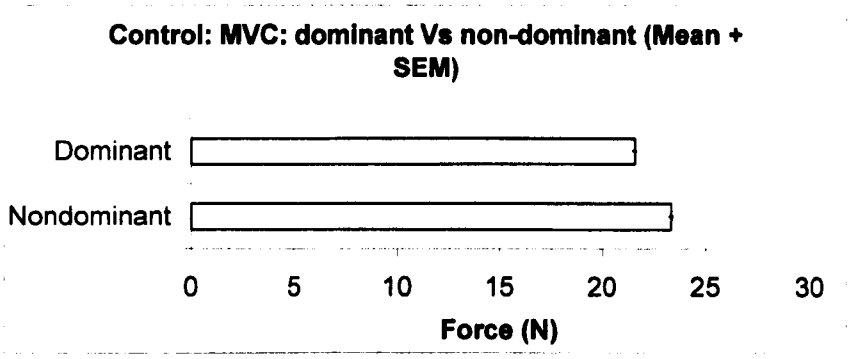


Figure 3.9 presents the absolute force exerted by dominant and non-dominant hands (MVC). No significant difference could be found.

3.4.2- Patients Group:

3.4.2.1- Fatigability of reinnervated Vs unaffected hands.

One of the main aims of this study was to examine fatigability of reinnervated first dorsal interosseous muscles compared to contralateral normal hands. Linear regression analysis of the decline in force during the continuous protocol of muscle exercises was – 0.5 s^{-1} for the unaffected hands, which was not significantly different from – 0.44 s^{-1} for reinnervated hands (P = 0.4). Fig. 3.10 presents the mean of relative force over a 100 s period. It can be seen that at 100 s the relative force had reached ~ 50% MVC. The rates of decline in force were not different between unaffected and reinnervated hands.

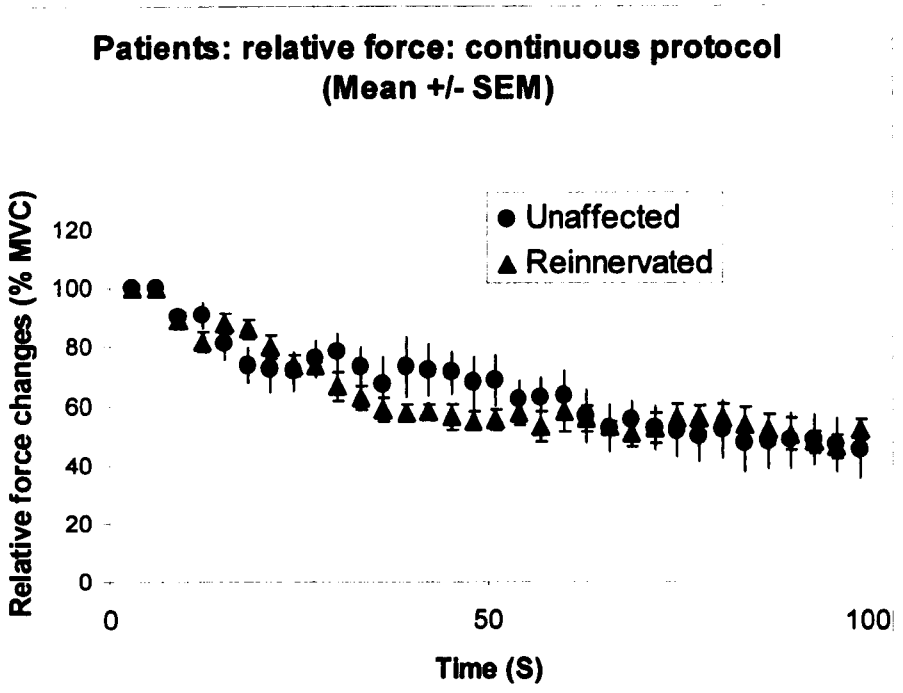


Figure 3.10 shows the decline of force through the continuous exercise protocol. No difference was found between the slopes of force between reinnervated and unaffected hands (P = 0.4). Data are presented as Mean ± SEM.

The intermittent protocol of exercises has also produced a decline in force that was observed in the reinnervated and unaffected hands. These rates of force decline were not different between the reinnervated and unaffected hands – 1.24 s^{-1} for the former and – 1.47 s^{-1} for the latter (P = 0.6) fig 3.11. These results confirmed the lack of evidence for any difference in fatigability between reinnervated and unaffected hands based on mechanical analysis of the decline in force in both fatigue exercise protocols used in this experiment.

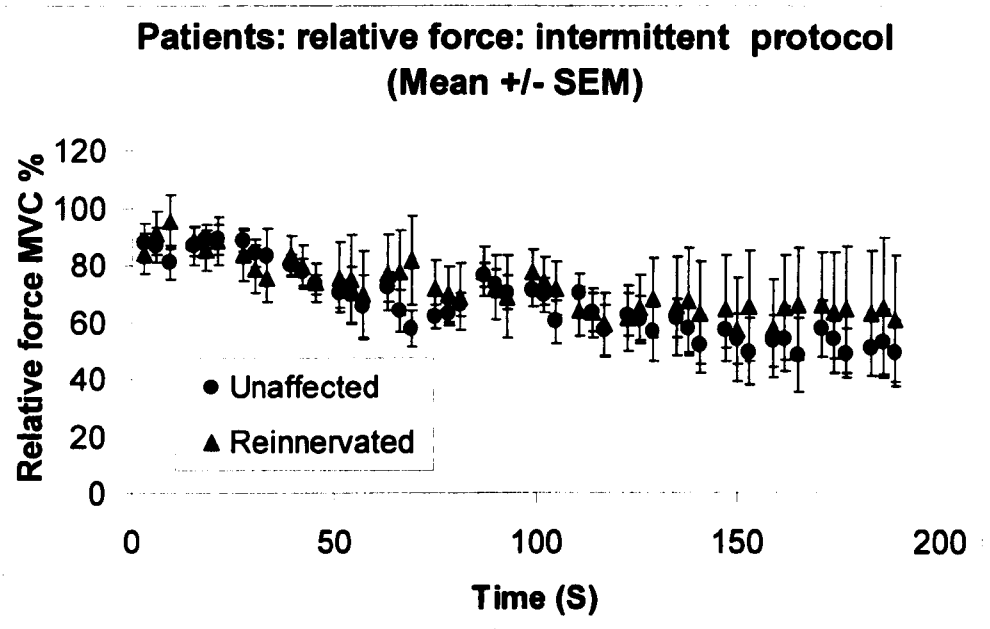


Figure 3.11 presents the changes in relative force during the intermittent protocol of exercises. No difference was found between the slope of force decline in reinnervated and unaffected hands (P = 0.6). Data are presented as mean ± SEM.

EMG parameters were also compared during the different fatigue protocols. During the continuous fatigue protocol EMG amplitude was found to decline. The rate of this decline in reinnervated hands was – 0.22 s^{-1} not significantly different from its rate of decline in unaffected hands - 0.28 s^{-1} (P= 0.6). Similarly, the slope of MDF was not different between the two sides - 0.26 s^{-1} for the reinnervated and – 0.36 s^{-1} for the unaffected hands (P = 0.5) fig 3.12 A & B.

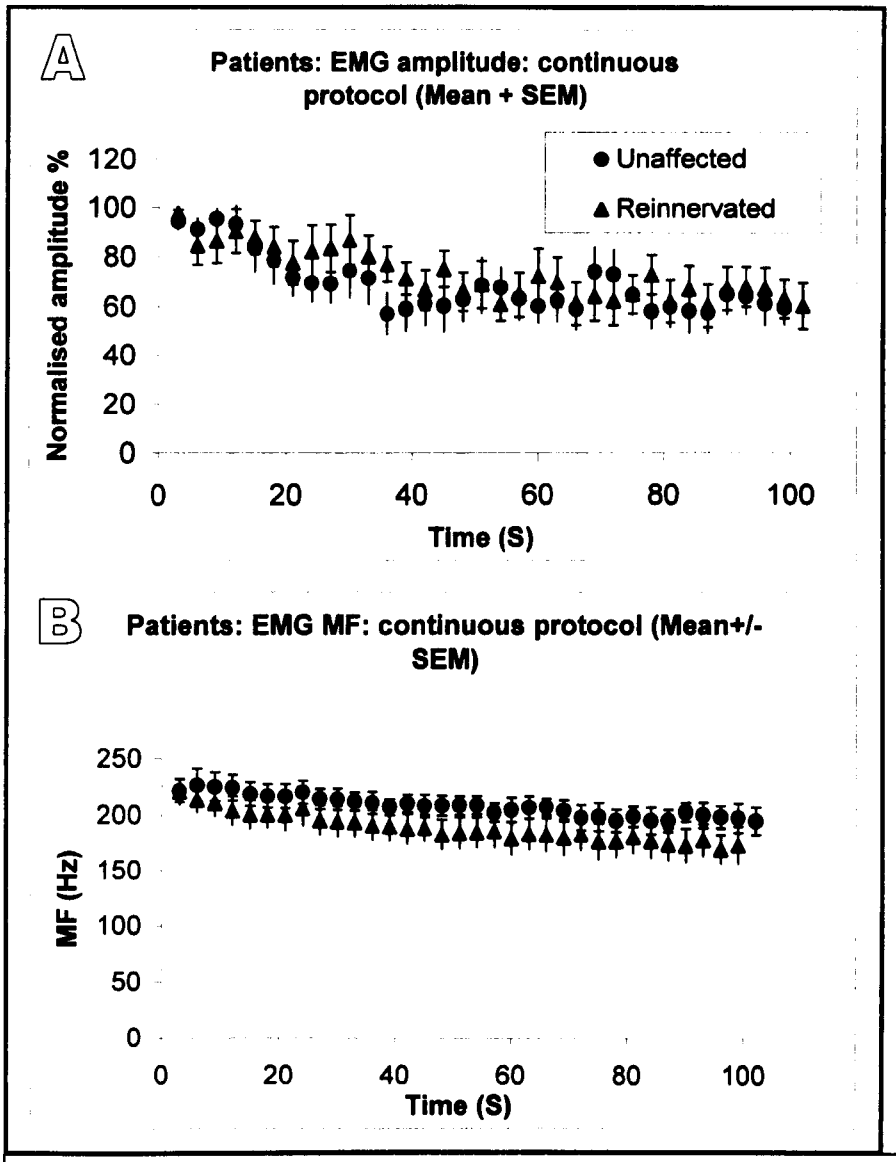


Figure 3.12 presents the myoelectric changes associated with the continuous protocol of exercises. A shows the decline of normalised amplitude (%) against time while B shows the change in MDF (Hz). These changes were not different between reinnervated and unaffected hands (P= 0.6) for the amplitude and (P= 0.5) for the MDF. Data are presented as Mean ± SEM. The legend in A applies to B.

The intermittent protocol revealed no difference in EMG amplitude or MDF slopes between the unaffected and reinnervated hands (P = 0.6) for amplitude and (P= 0.2) for the MDF fig 3.13 A&B.

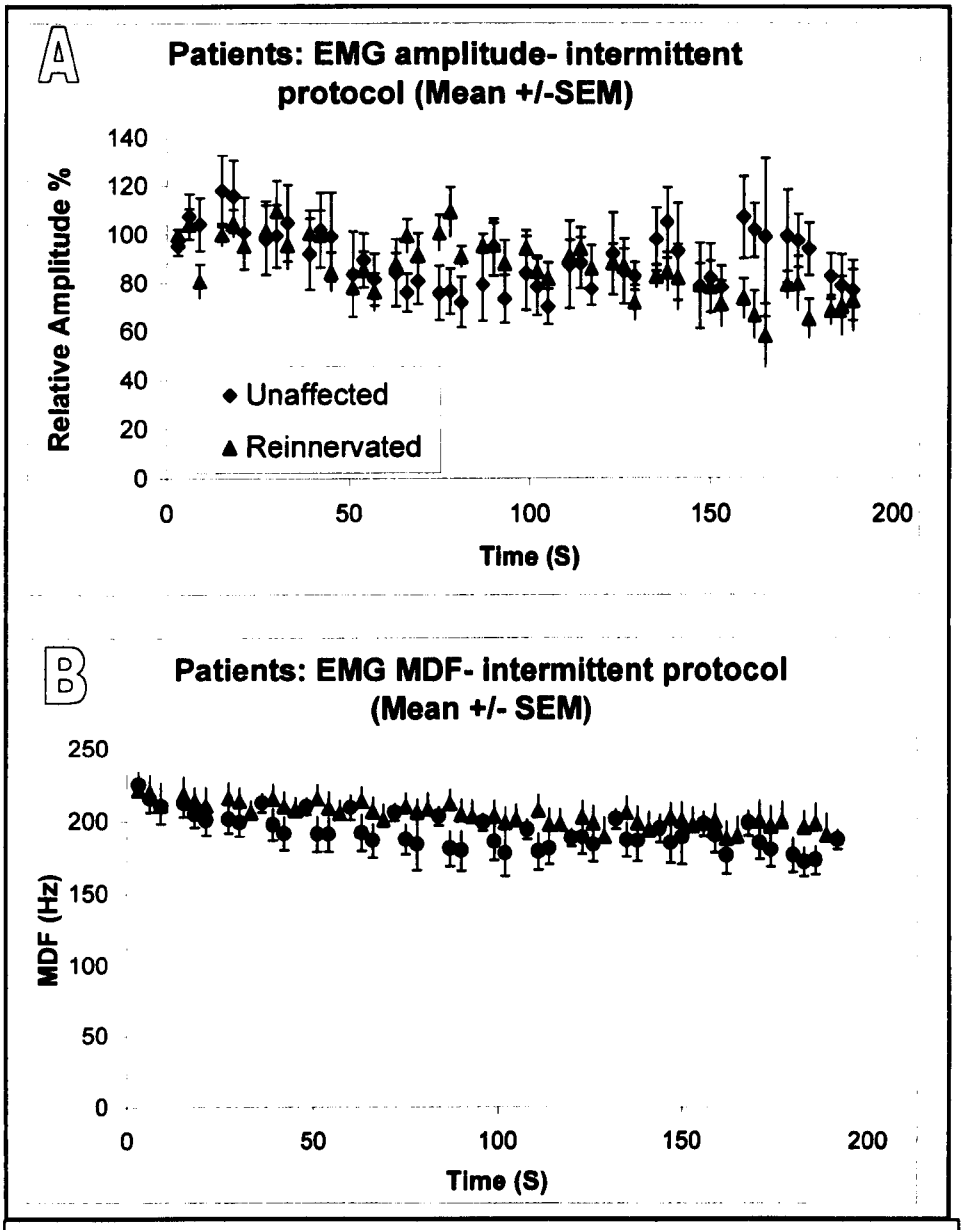


Figure 3.13 presents the EMG changes associated with the intermittent protocol of exercises. (A) shows the relative changes in the normalised amplitude while (B) presents the decline in MDF. No difference could be detected between reinnervated and unaffected hands. The legend in A applies to B. Data are presented as Mean + or – SEM for clarity.

3.4.2.2. Endurance of reinnervated Vs unaffected hands

Endurance or time to reach 75% MVC was compared between reinnervated and unaffected hands. The time to reach 75% MVC was 34.6 ± 12.4 s in unaffected hands 22.3 ± 3.3 s (mean ± SEM) in reinnervated hands. This slight difference was not statistically significant (P = 0.4) fig 3.14.

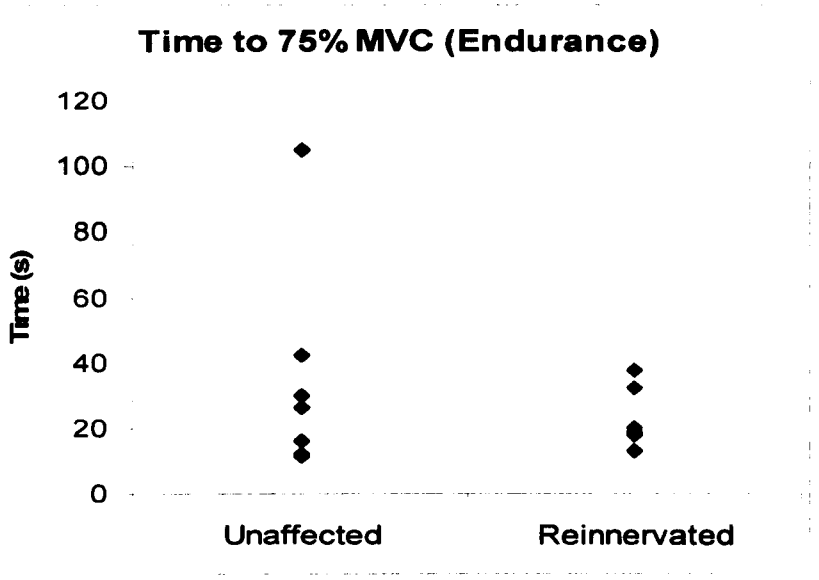


Figure 3.14 presents the time to 75% MVC in individual subjects divided into unaffected and reinnervated groups. No difference was found between the two sides.

To examine whether the muscles' endurance was correlated to their force generating capacity the linear regression and Pearson correlation coefficient were calculated between the hands' 100% MVC and the endurance (represented by the time to drop of force to 75% MVC). On the reinnervated hand r was – 0.2 and $r^2 = 0.028$ indicating a weak correlation (P = 0.6). A non-significant correlation was also found between the magnitude of the MVC and endurance of the unaffected hands with r = - 0.3, $r^2 = 0.13$ (P = 0.4) fig 3.15.

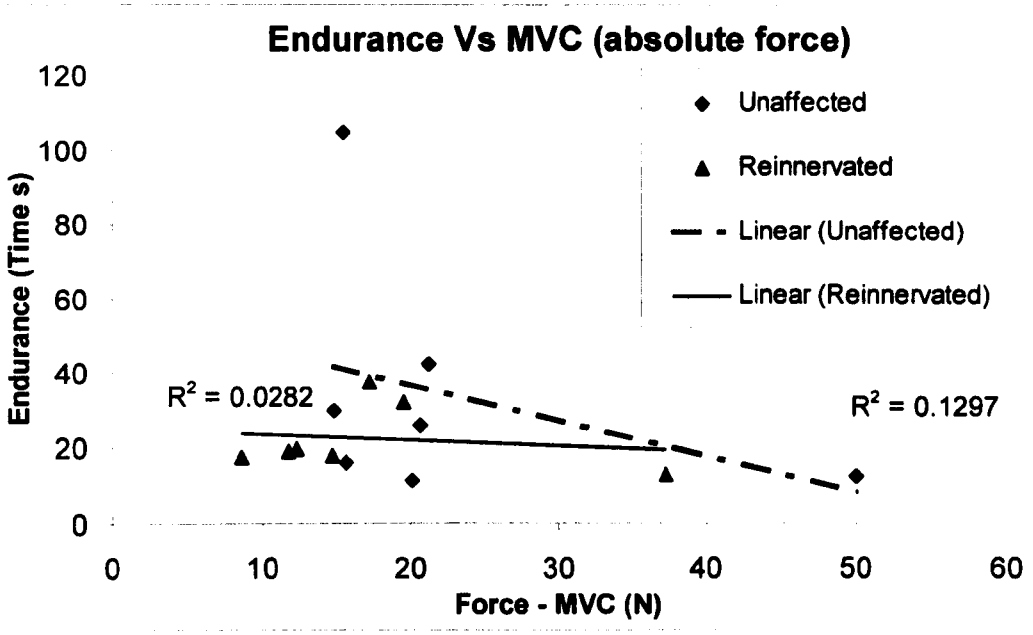


Figure 3.15 show the muscle endurance presented as time taken for the MVC to fall to 75% force plotted against MVC for reinnervated and unaffected hands. The best fitting line representing the correlation between these two variables is drawn. R^2 for the unaffected hands lies on the right of the graph and the R^2 for the reinnervated hands to the left.

3.4.2.3 comparing MVC (absolute force) of reinnervated to unaffected hands

At the beginning of these experiments subjects were asked to exert their MVC for brief periods of three seconds. The test was repeated three times with 5 seconds relaxation intervals in between. The highest level of force that was reached in these trials was considered the subjects MVC.

The MVC of reinnervated hands was 17.37 ± 3 N, lower than the MVC of unaffected hands 22.59 ± 4.8 (Mean ± SEM) fig 3.16. Using paired t-test this difference was found to be significantly different (P= 0.03). The 95% CI interval was calculated for the means of the MVCs and was 5.877 N for the reinnervated hands and 7.681 N for the unaffected hands (fig 3.17). Fig. 3.18 shows the mean difference between the unaffected and reinnervated hands and the 95% CI.

Using non-parametric analysis, the Wilcoxon's test revealed a significant difference between the MVCs of the unaffected and reinnervated hands (P = 0.02). The 95.3% CI for the difference between population medians was 1.6 to 12 N. These results provided further confirmation that reinnervated hands were significantly weaker than the unaffected.

MVC of the reinnervated and unaffected hands (Absolute Force)

	Mean	SEM	STD	95% CI	Number
Unaffected	22.59	4.785	12.4	7.6813	7
Reinnervated	17.37	3.075	9.5	5.877	7
P	0.032				

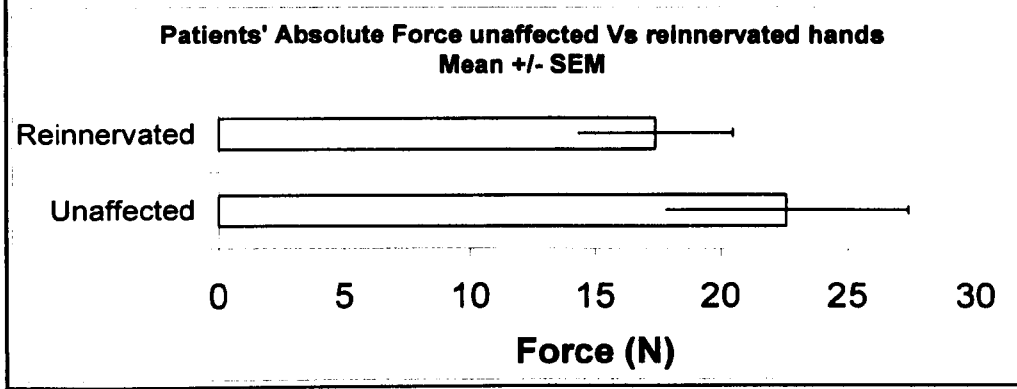


Figure 3.16 and attached table shows the difference in MVC (absolute force) generated by reinnervated and unaffected hands. Unaffected hands produced a higher level of force and the result was statistically significant P = 0.03.

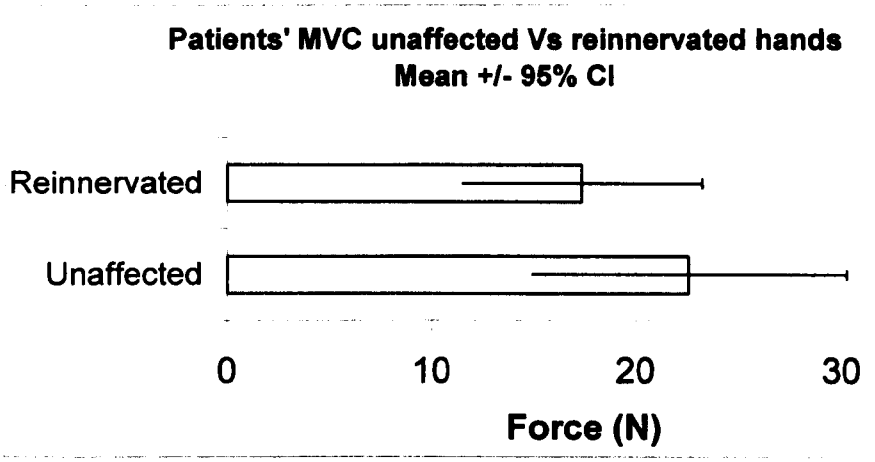


Figure 3.17 shows the mean MVCs generated by the reinnervated and unaffected hands. Data presented as Mean ± 95% CI. Although there was an overlap of the 95% CI the results were significant.

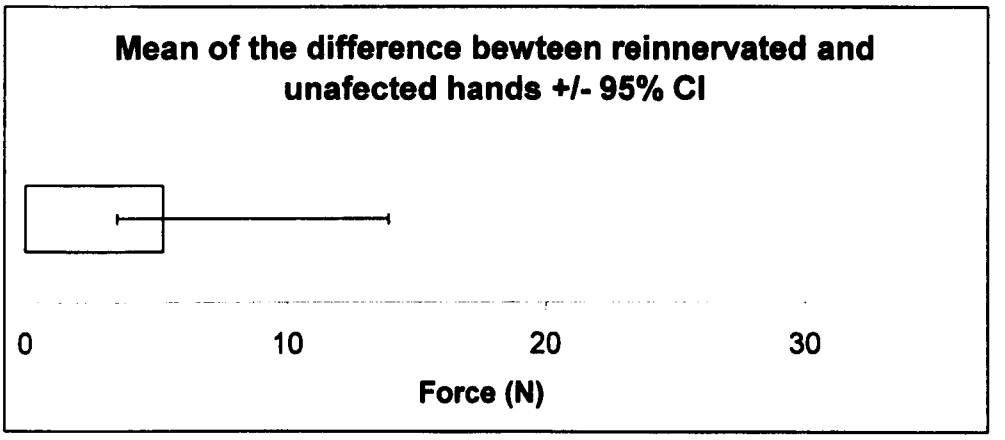


Figure 3.18 shows the mean difference in maximum strength of the first dorsal interosseous between unaffected and reinnervated hands ± 95% CI of the difference.

3.4.2.4 Continuous Vs intermittent fatigue protocols

The ability of different fatigue protocols on inducing fatigue on reinnervated and unaffected hands has been examined. For the purpose of this comparison the slopes of changes in force, MDF and EMG amplitude were calculated from start to 120 s in both protocols. Therefore, the fatigue protocols were comparable in level of force (100% MVC), mode of contraction (isometric) and duration and only different in the method of performing the task. The continuous protocol was in form of sustained MVC while the intermittent was alternating contractions and relaxations.

For the relative force the intermittent protocol has produced higher rates of decline in force decline compared to the continuous. This effect was observed in both reinnervated and unaffected hands table 3.2. EMG amplitude was also found to be reduced at higher rates during the intermittent compared to the continuous fatigue protocol. This effect was noted in reinnervated and unaffected hands.

For the MDF, significantly higher rate of decline was observed during the intermittent protocol compared to the continuous when unaffected hands were examined. Comparing the Slopes of decline in MDF between the intermittent and continuous protocols of reinnervated hands showed no difference (P = 0.6) Table 3.2.

Comparison between slopes of force and EMG with continuous and intermittent protocols

Patients' study	Continuous Protocol				Intermittent Protocol				
	Mean	SEM	STD	95% CI	Mean	SEM	STD	95% CI	P
Relative force									
Unaffected	-0.496	0.1951	0.07	0.135	-1.47	0.84	0.32	0.58	0.038
Reinnervated	-0.4	0.1333	0.05	0.087	-1.237	1.13	0.43	0.78	0.046
Median Frequency									
Unaffected	-0.36	0.2747	0.104	0.19	-1.94	0.53	0.2	0.368	0.016
Reinnervated	-0.26	0.2758	0.104	0.171	-0.892	1.95	0.73	1.352	0.61
EMG Normalised Amplitude									
Unaffected	-0.28	0.14	0.053	0.105	-0.68	0.37	0.13	0.25	0.031
Reinnervated	-0.22	0.37	0.138	0.227	-0.916	1.1	0.42	0.76	0.034

Table 3.2 provides summary of the rates of decline (slopes) of force, MDF and EMG amplitude in different fatigue protocols. Paired t-test was used to compare the different protocols.

3.5- Discussion

Control group results

In the healthy group of volunteers data from dominant and non-dominant hands were compared. No difference could be found between the dominant and non-dominant hands in their mechanical or myoelectric manifestations of fatigue. The fall in force against the time domain was accompanied by a similar fall in MDF and EMG amplitude.

In this study experimental protocols were designed to use 100% MVC and the decline in force was therefore accompanied by a decline in EMG amplitude. EMG amplitude declined by ~ 40% of its starting value in the intermittent and continuous protocols and the rate of change in amplitude was not different between the dominant and non-dominant hands.

Zijdwinde et al observed a similar decline in EMG (M-wave) by ~ 30% when the first dorsal interosseous was stimulated by electric impulses through the ulnar nerve (30 Hz stimulation, 0.33 s bursts, ten pulses per burst). In his study the magnitude of drop in M-wave was greater in the non-dominant compared to the dominant hands. However, Zijdwinde et al have compared the means of the actual EMG amplitude value, which was found to drop from 85 ± 11 at the beginning of the test to 73 ± 18 after the fatigue protocol in the dominant hand. In the non-dominant hand the M-wave amplitude was lower in the non-fatigued muscles (70 ± 15) and has dropped to 65 ± 13 at the end of the fatigue test (360).

The experiments presented in this chapter showed a difference between the slope of EMG amplitude of the non-dominant (- 0.27 ± 0.1 s^{-1}) and dominant (- 0.76 ± 0.2 s) hands. However, this difference was not significant (P = 0.1). What is more important is that Zijdewind et al could not find a difference in fatigability between the dominant and non-dominant hands as confirmed by the force level generated in the 1stDI as well as the ability of these muscles to recover their normal force generating capacity within a similar period of 14 minutes. In agreement with these previously published results, experiments presented in this chapter showed no difference in fatigability between dominant and non-dominant hands.

Comparing the MVC or the absolute force generated by dominant and non-dominant hands showed no difference (P = 0.3) fig 3.9. These results were similar to previously published results by Tanaka et al in 1984 when they examined electrically evoked and voluntary contractile properties of the first dorsal interosseous muscle on both hands in ten healthy adults. Maximal tetanic tension, maximal voluntary contraction strength, and maximal twitch tension were not significantly different (320).

Two fatigue protocols have been used in these experiments that had a similar level of force (100% MVC) but differed in their contraction period. The first protocol was a sustained MVC or continuous and the second consisted of intermittent short contractions. Data collected from each hand were compared separately and revealed significant differences between the rates of decline in MDF that were elicited by the different protocols on each side independent of the other fig 3.8. Comparing the rates of force decline that developed with the different protocols revealed a significant difference in the dominant side fig 3.7A. The difference did not reach statistical significant on the non-dominant side. When data from both hands were calculated as one group statistical significance was found between the rates of force drop with the intermittent protocol producing higher level of fatigability compared to the continuous

This finding agreed with other reports that compared the level of fatigue induced by repeated short periods of contractions compared to long contraction periods. Hultman et al stimulated the ischaemic quadriceps muscles at frequency of 20 Hz and intensity sufficient to elicit 25% MVC. The stimulation was either applied continuously for 52 s or intermittently with stimulation–rest durations of 0.8:0.8 s, 1.6:1.6 s or 3.2:3.2 s for 54 s of stimulations. The decline in force was least for the continuous protocol of stimulation and greatest for the intermittent protocol with alternate 0.8:0.8 s of stimulation and relaxation. Biopsies from the quadriceps muscles investigated in the Hultman's experiment revealed increased utilisation of ATP in the muscles stimulated with the intermittent protocol compared to the continuous. The difference observed in the fatigability induced by the two protocols was, therefore, attributed to higher rate of utilisation of ATP with the intermittent one (152). 40% of the energy expenditure in muscle contraction is spent in the development and relaxation process indicating that continuous contractions are more economic in terms of energy cost (20). Hogan et al (1998) demonstrated that repeated short duration contractions could induce

greater degree of force decline compared to sustained contractions of longer duration (144).

The experiments presented in this chapter showed a clear difference between the intermittent and continuous exercises. Intermittent exercises produced higher rate of fatigue development. This can be explained by the higher rate of ATP utilisation with intermittent compared to the sustained contractions. The rate of cross bridge formation is lower during the steady state of contraction than during its rising phase (96). In addition to the energy cost of cross bridge cycling, Ca^{2+} pump at the SR is known to consume a significant amount of energy (~40% of total energy consumption) (20). Although it is known that the energy cost of the Ca^{2+} cycling represents a significant portion of the total energy cost of muscle contractions and may explain our present results, it is also possible that the higher energy cost related to the time for the cross-bridge cycling to achieve full tetanic force was a contributing factor (96). During continuous contractions, this higher actomyosin ATPase demand at the onset of a single contraction diminishes but the magnitude of this fall with repeated contractions is unknown (144).

Another factor that would contribute to the observed changes between intermittent and sustained exercise is the accumulation of H^+. Lowered pH is known to reduce the number of attached cross bridges as well as the magnitude of force that develops from each attachment (89). Moreover, the increased H^+ concentration reduces muscle membrane conduction velocity, which accounts for the higher rate of decline in EMG power spectrum MDF (64). Other products of ATP hydrolysis like the P_i can modulate the cross-bridge behaviour. An increase in P_i reduces the maximum isometric force (55).

The intermittent protocol of contraction was also found to produce higher rates of decline in EMG amplitude. This indicates a greater rate of decline in motor unit discharge rate. Accumulation of metabolites particularly lactates and H^+ can initiate local reflexes through group III and IV muscle afferents with a consequent reduction of motoneuron discharge (116). As intermittent protocols were associated with higher rate of ATP turnover and consequently higher rate of lactate and H+ accumulation, group III and IV muscle afferents are likely to have been stimulated earlier and

possibly at higher rates than during the sustained contraction. This would initiate a reflex reduction in motoneuron discharge rate that would be observed as a higher rate of decline in EMG amplitude.

Patient group

In an earlier experiment (chapter II), fatigability of the reinnervated serratus anterior muscles could not be demonstrated when myoelectric manifestations of fatigue were compared between reinnervated muscles and a group of healthy controls. Smaller degree of increase in EMG amplitude was observed in reinnervated serratus anterior muscles compared to control. This difference in EMG amplitude was attributed to the lack of strong fast fatigue motor units in reinnervated muscles, and/or the disruption of motor unit order of recruitment. Mechanical fatigue of the serratus anterior could not be measured because of the unique anatomy of this muscle that made it impractical to separate its function or record its torque. In this study it was possible to record both mechanical and myoelectric fatigue of the first dorsal interosseous muscle.

Comparison of mechanical manifestations of fatigue both the rate of decline in force and endurance were not different between reinnervated and healthy unaffected hands. The lack of difference was present during intermittent and continuous protocols of exercises. Analysis of the myoelectric manifestations of fatigue, MDF and amplitude, also showed no difference between the reinnervated and healthy unaffected hands. When the muscles' force generating capacity represented by the absolute MVC was compared reinnervated muscles were found to produce less force (17.4 N ± 3) compared to control (22.5 N ± 4). This difference indicated that reinnervated hands were weaker but there was no evidence for being more fatigable fig 3.10 to 3.14.

The above results supported previous findings from animal and human experiments that demonstrated lower force generating ability of reinnervated muscles compared to control. Finkelstein et al (1993) investigated the effect of different periods of denervation and the effect of self (original nerve) or cross (foreign nerve) reinnervation on the medial gastrocnemius muscles of the rat. The authors found the reinnervated muscles' maximum tetanic force to be 50% less than normal after eight weeks of recovery. following repetitive stimulation, the fatigue index of normal muscles was 0.58 ± 0.28 while that of reinnervated muscles was 0.92 ± 0.45 which

was not significantly different (103). Chammas et al (1997) examined the strength and fatigue characteristics of the biceps and brachialis muscles reinnervated by a microsurgical nerve transfer following brachial plexus injury. Ten subjects were studied and compared to the contralateral unaffected sides using the Wilcoxon's rank test for statistical analysis. The maximum isometric force of the reinnervated muscles (49 N ± 19) was significantly lower than that of the contralateral healthy side (215 N ± 59). When the fatigue index of reinnervated muscles (35 ± 14) was compared with that of the contralateral side (32 ± 18) no significant result was found with $P = 0.3$.

Fatigability of reinnervated muscles has been a controversial subject for decades. Disuse and denervation have been found to change fibre type proportion of the affected muscles (159, 315). Chronically paralysed muscles have been shown to have a higher degree of fatigability compared to normal or acutely paralysed muscles (305). However, this conversion process has been usually found to be incomplete and largely dependent on the severity of nerve injury and characteristics of the reinnervating motor neuron (198, 283). In case of reinnervated first dorsal interosseous muscles following ulnar nerve compression it is unlikely that cross reinnervation had occurred. The more likely process to happen is the sprouting of motor axons to innervate surrounding denervated motor units. Motor axons that supply slow MUs were found to have a higher potential for sprouting compared to those supplying fast fatigue units (122). This would increase the chance of the reinnervated motor units to be of the slow, fatigue resistant type. Therefore even if the denervation process has induced some changes to the muscle fibre type with a resultant increase in their fatigability, through muscle fibre conversion, this effect may have been neutralised by the likelihood of the slow motoneurons to sprout and increase their innervation ratio. It would be logical to find no difference in fatigability between the reinnervated and unaffected first dorsal interosseous muscles.

Muscle wasting observed with denervation is the result of lack of stimulus from the motor axons as well as disuse. This muscle wasting has been expressed as a reduction in the muscle fibres cross sectional area. A correlation was drawn between the muscle's cross sectional area and its force generating capacity (19, 331). The group of patients examined in these experiments suffered from ulnar nerve palsy that was treated by surgical decompression. Wasting of the 1st DI was clinically observed in

some of these patients twenty months following surgery fig 3.19. Clinical assessment (MRC system) of the 1st DI power revealed an average grade IV. Deficient function of reinnervated muscles can at least partially explained by their weakness.

The modulation in motor unit discharge is dependent on the intensity and duration of the fatiguing contractions (40). Fatigue exercises that use maximum voluntary contractions are associated with a decline in EMG amplitude. In this study EMG amplitude was found to decline in normal and reinnervated hands. The first dorsal interosseous motor units are known to be fully recruited at force levels closer to 40% MVC (65), therefore in this study with the use of 100%MVC the motor units had been fully recruited at the beginning of the test with a subsequent reduction in the motor unit discharge rate as fatigue developed (271). Reduction of EMG amplitude during fatigue can be explained either by a total reduction in the motoneuronal activity arriving at the muscle "central fatigue"; or a reduction in the sarcolemmal action potential (362, 363).

Similar to the results from the control group of healthy subjects, the findings from the patients' group demonstrated a higher degree of fatigability to be induced by the intermittent protocol of exercises table 3.2. The difference between the two protocols of exercises was evident in the unaffected and reinnervated hands. The fact that reinnervated hands responded to the intermittent and continuous protocols of exercises differently, similar to the normal hands, adds an additional strength to the argument that fatigability of reinnervated hand was not different from normal.

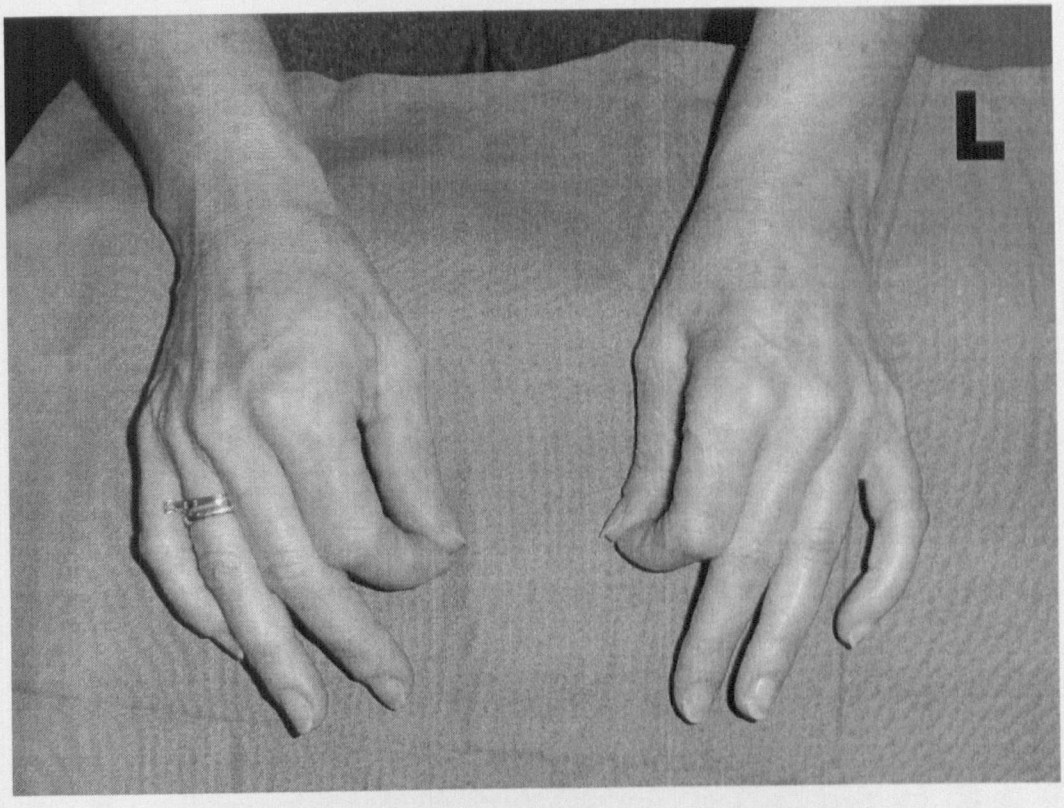


Figure 3.19 shows the hands of a 54 years old left handed female who suffered numbness, tingling and wasting of ulnar innervated muscles in her left hand for 12 months before having decompression of the ulnar nerve at the elbow level. These photographs were taken 8 months after the operation. Despite the subjective feeling of improvement in her sensory symptoms, the first dorsal interossous muscle remained wasted and weaker compared to the right side.

Recovery of reinnervated muscles was found to be affected by the denervation period. The longer the period of denervation, the less favourable would be the outcome in terms of regaining the muscle mass and force (103). With ulnar nerve entrapment, prognosis has been correlated to the preoperative findings. Recovery was found to be good for those presented with intermittent sensory symptoms, variable when there is persistent hyposthesia and some muscle wasting. Recovery was usually incomplete when the first dorsal interosseous was wasted and weak (278, 342). The patients included in this study have originally presented with persistent sensory symptoms and would fit under the second or moderate degree of nerve compression. The clinical

assessment of these patients seems to be reflecting the picture following ulnar nerve decompression. At the time of assessment (twenty months post surgery) sensory evaluation with 2PD test did not show a difference between reinnervated and unaffected hands ($P>0.05$). Using the Wilcoxon's rank test, motor MRC grading was significantly different between the two groups ($P = 0.03$), 95% CI – 1.0 to – 0.5. Patients with noticeable wasting of their first dorsal interosseous were those who suffered longer periods of denervation before coming to surgery and despite 20 months of recovery period there was a failure to regain muscle power (case example fig. 3.19).

In this study the clinically applicable key pinch grip was used instead of pure first dorsal interosseous abduction force in the testing protocol. However, great attention was paid to keeping the index finger in 5°-10° of abduction throughout the test period and the test was abandoned or terminated if the index finger failed to maintain its position to insure loading of the first dorsal interosseous muscle.

3.6- Conclusion:

In these experiments two protocols of muscle testing an intermittent (9 s contraction-3 s rest) and a sustained MVC were used to test two groups of subjects. No difference could be demonstrated between dominant and non-dominant hands in strength or fatigability. Intermittent protocol of exercises has been demonstrated to induce a higher degree of fatigability compared to the continuous.

In this study the hypothesis that reinnervated first dorsal interosseous muscles would be more fatigable than the unaffected contralateral side was tested. The results have not demonstrated any difference between reinnervated and normal hands in terms of mechanical and myoelectric manifestation of fatigue or endurance. However, reinnervated muscles were found to be significantly weaker than normal.

Chapter IV: Effect of cutaneous anaesthesia on hand sensory- motor performance

4.1- Introduction

4.1.1- Effect of denervation on cutaneous sensation

Peripheral nerve injury gives rise to varying degrees of nutritional and circulatory disturbances in the denervated skin and subcutaneous tissues. The pathophysiology of these changes is not just atrophic in nature but is related to the interference with sensory and sympathetic mechanisms (317).

Denervation leads to histological changes that affect the different types of sensory receptors. The outlines of denervated dermal plexus and fine nerve terminals, however, persist as trains of sheath and can be utilised by regenerating nerve axons for up to a year. Other encapsulated receptors like the Pacinian and Meissner's corpuscles and Merkel cells can survive into the 2nd year waiting for regenerating axons (181, 354). It has been suggest that the quality of sensory recovery following the reinnervation process is dependent more on the severity of nerve injury and the ability of regenerating axons to reach their target receptors rather than on the fate of denervated sensory end organs (112, 317).

Quality of cutaneous sensation is usually expressed by the subject's ability to detect different stimuli that would assess the different sensory modalities of touch, pain, and temperature in addition to other tests like two point discrimination that determines the innervation density and pressure threshold. These neurological tests, though valuable for communication in the medical field, have limited value when the actual Sensory-Motor function of the hand is questioned (171). As neurological tests would provide information about the threshold necessary for a stimulus to be detected this may not reflect the quality of the sensory function. It was Walshe who wrote in 1948 that "Although graduated punctate stimuli may be necessary in the investigation of sensory function, yet such stimuli are not physiological in the sense that the surface of the normal organism under normal conditions does not receive single stimuli of this nature, but more widely distributed and qualitatively multiple stimulations, often affecting simultaneously end organs of diverse kinds" (339) review (317).

Functional evaluation of the hand that relies on evaluating the overall sensori-motor performance has been introduced since 1930's. This form of hand evaluation employs more objective assessment methods and usually involves motor tasks that depend on finger manipulations and tactile sensation. Various forms of the functional hand evaluation tests have been developed over the years, for example Moberg's test (244) and the Mayo Dexterity Test (72). In Moberg's test subjects are asked to collect and identify common objects like (safety pins, paper clips, coins etc.) from the surface of a table without visual feedback then place them in a box. In the Mayo dexterity test different sizes of squares, cylinders, metal pins and collars are screwed into place while visual feedback is permitted (72). One common limitation of these tests is that they rely on testing the speed of the patient's performance rather than the quality of the task being performed (170).

Cutaneous afferent input plays an important role in coordinating the motor function of the hand; it is therefore not uncommon for patients with sensory loss to present with motor deficiencies as their main complaint (170). Westling and Johansson have confirmed the role of afferent input from the glabrous skin of the index and thumb in adjusting the static force necessary to hold a slippery object stationary in a pinch grip (348). More recently McNulty et al found excitatory and inhibitory spinal reflex responses acting on an ongoing EMG recorded from muscles of the thumb and index fingers by electrically stimulating cutaneous receptors (227, 228). A deafferented patient was found to lack the ability to maintain a specific level of force or to perform fine hand manipulations (292). Meanwhile, little is known about the coordinated function of skin and muscle sensation and the integration of these functions into the final skilled hand performances (166).

Lack of smoothness in hand functions following nerve injury can easily be differentiated from the effortless skilled performance of the normal hand. What may be needed are tests that concentrate on measuring the quality of hand movements and how they are accomplished; and instruments that evaluate how a hand produces these movements in terms of the fine movements, force generated and positions adopted. In this chapter the results of two experiments that were designed to test the influence of cutaneous sensory loss on the motor function of the hand will be presented.

4.1.2- Handwriting as a tool for investigating hand function

Specimens of handwriting and Archimedes spirals have been used in the evaluation of patients with various types of pathological tremors. Before the recent advances achieved in electronic technology, quantification of handwriting data was limited to the subjective assessment of clarity and smoothness (191). Accelerometers were the most popular methods of assessment of finger movements and tremors (124). However, these accelerometers, that usually work in one dimension, were limited and provided only partial assessment on movements that should be considered three dimensional (X,Y & Z), in addition to the rotational movements around the instantaneous axis of rotation (352).

Elble et al (1990) have presented their experience with the use of a digitising tablet for recording handwriting on an IBM PC and analysis of these trajectories using a purpose-designed software. The authors used this method for quantification of tremors and achieved good advances in the objective assessment of both amplitude and frequency of tremors. Digitised handwriting has been used to study fine motor control of various neurological disorders (140, 183, 210, 222, 250, 307). Mavrogiorgou et al have compared the hand motor performance of patients with obsessive-compulsive neurosis to normal controls using digitised handwriting techniques. Severity of the psychological disorder was correlated to the poorer performance in handwriting task (222). Smits-Engelsman et al (2001) have investigated a group of pupils who were considered slow learners and correlated their slow learning and poor handwriting to a fine motor control disturbance (307). Teulings et al have compared handwriting strokes between a group of patients with Parkinsonism and a second group of normal controls. Parkinsonism patients were found to have less coordination of their finger, wrist and arm movements that was reflected on the degree and magnitude of tremors that quantified in their writing strokes (324).

The handwriting movements in space were originally assumed to be homogeneous or identical in all directions. However, several investigators have described the handwriting apparatus as consisting of two main axes, a fast main axis and slow main

axis. Teulings et al have suggested that the direction of the fast main axis can be achieved mainly by wrist movements.

Movements parallel to the slow main axis are produced by simultaneously flexing and extending the index finger and thumb (325). It is generally thought that thumb and finger flexion and extension movements are responsible in producing the up and down strokes i.e away and towards the body; while wrist flexion-extension and radial-ulnar deviations generates the small right and left movements. Gross right and left movements usually arises from forearm movements (324).

4.1.2.1- Principle of recording handwriting movements

Beneath the writing surface of most tablets is a fine wire grid comprised of evenly spaced horizontal and vertical wires. A stylus "pen" or puck emits an electromagnetic field that excites neighbouring horizontal and vertical wires. The tablet computes the X and Y coordinates of the stylus by determining the vertical and horizontal wires that were most excited by the movement (94). Tablets differ in their speed of data transmission, resolution and sampling frequency. Handwriting and drawing have been reported to have a frequency content of 0-6 Hz, and pathological hand tremor of less than 20 Hz (93).

4.1.2.2- Analysis of hand-writing

Handwriting can be analysed in 2 ways: the first, is through global analysis of the written material in terms of average frequency spectrum, direction of movement and the running angles. The second method is by splitting the written material into strokes. These strokes can then be analysed in regard to various parameters like duration, length, detour (indirectness) and jerk.

Voluntary movements are naturally smooth and graceful. A measure of smoothness in movement is the mean squared jerk. Jerk is the rate of change of acceleration or the third time derivative of position. Therefore, the smoothest movement between 2 points would be the one path (among many possibilities) that keeps the jerk value to a minimum (145). As duration is an integral part in the calculation of jerk, the effect of duration on jerk magnitude has to be eliminated before it can be used reliably to

compare between different movements. Slow (but not necessarily smoother) movements may have a lower jerk values when compared to faster ones (58). To allow comparison between movements of various durations or sizes a normalisation process would be necessary (324).

Teulings et al used the normalised integrated squared jerk to quantify the lack of smoothness in hand writing caused by Parkinson's disease compared to a group of healthy volunteers (324). Cozen and Bhakta presented another form of normalisation called the normalised average rectified jerk (*NARJ*) (58). This measurement calculates the jerk at a second duration for any movement and is intended to compare movements of similar trajectory profile. Assuming that movements have similar trajectory profile, differences in spectrum can be attributed to lack of smoothness (58).

In this chapter two sets of experiments have been designed to test subjects' ability to perform fine finger manipulations and maintaining specific level of force in addition to the influence of cutaneous sensory block on the quality of performing these tasks. Purpose designed software macros have been used to evaluate various kinematic parameters of handwriting.

4.2- Subjects and Methods

This work was approved by the local ethics committee and all subjects signed an informed consent. Ten healthy volunteers (nine males and one female) with a mean age of 29 years (range 19-39) were studied. All were right handed except one and the dominant side was always tested. The experiments were performed with the subjects having intact hand function, and again after induction of median nerve block similar to a severe case of carpal tunnel syndrome. To measure reproducibility of these measurements, subjects (four for the first experiment, and five for the second) repeated the test with intact hand function twice, with a week interval between the two tests.

Median nerve block: median nerve block was performed with the use of 3-5 ml of the commercially available Lignocaine-HCl 0.2%. Aseptic measures were taken throughout the procedure. After the palmaris longus and flexor carpi radialis tendons were defined at the wrist level, a 22 gauge hypodermic needle was connected to a 5 ml syringe filled with Lignocaine. The skin was penetrated 2-3 cm proximal to the wrist crease between the two tendons. The local anaesthetic was infiltrated around the median nerve sheath (82).

4.2.1- Experiment one (maintaining isometric force)

Hypothesis: the hypothesis tested was that an altered median nerve function would reduce the ability to maintain a submaximal level of force.

Objective tested: the ability of the subjects to maintain a submaximal level of force without visual feedback. This test was performed before and after the induction of median nerve block to induce sensory block of the index finger and the thumb.

Aim: the aim was to maintain 20% of the maximum key pinch force without visual feedback.

Data acquired: the force level from a key pinch and EMG from the first dorsal interosseous muscle.

Equipment set up: a Biopac 100 A (BIOPAC Systems, Inc., Santa Barbara, California). machine was set up on 2 channels. Channel one for EMG acquisition with gain of 2000, sampling rate 2000/ sec. Two surface electrodes were used for recording with an outside diameter of 12.5 mm, hosting a contact area of 10 mm diameter. The electrodes were Ag-AgCl and shielded. The ground electrode was disposable foam with 38 mm outer diameter and Ag-AgCl 10 mm recording area. Differential input amplifiers were used with a common mode rejection ratio of 100 dB, minimum input impedance 10 Ω, bandwidth 10-4000 Hz and A/D resolution of 16 bits. The EMG signals were sampled at 2500 Hz.

A strain gauge was connected to the second channel. The strain gauge was calibrated to be able to covert the recorded volts directly into Kg, which was then converted to Newtons (N). One Newton on the surface of the earth is equal to 101.972 grams (290). The calibration process was repeated monthly, and the strain gauge was zeroed at the beginning of each test to avoid any noise that may influence the recorded data.

The experiment: with the subject seated comfortably on a chair facing the PC screen they were familiarised with the equipment. The two recording surface electrodes were filled with hypoallergenic conduit gel. The skin over the first dorsal interosseous muscle was prepared by shaving any hair and then cleaned repeatedly with alcohol to reduce the skin impedance. The same preparation was repeated for the site of the ground electrode over the tip of the olecranon process. The borders of the first dorsal interoseous muscle were defined by asking the subject to perform a key pinch and press the palmar aspect of the tip of the thumb against the radial side of the tip of the index finger (figure 4.1). The attachment site of the recording electrodes was then decided. Double-sided adhesive plastic rings with an inner diameter matching the recording area of the electrodes were used to stabilise them over the selected positions (figure 4.2). The ground electrode was then attached over the olecranon tip of the same limb. The leads were weaved together throughout their length until the connection to the recording machine.

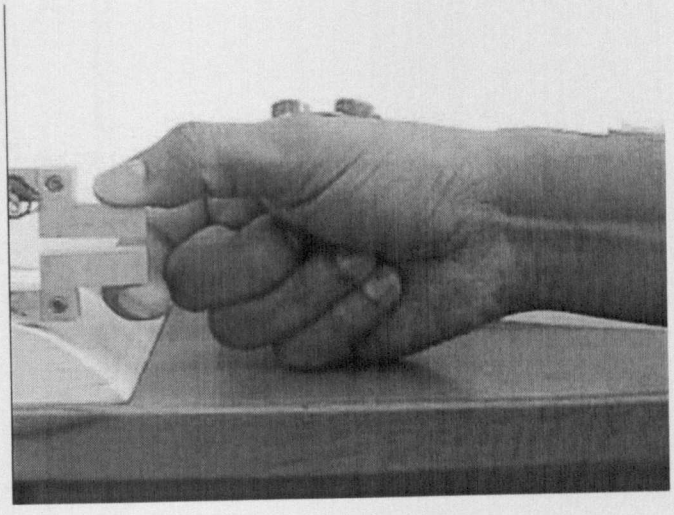


Figure 4.1. The strain gauge is held between the radial side of the index finger and the tip of the thumb (key pinch position).

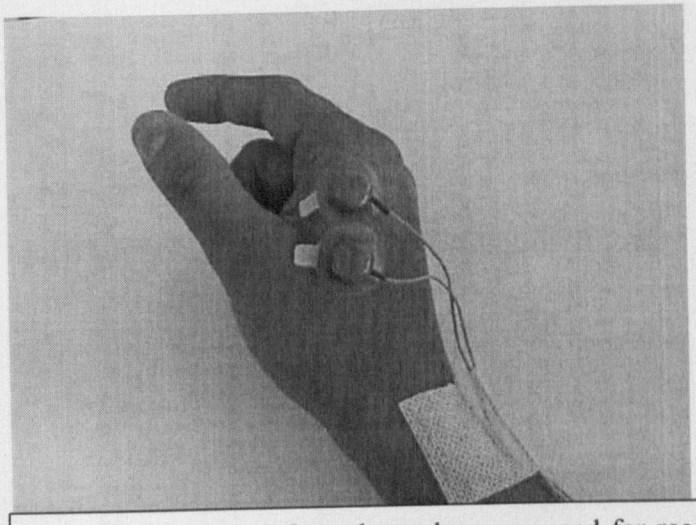


Figure 4.2. Two surface electrodes were used for recording with an outside diameter of 12.5 mm, hosting a contact area with a diameter of 10 mm. The electrodes were attached over the surface of the first dorsal interosseous muscle using double-sided adhesive plastic rings.

The subjects were then asked to hold the strain gauge between the index finger and the thumb (key pinch). They were familiarised with the set up and methods of recording while a check was made that good quality signals were recorded by loading the 1st. DIM. Subjects were asked to press the strain gauge using their maximum force for five seconds. This step was repeated three times and the highest recorded force was considered the subject's own maximum force. The value of 20% maximum force was defined for each subject who was given the chance to reproduce this level of force.

Following this preparation subjects were asked to exert the desired force (20% of their MVF) and maintain it while a visual feedback from the screen was allowed. Once the subjects had reached the target force, they were asked to maintain it for 30 seconds without looking at the screen (deprived of the visual feedback). Simultaneous recording of EMG and force was performed and data saved for later off line analysis. This experiment was performed before and after median nerve block.

Analysis of data

EMG: using Acqknowledge-35 software (BIOBAC Systems, Inc.) EMG signals were bandpass filtered at 10-500 Hz, mean value smoothed by 2000 and rectified by 400. The mean of the EMG amplitude signals throughout the whole cycle was then found. EMG amplitude was normalised to the mean of the test period by dividing the recorded signals from a single recording period by their mean. The outcome of each recording period was then multiplied by 100 to give a percentage (%) of the mean. Each of the 30 sec. cycles was divided into 10 equal periods. The mean of EMG amplitude for each of these 3 s periods was calculated. Data were transferred to an Excel spreadsheet for linear regression analysis.

Force: force delivered to the strain gauge was recorded simultaneously along with the EMG on a second channel. As we were interested more in the change of the level of force and the magnitude of this change compared to the target force, the force throughout the test period was normalised to the target force (20% MVC) to give a %

value from the subject's own target. Similar to EMG amplitude, force channels were divided into 10 intervals each of 3 s duration and the mean of force for each of these periods was calculated. Data were transferred for calculation of rate of change using linear regression analysis and paired t-test for comparison between trials.
For the linear regression analysis Excel 2000 spreadsheet's *linest* function was used. The slope of the data against time was calculated and the trend line was fitted using the least squares fit for a line represented by the following equation: $y = mx + b$ where m is the *slope* and b is the *intercept* (26).

Pearson correlation coefficient test was used to correlate between force and EMG relative changes. The test returns r value, which represent the strength of the linear relation between the two variables. The r value for the regression line is:
$r = n(\sum XY) - (\sum X)(\sum Y) / \sqrt{[n\sum X^2 - (\sum X)^2][n\sum Y^2 - (\sum Y^2)]}$. To produce a P value r^2 is then calculated as well as the degrees of freedom (26).

For repeatability experiments (repeated measurements of the same hand) a paired t-test was used to determine whether there was a statistically significant difference between the means.

The 95% confidence interval (CI) was calculated for statistically significant results with $P< 0.05$. Assuming alpha equals 0.05, the area under the standard normal curve that equals (1 - alpha), or 95 percent is ± 1.96. The formula for CI estimation is therefore: $X \pm 1.96 (\sigma / \sqrt{N})$ where X is the sample mean, σ is the standard deviation and N is the sample size. When the CI is calculated it gives a degree of certainty that the mean of the data falls within the range provided by the upper and lower limits of the CI. This degree of certainty is 95% when alpha equals 0.05 (26).

4.2.2- Experiment two: (writing task)

The hypothesis was that altered median nerve sensory function would reduce the subjects' ability to perform a fine hand movement task.

Objective tested: ability to perform a handwriting task before and after induction of median nerve block (similar to a severe case of carpal tunnel syndrome) using local anaesthetic.

Data acquired: handwriting strokes recorded through a digitizer.

Task to be achieved: was to follow a moving dot on a PC screen using an Intutous2 pen on a digitizer utilising their finger movements.

Equipment setup: a WACOM tablet digitizer 616 × 445.5 × 37 mm (active area 457.2 × 316.8 mm) was used. The digitizer was accurate in recording the pen position within ± 0.25 mm (Intuos2 Pens/4D Mouse); with a resolution of 100 line/mm; and connected to a PC through an RS-232C serial interface. The digitizer was covered with an A3 size sheet of paper. The elbow and shoulder were adjusted to allow a comfortable hand position at the centre of the digitizer, and the wrist rigidly splinted with a removable splint (figure 4.3).

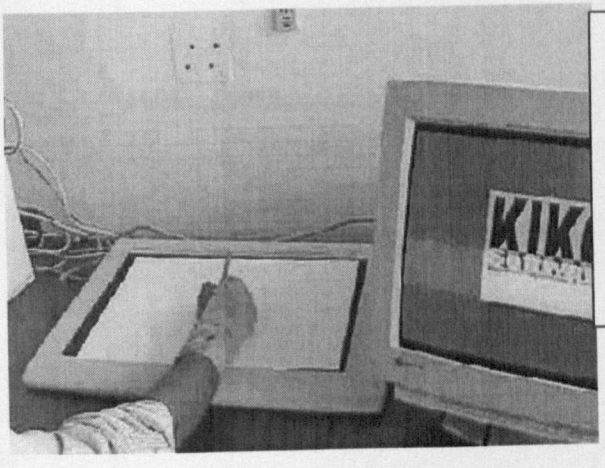


Figure 4.3. A subject seated facing the PC screen and holding the pen over a digitizer covered with A3 size paper sheet. The subject's wrist is splinted to eliminate wrist movement.

Pen calibration to measure force: Intuos2 pen was calibrated with regard to the force that would be delivered by its tip over the digitizer. As the digitizer has a 15° tilt from the horizontal, a plastic block had been prepared with a tangential tunnel in its middle that would accommodate the pen and allow a perpendicular position in relation to the digitizer surface fig. 4.4. The weight of the pen itself was calculated and the digitizer zeroed. Blocks of gradually increasing weights (25, 50, 100, 200, 400, 500, 600 and 750 gm) were fixed to the end of the pen and calibrated by the KIKO software. This calibration was stored in the computer memory to account for the amount of pressure placed by the pen on the digitizer during the writing task.

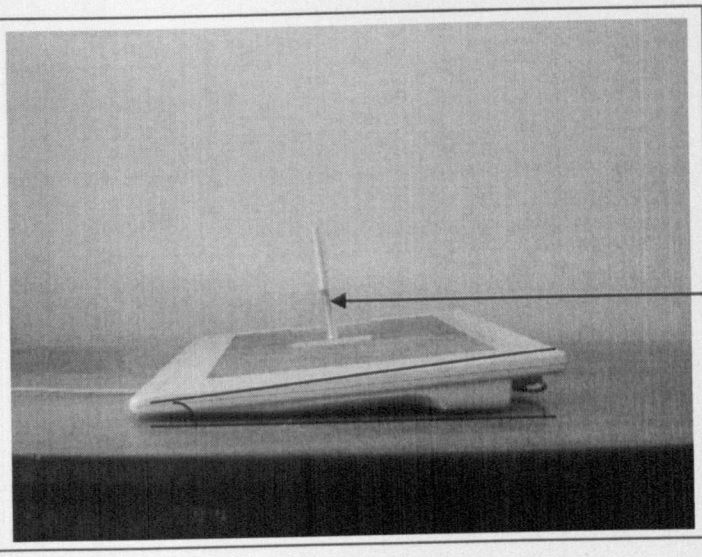


Figure 4.4 WACOM digitizer has 15° tilt angle from horizontal.
15°-angled tunnel drilled in a block to allow perpendicular position of the pen in relation to the digitizer surface during calibration of weights added to the pen base.

The experiment: Subjects were allowed to practice the task a few times until they felt familiar with the sequence of the steps before the actual recording started. The task used a purpose designed macro running in OASIS software (KIKO Software, Nijmegen, Netherlands): a 10×10 mm dot appeared in the centre of the screen, and subjects were asked to touch it by moving a modified Intuos pen (KIKO, Nijmegen, Netherlands), calibrated for force measurement up to 800 g, over the digitizer. Once touched, the dot moved in sequence around 4 positions, in a 'skewed N' shape (figure 4.5 & 4.6) in a 2×4 cm space, at each of which the subject was asked to follow it as fast as possible, avoiding movements of the rest of their upper limb. The writing task was designed to allow performing these writing strokes using finger manipulations

without a need for wrist or arm movements. As the strokes connecting the different dot positions were created by moving the pen away and towards the body; a movement that is normally created by finger movements during handwriting (325).

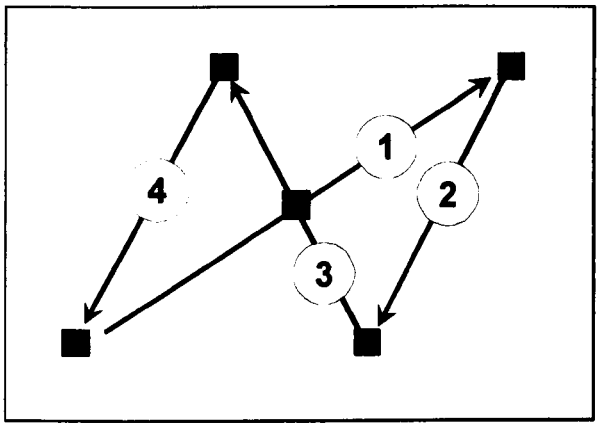


Figure 4.5 shows the consequent positions of the moving dot, and the 4 writing strokes constituting a skewed *N* shape.

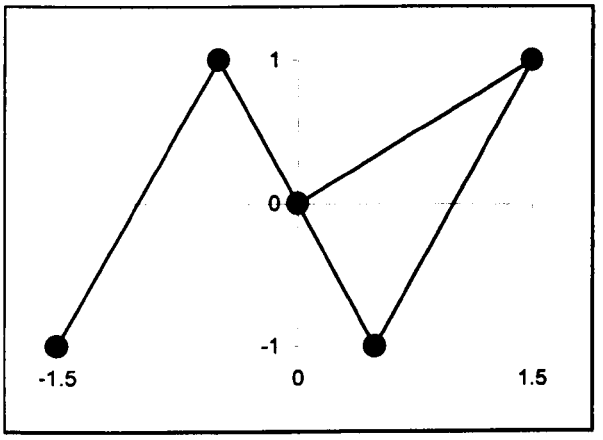


Figure 4.6. shows the relation of the different dot positions to each other and to the centre of the dizitizer within a space of 2x4 cm.

Pen movements between dot positions constituted 4 writing 'strokes'. The sequence was repeated 4 times, making 16 strokes in all. The test was repeated after induction

of median nerve block as described above. To examine repeatability, 4 of the subjects repeated the task on two occasions one week apart. Data were analysed using a KIKO macro that measured various parameters: the actual *length* of the trajectory expressed relative to the target length, the *Detour* (= [trajectory length]/[distance from start to finish] – 1). The *jerk* (J) is defined as the rate of change of acceleration, the third derivative of distance x with respect to time t ($J = d^3x/dt^3$), and the *integrated jerk* (= $\int|J|dt$) averages this over the whole movement segment (58); to correct for differences in duration we calculate the *normalised average rectified jerk* = $\int|J|dt \times duration^2$. We also measured the *force* delivered by the pen over the digitizer. Differences between pre to post induction of sensory block values were analysed by the paired t-test, $P<0.05$ being taken as statistically significant.

4.3- Results:

4.3.1- Reproducibility studies

4.3.1.1- Maintaining 20% MVC without visual feedback

The main observations from the analysis of the data were: first, EMG amplitude changes were very close to the force level exerted and was seen to reflect the changes in force values. Secondly, a good level of agreement between individual results in the two trials was seen. Finally, there was a tendency for the force to decline, despite the short test period.

Figure 4.7 shows the individual force and average rectified EMG results of the 4 subjects in the first trial. The force results are expressed as a % of the target force (20% MVC). EMG amplitude is expressed as a % after being normalised to the mean amplitude of the whole cycle. EMG amplitude tended to follow the level of force exerted. The results from both trials (trial 2 not shown as individual results) are similar in terms of level of activity and the tendency for the force to fall through the test period.

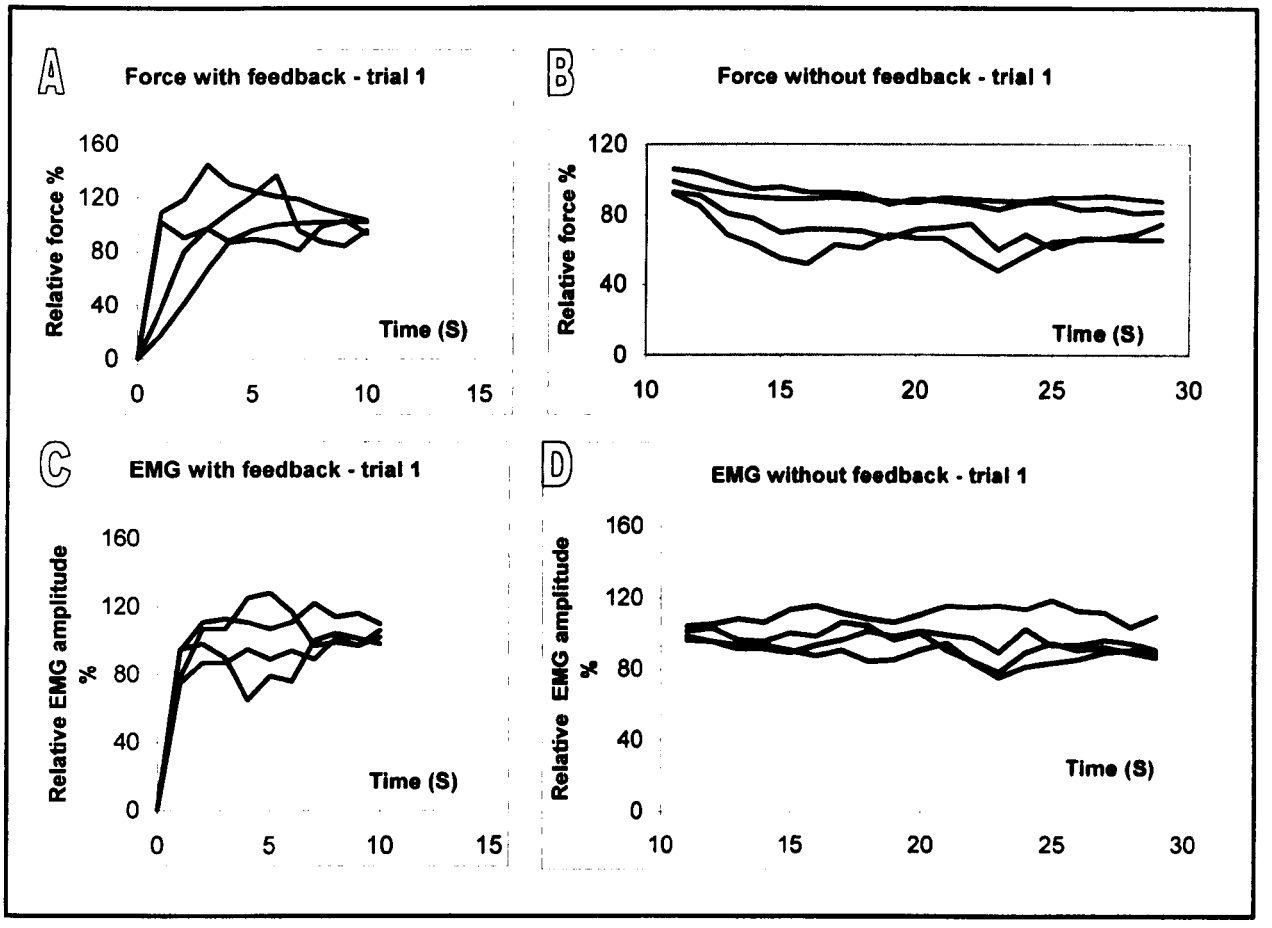


Figure 4.7 shows the individual results for the 4 subjects. Force is presented as a % of the task force and EMG amplitude as a % of the mean of whole test period. (A) Is the force from start till reached the target level with visual feedback. (B) Is the force without visual feedback. (C) Is the EMG amplitude for the period with visual feedback and (D) is the EMG activity for the period without feedback.

When the mean data from trials 1 & 2 were compared, similar levels of force and EMG amplitude were noticed (fig. 4.8). Trend lines were fitted for the second part of the test (without visual feed back). The regression lines for trials 1 & 2 were very close particularly for EMG where the lines overlapped. In force the equation for trial 1 trend line was (y = -0.6653x + 92.496) and trial 2 was (y = -1.064x + 105.49). For EMG trial 1 trend line equation was (y = -0.4256x + 104.58) and trial 2 was (y = -0.1898x + 99.752). Using a paired t-test trial 1 and 2 were compared and no significant difference was found between the means of force (P= 0.5), EMG (P= 0.7) or force/EMG (P= 0.5).

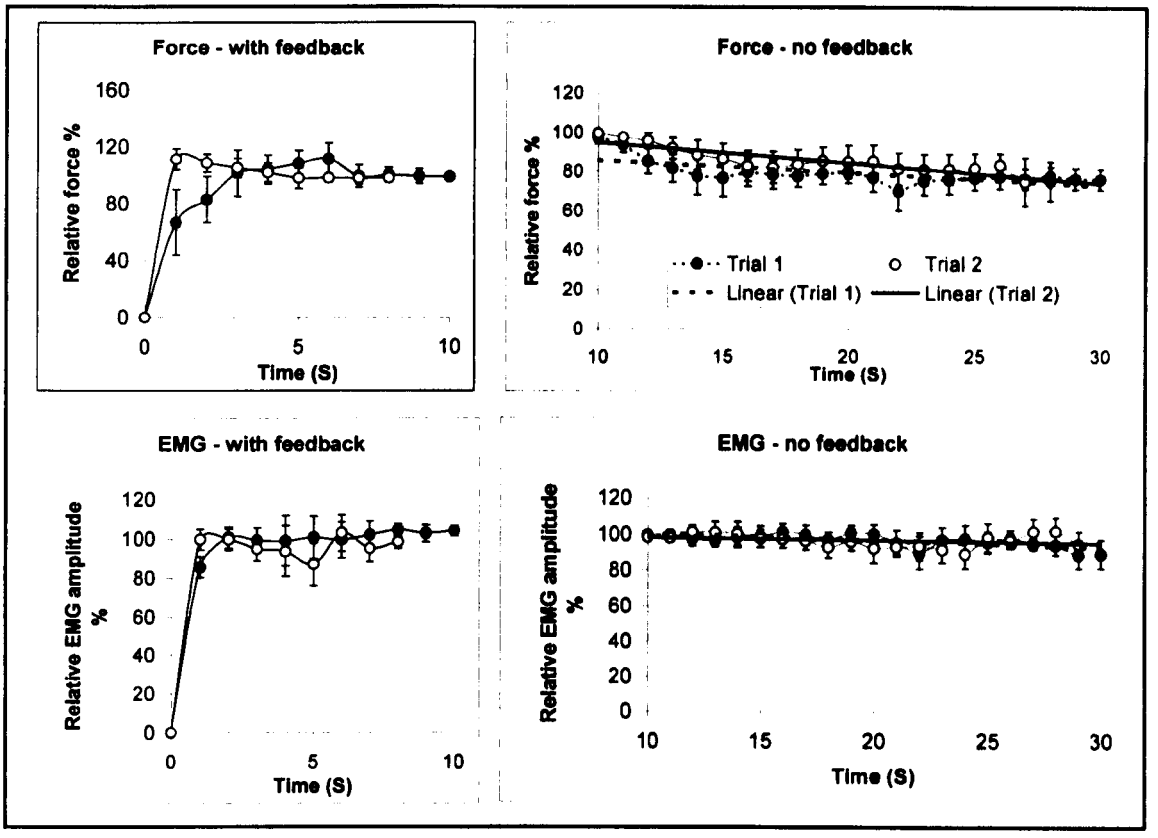


Figure 4.8 shows the mean data from both trials presented as % from the target force and normalised EMG. The key in (B) applies to the rest of the sub-figures. (A) represents the mean force with visual feedback. (B) represents the mean data from both trials and the regression lines without visual feedback. (C) & (D) presents the EMG amplitude for the same period compared to (A) and (B) respectively. Data are presented as Mean± SEM.

Using the Pearson correlation coefficient test the relation between force and EMG was studied. A significant correlation was found between force and EMG in the different trials. For trial 1 r^2 was 0.74 (P = 0.03) and for trial 2 r^2 was 0.75 (P = 0.03).

In addition to the significant correlation between force and EMG changes, trend lines for the two trials were very close as well as the squared R-values fig. 4.9. The close values of r^2 in trials 1 and 2 indicated good level of agreement between the results obtained in the two trials.

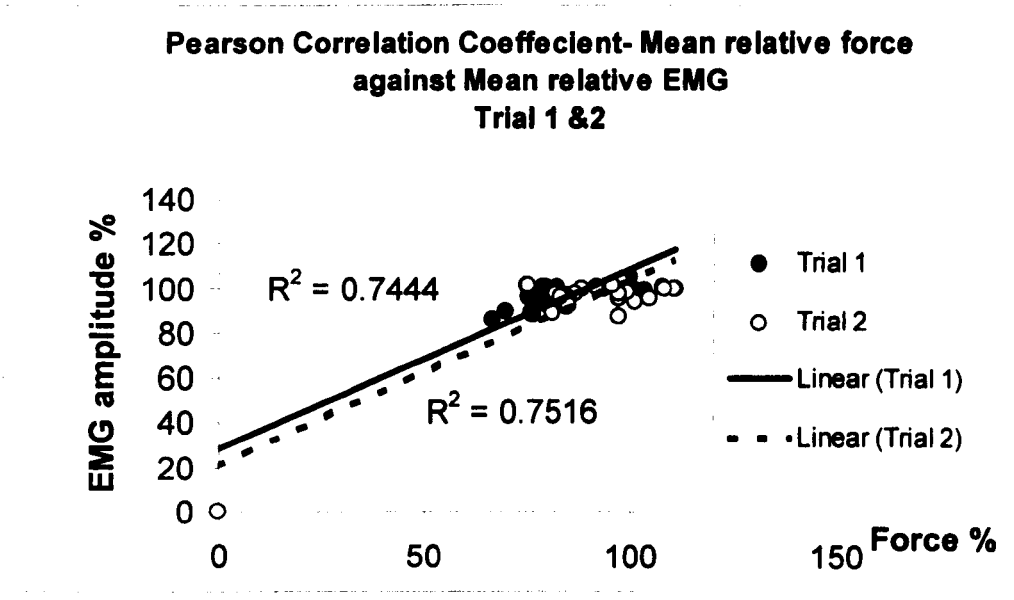


Figure 4.9 shows the correlation between relative force and EMG changes during repeatability trials (trial 1&2). A trend line was fitted using the least square method. r^2 values are presented: the top = trail 1, the bottom = trail 2.
The graph shows significant level of correlation between force and EMG. The close value of r2 in the two trials (parallel regression lines) indicates a high level of agreement between results obtained in both trials.

Comparing the slopes of force, EMG and force/EMG showed no significant difference between the 2 trials with P values of 0.8, 0.1 and 0.3 respectively.

To compare the variability of results around the regression line, the SE of slope and both residual (between trials) and total (between subjects and trials) variances for trial 1 and 2 were calculated. The mean residual variance value for trial 1 was 36.7 slightly higher than trial 2 of 30.2, but this difference was not significantly different P= 0.8. Similarly the difference between total variance and the SE of slope between the 2 trials were not significant with a P= 0.9 for both.

4.3.1.2- Reproducibility of handwriting task

Five subjects had the handwriting task repeated with a week interval between the two trials. Different parameters were calculated with regard to the pen movements over the digitizer. These parameters were particularly selected to clarify the difference in smoothness or clumsiness of manipulations. Good level of repeatability was found between the data collected in the two trials. Table 4.1 and fig. 4.10 show the mean and individual results obtained on the analysis of the various parameters of the writing stokes.

Table 4.1

Values compared / Parameter	Mean Trial 1	SEM Trial 1	Mean Trial 2	SEM Trial 2	Mean Ratio Trial 2 Vs Trial 1	t-test trial 1 to trial 2 P value
Detour (cm)	0.34	0.072	0.35	0.038	1.1	0.7
Fractional Length (cm)	1.2	0.08	1.2	0.04	1.02	0.8
Duration (s)	0.65	0.04	0.64	0.07	1.01	0.9
Integrated Jerk (cm/s^3)	968	171	950	204	0.97	0.8
Average Rectified Jerk (ARJ) (cm/s^3)	1655	318	1657	455	0.96	0.99
Normalised Rectified Jerk (cm/s^3)	464	97	443	54.1	1.17	0.8
Force (N)	1.6	0.16	1.9	0.25	1.1	0.2

Table 4.1 presents the data obtained in trial 1 and 2 and the P value of paired t-test between the two trails.

As illustrated in Table 4.1 and fig. 4.10 no significant difference could be found between individual or mean results during the repeatability studies.

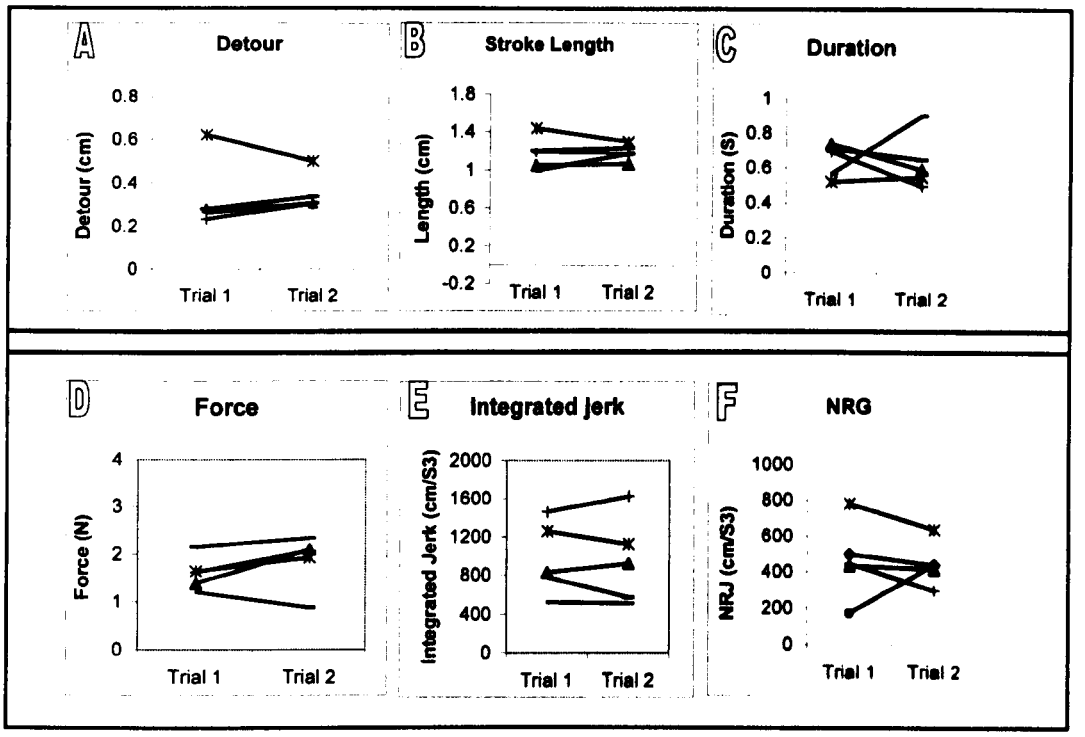


Figure 4.10 shows a comparison between individual results of the 2 trials performed to test reproducibility. The different panels from (A) to (F) gives the results of the various parameters that have been analysed. Good level of repeatability has been demonstrated at individual level.

4.3.2- Results of pre to post median nerve block

4.3.2.1- Maintaining submaximal level of force

In this section the results from the experiments comparing subjects' ability to maintain a submaximal level of force without visual feedback will be presented. Analysis of the results showed a significant non-linear decline in force that was present in both pre and post block experiments (fig. 4.11). This decline in force was significantly different from zero (P= 0.001) for pre-block trial and (P= 0.002) for the post-block. Although this force decline was different from zero, there was no significant difference when the two trials were compared. The mean slope of force in the pre block trial was -1.2 ± 0.5 compared to –1.4 ± 0.7 (Mean ± 95% CI) in the post block trial. The absence of significant difference between the pre and post nerve block trials denoted that nerve block made no difference to the subjects' ability to maintain a submaximal level of force.

An interesting observation was made in regard to the way subjects approached their target force level during the initial part of the test i.e. in the presence of visual feedback about the level of force exerted. For the pre-block trial, subjects overshot the target initially, and then reduced their force to reach the targeted level. In contrast, in presence of nerve block i.e. absence of cutaneous sensation, the target force was approached cautiously from below (fig. 4.11A).

The overshoot was quantified as the maximum force less 100%. The pre-block overshoot was 37.1% ± 12.6% (mean ± SEM), not significantly different from the post-block 13% ± 5% using t-test (P= 0.1). However, this difference was significantly different when the Wilcoxon test for non-parametric analysis was used (P= 0.03). The pre to post block overshoot difference is shown in the inset panel in fig. 4.11A.

EMG results were again qualitatively similar to the force changes. The EMG amplitude followed the level of force throughout the time course of the experiment and did not show any dissociation between the pattern of activity in the first dorsal interosseous muscle (small hand muscle) and the force generated by the long flexors

(forearm muscles). Comparing EMG amplitude results between the two trials (pre and post nerve block) showed no difference.

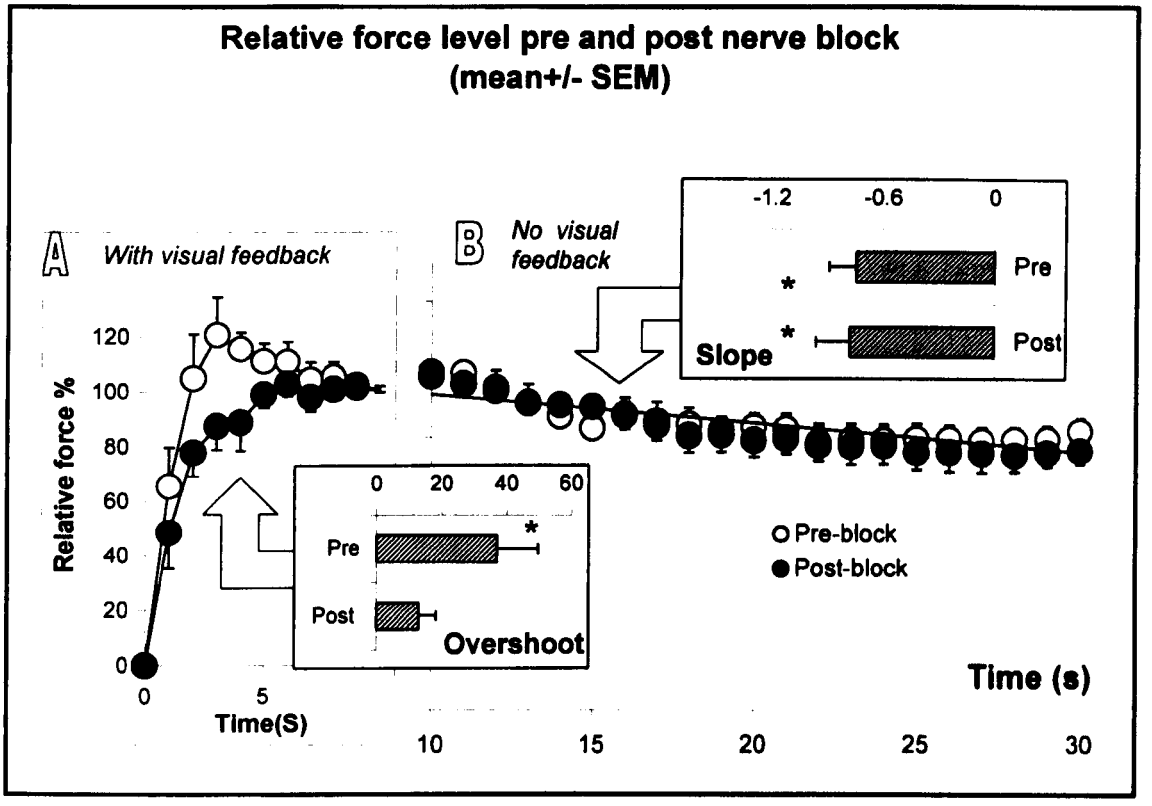


Figure 4.11. **The force-maintenance experiment.** (A) shows the time course of force (% of target value) during the 10 s in which subjects aim at and maintain pinch grip force in the presence of visual feedback. Results are shown for experiments pre- and post-block of the median nerve (see key). The inset panel shows the size of the overshoot in force: only the pre-block overshoot is significantly greater than zero (P=0.02). (B) shows the time course of force (% of target) during the subsequent 30 s in which subject try to maintain pinch grip in the absence of visual feedback. The inset panel shows the linear regression slopes of both sets of data (the line for pre-block data is shown in the main figure), which are both significantly below zero (P<0.003), and not significantly different. The overall mean rate of fall is -1.3±0.2 % s^{-1}. Data are shown as mean±SEM. The asterisk denotes a slope significantly different from zero (P<0.05).

4.3.2.2- Handwriting (fine manipulation) task

Figure 4.12 shows the principles of analysis of the writing experiment, taking length as an example. The inset panel shows the pattern of dots, with labels on the arrows corresponding to the 4 strokes. Results from all 16 segments from the sequence of 4 strokes (fig. 4.12A) are combined into four means, one for each stroke (fig. 4.12B), and then into a single mean value (fig. 4.12C), which was significantly larger post- than pre-block (but did not change in the control experiments). The same is true for duration (P=0.04, not shown).

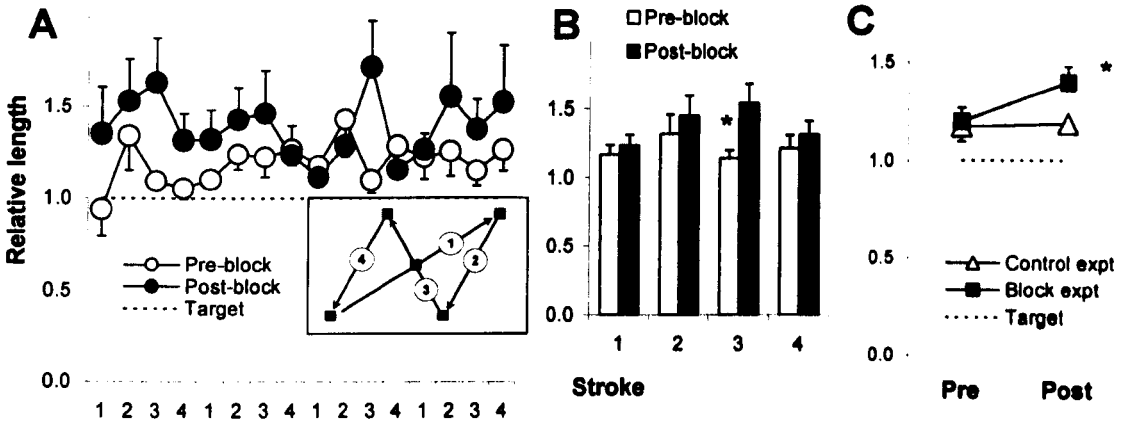


Figure 4.12. **The writing experiment**: principles of analysis, applied to stroke length, normalised to the target length (indicated by the dashed line). (A) shows the measurements made pre- and post-block (see key) in the block experiments, during four repeated sets of four strokes: the inset shows the pattern of the four points and the starting position, with the corresponding strokes numbered. (B) shows these 16 data points combined into means for the 4 strokes, pre- and post-block (see key). (C) shows data from the four strokes combined into a single mean, which is significantly larger post- than pre-block (P=0.02): corresponding data from the control experiments (see key) showed no significant pre-post difference. Data are shown as mean±SEM. The asterisk means that the post-block results differ significantly from pre-block results (P<0.05).

Figure 4.13 shows data on the smoothness of the movements. The overall integrated jerk did not differ significantly fig. 4.13A, but when this is corrected for the difference in duration, the normalised average rectified jerk was found to be increased post-block fig. 4.13B.

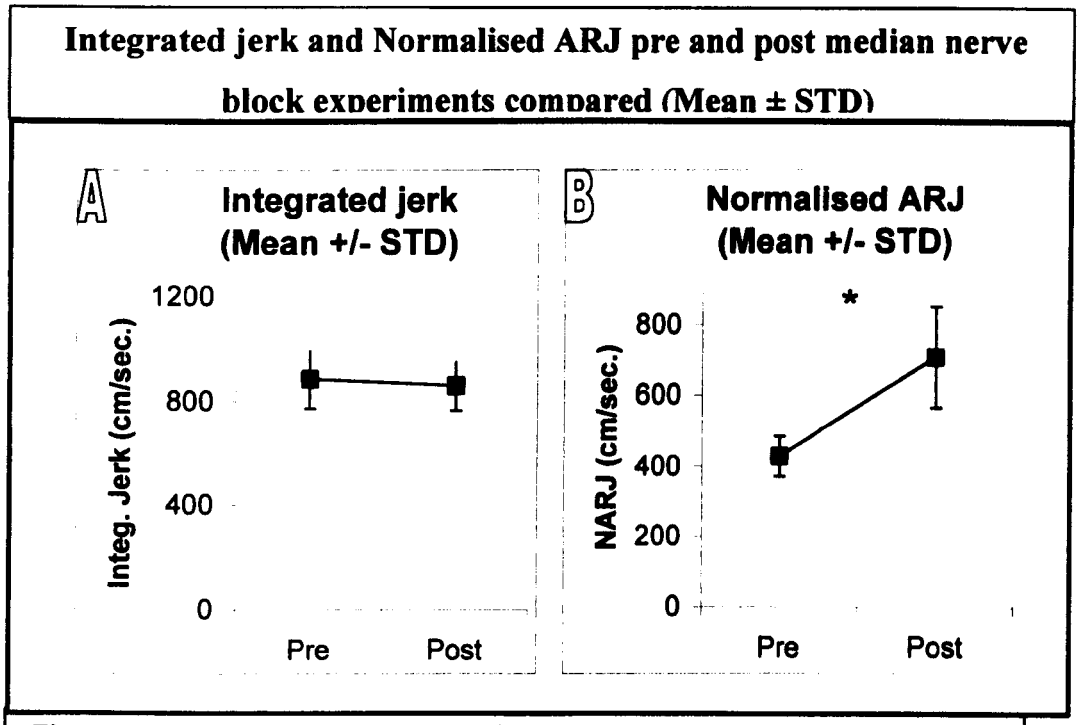


Figure 4.13. The writing experiment: functions of jerk. The figure shows the pre- and post-block data in (A) integrated jerk and (B) normalised average rectified jerk. Integrated jerk did not differ significantly pre- and post-block, but normalised average rectified jerk increased significantly post-block (P = 0.02). Data are shown as mean ± STD. The asterisk means that the post-block results differ significantly from pre-block results.

Figure 4.14 shows that the force exerted on the plate (Pen pressure on digitizer) has a rather different temporal pattern to the kinematic parameters such as trajectory length. In the absence of cutaneous block the force falls steadily throughout the pen movements; in its presence the force starts lower and stays constant. This fall in force that was noticed in the pre-block experiment dominates any differences between the two (pre to post) trials for the 4 strokes; so there was no significant pre-post difference in the mean force overall (P= 0.23).

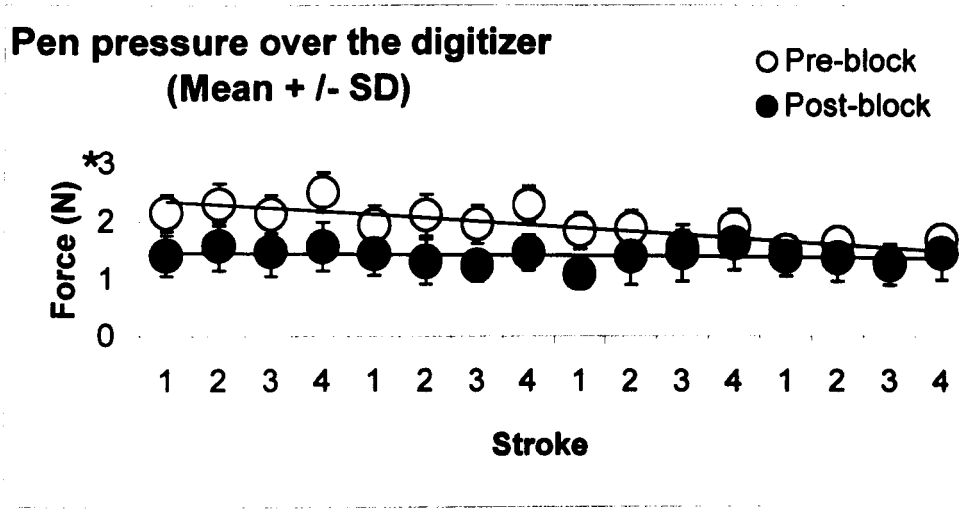


Figure 4.14. The writing experiment: force exerted on the plate. The figure shows the measurements made pre- and post-block (see key) in the block experiments, during consecutive intervals 1-16 of four sets of strokes (using the same format as Fig 10). The regression slopes (shown in the figure) and significantly less than zero (P = 0.02) for the pre-block data only (for post-block data, P=0.45, the difference being significant, P=0.02). There was no significant difference between the overall mean pre- and post-block values (P=0.23). The asterisk means that the slope of force pre-block was significantly different from zero (P<0.05).

Table 4.2 provides details of the different writing stoke parameters. The 95% CI is shown for differences between pre and post nerve block trials.

Table 4.2

Comparison of writing strok parameters

	Pre nerve block			Post nerve block			
	mean	STD	95% CI	mean	STD	95% CI	P value
Detour (cm)	0.417	0.083	0.05152	0.5865	0.084	0.05206	0.002
Length (cm)	1.199	0.07	0.04351	1.3991	0.083	0.05167	0.018
Duration (S)	0.684	0.059	0.0367	0.8059	0.058	0.03592	0.044
inegrated Jerk (cm/sec)	884	110.9	68.7143	858.23	93.56	57.9884	0.752
NARJ (cm/sec)	428.4	56.46	34.9921	708.51	142.8	88.4914	0.022
Force (N)	1.909	0.275	0.17042	1.4052	0.375	0.23232	0.213

The 95% CI was calculated for the different writing strokes parameters pre and post nerve block. Fig. 4.15 provides the 95% CI for length, NARJ and pen pressure over the digitizer. For NARJ and length, a wide gap can be clearly seen between the 95% CI of the pre and post nerve block trials. The narrow range of the 95% CI around the mean supports the statistical significance observed in these parameters and confirms the deleterious effect of nerve block on smoothness of fine movements of the hand. The wide difference between the 95% CI observed for the pre and post block trials was also noted for detour and duration as seen in the table 4.2.

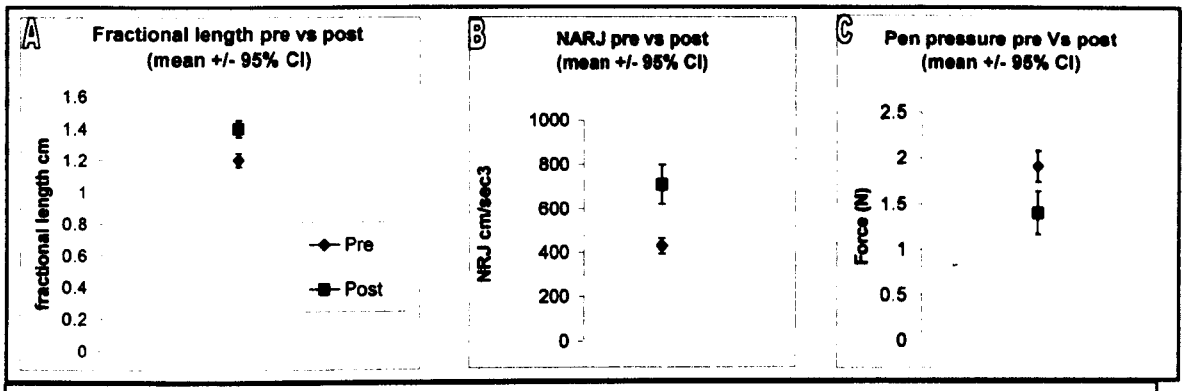


Figure 4.16 presents the mean ± 95% CI for stroke length (A), NARJ (B) and pen pressure (C). No overlap was seen between pre and post block results. Note the wide difference between pre and post results of NARJ and length supporting the statistically significant results. See text for discussion

No overlap of the 95% CI was noted for force (pen pressure over the digitizer). However, the differences between pre and post nerve block results did not reach statistical significance ($P > 0.05$).

4.4- Discussion

The first set of experiments in this chapter was directed to study the influence of abolishing feedback from cutaneous sensory receptors on motor function of the hand. Controversy still exists as to which of the sensory feedback sources (skin, muscle and joint) provides the most contribution to proprioception; particularly at the hand level (172).

Previous research has demonstrated a primary role for the muscle receptors in providing movement and position senses of large body joints like the hip and knee (48, 220, 221). However, this fact is questioned for the hand that is considered unique and does not represent the rest of the body (46, 47). Clark et al have tested the possible contribution from articular receptors on the ability of humans to detect movement of the distal interphalangeal joint in the middle finger. The authors concluded that the articular contribution to the movement sense of small hand joints is minimal (47). This finding was in agreement with Burke et al's results with the use of microneurographic techniques to record individual afferent discharges arising from cutaneous, muscle spindles and joint receptors (37).

Moberg (1983) reported that increasing the muscle length did not contribute to ability of patients to detect finger movement. Increasing muscle length to the extreme that leads to skin displacement in the forearm resulted in a vague subjective feeling by the patients that their fingers were moving in the direction of muscle lengthening (246).

Microneurographic studies have confirmed the value of the mechanoreceptors in the dorsal aspect of the hand on providing detailed kinematic information about finger movements (50). Electrical stimuli to skin on the dorsal aspect of the finger or skin stretch were found to produce illusory feeling of finger movement. Similarly, muscle vibration produced the same effect in a higher proportion of subjects (50).

Another aspect of motor coordination is whether the lack of skilful hand function following nerve injury is a reflection of the deficiency in muscle sensory recovery? The ability to recover a central nervous control over reinnervated muscles is usually

disappointing (263). Reinnervated muscles following nerve section suffer a massive reduction in the number of spindle and tendon organ afferents with the occurrence of various kinds of abnormally responsive afferents (11). However, this question would be difficult to cover in full, as controversy about the relative importance of the three types of receptors: skin, muscle and joint to hand proprioception still unresolved.

The experiments in this chapter tested subjects' ability on maintaining isometric submaximal level of force and perform fine hand manipulations, before and after median nerve block similar to a severe case of carpal tunnel syndrome. Sensory feedback from muscular, articular and cutaneous receptors contributes to the regulation and coordination of both tasks to a variable degree. The role of different receptor types could be tested by abolishing each of these in turn. Practically it is not possible to block the muscular or articular receptors separately. Therefore, this model of blocking the cutaneous and articular feedback while keeping the muscular feedback intact was chosen. In this model the interphalangeal joints and sensation of the dorsal aspect of the fingers that receive nerve supply from the radial nerve were intact. However, articular receptors are known to play little role in the hand (172), and keeping the model similar to the clinical situation of median nerve compression was preferred.

In experiment one, which needed the subjects to maintain a submaximal level of force, it was clear that absence of cutaneous sensation did not affect the subject's ability to perform the task. This may suggest that muscle receptors (in this case thumb flexors and first dorsal interosseous muscles) have provided enough sensory feedback to allow subjects to perform the task.

In a study of a deafferented man and a healthy control group, subjects were asked to maintain certain levels of pressure with their fingers against a lever. Healthy subjects were able to maintain force with minimal variation for periods of five to eight seconds. The deafferented patient could not perform this task and random responses were noted with either increase or decrease in the exerted force. The authors concluded that the deafferented patient lacked the ability to maintain a constant level of force without visual feedback while normal subjects could perform this task without difficulty (292). The effect of inability to maintain a constant level of force would be reflected in the

subject's performance of daily activities. For example, the deafferented man mentioned in the above study was not able to hold a cup or to maintain pressure against buttons for any length of time.

Interestingly, the experiments presented in this chapter showed a different response. Healthy subjects tested with or without cutaneous sensation had the same near-linear fall in force in absence of visual feedback. This cannot be attributed to motor fatigue, as the task required only 20% maximum force for 30 s. It reveals a shortcoming of the human motor coordination system; as humans would not normally have a visual feedback about the level of force exerted in their daily life.

The difference observed in this study from the earlier study by Rothwell et al is likely to be due to the difference in the test period (eight sec. in Rothwell's study and 30 sec. in this study). Moreover, although Rothwell has mentioned some variability in the control group results it was not clear from their paper how much this variability was.

The third observation from this experiment was that cutaneous block affected the performance with visual feedback: in absence of cutaneous sensation, when visual feedback has a larger role, subjects approached target force more slowly and, it appears, more cautiously. In the absence of cutaneous sensation subjects overshoot their target force level originally before they returned to the desired level. The pre nerve block overshoot was significantly different from zero.

The final observation in this study was in relation to the correlation between EMG amplitude and force level (fig 4.9). A significant correlation was found between these two variables.

Philipson and Larsson studied the relation between muscle force and EMG parameters of the biceps muscles in six healthy volunteers. The subjects recruited in this study were asked to perform isometric muscle contractions at different levels (%) of their MVC. The authors concluded that the ARV and RMS values of the EMG amplitude were the best descriptors of force from zero to 100 percent MVC, and that surface detected EMG can be used as a substitute measure of force (274).

Morris et al studied different normalisation techniques of the EMG acquired from the deltoid, supraspinatus, infraspinatus and trapezius muscles of healthy subjects during shoulder abduction, external and internal rotation. Normalised ARV of EMG was found to closely reflect the level of muscle activity (249). Clinically surface EMG is used in gait laboratories for detection and quantification of different muscles' level of activity during the different phases of the gait cycle.

The significant correlation between level of force and EMG amplitude as an ARV noted in this study agreed with previous research. This work confirmed that in non-fatiguing muscle activities normalised EMG amplitude closely reflects the level of muscle activity.

The results of the first set of experiments confirmed the subjects' ability to maintain a submaximal level of force relying mainly on muscular afferent feedback. This would suggest a more important role of muscle receptors in the overall coordination of hand motion.

It was important to establish the subjects' ability to maintain a certain level of force without cutaneous sensation. Many of the daily life activities need the subjects to maintain a constant level of force, for example holding a cup or a spoon. A clinical situation similar to the experimental set-up is a patient with reduced sensation in the median nerve distribution. A patient would be expected to perform in a way consistent with the findings in this test. It has to be emphasised that although gripping is considered a relatively simple motor task, cutaneous sensation is an essential element in the adjustment and maintenance of this function (348).

Sensory feedback is an essential element in the coordinated movements of the hand and in the ability to perform fine manipulation tasks. Neurological tests have been designed to measure isolated sensory modalities but failed to define the ability of the hand to perform fine manipulations and tasks of daily activities. These neurological tests have been called academic tests, as they may provide a good method of communication in the medical field but certainly lack the objectivity required to give a final conclusion about hand performance.

Functional methods of hand evaluation have significantly improved our understanding about the difficulties faced by patients with denervated hands. A shortcoming in common with these tests is that they rely on measuring how quickly patients perform the task without concentrating on the quality or smoothness of the performance. Skilled movements of a normal hand can easily be differentiated from the jerky, hesitant and clumsy movements of denervated hands but have hardly been quantified. What is necessary is to design a method for measuring the smoothness of hand manipulations, how movements are performed and tasks are executed.

Previously, handwriting has been used for objectively examining and quantifying incoordination in various neurological disorders (140, 183, 210, 222, 250, 307). Handwriting experiments tended to be in neurological patients (210, 214, 222), with the main aim of quantifying superimposed tremors or predicting psychological development(324).

The experiments in this chapter have for the first time presented the results of a handwriting experiment that was designed to quantify the kinematics of finger manipulations. Particularly, the smoothness and directness of the movement were of interest. Therefore, various parameters were analysed that relied on the length of the writing strokes e.g. length and detour, the rate of change in acceleration e.g. jerk, the timing or duration in performing the writing task; and finally the force exerted by the pen over the digitizer.

The repeatability study with 5 subjects repeating the test gave an assurance that these parameters can be objectively quantified. A good level of agreement was found between the data collected during the repeatability studies in the control group, with no statistically significant differences.

Having established the method, the test was conducted on 10 subjects pre and post block of median nerve with local anaesthetic. Statistically significant differences were found between the lengths of the strokes from pre- to post-block. Detour, which is a measure of indirectness, (Detour = [trajectory length]/[distance from start to finish] - 1) significantly increased between pre and post nerve block trials. The increased length and detour after nerve block can either be due to the increased irregularity of the

strokes or that subjects originally missed the target then readjusted their lines. Both types of inaccuracy in performing the writing strokes are a reflection of the reduction in the skill of performing this fine motor task.

When the integrated jerk (averaged over the whole movement segment) was compared, no difference was found. However, correcting for the difference of duration by calculating the Normalised Average Rectified Jerk (NARJ) showed a significant difference between pre and post-block trials. The findings from this experiment confirmed that altered cutaneous sensation impaired the ability of the hand in performing fine manipulation tasks.

Previous experiments have elaborated the importance of afferent input from the glabrous skin of the index finger and thumb in adjusting the force necessary to hold an object in a pinch grip (348). In this experiment cutaneous sensory block has influenced the adjustment of forces exerted by the pen on the tablet (fig 4.15). Though no significant difference could be demonstrated between pre and post-block tests the 95% confidence intervals were not overlapped when compared between pre and post trials (fig. 4.16). The gradual decline of force noted during the pre-block test may have reduced the chance of demonstrating any significant difference between pre and post-block trials. The actual force exerted on the pen (grip force) was not measured and can be considered in future developments of this method.

The results from the second set of experiments presented in this chapter agreed with previous observations (163, 166, 170, 172, 244-246) in demonstrating the essential role of cutaneous sensation in conducting fine hand movements.

In these experiments it was not possible at the beginning of these studies to conduct a formal power statistical calculation for the sample size. The degree of variability and effect size were not known. Therefore, from an ethical and statistical point of view these studies were conducted as pilot studies. It will be possible for future studies to conduct power calculations based on the findings of these experiments.

For the handwriting experiments where a statistically significant result was found and supported by the absence of overlap of the 95% CI between the two set of experiments

the null hypothesis of no difference between pre and post nerve block results was rejected. In the force maintenance experiment where no difference was noted between the pre and post nerve block trials and the clear overlap of the 95% CI the null hypothesis was accepted indicating that nerve block made no difference to the subject's ability on maintaining submaximal level of force. It is possible that type II error has occurred in interpreting the results of the hand force experiment, but the close range of the means of the results from both trials and the overlapped 95% CI make this possibility unlikely.

4.5- Conclusion

No difference could be demonstrated between subjects' ability on maintaining 20% MVC key pinch before and after median nerve block. However, the nerve block has significantly reduced the subjects' ability to perform fine hand manipulations. The findings from the first set of experiments suggest a bigger and more important role of the muscle sensory receptors in controlling motor performance of the hand than has previously been thought. However, cutaneous sensation is essential for conducting fine hand manipulations. Electronic methods have been used to record and analyse movements of the hand and likely to have a big role in future assessment and rehabilitation of hand function following nerve injury.

Chapter V Final conclusion

In this section, summary of the key findings and conclusions will be drawn.

Two methods of static loading of the serratus anterior muscle were tested (the isometric I and II protocols). The EMG response of the serratus anterior muscle to these fatigue protocols did not differ either in MDF or in amplitude. The third protocol that used a dynamic mode of muscle loading produced a similar degree of decline in MDF but a different amplitude response. The rise in EMG amplitude associated with dynamic mode of muscle testing was found to be less prominent than the increase in EMG amplitude observed with the static protocol of muscle contraction. This difference was significant. Skeletal muscles are known to follow a different strategy of MU recruitment as well as change their rate of discharge according to the contraction mode (dynamic or static). It has been suggested that during dynamic contractions a muscle tends to recruit additional motor units than to increase their rate of discharge in response to fatigue development. In contrast static contractions produce a different response and rely originally on the increase in motor unit rate of discharge before attemting to recruit additional units (309). In a fatigue protocol with a contraction level close to the MVC, as in these protocols, full muscle activation usually starts at the beginning of the contraction and this reduce the chance of recruiting additional units. Hence, static contractions that rely on increasing the MU discharge rate would have a greater potential to increase their EMG amplitude compared to the dynamic.

The MDF and EMG amplitude changes associated with the dynamic protocol of exercises showed an acceptable degree of repeatability. The use of a moderate velocity of $60^{o}\ s^{-1}$ on an isokinetic machine and the analysis of the EMG over short intervals of 3 s must have provided adequate control and minimised the variability that can result from the change in muscle length and level of force with dynamic contractions. It is still, however, too early to recommend the dynamic mode of contractions for general use in fatigue studies.

Surface electrodes have been found to produce less variable results compared to fine wires (173, 178). The recorded EMG with a surface electrode is more representative of the muscle's overall fatigue status than wire that tends to reflect the function of

isolated MUs (86). The data presented in this thesis support the use of surface electrodes when serratus anterior muscle fatigue is investigated.

EMG was found to be a useful tool for investigating muscle fatigue. Power spectrum analysis and amplitude of EMG signals have been widely used to examine muscle fatigability in different conditions and diseases (28, 200, 230). In this thesis the changes observed in MDF has been found to reflect the fatigue status of the muscle. A decline in MDF has been noted in the different fatigue protocols. Changes in EMG amplitude were found to be dependent on the level of force, type of contraction and duration of the exercise. With submaximal isometric contractions, EMG amplitude was found to increase gradually at the beginning of the test and then reached a plateau. This increase however, was less marked when the dynamic mode of exercise was used. For isometric contractions performed at a maximal level, EMG amplitude was consistently found to decline throughout the fatigue protocol.

The relation between force and EMG in non-fatiguing contractions has been previously investigated (274). In the experiments presented in chapter IV, a non-fatiguing (20 s) submaximal level of muscle contraction (20% MVC) was used. Force level and EMG amplitude were found to have a significant level of correlation. A finding that confirmed the role of EMG as a good indicator for the muscle's level of activity.

In chapter III, two fatigue protocols with a maximal level of muscle contraction were used. One of these protocols was intermittent and the other consisted of a sustained contraction. It was demonstrated in these experiments that the intermittent protocol of exercises elicited a higher rate of decline in force, MDF and EMG amplitude compared to the continuous one. These findings were attributed to the higher level of muscle load and energy consumption with intermittent contractions (144, 313). Although the relation between ATP consumption and the development of fatigue is not well understood, and may not be direct, the difference in the rate of Ca^{2+} uptake at the SR or proton accumulation can account for the greater fatigue inducing ability of the intermittent protocol (294).

A significant proportion of patients with long thoracic nerve palsy suffer from residual deficiency in their shoulder function (108). It has been our observation as well as others that despite the improvement in the control of scapular movements, many of these patients continue to complain about fatigability and a sense of instability (83, 259, 329). These symptoms are particularly common with overhead or strenuous activities. A hypothesis has been proposed that reinnervated muscles would be more fatigable. Three protocols of muscle fatigue have been used by patients with reinnervated serratus anterior and normal controls and EMG was analysed. Examination of the changes in MDF revealed no difference between reinnervated and normal serratus anterior muscle, indicating no difference in muscle fatigability. The rise in EMG amplitude was delayed and less marked in reinnervated muscles compared to normal. This difference can be attributed to the lack of the strong fast contracting MUs, difference in the central drive to the reinnervated muscles or a lower level of force exertion (86).

During the serratus anterior fatigue protocols subjects were instructed to produce their maximum voluntary force during forward flexion of their arms in the isokinetic machine and forward pushing against a wall. Encouragement was given during these exercises and visual feedback about level of force from the isokinetic machine. As these exercises have produced an increase in EMG amplitude it can only be assumed that the serratus anterior muscle was loaded at a submaximal level.

Experiments have been conducted to compare fatigability of reinnervated first dorsal interosseous muscles following ulnar nerve palsy compared to control using intermittent and sustained contractions. In these experiments no difference in mechanical or myoelectric fatigue parameters was observed between reinnervated and normal hands. Interestingly, reinnervated hands were found to have a lower force generating capacity when compared to the contralateral hand. This difference was significant and indicated weakness of the reinnervated muscles. Reduction in muscle cross sectional area and consequently its ability to develop force has been established in previous animal experiments (103). A comparison between human reinnervated biceps muscles and contralateral control revealed obvious weakness but similar fatigability when each side exerted the same percentage of its own MVC (41).

Therefore, it can be concluded that weakness is at least partially responsible for some of the deficiency observed in the function of reinnervated muscles.

The work presented in the second part of this thesis was directed to investigate the relation between sensory function and the motor performance of the hand. Two sets of experiments were designed to examine the effect of sensory loss on the ability of the subjects to maintain a submaximal level of force and to perform a fine motor task.

In the first experiment there was no difference in the subjects' ability to maintain a submaximal level of force before and after block of the median nerve function i.e. even in the absence of cutaneous sensation, muscle receptors have provided adequate feedback to allow the subjects to maintain their level of force. This would suggest a more major role for muscle receptors in controlling performance of the hand than previously thought. An interesting side observation has arisen from this study in regard to the subjects' ability to maintain a submaximal level of force. In the absence of visual feedback subjects were found to gradually drop their pinch grip force level with time (over 20 s). This drop of force was significantly different from zero and was present before and after median nerve block, which were not different. This represents a shortcoming of the human motor control system as visual feedback is not normally present in daily life activities (85).

The second experiment in chapter IV was designed to investigate the effect of cutaneous anaesthesia on the hand's ability to perform a fine motor task. In this chapter an electronic method for assessment of hand performance has been developed. Handwriting has previously been used in neurological patients to examine the severity of tremors and psychological development in children (140, 183, 210, 222, 250, 307). For the first time, in this experiment handwriting has been used to examine the kinematics of finger movements (85). Parameters of handwriting like jerk, detour, length of writing strokes and force exerted on the recording digitizer have been compared before and after median nerve block. Significant increase in NARJ, length of the writing strokes and duration of test was observed following nerve block. These findings have confirmed the pivotal role of cutaneous sensation to the control of fine hand manipulation.

Finally, the work presented in this thesis was an effort towards a better understanding of the effect of denervation-reinnervation process on muscle and hand sensory-motor functions. Like other research projects, this work has answered some questions, and presented ideas. However, in muscle fatigue and denervation there are many controversial issues that will take long before a consensus is reached. From ethical and statistical views the experiments on this thesis were conducted as pilot studies. The spread of the data and differences between groups were not available prior to this work, therefore, formal power calculation of the sample size was not possible. It will be possible for future research in this area to use data presented in this thesis.

VI References

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VII Appendix

```
{***********************************************
 **                                           **
 **    THIS IS AN OASIS EXAMPLE MACRO      **
 **                                           **
 **    VERSION 1.0  7-4-'95          **
 **    CHECKED      9-1-'96          **
 **                                           **
 **    USING THE DIGITIZER INTERACTIVELY    **
 **                                           **
 ***********************************************}


(Request
 Centered Off "Start"
 (ConCat
  "This FOLLOWIT example macro shows you how "
  "you can interact with OASIS through the digitizer.||"
  "Start it and follow the instructions. It is you task "
  "to follow the blue circle. When you hit it with the "
  "pen it will move to the next location."
 )
)

{global routines}
(SET

{get information; group, subject, and session numbers}
 GetInformation
 '(DO
   {build special purpose requester}
   (OpenWindow
    Centered
    "Experimental information"
    34 11 LightGray On
   )
   {start with defaults}
   (SET
    GroupNr  1
    SubjectNr 1
    SessionNr 1
   )
   {create all gadgets}
   (PutIntegerGadget 1 "Group Number   : " 4 3 26 GroupNr)
   (PutIntegerGadget 2 "Subject Number : " 4 4 26 SubjectNr)
   (SetGadgetHotKey  2 4)
   (PutIntegerGadget 3 "Session Number : " 4 5 26 SessionNr)
```

```
  (SetGadgetHotKey  3 9)
  (PutButtonGadget  4 "Accept"        4 8  8)
  (PutButtonGadget  5 "Stop"         24 8  6)
  {proces user actions}
  (ActivateGadget 1)
  (LOOP
   (SET HitID GetUserAction)
   (IF
    (= HitID 1) (SET GroupNr   (GetIntegerGadget 1 GroupNr   1  9))
    (= HitID 2) (SET SubjectNr (GetIntegerGadget 2 SubjectNr 1 99))
    (= HitID 3) (SET SessionNr (GetIntegerGadget 3 SessionNr 1  9))
    (= HitID 4) BREAK
    (= HitID 5) STOP
   )
  )
  {store information}
  (SetGroupNr   GroupNr)
  (SetSubjectNr SubjectNr)
  (SetSessionNr SessionNr)
  {close requester}
  CloseWindow
)

StandStill
'(DO (SET StandTime 0.0
       ReqTime   (Random 0.5 2.0)
    )
    (LOOP
     {get a new sample}
     NextPenSample
     {reset when not still}
     (IF (AND (< PenV 1.0)
              (> PenZ 10.0)
         )   (SET StandTime (+ StandTime PenDT))
         ELSE (SET StandTime 0.0)
     )
     (IF (> StandTime ReqTime) BREAK)
    )
)

RecordStroke
'(DO
    {target position}
    (Set
     Target  (Droplet (+ (MOD (- StrokeNr 1) 4) 1) TargetXY)
     TargetX (Droplet 1 Target)
     TargetY (Droplet 2 Target)
     TargetR (Droplet 3 Target)
    )
```

```
    {draw target}
    (FillMode Blue SolidFill CopyBlit)
    (Circle TargetX TargetY TargetR)

    {drop a marker}
    (SetMarker "New Target Shown")

    {record until a certain distance is covered}
    (LineMode Black SolidLn ThickWidth CopyBlit)
    (LOOP
     {remember old position and validity}
     (SET OldPenX   PenX
        OldPenY   PenY
        OldPenDown PenDown)
     {get a new sample}
     NextPenSample
     {draw it}
     (IF (AND OldPenDown PenDown)
       (Line OldPenX OldPenY PenX PenY)
     )
     {lift within target}
     (IF (AND
         PenDown
         (< (Abs (- TargetX PenX)) TargetR)
         (< (Abs (- TargetY PenY)) TargetR)
       ) BREAK
     )
    )

    {clear viewport}
    (ClearView White)
  )
)



{open the pen file}
(Set FileName "FOLLOW0.PEN")
(Loop
 (Set FileName (DestRequest FileName))
 (IF (= FileName "") StopRequest
   ELSE (BREAK (SetPathExtension FileName ".PEN"))
 )
)
(OpenNewPenFile OutputFile FileName)
(EnterPenFile OutputFile)

{start digitizer}
StartDigitizer
```

```
{get a pen space and enter it}
(CreatePenSpace MyPen)
(EnterPenSpace MyPen)

{invoer van de proefpersoon informatie}
(EVAL GetInformation)

{goto graphics mode}
DefaultVGAMode
(ClearView LightGray)
{put a large view on the screen without a title}
(PutViewGadget 1 ""  3 2 (- GetScreenWidth 4) (- GetScreenHeight 4))

(SET
  TargetXY
  '('(22.5 15.0 0.3)
    '(23.5 17.0 0.3)
    '(24.5 15.0 0.3)
    '(25.5 17.0 0.3)
   )
)

(PutWinTextBox
   2 (- GetScreenHeight 1) (- GetScreenWidth 2)
   "Start [FOLLOWIT] with the <Enter> key."
   JustifyCenter Black LightGray
)
{wait for enter}
(LOOP
  (IF KeyPressed (IF (= ReadKey "[Enter]") BREAK))
)

(PutWinTextBox
   2 (- GetScreenHeight 1) (- GetScreenWidth 2)
   "I am waiting for you to put the pen on the digitizer..."
   JustifyCenter Black LightGray
)

{enter view gadget and size it}
(EnterViewGadget 1)
FullDigitizerView
ExitViewGadget

{start after pen is on tablet}
StartTracking
(Eval StandStill)
StopTracking

(PutWinTextBox
   2 (- GetScreenHeight 1) (- GetScreenWidth 2)
```

```
  "I am now recording....."
  JustifyCenter Black LightGray
)

{start recording}
(EnterViewGadget 1)
StartRecording

{loop for some seconds}
(SET StrokeNr 1)
(LOOP
  {do a trial}
  (SetRecordNr  StrokeNr)
  (Eval RecordStroke)
  {increase trial number}
  (Inc StrokeNr)
  {stop after seven trials}
  (IF (> StrokeNr (* 4 (SizeOf TargetXY))) BREAK)
)

{stop recording and also exit tracking}
StopRecording

(PutWinTextBox
  2 (- GetScreenHeight 1) (- GetScreenWidth 2)
  "That was all, thank you....."
  JustifyCenter Black LightGray
)

{save it}
WriteNewPenRecord
```

Software Analysis Macro

```
{get pen space and enter it}
(CreatePenSpace MyPen)
(EnterPenSpace MyPen)

{open data output file with protection}
(LOOP
 (SET FileName (DestRequest "RESULT.DAT" "select destination for output"))
 (IF (= FileName "") StopRequest
   ELSE     BREAK
 )
)
(SetWriting DataFile FALSE FileName)

{Request the user to select files that are to be
analysed and create a complete list of those files}
(SET
 UserList (FileListRequest "*.PEN;*.INK" On GetCurrentDir)
 DirFiles (Until " " UserList)
 PenFiles (After " " UserList)
)
(IF (= DirFiles "") STOP)

{loop through all pen files and analyse them}
(LOOP
  {are we finished?}
 (IF (= PenFiles "") BREAK)
 {eat the first pen file name}
 (Set
   Name      (Trim (Word " " PenFiles))
   PenFiles  (Sentence " " PenFiles)
   Path  (ConCat DirFiles Name)
 )
 {open it for reading and enter it}
 (OpenOldPenFile MyFile Path)
 (EnterPenFile MyFile)
 {tell the world what we are doing}
 (PutWinTextBox
  1 10 GetScreenWidth
  (ConCat "Workin On [" Path "]")
  JustifyCenter Red White
 )
 {loop through all pen records and analyse them}
 (Set RecordNr 1)
 (LOOP
   ReadPenRecord
   (PutWinTextBox
     1 11 GetScreenWidth
     (ConCat "Working On Trial " (Text RecordNr))
```

```
 JustifyCenter Green White
)
(SET
 StrokeNr 1
 StartT 0.0
 Done FALSE
)
(LOOP
 {find a stroke}
 StartReplaying
 (GotoPenSample StartT)
 (LOOP
  NextPenSample
  (IF
   IsLastPenSample (BREAK (SET Done TRUE))
   (CheckForMarker "New Target Shown") BREAK
  )
 )
 (SET FinishT PenT)
 StopReplaying
 {analysis}
 (SET Result (GetSamplesPropertyRange StartT FinishT Directness))
 (SET Result2 (GetSamplesPropertyRange StartT FinishT TrajectLength))
 (SET Result3 (GetSamplesPropertyRange StartT FinishT Duration))
 (SET IntJerk 0.0)
 StartReplaying
 (GoToPenSample StartT)
 (LOOP
  (INC IntJerk (* (Abs PenJ) PenDT))
  (IF (>= PenT FinishT) BREAK)
  NextPenSample
 )
 StopReplaying
 {ceate output}
 (WriteText DataFile
  (Text GetGroupNr 6) " "
  (Text GetSubjectNr 6) " "
  (Text GetSessionNr 6) " "
  (Text RecordNr 6) " "
  (Text StrokeNr 6) " "
  (Text Result 16 8) " "
  (Text Result2 16 8) " "
  (Text Result3 16 8) " "
  (Text IntJerk 12 8) "|"
 )
 {next stroke?}
 (IF Done BREAK)
 (INC StrokeNr)
 (SET StartT finishT)
)
```

```
    {goto the next pen record}
    (IF IsLastPenRecord BREAK)
    NextPenRecord
    (Inc RecordNr)
  )
)
```