In the Name of God

the most Compassionate and the most merciful

Anterior Tibial Muscle Function Assessment Using Acoustic Myography and Electromyography

A Thesis Submitted for
the Degree of Doctor of Philosophy
in the Faculty of Medicine.

By

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Dedicated to my family

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Abbreviations

A/D	Analogue to Digital
AMG	Acoustic Myography
ANOVA	Analysis of Variance
CED	Cambridge Electronic Design
CNS	Central Nervous System
DC	Direct Current
EMG	Electromyography
FFT	Fast Fourier Transform
FM	Frequency Modulation
HP	Hewlett-Packard Microphone
Hz	Hertz
IAMG	Integrated Acoustic Myography
IEMG	Integrated Electromyography
Kg	Kilogram
LFF	Low Frequency Fatigue
MVC	Maximal Voluntary Contraction
μsec	Microsecond
mmHg	Millimetre Mercury
msec	Millisecond
mV	Millivolt
N	Newton
RMS	Root Mean Square
PC	Personal Computer

Sec	Second
SEM	Standard Error of Mean
V	Volt

Summary

This thesis describes a series of experiments comparing the electrical and acoustic signals recorded from the human tibialis anterior muscle.

Electromyography (EMG) is a widely used method of monitoring muscle activity. In many muscles its amplitude increases linearly with force and changes in the EMG/force ratio or shifts of the median frequency provide evidence of fatigue. However, in some circumstances it is difficult or impossible to record the EMG satisfactorily e.g. if there is sweat on the skin or in strong electrical fields or during electrical stimulation.

Acoustic myography (AMG) is a more recent development. It is a non-invasive technique which may also be used as an indicator of skeletal muscle activity. Transverse oscillations of the muscle surface are detected with microphones or accelerometers. Their performance is not affected by sweating or electrical stimulation artefacts. The AMG is a much simpler signal than EMG and because it has a very narrow bandwidth it is easy to filter out noise. Like EMG, it has been found that the AMG increases with force but it is also known that, contrary to the EMG, the amplitude of AMG declines with force during fatiguing activity.

The experiments described here investigate the relationships between the EMG, AMG amplitude and force in normal muscles and during muscle fatigue. In addition, the characteristics of the frequency spectra of EMG and AMG were investigated. The effect of muscle length on EMG and AMG characteristics were also studied. The contribution of blood flow to the AMG was studied by comparing the signals recorded with and without blood flow in the lower limb.

A linear relationship was found between rectified integrated EMG (IEMG) and force in fresh and fatigued muscle. The slope of the relationship increased with fatigue. A similar relationship was found between the IAMG and force in the range 0-75% of maximum voluntary contraction in control conditions. However, the slope of the relationship between IAMG and force declined after fatiguing exercise.

The EMG spectra from the tibialis anterior contained frequencies between 0 and 400 Hz. The median frequency increased linearly as the force of muscle contraction increased. The EMG median frequency decreased during sustained contractions as fatigue developed. However, the AMG contained a range of frequencies between 0 and 45 Hz. The median frequencies of the AMG also increased linearly with increasing force. However, the AMG frequency content was not significantly changed if fatigue developed at low forces such as 40% of maximum voluntary contraction but the median frequency declined significantly when the muscle was fatigued at forces above 60% of maximum.

Changes in the length of tibialis anterior affected the force development, EMG and AMG characteristics. At shorter muscle lengths, the maximal voluntary force is reduced compared to the intermediate and longer lengths and the slope of the relationships between force and IEMG and IAMG increases. There were no significant differences in force or the relationships between force and IAMG and force and IEMG between the intermediate and longer lengths. There were no significant changes in the median frequencies of EMG and AMG at different muscle lengths.

There were no significant changes in the characteristics of AMG and EMG when the blood flow to the lower limb was stopped by inflating an pressure cuff. It can be concluded that the contribution of blood flow to the AMG and EMG was insignificant.

In conclusion, the AMG represents a mechanical counterpart of the electrical activity in muscle fibres. The IAMG and the AMG median frequency may be used to provide indirect information about force. Analysis of changes in the IAMG/force ratio or AMG median frequency might be used to identify the development of fatigue during contractions above 60% of maximum voluntary force.

INTRODUCTION

General Introduction

Evaluation of muscle function is one of the most important features of muscle and nerve pathophysiology. It is also important in rehabilitation, medicine and sports in which a therapist, clinician or trainer must be easily able to evaluate the muscle at rest or throughout remedial strengthening exercises. One of their aims is to prevent or minimise the side effects of exercises or harmful diagnostic testing.

Many diagnostic tests require considerable training of personnel or the use of expensive equipment or are painful and time consuming for the patient. Typical examples of the these are: intramuscular needle electromyography, electrical stimulation to establish the strength duration curve or nuclear magnetic resonance spectroscopy. Each of these has some advantages and some disadvantages or difficulties. For instance, intramuscular EMG study is unpleasant and may be harmful for the subject. In addition, direct force recording can be very difficult in some muscles e.g. erector spinae or some cranial muscles. There is a clear need for a better monitor of muscle function.

Acoustic myography (AMG) is the study of transverse mechanical oscillation from contracting skeletal muscle (Barry, 1987, Frangioni, Kwan-Gett, Dobrunz and McMahon, 1987, Wee and Ashley, 1989). Its widespread scientific investigation started in the 1980s. In comparison with other testing techniques, AMG is non-invasive, cheap and may be easier to apply than EMG or force recording (Barry, Geiringer, and Ball, 1985, Barry, Leonard, Gitter and Ball, 1986).

In this thesis human muscle function was investigated in different experiments with different aims. It was carried out in combination with two reliable tools associated with studies of muscle contraction, namely surface electromyography (EMG) and measurement of force.
The tibialis anterior muscle was investigated. It is one of main dorsiflexors of the ankle joint. This muscle is characterised by parallel muscle fibres, simple mechanics, a superficial location and high radius of surface curvature. In addition, the common peroneal nerve which supplies this muscle is accessible and easy to stimulate. Another reason which led to the selection of this muscle is the high incidence of injury which occurs in this muscle and its nerve. Physiotherapists will be better able to manage the rehabilitation of a patient if they can understand the nature of problem.

Three variables, force, EMG and AMG, were investigated before and after intermittent and during sustained fatiguing contractions. In addition, they were studied at different muscle lengths and during ischaemia.

1. Review of literature

1.1. The background history of the study of acoustic myography

The first report about the generation of sounds by contracting skeletal muscle was by Francesco Maria Grimaldi, an Italian Jesuit priest, in his book Physicomatheis de Lummine (Grimaldi, 1665). His work was a treatise on light and he is famous for his description of the diffraction of light, but he was also interested in acoustics. He found that a low rumbling sound is audible when subject stops his ears with his thumbs and clenches his fist. He attributed this sound to "the hurrying motion of animal spirits".

Nearly 150 years later, in 1810, another report of muscle sound was made by William Hyde Wollaston, a physicist, chemist and physician. He attributed this sound to contraction of skeletal muscle and stated that the muscle sounds increased with strength of contraction. To estimate the frequency of sounds, he used two methods. The first method compared the muscle sounds he heard when he placed his thumb in his ears and clenched his fist with the noise generated by rubbing a round piece of wood over a notched board. The notches were of equal size, and by rubbing along them at different speeds, he was able to estimate the muscle sounds frequency by knowing the space of notch and rate of rubbing.

In the second method, he was also able to estimate the frequency by comparing the muscle sounds with the rumbling sounds of a horse carriage drawn over the regularly spaced bricks of London's streets at various speeds until the noise matched the rumbling sound he heard through his thumbs. The frequency range of muscle sounds from both of his methods, was between 14 and 36 Hz. He pointed out that all muscles of human body produce the same kind of sound. Another early report was by the German anatomist, Helmholtz in 1864. He stated that the rumbling sound appeared to relate to oscillations of muscle fibres and his estimation of muscle sound frequency was in the same range as that of Wollaston. Muscle sounds were recorded from jaw muscles by Marey (1874). He found that they were audible during clenching of teeth. He also pointed out that with increasing biting force, the intensity of sound increased.

In 1885, Herroun and Yeo compared the sounds from voluntary muscle contractions with those emitted during electrical stimulation of muscle. The first modern recordings were made by Gordon and Holborn (1948). They used a small piezo-electric microphone and suggested that the increase in diameter of muscle fibres during contraction was the origin of the muscle sounds. They believed that the radial expansion could cause a pressure wave that could spread to the skin surface.

1.2. Scientific historical study of acoustic myography

After 1810 the phenomenon of muscle sounds was largely ignored for about a century and a half. Occasionally, some scientific publications described their presence as noise that interfered with listening to other sounds in the body. The reasons for this lack of investigation were:

1- Muscle sounds had been detected by mechanical stethoscopes which are maximally responsive to sounds at about 200 Hz but are practically unresponsive at about 20 Hz. Newer stethoscope designs solved this problem.

2- Detection of the muscle sound was usually contaminated and complicated by confusion with ambient vibrations. The low frequency sounds associated with machinery, footsteps, and traffic noises are more difficult to filter out than are high frequency sounds (e.g. speech). These recording difficulties were solved by advances and availability of electronic sensors, e.g. piezoelectric transducers, condenser microphones and accelerometers. In addition, computerised signal processing techniques were introduced which allow time and frequency domain analysis of acoustic myogram signals.

The first report of scientific investigations into muscle sounds described experiments using an electronic stethoscope (Oster and Jaffe, 1980). The microphones in most electronic stethoscopes rely on piezoelectric crystals which can convert pressure waves directly into electricity. In the biceps brachii contracting under various loads they found the dominant AMG frequency was 25 ± 2.5 Hz.

They also found that there was no difference between sounds which were recorded during voluntary contraction and during electrical stimulation. They pointed out that muscle sounds were not affected by microphones scraping on the skin, by blood flow or by temperature effects. They showed that the intensity of sounds increased with increasing force. They claimed that the most intense muscle sounds were emitted by fast twitch fibres. They found a linear relationship between AMG, EMG and force. In his second paper, Oster (1984) hypothesised on the origin of muscle sounds and suggested that muscle sounds might be responsible for heart sounds. In this paper he pointed out that the sound from soleus muscle, which contains predominantly slow twitch fibres (Johnson, Polgar, Weightman and Appleton, 1973), is nearly ten times more intense than that from the gastrocnemius during normal standing. This might reflect the extent of muscle activation rather than the fibre types.

1.2.1. Technical problems during acoustic myogram recording

Recording of any bio-electric signal e.g., EMG, presents some difficulties such as extraneous environmental noise. These noise sources need to be minimised to optimise the signal-noise ratio. The simplest techniques are shielding or filtering of signals. Recording AMG presents fewer problems than recording EMG but there are some factors which make the sounds difficult to detect. These include excess adipose tissue which muffles the muscle sounds and prominent bony edges which prevent a good skin-microphone coupling (Barry et al, 1985).

Many different types of transducers have been used to record muscle sounds. The most important characteristic of the recording apparatus is the frequency response of the transducers. It has to be sufficiently sensitive to frequencies between 1 and 100Hz since almost all the AMG signal is in this range (Bolton, Parkes, Clark and Sterne, 1989).

An important guideline in choosing a transducer to record muscle sounds is the ratio between its mass and that of muscle under investigation. The transducer mass must not interface with the oscillations at the muscle surface. Very light accelerometers such as the Entran EGAY-25D or Dytran 3115A which weigh about 0.5g, have been very useful, particularly in studying of small muscles (Barry, Hill and Im, 1992, Keidel and Keidel, 1989). Another advantage in using accelerometers is that the measurement is made in physiological units (m/s^2) rather than transducer dependent units such as mV (Barry et al, 1992).

The larger piezoelectric contact transducers such as the Hewlett-Packard 21050-A, weighing 44g, are often used over the muscles with greater mass (Orizio, Perini, Veicsteinas, 1989, Wee and Ashley, 1989). It is important to mention that the output of the contact sensor depends on the magnitude of the applied force coupling the transducer to the muscle surface. The optimum force is usually thought to be about 200 g (Barry, 1992, Orizio et al, 1989). The transducer frequency response is flat across the bandwidth of the acoustic myogram.

Secure attachment is needed to avoid any movement of the transducer relative to the muscle surface (Stokes, Moffroid, Ruch and Haugh, 1988). Care has to be taken when the microphone or the sensor is strapped over the muscle. If the fixing band is applied too tightly, too much pressure may act on the sensor during muscle contractions, particularly at higher levels of force and this can cause saturation of microphone. These problems can be overcome by using a compliant strap.

Accelerometers are not influenced by contact pressure. However, they are less suitable than microphones for recording muscle sounds during sustained contractions because the AMG signals can be contaminated by limb tremor (Smith and Stokes, 1993). During repeated contractions, the variability of AMG is greater than that of EMG (Stokes et al, 1988; Lee, Stokes, Taylor and Cooper, 1992). The total signal intensity of AMG is much more variable than its frequency content (Orizio, Perini, Diemont, Figini and Veicsteinas, 1990).

The principal factors which can affect the magnitude of AMG signals are:

1. the relative positions of microphone and muscle. When the sensor is placed over the tendon instead of the belly of muscle, the magnitude of the AMG is reduced (Bolton et al, 1989; Stokes and Dalton, 1991).
2. the contact pressure between sensor and the belly of muscle (Bolton et al, 1989; Orizio et al, 1989).

1.3. Skeletal muscle structure and function

Skeletal muscles maintain posture and cause movement. They are activated by their motoneurones. Muscle fibres vary in length from a few millimetres up to 20 centimetres and their diameter ranges between 10 to 100 μm.

Anatomical muscles are formed by a number fibres bound together by connective tissue and usually connected to the bone via a bundle of collagen fibres, named tendons, which attach at either end of muscles. In some muscles, the muscle fibres may extend almost the entire length of the muscles. In most muscles the fibres are shorter than the apparent muscle length and may be oriented at an angle to long axis of muscles. The longitudinal arrangement of muscle fibres is more suitable for movement than force generation. The oblique orientation is more suited for force generation. Muscles vary in shape and size but they share in a common operation, namely contraction. Most muscles have a shape in which their fibres converge rather than lying parallel to each other. This convergent arrangement of muscle fibres tends to produce fusiform or pennate muscle shape. Penniform muscles, which are flat and sometimes bipennate or multipennate, seem to have the most mechanically efficient shape for force generation. This configuration also restricts the operating range of muscle lengths. Force is transmitted from muscle to bone via the connective tissue and tendons.

The tibialis anterior muscle, which is located on the lateral side of leg, arises mostly from the upper half of the lateral surface of the tibia and also from the adjoining part of anterior surface of the interosseous membrane. The fibres run downwards and terminate in a strong tendon on the anterior surface of the muscle at the lower third of the leg. The tendon reaches the dorsum of the foot. After passing through both extensor retinacula and turning round the medial side of foot it inserts into the medial and plantar surface of the medial cuneiform bone and into the base of first metatarsal bone of the great toe (Romanes, 1987, Gray, 1991).

The tibialis anterior muscle, which is one of main dorsiflexors of the ankle joint, can be fully activated by voluntary effort. Since its motoneurones receive relatively weak input from Ia fibres but relatively strong input from descending motor pathways, these can be completely activated during strong voluntary dorsiflexion (Belanger and McComas, 1981; Bigland-Ritchie, Furbush, Frank, Gandevia and Thomas, 1992). The tibialis anterior is composed of about 73% slow twitch fibres (Johnson, et al, 1973). This composition is very similar to soleus muscle, which is composed of more than 85% slow twitch fibres. This suggests that tibialis anterior might have a postural role. Furthermore, biochemical analysis has shown that this muscle is fatigue resistant (Jones, Turner, Newham, and McIntyre, 1993).

In an erect standing position, the line of gravity passes through a point just in the front of centre of the knee joints and also a point just in front of the ankle joints. To overcome and counteract this torque in the knee and ankle regions, the posterior capsule of the knee and soleus muscle, located in the posterior of leg, become tight and contract, respectively. Thus, the soleus muscle has to be a predominantly fatigue resistant, slow twitch fibre and tonic muscle. In contrast, from the bio-mechanical viewpoint the tibialis anterior might be expected to be used relatively little and so might be expected to have fast twitch and a majority of fatigable motor units. However, in situations which lead to a changed line of gravity in the lower limbs for a long time, the muscle may play an anti-gravity role. Perhaps co-contracting with soleus to stabilise the position of the ankle (Basmajian and De Luca, 1985). Dorsiflexing the ankle at about the time of heel strike during walking may contribute to its fatigue resistant nature.

1.3.1. Electrical activity in muscle

The surface of a resting skeletal muscle fibre displays no differences in electrical potential. However, the inside of the fibre is maintained at a negative potential of about -100mV. When the muscle membrane is depolarised a regenerative action potential is initiated in the muscle fibre membrane. This propagates along the length of the muscle fibre down the T tubules into the interior of muscle. An interaction with the sarcoplasmic reticulum causes release of calcium ions. This forms an important link in the chain of events leading from action potential in the surface membrane to the interaction of the actin and myosin and force development.

1.3.2. Motoneurone Size Principle

Motoneurone size plays an important role in the recruitment of motor units. The size of motoneurone refers to size of nerve cell body, which is usually correlated with the diameter of the its axon and does not refer to size of the motor units which it controls.

In most circumstances, motor units are recruited according to size, so that small units become active at low forces and larger units at higher forces. The recruitment sequences is maintained throughout a broad range of motor activity including isometric and isotonic contractions.

During weak effort small units are recruited, adding small force increments. During stronger efforts larger units are recruited so adding larger force increments. The different excitabilities of motor units ensure that the smaller units are active more often than the large units.

Henneman, Somjen and Carpenter (1965) stated the Size Principle as:

1. The small force, slow twitch motor units are innervated by small alpha motoneurones while larger, faster twitch muscle units are supplied by correspondingly larger motoneurones.
2. In a mixed muscle, recruitment gradually proceed from the smallest neurones, recruited first, to progressively larger neurones.

The human tibialis anterior muscle has about 445 motor units (Enoka and Stuart, 1985). The behaviour of motor units in the tibialis anterior has been studied in considerable detail (Macefield, Gandevia, Bigland-Ritchie, Gorman and Burke, 1993, Bigland-Ritchie, Furbush, Gandevia and Thomas, 1992). It seems that during isometric voluntary dorsiflexion two distinct neural mechanisms control the force development in the tibialis anterior, namely recruitment and firing rate. During isometric dorsiflexion, the motor unit which was recruited at the lowest force had a firing rate of 7.3 Hz. At 50% and 75% of maximal effort, its mean firing rate was 16.5 Hz and 22 Hz, respectively. At maximal voluntary contraction it was 28.2 Hz.

1.4. EMG characteristics during non-fatiguing contractions

The electromyogram recorded from active skeletal muscle results from summation of motor unit action potentials within range of the recording electrodes. Larger motor units make larger contributions to the EMG. Motor units farther from the recording site make only a small contribution to the surface signal. The EMG always increases with force but its relationship with force varies in different muscles. The presence of complex relationships between EMG and force can be due to physiological differences rather than differences in methodology (Woods and Bigland-Ritchie, 1983). The physiological phenomena contributing to the EMG/force relationship are the fibre type composition, fibre architecture, electrical cross-talk from adjacent muscles, or co-contraction of agonist and antagonist muscles. Motor unit recruitment patterns, firing rate properties and location of fast twitch fibres within a muscle can also alter the EMG force relationship.

Analysis of the EMG signal is complicated by the complex waveform of some motor unit action potentials.

Rectification of the EMG signal produces a uni-directional version of the original source signal and this simplifies the measurement of activity. Full-wave rectification is preferred because this method ensures that all the signal information is available for analysis. By combining the positive and negative halves of the source, any subsequent signal processing or analysis will take account of all variations in magnitude and shape of the elements contained in the source. Integration methods have been widely used for a considerable time (Basmajian and De Luca, 1985).

Complex waveforms such as EMG can be represented as a sum of sine waves with different frequencies. The Fast Fourier Transform (FFT) is commonly used to determine the frequency content of signals (Bergland,1969, Diemont, Maranzana-Figini, Orizio, and Veicsteinas, 1988). EMG has a wide frequency band, ranging from 0-400Hz. The precise frequency spectrum depends on the type of muscle under investigation (De Luca, 1984). In contrast, AMG contains a relatively narrower range of frequencies, between 0-50Hz (Orizio, et al, 1990, Mealing and McCarthy, 1991). Several parameters are already being used to analyse myoelectric signals. These are median, mean, mode and band frequency. The median frequency is defined as the frequency at which power spectrum is divided into two regions containing equal power.

The mean frequency is the average frequency and the mode frequency is the frequency at which peak energy is found in the spectrum. The median and mean frequency parameters were found to be most reliable and of these two the median frequency is preferred because it is less sensitive to noise (Stulen and De Luca, 1981). In the frequency bands analysis the energy concentration between two defined frequencies is measured. The frequency bands analysis seems to be reliable and can be more informative than the other parameters when working with complex waveforms.

1.5. IAMG and force relationship

The origin of muscle sounds is not yet known, but it is thought to reflect intrinsic mechanical activity of muscle (Oster and Jaffe, 1980; Barry et al, 1985) perhaps from transverse mechanical oscillations of muscle fibres (Barry et al, 1987; Frangioni et al, 1987). The relationship between AMG and force seems to vary in different muscles, but it is generally accepted that there is a positive correlation between IAMG and increasing contraction. This is shown in Table 1.

Table 1 shows a summary of the previous studies of the AMG-force relationship (1976-1993).

Author	Muscle	Transducer	%MVC	Relationship	Year
Stokes	AP	Microphone	0-100	Curvilinear	1992
Lammert	BB	Accelerometer	20-100	S-shaped	1976
Oster	BB	Microphone	0-50	Linear	1980
Barry	BB	Phonocardiograph	0-50	Linear	1985
Orizio	BB	Microphone	10-100	Parabolic	1989
Maton	BB	Microphone	10-100	Quadratic	1990
Zwarts	BB	Microphone	20-100	Linear	1991
Stokes	ES	Microphone	10-100	Quadratic	1988
Stokes	QF	Microphone	20-100	Linear	1991
Zhang	QF	Accelerometer	20-80	Linear	1992
Smith	QF	Microphone	20-100	Linear	1993
Smith	QF	Microphone	20-100	Non-Linear	1993
Rouse	TB	Microphone	20-100	Linear	1991

Table 1. The relationship between IAMG and force in different muscles. AP, BB, ES, QF and TB indicate adductor pollicis, biceps brachii, erector spinae, quadriceps femoris and triceps brachii, respectively.

The relationship between integrated AMG or root mean square AMG (rmsAMG) and force is described in several different ways by the authors listed in Table 1 on the previous page. There are 7 reports of a linear relationship and 6 of non-linear relationships of various sorts. This may be partly explained by experimental conditions, particularly by the type of transducer used. Many experiments were performed on the biceps brachii. In almost all experiments, subjects made isometric forearm flexion contraction between 0-100% MVC. Different AMG/force relationships were still reported. One major reason for this difference is the usage of different transducers with different characteristics and performance. The difference in AMG/force relationship may be also depend on a uniform or mixed composition and distribution within muscle. Several experiments were performed in a narrow range of contraction force (Oster and Jaffe, 1980, Barry et al, 1985). They found linear relationship between AMG and force. These results differ from some other experiments which carried out in whole range of contraction force of the same muscle. Thus, the range of contraction force can be the other reason for the AMG/force relationship. The AMG and force relationship seems to be less predictable in a smaller muscle, e.g., jaw elevator muscles. Stile and Pham (1991) showed that the amplitude of AMG of masseter and anterior temporalis increased to a maximum value at 5 or 10% MVC, and remained constant or decreased at higher forces. In general, all authors agree that AMG increases as force increases. The nature of the relationship is variously described as simple linear or more complex.

The increase in IAMG with force is thought to be due to recruitment of new motor units and increasing motor units firing rates (Orizio et al, 1989, Dalton and Stokes, 1991). The most controversial feature is the existence of a maximum in AMG at about 80% of MVC.

1.6. AMG frequency changes

The analysis of AMG frequency began early, by Wollaston (1810). He is the first one who estimate AMG frequency to be 14-36 Hz. The frequency range of the AMG was estimated by a frequency analyser and found that the greater part of the signal energy was below 100 Hz (Cerquiglini, Figura, Marchetti and Salleo, 1973). This result was reported for the gastrocnemius and quadriceps femoris.

With recent advances in laboratory instrumentation and computers more information has been obtained about AMG frequency content. The frequency content of AMG has been investigated by several authors. These are listed in Table 2.

Table 2 shows the summary of the previous studies in the AMG frequency characteristics of various muscles.

Author	Muscle	Transducers	MVC (%)	Frequency (Hz)	Year
Oster	BB	Microphone	10-50	25 ± 2.5	1980
Rhatigan	BB	Angiograph	0-100	15 ± 4.2	1986
Maton	BB	Microphone	0-100	15.5-22.2	1989
Wee	BB	Microphone	0-20	11.3	1989
Orizio	BB	Microphone	10-100	10-22	1990
Dalton	BB	Microphone	0-50	6-14.1*	1993
Dalton	BB	Microphone	0-50	6.9-10**	1993
Mealing	OO	Microphone	MVC	22 ± 5	1991
Dalton	QF	Microphone	10-100	7.1-16.9	1993
Zhang	RF	Accelerometer	20-80	11-19	1992
Herzog	RF	Accelerometer	70	25 ± 9	1994
Mealing	RF	Microphone	80	7.5-10	1990
Mealing	S	Microphone	MVC	10.8 ± 3	1991
Rouse	TB	Microphone	20-100	12-15	1990
Herzog	VL	Accelerometer	70	40 ± 7	1994

Table 2. The frequency measurement of AMG in different muscles. BB, OO, QF, RF , S, TB and VL reveal biceps brachii, orbicularis oris, quadriceps femoris, rectus femoris, soleus, triceps brachii and vastus lateralis, respectively. * and ** indicate concentric and eccentric contractions.

The differences in the AMG frequency measures between various investigations may be partly explained by experimental conditions, particularly by the type of transducer used. The difference may also be partly due to differences in muscle size which leads to a difference in resonant frequency. Alternatively, differences in fibre composition and distribution within muscle may be important. Variation in the AMG frequency content can also be explained by different types of frequency analysing software and different time windows. Several frequency parameters such as bands, mean, peak and median frequencies, were used by authors. In addition, using different types of contractions and different contraction forces led to different AMG frequency contents. Keidel and Keidel (1989) showed that during maximal isometric voluntary contraction of tibialis anterior, biceps brachii, masseter and wrist extensor muscles, the AMG frequency spectrum is spread from 1-49 Hz. They also found several peaks in this range which do not support the concept "of just one stable frequency of, e.g., 10 Hz".

The frequency of the AMG signal, may be affected by a combination of factors, including the physical properties, stiffness of muscle, the force developed, the motor unit firing rate, physiological tremor, the density and elasticity of tissue, muscle temperature and distortion of sound wave at the tissue/air interface.

1.7. Skeletal muscular fatigue

The term fatigue is itself complex and has a number of different meanings. Fatigue was defined as "failure to maintain the required or expected force " (Edwards, 1981). The definition which is most generally accepted in human exercise is "the failure to maintain an expected power output". Fatigue is also defined as "any reduction in the force-generating capacity of the entire neuromuscular system, regardless of the force expected" (Bigland-Ritchie and Woods, 1984). Vollestad, Serjersted, Bahr, Woods and Bigland-Ritchie, (1988) suggested a distinction between fatigue and exhaustion. Their definition of exhaustion was " an inability to sustain contractions/exercises at the target force/intensity".

Several experiments were performed to find the answer of two general question about fatigue:

1. Where is the site of failure during the contraction process?
2. What is the nature of change that causes the impairments in force development?

Fatigue can be caused by impairment of any one of, or combination of, the links in the command chain between CNS and muscle.

In general, fatigue has been subdivided into central and peripheral components. Central fatigue is the failure of neural drive which causes a reduction in the numbers of active motor units or a reduction in the firing rate of active motor units. Peripheral fatigue is the failure of force generation in muscle. It results from the failure of neuromuscular junction, muscle action potentials or impaired excitation-contraction coupling. Localised muscular fatigue has been defined by Chaffin (1973) as " an inability to maintain a desired force output with augmented muscular tremor and localised pain". This definition differentiates peripheral fatigue from central fatigue. Merton (1954) showed that fatigue can be the result of processes occurring entirely within muscle fibres. Peripheral fatigue can be divided into low and high frequency fatigue categories. These are associated with direct muscle stimulation frequencies of 20 and 80 Hz, respectively (Edwards, Hill, Jones, and Merton, 1977). Low frequency fatigue (LFF) thought to be a result of excitation contraction coupling failure. It is generally long lasting and also more pronounced after eccentric contractions. The activities of every day life are mostly the result of submaximal contractions induced by low frequency activity of motor units. High frequency fatigue processes have a more rapid onset and the force recovers more rapidly than that in low frequency fatigue. High frequency fatigue is most probably due to neuromuscular junction block (Stephens and Taylor, 1972) or impairment of muscle action potential propagation (Bigland-Ritchie, Jones, and Woods,1979, Jones, Bigland-Ritchie and Edwards 1979).

During all voluntary contractions the higher motor centres are active so that failure of some central mechanism during the course of sustained contraction can lead to a sense of fatigue or exhaustion. Motivation of subject and the provision of visual feedback during exercise is crucial.

Twitches or tetani superimposed on the voluntary contraction have been very useful to investigators for differentiating peripheral muscular fatigue from lack of motivation (Merton, 1954).

1.8. IEMG and IAMG changes during muscle activity

It has been shown for several skeletal muscles that as fatigue develops during submaximal isometric contractions, the amplitude of the rectified integrated EMG signals increases in order to maintain the same force output (Edwards and Lippold, 1956, Merlleti et al, 1990). This increase in amplitude is more pronounced near the end of sustained contraction and is a result of either recruitment of fresh motor units (Edwards and Lippold, 1956), or synchronisation of motor unit action potentials (Milner-Brown, Stein and Lee, 1975). The relationship between IEMG and force is shifted to the left when muscle becomes fatigued as EMG/force ratio rises (Komi and Vitasolo, 1977). Stephens and Taylor (1972) showed that the smoothed EMG of first dorsalis interosseous (FDI) declined with force during sustained MVC. They concluded that the decrement was due to neuromuscular junction failure.

Similar experiments have been performed to investigate the AMG during sustained contractions. In these experiments, subjects made isometric contractions of biceps brachii of an initial force of 75% MVC (Barry et al, 1985). This was sustained until fatigue had reduced the force to 35%

of the initial MVC. The AMG with surface EMG were compared to detect any dissociation of electrical from mechanical events, i.e. a loss of excitation-contraction coupling. They stated that the rmsAMG amplitude was affected and declined in parallel with the force, whereas the rmsEMG did not change. Their EMG results are in contrast with most generally accepted literature which describes an increase in EMG/force ratio with fatigue. They concluded that the rmsAMG correlates better with force, than does rmsEMG during sustained contractions. They also found that during prolonged intermittent exercise, the rmsAMG increases. They claimed that the increment in the rmsAMG during prolonged intermittent fatigue activity can be attributed to increased physiological tremor, better efficiency of the AMG transmission or perhaps recruitment of new motor units.

In other studies of sustained exhausting activity in a series of contractions at 20, 40, 60, or 80% MVC of biceps brachii, three different trends were identified (Orizio et al, 1989). EMG/force ratio, increased at all four force levels, which clearly showed fatigue had developed. However, the authors found that it is difficult to make any single statement about AMG/force ratio. During contractions at 20% MVC, the IAMG increased though the force remains constant. The IAMG fluctuated around a steady value during contractions at 40% of MVC. During higher force contractions, they observed that the IAMG decreased in a non-linear fashion. After exhaustion at 20% MVC, the AMG amplitude was 5 times greater than its control values, whereas after exhaustion at 60% and 80% MVC it was about 4.5 times less than their control values. These results show that if fatigue develops slowly, for instance at 20% MVC, the AMG amplitude tends to increase but if fatigue develops rapidly, e.g., at 60% MVC, the AMG reduces.

It has been found that the electro-mechanical dissociation is evident "not only when the force decreases with time as was shown by Barry et al (1985), but also when force output is maintained constant" (Orizio, et al, 1989).

The AMG changes were investigated during contractions at 10% (Keidel and Keidel, 1989) and 50% MVC (Zwarts and Keidel, 1991) in the biceps brachii sustained until exhaustion. These results were similar to Orizio's in that, at lower forces, the amplitude of the AMG increased but at higher forces it did not change significantly. In a study of the abductor digiti minimi (Goldenberg, Yack, Cerny and Bunton, 1991), the AMG was recorded during contractions at 15, 25, 50, and 75% MVC sustained to exhaustion. They reported a considerable increase in the rmsAMG at 15 and 25% MVC, whereas a clear reduction was shown at 50% MVC. There was only slightly decrement in the rmsAMG at 75% MVC.

Stokes and Dalton (1991) studied the IAMG and IEMG during intermittent fatiguing activity of rectus femoris. Fatigue was induced by repeated voluntary contractions, initially at 75% MVC continued until only 40% MVC could be maintained. The contractions each lasted 10 seconds with a 10 second interval. At the end of the period of activity, the slope of the regression lines between force and IEMG increased which confirmed fatigue had developed. However, the slope of regression line between force and IAMG was unchanged. Cooper, Stokes, and Jayson (1991) recorded the AMG and EMG during sustained contractions of the lumbar erector spinae. They showed that the AMG remains unchanged in the normal and low back pain groups, whereas the values of EMG increased significantly.

It can be concluded that a comparison between AMG and EMG may also supply reliable information about electro-mechanical dissociation during sustained and intermittent fatiguing activity at different force levels. It could be used as an indirect fatigue index.

1.8.1. The EMG and AMG frequency changes

It is clear that as fatigue develops there is a shift in the surface EMG median frequency towards lower values (Stulen and De Luca, 1982, Merletti et al, 1990). The frequency shift in EMG can be used to identify muscular fatigue. In particular, it has been shown that the frequency spectrum of the EMG signal becomes narrower with fatigue, losing parts of the high-frequency content and so shifting towards a lower frequency range. This phenomenon can be quantified by calculating the median or mean frequency. This shift is most pronounced near beginning of a sustained contraction. The decrease may be up to 50% in value from the beginning to the end of a sustained constant contraction. It is attributed to slowing of the muscle conduction velocity which is related to muscular fibre diameter and also to intramuscular pH.

Changes in EMG median frequency have also been shown to be influenced by blood flow occlusion within the sustained contracting muscle (Merletti, Sabbahi, and De Luca, 1983). The reduction of median frequency in contractions under ischaemic conditions is related to the accumulation of acidic by-products. There is a similar trend between the decrease of EMG median frequency and muscle pH during sustained contractions (De Luca, Sabbahi, Stulen and Bilotto,1982). They also showed that the shift of the median frequency in slow twitch muscle is less than in fast twitch muscle. When blood flow is restored to the muscle, median frequency recovers quickly (Merletti et al, 1983).

IAMG has also been used to measure force and monitor fatigue (Barry et al, 1985; Frangioni et al, 1987; Goldenberg et al, 1991; Orizio et al, 1989, Stokes and Dalton, 1991). However, there are few study which deal with the effect of fatigue on the AMG frequency content. Keidel et al (1989) observed an increase of the AMG power frequency in the bandwidths of 8-18 and 20-30 Hz during isometric contraction of biceps brachii. Goldenberg et al, (1991), showed a frequency shift in the AMG towards lower values from the beginning up to the end of 50% MVC of abductor digiti minimi sustained to exhaustion.

A new investigation of frequency content during sustained isometric exhausting contraction was carried out on the biceps brachii (Orizio, Perini, Diemont and Veicsteinas, 1992). In this study the mean frequency of the AMG was calculated at different forces.

During sustained contractions at 20% of MVC the mean frequency of the AMG remains unchanged. At higher force levels (e.g., 80% MVC), after an initial increase in its frequency content, the AMG spectrum shifted towards lower values. They observed that the changes in the AMG frequency spectrum are similar to changes in motor units firing frequency during contraction.

Zwarts and Keidel, (1991) did not find a clear frequency shift in the AMG during contractions of 50% MVC of biceps brachii sustained to exhaustion . However, at maximal voluntary contraction, they showed a shift in mean frequency of the AMG to lower values. Similar experiments were recently performed at 70% of MVC sustained isometric contraction in the quadriceps femoris (Herzog, Zhang, Vaz, Guimares and Janssen, 1994). The median frequency of AMG shifted towards lower values from the onset to the end of contractions. The average shift was from 40-19 Hz for rectus femoris and from 25-12 Hz for vastus lateralis.

1.9. Aims of investigation:

The present study is to extend the previous works by investigating the amplitude and frequency changes of the AMG and EMG over the human tibialis anterior.

The aims of investigation are as follow:

1. To study the relationship between force, IAMG and IEMG in fresh and fatigued muscle.
2. To investigate the relationship between AMG and EMG median frequency and force.
3. To investigate the changes of EMG and AMG amplitudes during intermittent and sustained contractions.
4. To study the EMG and AMG frequency during sustained contractions.
5. To investigate the EMG and AMG amplitude and frequency spectra at different muscle lengths.
6. To study the effect of blood flow on the amplitude and frequency spectra of EMG and AMG.

MATERIALS and METHODS

2. Materials and methods

2.1. Experimental subjects:

The experiments were performed on 46 subjects aged 19-44 years, both male and female, with no history of neurological disease or musculoskeletal abnormality. Each subject did not perform all experiments. Details of numbers of subjects in each type of experiments are given at the appropriate places in the result section. All subjects gave their informed consent in accordance with a protocol approved by the Western Infirmary research ethics committee.

2.2 Experimental set up:

Each subject was comfortably seated in a chair with their right leg held in a rigid frame. The leg was immobilised by adjustable clamps with the tibia vertical and the ankle and knee at 90°. The general experimental set up is shown in Figure 1.

A force transducer was mounted underneath the foot plate. This allowed the dorsiflexion force developed by the subject to be measured directly. The whole force frame was fixed to a heavy metal plate to stop any movement.

Figure 1. The general experimental set up. The instruments which include: 1. PC, 2. 1401 interface unit, 3. Force amplifier, 4. Opto-isolation EMG system, 5. Neurolog, 6. Multi channel chart recorder, are shown in the left side. The position of subject and the microphone and EMG electrodes are seen in the right side. The left leg of subject was fixed to a supporting rigid frame. The knee and the ankle were positioned at 90° by two clamps.

2.3. Instructions to subjects and visual feedback:

Prior to any experiments, the purpose of the work and the type of contractions required were described to subjects. They were also taught to use the monitor screen or oscilloscope to enable them to maintain the required force for a specified time. "Target" forces were indicated either on a monitor or on a storage oscilloscope or chart recorder to provide visual feedback for the subjects. In addition to the visual feedback the subjects were encouraged strongly to maintain the required contraction of muscle.

2.4. Recording protocol

Throughout of all experiments force, EMG and AMG from the anterior tibial muscle were recorded simultaneously.

2.4.1 Force

The isometric dorsiflexion torque at the ankle joint was measured with a load cell (maximum load 250 Kg, RS Components, UK).

At the beginning of all experiments the maximal voluntary dorsiflexion force was measured. Subjects were asked to perform three maximum isometric contractions, each of which should be maintained at least for 2 seconds (Edwards et al, 1977). The greatest force was identified as the maximal voluntary contractions (MVC) and then the submaximal contractions were expressed as percentages of MVC.

The strain gauge was calibrated to confirm its linearity. The temperature of the lab was controlled between 22-25°C. Figure 2 shows the calibration curve obtained.

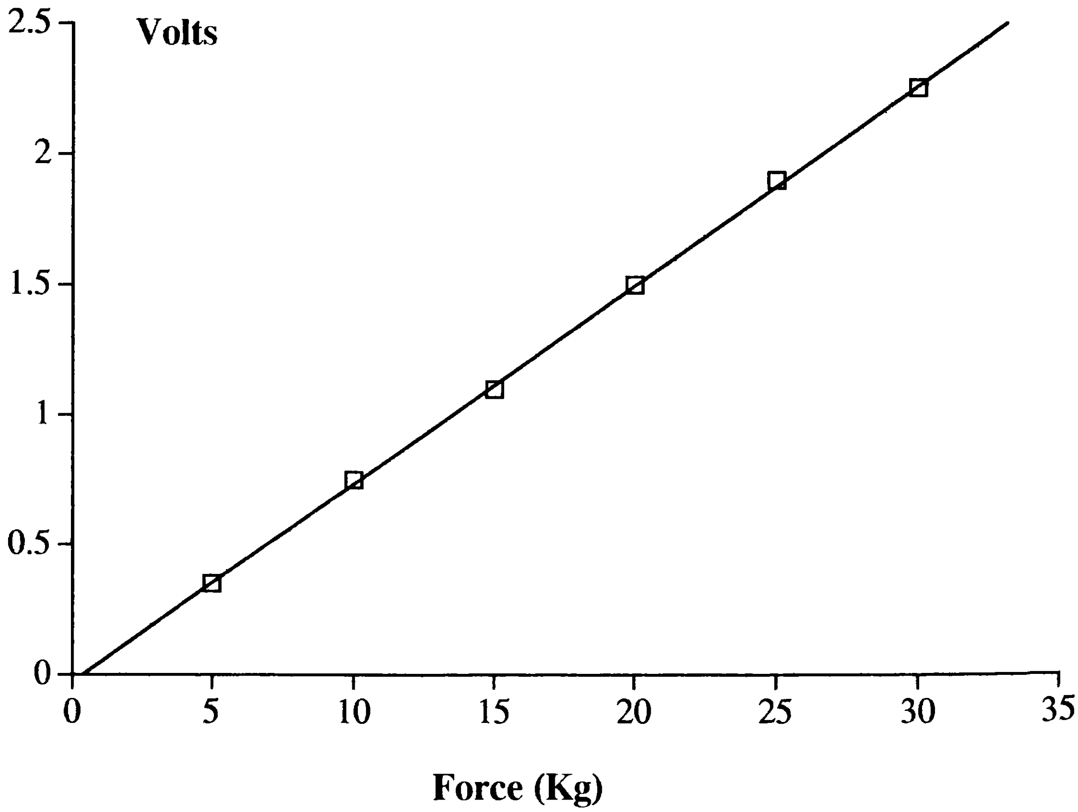

Figure 2- The calibration curve of the strain gauge.

2.4.2. Electromyogram

EMG was recorded using three surface metal foil electrodes (Littman 2325VP 3M Ltd). These are disposable single use adhesive electrodes for diagnostic purposes.

Figure 3 shows the locations of these electrodes. The skin lying over tibialis anterior was shaved, cleaned with alcohol and abraded with fine sand paper. Two recording electrodes were placed immediately adjacent to the sensor and one earth electrode was placed over the head of fibula. The EMG signal was pre-amplified (x1000) by an optically isolated amplifier. The pre-amplifier was placed close to the subject so as to keep the wires from the EMG electrodes as short as possible.

2.4.3. Acoustic myogram

The acoustic myogram was recorded with a Hewlett Packard (21050-A) heart sound microphone (Figure 3). This is a relatively large microphone, and contains a piezoelectric, crystal microphone. It has a contact surface about 14mm in diameter and weighs about 44 grams. The microphone is sensitive in the frequency range 0.02-2000 Hz. The subject was asked to contract the tibialis anterior and the middle of muscle belly was identified and marked. The middle of the of belly the muscle was the best point to obtain good contact between the sensor and the skin which lies over the muscle (Bolton et al, 1989, Stokes and Dalton, 1992). The microphone was strapped over the middle of the tibialis anterior using a rubber band. The surface of tibialis anterior is not greatly curved, neither does the profile change much on contractions.

Thus, microphone position is not much changed during isometric contractions. The microphone records transverse accelerations of muscle during activity.

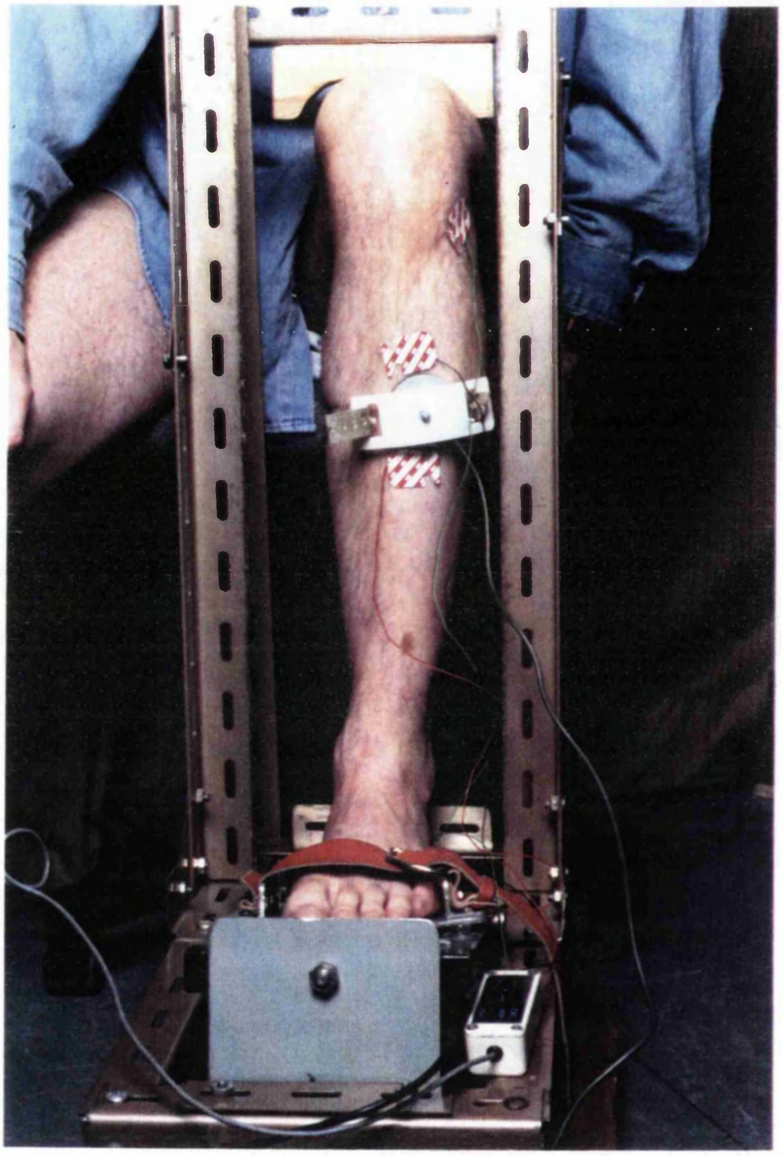

Figure 3- Shows the location of the microphone and the recording electrodes of EMG on the belly of the tibialis anterior muscle.

2.5. Signal processing and analysis:

The raw signals of force, EMG and AMG were stored on magnetic tape using a PCM-8. FM tape recorder. This provided a bandwidth of DC-3.5kHz in the 8-channel mode and DC-7kHz in the 4 channel mode. In addition, data was conditioned by further amplification and filtering before being analysed on line or off line using a CED 1401 interface unit and a Viglen 25MHz PC.

2.5.1.Force

Earlier experiments used a PC26AT card (Amplicon Liveline Ltd) to digitise signals. Later experiments used a CED 1401. Isometric force was displayed on a chart recorder or after a 12 bit A/D digitisation, stored in a PC. The minimum resolution of time and force was 8μsec and 0.005% MVC, respectively. Forces were measured using a cursor positioned on the force record of the computer screen.

2.5.2. EMG

EMG signals were subjected to two different types of analysis.

a. Rectified Integrated EMG

The EMG signal was band pass filtered between 10Hz-3kHz bandwidth and amplified using Neurolog NL 106 and NL 125 modules. A 50Hz notch filter could be switched into circuit to reduce unwanted mains noise.

The amplified EMG was subsequently full wave rectified and integrated using a Neurolog NL703 integrator unit. The time constant was set to 0.5 seconds. The rectified integrated signal was then digitised with a PC26AT A/D card (Amplicon Liveline UK Ltd) and displayed on a PC. The cursors were used to measure the amplitude of the rectified integrated EMG during periods when the muscle force was stable.

b. EMG frequency spectrum

Frequency spectra were calculated off line using CED Waterfall software.

Unfiltered EMG signals were stored on a PCM-8 magnetic tape recorder and replayed to a CED 1401 interface unit where they were digitised at 1024 Hz. The Waterfall package uses the Fast Fourier Transform (FFT) to establish the frequency content. The expected upper frequency limit of the EMG was about 400 Hz. A section of EMG lasting about 0.5 sec was selected for analysis using the vertical cursors.

The FFT calculation was performed on 512 points. A specimen spectrum is shown in Figure 4. Two variables were measured from the frequency spectrum: the median frequency and the bands frequencies.

The median frequency is defined as the frequency at which the spectrum is divided into two parts with equal power.

The median frequency was found to be less sensitive to noise and this was particularly useful for the signals that are recorded at low force level. In the frequency band analysis the energy concentration between two defined frequencies is measured.

The area of the spectra to be used for bands and median frequencies were specified with two vertical cursors. These frequencies lay between 0-500Hz. The percentage of energy in 50Hz bands was calculated so that the distribution of the energy at different frequencies could be displayed.

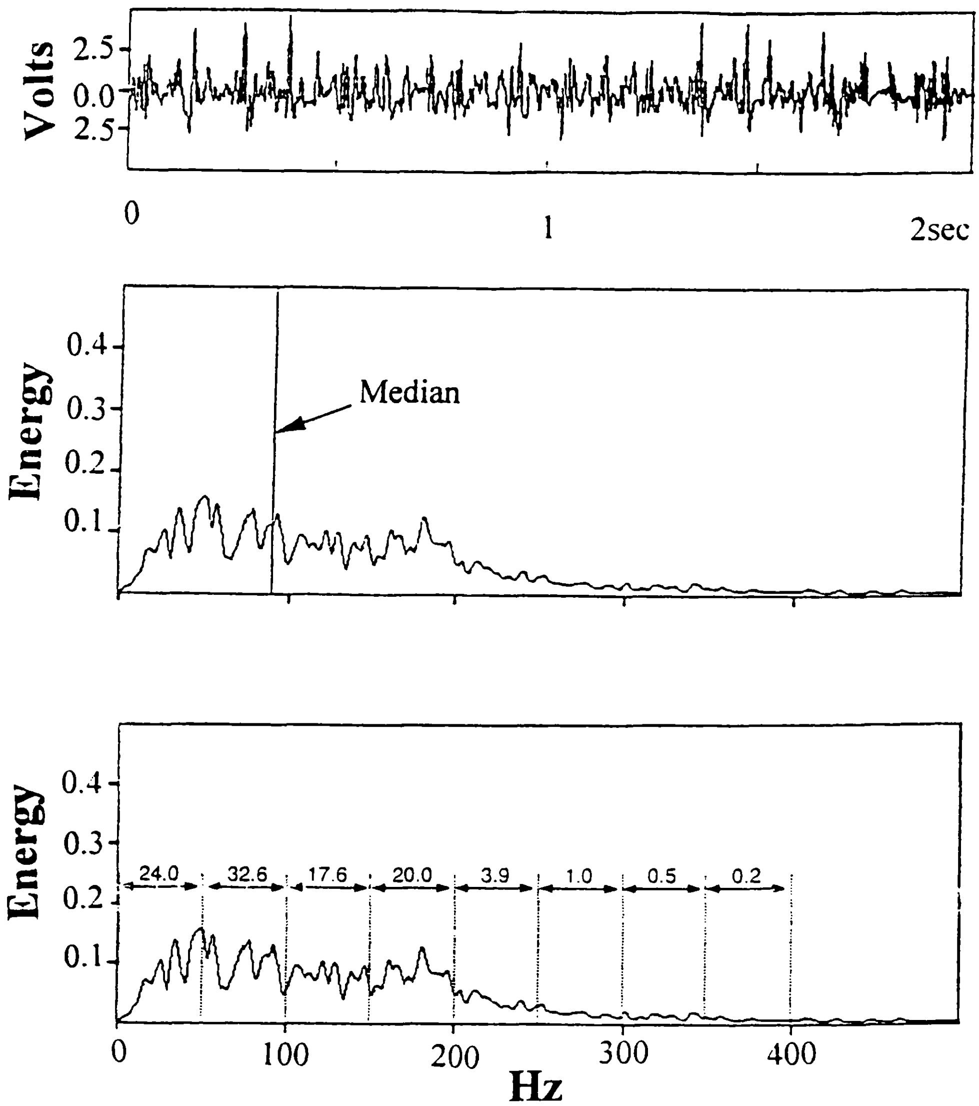

Figure 4. Typical spectral analysis of EMG. The upper panel shows two seconds of unfiltered, raw EMG data. Middle panel shows the median frequency at the cursor. The EMG frequency bands analysis is shown in the lower panel .The figure show the percentage of energy spectrum of EMG in each frequency bands.

2.5.3. AMG

Two different kinds of analysis were carried out on the AMG signals. They will be described in sequence.

a. Rectified Integrated AMG

The AMG signal was band pass filtered between 2 and 160Hz and amplified using Neurolog NL 106 and NL 125 modules. The filtered, amplified AMG was subsequently full wave rectified and integrated using a Neurolog NL703 integrator unit. The time constant was set to 0.5 seconds.

The rectified integrated signal was then digitised with a PC26AT A/D card (Amplicon Liveline UK Ltd) and displayed on a PC. The cursors were used to measure the amplitude of the rectified integrated AMG during periods when the muscle force was stable (Stokes and Dalton, 1991).

b. AMG frequency spectrum

The energy spectrum of unfiltered AMG signal was processed and analysed in the similar way to the EMG which was described in the 2.5.2 EMG (b) section.

Since the expected upper frequency limit of the AMG was below 50Hz (Goldenberg et al, 1991, Dalton and Stokes, 1993), a sampling rate of 256 Hz was chosen.

A section of AMG lasting about 0.5 sec was used for analysis using the vertical cursors. The FFT calculation was performed on 128 points. A specimen spectrum is shown in Figure 5.

Two parameters, the median frequency and the band pass filtered frequencies, were measured from the AMG frequency spectrum.

The area of the spectra to be used for bands and median frequencies were specified with two vertical cursors, this area was between 0-60Hz.

The median frequency of the AMG was calculated and displayed as described by Marchetti, Felici, Bernardi, Minasi, and Di Filippo (1992).

The percentage of energy in 5Hz bands was calculated so that the distribution of the energy at different frequencies was displayed (Orizio et al, 1990).

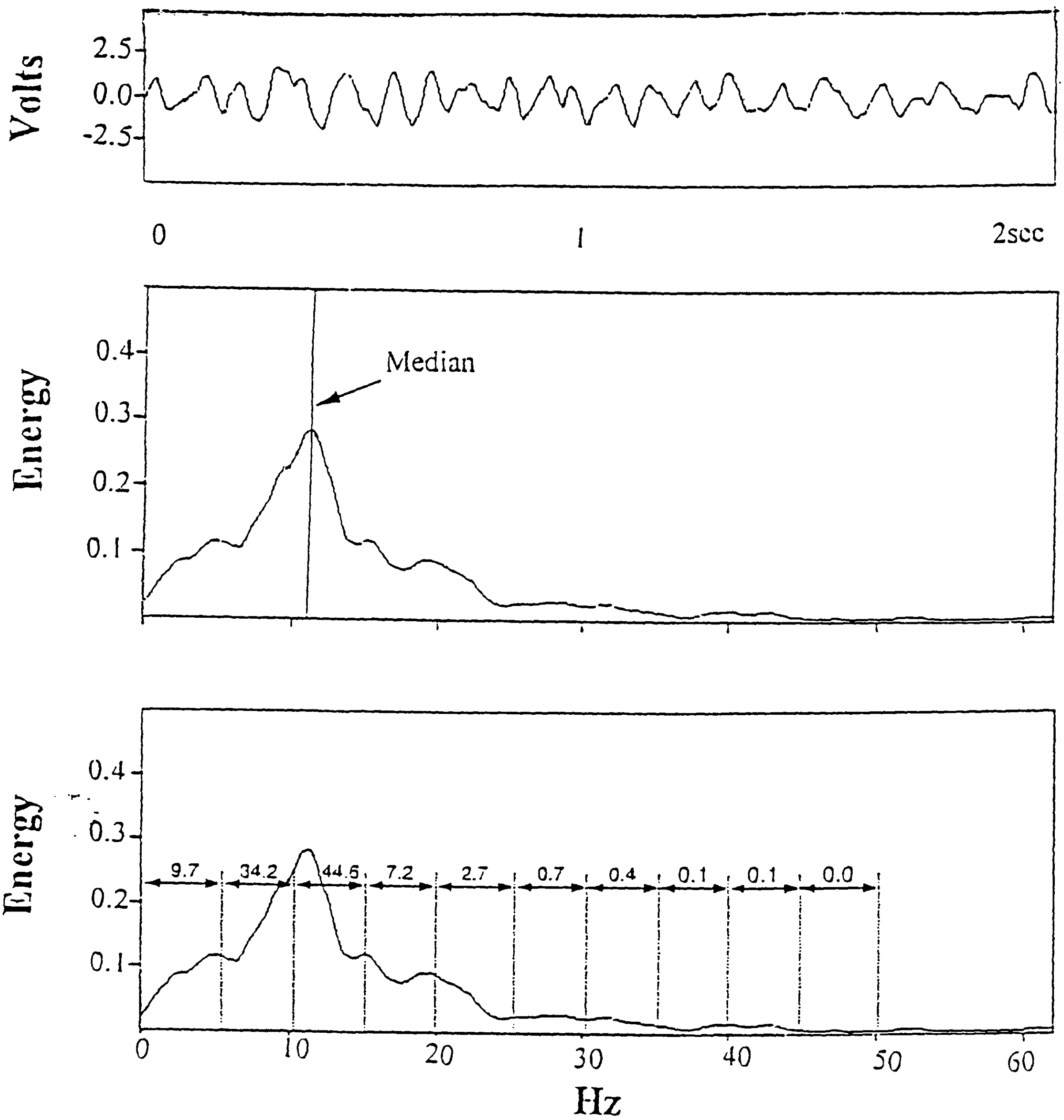

Figure 5. Typical spectral analysis of AMG is shown. The upper panel shows two seconds of unfiltered, raw AMG data. The middle panel shows the median frequency at the cursor. The AMG frequency bands analysis is shown in the lower panel. The figure show the percentage of the AMG energy spectrum in each frequency band.

2.6. Pattern of muscle activity

Several types of isometric muscle activity were investigated. Short periods of contraction lasting a few seconds with long rest periods at different force levels (25-100% MVC) were used to avoid fatigue. Contractions at 75% of MVC lasting a few seconds with short rest periods were performed to produce intermittent fatigue. Longer sustained contractions lasting up to 330 seconds were also used to investigate fatigue. In addition, contractions were made at different muscle lengths at different forces (20-100% MVC). Each of these will be described in detail in turn.

2.7. Isometric contractions of fresh muscle

After a period of familiarisation with equipment, an ascending series of contractions were made at 25, 50, 60, 75, and 100% of MVC. This was followed by a descending series at the same force levels. Each contraction lasted 6 seconds with 30 to 90 seconds intervals allowed for complete recovery. Longer rest periods were used with higher force levels to avoid fatigue. The absence of fatigue was confirmed by a constant EMG/force ratio.

2.8. Fatiguing activity:

Two types of fatiguing exercise were studied: intermittent and continuous contractions.

2.8.1. Intermittent fatiguing exercise at 75% MVC

The subject was asked to perform isometric dorsiflexion contractions at an initial target force of 75% of MVC. The contractions were held for 6 sec with 4 sec rest. The exercise continued after the subject could no longer maintain 75% of the starting MVC and was stopped when the force reduced 60% of initial MVC. The duration of this exercise was between 5 and 8.5 minutes. After the period of activity, a series of graded contractions, as described in 2.7, was repeated. Then, the AMG/force and EMG/force relationships was compared in fresh and fatigued muscle. Fatigue development was confirmed by increasing EMG/force ratios.

2.8.2. Continuous fatiguing exercise

Subjects were also asked to make sustained isometric contractions to exhaustion at different starting forces. Every volunteer visited the laboratory on 4 days over 2 weeks. At least 3 days of recovery was allowed between experiments. Good co-operation and motivation from the subjects was essential.
The experiments were performed on 8 healthy volunteers, aged 19-41 years. Each subject made three pre-trial tests to to determine the maximal voluntary contraction.

The subjects were required to exert and maintain a force corresponding to 40%, 60%, 80%, of their own MVC up to exhaustion. The contractions sequences were randomised. Each effort was sustained up to exhaustion and the subject was strongly encouraged to keep the force within 5% of given value. The experiment was repeated whenever greater fluctuations were present The contraction was stopped when subject was unable to maintain the required force.

2.9. Effect of blood flow

The blood flow in the lower limb could be stopped by inflating a blood pressure cuff placed around mid-thigh to 200mmHg. This was used to speed up the rate of onset of fatigue or to delay recovery after periods of exercise. A series of contractions, described in 2.8.1, was again repeated. Then, the relationships between EMG/force and AMG/force were compared in fresh and the occluded muscle.

2.10. The influence of muscle lengths

Experiments were performed using isometric dorsiflexion contractions at different muscle lengths to investigate any changes in EMG or AMG associated with longer or shorter muscle lengths.

2.10.1. Position of subject during experiments

The subject sat in the chair with left leg hanging freely and the other mounted inside a modified device for supporting the lower limb rigidly. A photograph of this is shown in Figure 6. The isometric dorsiflexion force of ankle joint was measured with the load cell, as described in section 2.4.1. The load cell is securely housed underneath a rectangular steel/aluminium footplate which is capable of moving through angles of 75°, 90° and 105° to the horizontal. The footplate is fixed into a very strong and rigid steel angle frame by two bearings which allow the footplate to swing and lock to the required angle for experimentation. The subjects' foot is securely strapped into the footplate by a strong leather band. The subjects lower leg is held at a 90° angle to the ankle by means of uprights which lock into place at the subjects' knee. Additional clamps to prevent the knee moving antero-posterior when knee is held at 90° and tibia is vertical. The subjects' right lower leg is now firmly locked into place and all voluntary motion of limb is negated.

The exercise protocols already described for the ankle at 90°, were repeated with the joint locked in the plantar or dorsiflexed positions.

Figure 6- The experimental set up for experiments at different muscle lengths. The subject was seated comfortably and the left leg was secured in a rigid supporting frame. The knee was fixed at 90° throughout of experiments and the angle of ankle joint could be fixed at 90° or 75° dorsiflexion or 105° plantarflexion.

2.11. Statistical methods:

Force, IAMG and IEMG were normalised to control values for each individual, expressed as percentages and then, the data from all trials at each level were combined to produce an overall mean value. Values indicated means ± SEM of the IAMG and IEMG and force for all subjects. During sustained contractions, force, EMG and AMG values of the beginning, midpoint and the end of contraction time were normalised to the maximum value of unfatigued muscle for each individual. Thus, the IAMG and IEMG were normalised to normal values at 80% and 100% of MVC, respectively.

In addition, when the ankle position is changed, the EMG, AMG and force are normalised to those at 90°. An analysis of variance (ANOVA) was applied to compare the results obtained during different muscle lengths.

Coefficient of correlation analysis was used to examine the IAMG/force and IEMG/force relationships before and after intermittent fatiguing exercise. The analysis was then performed to determine the relationship of AMG to force and EMG to force before and after arterial occlusion in fresh and fatigued muscle. The Student's paired t test was used to compare results obtained before and after exercise. Analysis of variance also was used to compare results obtained at different muscle lengths or to compare frequency spectra at different bandwidths. The formula $Y= aX+b$ provides a method of determining the slope, where a is gradient and b is constant.

RESULTS

3. Results

Results will be presented for 8 types of experiments:

1. The relationship between isometric force, AMG and EMG.
2. The extent to which muscle blood flow sounds contribute to AMG.
3. IAMG and IEMG changes during fatiguing exercises
4. Analysis of the frequency content of EMG and AMG in fresh muscle.
5. Analysis of frequency content of EMG and AMG in occluded muscle.
6. Analysis of frequency content of EMG and AMG in fatigued muscle.
7. Influence of different muscle lengths on the AMG and EMG.
8. A comparison of the performance of two types of transducers.

3.1 The relationship between isometric force, EMG and AMG

Seven healthy adult male subjects took part in this series of experiments. They gave their informed consent to the research and agreed fully to participate with the understanding that they could withdraw at any point during experiments. Experiments were performed after subjects had became familiar with the techniques. The design of the experiments is described in section 2.2 of the Materials and Methods. Each contraction lasted 6 seconds. The signals were filtered and full wave rectified and integrated with a time constant of 0.5 seconds.

Typical records of force, EMG and AMG as the subject makes a series of contractions up to maximal voluntary contraction, are shown in Figure 7.

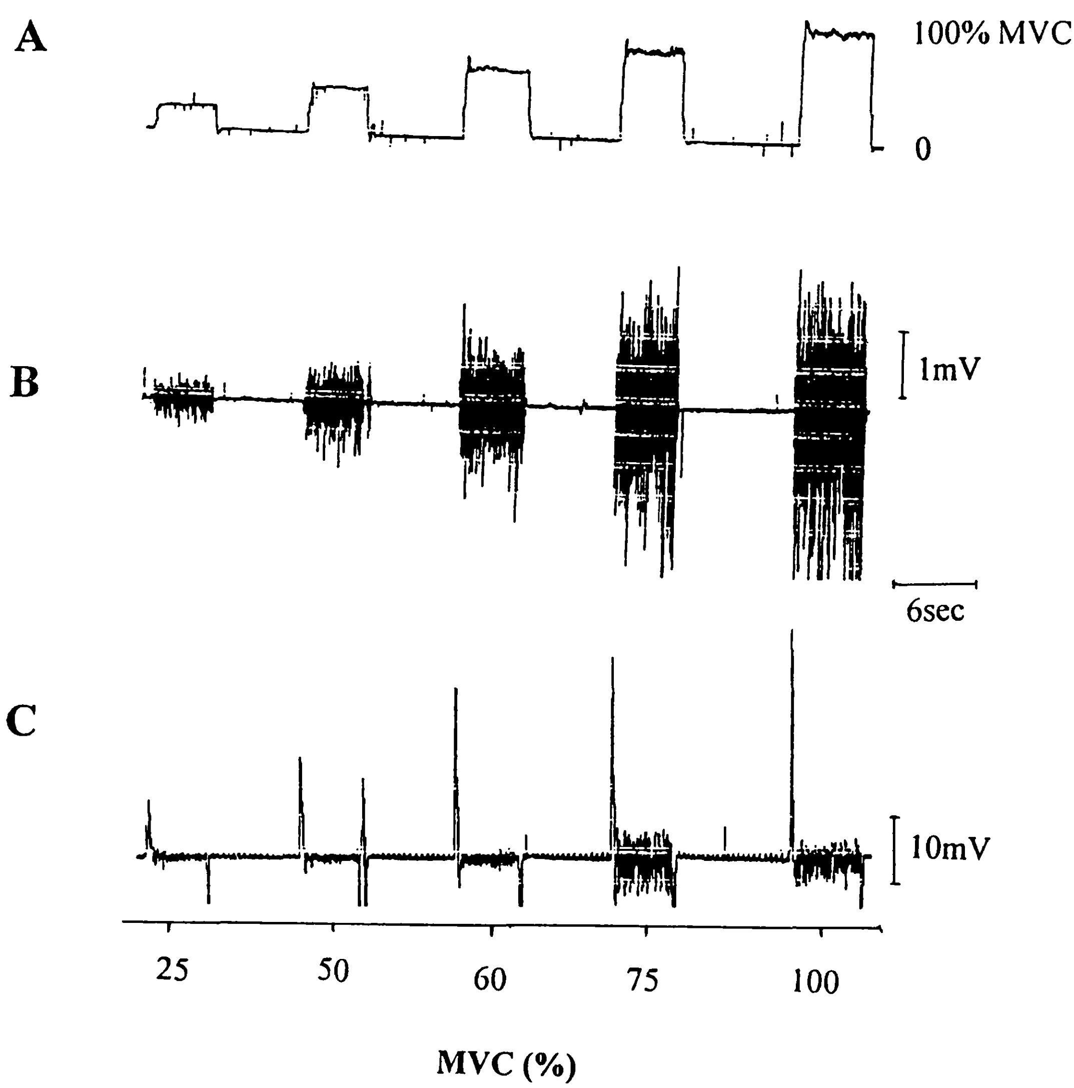

Figure 7. Simultaneous force (A), EMG (B) and AMG (C) recordings during a series of graded isometric contractions in one subject. Contractions were made at 25, 50, 60, 75 and 100% MVC. Each contraction lasted 6 seconds. Movement artefacts are seen at the beginning and the end of each contraction in the AMG trace. Arterial pulse waves can be seen between muscle contractions.

In this case the subject maintained the required force well in the lower force range. But at the highest force level some small oscillations were seen. The raw EMG peak to peak amplitude rises with increasing force up to MVC.

Before the first contraction begins, small regular oscillations synchronised with the heart beat, and probably with arterial pulse wave can be seen. The pulses at the end of contractions are larger than those at beginning of contractions. At onset of contraction, there is a rapid transient artefact as the muscle begins to move. This passes off in about one second and is then followed by the AMG. This is superficially very similar to EMG. There is a second movement artefact at the end of the contraction. Similar events can be seen in each of the subsequent contractions. The peak to peak amplitude of AMG increases with force up to 75% MVC so that the biggest AMG can be seen at 75% of MVC but beyond this force the amplitude of AMG declines.

The rectified integrated EMG and AMG were calculated at the mid-portion of isometric forces where they expected to be stable. Typical measurements of force, IEMG and IAMG are shown in Figure 8.

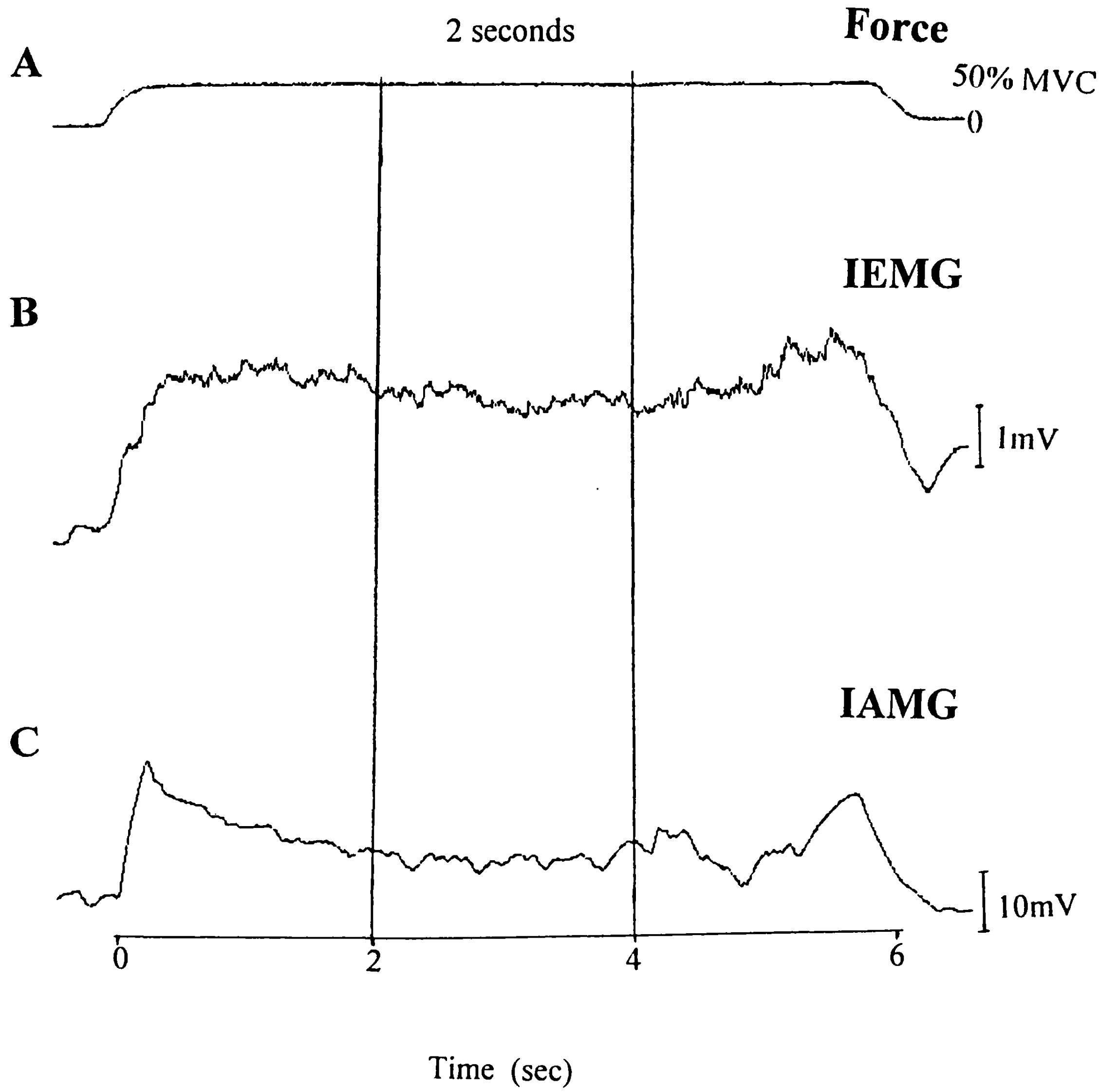

Figure 8. Typical measurement of force (A), IEMG (B) and IAMG (C) at 50% MVC. To avoid transient movement artefacts, the first and the last two seconds of 6 the seconds contractions were discarded. Section of EMG and AMG signals lasting 2 seconds were selected near the middle of contraction. This provides the most stable records for the IEMG and IAMG analysis.

The relationships between IEMG, IAMG and force in one subject are shown in Figure 9. The IEMG rises monotonically with force up to 100% MVC. The IAMG rises progressively up to 75% MVC and it declines when a maximal contraction is made.
Figure 10 shows summary data of mean (± SEM) of IEMG and IAMG for 14 contractions in 7 subjects. It shows a linear relationship between force and rectified integrated values of IEMG with correlation coefficient of 0.999.

The IAMG rises with increasing force up to 75% MVC and then it falls with larger force. There is a linear relationship between IAMG and force up to 75% MVC with correlation coefficient of 0.973. The IAMG includes an increased frequency of AMG spikes as well as the increased amplitude as seen in Figure 7.

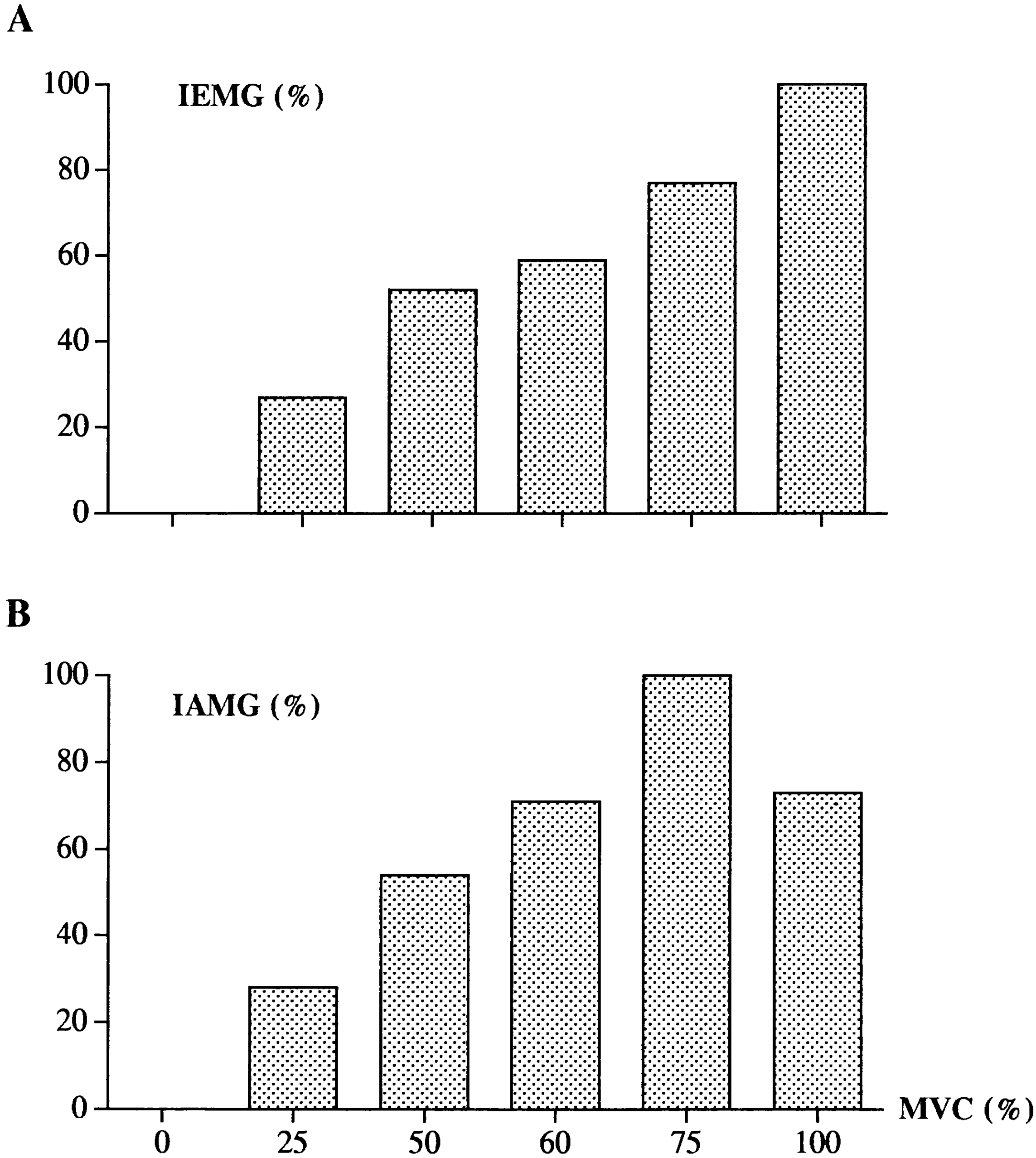

Figure 9. Plot shows the rectified integrated EMG (A) and AMG (B) against MVC in a series of contractions in one subject. The amplitude of IEMG and IAMG rise progressively up to 75% of MVC. At MVC, the IEMG increases whereas the IAMG declines.

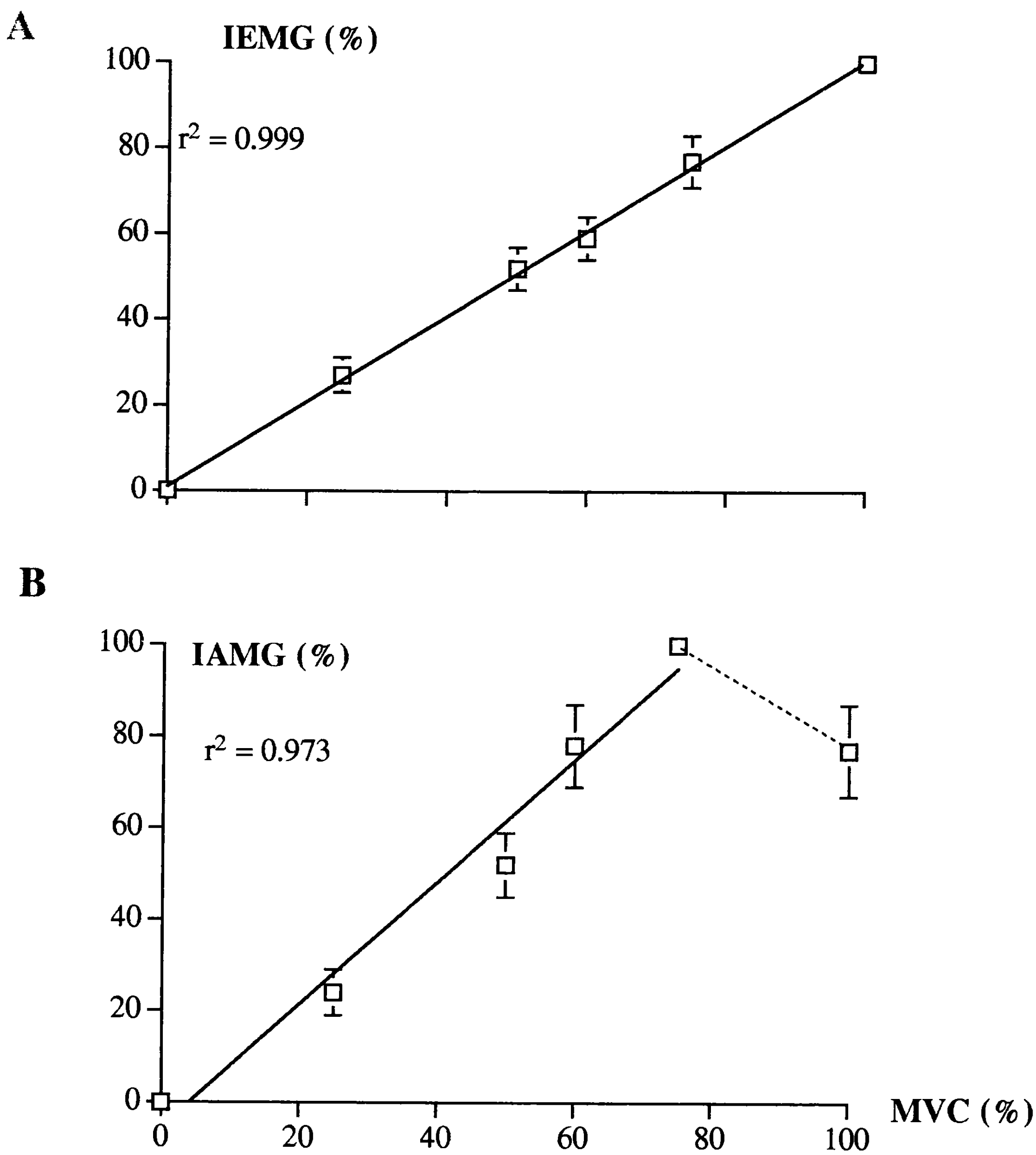

Figure 10. Mean of IEMG (A) and IAMG (B) plotted against force. Pooled data obtained from 14 contractions in 7 subjects. The error bars represent the SE of the mean. There was a linear relationship between IEMG and force up to MVC. The IAMG/force relationship also showed up to 75% of MVC. Beyond this force the IAMG declined.

3.2 Effect of blood flow on IEMG and IAMG

Figure 11 shows typical recordings of raw EMG as the muscle contracted at different forces before and after arterial occlusion. The contraction lasted 6 seconds with appropriate rest to prevent fatigue. The peak-peak amplitude of raw EMG signals increases continuously with force under both conditions.

Typical recordings of raw AMG with normal blood flow and in occluded conditions are shown at Figure 12. Arterial pulse waves can be seen between contractions in the AMG traces in Figure 12A. When blood flow to the muscle is stopped the arterial pulse waves disappears. There are two movement artefacts at the beginning and the end of contractions. The amplitude of raw AMG rises in parallel with increasing force up to 75% of MVC. Above this force the amplitude of AMG decreases in both conditions. After blood flow occlusion the peak-peak AMG amplitude is reduced. Figure 13 shows the IEMG and IAMG during a series of isometric contractions in one subject with normal blood flow and during occlusion. The IEMG rises with increasing force monotonically under both conditions. However, at all force levels there is a decrease in IAMG after occlusion but slopes of regression lines between IAMG and force are similar ($p > 0.05$). Figure 14 shows the mean IEMG and IAMG for 14 contractions in 7 subjects.

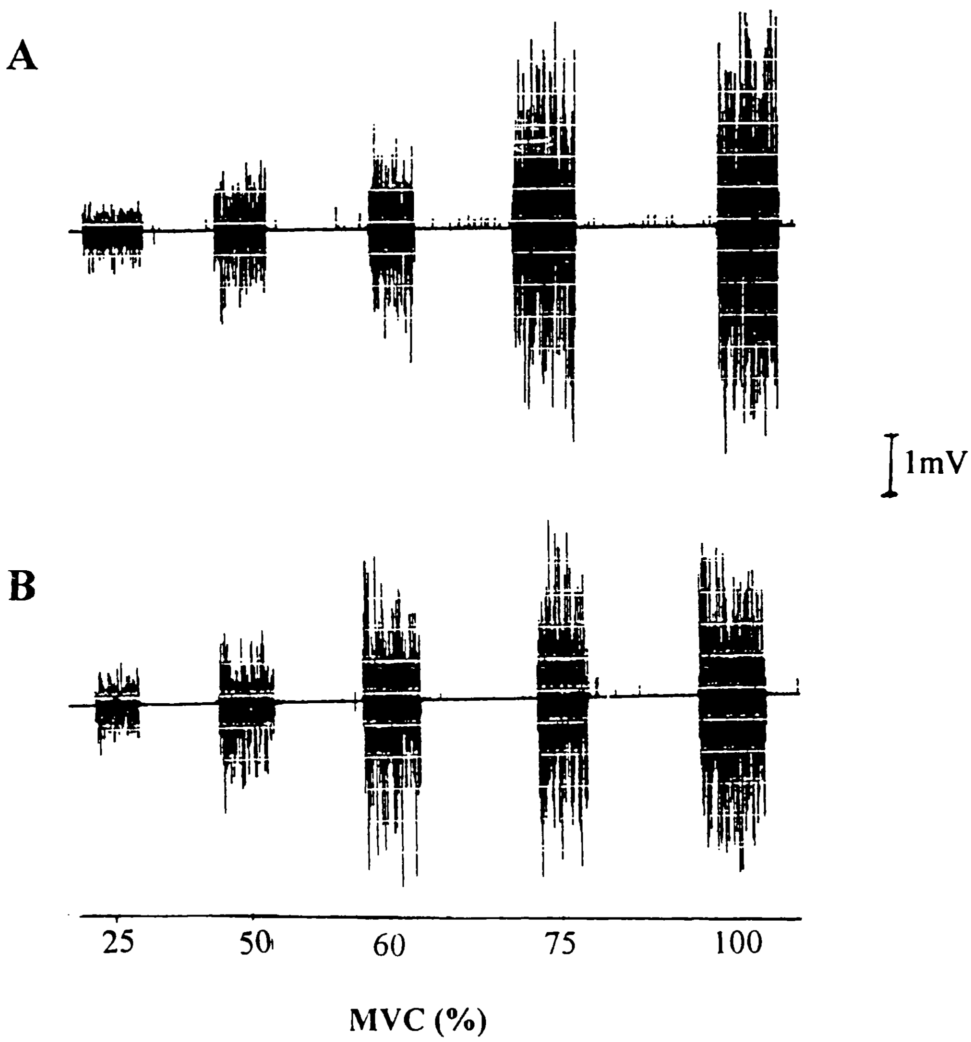

Figure 11. Typical recordings of raw EMG in normal muscle (A) and after blood flow has been stopped (B) are shown. Each contraction lasted 6 seconds. Contractions were made at 25, 50, 60, 75 and 100% MVC. When blood flow stopped to the muscle, the peak to peak amplitude of the raw EMG decreased at higher forces.

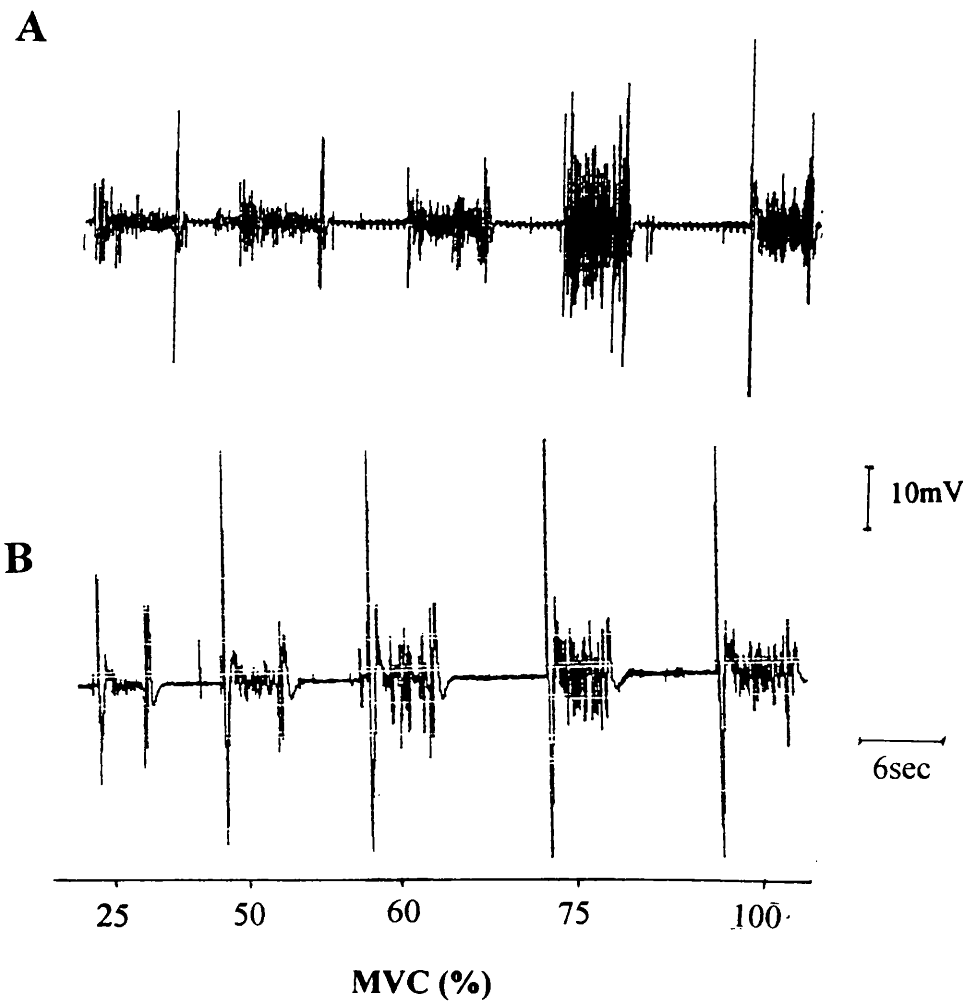

Figure 12. Typical recordings of raw AMG in normal muscle (A) and when blood flow to the muscle stopped (B). Each contraction lasted 6 seconds. Contractions were made at 25, 50, 60, 75 and 100% MVC. Arterial pulsation can be seen when the muscle is relaxed but it tends to disappear after blood occlusion. There are also two movement artefacts at onset and the end of contractions.

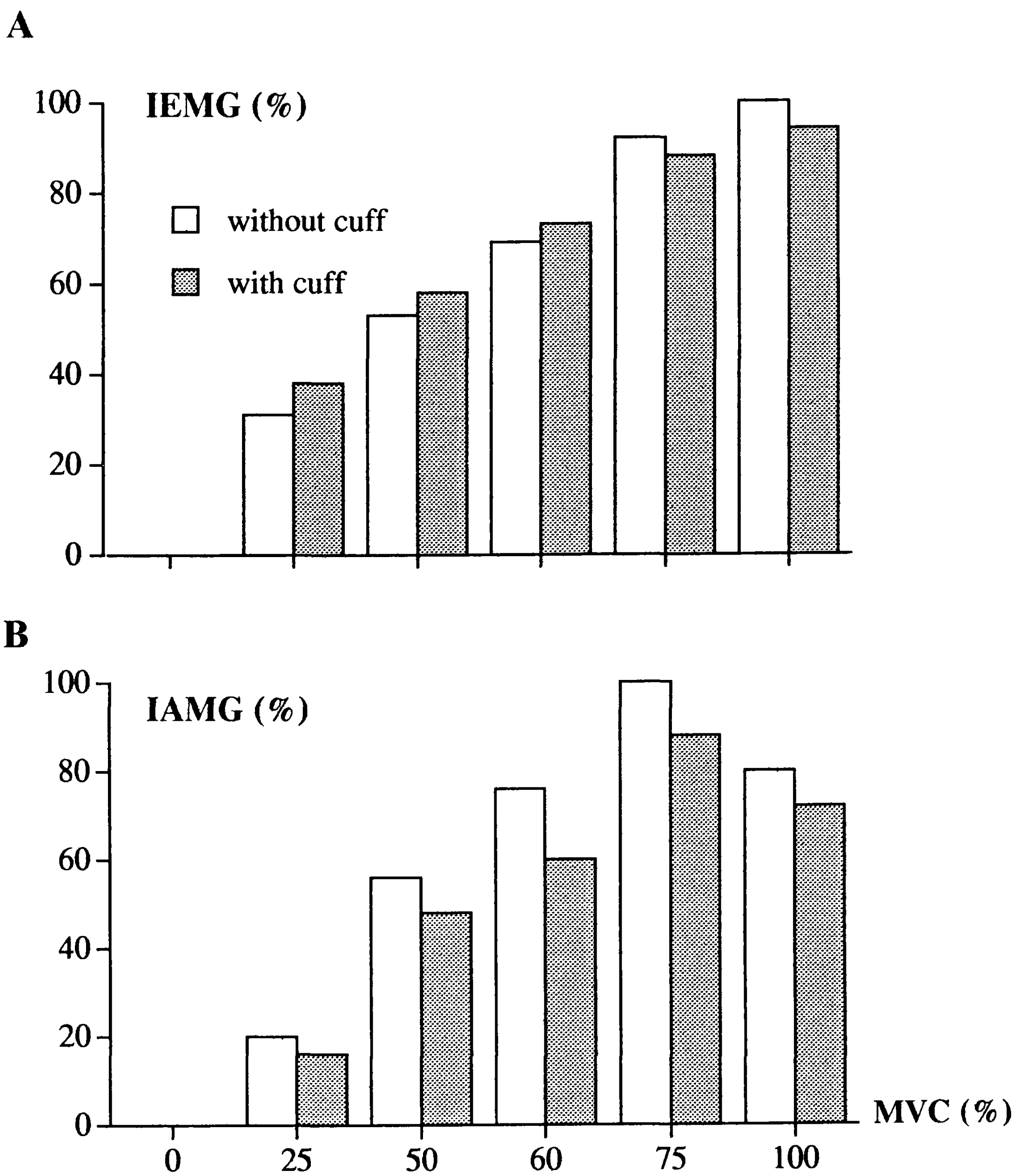

Figure 13· Rectified integrated EMG and AMG in a series of contractions before and after arterial occlusion are shown for one subject. A- IEMG increases continuously with force in fresh and occluded muscle. B- IAMG shows the similar trends up to 75% MVC under both conditions. It declines after this force level. Contractions were made at 25, 50, 60, 75 and 100% MVC.

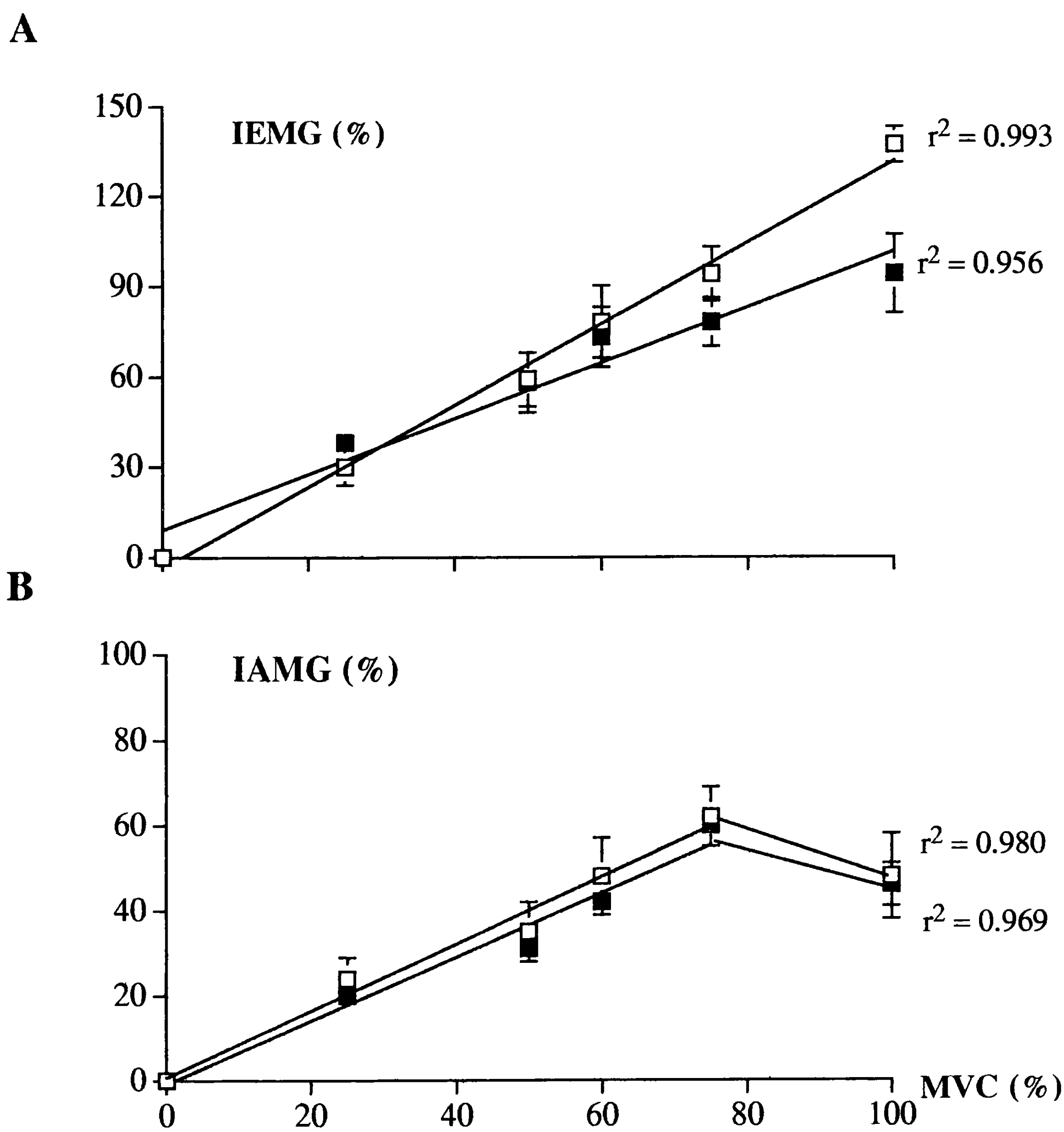

Figure 14- Mean (± SEM) of IEMG (A) and IAMG (B) plotted against force for 14 contractions before (open square) and after (closed square) arterial occlusion in 7 subjects. The similar slope of regression lines between IEMG, IAMG and force was found under both conditions. Contractions were made at 25, 50, 60, 75 and 100% MVC.

The IEMG increases linearly with force but the slopes of regression lines between IEMG and force did not change significantly when blood flow was stopped to the lower limb. There is a linear relationship between mean IAMG and force up to 75% MVC in normal condition and after blood flow occlusion. The mean IAMG declines at maximal voluntary contraction. No significant difference was found between IAMG in normal and occluded flow conditions.

3.3. IEMG and IAMG changes during fatiguing exercises

Experiments were performed to investigate how IEMG and IAMG change during or after fatiguing activity of tibialis anterior. The effects of intermittent and continuous fatiguing exercises were investigated. They will be described in sequence.

3.3.1 The effect of intermittent fatiguing exercises

As described in section 2.8.1 of the methods section, the muscle was fatigued by repeated contractions at 75% of MVC.

Fatigue was developed by repeated isometric dorsiflexion initially at 75% MVC, (6sec on, 4sec off), continued until only 60% MVC could be maintained. Then, EMG, AMG and force were re-investigated at different forces.

Typical recordings of raw EMG obtained during a series of progressively stronger contractions in fresh and fatigued muscle are shown in Figure 15. The peak-peak EMG amplitudes were increased after fatiguing exercises. The increment is considerable, particularly at higher forces. Figure 16 shows typical recordings of raw AMG in fresh and fatigued muscles. At similar forces, peak-peak AMG amplitudes were reduced after fatiguing exercises. This reduction is evident even at 75% of MVC where the AMG is normally most intense.

The amplitudes of IEMG and IAMG were measured at the mid-part of each contraction where the signal was most stable and the effect of movement artefacts were diminished. The IEMG and IAMG amplitude were normalised to maximal values in unfatigued muscle. The relationships between IEMG, IAMG and force were investigated.

Figure 17 shows IEMG and IAMG during a series of contractions from one subject before and after muscle fatigue. At similar force levels after fatiguing activity, the IEMG was increased but IAMG was reduced.

The mean IEMG and IAMG were then calculated for 14 contractions in 7 subjects and plotted against force. These data are shown in Figure 18. There is a strong linear relationship between IEMG and force in fresh and fatigued muscle. The slope of the regression line between IEMG and force is significantly greater after fatiguing exercises ($p < 0.05$).

A linear relationship between IAMG and force at submaximal contractions was found. A significant difference in the IAMG between fresh and fatigued muscle was found at 75% of MVC ($p < 0.05$). However, when all the data are considered, the slope of regression line between IAMG and force showed a significant reduction after fatiguing activity ($p < 0.05$).

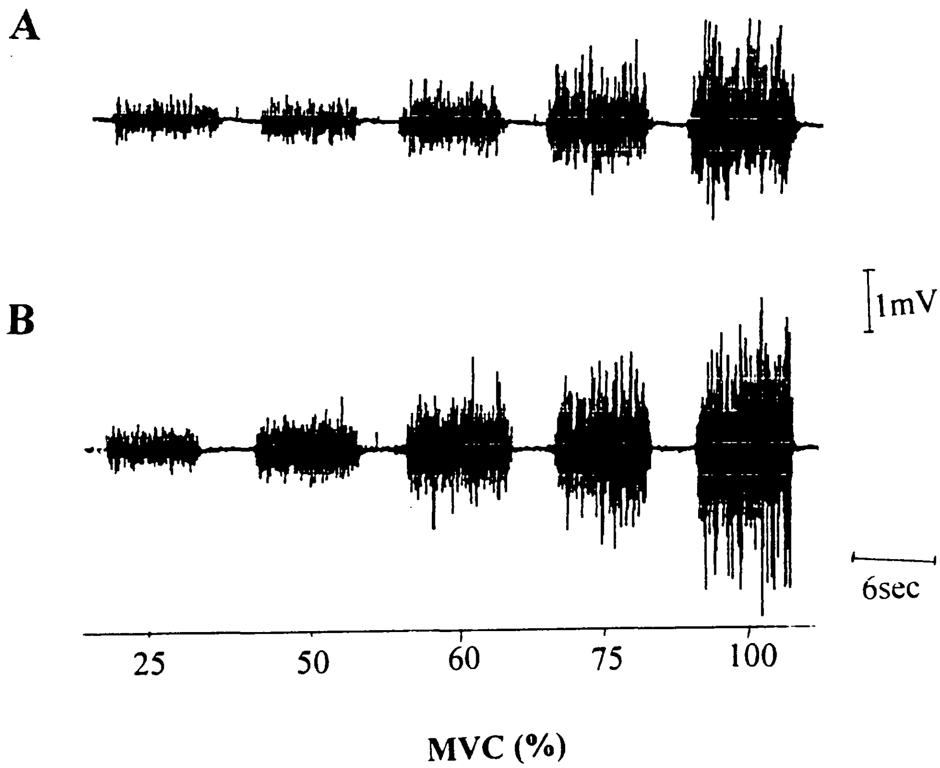

Figure 15. Typical recordings of raw EMG in fresh (A) and fatigued (B) muscle. The intermittent exercises were performed at 75% of MVC. EMG was recorded at 25, 50, 60, 75 and 100% of MVC. Each contraction lasted 6 seconds. Progressive increase in the EMG can be seen with force in fresh and fatigued muscle. At similar forces the peak-peak EMG amplitude is greater after exercises.

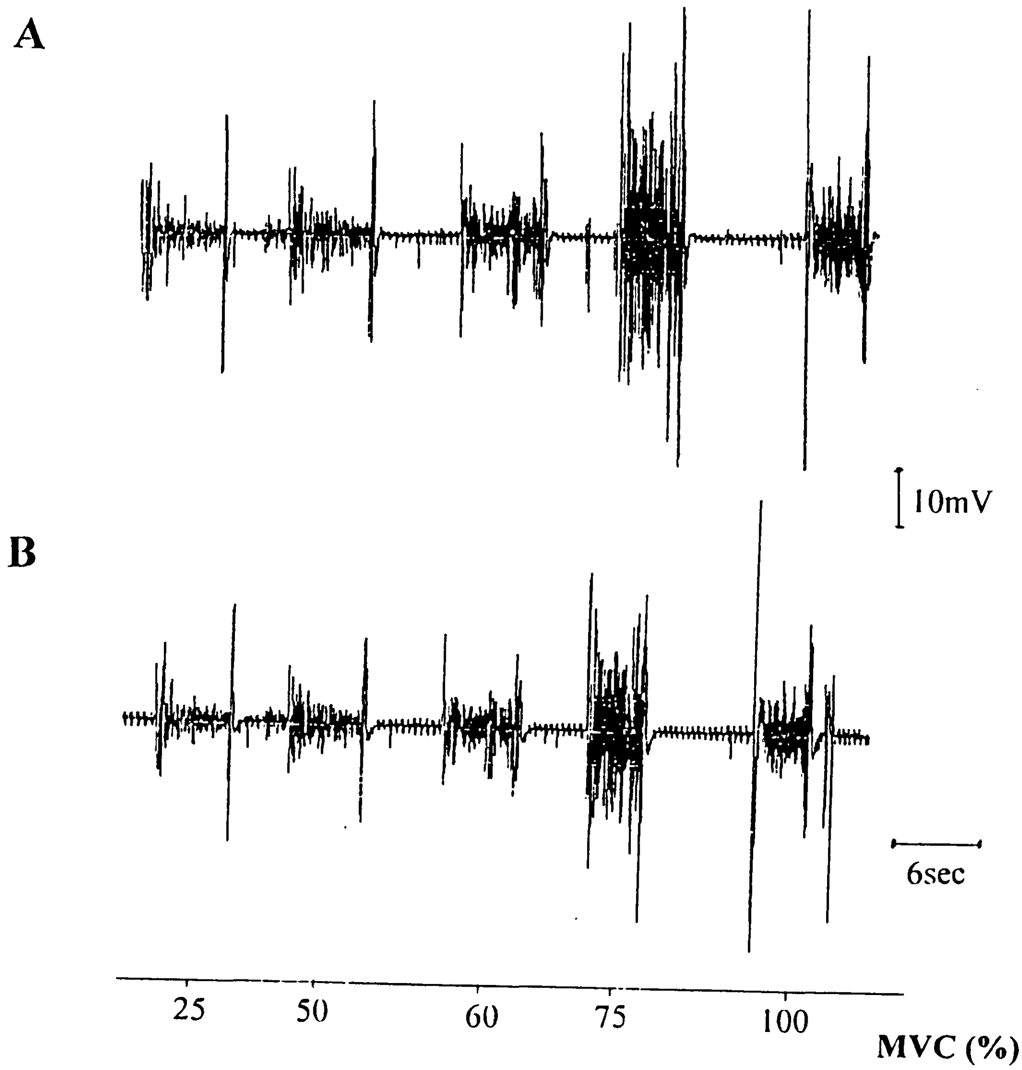

Figure 16. Typical raw AMG recordings in fresh (A) and fatigued (B) muscle are shown. Each contraction lasted 6 seconds. Contractions were made at 25, 50, 60, 75 and 100% MVC. The peak to peak amplitude of raw AMG increased progressively with force up to 75% of MVC in fresh and fatigued muscle. After intermittent fatiguing activity at 75% of MVC, the peak-peak raw AMG amplitude decreases at the similar forces. Arterial pulse waves and movement artefacts can be seen under both conditions.

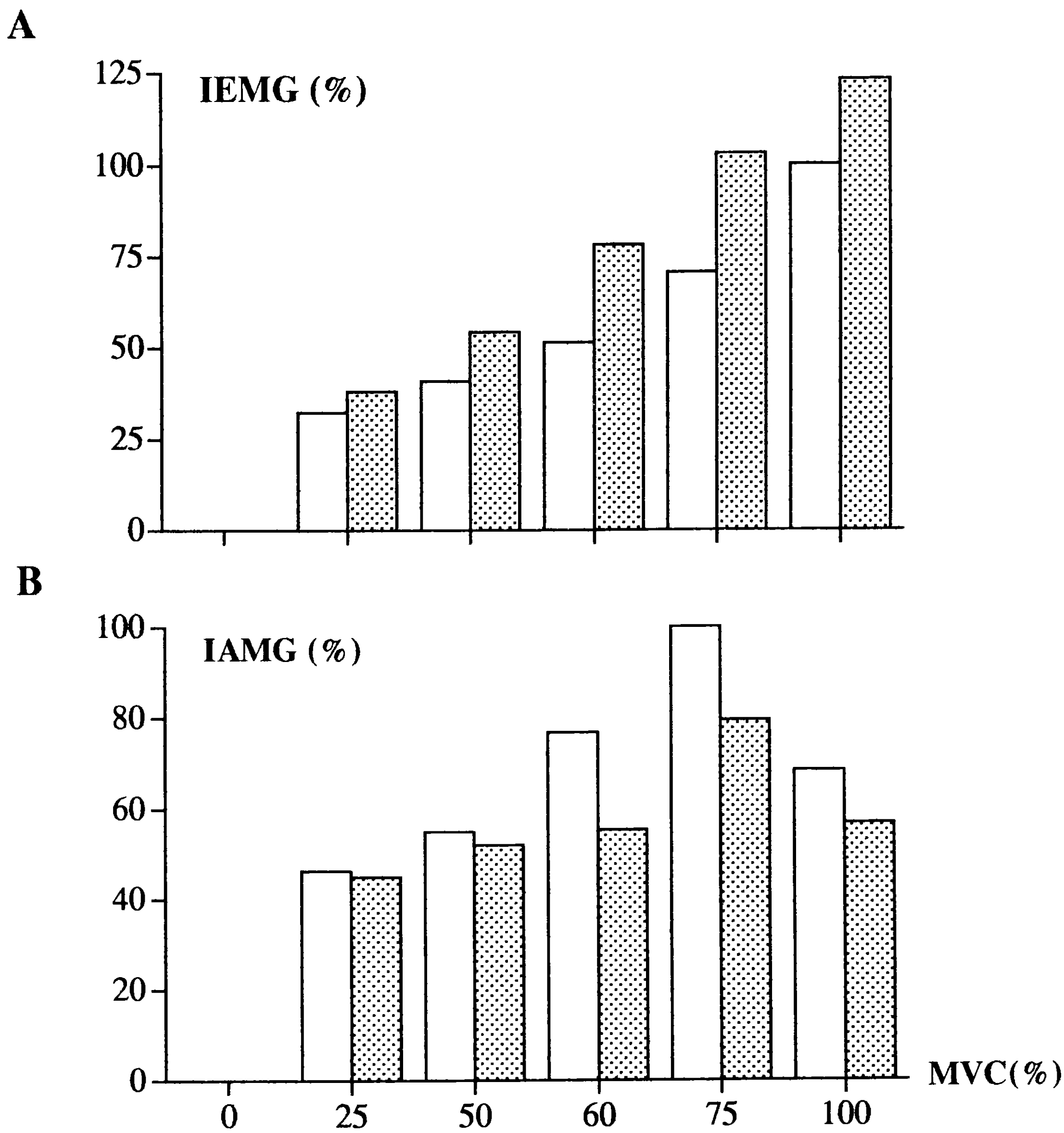

Figure 17. IEMG (A) and IAMG (B) in a series of contractions in fresh (open bar) and fatigued (dotted bar) muscle in one subject. IEMG increases progressively up to MVC whereas IAMG rises up to 75% of MVC and it declined with further force. At similar forces, after fatiguing exercises IEMG increases but IAMG decreases.

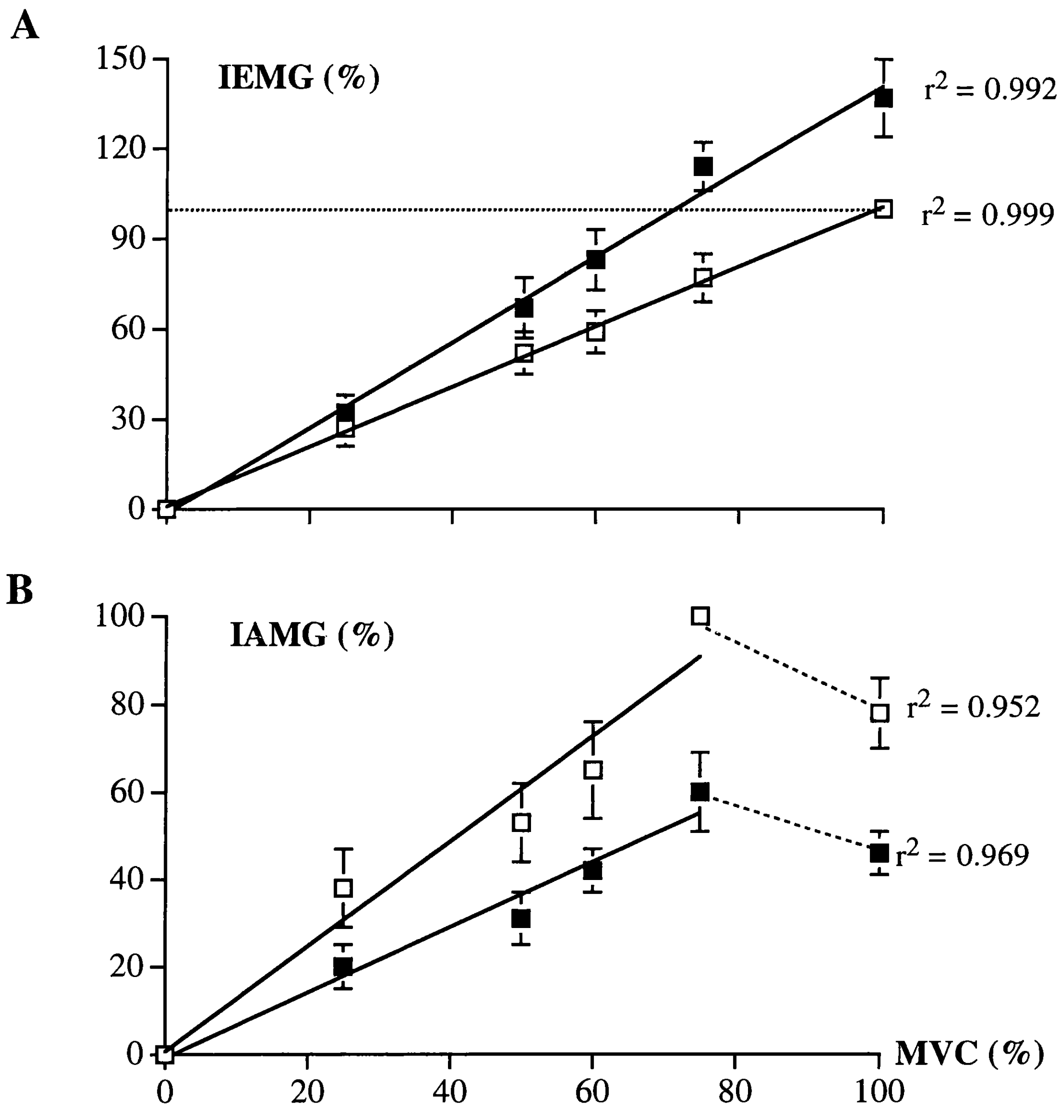

Figure 18. The mean ± SEM of IEMG (A) and IAMG (B) in fresh (open square) and fatigued (closed square) muscle are shown. After fatiguing activity, the IEMG rises above control values and IAMG falls below control values. The slope of regression lines between IEMG and force increases in fatigued muscle in compare with that in fresh muscle ($p < 0.05$). The slope of regression lines between IAMG and force in fatigued muscle in compare with that in fresh muscle decreases ($p < 0.05$). In graph A, the dotted line represents the 100% MVC.

3.3.2. The changes of the IEMG and IAMG during sustained fatiguing contractions

The design of these experiments was described in the section 2.8.2 of the Methods. The subjects sustained a contraction at 40%, 60%, or 80% MVC until exhaustion. During contractions, force was maintained within ± 5% of given value. EMG and AMG were recorded throughout the experiments.

Figure 19 shows a typical recording at 80% of MVC. Prior to the contraction, the EMG trace is silent and its amplitude increases at the beginning of contraction. During activity, the EMG amplitude rises progressively though the force remains constant. The rise in EMG/force ratio is a clear indication of the development of muscle fatigue. Before the onset of muscle contraction, the AMG trace has a low background level. The peak to peak AMG amplitude reaches a maximum within a few seconds of the beginning of the contraction. After this it falls progressively throughout the contraction. Thus, during an isometric contraction at 80% MVC the IEMG rises whereas the IAMG decreases.

IEMG and IAMG and force were calculated at the beginning, middle and the end of each trace, and then normalised to values obtained in control conditions. Data were collected from 8 subjects with different endurance levels.

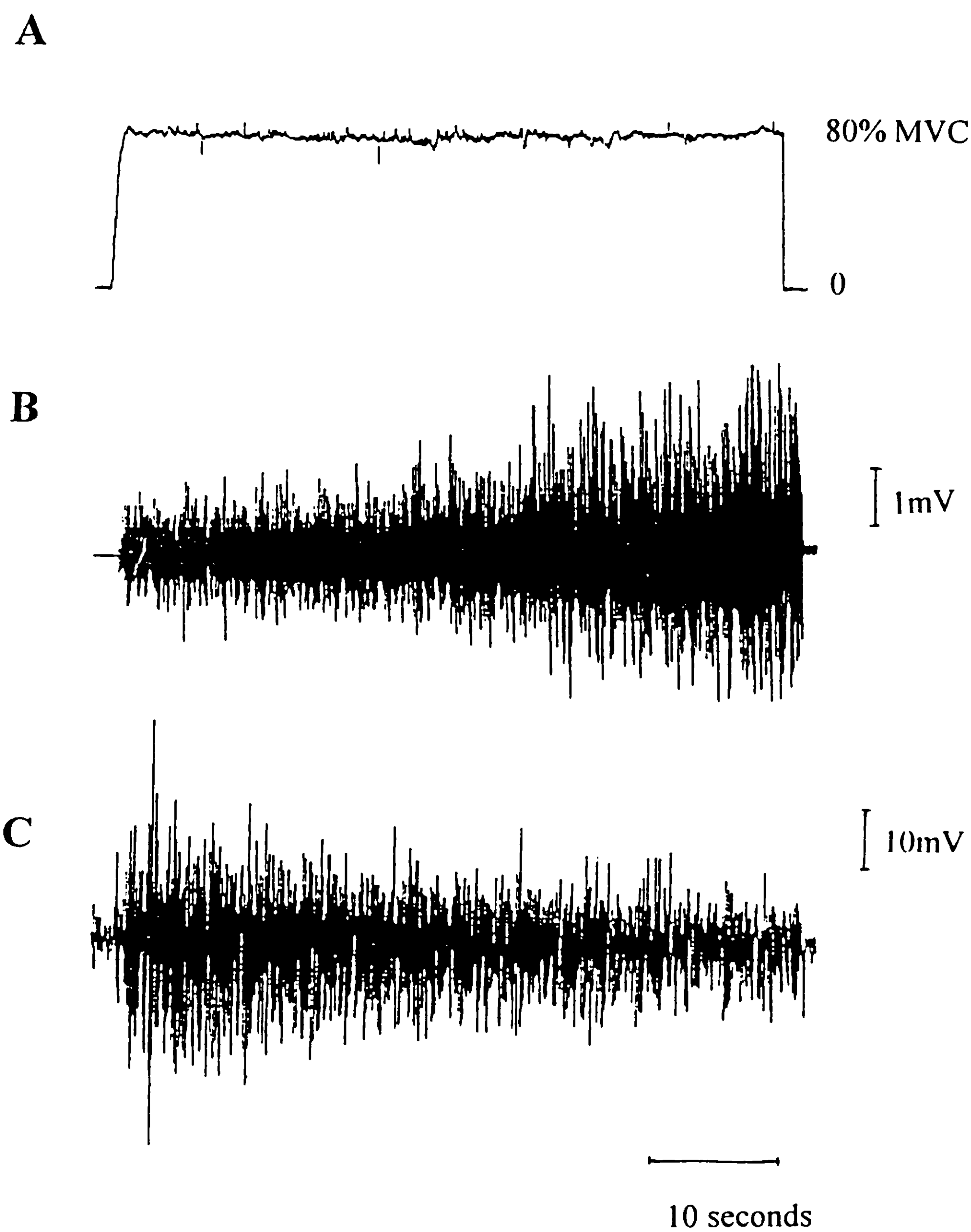

Figure 19. Force (A), EMG (B) and AMG (C) during a voluntary contraction at 80% of MVC. The peak to peak amplitude of raw EMG increases from the middle of contraction time but the peak to peak amplitude of raw AMG progressively decreases throughout the contraction. The contractions sustained 56 seconds to exhaustion.

The IEMG and IAMG in a series of contractions in one subject at 80% of MVC are shown in Figure 20. The mean and SEM of the IEMG and IAMG during a sustained contraction at 80% of MVC were calculated and plotted as functions of contraction duration. Figure 21 shows these data. The IAMG declined by about 60% of initial values, whilst the IEMG had risen by 40%. The differences in IEMG and IAMG between the beginning and middle and beginning and end of the contraction were investigated by analysis of variance. There were statistically significant differences ($p < 0.03$) in the mean amplitudes of both IEMG and IAMG between the beginning and the end of contractions. When the experiment was repeated with the subjects sustaining a force of 40% or 60% MVC, the IEMG showed the same behaviour. Figures 22 and 23 show the progressive rise in raw EMG as time passes. However, the raw AMG at 40% MVC behaves differently. The mean rectified integrated EMG and AMG at lower forces were also calculated. The differences in IEMG and IAMG between the beginning and middle and beginning and end of the contraction were investigated by analysis of variance. There were statistically significant differences ($p < 0.05$) in the mean amplitudes of both IEMG and IAMG between the beginning and the end of contractions at 60% MVC. However, no significant differences were detected in mean of IAMG amplitude throughout the 40% fatiguing contractions, even though the significant difference in the mean IEMG amplitude between beginning and the end of contractions at 40% MVC ($p < 0.05$) provides a clear indication that the muscles are fatiguing. These data are shown in Figure 24 and 25

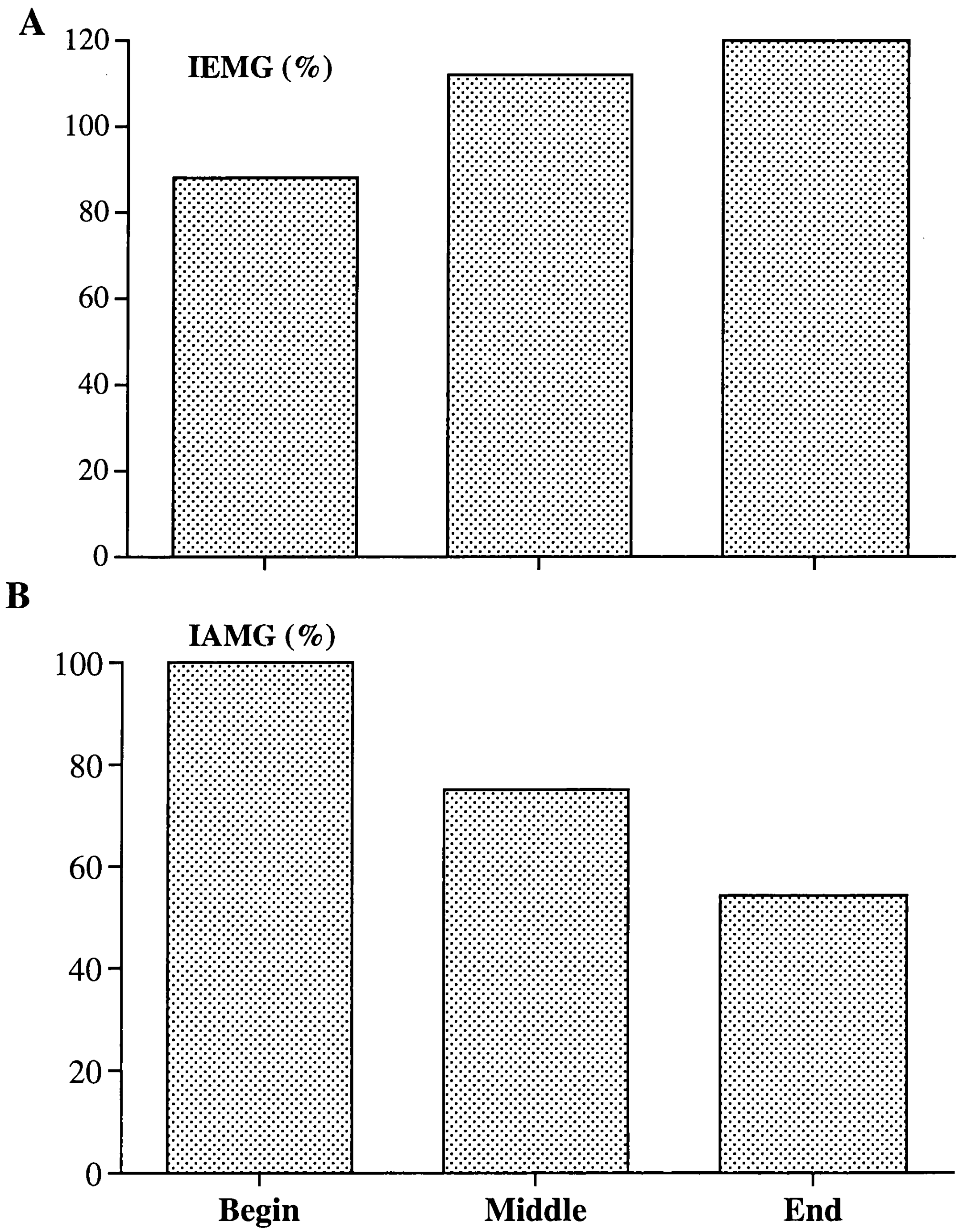

Figure 20. IEMG (A) and IAMG (B) at the beginning, middle and end of a sustained contraction at 80% of MVC in one subject. Each signal is normalised to 100% at the beginning of the contraction. The IEMG increases above control values whereas the IAMG decreases below control values.

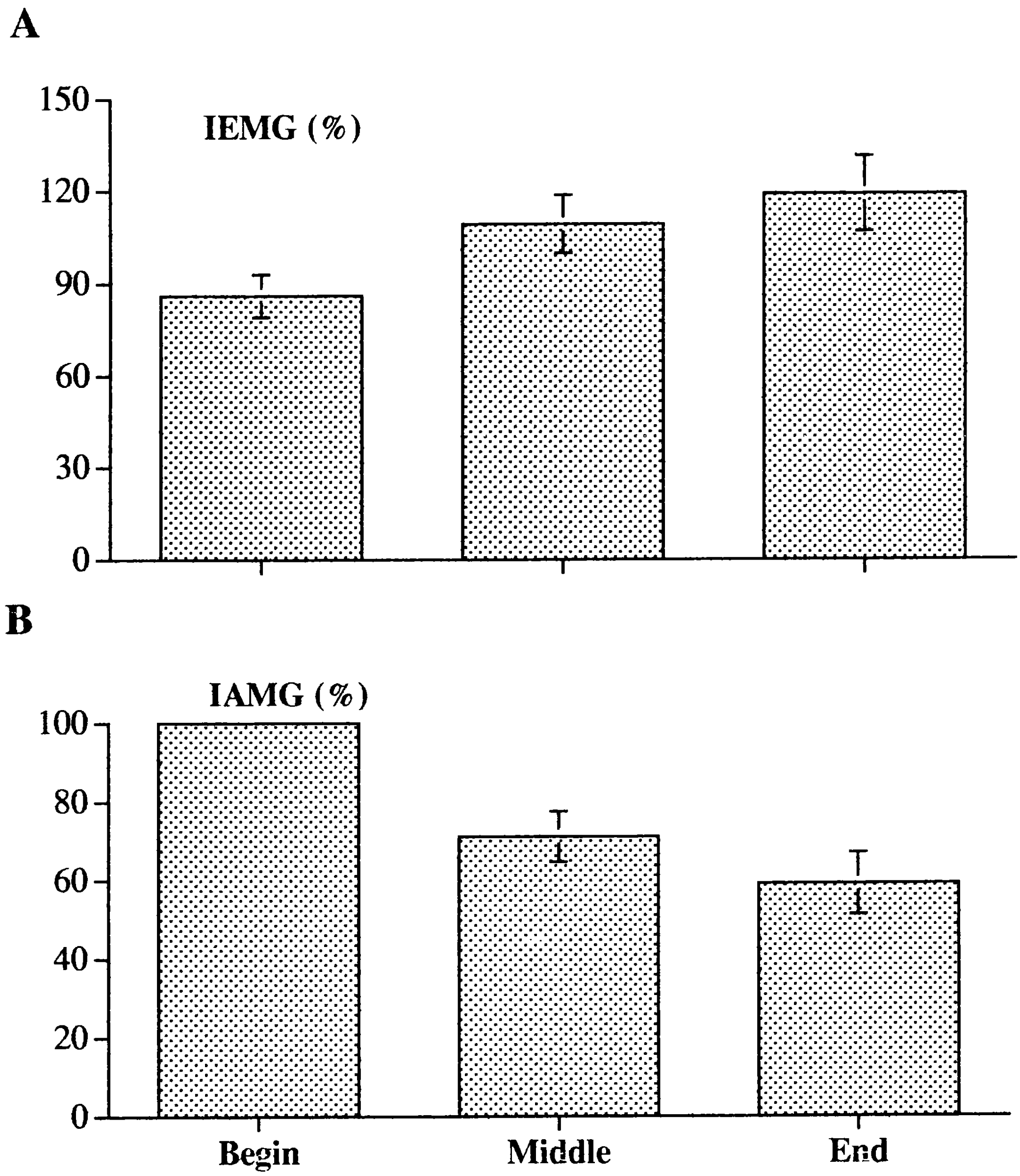

Figure 21· Mean ± SEM of IEMG (A) and IAMG (B) plotted against time. At 80% of MVC exhaustion, the IEMG rises and IAMG falls. The increment in the IEMG and decrement in the IAMG were significant between onset and the end of contractions ($p < 0.03$). No error bar is shown at the beginning of the IAMG because whole data was normalised to 100% at the start of each contraction. N = 8.

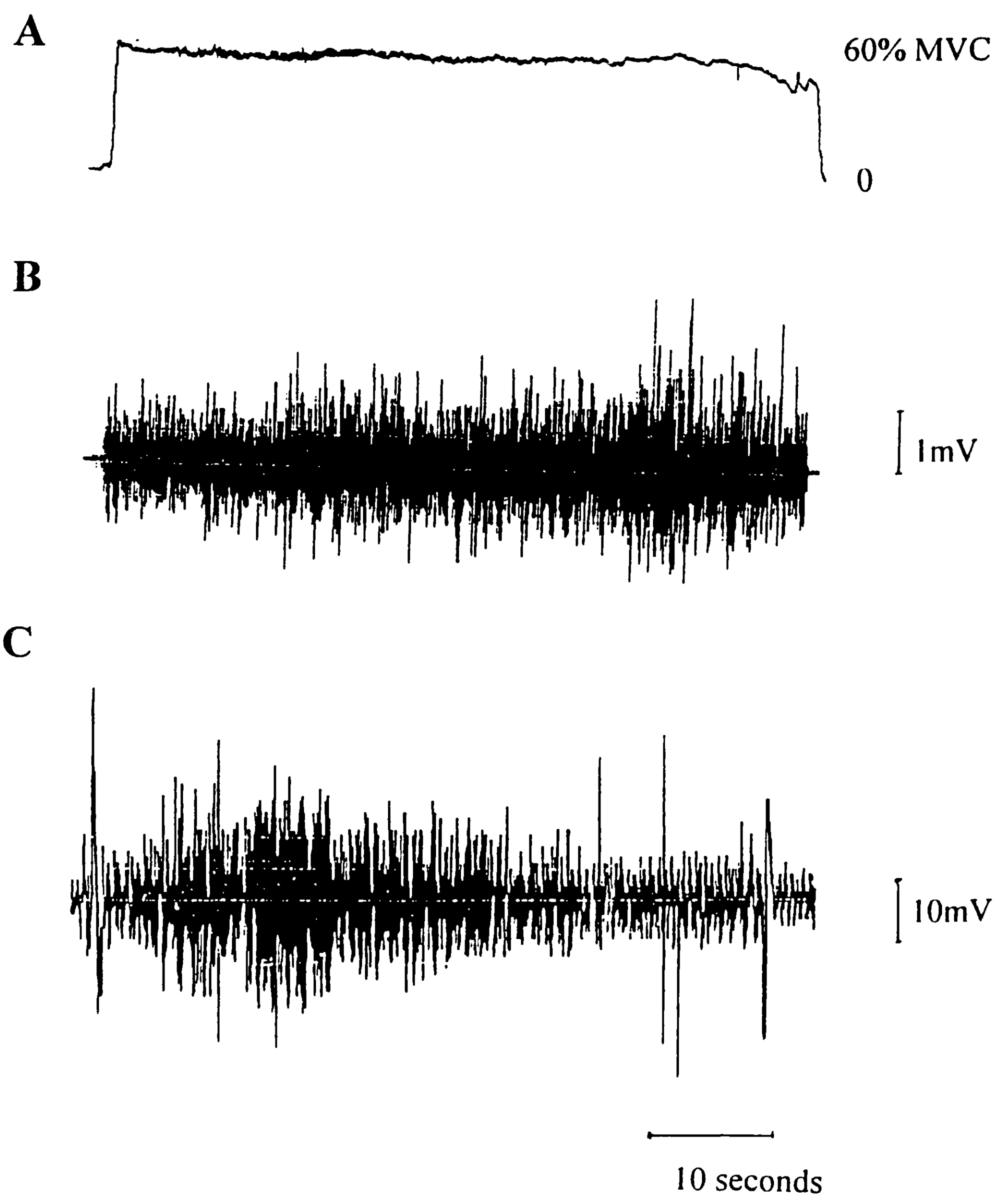

Figure 22. Typical recording of force (A), EMG (B) and AMG (C) during sustained contraction at 60% MVC. The peak to peak amplitude of raw EMG rises but the peak to peak amplitude of raw AMG falls. The changes are more pronounced at the end of contraction.

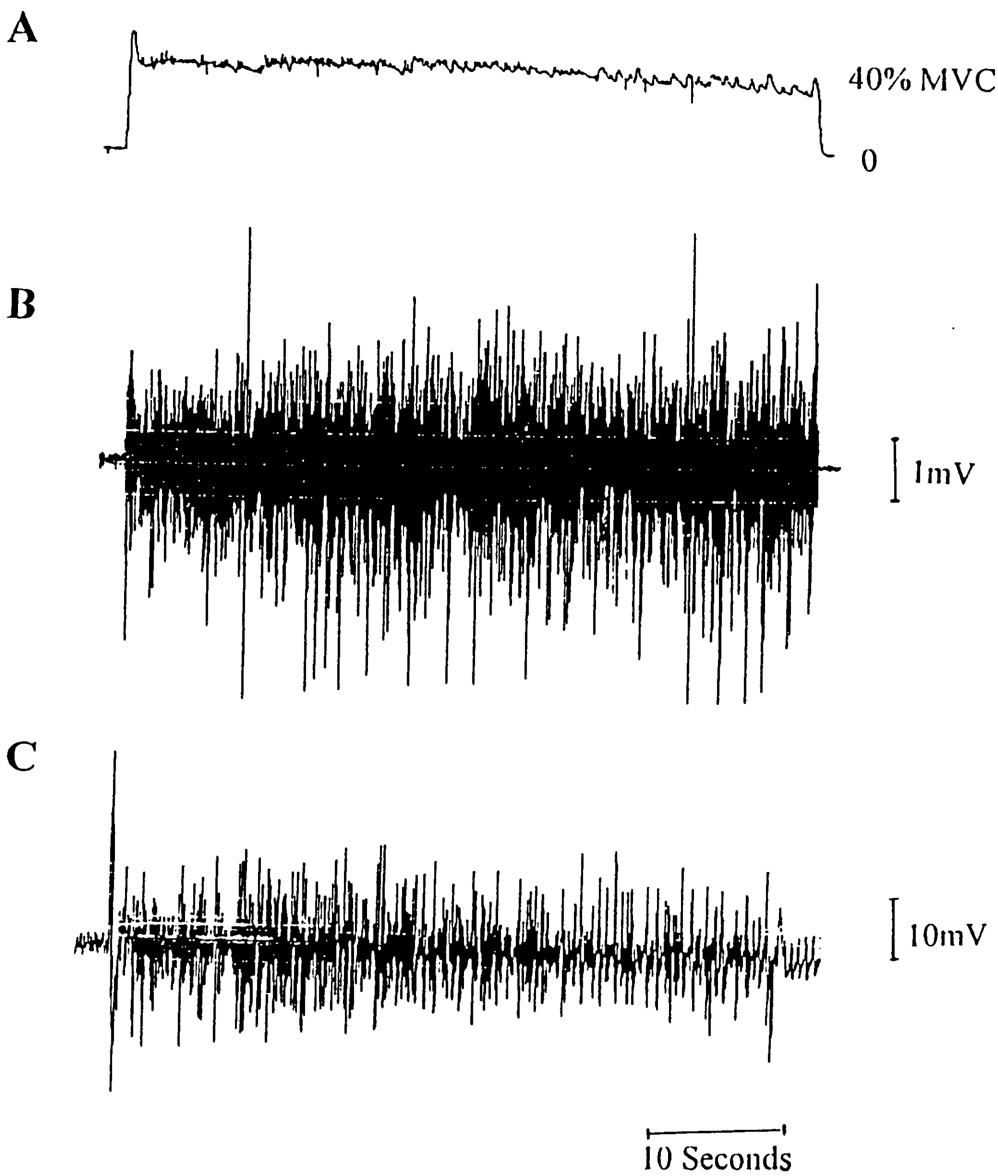

Figure 23. Typical recording of force (A), EMG (B) and AMG (C) during sustained isometric contraction at 40% MVC. The peak to peak amplitude of EMG tends to rise but the peak to peak amplitude of AMG tends to be constant.

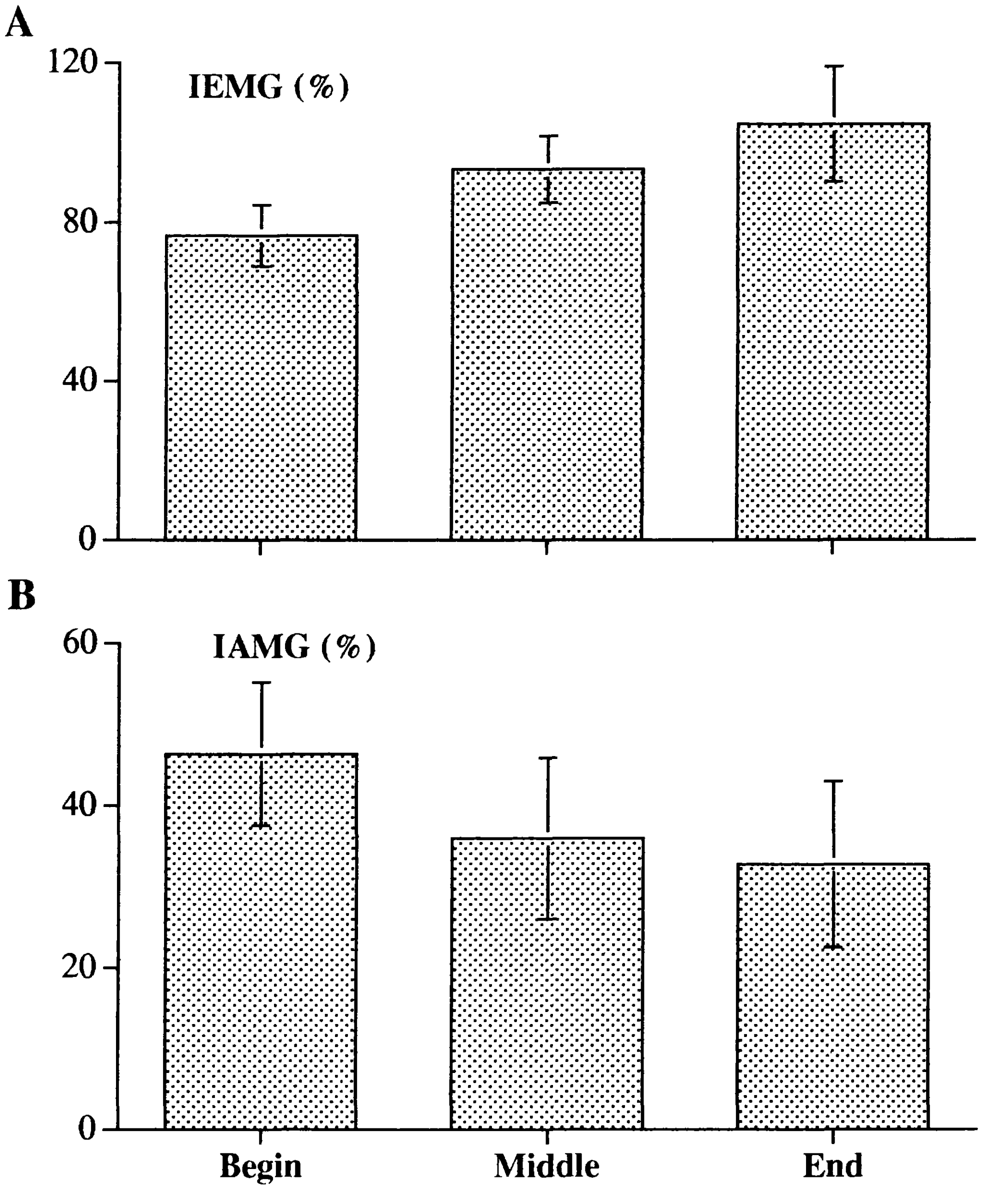

Figure 24. Mean ± SEM of IEMG (A) and IAMG (B) at the beginning, middle and end of a sustained contraction at 60% of MVC. The amplitude of IEMG increases above control values but the amplitude of IAMG decreases below control values. The changes in amplitude of IEMG and IAMG between the beginning and the end of contractions were significant ($p < 0.05$). N = 8.

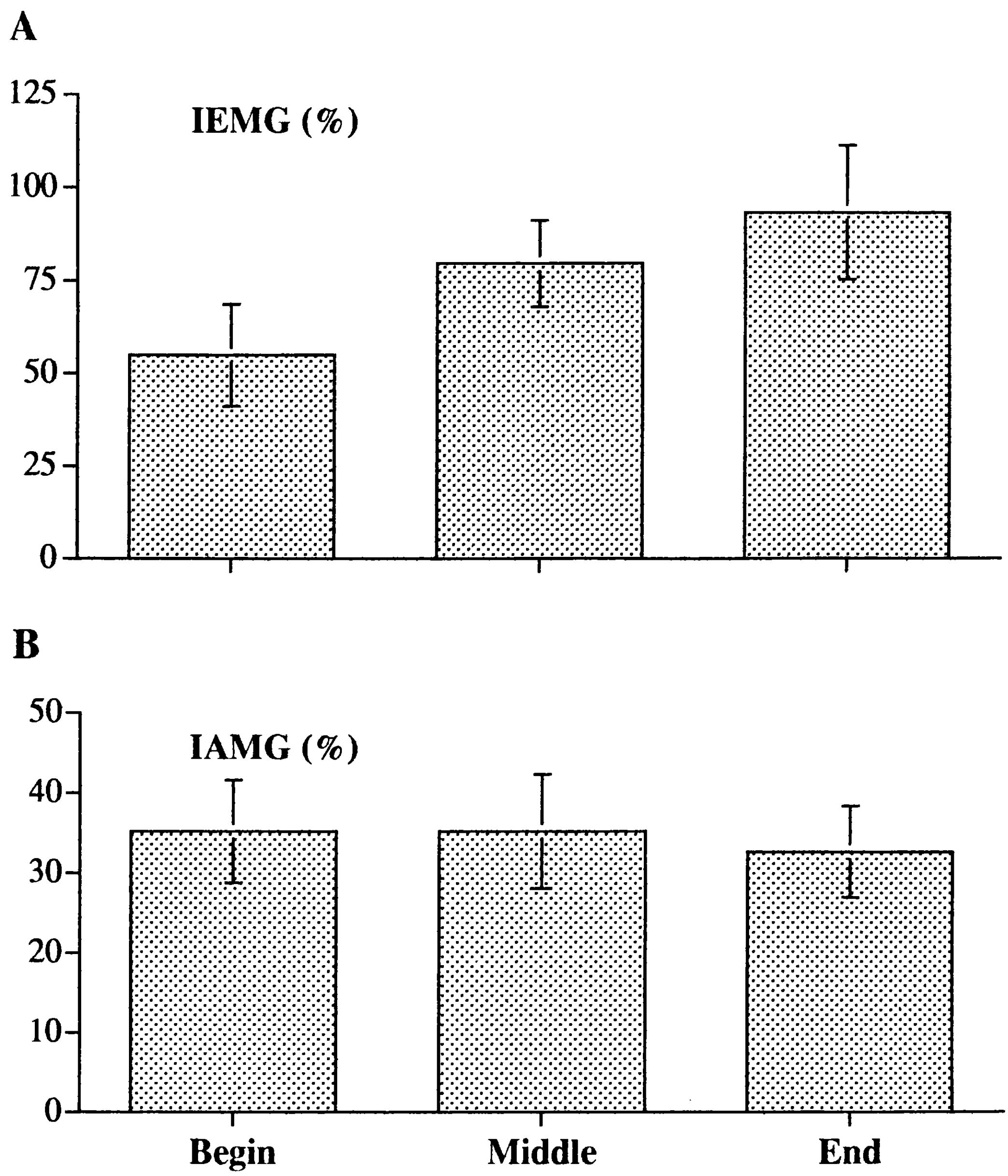

Figure 25. Mean ± SEM of IEMG (A) and IAMG (B) at the beginning, middle and end of a sustained contraction at 40% of MVC. The amplitude of IEMG shows a significant difference between the beginning and the end of contractions ($p < 0.05$) whereas the amplitude of IAMG remains constant. N = 8.

3.3.3. Exhaustion times of IEMG and IAMG

Figure 26 shows mean of exhaustion times as function of different forces. The exhaustion times declined at higher force levels. Contractions at 40% of MVC were sustained on average for 330 ± 75 seconds. At 60% MVC the mean duration was 178.8 ± 42 seconds and this fell again to 81 ± 53 at 80% MVC. Attempts to sustain higher forces were always problematic. The force oscillations were unacceptably large and this introduced artefacts into the AMG and EMG records which prevented the calculation of worthwhile integrated values.

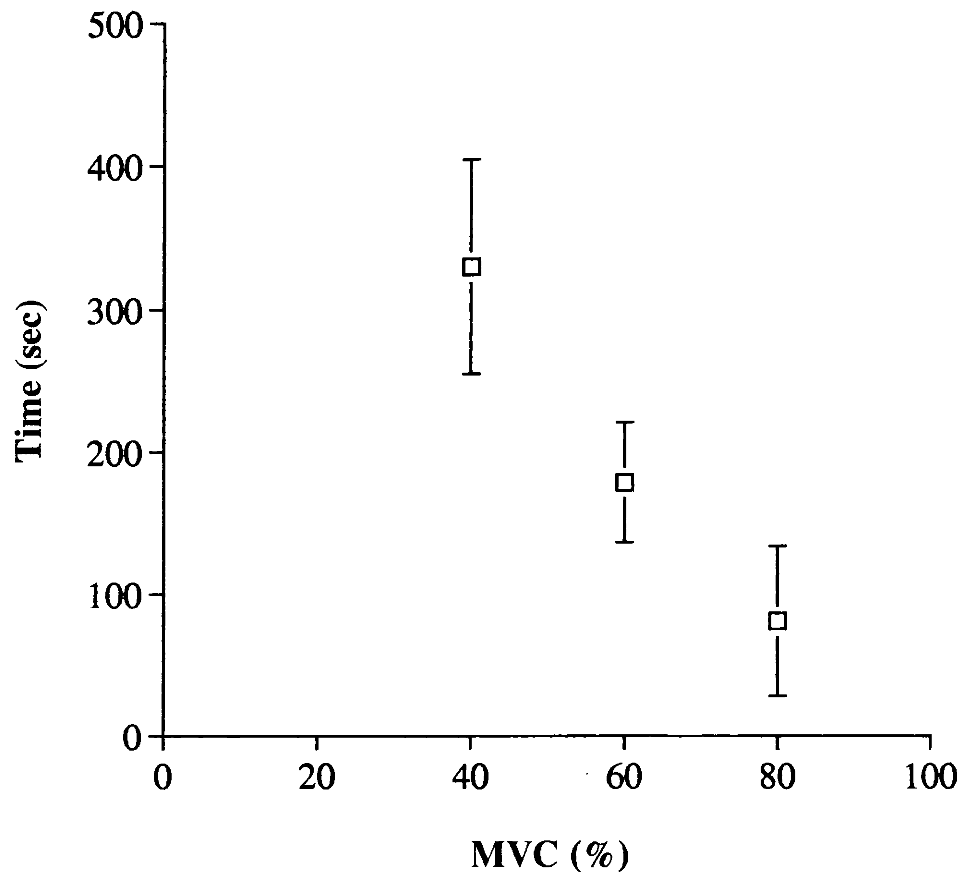

Figure 26- Mean ± SD of exhaustion times plotted against different forces. Higher forces are sustained for about one minute whereas lower forces held to exhaustion more than 5 minutes. N = 8.

3.4. Analysis of the frequency content of EMG and AMG

In addition to studies of the raw and integrated acoustic and electromyograms a second series of experiments was performed to investigate the characteristics of the frequency spectrum of both signals. The experimental protocols are essentially similar to those described in sections 3.1-3.3, but the analysis of EMG and AMG is quite different. Details of the calculation of the frequency spectra are given in the Materials and Methods section 2.5.2b and 2.5.3b. Briefly the spectra were analysed in two ways:

1. to identify the median frequency, i.e. that frequency which divides the spectra into two equal energy components.
2. to identify the energy associated with specified frequency bands with the spectrum. The bandwidth analysis provides a more detailed description of the spectrum. The median frequency analysis is less informative but quicker to do. Its application to EMG signals for the early identification of fatigue is well established (De Luca, 1984). The frequency analysis of the EMG and AMG were performed with normal blood flow to the muscle and with the blood flow occluded, in fresh and fatigued muscle and at different muscle lengths. These will be described in turn.

3.4.1. EMG and AMG median frequencies in unfatigued muscle

The median frequencies were calculated for sections of the EMG and AMG signals lasting 2 seconds which were selected near the middle of contractions lasting 6 seconds. This provides the most stable records for analysis. Details of the data sampling are shown in Figure 27.

EMG yields a spectrum with components between 0-400 Hz. The spectrum was calculated using an FFT. A typical EMG spectrum is shown in Figure 28 A. The median frequency is just above 100 Hz. The median frequency of the AMG was calculated in similar way except that the AMG was digitised at 512 Hz because its bandwidth is narrower. It also has a relatively simpler spectrum ranging between 0-50 Hz. Figure 28D shows a typical AMG spectrum. The median frequency of the AMG is about 11Hz.
The median frequencies of EMG and AMG were calculated at various forces between 20-100% MVC. Mean ± SEM of the median frequencies of EMG and AMG are shown in Figure 29. The EMG median frequency ranging from 71 Hz at 20% of MVC to 112 Hz at 100% of MVC. The median frequencies of EMG increased linearly with increasing force. The correlation coefficient of the fitted line is 0.976. The AMG median frequencies were between 5 Hz at 20% MVC and 15 Hz at 100% MVC. There is also a strong linear relationship between AMG median frequency and force. The correlation coefficient of fitted line is 0.981. Thus, the median frequency of EMG spectrum is about 10 times greater than the median frequency of AMG spectrum.

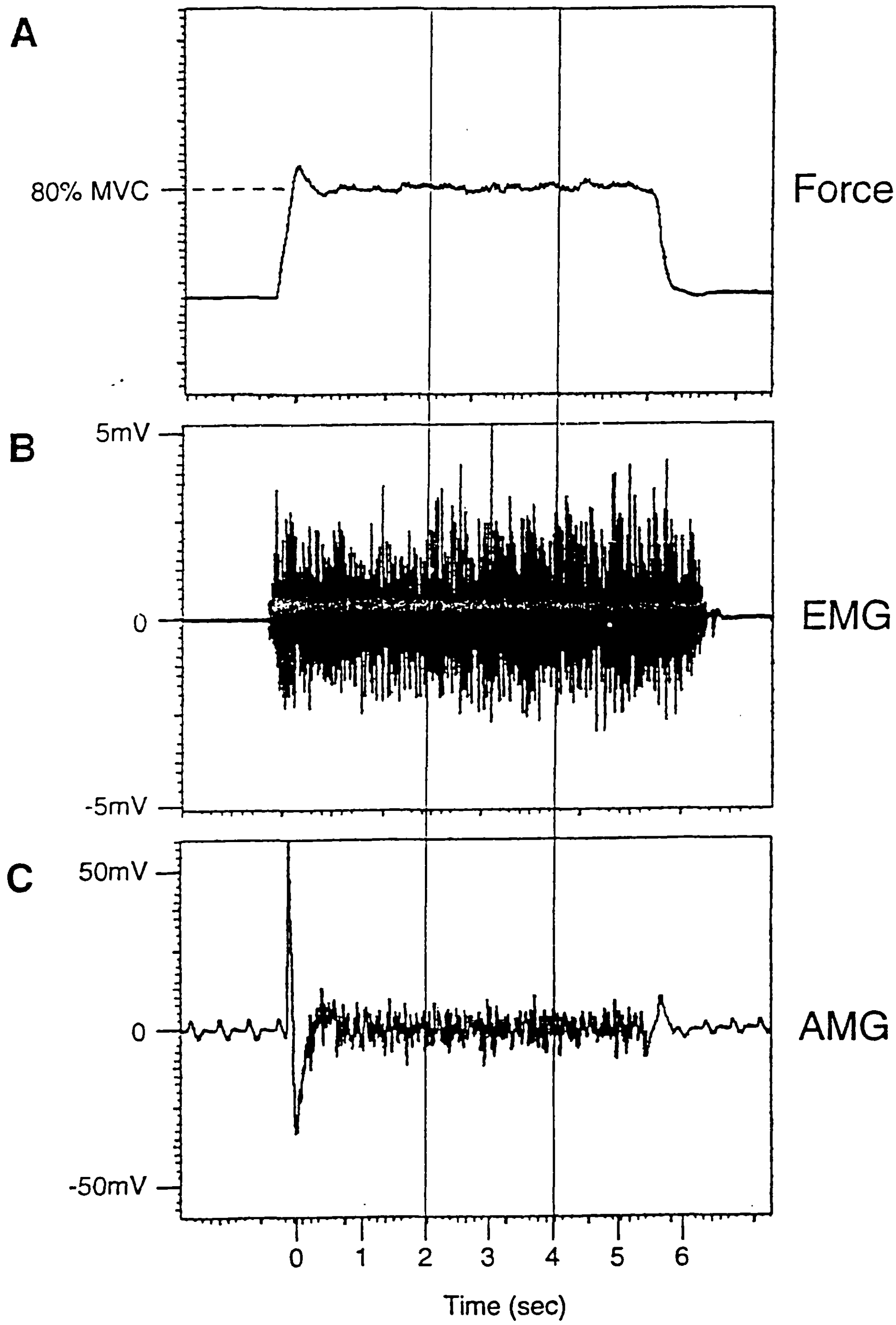

Figure 27. Typical simultaneous recordings of force (A), EMG (B) and AMG (C) at 80% of MVC. Sections of EMG and AMG signals lasting 2 seconds which were selected near the middle of contractions lasting 6 seconds.

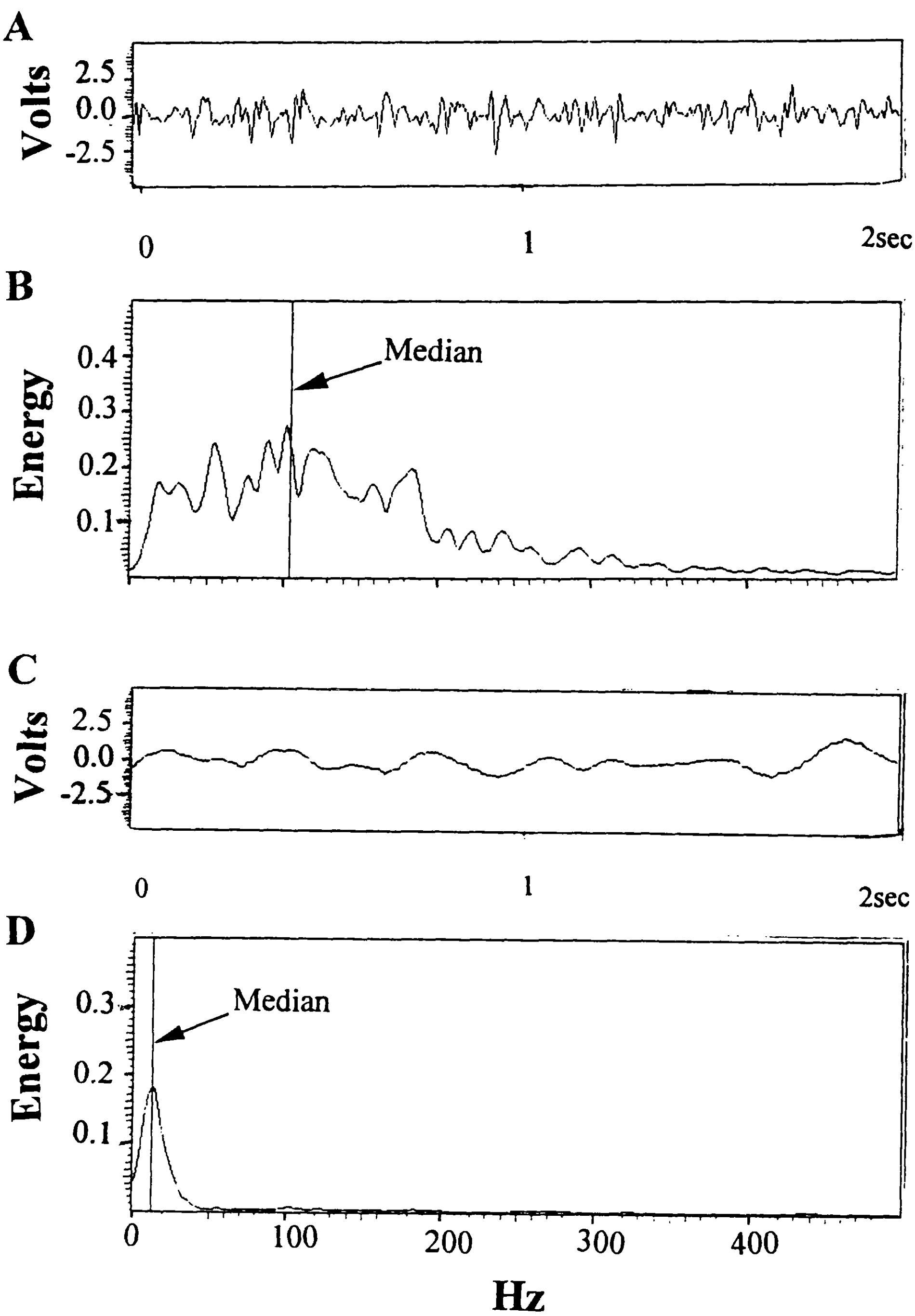

Figure 28. A typical analysis of the median frequency of EMG (B) and AMG (D). The raw EMG (A) and AMG (C) are also shown. The vertical lines in B and D indicate the median frequency of the signals. The EMG frequency range is wider than the AMG. The EMG median frequency is about 10 times greater than in the AMG.

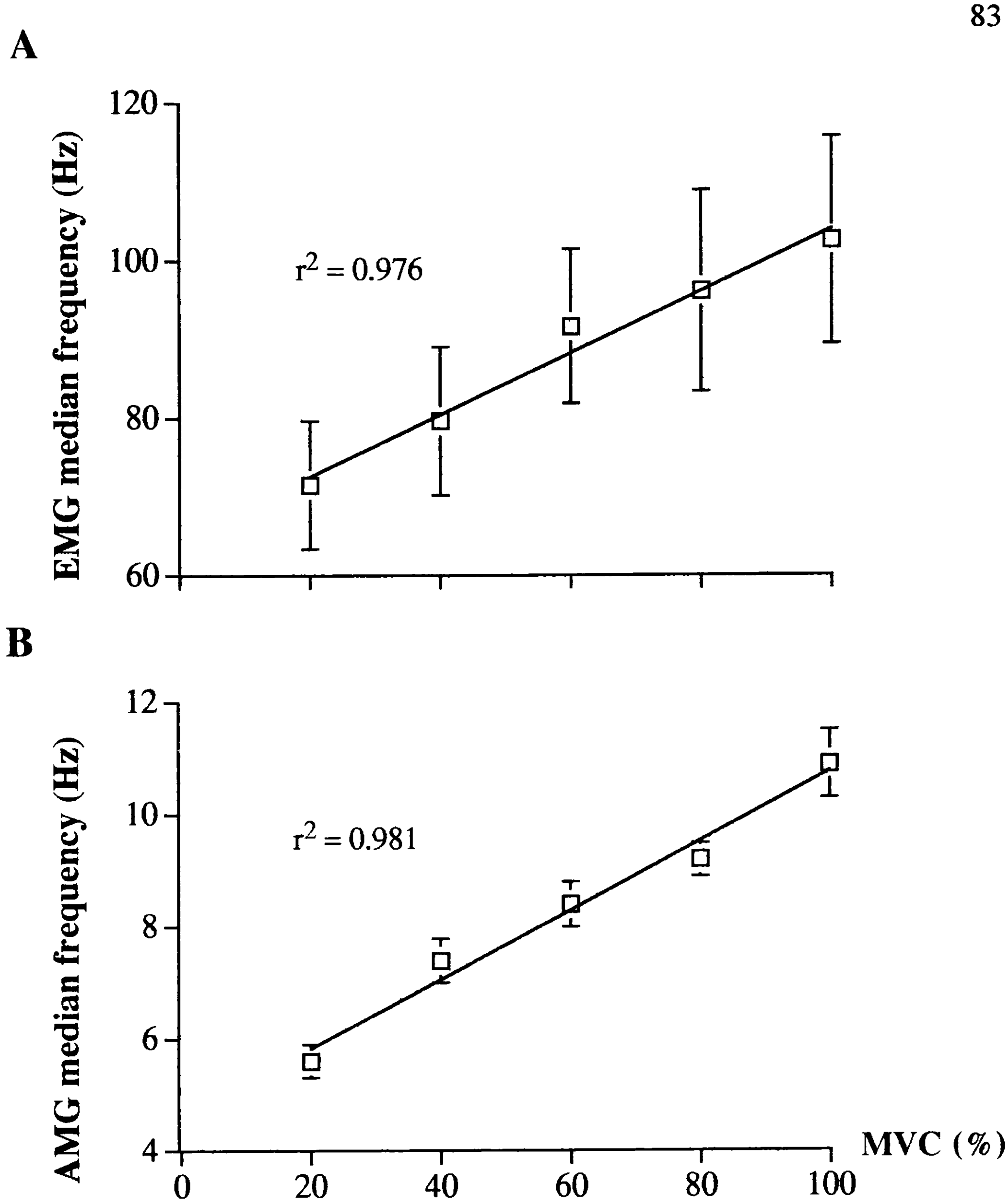

Figure 29. Mean ± SEM of the median frequencies at different forces are shown. A- Median frequency of the EMG. B- Median frequency of the AMG. The EMG median frequency is about ten times higher than in the AMG. The relationship between EMG, AMG median frequencies and force are linear. The larger SEM in the EMG median frequency reveals higher inter-subject variations.

3.4.2. Frequency bands analysis of EMG and AMG in unfatigued muscle

The percentage of total signal energy of the EMG was measured in frequency bands of 50 Hz between 0 and 400Hz. Figure 30B shows a typical bands analysis. Frequency, measured in Hertz (Hz) is shown along the x axis and the linear amplitude of signal (volts) is along y axis. About 95% of energy spectrum of EMG is concentrated below 200 Hz. The AMG frequency analysis was done using narrower bands of 5 Hz up to 50Hz. A typical bands analysis of the AMG signal is shown in Figure 30D. The energy above 35 Hz is negligible. The principle concentration of the AMG energy is between 5-15 Hz. The frequency bands analysis was carried out at different forces.

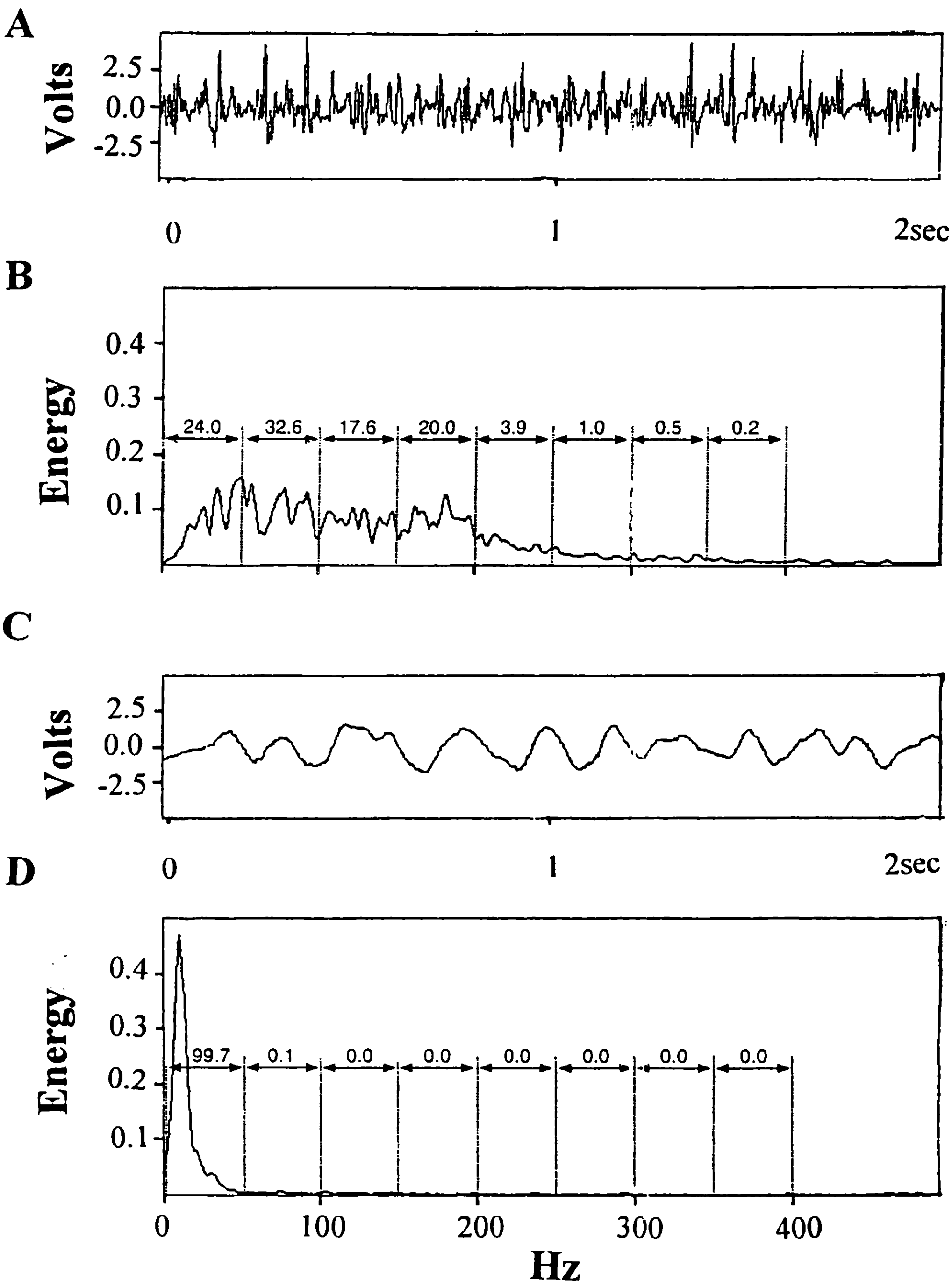

Figure 30. The distribution of energy in the EMG (B) and AMG (D) in a range of frequency bands. The frequency bands analysis were applied to two seconds of the raw EMG (A) and AMG (C). The percentage of EMG energy mostly concentrated below 200Hz whereas the AMG energy is mostly distributed below 30Hz.

Table 3 shows distribution of mean ± SEM of percentage of EMG frequency spectrum in a series of isometric dorsiflexion contractions in fresh muscle in different bands. These results were obtained from experiments in 5 subjects.

MVC	0-50 Hz	100 Hz	150 Hz	200 Hz	250 Hz	300 Hz
20%	33.9 ± 5.5	40.6 ± 1.8	16.4 ± 3.7	5.2 ±1.2	1 ± 0.4	0.2 ± 0.1
40%	29.7 ± 5.4	34.9 ± 5.6	20.5 ± 3.9	6.9 ± 2	2.5 ± 0.9	1.2 ± 0.2
60%	28.4 ± 5.5	35.2 ± 3	20.7 ± 4	9.3 ± 2.5	3.3 ± 1.1	1.4 ± 0.4
80%	26.4 ± 5.5	35.6 ± 4.8	20.7 ± 4.5	11.2 ± 3.5	3.6 ± 1.3	1.3 ± 0.5
100%	25.5 ± 5.8	34.1 ± 5.2	23.2 ± 4.6	11.5 ± 3.6	4.4 ± 1.9	1.1 ± 0.4

Table 3. The distribution of energy in the EMG in a range of frequency bands. Each cell contains the mean (± SEM) energy expressed as a percentage of the total energy recorded.

As shown in Table 3, the EMG frequency spectrum shifted towards higher frequency range as force increases. The greater concentration of EMG energy spectrum occurs between 0-150 Hz. The differences in the EMG energy during contractions at different forces were not significant.

The distribution of energy in the AMG across a range of frequency bands was also calculated. The bands are narrower in this case due to the much tighter distribution of AMG frequencies. These data are shown in Table 4.

MVC	0-5 Hz	10 Hz	15 Hz	20 Hz	25 Hz	30 Hz
20%	52 ±10	29.6 ± 2	14 ± 4.3	2.1 ± 0.8	1.1 ± 0.0	0.4 ± 0.1
40%	40 ± 6.5	34.6 ± 2	17.4 ± 5	5.4 ± 1.5	1.1 ± 0.2	0.5 ± 0.1
60%	31 ± 4.5	33 ± 4.5	20.5 ± 2	8.6 ± 3	2.5 ± 0.5	1 ± 0.2
80%	28 ± 3.4	39.6 ± 4	20 ± 2.1	7.1 ± 1	3 ± 1.1	1 ± 0.2
100%	25± 3.6	28 ± 5.4	24 ± 2.6	13.2 ± 3	4.3 ± 0.9	1.3 ± 0.3

Table 4. The distribution of energy in the AMG in a range of frequency bands. Each cell contains the mean (± SEM) energy expressed as a percentage of the total energy recorded.

Table 4 shows a stronger concentration of AMG energy in lower frequency bands than for EMG. Like EMG, as force increases the AMG frequency spectrum is shifted towards higher frequency. As force increases the AMG energy distributes in a wider range bandwidths. Dominant energy in the AMG spectrum is below 20Hz. The reduction in energy in the 0-5 Hz is statistically significant at the higher forces. Analysis of variance confirmed that the energy in the 0-5 Hz band at 80% and 100% MVC was less than at 20% ($p < 0.05$).

The difference in AMG energy was non-significant among other forces or bands. The energy of AMG spectrum was always negligible beyond 35 Hz.

3.5. The effect of blood flow on EMG and AMG frequency spectra.

The design of experiments was described in section 2.9 of the Materials and Methods section.

3.5.1. EMG and AMG median frequency in occluded muscle

Section 3.4 showed that the EMG and AMG median frequencies increase linearly with increasing force. Similar experiments were performed to measure the median frequency of the EMG before and after blood flow was occluded. The data is shown in Figure 31A. No significant difference was found in the EMG median frequency when blood flow was stopped. The median frequency of the AMG in the two conditions was also measured. As shown in Figure 31B the AMG median frequency was not significantly different when blood flow was stopped.

3.5.2. EMG and AMG frequency bands analysis.

The frequency bands analysis was applied to the data shown in Figure 29 and no significant differences were found when blood flow was temporarily stopped.

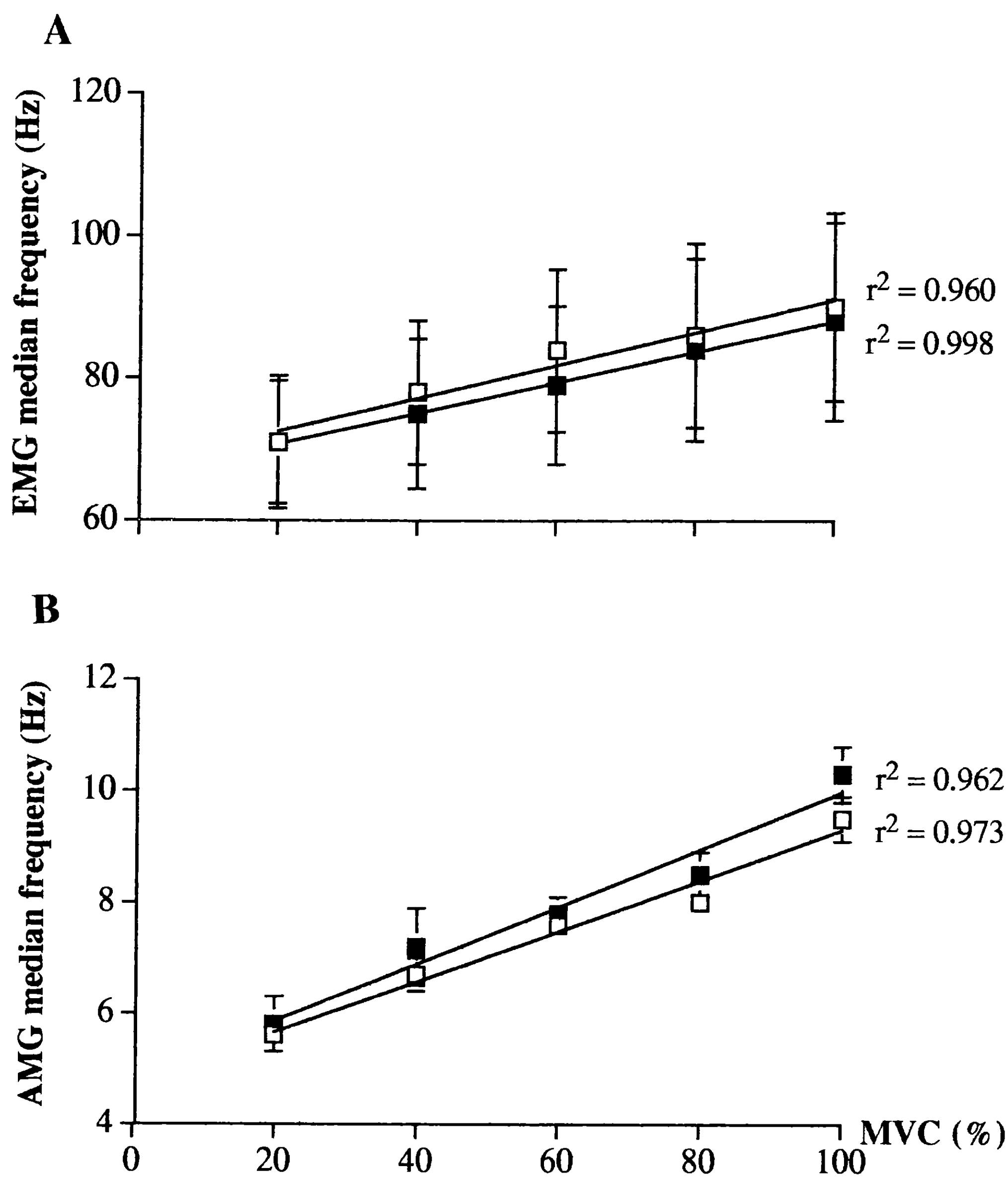

Figure 31. The mean ± SEM of the EMG (A) and AMG (B) median frequencies in normal condition (open square) and after (closed square) blood flow was stopped. No significant differences were found in the EMG or AMG median frequency after blood flow occlusion at similar forces. Larger SEM in the EMG median frequency indicate higher inter-subject variations. N=7.

3.5.3. Sounds from resting muscle

The experiments were performed on 5 male subjects with no history of cardiovascular or neuromuscular problems. The Hewlett-Packard microphone (21050-A) was strapped over the midpoint of the tibialis anterior. Experiments were carried out in three stages:

1. Sounds were recorded from the relaxed muscle, the frequency spectrum was calculated and its area was expressed as 100% (control).
2. The recording was repeated when a blood cuff pressure was applied at mid-thigh and inflated to 260 mmHg. This was expected to occlude blood flow in the leg.
3. After deflation of the cuff and during a period of recovery.

The area under the frequency spectrum during these three stages was normalised to control values. Typical data are shown in Figure 32. The dominant frequencies in resting muscle lie below 5 Hz. After cuff inflation, the signals were almost all abolished. During the recovery period, the sounds re-appeared and the area became about two times larger than in controls. This is probably due to the reactive hyperaemia.

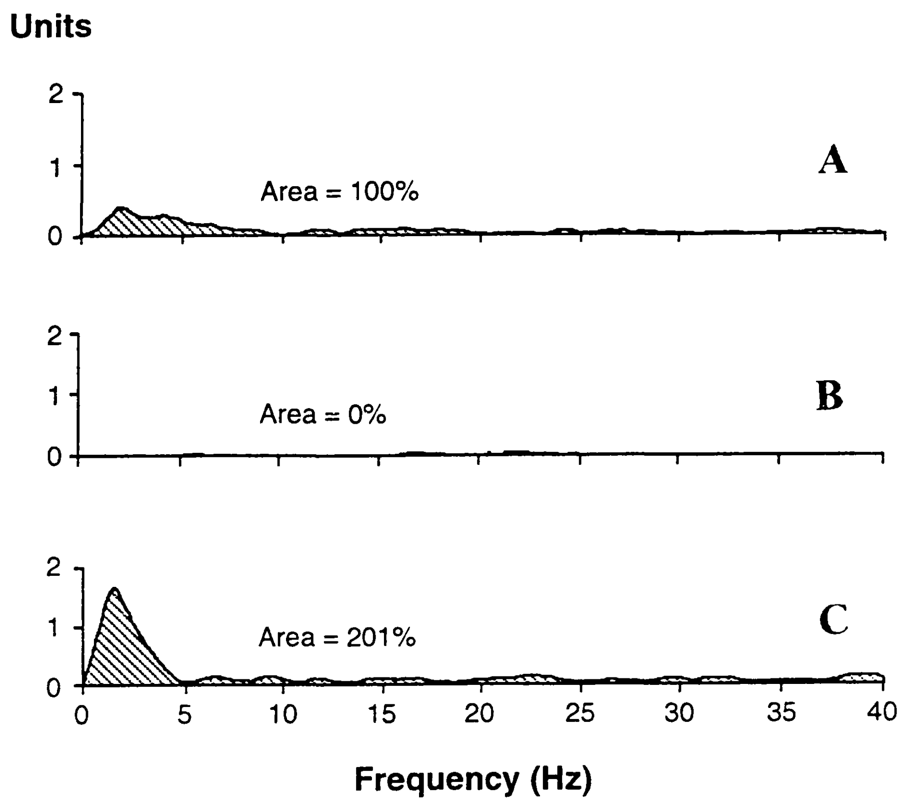

Figure 32. Shows changes of sounds of muscle: A. at resting muscle. B. after inflating blood cuff pressure. C. After deflation and during period of recovery.

3.6. Changes in the frequency spectrum with fatigue.

Spectral analysis of EMG and AMG signals was performed during sustained fatiguing activity. The changes in the EMG frequency spectrum are already well established as an important index of fatigue (De Luca, 1984). Any shift of the median frequency to lower values is an early indication of fatigue.

The spectra of the EMG and AMG was analysed in two ways: a. median frequency analysis. b. frequency bands analysis. They will be described in turn.

3.6.1. Analysis of EMG and AMG median frequency in fatigued muscle

The median frequencies of EMG and AMG were measured at the beginning , middle and the end of a sustained constant force contractions. During contractions at 40% of MVC, the EMG median frequency shifted towards lower frequencies indicating a fatigue of the muscle even though the force was constant. The mean EMG median frequencies measured in experiments in 8 subjects is shown in Figure 33A. Analysis of variance showed that differences in the median frequency between at onset and the end of contraction was significant ($p < 0.05$).

However, in the same experiments the AMG median frequency was not significantly different at the end of the contractions. AMG data are shown in Figure 33B. This is similar to the earlier observation in

Figure 25, that during sustained contractions at 40% MVC, IEMG rose but IAMG was unchanged.

The experiments were repeated at 60% and 80% of MVC. Figure 34A shows that the mean of EMG median frequency shifted towards lower values during contractions at 60% of MVC. The difference between the beginning and the end of fatiguing contractions was significant ($p < 0.01$). However, the AMG median frequencies also drifted towards lower frequencies during 60% of MVC. A significant difference in the AMG median frequency was found between onset and the end of sustained contraction ($p < 0.05$). The mean AMG median frequency changes are shown in Figure 34B.

The changes in mean of median frequency of EMG during 80% of MVC are shown in Figure 35A. The EMG median frequencies shifted towards lower values. The changes of EMG median frequency were significant during contraction ($p < 0.01$).

As shown in Figure 35B, mean of the AMG median frequency also drifted towards lower frequency at 80% of MVC. The difference in AMG median frequency between the onset and the end of contraction time, was significant ($p < 0.01$). The magnitude of this shift in AMG median frequency is greater than that seen during contractions at 60% MVC.

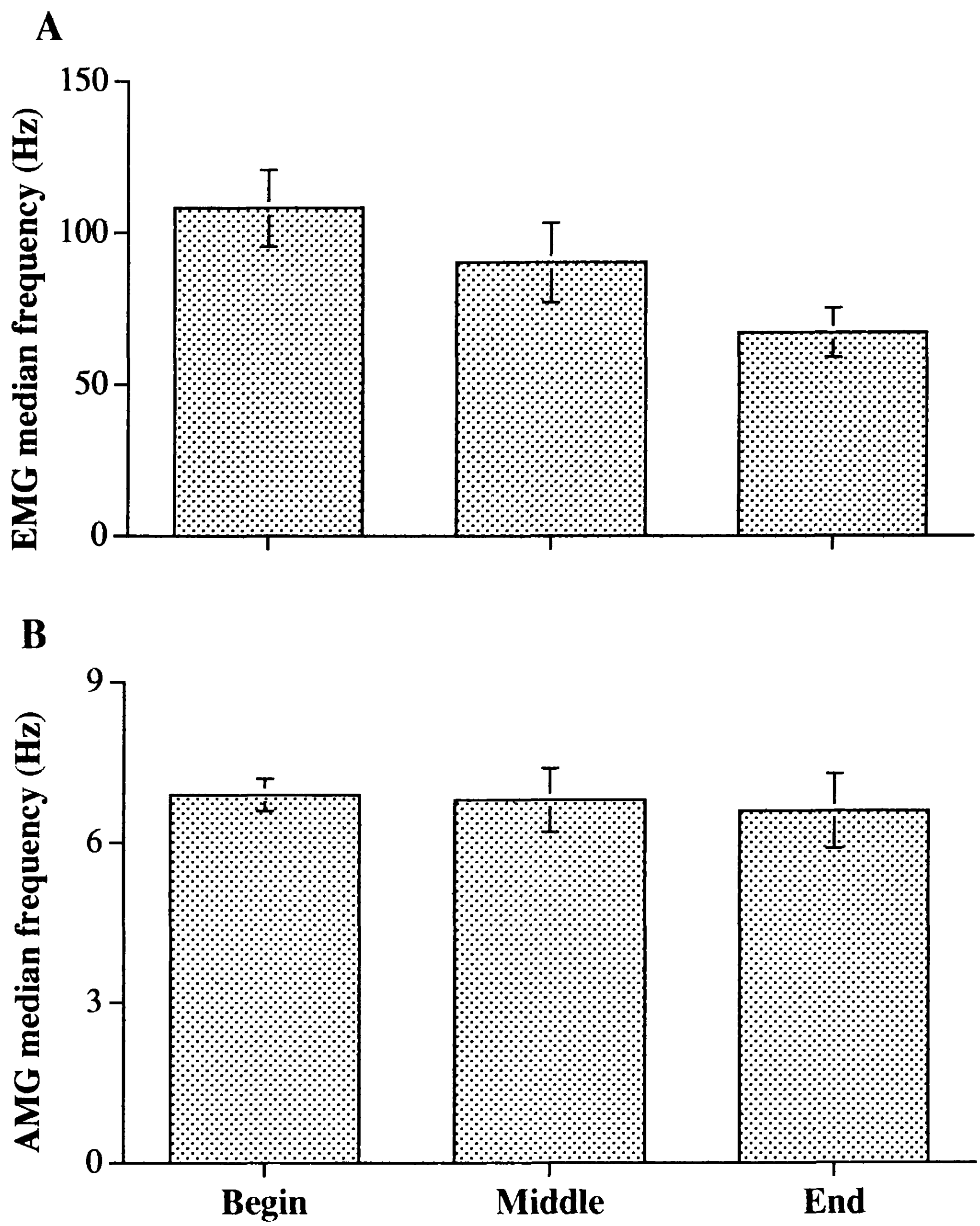

Figure 33. Mean (± SEM, n=8) of the EMG (A) and AMG (B) median frequency changes during a sustained contraction at 40% of MVC. The EMG median frequency shifted towards lower values significantly (P< 0.05), whereas the AMG median frequency was unchanged.

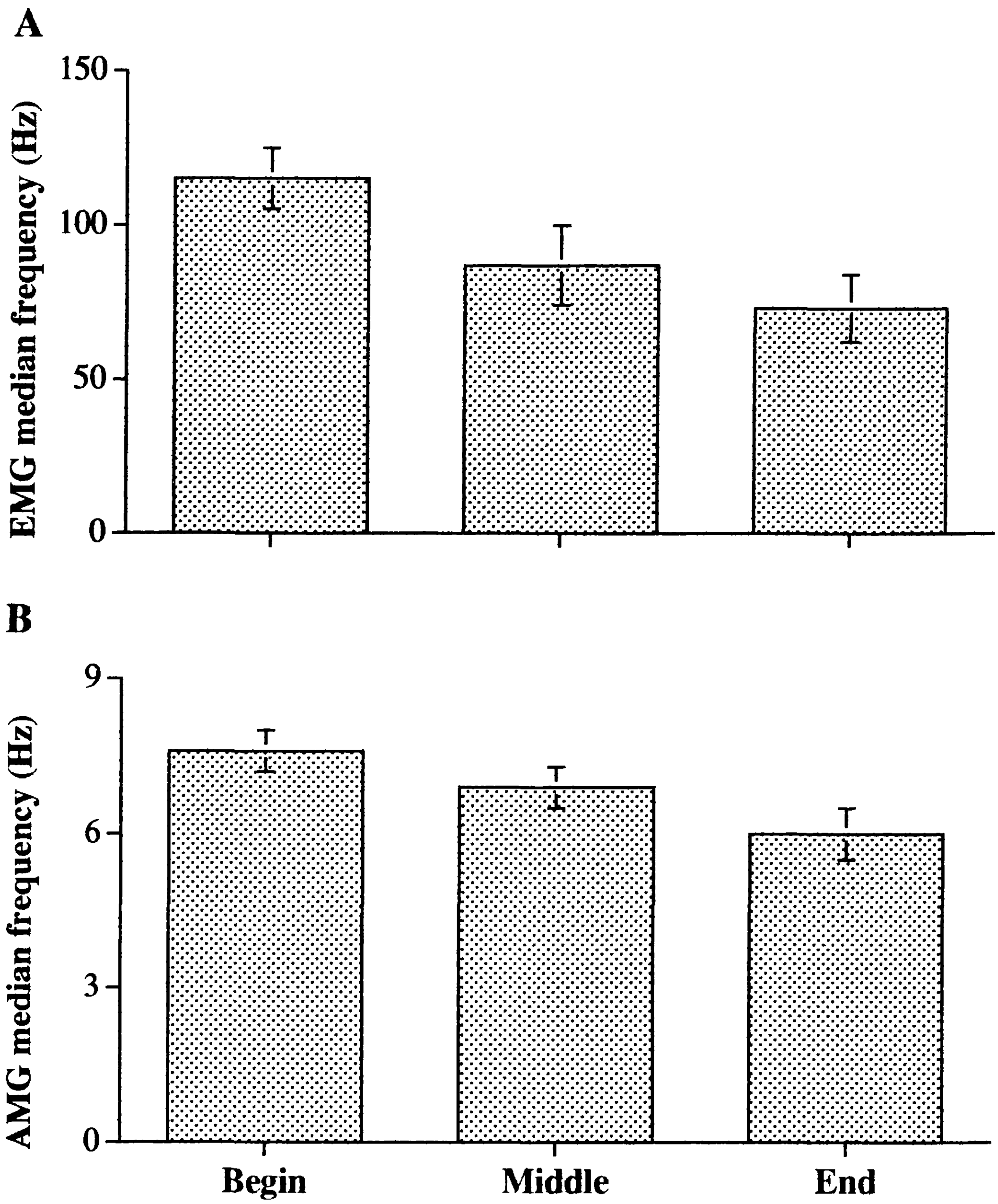

Figure 34. The changes of median frequencies of EMG (A) and AMG (B) during a sustained contraction at 60% of MVC are shown. The EMG and AMG median frequencies were shifted towards lower values. The changes in the EMG and AMG median frequencies between the beginning and the end of contractions were significant. ($P<0.01$ and $P<0.05$ for EMG and AMG, respectively). n=8.

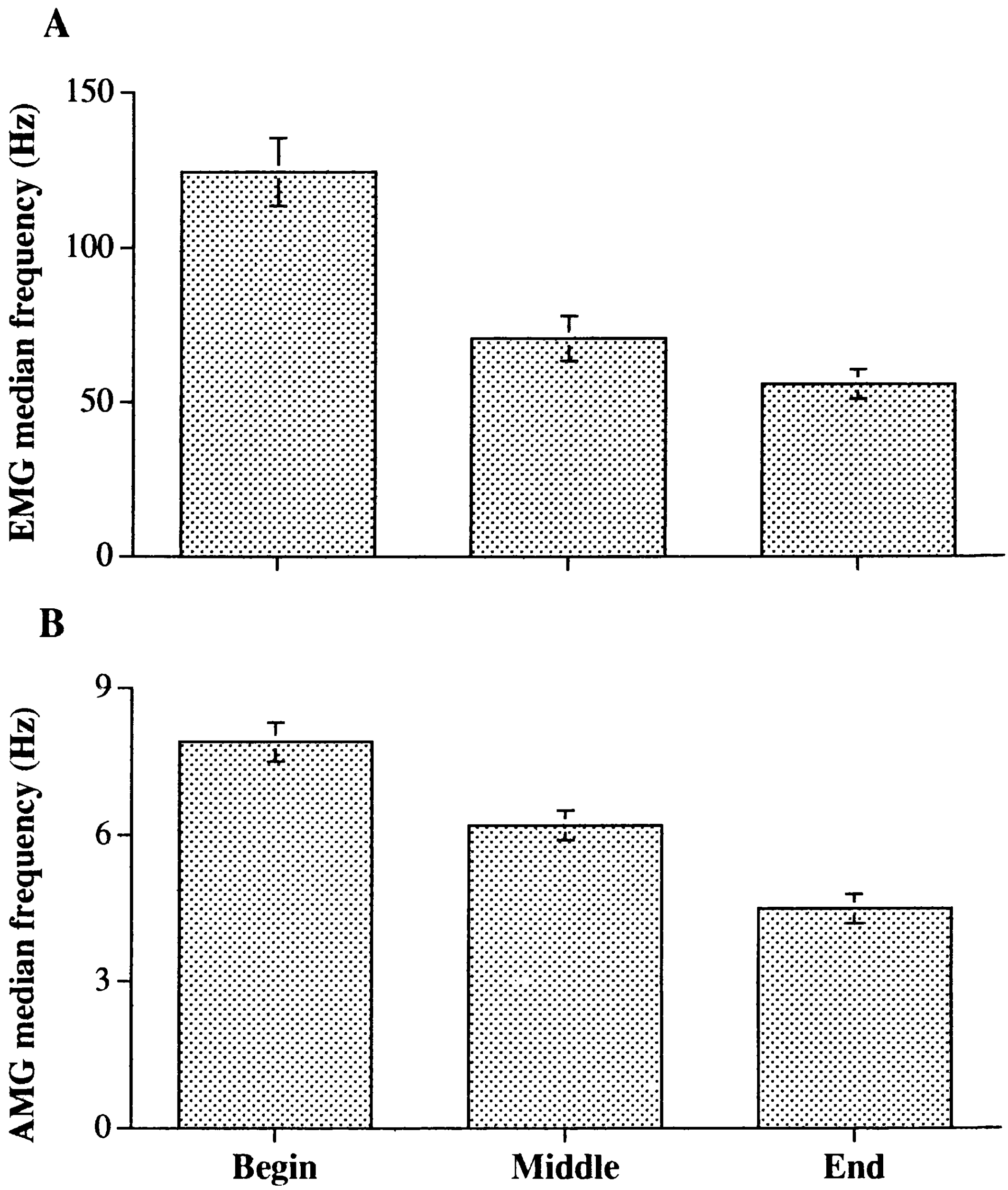

Figure 35. Changes of median frequencies of the EMG (A) and AMG (B) during a sustained contraction at 80% of MVC. The EMG and AMG median frequencies shifted towards lower values during contraction. The changes in the EMG and AMG median frequencies between the beginning and the end of contractions were significant ($p < 0.01$, n=8).

3.6.2. EMG and AMG bands frequency analysis during fatiguing contractions.

Bands frequency analysis is more informative than median frequency in the description of signals spectra. The percentage of the total signal energy present in each frequency band of the EMG was measured at the beginning, middle and the end of fatiguing contractions at different forces.

During sustained contractions at 40% MVC, the changes in frequency bands of EMG from 8 subjects were calculated. The data are shown in Table 5.

Bands	0-50Hz	100Hz	150Hz	200Hz	250Hz	300Hz
Begin	23.5 ± 5	23.2 ± 2	23.6 ± 3	16.6 ± 3	6.9 ± 1	3.1 ± 0.8
Middle	27.8 ± 7	24.9 ± 2	26.4 ± 4	12.7 ± 3	4.1 ± 1	2.3 ± 0.5
End	38.4 ± 6	31.2 ± 3	15.5 ± 2	9.5 ± 3	2.9 ± 0.7	1.5 ± 0.4

Table 5. The distribution of energy in the EMG in a range of frequency bands during 40% of MVC. Each cell contains the mean (± SEM, n=8) energy expressed as a percentage of the total energy recorded.

As indicated earlier in the median frequency analysis, during fatiguing contractions at 40% of MVC, the EMG frequency spectrum shifted towards lower values.

There is an increase in energy in the 0-50 Hz band from the beginning to the end of the contraction. This increase is significant (P< 0.05). There is a similar but smaller rise in the 50-100 Hz band, but this is not significant.

Frequency band analysis was applied to the AMG data at 40% of MVC and no significant difference was found with fatiguing contraction during this force level. The changes of AMG frequency spectrum during sustained contraction at 40% MVC were calculated. Table 6 shows the mean of the energy in AMG frequency bands at 40% of MVC.

Bands	0-5Hz	10Hz	15Hz	20Hz	25Hz	30Hz
Begin	33 ± 3.8	40.5 ± 5	16.4 ± 3	7.± 1.4	1.5 ±.4	0.4 ±.1
Middle	39 ± 4	40 ± 3.5	15.3 ± 3	3.5 ± 1	1 ± 0.2	0.5 ±.1
End	40.3 ± 7	41.5 ± 5	12 ± 2.8	3.8 ± 2	1.4 ±.6	0.5 ±.2

Table 6. The distribution of energy in the AMG in a range of frequency bands during 40% of MVC. Each cell contains the mean (± SEM, n=8) energy expressed as a percentage of the total energy recorded.

The frequency bands analysis was applied to EMG data at 60% MVC. Table 7 shows the changes of energy spectrum of EMG during a series of contractions at 60% of MVC.

Bands	0-50Hz	100Hz	150Hz	200Hz	250Hz	300Hz
Begin	26.8 ± 6	23.3 ± 2	21.9 ± 2	15 ± 2	7. 7 ± 2	3 ± 0.8
Middle	34.4 ± 8	25.9 ± 2	21.6 ± 4	11.4 ± 3	3.7 ± 1	2 ± 0.5
End	41.3 ± 7	27.6 ± 3	17 ± 3	8.4 ± 2.7	3.3 ± 1	1 ± 0.4

Table 7. The distribution of energy in the EMG in a range of frequency bands during 60% of MVC. Each cell contains the mean (± SEM, n=8) energy expressed as a percentage of the total energy

The EMG frequency spectrum changes and shifts towards lower values during 60% of MVC. The pattern of changes is similar to that in Table 5.

ANOVA showed that the change within 0-50 Hz bands was significant ($P< 0.05$) between the beginning and the end of contraction.

The frequency band analysis was applied to the AMG data at 60% of MVC. The changes of AMG frequency spectrum were calculated during sustained contraction at this level.

The mean of changes of AMG frequency spectrum is shown in Table 8. Whilst no changes in the AMG were seen at 40% MVC, when the sustained force was 60% there were significant changes in 0-5 Hz band energy ($p < 0.01$).

Bands	5Hz	10Hz	15Hz	20Hz	25Hz	30Hz
Begin	29.3 ± 3	46.2 ± 4	17.8 ± 4	4.5 ± 0.8	1.1 ± 0.5	0.5 ± 0.2
Middle	37.4 ± 3	38.2 ± 4	17.5 ± 3	4.1 ±.9	1.7 ±.5	0.4 ±.1
End	45.3 ± 7	32.3 ± 3	15.7 ± 2	3.9 ±.8	1.3 ±.4	0.8 ±.3

Table 8. The distribution of energy in the AMG in a range of frequency bands during 60% of MVC. Each cell contains the mean (± SEM, n=8) energy expressed as a percentage of the total energy

This will contribute to the fall in median frequency of the AMG reported earlier. There must be a fall in the energy in the higher bands but this is too small to be significant on statistical testing.

The frequency bands analysis was applied to the EMG median frequency data. The changes of EMG frequency spectrum were calculated during sustained contractions at 80% MVC.

The changes in the EMG frequency spectrum are shown in Table 9.

Bands	**0-50Hz**	**100 Hz**	**150 Hz**	**200 Hz**	**250 Hz**	**300Hz**
Begin	24 ± 4	32.5 ± 4	20.4 ± 2	13.9 ± 3	5.2 ± 1.1	1.9 ± 0.4
Middle	36.7 ± 4	29.4 ± 2	19.3 ± 3	10 ± 2	2.3 ± 0.3	1.1 ± 0.2
End	46.6 ± 5	30.2 ± 2	14.7 ± 3	5.3 ± 1.2	1.7 ± 0.2	0.8 ± 0.2

Table 9. The distribution of energy in the EMG in a range of frequency bands during 80% of MVC. Each cell contains the mean (± SEM, n=8) energy expressed as a percentage of the total energy

The frequency spectrum shows similar but greater changes to those seen in Tables 5 and 7. A significant difference in the EMG energy spectrum was found between beginning and end of contraction within the 0-50 Hz band ($p < 0.001$). There were no significant differences between the other bands.

The changes of AMG frequency spectrum during sustained contractions at 80% MVC were also calculated. These are shown in Table 10.

Bands	0-5 Hz	10 Hz	15 Hz	20 Hz	25 Hz	30 Hz
Begin	22.6 ± 2	49.8 ± 6	16.8 ± 4	4.6 ±1	1.5 ± 0.3	0.6 ± 0.1
Middle	41.8 ± 3	31.6 ± 3	14.4 ± 2	4 ± 0.4	1.8 ±.4	1 ± 0.2
End	54± 3.2	26 ± 4.2	11.8 ± 2	2.5 ± 0.3	1.1 ± 0.2	0.7 ± 0.2

Table 10. The distribution of energy in the AMG in a range of frequency bands during 80% of MVC. Each cell contains the mean (± SEM, n=8) energy expressed as a percentage of the total energy

The concentration of the energy in the 0-5 Hz band is prominent at the end of contractions. ANOVA showed that significant differences between the beginning and the end of contractions in the 0-5 and 5-10 Hz bands. The p values for these differences in the 0-5 Hz and 5-10 Hz bands are 0.001 and 0.01, respectively.

3.7. Analysis of EMG and AMG median frequency at different muscle lengths

Experiments were performed to investigate how EMG and AMG changed with changing of muscle lengths. The experiments were carried out on nine subjects at ankle joint angles of 75°, 90° and 105°.

The design of the experimental protocol was described in section of 2.10 of Methods.

The maximum voluntary contraction was significantly reduced by 33% when the experiments were performed at the shorter muscle lengths. Longer muscle lengths did not significantly change the voluntary force developed. The IEMG and IAMG did not show any change as muscle shortened or lengthened when they were normalised to values obtained in optimal length. This is shown in Figure 36. Linear relationships between IEMG, IAMG and force were found at three different muscle lengths.

The process of normalising forces conceals a change in the EMG/force and AMG/force relationships with muscle length. This shift is seen more obviously in Figure 37 where data were plotted against absolute force rather than MVC. The slope of the regression lines between IEMG, IAMG and force at shorter lengths with respect to optimal muscle length increased significantly ($p < 0.05$) whereas at the longer length it was not significant. The relationship between IEMG, IAMG and force are still linear at three muscle lengths.

The spectral analysis was similar to those detailed in section of 3.4.

In spite of significant differences in force between the optimal muscle length and the shorter length, the EMG median frequency was similar. This was true for submaximal forces as well as at MVC. At the longer length the force was unchanged as was the EMG median frequency. These data are shown in Table 11.

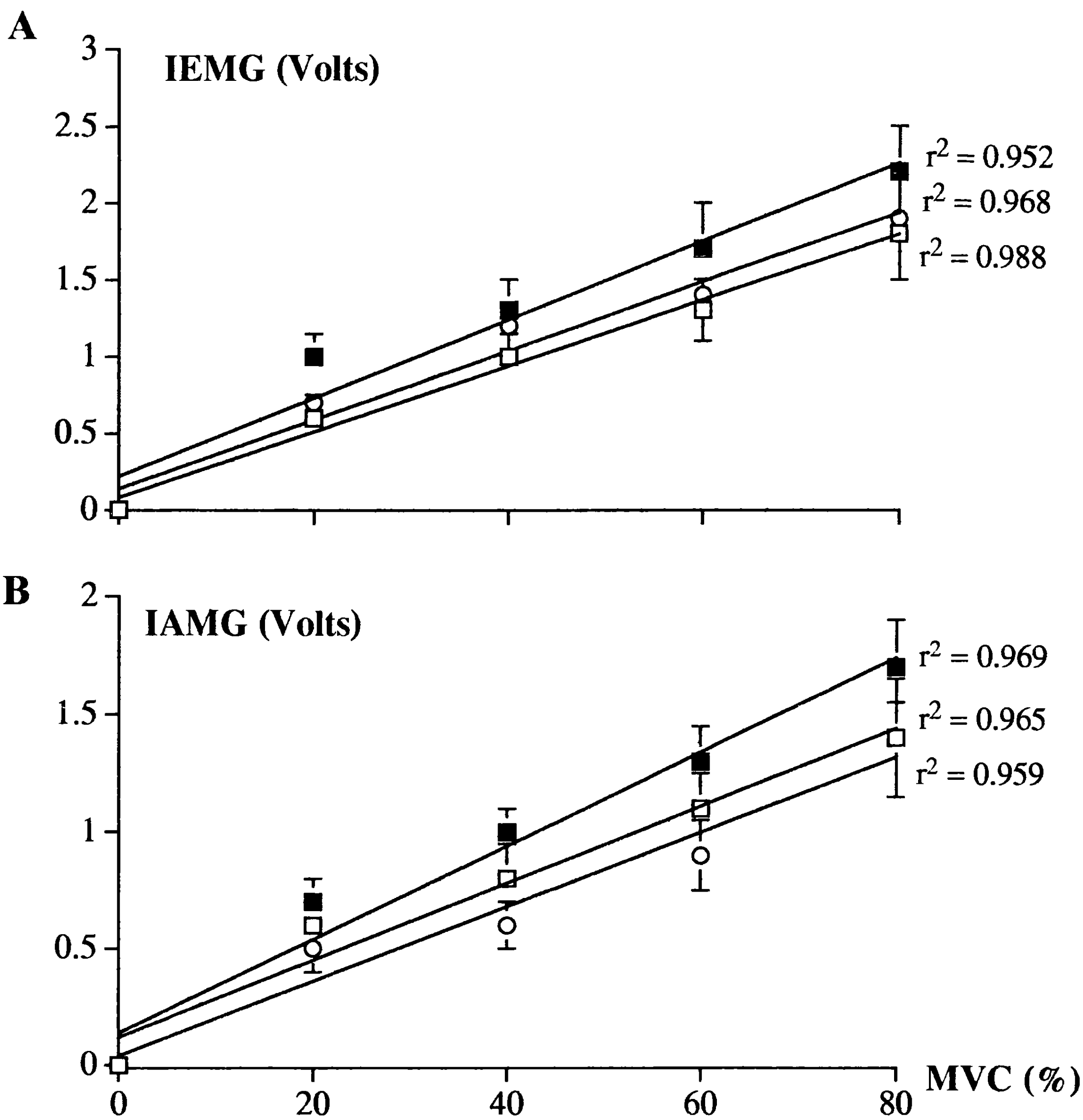

Figure 36- Mean (± SEM) of IEMG (A) and IAMG (B) at 75° (■), 90° (□) and 105° (O) of the ankle joint plotted against MVC. Each contraction lasted 6 seconds. Contractions were made at 20, 40, 60 and 80% MVC. The IEMG and IAMG show the similar trends with increasing force at three muscle lengths. The relationship between IEMG, IAMG and force was linear at three muscle lengths.

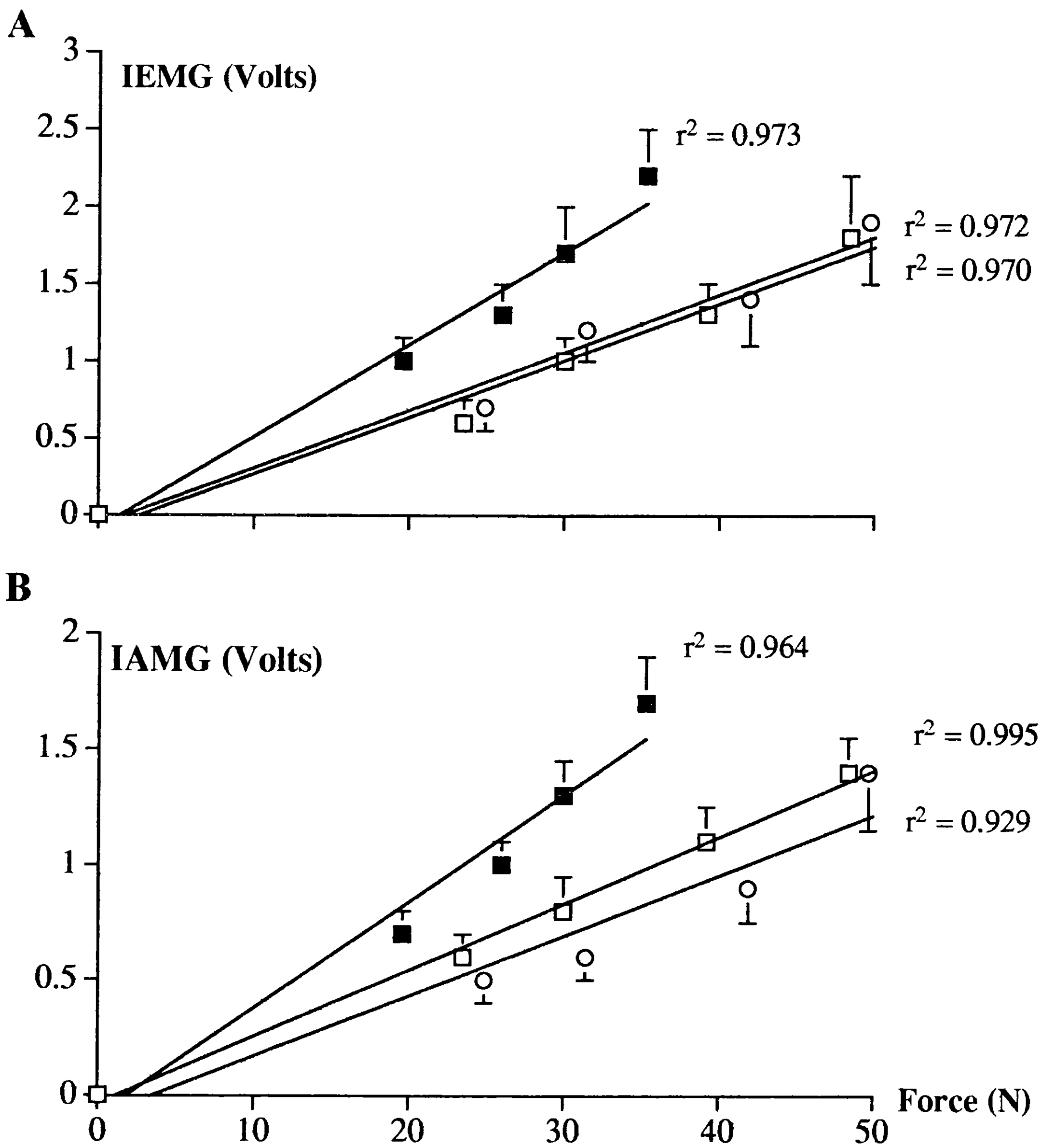

Figure 37. Means (± SEM) of the IEMG (A) and IAMG (B) plotted against absolute force at 75° (■), 90° (□) and 105° (O) of the ankle joint were shown. Contractions were made at 20, 40, 60 and 80% MVC. At the shorter lengths forces are decreased by one third of their maximum values, and the IEMG and IAMG values are shifted to the left in spite of increasing amplitude.

Table 11 shows the mean of median frequency of EMG for 9 subjects.

%MVC	EMG Median Frequency (Hz)		
	75°	90°	105°
20	78.8 ± 8.2	71.5 ± 8.1	72.1 ± 7.6
40	86.3 ± 8.2	79.6± 9.4	78.6± 8.8
60	94.4 ±10.2	91.6 ± 9.8	85 ± 9.4
80	102.3 ± 10	96.9 ±13	92.3 ± 9.6
100	107 ± 11.5	102.5 ± 13	95.5 ± 9.5

Table 11. The EMG median frequency at three muscle lengths. Each cell contains the mean (± SEM, n=9) EMG median frequency at various forces between 20-100% MVC.

The EMG median frequency increases with force at different muscle lengths. Analysis of variance showed that there is no significant difference in EMG median frequency at different lengths.

The AMG median frequency also was measured. These data are shown Table 12. It behaved in a similar way to EMG median frequency, except that the frequency was substantially lower. Despite reducing absolute force at the shorter length, the AMG median frequency remained similar to that at optimal length. At longer lengths, the AMG median frequency was also not significantly changed.

Table 12 shows mean of AMG median frequency changes at different muscle lengths.

%MVC	AMG Median Frequency (Hz)		
	75°	90°	105°
20	6.3 ± 0.2	5.6 ± 0.2	5.2 ± 0.2
40	7.9 ± 0.3	7.4 ± 0.3	7 ± 0.4
60	9.2 ± 0.3	8.4 ± 0.3	8.5 ± 0.4
80	9.9 ± 0.4	9.2 ± 0.2	9.5 ± 0.4
100	11.7 ± 0.4	10.9 ± 0.5	10.8 ± 0.5

Table 12. The AMG median frequency at three muscle lengths. Each cell contains the mean (± SEM, n=9) AMG median frequency at various forces between 20-100% MVC.

3.8. A comparison of the performance of two types of transducers:
In almost all the experiments described in this thesis, the acoustic myogram was recorded by the Hewlett-Packard heart sounds microphone. The results show that IAMG increases linearly with force up to 80% of MVC. It declined at higher force.
The response of this microphone was compared with that of a newer type of transducer.

The experiments were performed on 5 normal subjects. The AMG was recorded using the microphone and an Entran (EGAX-F-100) accelerometer. Prior to the experiments, two points over the belly of tibialis anterior muscle were chosen and marked. The transducers were placed at these points during successive non-fatiguing isometric contractions at different forces. After four trials the transducers were swapped with each other. Each contraction lasted 6 sec with a 30-90 seconds interval between contractions. At least 10 minutes were allowed between each set of trials to avoid fatigue. The signals were band pass filtered (2-160 Hz) and where necessary amplified using NL 106, 125 modules and then full wave rectified and integrated. The mean and standard error of mean of the rectified, integrated values for each point for both sensors was calculated and plotted against force.

Figure 38 shows that the IAMG recorded by the Entran rises with increasing force as does the IAMG recorded with a Hewlett-Packard sensor up to 80% of MVC. After this force the latter declines. There was a linear relationship ($r^2 = 0.978$) between force and the IAMG recorded by Entran . A linear relationship between force and IAMG recorded by the microphone was found up to 80% of MVC ($r^2 = 0.979$).

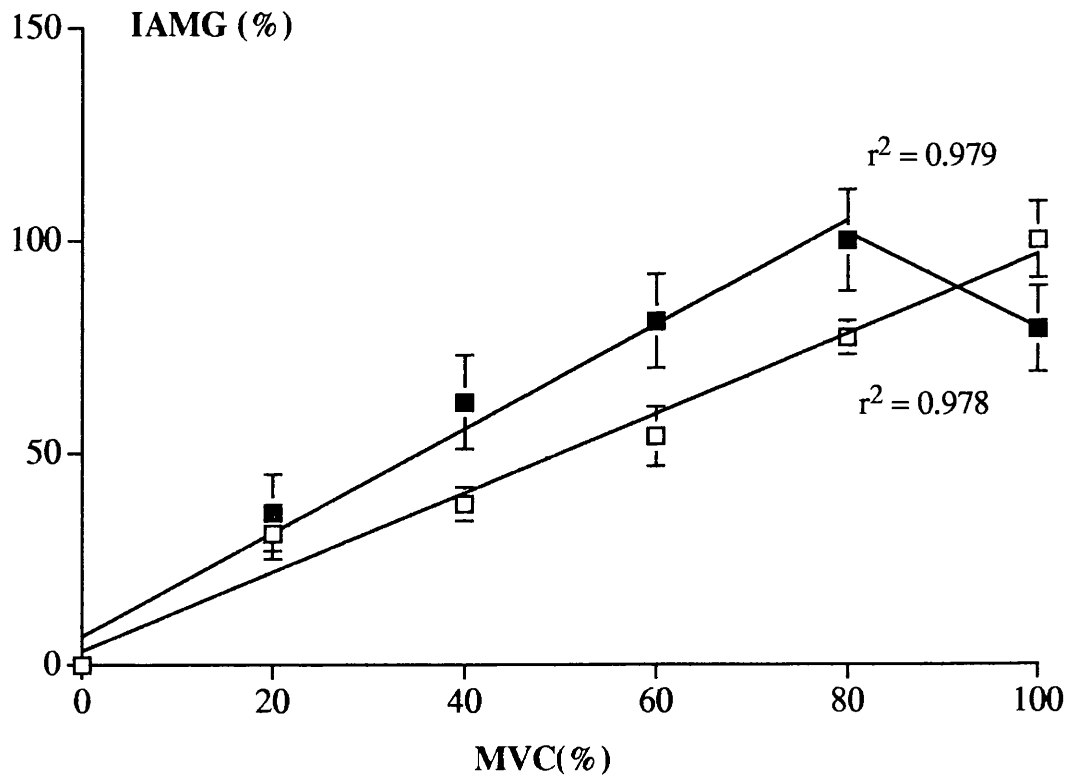

Figure 38• A comparison of the performance of the Hewlett-Packard heart sounds microphone (■) and the Entran accelerometer (□). Each contraction lasted 6 seconds with 30-90 seconds rest between contractions. Contractions were made at 20, 40, 60, 80, and 100% MVC. The IAMG recorded by the Entran increases with force linearly up to MVC (r^2=0.978). The similar linear relationship between IAMG, recorded by the microphone, and force up to 80% of MVC was found (r^2=0.979). Above 80% of MVC the IAMG declines.

DISCUSSION

4. Discussion

The EMG and AMG were recorded from the tibialis anterior during isometric contractions. These recordings have been made in unfatigued muscle at different force levels and different muscle lengths and also during a range of fatiguing contractions. The EMG and AMG have been subjected to conventional analysis to show changes in intensity and frequency content during various types of muscle contraction. These data have been used to address several previously unanswered questions about AMG characteristics and muscle performance:

1. What are the relationships between IEMG, IAMG and force during isometric contractions?
2. What are the relationships between EMG, AMG median frequencies and force?
3. Can AMG be used as an indicator of muscle fatigue?
4. Are EMG and AMG characteristics influenced by changes in muscle length?
5. Are the EMG and AMG characteristics affected by blood flow?

Experiments in this thesis were performed to obtain answers to these questions.

4.1. The relationship between IAMG, IEMG and force in the unfatigued tibialis anterior.

The tibialis anterior muscle, which is one of main dorsiflexors of the ankle joint, can be fully activated by voluntary effort. Since its motoneurones receive relatively weak input from Ia fibres but relatively strong input from descending motor pathways, these can be completely activated during strong voluntary dorsiflexion (Belanger and McComas, 1981; Bigland-Ritchie, Furbush, Frank, Gandevia and Thomas, 1992). The tibialis anterior is composed of about 73% slow twitch fibres (Johnson, et al, 1973). This composition is very similar to soleus muscle, which is composed of more than 85% slow twitch fibres. This suggests that tibialis anterior might have a postural role. Furthermore, biochemical analysis has shown that this muscle is fatigue resistant (Jones, Turner, Newham, and McIntyre, 1993).

Muscle is composed of motor units, the number of these depend on the size and type of muscle. The number of muscle fibres in each motor unit depends on how the muscle is controlled. They can be as few as 3-6 in the muscles of the hand and face where fine and delicate movements are made or as many as 2000 in the gastrocnemius muscle where the control is coarse. Motor unit activity is controlled by the interaction of afferent inputs causing them to be recruited or changing their firing rate in relation to the required force. Two neural mechanisms control the force output. These are recruitment of motor units and modulation of firing rate of active motor units (Milner-Brown, Stein and Yemm, (1973a, b), De Luca, Lefever, McCue and Xenakis, (1982a, b)).

During relatively low force isometric contractions the smaller, slower contracting motor units are activated (Burke, 1980). As force output increases, in addition to an increase of firing rate of smaller motor units, a number of larger faster motor units are recruited. This orderly recruitment of motor units is known as the 'Size Principle' (Henneman et al, 1965). When the force of contraction is reduced voluntarily, motor units are derecruited in the reverse sequence (De Luca et al, 1982a,b). It has to be noted that the contribution of recruitment and firing rate are different in different muscles. Smaller muscles such as those in the hand, recruit their motor units between 0-50% MVC and beyond this force level the firing rate of active motor units is the sole mechanism for the increase of force output. In the larger muscles, e.g., the soleus muscle, the increase in force is mainly due to a recruitment of additional motor units and depends less on changes in the rate of firing.

The EMG is the summation of all the action potentials from the muscle fibres contracting within the muscle and clinically is used as an index of force measurement.

Although the relationship between force and EMG is known to vary in different muscles it has been well documented in biceps brachii, first dorsal interosseous, deltoid, quadriceps femoris, triceps brachii and soleus muscles (Basmajian and De Luca, 1985).

Different relationships between AMG and force have been reported in different muscles (Oster and Jaffe, 1980; Stokes et al, 1988; Orizio et al, 1989 et al, 1989; Stokes and Dalton, 1990; Rouse and Baxendale, 1990). These differences can be related to several factors e.g., different types of the transducers, muscles, contractions and range of forces were being used.

In the experiments described in this thesis, the middle of the belly of the tibialis anterior was chosen to obtain a good contact between the AMG detector and the skin which lies over the muscle. Furthermore, it is consistent with the theory that AMG reflects the mechanical events of muscle contraction, with the majority of the fibres bundled together in the middle of muscle belly. In addition, if AMG signal is spreading out like a wave from active muscle fibres to the surfuce of the leg, the best postion for recording AMG with larger magnitude will be around the middle of the muscle.

The raw EMG and AMG were full wave rectified and integrated to overcome some transient movement artefacts of raw signals. Inman, Ralston, Sanders, Feinstein. and Wright, (1952) found a linear relationship between IEMG and isometric tension of the tibialis anterior but they could not find the similar relationship between IEMG and muscle power. To date, there have been no reports concerning the AMG and force relationship in the tibialis anterior.

The results described in this thesis show that EMG and AMG behave similarly at submaximal contractions. Figure 7 shows that during a series of graded isometric contractions, the peak-peak amplitude of raw EMG increases progressively with force but the amplitude of raw AMG increases up to 75% of MVC. The amplitude of the AMG declines between 75% and 100% of MVC. During investigations of isometric contractions, the experimental set-up permitted certain amount of movements of the muscles at the beginning and end of the contractions. These movement artefacts at both ends of contraction were the result of the muscle shortening to pull the tendon taught from slack state. This is evident in Figures 7, 12, 16, 23 and 27.

Figure 10 clearly presents the relationships between IEMG, IAMG and force. These results are in agreement with those were reported by Orizio et al (1989) using the Hewlett-Packard heart sounds microphone in experiments on the biceps brachii. They found that IEMG rose linearly with force, as did IAMG up to 80% of maximal voluntary contraction, but it declined thereafter as isometric force increased. The variability in the IAMG above 75% of MVC might be due to the performance of the Hewlett-Packard microphone since it is not seen in the experiments with Entran accelerometer shown in Figure 38.

The progressive increment in the EMG magnitude with force is due to the recruitment of motor units and increased firing rate in active motor units (Edwards, 1981).

The increment in the AMG magnitude with force may be due to the same mechanisms. The force output of tibialis anterior seems to rely on both neural mechanisms, namely recruitment and firing rate of active motoneurones (Macefield et al, 1993). In addition, force and AMG are both mechanical characteristics of muscle, and the IAMG is quite similar to force in total time course. Bolton et al (1989) pointed out that following electrical stimulation of the thenar muscles at shorter or longer muscle lengths, the force tended to decrease but the amplitude and duration of muscle sounds seemed to be more constant. They speculated that the series elastic properties may act only for force recording whereas, the sound recording is influenced by the contractile and parallel elastic components.

In other words, during contraction, longitudinal and transverse oscillation would be produced. The former is detected by the strain gauge and the latter is recorded by the microphone. They concluded that the transverse mechanical oscillations of muscle fibres during contractions could produce the muscle sounds. This was first suggested by Barry, (1987) and Frangioni et al, (1987). Transverse oscillations might occur at a frequency related to the resonant frequency of the muscle (Barry and Cole, 1990). The resonant frequency of the muscle is determined by several parameters such as mass, length, geometry and

stiffness. During isometric contraction the change in the stiffness is much greater than the change in any of the other parameters (Dobrunz, Pelletier and McMahon, 1990).
There are several other possible origins of muscle sounds. Artefacts and non-myogenic sources such as heart sounds and arterial pulse waves, microphone movements on the skin, body movements and tremor might contribute.

In the present study a high-pass filter at 2 Hz was chosen to eliminate low frequency noise related to the external environment such as low frequency vibration generated in the walls and floor of building. This filter setting also reduced some of low frequency physiological signals caused by body and lower limb displacement unrelated to the contraction in the anterior tibial muscles.
Since the microphone was strapped over the muscle with a rubber band, there should have been minimal relative movement of the transducer with respect to the surface of muscle. As described in the Material and Methods in this thesis, any gross movement of lower limb of subjects during experiments was prevented by a very rigid and heavy frame.
Any significant movement generated audible, scraping sounds and these were very effectively cut off by the upper limit of the band pass filter at 160Hz.
Tremor is a low frequency shaking of the limb. It is produced by oscillation of muscular force. Therefore the AMG and tremor both originate from muscle. The AMG frequencies recorded during voluntary

contractions are similar to physiological tremor frequencies i.e. about 10 Hz (Lippold, 1957) and 6-12 Hz (Allum, Dietz and Freund, 1978). In addition, both AMG and tremor increase as contractions become stronger (Rhatigan et al, 1986; Goldenberg et al, 1991). Thus, the role of tremor in the origins of AMG must be considered. Whilst the frequency ranges of tremor and AMG are similar it is clear that AMG contains many frequency components which are not associated with tremor. During fatiguing contractions it is well known that tremor increases but the results shown in Figure 18 show that AMG amplitudes decrease. Both AMG and tremor tend to shift towards lower frequency ranges as fatigue develops i.e., 4-6 Hz for tremor (Lippold, 1981) and 4.5-6 Hz for AMG shown in Figures 33, 34 and 35.

In addition, there are other factors which support the belief that tremor makes little contribution to the results reported in the thesis. Tremor is an oscillation in force developed along the long axis of a muscle whilst AMG is a transverse oscillation. Tremor is mostly associated with the unsupported upper limb whilst the records shown here come from rigidly supported lower limbs.

None of the subjects displayed any resting tremor and AMG is recorded even in low force, non-fatiguing contractions where tremor is unlikely to be significant.

On the occasions where tremor was observed, most usually at high forces, its contribution to the AMG was obvious and the records were discarded. Thus it is safe to conclude that tremor makes at most a very small contribution to AMG in some circumstances.

The changes to the AMG signal when blood flow to lower limb was temporarily stopped is shown in Figure 12. The background noise was reduced by 200 mmHg of cuff pressure. The abolition of sounds after inflation of the cuff and its re-appearance during the period of reactive hyperaemia suggest that the background noise in the muscle is due to blood flow. This can be seen in Figure 32. Figure 14 confirms that the difference in the AMG was not significant in normal flow and when blood flow stops to muscle. Thus, it can be concluded that muscle sounds during contraction do not have a vascular origin. This finding agrees with the observations of Oster and Jaffe, (1980) and Keidel and Keidel (1989).

The amplitude of the AMG declines as the force increases from 75-100% MVC in spite of increasing EMG. Such a reduction in the raw and IAMG above 75% MVC, was also reported by some other investigators (Orizio et al, 1989; Smith and Stokes, 1992). The reduction in the AMG magnitude may be related to some of following factors:

1. It could be due to completely fused tetanic contractions. Thus, force output fluctuations and the transverse mechanical oscillation, implicated in the generation of the muscle sounds are reduced (Gordon and Holbourn, 1948; Brozovich and Pollack, 1983).
2. From 75% to 100% MVC, force output is controlled mainly by increasing motor unit firing rate (Freund, 1983, Macefield et al, 1993) since no new motor units remain to be recruited in this range. Therefore, the firing rate of motor units increases. As well as increasing force this

also increases the stiffness of the muscle The increase of muscular stiffness may reduce or eliminate muscle sounds, or increase their frequency. This last possibility might explain the increase in AMG median frequency seen in Figure 29.

3. The decrease in the IAMG above 75% MVC might indicate a greater synchronisation of motor units reducing transverse mechanical vibrations in this range.

4. The reduction may be due to difference in applied pressure between the contact transducer and the skin over muscle (Smith and Stokes, 1993). This might change the efficiency with which muscle oscillations are transferred to the transducer.

5. The reduction of the IAMG at MVC could be the increased intramuscular pressure and reduced muscle compliance (Sadamoto et al, 1983).

6. The reduction in the AMG might be due to the contact transducer characteristics. It is commonly found that the acoustic myogram recorded by heart sounds microphones falls in amplitude between 75-100% MVC (Orizio et al, 1989; Smith and Stokes, 1992). This may be due to a relative insensitivity to lower amplitude or higher frequency oscillations. However, during isometric voluntary contraction of the biceps brachii, a similar variability in IAMG has been seen when an accelerometer was used (Zwarts and Keidel, 1991).

In the pilot experiments described in this thesis the AMG recorded concurrently with a heart sounds microphone and an accelerometer deviated at forces above 75%. This suggests that the fall in AMG when the microphone is used may be an artefact introduced by the sensor characteristics rather than a true feature of the muscle. Thus it is safe to conclude that the performance of heart sounds microphone (HP 21050A) makes a large contribution to fall of IAMG at higher forces. However, other factors may still be significant.

4.2. Analysis of the frequency components of EMG and AMG

Recent developments in computers and analysis programs have led to further investigation of myoelectric signals.
The analysis of frequency content in the biological signals seems to offer additional information about tissue function.
The results described in this thesis show that the EMG median frequency rises progressively with increasing force in unfatigued muscle. The shift in the median frequency of EMG can be explained by an increase in the conduction velocity of action potentials along the muscle fibres. The increase in the conduction velocity is accompanied by a reduction of action potential duration (Stulen and De Luca, 1981). The recruitment of larger motor units activate larger muscle fibres with faster conduction velocities and shorter action potential duration.
The AMG median frequency also shifts toward higher frequencies at higher forces. This is shown in Figure 29. The AMG median frequency showed a linear relationship with force up to 100% MVC. In addition, the intra- and inter-subject variability in median frequency is less than

that in the IAMG. The strong linear relationship and reduced variability make median frequency a better monitor for force than IAMG. The increase in the AMG median frequency could be due to higher motor unit firing rates or changes in muscle contractile properties e.g., muscle stiffness (Barry and Cole, 1988, Orizio et al, 1990). The present study was not able to show a steeper increase in the AMG median frequency in the range 75-100% MVC at which IAMG reduces. This finding is not in agreement with the observation of Orizio et al, (1992).
When EMG and AMG median frequencies at different isometric forces were compared, the results showed that the AMG median frequency was about 10 times lower than that in the EMG. The range of median frequency was variable between 5 and 15 Hz whereas, the median frequency of EMG was between 71 and 112 Hz. This indicates the origins of the EMG and AMG are different and each represents different features of contracting muscle.

More useful information was obtained when a frequency band analysis was applied to the AMG data. Figure 39 shows a similarity between the distribution of the AMG energy spectrum and motor units firing rates. The motor unit firing rates are taken from a recently published by Bigland-Ritchie et al, (1992). Both values rose with increases in force and they are both distributed between 0 and 35Hz. However, their distributions are different. This could be explained by the fact that of motor units of different sizes fire at different frequencies (Henneman et al, 1965, Macefield et al, 1993). Thus small units, which make little contribution to the force, might reach the highest rates whilst larger units, which contribute more force, might be expected to fire more slowly. Whatever the cause, there is no simple relationship between motor unit firing rates and AMG frequency content other than that they share a common frequency range.

In summary, there are linear relationships between IEMG, IAMG and force although at higher forces the variability of the IAMG became rather high. The EMG and AMG median frequencies also showed a clear linear correlation with force.

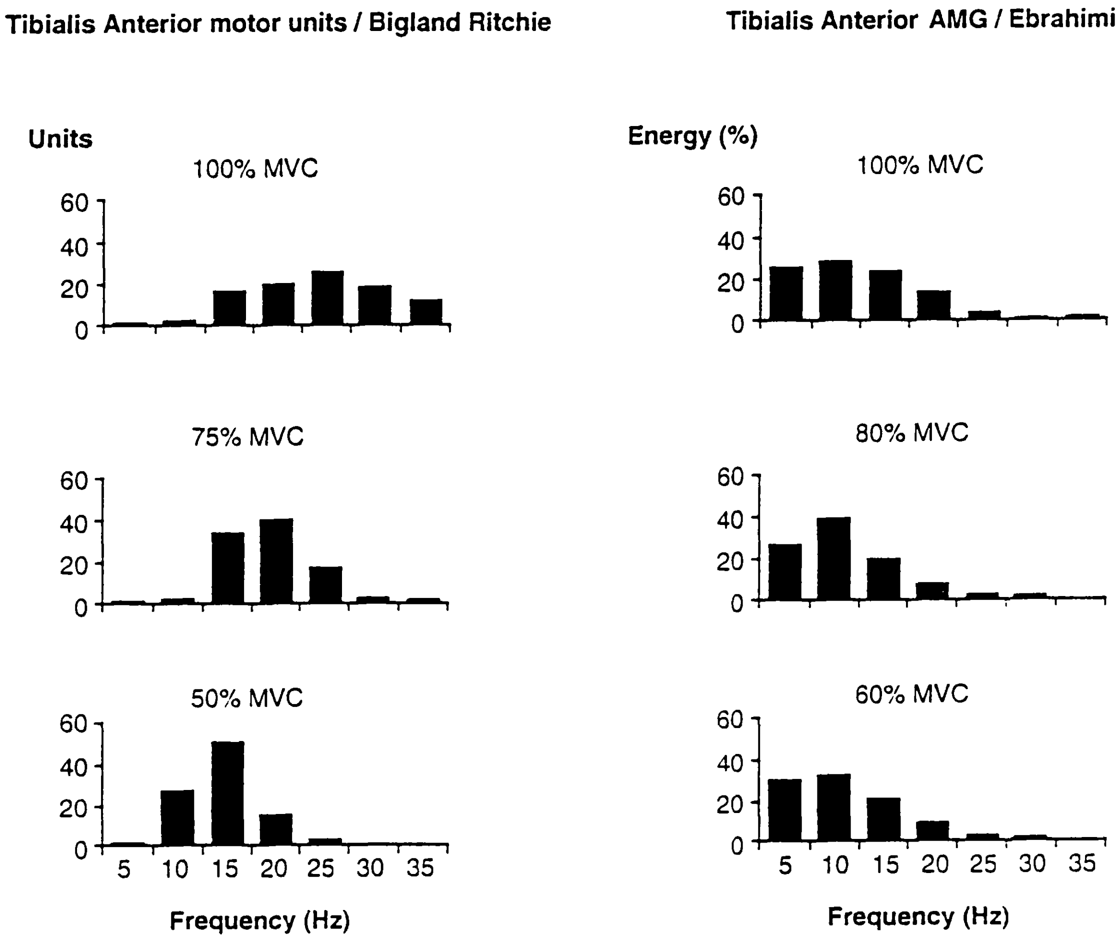

Figure 39. The mean motor units firing rates (left-hand figures) and AMG frequency distributions (right-hand figures) during a series of voluntary contractions in tibialis anterior muscle. The motor units firing rates are reported from work recently published by Bigland-Ritchie et al, (1992).

4.3. Changes of the AMG and EMG in fatigued muscle.

Changes of EMG and AMG were investigated in two different types of fatiguing exercises. Time and frequency domains analysis were performed. They will be discussed in turn. In addition, changes of IEMG and IAMG after intermittent and during sustained contractions will be described.

Intermittent fatiguing activity

During fatigue produced by submaximal intermittent isometric contractions, the loss of force is thought to result mainly from failure of the muscle contractile apparatus (Bigland-Ritchie, Furbush and Woods, 1986).

Figure 18 shows that the relationship between force and IEMG was still linear after fatiguing exercise but the slope of the regression line had increased. This increase confirmed the presence of fatigue. The increase in EMG amplitude has been attributed to recruitment additional motor units, since more motor units are activated to achieve the given force, (Edwards and Lippold, 1956). It seems that low frequency fatigue develops because of impairment of excitation-contraction coupling, and this explains the dissociation of force and IEMG.

Therefore, the IEMG alone is not accurate enough to be used as a indicator in fatigue which develops with intermittent exercise though the IEMG/force ratio still can be used as a fatigue index.

There is a clear reduction in the slope of IAMG/force relationship after intermittent fatiguing exercise shown in Figure 18. This reflects the changes in raw AMG shown in Figure 16. This reduction in AMG might be a consequence of fatigued motor units contracting and relaxing more slowly and so being more fully tetanised even at lower forces. There are the additional possibilities that motor units could fire more slowly, though still maintaining a fused tetanus (Bigland-Ritchie et al, 1983) and changes in muscle stiffness, due to swelling of muscle fibres, changing the AMG transmission characteristics. Thus, it can be noted that similar reasons may explain the force reduction and the IAMG reduction.

The results described in this thesis are in agreement with those during the early stages of fatigue intermittent activity of the quadriceps reported by Dalton, Comerford and Stokes (1991). They examined the AMG during intermittent contractions repeated until a profound fatigue developed. The contractions began at 75% of the initial MVC and continued until only 35% of the initial MVC could be developed. They described reductions in IAMG amplitude with force reductions during the early stages of fatigue, until the force falls to 60% MVC. In the later stages of fatigue the IAMG rises again.

The reduction in force in early fatigue must mean that even though a force of 60% of the initial MVC is produced, the same force is now the maximum which that muscle can produce. Thus, the decline in the IAMG may have the same origins as those described earlier for the

AMG reduction at higher forces, i.e., complete fusion of motor unit contraction, slowing of force rise times and stiffening of the muscle.

It is interesting to note that Stokes and Dalton (1991) did not observe a shift in the slope of the IAMG/force relationship in fatigue. This shift in slope is clearly found in the experiments described in this thesis (Figure 18). This difference is most probably due to a delay of 15 minutes between the fatiguing exercise and the AMG measurements in their experiments. It is likely that the quadriceps will have recovered significantly in this time. The experiments described here did not continue the fatiguing process for the same duration as Stokes and Dalton (1991). Their exercise period lasted longer and produced much greater fatigue. In these circumstances the AMG is likely to be severely affected by fatigue tremor, reducing the significance of any observation.

In addition, subjects often recruit additional muscle groups to help maintain the force and the possibility exists that sounds associated with gross body movements and AMG from other muscle groups might also influence the recorded signals (Wee and Ashley, 1990). All of the factors might help to explain the rising AMG seen in late fatigue in Dalton's experiments.

Sustained fatiguing contractions

The results in this thesis show that IAMG and IEMG behave differently during sustained contractions. Figure 19 shows that when a force of 80% MVC is maintained the EMG increases after 20 seconds whereas the AMG falls progressively during the contraction. These trends are clearly present in all subjects tested and Figure 21 confirms that the changes are statistically significant.

When lower forces are sustained, the behaviour of the AMG is quite different. Figure 23 shows a sustained contraction at 40% MVC during which the AMG does not appear to change. Figure 25 illustrates summary data for eight subjects which shows that even though fatigue clearly developed, as seen by a rising EMG/force ratio, the IAMG was not significantly changed. The explanation for these observations might lie in the motor units contractions remaining unfatigued even after several minutes activity at 40% MVC and so maintaining the AMG signal. In the higher force range the muscle must be almost maximally contracted. 80% of initial MVC must be close to maximal force quite soon after the contraction begins .

Thus the fully fused tetanic contractions and increased muscle stiffness which explained the reduced AMG at 100% MVC in fresh muscle probably also apply in the later stages of fatigue at 80% MVC. The fall in AMG during high force contractions also suggests that fatigue induced tremor was not a problem during these experiments.

The reduction in AMG during these experiments is in agreement with data from similar experiments reported by Barry et al (1985), during contractions at 75% MVC and by Orizio et al, (1989) during contractions at 60% and 80% of MVC in the biceps brachii.

It has to be noted that the EMG/force ratio, which is a clear indication of fatigue, increased during sustained constant forces at different levels.

The increase in amplitude of IEMG, was greater at higher force which indicates more recruitment of larger, faster contracting fibres. Voluntary contractions in the higher force range are associated with higher percentage activation of larger motor units. Henreksson-Larsen et al, (1985) have reported that the larger motor units are located in the deeper portions of the human tibialis anterior. This does not agree with the observation of Burke (1981) that smaller motor units have deeper locations in muscle.

As seen in Figure 26, the duration of exercise is progressively shortened as contraction intensity increases. The IEMG rises up to exhaustion regardless force levels. The IEMG/force ratio tends to increase with exhaustion and this ratio is already used as an indicator of muscle fatigue. This increase can be attributed to the recruitment of larger fresh motor units, synchronisation and slowing of conduction velocity (Bigland-Ritchie and Woods, 1984).

In summary, the IEMG and IAMG indicate two different features of fatigue during sustained contractions especially at higher forces.

During isometric sustained contractions, the recording of EMG and AMG can be helpful to identify the electromechanical dissociation. The IEMG alone will not be an accurate monitor of sustained contraction, whereas the IAMG, particularly at higher force levels, could be considered as a reliable indicator of localised muscular fatigue.

It is well known that the median frequency of EMG shifts towards lower frequencies during sustained contractions and this change is already widely used as an indicator of localised muscle fatigue (De Luca, 1984). The median frequency may decrease by more than 50%. However, the decrement depends on the muscle being investigated (De Luca et al, 1983).

In this thesis, the changes of EMG frequency spectrum were studied at the beginning, middle and the end of sustained contractions at different forces.

The EMG median frequency shifted to lower values at all forces tested but its reduction was more pronounced at higher forces. Figure 33 shows that when a force of 40% MVC is sustained the EMG median frequency shifts progressively towards lower values from the beginning of contraction. Frequency bands analysis seems to be much more valuable than median frequency during fatiguing activity particularly in fluctuating signals. Under these conditions, the median frequency does not describe the spectrum as well as the frequency band analysis does. The shift of EMG energy spectrum from higher frequency bands into lower frequency can be seen in Tables 5, 7 and 9. This confirms that fatigue developed at all forces. It agrees with Merletti, Knaflitz and De Luca (1990) who showed that during submaximal prolonged voluntary contractions of the tibialis anterior the mean and median frequencies of the EMG spectra decreased. It is likely that the range of muscle conduction velocities is compressed during the fatigue process.

The significant leftward shift of EMG median frequency at all force levels seen in Figures 33, 34 and 35 could be due to change in shape of muscle action potentials and slowing conduction velocity of muscle fibres. The slow conduction velocity could be related to accumulation of some types of metabolites such as the hydrogen ions in the muscle. The greater reduction in the EMG median frequency at higher forces could be due to the larger motor units which are faster but more fatigable than the smaller motor units.

There are relatively few descriptions of changes in the AMG frequency spectrum during fatigue available in the literature (Zwarts and Kiedel, 1991, Goldenburg et al, 1991 and Orizio et al, 1992). The study of AMG frequency spectrum in this thesis, showed a different behaviour at different sustained forces. Figure 35 shows that when a force of 80% of MVC is maintained the median frequency of AMG shifts significantly towards lower values.

Figure 33 illustrates pooled data from 8 subjects which shows even though fatigue clearly developed, as seen by rising EMG/force ratio and shift of EMG median frequency towards lower values, the AMG median frequency was not changed. Frequency bands analysis of AMG also shows the similar trends of AMG median frequency during higher and lower force sustained contractions. A shift of AMG median frequency towards lower values might be related to the decreased firing rate of motor units (Barry et al, 1985, Orizio et al, 1992). A greater reduction in the AMG median frequency at higher forces can be related to the use of larger motor units which are easily fatigable. Perhaps, only small motor units, remain active at high frequency to maintain the required force.

One possibility for the stability of AMG frequency spectrum during contractions at lower forces could be due to stability of firing rate of active motor units (Maton and Gamet, 1989).

In summary, it appears that analysis of IAMG and IEMG and their frequency spectra during isometric sustained contractions gives additional information about feature of muscle fatigue. The presence of fatigue was confirmed by three well known indicators. They were the increase of EMG/force ratio, IEMG (particularly at higher force of intermittent activity or at the end of sustained contractions) and the decrease and shift of median and bands frequency towards lower values. Under these conditions, the AMG/force ratio or IAMG decreased and the frequency spectrum shifted towards lower values. This was more pronounced at higher force levels. However, in fatigued muscle the IEMG rises but the IAMG falls, which indicates an electromechanical dissociation and failure in excitation-contraction coupling.

4.4. Changes of AMG and EMG at three muscle lengths.

The changes in AMG and EMG characteristics at three different muscle lengths was investigated.:

a- IAMG and IEMG changes

At the shorter muscle length, the absolute force decreased by 33%. This is in agreement with data reported by Marsh, Sale, McComas and

Quinlan (1981). The reduction of force at shorter muscle length indicates a mechanical disadvantage. For a given submaximal force the muscle under investigation needs to recruit more motor units or increase the firing rate of active motor units to compensate. When muscle shortens more muscle fibres moves close to the surface EMG electrodes. Thus, despite the force reduction at the shorter length, the amplitude of IEMG rose.

This probably explains a steeper IEMG/force relationship at shorter muscle lengths shown in Figure 37. At longer muscle lengths, the slope of regression line between IEMG and force was similar to that in the optimal length. This differs from the results reported by Inman et al (1952). The increase in slope of regression line between IAMG and force at the shorter length when compared to that at the optimal length suggests that more fresh motor units are recruited to compensate mechanical deficiency. It is interesting to note that tibialis anterior can be maximally activated by volitional effort in spite of changes in its overall mechanical behaviour (McKenzie and Gandevia, 1986). The similarity of slopes between IAMG and force between longer and optimal lengths might be due to activation of the same motor units.

b- Frequency changes

The similarity of the relationship between EMG median frequency and force in the shorter or longer and optimal lengths suggests that muscle action potential duration and conduction velocity are little affected by the muscle length changes.

The similarity of the relationship between AMG median frequency and force at the optimal, longer and shorter muscle lengths suggests that the muscle does not resonate at a different frequency as it lengthens or shortens. It is interesting to note that there is no change of motor unit firing rates at different lengths of the tibialis anterior (Bigland-Ritchie et al, 1992). There is also no clear correspondence between the AMG median frequency and motor units firing rates. The nature of the AMG mechanism, is still unknown but it is not a simple resonance phenomenon.

4.5. The influence of blood flow on EMG and AMG

The results shown in Figure 31 indicate that there is no significant difference between EMG median frequency under normal and occluded conditions. The explanation may be that the EMG median frequency is not affected by occlusion during brief non-fatiguing isometric contractions. This finding differs from the observation of Merletti et al, (1983) and De Luca (1984). Their experiments were performed with longer periods of contraction. The difference between the results described in this thesis and theirs can be related to the duration of muscle contraction or could be due to the effect of fatigue and type of muscle under investigation. But the similar slopes between IEMG and force under normal and occluded conditions, shown in Figure 14, indicate that results in this thesis were obtained from non-fatigued muscle.

The similarity of the IAMG/force relationship in normal and occluded muscle, suggests that the effect of blood flow on the AMG is negligible.

In the AMG and EMG, the similarity of relationship between median frequency and force in normal and occluded conditions, indicates that the motor units are little influenced by lack of blood flow during brief isometric contractions.

4.6. Potential applications:

Although there are many unknown factors concerning the AMG, there are several practical applications for its use in medicine, sports sciences and rehabilitation:

1. The AMG could be used as an indicator of mechanical activity, particularly in those muscle groups in which force measurements are difficult, e.g., in paraspinal muscles that are often involved in myopathies, and used to estimate their electro-mechanical efficiency.
2. The AMG is already being used as a monitor of muscular fatigue. If during the fatigue process, the EMG and AMG are recorded simultaneously, the IEMG rises but the IAMG decreases. This can indicate an excitation-contraction coupling failure.

3. The AMG characteristics might play a role in distinguishing muscle fibres types, i.e., the dominant frequency of orbicularis is about 22 Hz whereas in soleus it is about 11 Hz. This possible advantage of the AMG can be used in sport medicine.
4. AMG can be used as a non-invasive method for diagnosis of peripheral neuropathy. In addition, it is useful for assessment of the functional state of the nerve terminal and end plates (Hufschmidt, Schubnell and Schualler, 1987).
5. AMG is already being used as an externally powered prosthesis in rehabilitation (Barry, Leonard, Gitter, and Ball, 1986).
6. AMG might be used in the assessment of muscle function in cardiac muscle and paediatric investigation in health and disease.

4.7. Future plan

Parts of the results in this thesis, even if related to a biological signal which is still in its "infancy" indicate that the acoustic myogram is an important low frequency signal with a mechanical origin which can be detected from a contracting skeletal muscle. The most important feature of the AMG is that these studies can be made on muscles whose anatomical position makes access difficult.

Guidelines for appropriate use of AMG need to be established before it can be used to assess muscle function.

Different force/AMG relationship may be due to technical factors rather than physiological differences between muscles and individual subjects. Technical considerations such as the type detector used, different types of contraction, different ranges of joint motion, analysis of signals, coupling with the skin and repeatability of recording are necessary before AMG can be accepted for clinical use, either as a diagnostic and monitoring tool, or for rehabilitation research.

The present work will be extended by looking at the AMG in the tibialis anterior following electrical stimulation and dynamic contractions. It will be also considered in different types of muscle contraction in other muscles. Further work is required to determine the effect of type of recording used and the method of securing the sensor to the skin. The performance of various types of transducers will be compared. The pressure with which the AMG detector is applied over the muscle influences the amount of AMG activity recorded. This pressure has to be standardised to reduce variability during repeated recordings.

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