SITE SPECIFIC MOTOR UNIT RECRUITMENT DURING FATIGUE IN HUMAN SOLEUS MUSCLE: A QUANTITATIVE EMG ANALYSIS

by

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ABSTRACT

The purpose of this study was to determine whether motor unit recruitment is site specific in the human soleus muscle, particularly during muscular fatigue. Needle electrodes measured the electrical activity from the muscle at sites 1 and 2, located on the lateral border of the soleus, and site 3, located on the medial border. Turns analysis was used to determine if there were differences in recruitment among the three sites during isometric plantarflexion at various intensities. Contractions of 20%, 40%, and 60% MVC were each maintained for 10 seconds. Next, a gradual increase in torque (ramp contraction) from 0-80% MVC was completed in approximately 40 seconds. Finally, a 70% MVC fatiguing contraction was sustained for three minutes. During each of the 10 second contractions, torque, numbers of turns, and amplitude remained constant throughout each contraction. There were significant differences in turn counts among the sites, with the largest differences observed at the lowest intensity (20% MVC). During the ramp contraction, as the torque continued to increase to 80% MVC, the amplitude of the EMG signal showed a corresponding increase. The numbers of turns, however, leveled off at sites 1 and 2 at 60% and 50% respectively. At site 3, the number of turns had not yet reached a plateau at 70% MVC. There were significant differences observed among the sites, with the largest differences at the lowest intensities. During the 70% MVC fatiguing contraction, torque declined in every subject, with an average decline of 59%. Numbers of turns decreased at all sites by approximately 43% at site 1, 35% at site 2, and 25% at site 3. There was a significant difference among the sites, primarily at the lower torque levels, when torque had decreased as a result of fatigue. Site 3, located at the medial site in the muscle, was significantly different from the others. Fast Fourier transformation (FFT) was used along with turns analysis to analyze the fatigue data. The FFT showed a similar trend to the turns analysis, but with more pronounced differences among the sites. FFT decreased significantly by approximately 20% at site 1, 31% at site 2, but showed no significant decrease at site 3. Once again, site 3 was quite different from sites 1 and 2 and the greatest site differences were observed at the lower intensities. These data suggest that recruitment can be site specific in the human soleus muscle during fatigue and isometric contractions of various intensities. These recruitment differences were most evident at the lower intensities, suggesting that more recruitment "options" are available to the neuromuscular system at lower torque levels. During fatigue, the medial site was markedly different from the other sites, which were located laterally. The presence of such differences among muscle sites highlights the importance of consistently sampling from different regions of a muscle.

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INTRODUCTION

Muscular fatigue has been defined as the reduction in the force-generating capacity of the neuromuscular system that occurs during sustained activity (Bigland-Ritchie, Johannson, Lippold, & Woods, 1983). Fatigue has been attributed to many factors, which are usually classified as either central or peripheral mechanisms. Central fatigue refers to those mechanisms of the central nervous system that control the drive to motoneurons (Enoka & Stuart, 1992). Peripheral fatigue sites and processes include the motoneuron, neuromuscular junction, sarcolemmal membrane, excitation-contraction coupling, accumulation of metabolites, depletion of fuels, and impaired blood flow (Kirkendall, 1990). It has been difficult to specify the exact mechanisms of fatigue because it is likely that several processes may be responsible, depending upon the type of contraction, its intensity, and duration.

It is also possible that different sites within a muscle may not be equally affected by the same mechanisms (Enoka et al. 1992). Therefore, one site might be more fatigued than another at any given time during a contraction. In a study as early as 1964, Willison demonstrated that activity measured in terms of counts per second (turns analysis) could vary considerably from one area to another in a single muscle and indicated that it was necessary to take samples from many areas of one muscle. This concept has not since received much attention, or been previously demonstrated during fatigue, but there are several reasons for believing that fatigue may be site specific.

To increase the force of muscle contractions, it is necessary to raise the firing frequency of the motoneurons and/or "recruit" additional motor units (Adrian & Bronk, 1929). Since recruitment of motor units is based on the size principle (Henneman, Somjen, & Carpenter, 1965), and different motor unit types are recruited in different sequences according to the intensity of contraction, it would be logical that recruitment patterns would differ among sites in a muscle. This would also be expected because muscles have been shown to have distributions of motor units that vary among sites of a muscle (Elder, Bradbury, & Roberts, 1982). Furthermore, since motor units have different thresholds of recruitment and different rates of fatigue, it would not be unexpected to see patterns of recruitment and fatigue that are site specific.

Studies have also shown that recruitment of motor units can be site and task specific (Ter Haar Romeny, Denier Van Der Gon, & Gielen, 1984; Enoka et al. 1992; Hensbergen & Kernell, 1992), and that muscle length, the force exerted by the muscle, and whether the muscle is shortening, lengthening, releasing, or maintaining a contraction (Enoka et al. 1992) can all influence recruitment. It has been concluded from these studies that there is preferential recruitment of motor units in certain parts of a muscle for specific tasks. The territory of the muscle fibers of a motor unit usually occupies only a fraction of the cross-sectional area of the muscle, thereby supplying a means for selective use of part of the muscle (Ter Haar Romeny et al. 1984). It is also possible that motor units could be recruited in rotation during fatiguing activity (Enoka et al. 1992), which would provide periods of inactivity that could be used for replenishment of substrates.

Another important reason for suspecting that fatigue can be site specific is related to the influence of force of contraction on muscle blood flow. During isometric contractions, the intramuscular pressure (IMP) is determined by the intensity of contraction. With increasing intensities, IMP increases, causing blood flow to be impeded until arterial pressure is exceeded, at which point flow is occluded. If IMP is not uniform in different parts of a muscle during a contraction, the amount of blood flow that each site receives could have an effect on the rate of fatigue, regardless of the motor unit types found at that site. It is known that muscle structure, which includes shape, pennation, proximity to other contracting muscles, and the degree of vascularization can affect IMP (Sadamoto, Bonde-petersen, & Suzuki, 1983; Sejersted, Hargens, Kardel, Blom, Jerson, & Hermansen, 1984). Since the soleus does not have a uniform shape and has a very complex fiber arrangement, it is reasonable to assume that blood flow may not be equal to all regions of the muscle during isometric contractions.

In a study by Sjogaard et al. (1986), isometric knee extension was performed continuously for one hour at very low tensions of 5% maximal voluntary contraction (MVC) (Sjogaard, Kiens, Jorgensen, & Saltin, 1986). Intramuscular pressure and electromyography (EMG) recordings were examined at four sites within the quadriceps muscle: vastus lateralis (proximal), vastus lateralis (distal), rectus femoris, and vastus medialis. It was found that the blood flow was distributed heterogeneously throughout the muscle during low level static contractions. Through EMG recordings, it was confirmed that this heterogeneity was related to the alternating recruitment of muscle fibers. Sjogaard et al. (1988) also found that there was increased pressure deeper in the muscle (Sjogaard, Savard, & Juel, 1988). Similarly, Wisnes and Kirkebo (1976) demonstrated that in rat calf muscles, blood flow was first compromised deep in the muscle where pressure is the highest, and that anaerobic conditions may exist in interior parts of the muscle during contraction.

Although there has been much research conducted into the mechanisms responsible for fatigue, there has been very little attention focused specifically on whether fatigue is site specific within a single muscle. Previous research that has incorporated EMG analysis (Bigland-Ritchie, 1981) and muscle biopsy data (Karlsson, Funderburk, Essen, & Lind, 1975) has predominantly investigated single sites within a muscle. For the many reasons outlined above, the findings at one site may not be representative of the entire muscle. It is therefore possible that recruitment patterns and fatiguability may vary from site to site. The purpose of this study was to test the hypothesis that recruitment patterns differ at three sites in the human soleus muscle during short duration submaximal contractions (20%, 40%, 60% MVC), a ramp contraction (0-80% MVC), and particularly during a 70% MVC fatiguing contraction.

REVIEW OF LITERATURE

It has been established that a number of mechanisms may contribute to fatigue in a muscle. The degree to which each contributes to fatigue depends upon such factors as fiber arrangement, fiber type distribution, intramuscular pressure, blood flow, and intensity of contraction. Considering that the above factors are known to vary among sites in a muscle (Enoka et al. 1992), it is possible that the level of fatigue may differ among sites at any given time during a contraction. This would be indicative of site specific motor unit recruitment in a muscle. This concept has not received much attention, but there is much evidence in support of heterogeneous recruitment patterns. This review will examine the possibility of site specificity of motor unit recruitment, focusing on fiber type distribution, fiber arrangement, IMP and blood occlusion, and site and task specificity. In addition, it will review fatigue mechanisms and the EMG methodology used to quantify the muscle activity levels.

Electromyography

The smallest functional unit of the neuromuscular system is the motor unit, which is comprised of an anterior horn cell, its axon, and all of the muscle fibers innervated by the axon. The anterior horn cell is located in the ventral horn of the spinal cord and its axon exits the spinal cord and travels to the muscle. Once it has entered the muscle, the axon branches several hundred times or more and each branch of the axon terminates on a single muscle fiber.

Several events must occur before a muscle fiber contracts. An action potential is propagated along the motoneuron to the motor endplate. At the endplate, a chemical substance, acetylcholine, is released that diffuses across the synaptic cleft, causing a rapid depolarization of the muscle fiber under the motor endplate. This rapid depolarization, and the subsequent repolarization of the muscle fiber, is an action potential (Lamb & Hobart, 1992).

The recording of action potentials is the basis of electromyography (EMG). The electrical activity approaching, reaching, and leaving the recording electrode generates a motor unit action potential (MUAP) that is usually triphasic (positive-negative-positive) in configuration. The selectivity of the recording electrodes will determine how many muscle fibers or motor units will be within the recording field. The amplitude and the shape of individual motor unit potentials are generally determined by approximately one to three muscle fibers that are situated close to the leading-off surface of the recording electrode (Buchthal, Guld, & Rosenfalck, 1957). The total duration of the motor unit potential reflects the activity of all fibers of the motor unit (Buchthal & Rosenfalck, 1963). The pattern of electrical activity during full recruitment is produced when all motor units are active, so that their action potentials can no longer be individually identified (Fuglsang-Frederiksen, 1981). The resultant signal, or complex interference pattern (IP), is the spatial and temporal summation of the action potentials from active motor units within the muscle.

Quantitative EMG

The most basic information that can be derived from an EMG signal is knowledge of whether the muscle of interest was active, and if so, the degree of activation (Gerleman & Cook, 1992). EMG-Force information can also be derived from the processed EMG signal. This processed signal can include information on the number of active motor units and their firing rates during contractions of a known force. However, the accuracy obtained from this signal processing is not always very high. Therefore, the aim of signal processing in most studies is to assign a numerical value (usually a percentage of MVC) to the level of EMG activity associated with the generation of a corresponding force. With increasing intensity of contraction, more and more units are recruited, and their firing frequencies increase. The summated motor unit activity reflects these changes as the resulting interference pattern becomes more dense and of greater amplitude (Gerleman et al. 1992). The relationship between force and EMG is dependent on factors such as muscle length (Grieves & Pheasant, 1976), velocity (Bigland & Lippold, 1954), cross talk between electrodes (Hof & VandenBerg, 1981), and co-contractions of synergistic and antagonistic muscles (Psek & Cafarelli, 1993; Sirin & Patla, 1987).

Several methods have been developed to quantitate the interference pattern, including the following:

Duration of Waveform

The duration of a waveform is the time from the onset to termination. Analysis of the shape and duration of a waveform can provide an estimate of the distribution of conduction velocities of the fibers contributing to the potential (Daube, 1996). Estimates of the number of fast and slow conducting axons can also be made from the change in size with paired stimuli at decreasing intervals between the stimuli (Daube, 1996).

Rise Time

Rise time is the duration of the rising phase (negative peak to positive peak). The moving region of depolarization in nerve or muscle fibers has reached the active electrode when this reversal of potential from negative to positive occurs (Daube, 1996). The rate of rise (slope) is related directly to the distance of the motor unit from the electrode and, to a lesser extent, to fiber conduction velocity (Daube, 1996).

Area

The integrated EMG (IEMG) which represents the "area under the curve" of the waveform has been a commonly used method of quantifying the EMG. Area provides the most direct estimate of the functioning tissue that generates the waveform. Area measurements, similar to amplitude measurements, also provide the most information about the number of fibers close to the site of activation (Daube, 1996).

The simplest form of integration, measured in mv·s, starts at some preset time and continues during the total time of muscle activity. A second form of integrator involves a resetting of the integrated signal to zero at regular intervals of time (usually from 50 to 200 ms) and the time should be specified. Such a scheme yields a series of peaks which represent the trend of the EMG amplitude with time. Each peak has units of mv·s (or μv·s). The sum of all the peaks in any given contraction should equal the IEMG over that contraction (Gerleman et al. 1992).

Fast Fourier Transformation

Frequency analysis, most commonly performed by a fast Fourier transformation (FFT), determines the frequency components in a limited segment of a recording, usually a few seconds (Bergmans, 1977). The IP, like any other signal or waveform, can be mathematically described as a sum of sine waves of different frequencies, using FFT. This provides a method for characterizing the IP according to the amount (density) of power it contains at various frequencies: the so-called power spectrum. In general, the IP power spectrum resembles an inverted "u" with a peak at about 100-200 Hz and range from about 10-2000 Hz. There is some evidence that the lower frequency components of the power spectrum (10-50 Hz) tend to reflect MUAP firing rates, whereas the higher frequency components are more related to the MUAP shapes (Dorfman & McGill, 1988).

Spectral analysis has been used extensively to study muscle fatigue. The distribution of frequencies in the EMG has been used to describe the recruitment pattern during fatigue, with the implication that changes in the duration and shape of individual motor unit potentials are reflected in the content of different frequencies (Richardson, 1951; Walton, 1952). Fatigue increases the content of the low frequencies (Kadefors, Kaiser, & Petersen, 1968). The shift in distribution of frequency to lower frequency during sustained contraction has been explained by a slowing in conduction velocity along the fatigued muscle fiber (Lindstrom, Magnusson, & Petersen, 1970)

Turns Analysis

Counting the number of spikes per unit time was one of the earliest attempts to quantify the recruitment pattern (Haas, 1926; Travis & Lindsley, 1931; Bergstrom, 1959; Close, Nickel, & Todd, 1960; Person & Kushnarev, 1963).

Willison (1964) developed a quantitative method of EMG analysis based on measuring the "turns" in the interference pattern per unit time. This has been reported as the "best studied and most widely used method of automatic IP analysis" (Dorfman et al. 1988). A turn occurs at a local minimum or maximum of the signal (peak) where the signal changes its direction and the signal amplitude changes by at least 100 μv compared to the preceding and subsequent turns (Willison, 1964). A turn in the IP may correspond to a peak in a motor unit action potential (MUAP), to an interaction between overlapping MUAPs, or to noise: to the extent that turns derive from MUAP peaks, their count reflects the number of active MUAPs, the proportion of polyphasic MUAPs, and MUAP firing rates (Dorfman et al. 1988). Turns analysis provides a means of quantifying the EMG, describing the interference pattern seen during voluntary effort in simple numerical terms. It is objective, reproducible, and fast, and allows the data to be analyzed at very specific time increments. Whereas IEMG gives the sum of electrical activity, the turns analysis provides information as to what exactly is contributing to the electrical signal (ie. few motor units or many motor units firing).

Attempts have been made to establish a well defined relationship between force and turns analysis. In 1926, Haas related the number of spikes in the deltoid muscle with the force. He recorded with needle electrodes and found an increase in the number of spikes with increasing force up to about 10-15% MVC; with greater force, the number of spikes decreased. Others found a linear increase up to 60-70% MVC, followed by a decrease

(Travis et al. 1931; Person et al. 1963). In the soleus muscle, Close et al. (1960) did not find the decrease in the spike counts during strong effort. Close et al. (1960) studied the relation of the number of spikes to the integrated electromyogram. Up to a force of "moderate intensity" the number of spikes increased linearly with the integrated electromyogram (Close et al., 1960). In the brachial triceps, the number of turns increased linearly up to a force of two kilograms, corresponding to approximately 12-30% MVC (Willison, 1964; Fuglsang-Frederiksen & Mansson, 1975). In the brachial biceps muscle, above a force of more than 50% MVC, the number of turns decreased (Fuglsang-Frederiksen et al. 1975). In addition to turns, Willison (1964) and Fitch & Willison (1965) determined the mean amplitude between turns. Unlike turns, the mean amplitude increased almost linearly up to maximum force (Fuglsang-Frederiksen et al. 1975).

This method has since been extended and modified by others (Christensen, LoMonaco, Dahl, & Fuglsang-Frederiksen, 1984; Fuglsang-Frederiksen, Dahl, & LoMonaco, 1984; Garcia, Milner-Brown, & Fisher, 1980; Nandedkar, Sanders, & Stalberg, 1986). Stalberg et al. plotted mean amplitude as a function of turns frequency without measuring force (Stalberg, Chu, Bril, Nandedkar, & Ericsson, 1983). The resultant "Stalberg cloud" has now become a clinical tool of quantitative EMG.

Amplitude

Amplitude is the voltage from the baseline to the maximum positive peak or from the maximum negative peak to the maximum positive peak. The amplitude of the positive component is proportional to the number and size of fibers that are depolarized (Daube, 1996). The amplitude also depends on the distance of the recording electrode from the motor unit. This distance can be judged by the rise time (Daube, 1996). Amplitude measurements are the most reliable estimates of active fibers when amplitude is recorded close to the site of stimulation (Daube, 1996).

One of the simplest ways to characterize electrical activity is the average peak-to-peak amplitude of the recruitment pattern obtained from the envelope curves during full effort (Fuglsang-Frederiksen, 1981). The peak-to-peak amplitude is defined by the positive and negative peaks of the pattern excluding solitary high peaks. An approximate value for the numerical average can be obtained by full wave rectification and summation (Fuglsang-Frederiksen, 1981).

The diagnostic yield of all of the above methods are not exactly the same, but they supplement and complement each other, improving the discriminant index when used together (Preece, Wimalaratna, Green, Churchill, Morgan, 1994).

Sampling Frequency

The EMG signal has an upper limiting frequency for electrophysiological reasons and due to the geometric properties of the needle (Jorgensen & Fuglsang-Frederiksen, 1991). According to Buchthal et al (1959), the amplitudes of motor unit potentials picked up by concentric needles are almost constant at upper limiting frequencies of 10 KHz or higher (Buchthal, Erminio, & Rosenfalck, 1959). The Nyquist sampling theorem states that it is possible to reproduce a signal which is sampled with a frequency satisfying the following criterion: Sampling frequency is greater than or equal to two times the upper frequency of the signal. This sampling frequency is the minimum necessary to avoid distortion of the signal. Linear signal processing is correct as long as the calculations are performed with at least this frequency. However, non-linear calculations must be performed at a higher frequency (Jorgensen et al. 1991).

The influence of sampling frequencies from 6 to 200 KHz on turns, mean amplitude, and ratio of turns to mean amplitude analyzed on the IP at a force of 30% MVC was examined at ten sites in each of five control muscles, five myopathic muscles, and five neurogenic muscles (150 sites in all) (Jorgensen et al. 1991). In this small group of muscles, low sampling frequency did not tend to reduce the diagnostic yield of the measurements.

Muscle Fatigue

Fatigue Mechanisms

Muscular fatigue has been defined as a reduction in the force generating capacity of the neuromuscular system that occurs during sustained activity (Bigland-Ritchie et al. 1983). The etiology of fatigue is complex and different mechanisms are involved depending upon the intensity, duration, fiber type composition of the muscle, recruitment pattern, state of training, and nature of the exercise (Sjogaard, 1990). Each step in the chain of events that leads to voluntary contraction of skeletal muscle could be a contributing factor to fatigue. Research has broadly characterized fatigue into central and peripheral mechanisms. Central fatigue refers to reduced motivation or altered central nervous system transmission to motoneurons (Enoka et al. 1992).

Peripheral fatigue sites and processes can involve a loss of electrical transmission and/or a failure in excitation contraction coupling (ECC) mechanisms. Electrical transmission involves the cell body, axon, nerve branches going to individual muscle fibers, muscle membrane, and the motor end plate. Neuromuscular junction failure may involve reduced neurotransmitter release (acetylcholine), or an inability of the post synaptic membrane to become excited (Kirkendall, 1990).

The sarcolemma is a potential site of fatigue. There has been speculation that changes in electrical properties of muscle might affect the tension development of muscle. The membrane potential is dependent on intra- and extracellular sodium and potassium concentrations and these concentrations are affected by water content (Kirkendall, 1990). Thus, any shifts of water and electrolytes may influence the membrane potential. If the membrane undergoes some alteration in its electrical potential, then this may lead to a failure in propagation, leading to fewer muscle fibers contributing to force (Kirkendall, 1990).

Fatigue may also take place within the ECC mechanism. The t-tubule system may undergo ionic changes, causing a reduction in force. Even if the action potential invades the t-tubular system, the sarcoplasmic reticulum may not be triggered properly or calcium release may not proceed normally (Clamann, 1987; Lenman & Ritchie, 1983). Another possible cause of fatigue at the level of the t-tubule is failure of the inward spread of activation into the muscle fiber. This failure of activation may arise because potassium accumulates in the transverse tubules under extreme fatigue conditions (Marconnet, Komi, & Saltin, 1990). An increased concentration of potassium in the t-tubules, possibly in combination with osmotic changes in the t-tubular membrane will impair the inward spread of the action potential and therefore make the inner parts of the fiber inaccessible for the stimulus (Marconnet et al. 1990).

During high intensity exercise, there is an eventual accumulation of metabolism by-products which also contributes to fatigue. After a period of maintained muscle contraction, several metabolic changes occur: High energy phosphates are reduced, lactate and hydrogen ions accumulate, resulting in a decrease in intracellular pH, and K^+/Na^+ gradients across muscle membranes are altered (Milner-Brown & Miller, 1986). Hydrogen ion accumulation can also produce fatigue by inhibiting glycolysis, inhibiting the contractile process itself, and interfering with several important equilibrium reactions. The combined effect of these changes result in fatigue and an interruption of continued contraction (Milner-Brown et al. 1986).

During fatiguing exercise, depletion of fuels is also an important consideration. Energy supplies such as high energy phosphagens (adenosine triphosphate and creatine phosphate), glucose and glycogen, which are used at high intensities, may be depleted to a point that causes power output to decrease (Kirkendall, 1990). When the energy demands of the contracting muscle exceed metabolic capacity, fatigue occurs.

Much of the present evidence suggests that peripheral mechanisms play a more dominant role in the fatigue process, however, opinions have varied. In 1954, Merton concluded that failure of electrical transmission from nerve to muscle was not a major factor, since he found no reduction in the muscle surface mass action potential (M-wave) evoked by a single maximal shock to the motor nerve during sustained MVC's, despite near total loss of force. In 1972, Stephens and Taylor reported a decline in both the M-wave and in the voluntary electromyogram (EMG). These two observations led them to propose that the loss of force during the first minute of a sustained MVC resulted mainly from increasing neuromuscular block. In 1979, Bigland-Ritchie, Jones and Woods, studied the adductor pollicis and confirmed the decline in the voluntary EMG, but like Merton (1954), they found no parallel reduction in the evoked M waves. Bigland-Ritchie and Lippold (1979) found that the declining surface EMG could be accounted for by a corresponding reduction in motoneuron firing rate, but also found no evidence of neuromuscular block.

EMG and Fatigue

EMG is also a valuable tool in assessing muscular fatigue. As mentioned above, several investigators have demonstrated a decrease of power density in the high frequency region of the EMG signal and an increase in the low frequency region during fatiguing contractions. In a study by Moritani, Muro, and Kyima (1985), the simultaneously recorded surface EMG and intramuscular spikes from the gastrocnemius and soleus were compared at the beginning and at the end of sixty seconds of MVC. A marked reduction in the surface EMG amplitude was seen at the end of a fatiguing MVC, signifying dropping out of motor units. The intramuscularly recorded motor unit spikes from the gastrocnemius muscle also showed a marked reduction in the firing frequency, whereas the soleus muscle showed very little changes to the intramuscularly recorded motor unit activity. Factors influencing the amplitude of the EMG signal include the number and size of active muscle fibers, the size and orientation of the electrode detection surfaces relative to the active muscle fibers, and the distance between the active fibers and the detection electrodes. The frequency content of the EMG is influenced by factors such as the distance between electrodes and the distance between the active fibers and the detection electrodes (Gerleman et al. 1992). EMG can also be used to determine the specific mechanism(s) contributing to fatigue in the muscle. For example, when force loss is accompanied by a parallel decline in electrical activity, fatigue is generally attributed to failure of excitation, but when the electrical activity is undiminished, the failure is considered to be in the muscle contractile system (Bigland-Ritchie, 1981).

Fatigue and Coactivation

Coactivation is antagonist muscle activity that occurs during voluntary contraction (Psek et al. 1993). Psek et al. (1993) conducted a study which determined the behaviour of coactivation in knee flexors during the time course of fatigue in knee extensors and related these changes to the agonist electrical activity present during the same period. The results of the experiment showed that biceps femoris coactivation increased, along with increased EMG activity in vastus lateralis, during fatiguing contraction (Psek et al. 1993). They concluded that a small but significant force loss was due to an increase in antagonist activity. The close correlation between the antagonist and agonist EMG supports the notion of a "common drive" to both motoneuron pools (Psek et al. 1993). An increase in antagonist force caused by coactivation detracts from the force produced by the agonist. Consequently, recruitment beyond what is required to compensate for fatigue is necessary to counteract coactivation and to maintain constant force output (Psek et al. 1993). Thus, an earlier recruitment of fast fatiguing motor units, as well as increased metabolic cost of active antagonist muscles occurs. This suggests that increasing coactivation may shorten the time to the limit of endurance and thereby contribute to the fatigue process (Psek et al. 1993).

Fatigue and Synergistic Muscle Activity

According to Basmajian and DeLuca (1985), a synergist muscle is one which actively provides an additive contribution to a particular function during a contraction. Synergistic behaviour of the triceps surae muscles (medial gastrocnemius, lateral gastrocnemius, and soleus) during sustained submaximal plantarflexion was investigated (Sirin et al. 1987). During the contractions, EMG was recorded from the surface of the triceps surae muscles. Two types of synergisms were identified: trade-off and coactivation. Trade-off and coactivation synergies were defined by significant ($p< 0.05$) positive and negative correlations respectively (Sirin et al. 1987). Coactivation synergism was found to occur predominantly under conditions of high load or reduced length of the triceps surae and increased with the duration of the contraction. Trade-off synergism was evident when the muscles were at their optimum length and the loads sustained were submaximum (Sirin et al. 1987).

The fatigue process becomes even more complicated when we consider that fatigue may be site specific within a muscle. Since the majority of past studies have examined only one site within a muscle during fatigue, it is not yet known whether the motor unit activity recorded from one site is representative of the entire muscle. However, much evidence exists which supports the argument that there may be site specificity of muscular fatigue.

Site Specificity

Fiber Type Distribution

Muscle fibers may be classified into at least two main types: type 1 (slow twitch) and type II (fast twitch). The proportion of the two fiber types varies between different muscles and between different sites within a muscle (Lexell, Henriksson-Larson, & Sjostrom, 1983). However, the value for the proportion of the two fiber types, type I and II, in a sample is frequently considered to be representative of the whole muscle under investigation. These values are compared with biochemical and physiological variables and used to determine such things as "performance capacities" (Lexell et al. 1983). Although some studies have found a correlation between these parameters, it has been suggested that the fiber type proportion determined from a small sample is not a reliable predictor of fiber distribution throughout the entire muscle (Lexell et al. 1983). Studies analyzing cross sections of whole human muscles have provided an explanation. A single biopsy is a poor estimator of the proportion of the fiber types for the whole muscle because of the large variability in the proportion of the fiber types within a muscle (Lexell et al. 1983). Results of autopsy studies have also shown that there can be significant differences between sites in terms of fiber type distribution (Elder et al. 1982). Both the soleus and biceps brachii were found to have significantly more type II fibers at their insertions than at their origins. Such an arrangement would be clearly possible in the soleus, which has a complicated multipennate arrangement of its fibers (Elder et al. 1982). Additionally, there was strong evidence of within site variability in the soleus, biceps, and vastus lateralis muscles (Elder et al. 1982).

Differences in fiber composition between superficial and deep sites might have been anticipated from animal studies in which fast twitch fibers were found to be more prevalent superficially (Denny-Brown, 1929). Similar observations have been made in some human muscles. In studies of the vastus lateralis, Lexell et al. (1983) and Sjostrom, Downham, & Lexell (1986) found differences in the proportion of muscle fibers with different properties between the periphery and the more central parts of a fascicle or muscle. There was a gradual increase in the proportion of type 1 fibers with depth and a corresponding decrease in type II fibers. Other studies (Elder et al. 1982; Edgerton, Smith, & Simpson, 1975), however, did not find evidence of differences with depth. Their methods may not have been as well suited to find specific differences as those of Lexell et al. (1983) and Sjostrom et al. (1986).

Intramuscular Pressure (IMP)

During an isometric contraction, a muscle develops tension which appears externally as a force and internally as an increase in IMP. This pressure compresses the walls of the capillaries, venules, and arterioles within the muscle tissue, which are able to remain open due to the pressure of the blood which the heart is sending through them (Start & Holmes, 1963). However, during contraction, a muscle is capable of developing IMP far in excess of the blood pressures generated by the heart. As a result, when muscular tension rises, the intramuscular circulation is at first impeded, and finally becomes occluded when systolic pressure is exceeded (Start et al. 1963). The importance of sufficient blood flow through the exercising muscle is essential to maintain homeostasis within muscle fibers. The increased energy turnover during muscle contraction calls for increased supply of substrates. Metabolites must be eliminated and heat has to be transferred from the muscle. Such homeostasis is necessary for the transfer of chemical into mechanical energy (Sjogaard et al. 1988). A decrease in blood flow will therefore affect the level of force and the ability to deliver the energy required to maintain that force. There is a balance between the energy required to maintain a given level of muscle contraction and the amount supplied by the working muscle. When demand exceeds supply, as may occur when blood flow becomes impaired, fatigue results and this limits the time for which the contraction can be maintained.

However, it is not possible to determine at what percentage of MVC blood flow restriction causes fatigue, as IMP may vary considerably within a muscle group and between different parts of a single muscle (Sjogaard et al. 1988). Heterogeneity of flow within a muscle was demonstrated by Laughlin and Armstrong (1982) and may be caused by an alternating recruitment of muscle fibers within a muscle. Similarly, Sjogaard et al. (1986) concluded that during low-level static contractions, the blood supply to the exercising muscle is maintained at a sufficiently high level and that the alternating recruitment of muscle fibers may result in a heterogeneously distributed blood flow within the contracting muscle. This was confirmed with EMG recordings which showed that a decrease in intramuscular pressure was related to decreased muscle activity (Sjogaard et al. 1986). It was also demonstrated that when EMG amplitude and IMP decreased in one area of the muscle, then activity in another portion of the muscle was increased simultaneously. Sjogaard et al. (1988) found that there was increased pressure deeper in the muscle, and in agreement with this finding, Sejersted et al. (1984) discovered that blood flow to central parts of the muscle will be compromised first during contraction. IMP within a muscle may also be affected by surrounding muscles. Pressure within a muscle can be increased by the contraction of surrounding muscle groups, the compression of fascia, and the compression of muscle against bone (Hargens, Romine, Sipe, Evans, Mubarak, & Akeson, 1979). Barcroft and Millen (1939) stated that the reduction in blood flow was due to anatomical "nipping off" of arteries in the layers of fascia surrounding the muscles.

Anatomical arrangement of fibers

The pennation of the muscle fibers will have implications for the IMP developed during a contraction. This was confirmed in studies where the smallest pressure increments were found in muscles which had more parallel fiber arrangements than other muscles investigated (Sadamato et al. 1983). Muscle shape can also affect the amount of IMP produced during a contraction. An almost spherical muscle with a great degree of curvature will transfer little stress to its tendons and most of the stress will be intramuscular. A long slender muscle with parallel fibers and very little curvature will produce less intramuscular pressure and would therefore be less susceptible to fatigue induced by blood flow impairment (Sejersted et al. 1984). Since differences in fiber pennnation and shape exist within single muscles, regional differences in IMP might be expected. This would affect recruitment differently according to site (Sejersted et al. 1984).

Recruitment Patterns - Site and Task Specificity

The force of muscle contraction can be increased by raising the firing frequency of the motoneurons and by calling additional motor units into activity (*recruitment*). The size principle of motor unit recruitment was introduced by Henneman et al. (1965). This was based on their finding that the threshold of recruitment was related to the size of the anterior horn cell. Smaller anterior horn cells were recruited first, had smaller axons, of slower conduction velocity, and innervated fibers which produced lower forces. Small motoneurons participate in most functional activities because they tend to innervate slow twitch, type I muscle fibers that fatigue slowly. Motor units with large anterior horn cells, which innervate large, fast twitch fibers, were recruited last in sequence and often only during very strong contractions. Smaller potentials appear first with a slight contraction and as the force is increased, larger and larger potentials are recruited and all motor units increase their frequency of firing (Henneman et al. 1965; Grimby & Hannerz, 1968). The stimulation thresholds for human motor fibers in the same nerve were found to be lower for those of small diameter than for those of large diameter. The smaller fibers supply the smaller motor units which appear to be the most easily recruited in normal voluntary contraction. It was the sequential recruitment based on size which became accepted as the size principle of recruitment (Henneman et al. 1965).

More recently, studies have found evidence to suggest that recruitment does not always follow this principle. Although it has never been examined extensively, the switching of activity during fatigue may occur within parts of a muscle, since it is known that some muscles consist of discrete compartments (Enoka et al. 1992). This seems most likely in muscles with distributed attachments, in which changes in the direction of the force vector can be associated with changes in motor unit activity. Motor unit rotation would provide periods of reduced activity or inactivity that could be used for the replenishment of metabolic needs (Enoka et al. 1992). Enoka, Robinson, and Kossev (1989) examined motor unit behaviour in the first dorsal interrosseus muscle of human subjects during a ramp and hold task that was performed before and after a fatiguing contraction. Motor unit activity was characterized before and after fatigue by the use of a branched bipolar electrode that was positioned subcutaneously over the muscle. Activity was characterized in terms of recruitment and derecruitment and the discharge pattern. Motor unit behaviour during and after fatigue showed that the relatively homogeneous behaviour seen before fatigue could be severely disrupted. After a fatigue test, when the initial threshold tasks were repeated, the behaviour of most motor units changed. Some low threshold motor units were not active after the fatiguing activity although the task was identical to that performed before the fatigue task. This variation in motor unit recruitment was interpreted as reflecting some degree of history dependent flexibility in recruitment order among these motor units, which is consistent with the concept of motor unit rotation (Enoka et al. 1989).

As early as 1964, Willison noted that there may be variability among sites in terms of motor unit recruitment. In a study, five or more sites in a muscle were measured electromyographically, while standard weights equal to 20% MVC or less were maintained by the triceps. Activity measured in terms of counts per second varied considerably from one area to another in a single muscle. It was therefore recognized that it was necessary to take samples from many areas of each muscle. Since this time, other researchers (ie. Preece et al. 1994) have also recognized that the most useful results are obtained by sampling several muscles at many different sites to give a more objective view of the distribution of activity within the muscle.

There are also studies which have shown that the muscle activation patterns may alter depending on the type of task being performed. In a study by Zijdewind, Kernell, and Kukulka (1995), multiple bipolar recordings were taken of the first dorsal interosseus muscle (FDI). This muscle would be expected to be used in an homogeneous manner as FDI units are assumed to be uniform in their task recruitment behaviour (Thomas, Ross, & Stein, 1986; Enoka et al. 1989) The amount of EMG activity was expected to show a continuous rise during an endurance test at 50% of MVC. However, in all four subjects, recordings were obtained in which EMG activity at different sites simultaneously decreased during the test. Such variability in the individual EMG reactions indicated that FDI motor units positioned in a given area might be recruited differently than other sites, affecting the level of total muscle activation.

In a study by Ter Haar Romeny et al. (1984), critical firing levels of single motor units in the long head of the human biceps brachii muscle (BLH) were determined in combinations of isometric tasks. Motor units recorded at different sites in the BLH responded differently on performing the assigned tasks. For example, motor units recorded on the medial side were preferably recruited during isometric supination, whereas laterally recorded motor units were recruited mainly during isometric flexion, independent of supination. It was concluded that the order of recruitment can be changed consistently by changing the task and thus the direction of the exerted force.

Buchanan (1995) examined muscle activation levels during two different static tasks which required the same joint angles and the same joint moments. In the isometric case, joint angles were fixed and subjects were required to match forces. In the isoinertial case, a constant load was imposed across the joint and the subject was required to match position. It was observed that for a specific posture and for specified load conditions, EMG activity varied depending on whether the limb was loaded isometrically or isoinertially (Buchanan, 1995). Different coactivation relationships were observed for position control versus force control tasks during otherwise similar conditions. These results imply that the neural command for static tasks depends on more than joint angles and load magnitude (Buchanan, 1995).

Conclusion

Previous studies have reported that site differences exist during non-fatiguing contractions. Although little research has addressed the topic of site specific motor unit recruitment in a muscle during fatigue, there is much evidence which supports this theory. Based on the heterogeneity of fiber type, fiber arrangement, motor unit distributions, IMP, and blood flow, it would be expected that recruitment would be affected differentially at various sites of a muscle during fatigue.

METHODS

Subjects

Experiments were conducted on the soleus muscles of ten healthy female volunteers, aged 21-26 years (Appendix A), who had no prior injuries or neuromuscular problems of the right leg. Ten subjects were a manageable number for this study and was a sample size from which statistical conclusions could be drawn. The data was collected from a female population because of the relative lack of female subjects in previous physiological studies. All subjects were well trained in at least one form of endurance exercise involving the lower leg muscles and had the endurance capabilities to maintain muscular contractions of various intensities and durations. Subjects were given detailed information about the nature of the study prior to participation and gave their informed consent. Ethical approval was granted by the Faculty of Graduate Studies at Dalhousie University and the I.W.K. Children's Hospital Research Ethics Board, where the studies were carried out.

Procedures

Measurement of Plantarflexion Torque

Subjects were seated and their right leg was held in a torque measuring device while the foot was attached to a stainless steel footplate by Velcro straps. The footplate rotated about the axis of rotation of the ankle joint and could be locked at any angle of plantar or dorsiflexion. No external movement of the footplate occurred during the isometric contraction. Plantarflexion torque was measured at 10 degrees dorsiflexion, which has been shown to produce the optimal length of the plantarflexors for force production (Sale, Quinlan, Marsh, McComas, & Belanger, 1982). The leg was fixed in an upright position with the knee joint flexed and held at 90 degrees, at which angle the soleus contributes approximately two-thirds of the maximum plantarflexion torque (Toft, Sinkjaer, Andreassen, & Larsen, 1991). An adjustable knee clamp prevented leg movement as well as the heel from lifting off the footplate. The apparatus was calibrated pre and post testing by measuring the voltage developed when known weights were suspended from the footplate and the unit of torque was calculated by multiplying each weight by the distance between the axis of rotation and the point of weight attachment. A limitation of the torque measuring device was that the footplate was not adjustable in height so that the axis of rotation of the device was not always colinear with that of the ankle. However, this did not appear to be a significant limitation when calculating torque, since the torques measured in this experiment matched the expected range (Belanger, McComas, & Elder, 1983).

Prior to beginning the experiment, subjects were familiarized with the testing procedures, and practiced maintaining torques at different percentages of MVC. To measure the MVC, a contraction was performed to maximum and held only momentarily. The subjects' maximal isometric plantarflexion torques were determined from several trials, during which time verbal motivation was provided. The measures of MVC were taken by one examiner and then another to ensure that consistent results were obtained over a series of trials.

Electromyography (EMG)

Concentric reusable 26 gauge, 30 mm needle electrodes (Chalgren 235-630) were used to measure the electrical activity from the muscle. The electrodes were sterilized prior to use, by steam autoclave at approximately 120 degrees Celsius for twenty minutes. Needle electrodes were used because of their ability to provide information on recruitment from a limited number of motor units within a region of muscle. Concentric needle electrodes consist of an outer cannula through which runs a single wire that is exposed at its tip. The inner wire serves as the recording electrode, the outer wire serves as the reference electrode, and the potential difference between the two is recorded. Medi Trace Pellet surface electrodes were used as a ground for each site.

The skin was first cleaned with alcohol swabs and then the electrodes were inserted approximately two centimetres. The exact depth of the needle in the muscle depended upon the thickness of the tissue overlying the muscle. The sites were standardized in the following manner: The distance from the fibular head to the center of the lateral malleolus (lower leg length) was measured. Sites 1 and 2 were located at 16% and 32% respectively of this distance from the fibular head, along the lateral border of the soleus. The third electrode was placed on the medial border of the soleus, approximately 50-60% of the lower leg length from the center of the medial malleolus (Appendix C - Figure C1). These three sites were chosen with the assumption that sites 1 and 2 were within the same compartment and that site 3 was in a completely different compartment. We could therefore assess the effect of compartment on whether the sites were the same or different. EMG electrodes were connected to a Grass Model 7P5 pre-amplifier by a five lead input cable. The input impedance was 20 MΩ and the common mode rejection was greater than 1600:1 from 10 to 100 Hz. Needle depths were measured prior to testing and several times throughout the experiment to ensure a consistent depth, since intramuscular pressure could cause movement of the needle.

Protocol

Recruitment (EMG) was recorded simultaneously at three sites of the soleus during the following tests:

Test 1: 20%, 40%, & 60% MVC Contractions

Subjects maintained 20%, 40%, and 60% of their MVC for ten seconds. While the subjects were plantarflexing, a target torque was displayed on a digital oscilloscope. Since recruitment levels may vary due to even slight changes in torque, the amplifier of the oscilloscope was set at a very sensitive level so that even slight deviations in torque were visible to the subject and the examiner. This ensured that the desired torque was more accurately maintained. When the target torque was reached and stable, the computer recording of the EMG signal was started. If the torque level was not accurately maintained, the data was discarded. Three trials were recorded at each torque level to assess reliability of the measurements.

Test 2: Ramp Contraction to 80% MVC

Recruitment was recorded during a slow (30-40 s) ramp contraction, from 0-80% of MVC. A target (80% MVC) was set on the oscilloscope and subjects were given both visual and verbal cues as to the speed of their ramp contraction. Subjects then rested for 30 minutes to recover from any fatiguing effects of the ramp contraction, during which time the electrodes remained inserted in the muscle. A 30 minute rest period was determined during pilot testing to be adequate for the muscle to recover. Following this rest period, the numbers of turns recorded during a ten second 60% MVC contraction were the same as those recorded prior to the ramp contraction.

Test 3: Fatigue Test

EMG was recorded while subjects attempted to maintain 70% of their MVC for three minutes. Subjects could observe their torque levels in relation to their target torque. They were given verbal encouragement to motivate them to sustain the highest torques possible.

The various tests were ordered in this manner, separated by standard rest periods, in an attempt to minimize fatigue throughout the course of the experiment. However, it is recognized that this may have been a limitation if an order effect was present when comparing data across tests. It is possible, for example, that the torque production and the resulting EMG during each test may have been somewhat affected by the preceding test.

Data Recording System

The electrical signal from the muscle was amplified by 7P5 Differential Grass Amplifiers and Driver Amplifiers and displayed on a Nicolet digital oscilloscope (#2090) (Figure 1). The gain of the EMG signal for each channel was adjusted so that no potentials exceeded one volt. This was the recording range set for the A/D board. To ensure that no potentials exceeded one volt, subjects were asked to maintain 60% of MVC while their EMG was recorded. The gain of the amplifier was adjusted so that the highest potentials did not exceed 800 mv. This allowed for any large potentials that may occur during the ramp contraction or other tests. This procedure allowed the full 1.0 V recording range to be used without it being exceeded and it also standardized the procedure for all subjects.

The signal was analyzed using Labview Software on a Macintosh Centris 650 computer and stored on an optical disc drive. The Centris computer used a National Instruments MIO-16 analog to digital (A/D) board and an accelerator board. Four channels were sampled continuously at 6 KHz and the signal was low and high pass filtered between 10 Hz and 40 KHz respectively. A sampling rate of 6 KHz was found, during equipment and methodology validation, to accurately analyze the EMG signal.

Analysis

The complex interference pattern was analyzed using a modification of the Turns Analysis Technique, first used by Willison (1964). A turn was defined as any potential showing a reversal of polarity by a given amplitude. A modification of this method, first used by Blewett and Elder (1993) was also used in the present study to measure the numbers of turns and the peaks of their amplitudes. Only positive waves of the interference pattern were analyzed in a 0-1.0 V range, divided into ten bins of 100 mv each (ie. Bin 1=0-100 mv; Bin 2=101-200 mv, etc.). Numbers of turns and their corresponding amplitude ranges could then be identified for every second of the contraction period. A turn was defined, for the purposes of this investigation, as any peak potential that declined in amplitude by at least 30 mv (amplified signal) (Figure 2). A baseline threshold was also set at 30 mv (amplified signal) to prevent noise from being included in the data.

The fatigue data were also analyzed using FFT. It characterizes the IP according to the amount (density) of power it contains at various frequencies: the "power spectrum". In general, the IP power spectrum resembles an inverted "u", with a peak at about 100-200 Hz and range from about 10-2000 Hz.

Statistics

The 20%, 40%, and 60% MVC and ramp data were analyzed using two-way (site and intensity) analyses of variance (ANOVA) with repeated measures. The amplitude data was analyzed with a three-way (site, time, and bin) ANOVA with repeated measures. The fatigue data (turns analysis and FFT) were analyzed using two-way (site and intensity) analyses of covariance (ANCOVA). ANCOVA was used in this analysis because one of the variables (intensity) was a continuous variable, meaning that at any given time period, the intensity levels (% MVC) of subjects may have all been different. A SAS statistical package was used to compute these statistics.

TORQUE & EMG RECORDING SYSTEM

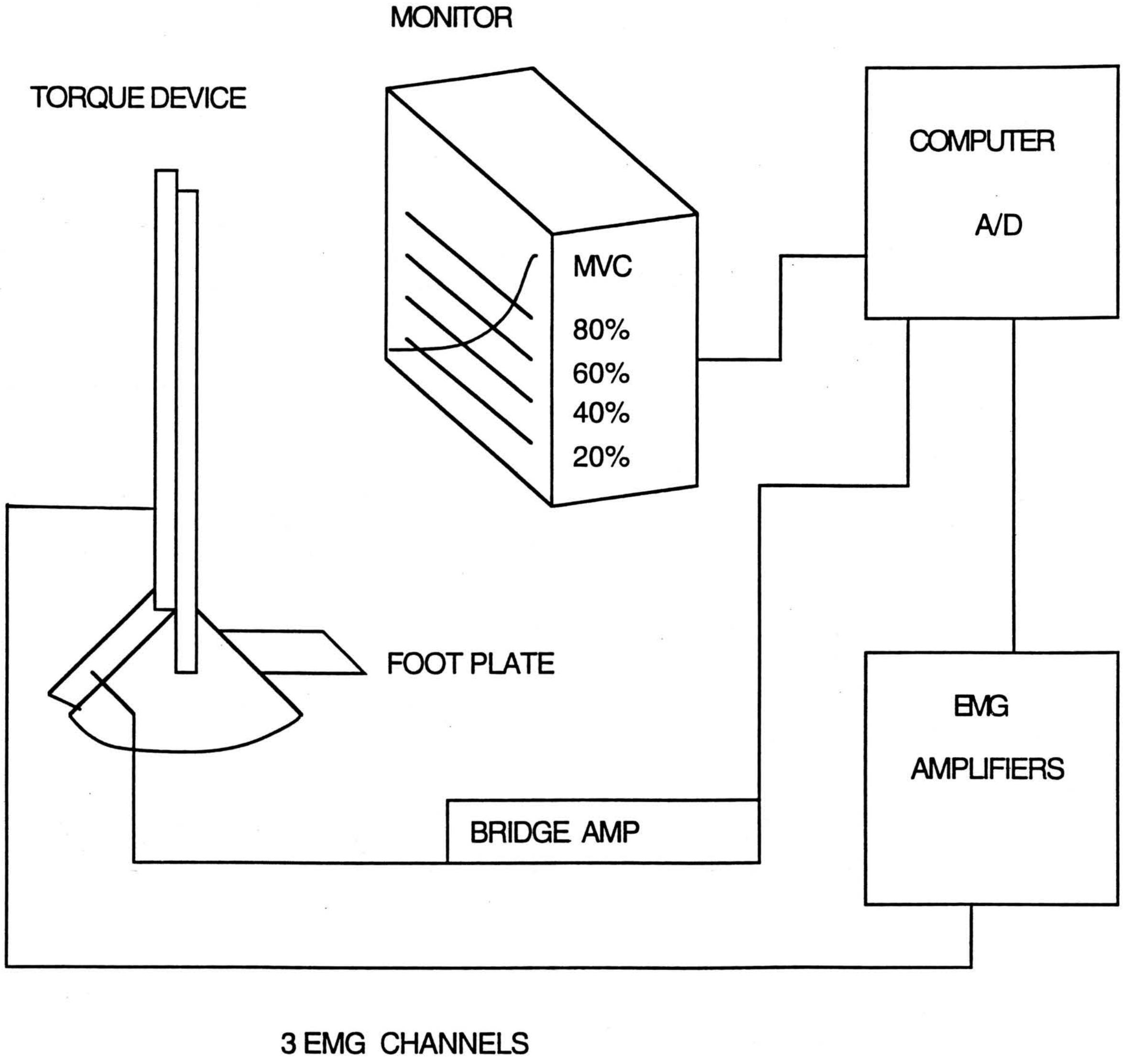


Figure 1: Torque and EMG data recording system

TURNS ANALYSIS

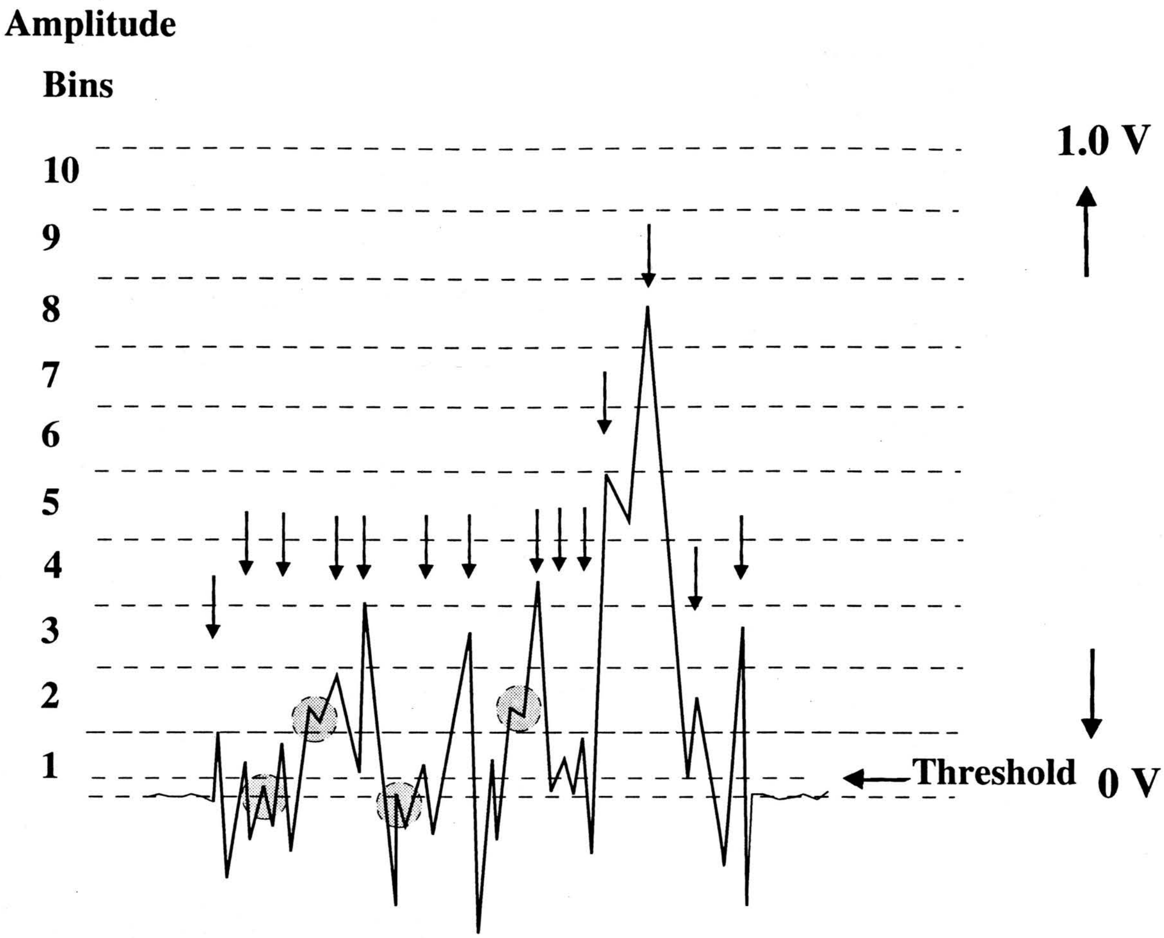


Figure 2: EMG complex interference pattern and recognition of "turns" by computer analysis. The threshold was set 0.03 V above the baseline so that only potentials above this would be recorded. A turn was identified by the program to be any peak potential which was preceded by a change in polarity exceeding 30 mv. Each new turn is indicated by an arrow placed above it. The circles indicate potentials which will not be counted as turns. The potentials were placed in bins (1-10) according to their respective amplitudes. Amplitudes indicated are amplified values, not actual amplitudes of motor unit action potentials.

RESULTS

The following results provide evidence which suggest that there are differences in EMG among sites of the soleus muscle during isometric contractions of different intensities, based on differences in the frequency and amplitude of turns from the interference pattern. These include submaximal contractions at 20%, 40%, and 60% MVC, linearly increasing ramp contractions from 0-80% MVC, and during fatiguing contractions, when the muscles attempted to maintain 70% MVC for three minutes. Maximum voluntary torque achieved by each subject is summarized in Appendix A and all contractions in this study are expressed as a percentage of these values.

Recruitment in each site was based on the approximate numbers of motor unit action potentials (represented by turns) recorded by the needle electrodes at three sites. Included in this section are reliability measures of the methods used. These include measurements of the numbers of turns at different torque levels from trial to trial and the influence of needle electrode depth on the numbers of turns recorded at each site (depth is addressed in Appendix E).

Reliability

Each subject performed three contractions at 20%, 40%, and 60% of MVC to test for reliability among the trials. A two-way analysis of variance (ANOVA) with repeated measures (Tables B1-B9) indicated that there were no significant differences between the three trials at each intensity and each site (Figures 3 - 4). The measurements were deemed reliable and in subsequent analyses of the 20%, 40%, and 60% MVC data, each subjects' results were first averaged within each trial over the ten second period and then the three trials were averaged.

RELIABILITY AT 20% MVC

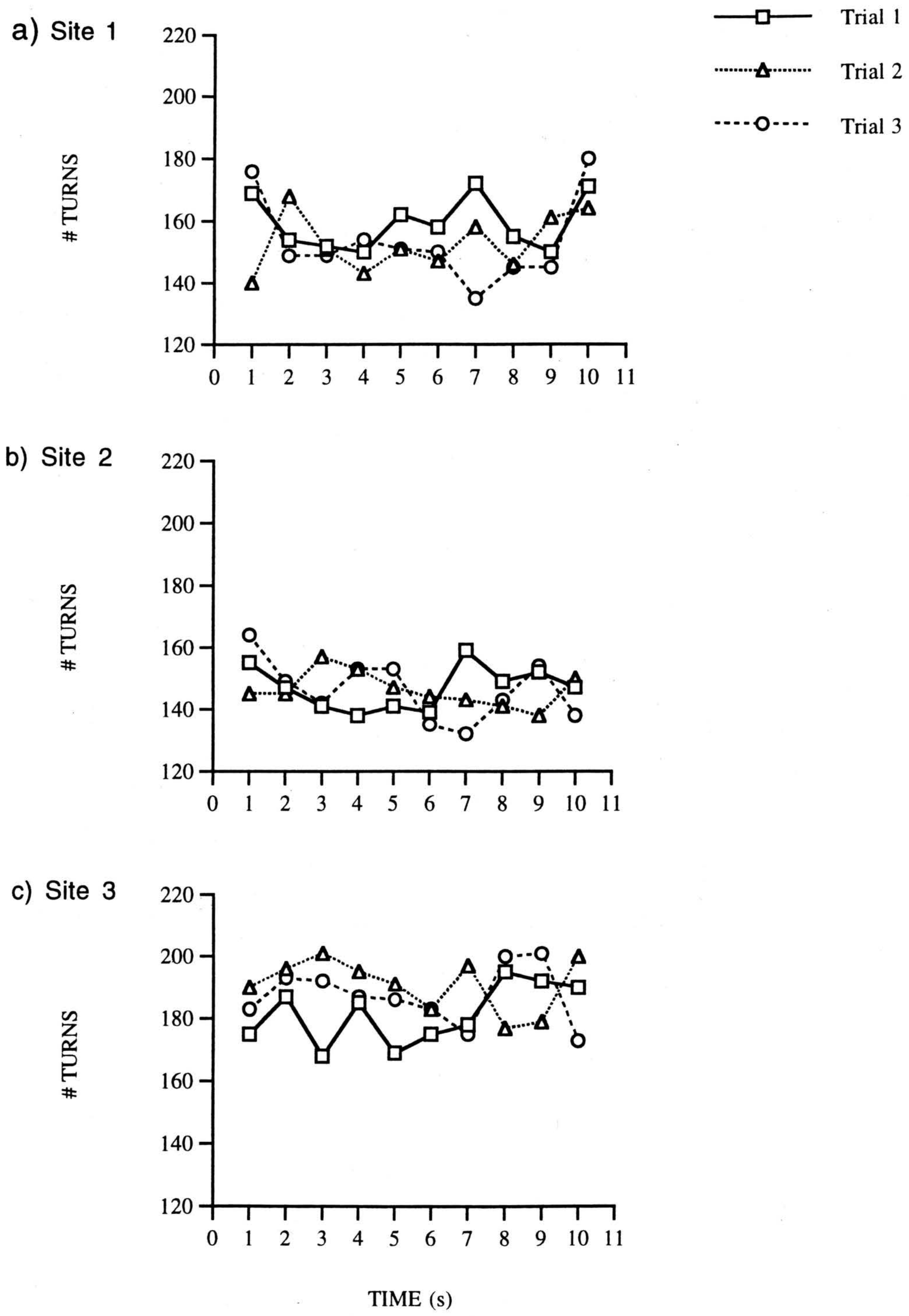
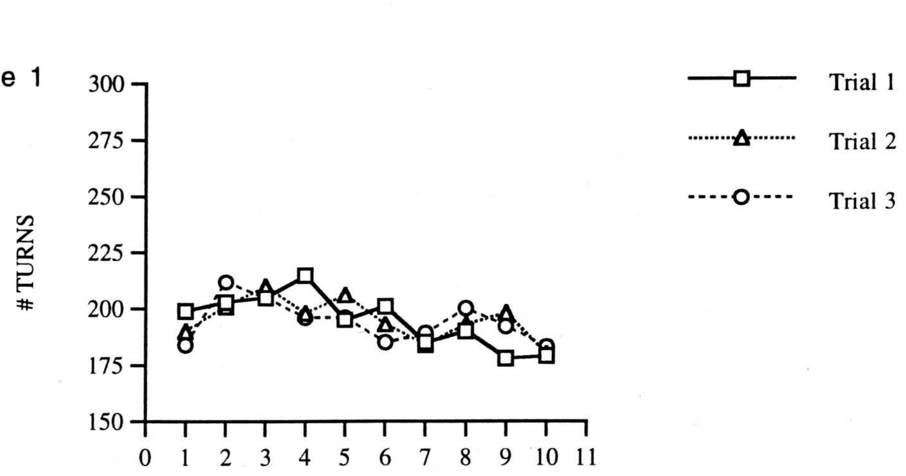


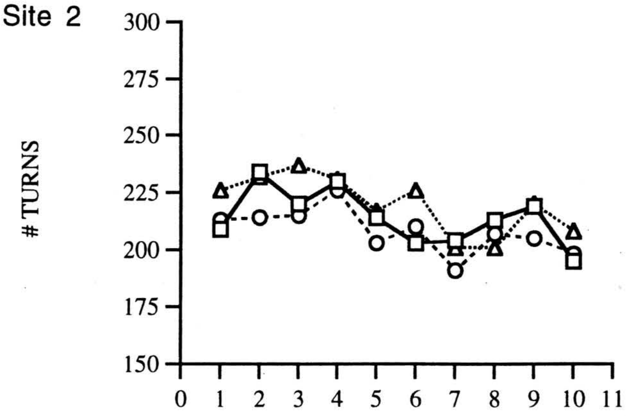
Figure 3 (a,b,c): Example of numbers of turns recorded during 3 10-second trials at 20% MVC (Subject 10)

RELIABILITY AT 60% MVC

a) Site 1



b) Site 2



c) Site 3

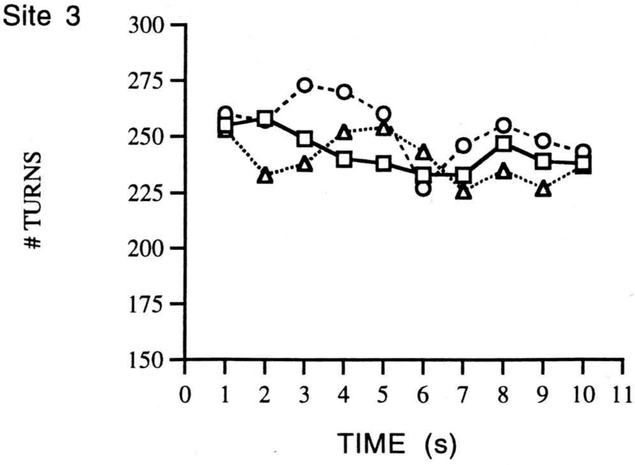


Figure 4 (a,b,c): Example of numbers of turns recorded during 3 10-second trials at 60% MVC (Subject 10)

Validity of Turn Count Methodology

The Labview software used to calculate the numbers of turns has previously been validated in our laboratory. A function generator was used to produce known frequencies of sine waves of varying amplitudes. Frequencies exceeding those of actual motor unit potentials were used to determine necessary sampling frequencies. A sampling rate of 6 KHz used in this study was found to accurately count the numbers of turns found in the most complex interference patterns recorded during maximal contractions. The accuracy of assigning the correct bin for each potential is dependent upon the amplitude of the potential recorded, the frequency of potentials, and the sampling rate. A sampling rate of 6 KHz could accurately determine the correct bin for each MUAP with an accuracy to within one bin of the expected bin. Those MUAP's that failed to be accurately placed would have been allocated to the bin/s immediately below the expected bin. This did not affect the total numbers of turns and the same limitation applied to all sites equally.

Submaximal Contractions at 20%, 40%, and 60% MVC

Electromyography (Turns Analysis)

Submaximal contractions were maintained at 20%, 40%, and 60% of MVC for 10 seconds. A two-way ANOVA with repeated measures was performed on the turn counts of the submaximal trials which were averaged over a ten second period. There was a significant difference in the numbers of turns among sites ($p < 0.05$) at certain intensities. The differences among sites were not consistent, but changed as the intensity of the contractions increased. These differences were greater at the lower intensities (Figure 5). At 20% MVC, sites 1 & 2 and 2 & 3 were significantly different from each other ($p < 0.05$), but there was no significant difference between sites 1 & 3 ($p > 0.05$). At 40% MVC, there was a significant difference between sites 1 & 2 ($p < 0.05$), but no significant differences between sites 1 & 3 or 2 & 3 ($p > 0.05$). At 60% MVC, none of the sites were significantly different from one another ($p > 0.05$).

At site 1, there were significantly more turns at 40% than at 20% MVC and significantly more turns at 60% than at 40% MVC ($p < 0.05$). At site 2, the numbers of turns significantly increased from 20-40% MVC ($p < 0.05$), but did not significantly increase from 40-60% MVC ($p > 0.05$). At site 3, the numbers of turns significantly increased from 20-40% MVC ($p < 0.05$) and from 40-60% MVC ($p < 0.05$).

NUMBERS OF TURNS / SECOND AT 20%, 40%, & 60% MVC

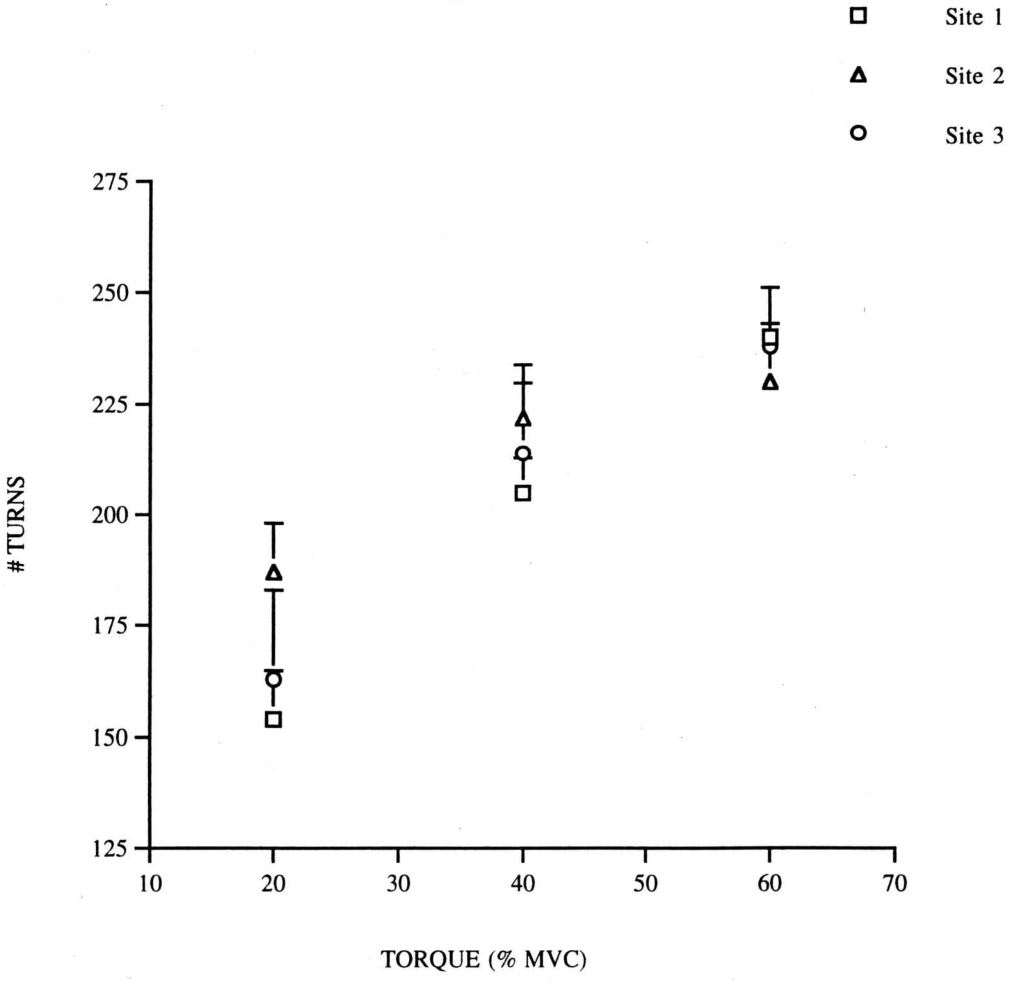


Figure 5 : Mean turn counts (+ SE) at 3 sites during submaximal contractions of 20%, 40%, and 60% MVC
20% - s1 & s2 and s2 & s3 are sig. diff. ($p < 0.05$); s1 & s3 are not sig. diff. ($p > 0.05$)
40% - s1 & s2 are sig. diff ($p < 0.05$); s1 & s3 and s2 & s3 are not sig. diff. ($p > 0.05$)
60% - none of the sites are sig. diff. from each other ($p > 0.05$)

Ramp

During the ramp contraction, subjects gradually increased their torque from zero to approximately 80% of their MVC in approximately 40 seconds. The numbers of turns increased with increasing torque at all sites, and in sites 1 and 2, reached a plateau at 60% and 50% MVC respectively. However, at site 3, the number of turns continued to increase and had not yet leveled off at 70% MVC (Figure 6).

An analysis was performed to determine if there were significant differences in numbers of turns among the three sites during one second periods at intervals of 10% MVC (10%-70% MVC). Results of a two-way ANOVA with repeated measures indicated that there was a significant difference ($p < 0.05$) among the three sites at particular intensities, indicating that as the torque levels increased, the site differences did not remain consistent. Between 10%-30% MVC of the contraction, site 1 was significantly different from sites 2 & 3 ($p < 0.05$), but sites 2 & 3 were not significantly different from each other ($p > 0.05$). From 40%-60% MVC, no sites were significantly different from each other ($p > 0.05$). At 70% MVC, site 3 was significantly different from sites 1 & 2 ($p < 0.05$), but sites 1 & 2 were not significantly different from each other ($p > 0.05$).

NUMBERS OF TURNS / SECOND DURING A RAMP CONTRACTION

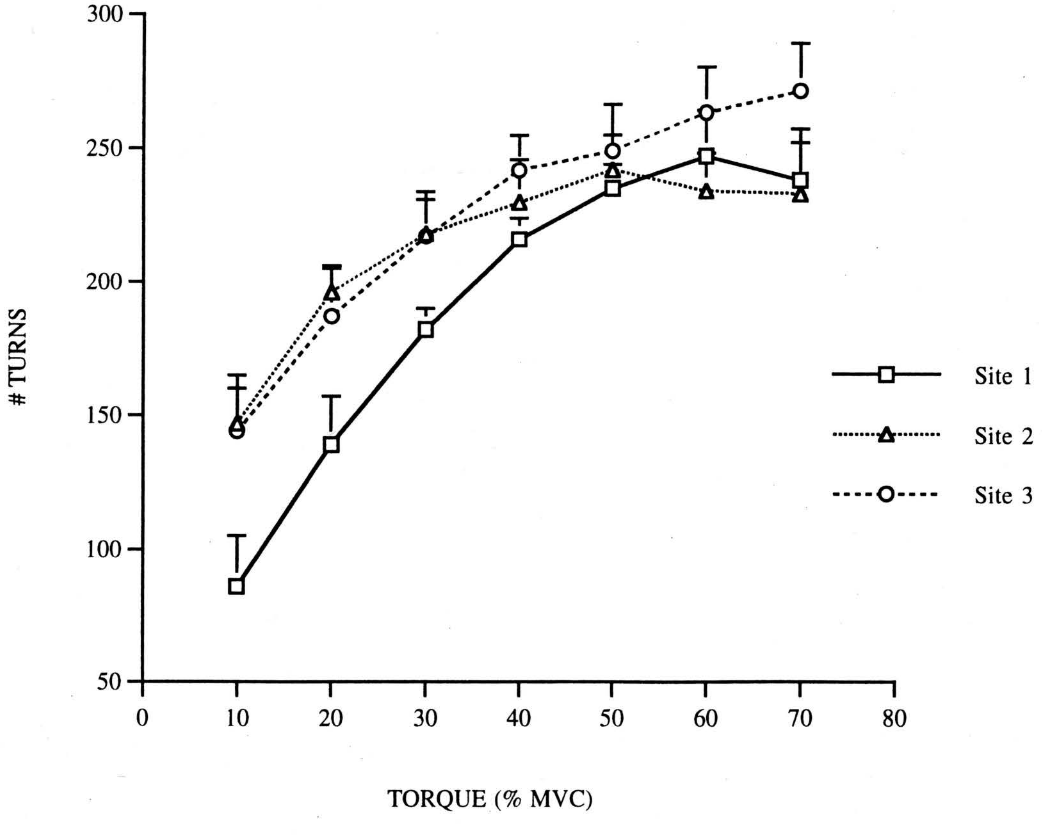


Figure 6 : Mean numbers of turns (+ SE) at 3 sites during a ramp contraction (n=10).
10-30% MVC - Site 1 is sig. diff. from sites 2 & 3 ($p < 0.05$); sites 2 & 3 are not sig. diff. from each other ($p > 0.05$)
40-60% MVC - none of the sites are sig. diff. from each other ($p > 0.05$)
70% MVC - Site 3 is sig. diff. from sites 1 & 2 ($p < 0.05$); sites 1 & 2 are not sig. diff. from each other ($p > 0.05$)

Fatigue

Torque

Subjects attempted to maintain 70% of their MVC continuously for three minutes. Torque levels decreased throughout the contraction for all subjects, but the slopes of the fatigue curves varied. The amount of time for which the original 70% MVC could be maintained ranged from 15 to 80 seconds, with an average of 51 seconds. Resultant torque levels, after three minutes, had fallen by 50-73%, with a group mean of 59% (Figure 7).

Electromyography (Turns Analysis)

The numbers of turns decreased during the three minute contraction in all subjects. An analysis of covariance (ANCOVA) was used in this particular analysis because one of the variables (intensity) was a continuous variable, meaning that at any given time period, the contraction intensity levels of the subjects may have all been different. A two-way ANCOVA with repeated measures (Table B12) found that the numbers of turns were significantly different at the three sites ($p < 0.05$). These differences varied depending upon the intensity of the contraction. As torque levels and numbers of turns decreased, the site differences were not consistent. For example, at the beginning of the contraction when the intensity was the greatest, site 1 recorded the greatest number of turns. However, at the end of the contraction, at the lowest intensities, site 1 had the least number of turns. Figure 8 illustrates the means, as well as upper and lower confidence limits. The confidence interval was established at the .95 level and represents the limits within which the population mean resides. The graphed confidence intervals are useful to identify where site differences exist; when the lines cross each other, there are no significant differences between the sites, and when they do not cross, there is a significant difference.

Electromyography (FFT)

The turns analysis method has frequently been used in clinical studies when needle electrodes were used, but it has not previously been used in the analysis of fatigue. FFTs have been used extensively to analyze fatigue, and therefore FFT analysis was included in this study so that a comparison between the two methods could be made.

During the fatiguing contraction, the FFT decreased significantly with time at sites 1 and 2. There was, however, no significant decline at site 3 (Figure 9). A two-way ANCOVA with repeated measures found that the FFTs recorded at the three sites were significantly different from each other ($p < 0.05$). There was also a significant difference found in the relationship between intensity and the FFT at each site ($p < 0.05$); as torque decreased, the differences between the sites were not consistent. For example, the site differences were greater at the end of the contraction, at the lower torque levels, than at the beginning of the contraction. This variation which depends upon intensity is in agreement with the Turns Analysis method which showed similar trends.

TORQUE CURVE DURING A 3-MINUTE 70% MVC CONTRACTION

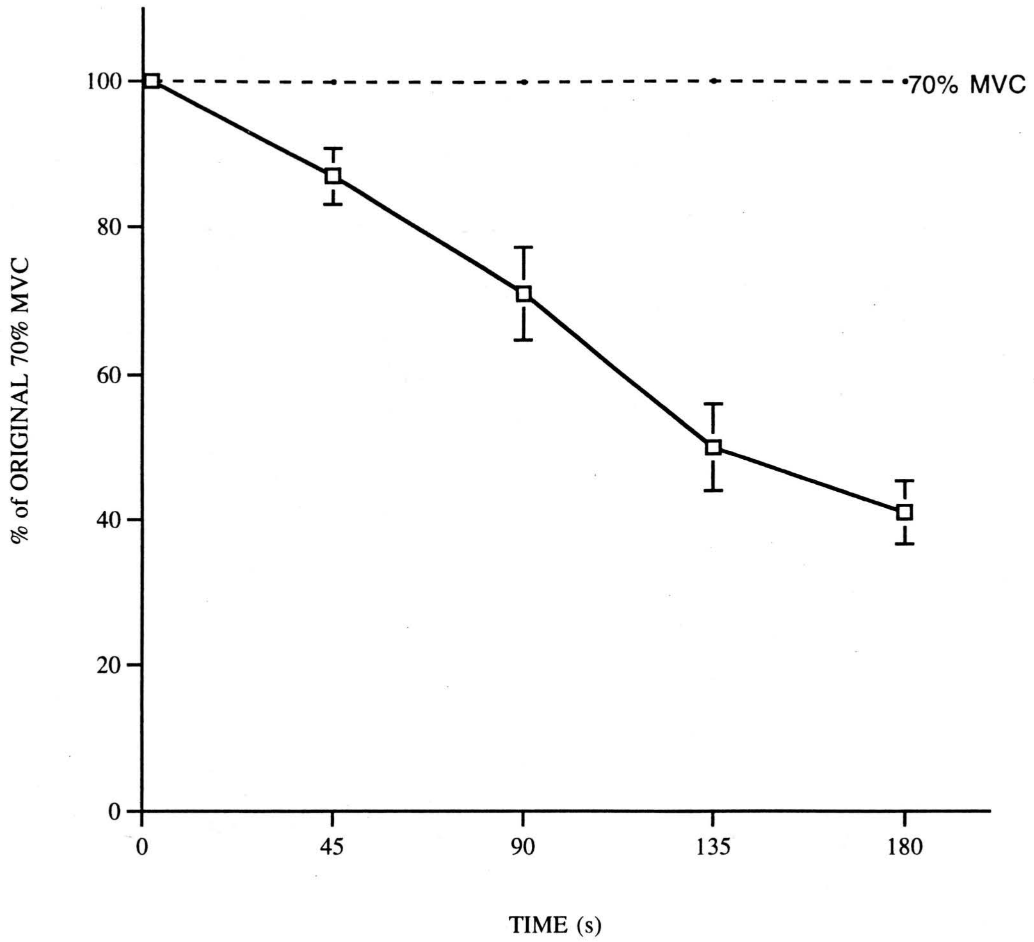


Figure 7 : Mean fatigue torque curve (±SE) during a 3 minute 70% MVC contraction (n=10)

NUMBERS OF TURNS / SECOND DURING A 3-MINUTE 70% MVC CONTRACTION

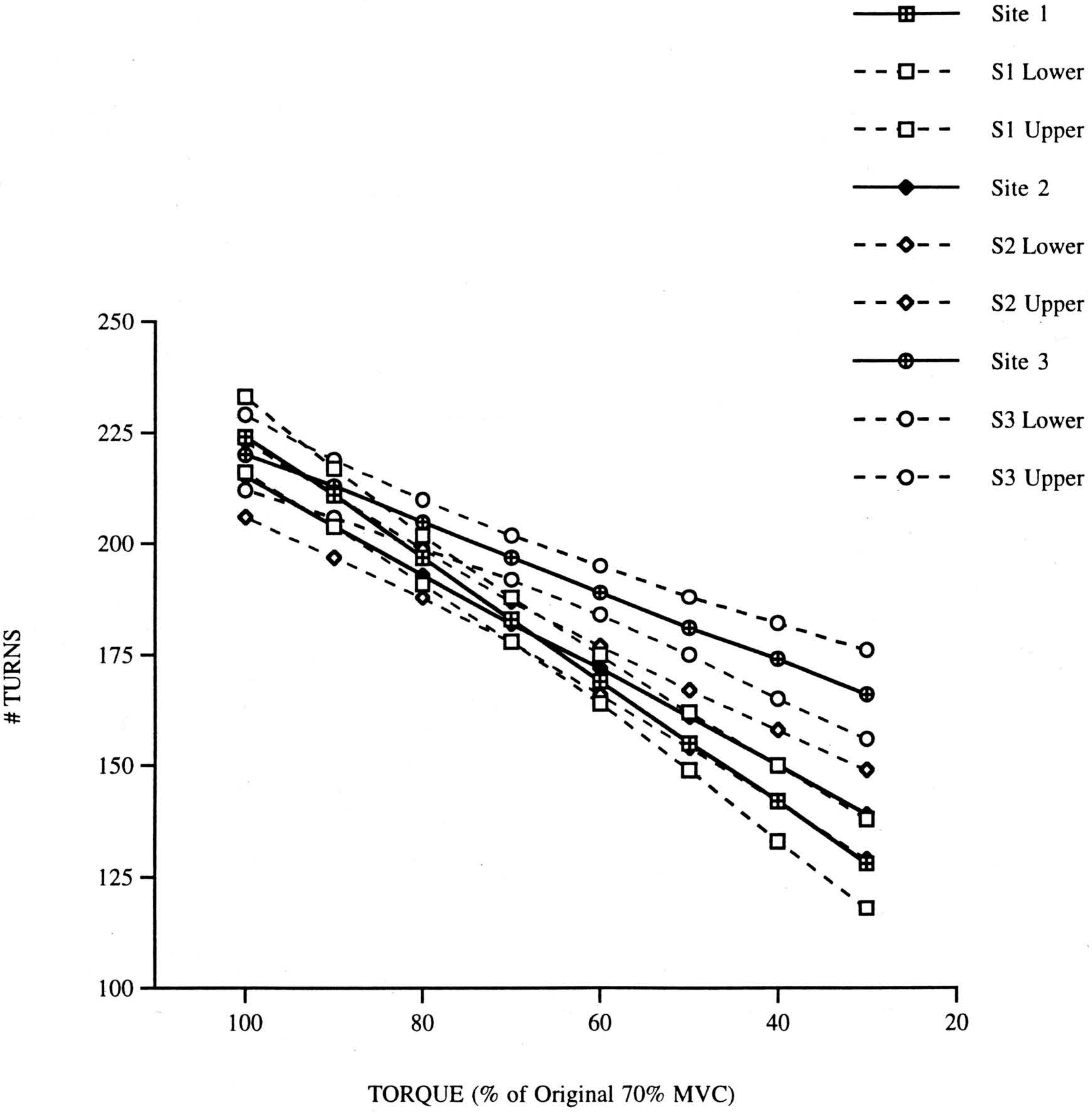


Figure 8 : Mean numbers of turns during a 70% MVC contraction (n=10). Means are illustrated with solid lines and filled symbols. Upper and lower confidence intervals are illustrated with broken lines and empty symbols. When the confidence level lines cross each other, there are no significant differences between those sites and when the lines do not cross, there is a significant difference.

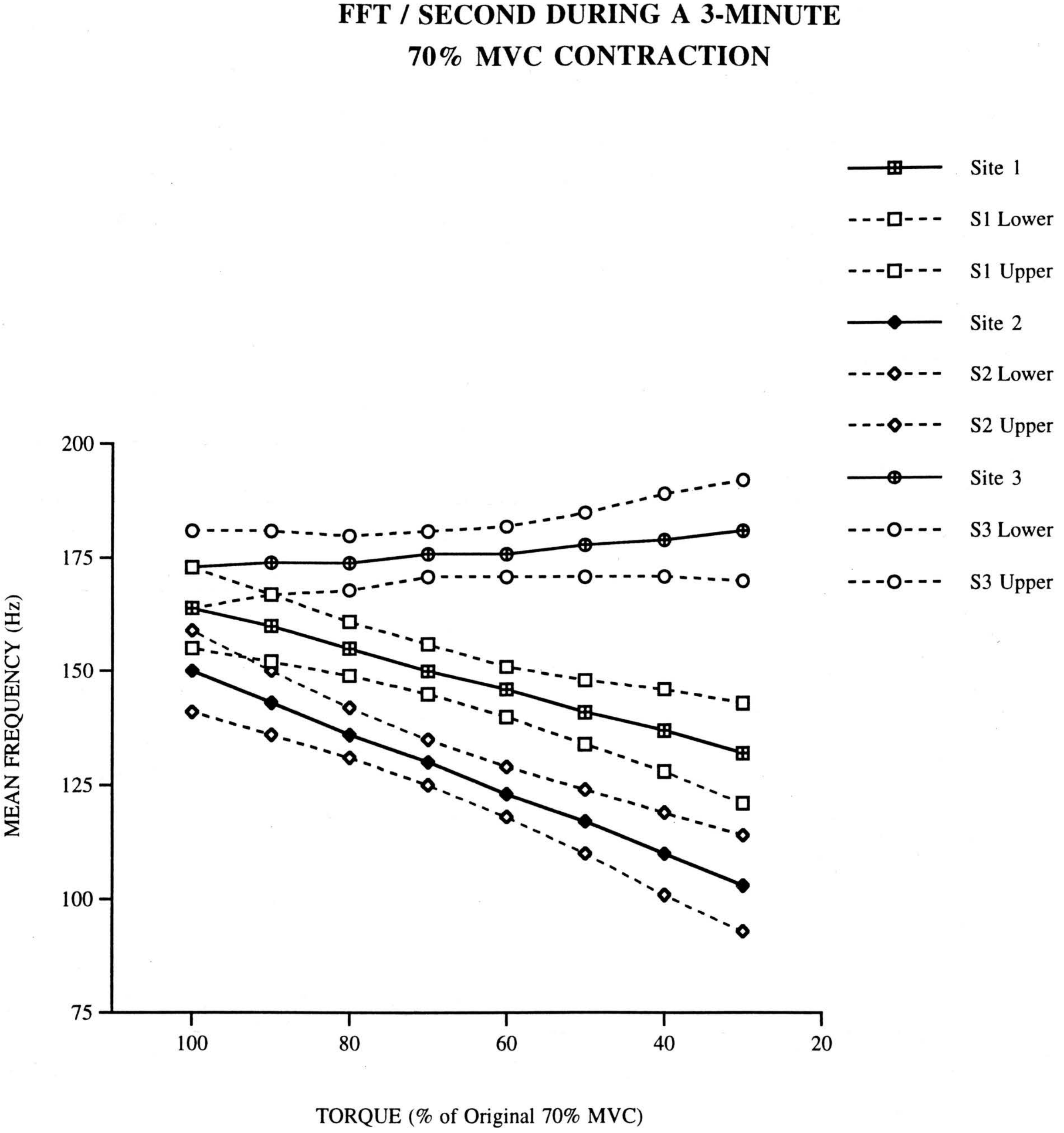
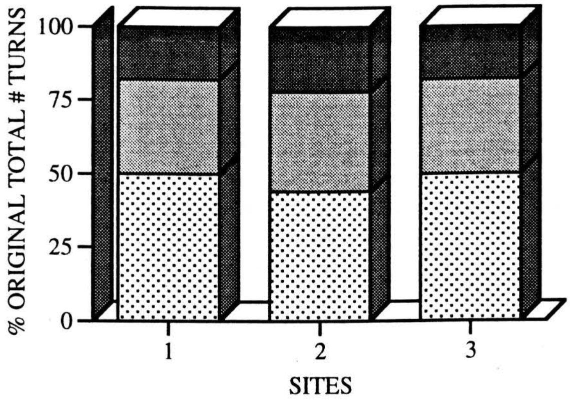


Figure 9: FFT during a 70% MVC contraction (n=10). Means are illustrated with solid lines and filled symbols. Upper and lower confidence intervals are illustrated with broken lines and empty symbols. When the confidence level lines cross each other, there are no significant differences between those sites and when the lines do not cross, there is a significant difference.

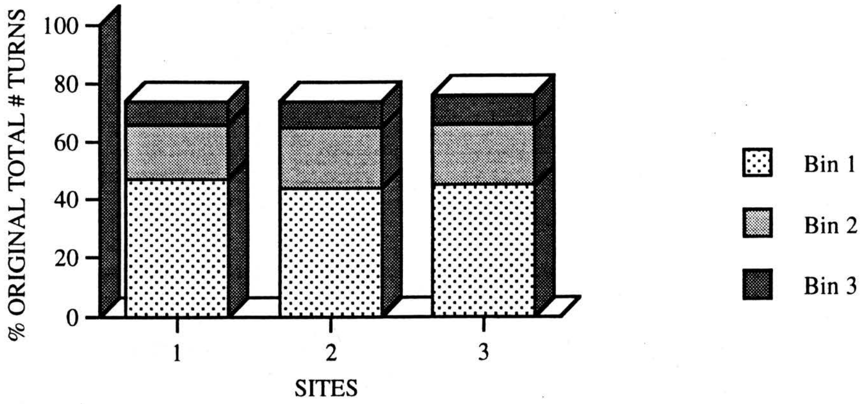
Amplitude

The EMG signal was amplified so that the highest amplitude potentials did not exceed 1.0 V. The amplitude of each turn during the fatiguing contraction was measured and sorted according to their amplitude into one of ten 100 mv bins (ie. bin 1=0-100 mv; bin 2=101-200 mv, etc.). Considering the distribution of turns found, it was decided to group some of the bins for the purposes of data analysis. Most turns fell in the first two bins and these were grouped to form the revised bin 1. The majority of the remaining turns fell in bins 3 & 4, and these were grouped, forming the revised bin 2. Bins 5-10 were grouped together since the fewest turns were recorded in these higher bins (revised bin 3). A three-way ANOVA with repeated measures found that the sites were significantly different from each other in terms of how the turns were distributed in the three bins ($p < 0.05$). There was also a significant interaction between bin and time, meaning that the numbers of turns in the different bins changes with time. There was an overall decrease across time in the numbers of turns per bin, but the most pronounced decrease was seen in bin 3 (higher amplitude turns). The total numbers of turns at site 1 decreased by approximately 40%, with a decrease of 20% in bin 1, 53% in bin 2, and 67% in bin 3. At site 2, total numbers of turns decreased by approximately 30%, with an increase of 9% in bin 1, a decrease of 50% in bin 2, and 67% in bin 3. At site 3, total numbers of turns decreased by approximately 30%, with a decrease of 6% in bin 1, 44% in bin 2, and 63% in bin 3 (Figure 10).

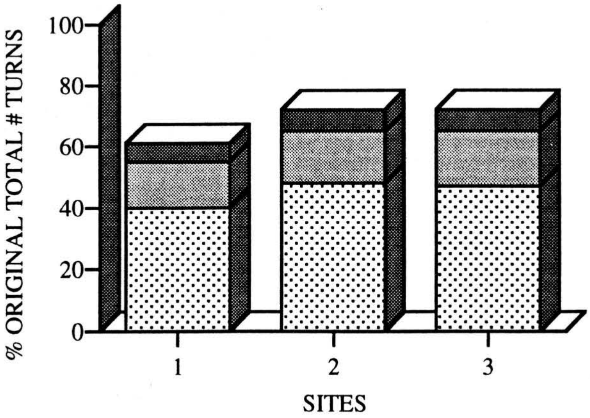
AVERAGE AMPLITUDE DISTRIBUTION DURING FATIGUE



a) First 30 seconds (0-30 s)



b)Middle 30 seconds (75-105 s)



c) Last 30 seconds (150-180 s)

Figure 10 (a,b,c): Distribution of turns to bins at three time periods (30-second averages) during a 3-minute 70% MVC contraction. There was a significantly larger ($p < 0.05$) decrease in the numbers of turns in Bin 3 than in Bins 1 & 2.

DISCUSSION

The purpose of this study was to determine whether motor unit recruitment is site specific in the human soleus muscle, particularly during fatigue. EMG was examined at three different sites in the muscle during isometric plantarflexion and site differences were observed during fatiguing contractions, as well as during various types of non-fatiguing contractions. These findings were significant because it has not been previously demonstrated that there are differences in recruitment among sites in a single muscle during fatigue. These site differences were most evident at lower torque levels in the different types of contractions. It was also found that recruitment levels continued to increase during ramp contractions at much higher percentages of MVC than previously reported.

Turns Analysis, Mean Frequency (MF), and Amplitude

It was important in this study to use a method of EMG analysis which provided quantitative information about muscle activity. Therefore, the complex interference pattern was analyzed using a modification of the Turns Analysis technique first introduced by Willison (1964). This method has been primarily used to analyze the IP in muscles of patients to distinguish between myopathies and neuropathies. Turns analysis has been deemed the "best studied and most widely used method of automatic IP analysis" (Dorfman et al. 1988). It is objective and provides very specific information regarding the frequency and amplitude of motor unit action potentials. During low levels of recruitment when up to four or five motor units are active, individual potentials of each unit can be identified. When additional motor units are recruited and their firing frequencies are increased, potentials of individual units can no longer be identified and a complex IP results. Turns analysis has the capacity to "count" each of these potentials and sort them according to amplitude. As more units are recruited and their firing frequencies increase, the numbers of turns increase up to a given percentage of MVC. Beyond that, numbers of turns plateau, but the amplitudes of turns can increase, possibly due to summation. The "ramp" contraction in the present study clearly demonstrated this continuing increase in amplitude (Appendix C - Figure C3), while the numbers of turns simultaneously plateaued, with the exception of site 3 (Figure 6).

During the three minute fatiguing contraction, however, amplitude decreased at all sites and was characterized by a more pronounced decline of high amplitude turns (500 mv - 1.0 V) (Figure 10). Similar decreases in amplitude have been reported in other studies during fatiguing contractions (Stephens et al. 1972; Milner-Brown et al. 1986; Moritani, Muro, Nagata, 1986; Linssen, Stegeman, Joosten, van't Hof, Binkhorst, & Notermans, 1993). In some cases, the amplitude of the raw signal appeared to increase initially and then decrease later in the contraction. It has been proposed that this increase in amplitude was due to increased motor unit recruitment, but this seems unlikely since all motor units are believed to be active at the higher intensities when this increase has still been observed. Linssen et al. (1993) reported that during intermittent contractions at 80% MVC (rate of 30/minute), the amplitude increased initially and then declined. They suggested that the increase could be accounted for by changes in the firing frequency of individual motor units, recruitment of additional motor units, and synchronization between motor unit firing patterns.

While only a small degree of synchronization occurs between units during normal contraction, motor units fire more synchronously during powerful contractions and after fatigue (Rothwell, 1994). There was evidence of synchronization in this study, which became more pronounced as the intensity of the contraction increased (Figure C2). The reason that units fire at the same time is that their parent motoneurons in the spinal cord receive a common synaptic input which brings them to threshold at approximately the same time (Rothwell, 1994). Another possible explanation for this apparent synchrony may be cross-talk from synergistic and antagonistic muscles. Muscles that are frequently used together as synergists often have greater input than other muscle pairs (Rothwell, 1994). Sometimes synchrony of motor unit recruitment can be misinterpreted because the MUAP appearing in different sites of a muscle or in different muscles at the same time may be due to the MUAP from a given unit being recorded by more than one recording electrode. While it is possible that this might have occurred between sites 1 and 2 in this study, it is unlikely, and even less likely that it could have occurred between site 3 and the first two sites. The first reason has to do with the size of motor unit territories which is discussed later. The reason why site 3 was unlikely to be recorded by electrodes at sites 1 and 2, or vice versa, has to do with the sites being in quite different compartments, which were separated by much larger distances than the known territories for human motor units.

Only positive waves of the IP were used in our study to simplify data analysis. However, based on comparisons between the present results and those from other studies, it appears that the numbers of turns reported are comparable when the fact that only positive waves were counted is considered. The numbers of turns during the fatiguing contraction in this experiment ranged from approximately 250 at the beginning of the contraction to approximately 130 at the end of contraction. While fatigue has not previously been analyzed using turns analysis, similar numbers of turns have been found at high intensities, comparable to the numbers at the start of the fatigue contraction in this study, when taking into consideration only turns in the positive phase were counted (Nandedkar, Sanders, & Stalberg, 1991; Liguori, Dahl, & Fuglsang-Frederiksen, 1992). Near the end of the fatigue contraction in this study, when torque had decreased to approximately 20% MVC, the turns had decreased to approximately 130-180 per second. Other studies have found that the numbers of turns at these percentages were approximately 100-200 per second in the positive phase (Nandedkar et al. 1991; Christensen & Fuglsang-Frederiksen, 1986).

Since turns analysis has not been previously used in fatigue studies, a form of spectral analysis which has been used extensively in fatigue experiments was used in this study to examine fatigue, to allow comparison between methods. The FFT range for the three sites was approximately 110-180 Hz, at various times throughout the 70% MVC contraction. This is comparable to previous reports of the FFT range from about 10-2000 Hz, with a peak at about 100-200 Hz (Dorfman et al. 1988).

Fatigue is associated with a shift to the lower frequencies, perhaps due to increased MUAP duration, resulting from slowing of muscle fiber conduction velocity (Dorfman et al. 1988). There is some evidence that the lower frequency components of the power spectrum (10-50 Hz) tend to reflect MUAP firing rates, whereas the higher frequency components are more related to the MUAP shapes. During fatigue, the firing frequencies of motor units decrease to accommodate the increase in duration of a unit's contraction time. This would also explain the decline in the power spectrum and the reduced numbers of turns during fatigue. However, neither the turns analysis nor the power spectral density "correspond directly to any physiologic property of the IP signal" (Dorfman et al. 1988).

The two analysis methods have proven to be quite comparable. During the fatiguing contraction, both the numbers of turns and the FFT decreased significantly at all sites. There was one exception, however, with the average FFT at site 3 showing no significant decline. Although site 3 was consistently different from sites 1 & 2 throughout the experiment, this deviation appeared to be the result of only two subjects whose FFT actually increased and therefore increased the mean of the entire group.

Site Specific Recruitment

Willison (1964) first provided data which indicated that recruitment patterns may differ among sites of the same muscle. It was shown that activity measured in terms of counts per second varied from one area to another in a single muscle. In the present study, there were differences in recruitment patterns among sites during *both* fatiguing and non-fatiguing contractions.

Low vs High Intensities

Site differences varied depending upon the intensity of the contraction and the greatest differences were observed at the lowest intensities. Differences in the numbers of turns at low intensities might be explained in several ways. Muscles can increase the force of contraction by recruiting more motor units and by increasing the firing frequencies of individual motor units (Adrian et al. 1929). As each occurs, the numbers of turns will increase. Whether one or both of these options are used will be dependent upon a number of factors. Adrian et al. (1929) were of the opinion that at relatively low forces, recruitment was the more important mechanism to sustain or increase contraction levels and increased firing rates were increasingly employed to bring contractile force closer to the maximum. These conclusions were later supported by the spike-triggered averaging study of Milner-Brown, Stein, and Yemm (1973); in the FDI muscle, they observed that half of the motor units had already been recruited when only 10% of the maximal force had been developed. If this is the case, it would suggest that more site specific variability is present as a result of recruitment of new motor units than when firing rates are increased.

In addition, the distribution of these motor units is not homogeneous at all sites in a muscle (Bodine-Fowler, Garfinkel, Roy, & Edgerton, 1990). Motor unit type determines the threshold of recruitment and even motor units of the same type will have different thresholds of recruitment which will fire at different frequencies. Therefore, at lower levels of force when not all motor units are being recruited, and some are firing at different frequencies, differences in the numbers of turns would be expected.

During the fatiguing contraction, the site differences also became more pronounced at the lower torque levels. This would suggest that variability at low torque levels is present not only during recruitment of motor units, but also during derecruitment which may occur during fatigue.

It may be argued that the low and high intensities were different due to the turns analysis not being highly sensitive above 40-50% MVC because of the findings of previous studies. Christensen et al. (1984) measured electrical activity in the biceps brachii during a gradual increase in force from zero to maximum within 10 seconds. The numbers of turns increased with gradually increasing force up to 40%-50% MVC and was unchanged or decreased with further increasing force. Nandedkar et al. (1986) also reported that the numbers of turns in the normal IP measured in biceps muscle becomes relatively constant or actually decreases when the force of contraction is greater than 30-50% of maximum, whereas the mean amplitude continues to rise at these force levels. However, our results have shown that this may not always be the case. During the ramp contraction, sites 1 and 2 plateaued at 60% and 50% respectively, but site 3 had not yet leveled off at 70% MVC. This provides evidence which demonstrates that the numbers of turns can increase beyond 50% MVC, which is contrary to present belief. Therefore, the conclusion from this study that there is greater variability in recruitment patterns at lower intensities can be supported and the argument that the turns analysis is unable to discern differences at higher intensities is untenable.

Location of Recording Sites in the Soleus Muscle

Of the three sites examined in the soleus muscle, site 3 was generally different from sites 1 and 2 during the ramp and fatigue contractions. Site 3 had the greatest number of turns and the highest FFT (FFT was measured during fatigue only) for the majority of the contraction times and these differences were more pronounced at the lower intensities (Figures 8-9).

Since the sites nearest to each other (sites 1 & 2) were more than five centimetres apart, there is no reason to believe that the muscle fibers sampled at the sites belonged to the same motor unit. Buchthal et al. (1957) reported that the territories of individual human motor units have cross-sectional diameters of approximately 5-10 millimetres. Animal studies have also indicated that, in general, fibers belonging to a motor unit were not distributed across the entire cross-section, but were localized to a particular region of the muscle (Bodine-Fowler et al. 1990). However, the soleus muscle has a very complex architecture, divided into several distinct compartments, each with its own nerve supply (McComas, 1996). Motor axons belonging to a specific muscle nerve appear to selectively innervate specific regions of a muscle from the initial stages of the innervation process (Bodine-Fowler et al. 1990). Given the location of sites 1, 2, and 3 in the muscle, it would be most likely that sites 1 and 2 belonged to the same compartment and that site 3 was in a different compartment. Each compartment would also likely have varying fiber arrangements, fiber type distributions, and blood supply. Results of autopsy studies have shown that there can be significant differences between sites of a single muscle in terms of fiber type distribution (Elder et al. 1982). For example, the soleus and biceps brachii were found to have significantly more type II fibers at their insertions than at their origins. Such an arrangement would be possible in the soleus which has a complicated multipennate arrangement of its fibers (Elder et al. 1982). Differences in fiber pennation and shape also exist within single muscles (Sejersted et al. 1984). Since the pennation and/or shape can affect the IMP developed during a contraction (Sadamoto et al. 1983), regional differences in IMP might be expected. This would affect recruitment differently according to site.

Another possible explanation for the differences observed at site 3 may be related to the recruitment of fresh motor units or rotation between units (Arendt-Nielsen & Mills, 1988; Enoka et al. 1992). The switching of activity during high intensity contractions may occur within parts of a muscle since it is known that some muscles consist of discrete compartments (Enoka et al. 1992). Motor unit rotation would provide periods of inactivity or reduced activity that could be used for the replenishment of metabolic needs and thereby minimize fatigue. It may therefore be possible that site 3, located in the medial soleus, recruited some of its motor units or increased its firing frequency later than the other sites as a "compensatory" method to prevent or delay muscle fatigue.

Fatigue

Fatigue was demonstrated in all subjects by an average 52% decrease in torque after three minutes. The absolute torque levels agreed with previous findings for female subjects (Belanger et al. 1983) and the percentage decrease matched the findings of Moritani et al. (1985) and Bigland-Ritchie et al. (1983). Seventy percent MVC could be maintained for an average of 44 seconds (range 10-80 seconds), which is comparable to that found by Arendt-Nielsen et al. (1988). While it can be assumed that maximal recruitment was not required at the start of the contraction, when fatigue began to occur and torque fell below the target level, subjects would have been producing maximal effort to return to 70% MVC.

Effects of Fatigue or "Prior History" of Recruitment

A comparison was made of the actual turn counts to determine if the same numbers of turns were generated at a given percentage of MVC, but during different types of contractions. For example, does 20% MVC produce the same numbers of turns when it is held for 10 s, as when 20% MVC is reached during a ramp contraction, or when torque falls to 20% MVC during a fatiguing contraction? There was a significant difference in the numbers of turns at a given percentage of MVC, depending upon the type of contraction (Table B16). This variability in numbers of turns at the same percentage of MVC suggests that "prior history" of recruitment influences how motor units are recruited later in a contraction or in subsequent contractions. This theory has been supported elsewhere by Enoka et. al. (1989). They examined motor unit behaviour in the first dorsal interosseus muscle of human subjects during a ramp and hold task that was performed before and after a fatiguing contraction. Activity was characterized in terms of the forces of recruitment and derecruitment and the discharge pattern. Motor unit behaviour during and after fatigue showed that the relatively homogeneous behaviour seen before fatigue could be severely disrupted. After a fatigue test, when the initial threshold tasks were repeated, the behaviour of most motor units changed. Some low threshold motor units were not active after the fatiguing activity although the task was identical to that performed before the fatigue task. This variation in motor unit recruitment was interpreted as reflecting some degree of history dependent flexibility in recruitment order among these motor units (Enoka et al. 1989).

This also supports the theory that recruitment is task specific. Previous studies (Enoka et al. 1989; Ter Haar Romeny et al. 1984) have found that recruitment at a given site in a muscle can be changed consistently by changing the task performed. Although the task itself was not dramatically changed in the present study, the details of the task changed, which may have been sufficient to cause variations in recruitment patterns.

Intramuscular Pressure (IMP) and Blood Occlusion

An IMP study was included in preliminary testing, but was not directly part of the main experiment. Data was included for supporting evidence of site variability in the muscle. IMP was measured during a 70% MVC fatiguing contraction and it (IMP) increased immediately to a peak level as torque simultaneously reached its peak (Appendix D). IMP was measured at two sites (depths) and was greatest at the site which was nearest to the central region of the muscle. Sjogaard et al. (1988) also found that there was increased pressure deeper in the muscle, and in agreement with this, Sejersted et al. (1984) discovered that blood flow to central parts of the muscle was compromised first during contraction and that anaerobic conditions may exist in interior parts of the muscle. This is an important consideration since IMP may affect the distribution of blood flow within the contracting muscle (Sjogaard et al. 1986), and therefore contribute to recruitment differences among muscle sites. During high intensity sustained isometric contractions, blood flow impediment and occlusion leads to muscle fatigue. The degree of impediment to flow is related to the IMP generated by the contracting muscle. Heterogeneity of flow within a muscle was demonstrated by Laughlin et al. (1982) who believed it may have been caused by an alternating recruitment of muscle fibers within a muscle. Similarly, Sjogaard et al. (1986) concluded that during low level static contractions, blood supply to exercising muscle is maintained at a sufficiently high level and that the alternating recruitment of muscle fibers may result in a heterogeneously distributed blood flow within the contracting muscle. This was confirmed with EMG recordings which showed that a decrease in IMP was related to decreased muscle activity (Sjogaard et al. 1986). It was also demonstrated that when EMG amplitude and IMP decreased in one area of the muscle, then activity in another portion of the muscle was increased simultaneously.

Summary

It can be concluded that during both fatiguing and non-fatiguing contractions, there was site specificity in the EMG recordings from three sites in the soleus muscle. This indicates that different sites of a muscle can be recruited differentially at a given time during a contraction. Site specific motor unit recruitment has not previously been demonstrated during fatiguing contractions. These site differences, in every case, were greatest at the lower intensities of contraction which suggests that the neuromuscular system has more recruitment options available at the lower intensities, in terms of modifying either the numbers of motor units recruited or their firing frequencies. A second important finding was revealed during the ramp contraction, when force increased up to approximately 70-80% MVC and the numbers of turns also continued to increase at one site without ever leveling off. These results are different from those previously found by others and they suggest that the patterns of recruitment can continue to change beyond force levels that the current literature would suggest. It is also possible that patterns of recruitment during complex movements could be even more variable than is presently believed. Observation of such differences helps us to understand more clearly the manner in which muscles function under extreme conditions, such as fatigue. It is important to recognize that muscle structure and function are very complex and that analyses based on a single site or even a single intensity may be poor indicators of the activity of the entire muscle.

APPENDICES

Appendix A: Subject Characteristics

Appendix B: ANOVA Summary Tables

Appendix C: C1: Electrode sites in the soleus muscle
C2-C5: EMG activity patterns

Appendix D: Pilot Study - Variability of IMP During Isometric Contraction

Appendix E: Pilot Study - EMG Recorded at Different Muscle Depths

APPENDIX A

Table A1: Subject Characteristics

SUBJECT	AGE	HEIGHT (cm)	WEIGHT (kg)	MAX. TORQUE (Nm)
1	22	152	55	94.64
2	25	178	68	152.46
3	22	170	52	138.02
4	21	170	66	141.19
5	26	165	66	129.71
6	24	168	59	164.03
7	22	170	57	139.28
8	21	168	55	114.41
9	25	163	53	135.36
10	21	168	61	118.74
Mean	23	168	59	132.78

APPENDIX B

Tables B1-B3. ANOVA summary tables for two-way (Trial and Time) analyses of reliability measures at 20% MVC.

Table B1. Site 1

Source of Variations	Sums of Squares	Mean Square	DF	F	P	Error
Trial (Tr)	699	349	2	.212	.8113	1652
Time (T)	2478	275	9	1.42	.1928	194
TTr	1675	93	18	.940	.5314	99

Table B2. Site 2

Source of Variations	Sums of Squares	Mean Square	DF	F	P	Error
Trial (Tr)	2376	1188	2	.572	.5745	2078
Time (T)	1291	143	9	1.72	.0967	83
TTr	1343	75	18	.861	.6257	87

Table B3. Site 3

Source of Variations	Sums of Squares	Mean Square	DF	F	P	Error
Trial (Tr)	2238	1119	2	.459	.6389	2436
Time (T)	843	94	9	.939	.4965	100
TTr	936	52	18	.541	.9347	96

Tables B4-B6. ANOVA summary tables for two-way (Trial and Time) analyses of reliability measures at 40% MVC.

Table B4. Site 1

Source of variations	Sums of Squares	Mean Square	DF	F	P	Error
Trial (Tr)	917	458	2	.150	.8622	3068
Time (T)	1056	117	9	.661	.7415	178
TTr	2877	160	18	1.35	.1639	118

Table B5. Site 2

Source of variations	Sums of Squares	Mean Square	DF	F	P	Error
Trial (Tr)	10775	5387	2	4.21	.0317	1280
Time (T)	1171	130	9	1.46	.1766	89
TTr	1400	78	18	1.04	.4204	75

Table B6. Site 3

Source of variations	Sums of Squares	Mean Square	DF	F	P	Error
Trial (Tr)	459	229	2	.075	.9279	3052
Time (T)	1784	198	9	.938	.4971	211
TTr	2077	115	18	.862	.6252	134

Tables B7-B9. ANOVA summary tables for two-way (Trial and Time) analyses of reliability measures at 60% MVC.

Table B7. Site 1

Source of variations	Sums of Squares	Mean Square	DF	F	P	Error
Trial (Tr)	14534	7267	2	2.37	.1218	3063
Time (T)	883	98	9	.614	.7816	160
TTr	2270	126	18	.678	.8292	186

Table B8. Site 2

Source of variations	Sums of Squares	Mean Square	DF	F	P	Error
Trial (Tr)	3202	1601	2	2.07	.1555	774
Time (T)	4645	516	9	5.97	.0001	86
TTr	1660	92	18	1.33	.1737	69

Table B9. Site 3

Source of variations	Sums of Squares	Mean Square	DF	F	P	Error
Trial (Tr)	2843	1421	2	.727	.4973	1956
Time (T)	5323	591	9	3.45	.0012	172
TTr	3228	179	18	1.55	.0795	116

Table B10 . ANOVA summary table for two-way (Site and Intensity) analysis of the turn count differences among three sites at 20%, 40%, 60% MVC.

Source of variations	Sums of Squares	Mean Square	DF	F	P	Error
Site (S)	2778	1389	2	.393	.6808	3536
Intensity (I)	72541	36270	2	34.09	.0001	1064
SI	5349	1337	4	3.28	.0215	407

Table B11. ANOVA summary table for two-way (Site and Intensity) analysis of the turn count differences among three muscle sites during a Ramp contraction.

Source of variations	Sums of Squares	Mean Square	DF	F	P	Error
Site (S)	39721	19861	2	2.47	.1128	8044
Intensity (I)	379043	63174	6	34.69	.0001	1821
SI	28795	2400	12	1.93	.0380	1242

Table B12. ANCOVA summary table for two-way (Site and Intensity) analysis of the turn count differences among three muscle sites during a 70% MVC fatiguing contraction.

Source of variations	Sums of Squares	Mean Square	DF	F	P	Error
Site (S)	34679	17340	2	4.81	.0172	3607
Intensity (I)	695357	695357	1	1193	.0001	583
SI	27404	13702	2	23.51	.0001	583

Table B13. ANCOVA summary table for two-way (Site and Intensity) analysis of the FFT differences among three sites during a 70% MVC fatiguing contraction.

Source of variations	Sums of Squares	Mean Square	DF	F	P	Error
Site (S)	68701	34351	2	8.53	.0015	4027
Intensity (I)	152527	152527	1	241.3	.0001	632
SI	21397	10698	2	16.93	.0001	632

Table B14. ANOVA summary table for three-way (Site, Time, and Bin) analysis of the differences in distributions of turns to bins among three muscle sites during a 70% MVC fatiguing contraction.

Source of variations	Sums of Squares	Mean Square	DF	F	P	Error
Site (S)	149	74	2	4.51	.0259	17
Time (T)	5238	5238	1	87.74	.0001	60
Bin (B)	34303	17151	2	100.6	.0001	170
ST	139	69	2	.921	.4010	75
SB	96	24	4	.318	.8652	75
TB	1411	705	2	9.37	.0002	75
STB	527	132	4	1.75	.1445	75

Table B15. ANOVA summary table for three-way (Site, Depth, and Time) analysis of the turn count differences between two muscle depths at 20% MVC.

Source of variations	Sums of Squares	Mean Square	DF	F	P	Error
Site (S)	529749	264874	2	1.10	.4169	241301
Depth (D)	234834	234834	1	1.84	.3073	127291
Time (T)	7618	263	29	1.57	.0736	168
SD	62403	31201	2	.676	.5586	46160
DT	6294	217	29	.791	.7512	274
ST	12603	217	58	1.41	.0593	154
SDT	5752	99	58	.586	.9875	169

Table B16. ANOVA summary table for three-way (Site, Intensity, Treatment) analysis of the turn count differences at a given intensity among 20%, 40%, 60% (Treatment 1), Ramp (Treatment 2), and Fatigue (Treatment 3) data.

Source of variations	Sums of Squares	Mean Square	DF	F	P	Error
Site (S)	1423	711	2	1.07	.3867	663.5
Intensity (I)	20475	10237	2	15.43	.0018	663.5
Treatment (T)	19094	9547	2	14.39	.0022	663.5
SI	2812	703	4	1.06	.4355	663.5
ST	1089	272	4	.41	.7968	663.5
IT	1378	344	4	.52	.7248	663.5

APPENDIX C

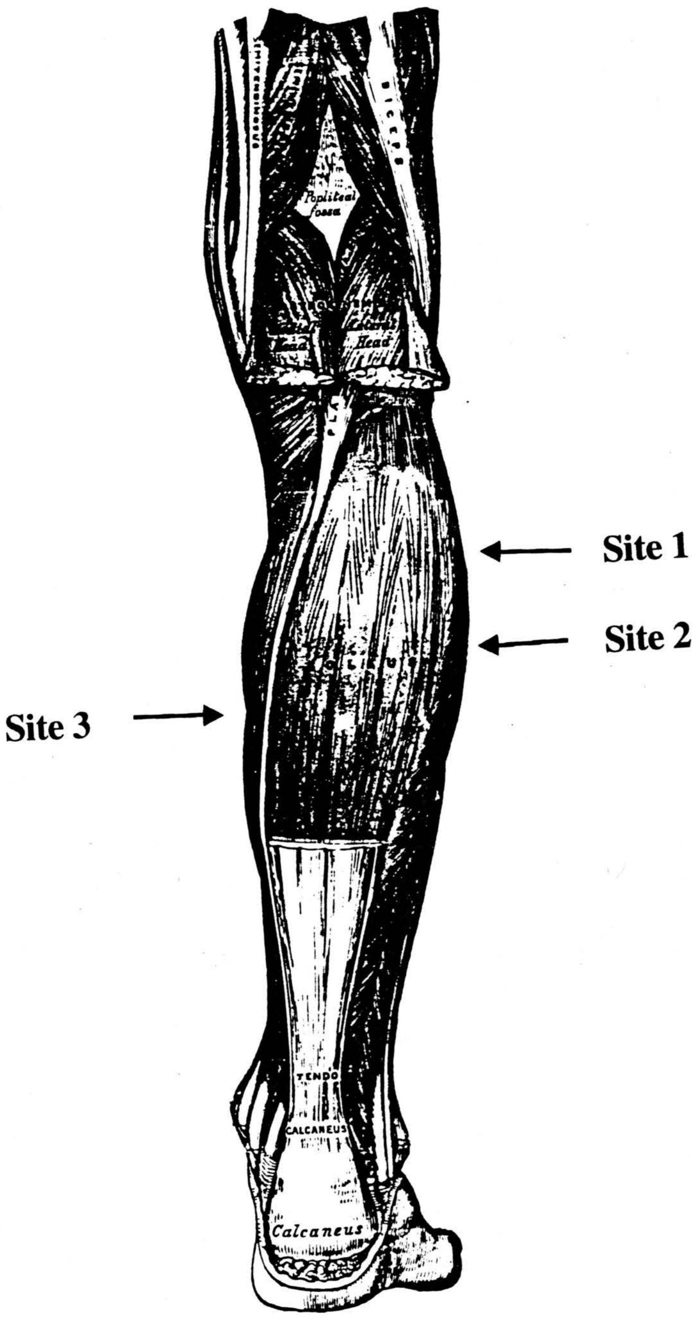
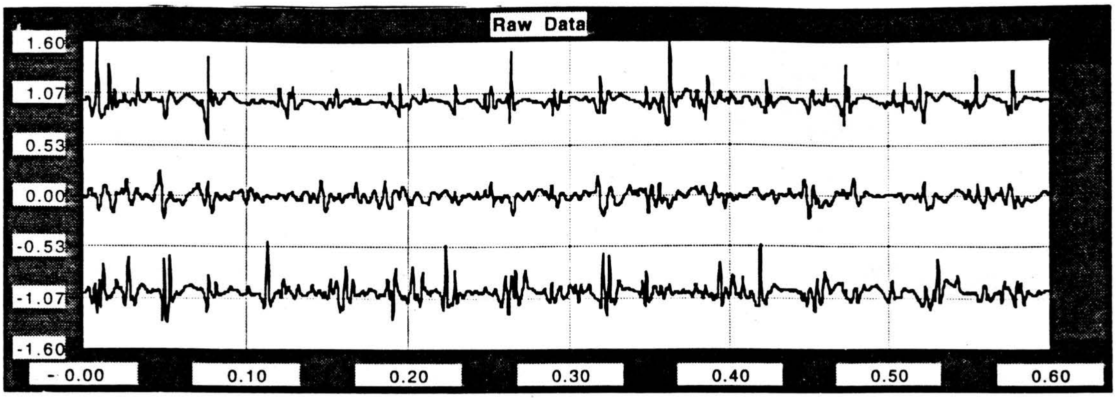
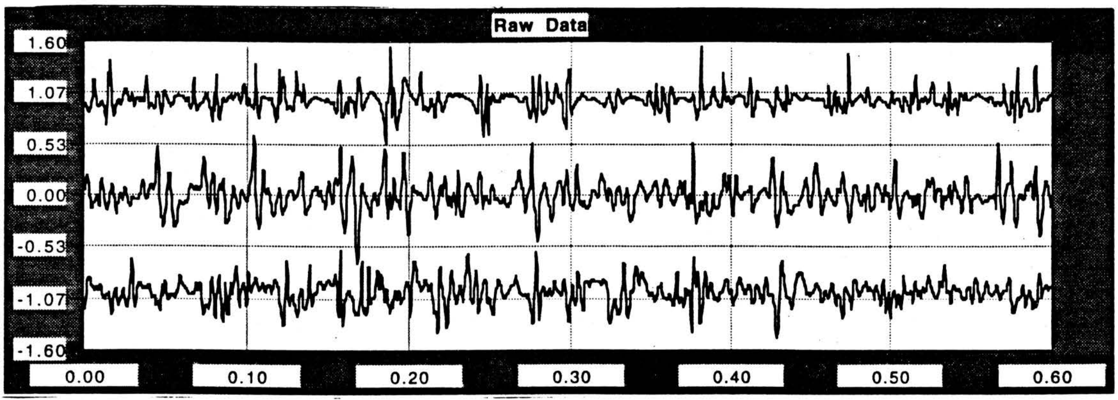


Figure C1: Needle electrode sites in the soleus muscle.

20% MVC



40% MVC



60% MVC

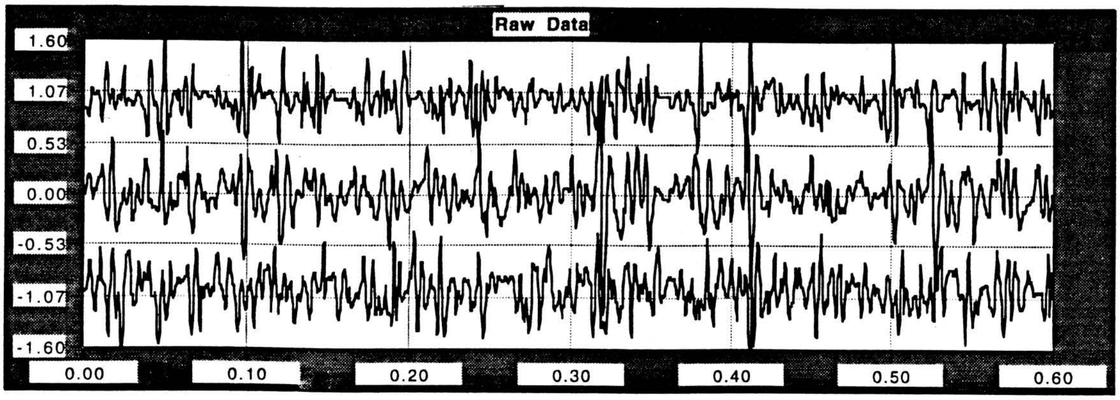
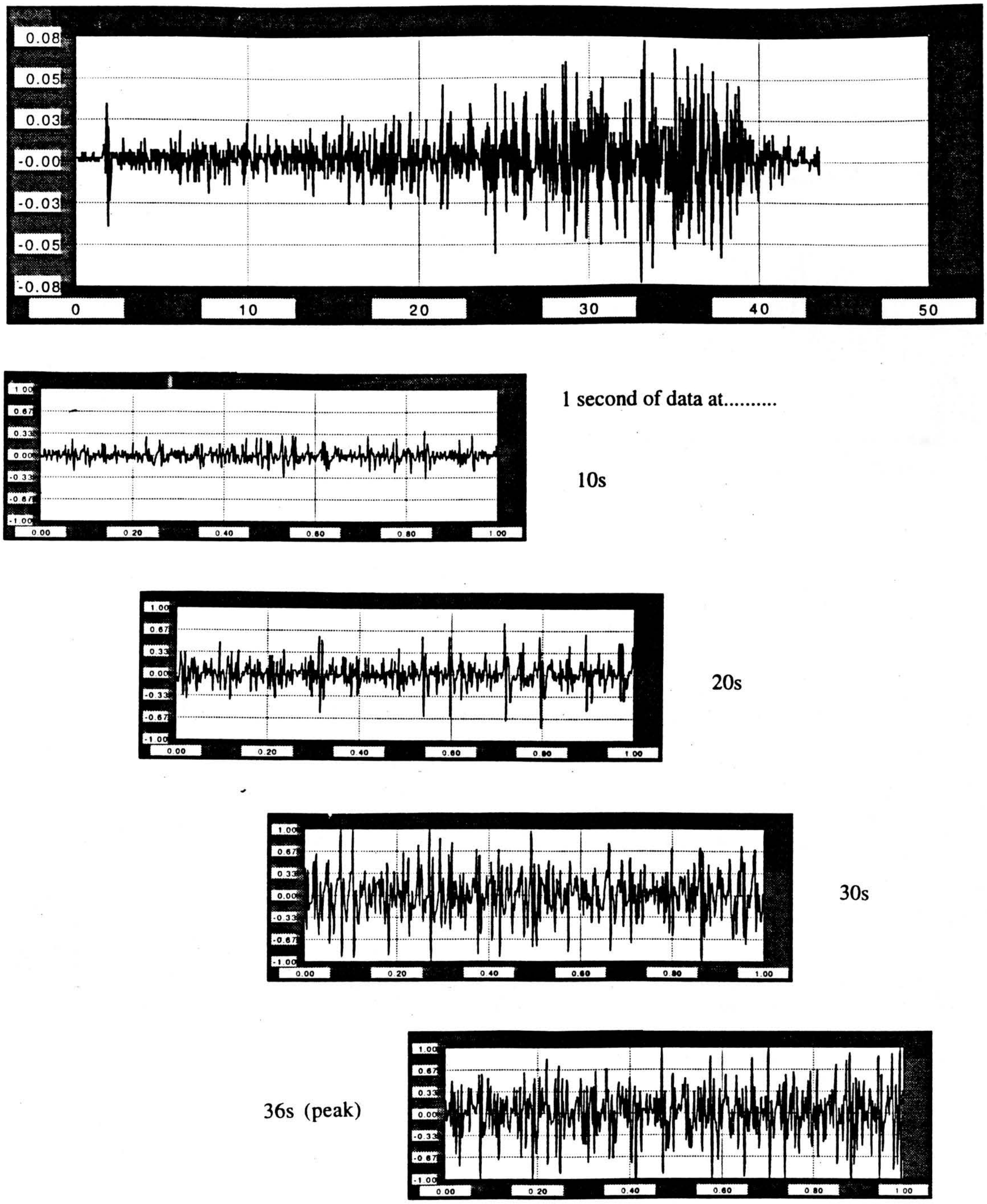


Figure C2: EMG activity at 3 sites at 20%, 40%, and 60% MVC. Voltage measurements appear on the y-axis and time on the x-axis.



1 second of data at..........

10s

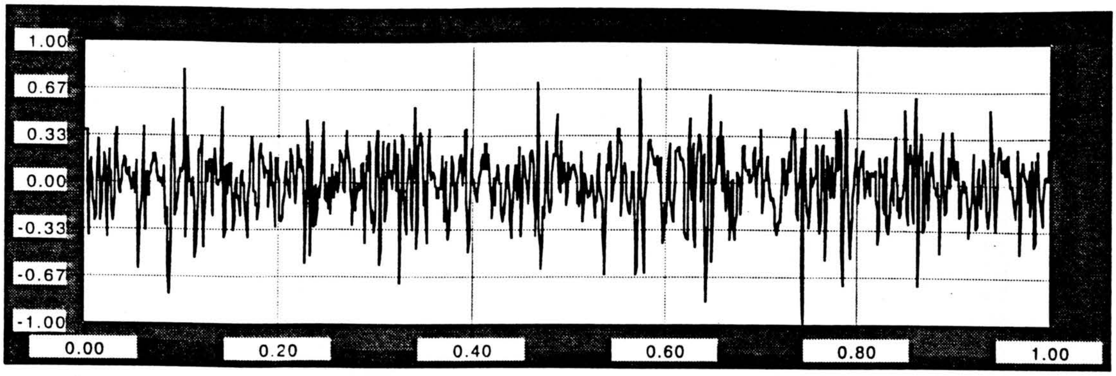
20s

30s

36s (peak)

Figure C3: EMG activity during a Ramp contraction. An interference pattern is shown for the full contraction (~ 40 seconds), along with one second portions of data at approximately every 10 seconds. Voltage appears on the y-axis and time on the x-axis.

First second



Last second

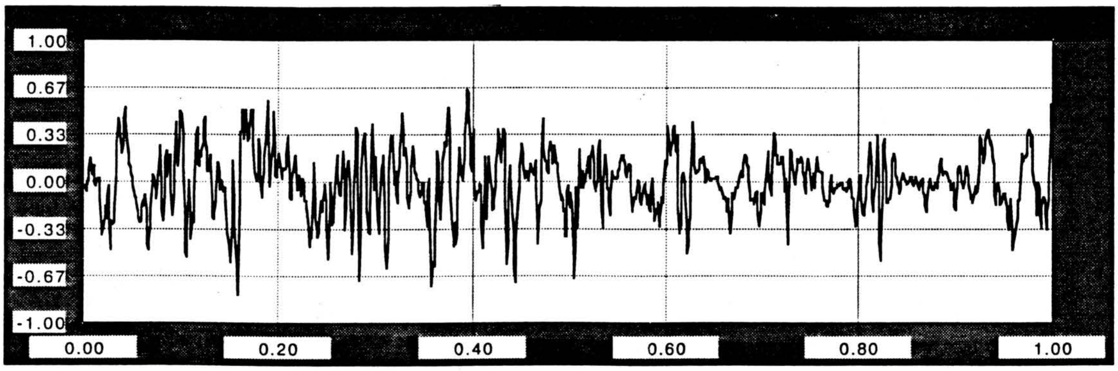


Figure C4: EMG activity during the first and last second of a 3-minute 70% MVC contraction. Voltage appears on the y-axis and time on the x-axis.

1st 30 seconds of 70% MVC

0sec.	72	75	31	19	8	3	0	0	0	0	208
1sec.	69	89	40	23	7	4	2	0	0	0	234
2sec.	76	69	41	24	10	1	3	2	1	0	227
3sec.	63	67	39	22	8	5	4	1	0	1	210
4sec.	50	53	44	27	9	8	3	2	1	0	197
5sec.	65	80	37	16	6	3	1	1	0	0	209
6sec.	64	79	42	15	9	3	1	2	0	0	215
7sec.	66	76	43	8	10	5	1	1	0	0	210
8sec.	61	73	51	22	9	5	1	0	0	0	222
9sec.	54	83	52	17	10	4	1	0	0	0	221
10sec.	65	62	35	21	10	12	2	0	1	0	208
11sec.	47	46	47	26	11	8	5	3	0	0	193
12sec.	54	63	45	31	12	8	6	0	0	0	219
13sec.	54	74	54	20	9	6	3	1	0	2	223
14sec.	67	70	48	30	11	4	1	2	0	0	233
15sec.	48	82	48	16	10	8	4	2	0	0	218
16sec.	62	72	50	23	4	6	3	1	0	0	221
17sec.	47	75	46	27	15	9	1	0	0	0	220
18sec.	61	70	64	23	10	6	2	2	1	1	240
19sec.	46	54	46	27	12	6	4	0	1	0	196
20sec.	57	63	46	22	12	2	1	3	0	0	206
21sec.	48	60	59	28	8	8	4	4	0	0	219
22sec.	37	69	57	25	14	10	5	1	1	0	219
23sec.	40	66	56	32	18	8	0	1	1	0	222
24sec.	62	61	55	25	17	5	4	2	0	0	231
25sec.	51	68	46	28	14	6	4	0	0	0	217
26sec.	40	71	43	23	15	7	4	3	3	0	209
27sec.	42	52	34	38	22	5	3	2	3	0	201
28sec.	36	64	51	27	16	5	3	4	0	2	208
29sec.	32	61	38	31	26	8	2	2	1	0	201

Last 30 seconds of 70% MVC

150sec	63	64	23	15	0	0	0	0	0	0	165
151sec	49	61	33	8	8	3	0	0	0	0	162
152sec	44	48	36	9	5	1	0	0	0	0	143
153sec	58	47	28	9	4	1	0	0	0	0	147
154sec	24	47	26	21	9	5	2	0	0	0	134
155sec	32	66	30	11	7	7	2	0	0	0	155
156sec	64	40	33	9	6	2	1	0	0	0	155
157sec	44	74	33	11	7	2	0	0	1	0	172
158sec	60	47	37	16	5	3	1	0	0	0	169
159sec	53	54	35	15	8	1	1	0	1	0	168
160sec	61	57	34	7	5	1	0	0	0	0	165
161sec	69	49	26	9	3	2	2	0	0	0	160
162sec	53	68	30	14	3	0	1	0	0	0	169
163sec	47	57	32	17	8	1	0	0	0	0	162
164sec	52	52	34	16	5	0	0	0	0	0	159
165sec	52	57	40	11	6	0	0	0	0	0	166
166sec	47	46	30	23	5	3	2	2	0	0	158
167sec	55	41	21	20	6	1	0	1	0	0	145
168sec	45	41	22	10	8	0	0	0	0	0	126
169sec	68	46	28	13	5	5	0	1	0	0	166
170sec	54	47	36	17	8	2	0	0	0	0	164
171sec	48	50	31	10	3	0	0	0	0	0	142
172sec	39	45	24	13	2	0	0	0	0	0	123
173sec	51	51	12	4	3	1	0	0	0	0	122
174sec	42	62	30	10	2	1	1	0	0	0	148
175sec	49	53	34	10	7	1	0	0	0	0	154
176sec	49	42	34	9	12	5	2	1	0	0	154
177sec	54	61	31	10	8	1	0	0	0	0	165
178sec	50	40	27	12	5	2	2	0	0	0	138
179sec	41	61	23	13	1	0	0	1	0	0	140

Figure C5: Example of a spreadsheet which includes the total numbers of turns per second and the numbers of turns in each of the ten 100 mv amplitude bins. This data compares the first 30 seconds of a 3-minute contraction to the last 30 seconds.

APPENDIX D

Appendix D - Pilot Study
Variability of IMP During Isometric Contraction

Muscle fatigue can be attributed to many factors, but during sustained isometric contractions, blood flow is believed to be impeded and even occluded during contractions starting at 40% MVC in the soleus muscle. The degree of impediment to flow is related to the intramuscular pressures (IMP) generated by the contracting muscle.

The objectives of this preliminary experiment were to determine the effect of increasing torque IMP in the human soleus muscle and to observe whether the intramuscular pressures created during a constant isometric contraction varied among different sites of a muscle. If it can be shown that IMP varies according to site, it would provide evidence to support the theory that fatigue effects are site specific in a single muscle.

Methods

Experiments were conducted on the soleus muscle of two of the female subjects studied in the main experiment, along with one male.

The procedure for measuring plantarflexion torque, electromyography techniques, protocol, and the data recording system were identical to those used in the primary experiment and have been previously described in the Methods section (exceptions are noted).

<u>Measurement of IMP</u>:

A local anesthetic (2 cc of xylocaine 2%) was first injected into the fascia of the lateral soleus at approximately 50% of leg length from the fibular head to the lateral malleolus. A 14 gauge catheter (Medicut) was inserted through the fascia into the muscle. The needle was then withdrawn and the catheter remained in the muscle. A solid-state pressure transducer (Millar) was inserted with the recording site near the distal end of the catheter (through which it had been placed). This recorded the pressures exerted as the subjects produced different percentages of maximum.

Initially, the catheter, which entered the lateral side of the muscle, was at a depth of approximately five centimetres, which would place the recording end slightly beyond the central posterior region of the muscle. With the catheter in this position, the subject performed the experimental procedure listed below, excluding the 70% MVC fatigue trial. The catheter was then withdrawn approximately 1.5 cm. and the exact protocol was repeated, including a 70% MVC fatigue trial at the end.

Analysis:

Differences in intramuscular pressures among sites, subjects, and types of contractions were examined. These comparisons were made by examining percentage differences, percentage changes, and observing relationships in the data. Statistics were not performed because of the very small sample size. Within subject reliability was assessed using an analysis of variance with repeated measures.

Results

The results presented are the major observations from the preliminary data on IMP recordings in the soleus muscle during isometric contractions of various intensities. It should be noted that while testing Subject 2, the pressure transducer did not provide reliable measures in the first position and results are therefore available for the second position only. Subject 1 (male subject) did not complete the Ramp or 70% MVC Fatigue contractions.

Torque vs. Pressure

Intramuscular pressures were recorded during ten second contractions at 20%, 40%, and 60% of MVC. The relationship between torque (% MVC) and IMP was examined in all subjects. In each case, with increasing torque production, there was a simultaneous increase in IMP. This trend was evident at both intramuscular recording positions.

IMP during Ramp contractions

Two of the three subjects performed a Ramp contraction and the relationship between linearly increasing torque and IMP was examined. It was observed that with increasing torque, the level of pressure in the muscle showed a corresponding increase. This relationship was observed in all subjects at all sites.

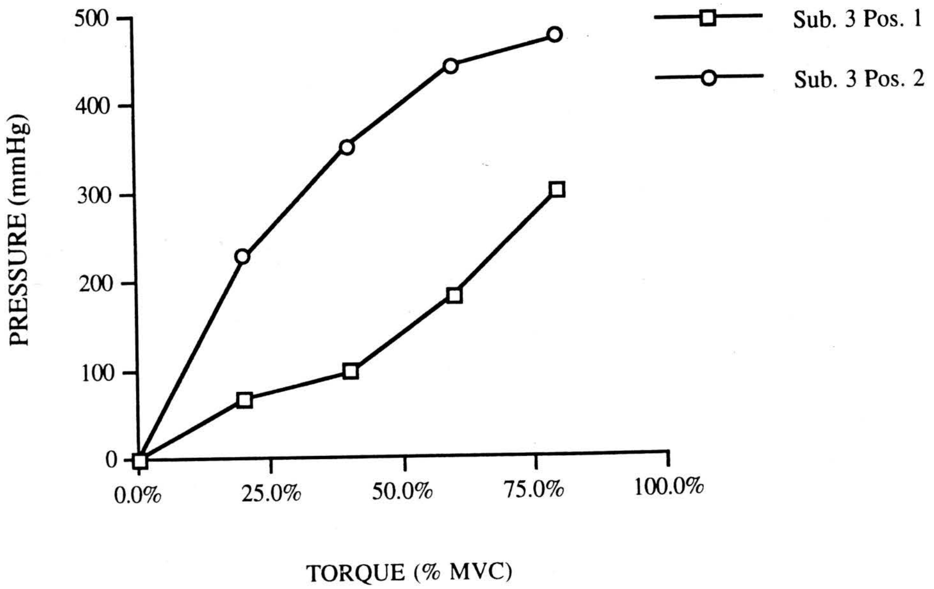


Figure D1: Intramuscular pressures produced during a ramp contraction at 2 sites (n=1)

Torque and IMP during Fatigue

Two of the three subjects performed a 70% MVC fatiguing contraction. One subject held a contraction for three minutes and the other for two minutes. Although torque gradually declined throughout the contraction period, subjects attempted to maintain a torque level as high as possible. In subject 2, torque levels decreased by 65% in two minutes. Pressure decreased by 83% and showed a more rapid decline than torque. In subject 3, torque decreased by 49% in three minutes, while pressure showed a decrease of 81% in the same time period (Figure D3). This contraction was performed with the IMP recording device in the second position only.

Comparison of 2 Recording Sites

In two of the three subjects, data was obtained from two sites in the muscle. The first site (as explained in Methods) was near the medial side of the muscle, while the second site was in the central posterior region of the muscle. In each of the different contractions, the pressures recorded from Position 2 were consistently higher than those recorded from Position 1 (Figs. 1 & 2). In some cases, at 20% MVC, the lowest percentage of MVC recorded, the pressures between the two sites were very similar. However, as the torque increased to 40% and 60% MVC, there was a more pronounced difference in the pressures between the two position.

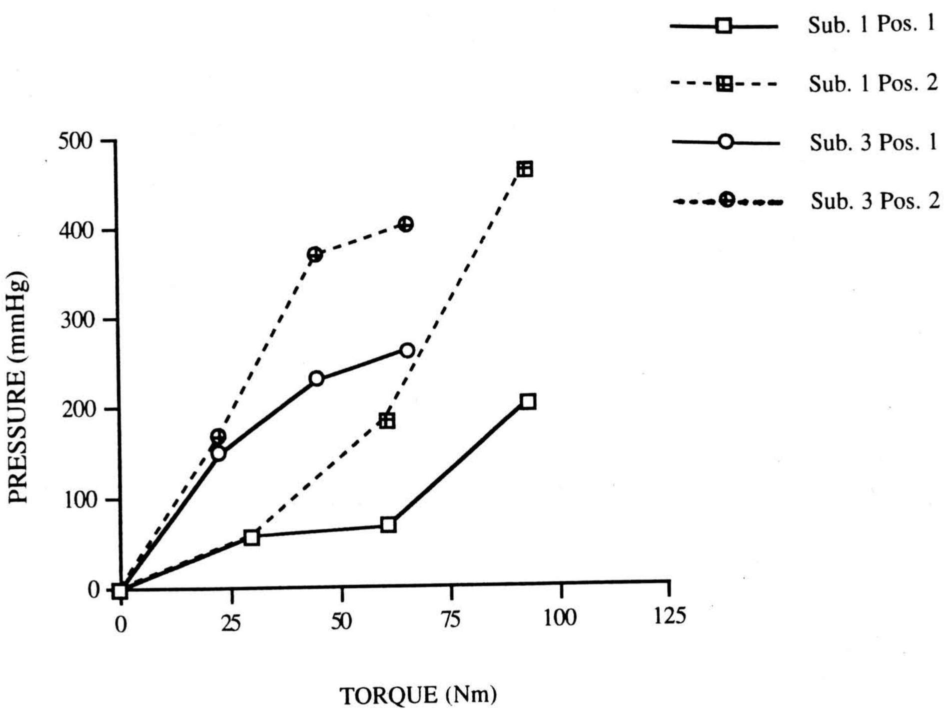
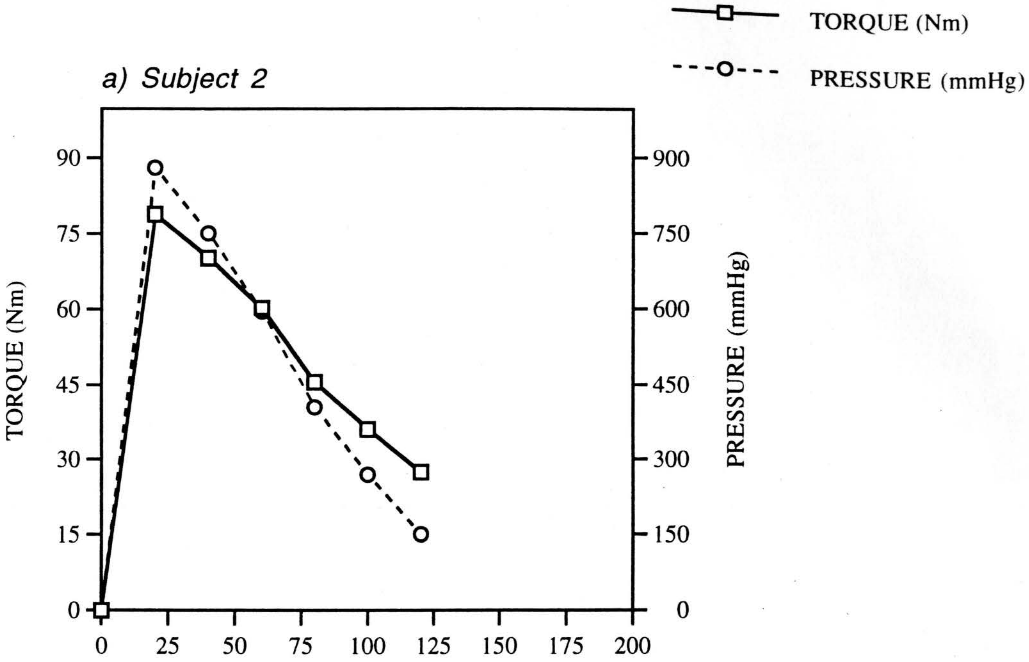


Figure D2: Intramuscular pressures produced at 2 sites at 20%, 40%, & 60% MVC (n=2)

Reliability

Each contraction was performed three times at each catheter position. It was necessary to determine if the pressure recordings were reliable at a given position as well as among trials at different positions. An analysis of variance with repeated measures was performed and it was found that the measures were reliable among trials at the same recording position as well as among trials at the different positions. The measurements at the two catheter positions could therefore be compared.


a) Subject 2

b) Subject 3

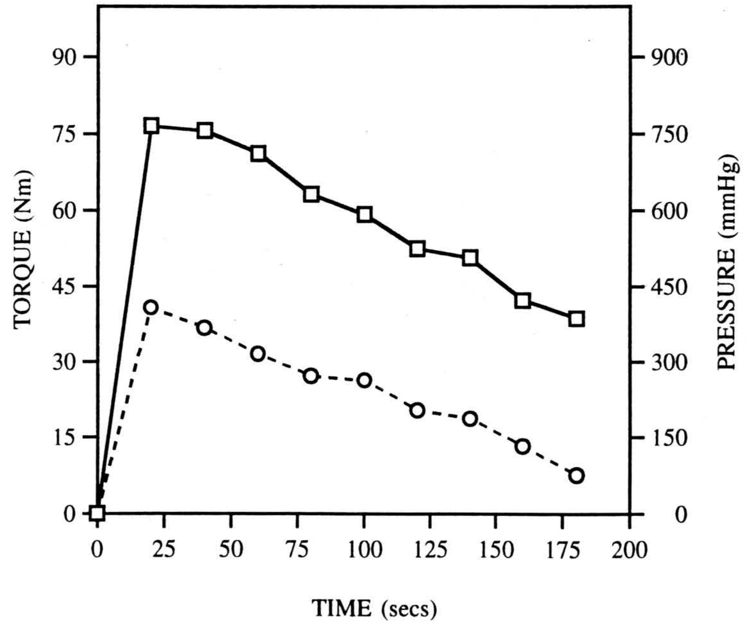


Figure D3 (a,b): Torque and intramuscular pressure during a 70% MVC fatiguing contraction

APPENDIX E

Appendix E - Pilot Study
EMG Recorded at Different Muscle Depths

Needle electrodes are designed to record the recruitment patterns of a relatively small population of motor units. Small movements in the fine recording tip may result in a new population of motor units being sampled. It was therefore important that the depth of the electrodes be controlled during the studies. This was particularly important because muscular contraction causes an increase in intramuscular pressure (IMP), which can, during stronger effort, cause the needle to be pushed out of the muscle, or moved to a new position. It was necessary to determine the effect of small depth changes on motor unit recruitment at different sites. Variability of needle EMG data has previously been found when recording from different depths of the same muscle (Preece et al. 1994). It was observed that changing the position of the needle tip within some muscles may give variable results, possibly due to anatomical and architectural variations in the organization, as well as possible fiber type variations of certain muscles.

A preliminary study, presented below, determined the effects of needle electrode depth on motor unit recruitment patterns.

Methods

Experiments were conducted on the soleus muscles of three healthy female volunteers, aged aged 22-24 years.

The procedure for measuring plantarflexion torque, electromyography techniques, the data recording system, and analysis were identical to those used in the primary experiment and have been previously described in the Methods section.

Protocol

The same three sites of the soleus muscle were examined as in the primary experiment (two lateral sites and one medial). With the needle electrodes at specific measured depths in the muscle, motor unit recruitment (EMG) was recorded at three sites during the following tests. Subjects performed an isometric contraction at 20% MVC for one minute. A line representing the required torque was visible on a digital oscilloscope. The amplifier of the oscilloscope was set at a very sensitive level so that even slight deviations in the target torque were visible to the subject and the examiner. When the target torque was achieved, the computer recording of the EMG signal was started. If the torque level was not accurately maintained, the data was discarded. Three trials were recorded at each torque level to assess reliability of the measurements. The needle electrodes were then moved approximately 0.3-0.5 centimeters deeper into the muscle. The same protocol was repeated with the electrode tip at the new depth.

Statistics

The data was analyzed using two and four way analyses of variance (ANOVA) with repeated measures. A SAS statistical package was used to compute these statistics.

Results

Reliability

A two way analysis of variance was run to determine if the measurements of numbers of turns were reliable between trials. There was no significant difference ($p>0.05$) in these values, confirming reliability of the measurements.

Depth

Two needle electrode depths were examined at three sites in the muscles of three subjects to determine the effects of small depth changes on recruitment at different sites. A four way analysis of variance was performed to determine if there was a difference between the two measured electrode depths during a one minute contraction. At each of the three sites, there were no significant differences ($p>0.05$) between the two needle depths throughout the one minute contraction. This would suggest that if the needle moved slight amounts within the muscle, then it would not significantly alter the signal coming from the muscle. However, the large variability among the three subjects must be considered (Fig. E1). It is therefore possible that if such variability did not exist, there would indeed be differences between the recordings at the different muscle depths. It can be seen in Figure E1, that there does appear to be differences in these recordings. Therefore, in the primary experiment, every effort was made to stabilize the electrodes during muscle contractions.

20% MVC
Turn Counts at Two Muscle Depths

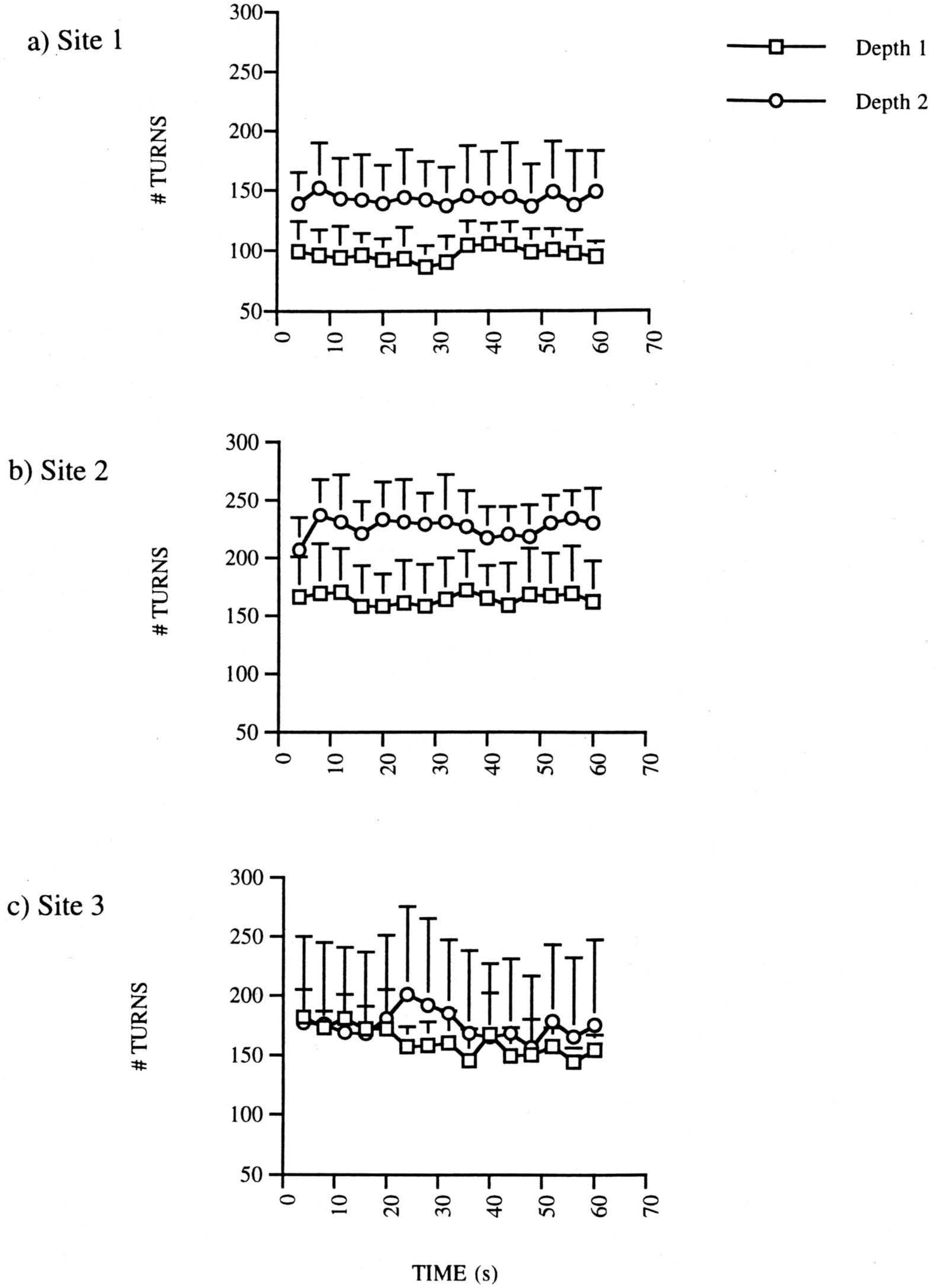


Figure E1 (a,b,c): Mean turn counts at two muscle depths during a 20% MVC 1-minute contraction (n=3).

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