DOCTOR OF PHILOSOPHY

Neural and mechanical factors associated with exercise-induced muscle damage and the repeated bout effect.

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NEURAL AND MECHANICAL FACTORS ASSOCIATED WITH EXERCISE-INDUCED MUSCLE DAMAGE AND THE REPEATED BOUT EFFECT

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CONTENTS

SUMMARY

Unfamiliar, predominantly eccentric exercise frequently results in muscle damage. Following recovery, a repeated bout of the same exercise results in minimal symptoms of damage. This has been referred to as the "repeated bout effect" and a specific mechanism has not been identified. Possible neural and mechanical factors associated with symptoms of exercise-induced muscle damage and the repeated bout effect were examined. Surface electromyographic (EMG) signals were recorded from the hamstring muscles during two bouts of sub-maximal isokinetic eccentric (11 men, 9 women) or concentric (6 men, 4 women) contractions separated by two weeks. EMG/torque and median frequency (MF) were analysed. Hamstring muscle stiffness, estimated from torque/ROM curves during passive stretch, was measured prior to both the initial and repeated exercise bouts and on each of the subsequent three days. The initial bout of eccentric exercise resulted in strength loss, pain, muscle tenderness and elevated plasma CK activity while the repeated eccentric bout resulted in a strength gain, minimal pain, no muscle tenderness and minimal plasma CK elevation. Minimal symptoms were seen following either bouts of concentric exercise. EMG analyses suggested that a small number of primarily fast-twitch motor units were recruited for eccentric exercise. This recruitment pattern was similar between eccentric bouts despite the fact that the initial bout resulted in symptoms of muscle damage while the repeated bout did not. Symptoms were greater in subjects with stiffer muscles but no change in passive stiffness occurred between the repeated bouts. Greater symptoms in stiffer muscles are attributed to the inability of the tendon-aponeurosis complex to absorb the strain imposed by

eccentric contractions. However, the repeated bout effect could not be explained by an adaptation in passive stiffness. Future studies on possible mechanisms for the repeated bout effect should address adaptations which might limit sarcomere strain such as increased myofibrillar cross-bridge stiffness.

LIST OF ABBREVIATIONS

ANCOVA=analysis of covariance
BF=biceps femoris
CC=contractile component
CK=creatine kinase
CSA=cross-sectional area
CV=coefficient of variation
CV=conduction velocity
Df=degrees of freedom
DOMS=delayed onset muscle soreness
E-C coupling=excitation-contraction coupling
EMG=electromyographic
FFT=Fast Fourier Transform
GG=Greenhouse-Geisser corrections
GOT=glutamic oxaloacetic transaminase
ICC=intraclass correlation coefficients
iEMG=integrated electromyographic activity
LDH=lactate dehydrogenase
MF=median frequency
MPF=mean power frequency
MRI=magnetic resonance imaging
MVC=maximum voluntary contraction
PEC=parallel elastic component
RMS=root mean square
ROM=range of motion
SEC=series elastic component
SLR=straight leg raise
SM=semimembranosus
ST=semitendinosus

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CHAPTER 1

INTRODUCTION

1.1 TERMINOLOGY

The terms "eccentric contraction" (Armstrong *et al.*, 1991; Friden and Lieber, 1992), "pliometric contraction" (Hunter and Faulkner, 1997), "lengthening contraction" (McCully and Faulkner, 1986),"eccentric activation" (Eston *et al.*, 1995) and "eccentric action" (Clarkson *et al.*, 1992) have been used synonymously to describe what happens when the force generated by an active muscle is less than the opposing load. In agreement with the position adopted by the American College of Sports Medicine the term "eccentric contraction" is used here (Raven, 1991). Exercise which primarily involves eccentric contractions frequently results in damage to individual muscle fibres. This condition has been referred to as exercise-induced muscle damage (Armstrong *et al.*, 1991; Clarkson *et al.*, 1992), contraction-induced muscle injury (Faulkner *et al.*, 1993) and delayed onset muscle soreness (DOMS) (Armstrong, 1984; Cleak and Eston, 1992a). It has been described in humans with downhill running (Byrnes *et al.*, 1985; Pierrynowski *et al.*, 1987; Maughan *et al.*, 1989; Westerlind *et al.*, 1992; Eston *et al.*, 1995; Eston *et al.*, 1996a; Eston *et al.*,1996b), eccentric cycling (Fridén *et al.*, 1983a; Fridén *et al.*, 1983b; Fridén, 1984), stepping exercise (Newham *et al.*, 1983a; Newham *et al.*, 1983b; Gleeson *et al.*, 1995a; Gleeson *et al.*, 1995b; Gleeson *et al.*, 1998) and voluntary eccentric contractions of isolated muscle groups (Clarkson, 1987; Jones *et al.*, 1987; Clarkson *et al.*, 1992; Cleak and Eston, 1992b; Golden and Dudley, 1992; Mair *et al.*, 1995; Brown *et al.*, 1997a; Brown *et al.*, 1997b). The condition has also been observed in animal models with downhill running (Armstrong *et al.*, 1983; Schwane and Armstrong, 1983) and forced lengthening of stimulated whole muscle (McCully and

Faulkner, 1986; Lieber and Fridén 1993; Wood *et al.*, 1993) and single fibre preparations (Macpherson *et al.*, 1996). Although various terms have been used to describe this condition, in humans it is most commonly referred to as exercise-induced muscle damage, even when damage is measured indirectly (Clarkson *et al.*, 1987; Clarkson and Tremblay, 1988; Balnave and Thompson, 1993; Mair *et al.*, 1995; Nosaka and Clarkson, 1995; Brown *et al.*, 1997a; Brown *et al.*, 1997b; Nosaka and Clarkson, 1997). The term DOMS has also been used to describe the symptoms in humans in the absence of direct evidence of muscle damage (Byrnes *et al.*, 1985; Pierrynowski *et al.*, 1987; Eston *et al.*, 1996a). However, this term refers to only one of several indirect measures of muscle damage i.e. soreness.

1.2 INDICES OF MUSCLE DAMAGE

Exercise-induced muscle damage can be observed in electron micrographs from needle biopsies of muscles which have performed eccentric exercise. This technique has been used in both animals (Lieber *et al.*, 1991; Wood *et al.*, 1993; Brooks *et al.*, 1995; Jones *et al.*, 1997) and humans (Fridén *et al.*, 1983a; Fridén *et al.*, 1983b; Fridén, 1984; O'Reilly *et al.*, 1987; Manfredi *et al.*, 1991; Hortobágyi *et al.*, 1998). However, damage is more commonly described in the absence of electron micrographic evidence according to various symptoms which include strength loss, pain, muscle tenderness, stiffness, swelling and elevated levels of specific muscle enzymes and proteins.

1.2.1 Electron Micrographs of Muscle Biopsies

Electron micrographs of biopsy samples from muscles at various time intervals following eccentric exercise have identified several specific characteristics of damage:

a) Damage involves Z bands streaming and loss of registration of Z bands in parallel myofibrils (Fridén and Lieber, 1992);

b) Myofibrillar disruption is typically distributed sporadically throughout a muscle fibre (Lieber *et al.*, 1991; Jones *et al.*, 1997);

c) Z band streaming is usually limited to a couple of parallel and serial sarcomeres (Lieber *et al.*, 1991; Jones *et al.*, 1997);

d) Disruption rarely extends along the complete width of a fibre and never extends the entire length of the myofibril (Lieber *et al.*, 1991; Jones *et al.*, 1997);

e) Z band abnormalities are three to four times more prevalent in fast twitch compared to slow twitch fibres (Fridén *et al.*, 1983b; Fridén, 1984); and

f) Myofibrillar disruption occurs with eccentric contractions but not with equivalent passive stretches or isometric contractions (Lieber *et al.*, 1991; Wood *et al.*, 1993).

Electron micrographs have also been used to quantify damage and repair. Three days following 30 minutes of eccentric cycling, 20% of micrographs from vastus lateralis biopsies showed Z band streaming (Fridén *et al.*, 1983b). Two days following eccentric isotonic quadriceps contractions, 65% of micrographs from vastus lateralis biopsies showed evidence of damage (Hortobágyi *et al.*, 1998). Similarly, damage was seen in 63% of micrographs immediately following eccentic contractions of isolated toad sartorius muscles (Wood *et al.*,

1993). However, quantification of the true extent of damage from electron micrographs of muscle biopsies is somewhat limited since biopsies represent only a small portion of the involved muscle.

1.2.2 Strength Loss

Strength loss can occur immediately following prolonged exercise involving eccentric, concentric or isometric contractions. This acute loss of force-generating capacity represents muscle fatigue (Enoka, 1992). However, strength loss on days following an exercise bout is indicative of muscle damage and is usually specific to activities involving eccentric contractions (Clarkson and Newham, 1995). It is typically documented using voluntary isometric contractions (Clarkson and Tremblay, 1988; Ebbeling and Clarkson, 1990; Cleak and Eston, 1992b; Howell *et al.*, 1993; Chleboun *et al.*, 1995; Nosaka and Clarkson, 1995, Teague and Schwane, 1995; Saxton and Donnelly, 1996; Nosaka and Clarkson, 1997; Child *et al.*, 1998) but has also been documented using voluntary isotonic and isokinetic contractions (Golden and Dudley, 1992; Eston *et al.*, 1996a; Ploutz-Snyder *et al.*, 1996; Hortobágyi *et al.*, 1998). In the elbow flexors (Clarkson and Tremblay, 1988; Ebbeling and Clarkson, 1990; Cleak and Eston 1992b; Howell *et al.*, 1993; Chleboun *et al.*, 1995; Nosaka and Clarkson 1995; Teague and Schwane 1995; Saxton and Donnelly 1996; Nosaka and Clarkson 1997) and knee extensors (Fridén *et al.*, 1983b; Golden and Dudley, 1992; Eston *et al.*, 1996a; Ploutz-Snyder et al 1996; Child *et al.*, 1998; Hortobágyi *et al.*, 1998) strength loss was greatest either immediately post-exercise or within the subsequent 48 hours, and took more than five days to return to baseline. Knee extension strength loss was similar with eccentric

compared to concentric contractions (Golden and Dudley, 1992; Ploutz-Snyder *et al.*, 1996; Hortobágyi *et al.*, 1998) and also similar between isometric, isotonic and isokinetic contractions (Golden and Dudley, 1992; Hortobágyi *et al.*, 1998). Isometric strength loss immediately following eccentric exercise has been shown to be length dependent, with greater strength loss at short versus long muscle lengths (Saxton *et al.*, 1996; Child *et al.*, 1998). These effects were attributed to a shift to the right in the length-tension curve following eccentric exercise.

Strength loss has also been examined with low and high frequency electrical muscle stimulation (Newham *et al.*, 1983b; Newham *et al.*, 1988; Brown *et al.*, 1997a; Brown *et al.*, 1997b). Eccentric exercise is associated with a disproportionate loss of force with low versus high frequency stimulation (low frequency fatigue). Low frequency fatigue following eccentric exercise is thought to be due to excitation-contraction coupling failure and a shift to the right in the force-frequency curve secondary to shortening of intact sarcomeres (Newham, 1996). Peak decrements occurred immediately post-exercise and returned to normal prior to return of voluntary strength in the knee extensors (Brown *et al.*, 1997a; Brown *et al.*, 1997b) and at the same rate as voluntary strength in the elbow flexors (Newham *et al.*, 1983b; Newham *et al.*, 1988).

In animal models, strength loss has been demonstrated using stimulation to the peripheral nerve (McCully and Faulkner, 1986; Lieber *et al.*, 1991; Lieber *et al.*, 1993; Wood *et al.*, 1993; Brooks *et al.*, 1995; Hunter and Faulkner, 1997; Lapier *et al.*, 1995), direct stimulation to isolated muscles (Warren *et al.*, 1993) and by immersing single skinned muscle fibre segments in calcium activating solutions (Macpherson *et al.*, 1996). With these experimental techniques, strength measurements are typically made immediately following the eccentric

contraction protocol but not on subsequent days. However, McCully and Faulkner (1986) documented strength loss in mice extensor digitorum longus muscles three days following a series of eccentric contractions. The distal tendon was detached for imposing eccentric contractions and subsequently reattached. Although the experimental procedure resulted in reduced strength (approximately 25%) greater strength loss was seen in muscles which also performed eccentric contractions (30-50%).

1.2.3 Pain

The discomfort experienced on the days following a bout of unfamiliar eccentric exercise has been assessed by visual analog pain scales (Clarkson *et al.*, 1986; and Eston, 1992b; Teague and Schwane, 1995; Nosaka and Clarkson, 1997), pain questionnaires (Byrnes *et al.*, 1985; Clarkson *et al.*, 1987; Jones *et al.*, 1987; Pierrynowski *et al.*, 1987; Clarkson and Tremblay, 1988; Ebbeling and Clarkson, 1990; Balnave and Thompson, 1993; Howell *et al.*, 1993; Chleboun *et al.*, 1995; Gleeson *et al.*, 1995a; Gleeson *et al.*, 1995b; MacIntyre *et al.*, 1996; Saxton and Donnelly, 1996; Gleeson *et al.*, 1998; Hortobágyi *et al.*, 1998) and subjective assessment of pain with palpation (Nosaka and Clarkson, 1995; Brown *et al.*, 1997a; Brown *et al.*, 1997b Child *et al.*, 1998). Peak pain was experienced one to two days following eccentric knee extension contractions (Brown *et al.*, 1997a, Brown *et al.*, 1997b; Child *et al.*, 1998; Hortobágyi *et al.*, 1998) and two to three days following downhill running (Pierrynowski *et al.*, 1987) and eccentric exercise of the elbow flexors (Clarkson and Tremblay, 1988; Ebbeling and Clarkson, 1990; Cleak and Eston, 1992b; Howell *et al.*, 1993; Chleboun *et al.*, 1995; Nosaka and Clarkson, 1995; Saxton and Donnelly, 1996;

Nosaka and Clarkson, 1997). In most studies it took approximately one week for pain to subside.

1.2.4 Muscle Tenderness

Muscle tenderness has been documented objectively by recording the force required to elicit pain by applying direct pressure to the muscle with a probe instrumented with a force transducer (Newham *et al.*, 1983b; Jones *et al.*, 1987; Newham *et al.*, 1987; Newham *et al.*, 1988; Cleak and Eston, 1992b; Teague and Schwane, 1995; Edwards *et al.*, 1996; Eston *et al.*, 1996a; Baker *et al.*, 1997; Eston and Peters, 1999). A tenderness value is recorded if the subject reports discomfort at an applied force level below a previously determined maximum (30 or 40 N). Tenderness measurements are typically made at proximal, mid-portion and distal sites along the target muscle or muscle group. Greater tenderness has been shown in the distal portions (Newham *et al.*, 1983b; Cleak and Eston, 1992b) but high variability between sites has been demonstrated (Baker *et al.*, 1997). Tenderness typically peaks two to three days following eccentric exercise returning to baseline within three to seven days. Similar results were seen in the quadriceps following stepping (Newham *et al.*, 1983b) or downhill running (Eston *et al.*, 1996a; Baker *et al.*, 1997) and in the elbow flexors following eccentric isotonic contractions (Newham *et al.*, 1988; Cleak and Eston, 1992b) and eccentric isokinetic contractions (Eston and Peters, 1999).

1.2.5 Stiffness and Swelling

Stiffness and swelling following eccentric exercise have been assessed by changes in joint range of motion (ROM) without pain (Howell *et al.*, 1985; Clarkson and Tremblay, 1988; Ebbeling and Clarkson, 1990; Cleak and Eston, 1992b; Chleboun *et al.*, 1995; Nosaka and Clarkson, 1995; Teague and Schwane, 1995; Saxton and Donnelly, 1996; Nosaka and Clarkson 1997; Eston and Peters, 1999), increase in limb circumference (Cleak and Eston, 1992b; Howell *et al.*, 1993; Chleboun *et al.*, 1995; Nosaka and Clarkson, 1995; Teague and Schwane, 1995; Saxton and Donnelly, 1996; Eston and Peters, 1999), increase in passive muscle stiffness (Howell *et al.*, 1993; Chleboun *et al.*, 1995), magnetic resonance imaging (MRI) (Sorichter *et al.*, 1995; Nosaka and Clarkson, 1996), ultrasonography (Howell *et al.*, 1993; Nosaka and Clarkson, 1995; Nosaka and Clarkson, 1996) and cellular markers of inflammation (Gleeson *et al.*, 1995a; Gleeson *et al.*, 1995b; Pizza *et al.*, 1996). Elbow ROM has been assessed by the decrease in relaxed flexion angle and increase in maximum active flexion angle (maximum flexion angle being with the elbow in full extension i.e. 180°). The decrease in relaxed flexion angle peaks two to four days following eccentric exercise and returns to baseline by the fifth to tenth day (Clarkson and Tremblay, 1988; Ebbeling and Clarkson, 1990; Cleak and Eston, 1992b; Chleboun *et al.*, 1995; Saxton and Donnelly, 1996; Nosaka and Clarkson, 1997). The increase in maximum active flexion angle peaks immediately post-exercise and returns to baseline approximately five days post-exercise (Clarkson and Tremblay, 1988; Ebbeling and Clarkson, 1990; Nosaka and Clarkson, 1997). Circumferential measurements following eccentric exercise of the elbow flexors show peak increases four days post-exercise with full recovery by 10 days post-exercise (Cleak and Eston, 1992b; Howell *et al.*, 1993; Chleboun *et al.*, 1995; Saxton and

Donnelly, 1996). Passive stiffness of the elbow flexors following eccentric exercise has been measured according to the torque/ROM relationship during passive stretch (Howell *et al.*, 1993; Chleboun *et al.*, 1995). Elbow flexor stiffness was increased by more than 100% immediately post-exercise remaining significantly elevated for up to four days (Howell *et al.*, 1993).

MRI and ultrasonography have been used to document muscle oedema in the elbow flexors (Howell *et al.*, 1993; Nosaka and Clarkson, 1995; Nosaka and Clarkson, 1996) and knee extensors (Sorichter *et al.*, 1995) following eccentric exercise. Ultrasound estimates of muscle cross-sectional area have shown swelling in the elbow flexors to peak three to four days post-exercise (Howell *et al.*, 1993; Nosaka and Clarkson, 1996) and remain swollen up to 10 days post-exercise (Howell *et al.*, 1993). The increase in MRI T_2 weighted relaxation times has been used to indicate oedema. Relaxation times peaked three to six days following eccentric exercise (Nosaka and Clarkson, 1996; Sorichter *et al.*, 1995) and remained increased up to 31 days post-exercise (Sorichter *et al.*, 1995).

Immunological markers of inflammation have also been documented following eccentric exercise (Gleeson *et al.*, 1995a; Gleeson *et al.*, 1995b; Pizza *et al.*, 1996). Circulating neutrophils increased up to 12 hours following eccentric exercise of the elbow flexors returning to baseline by three days (Pizza *et al.*, 1996). Following stepping exercise, neutrophils were elevated four hours post-exercise, falling below baseline by three days and gradually returning to baseline thereafter (Gleeson *et al.*, 1995a). Despite an acute inflammatory response in the first 24 hours following eccentric exercise, a chronic inflammatory condition does not ensue (Gleeson *et al.*, 1995a).

1.2.6 Elevated Muscle Enzymes and Proteins in the Circulation

Measurements of muscle enzymes and proteins released into the circulation following exercise have been used as indirect indicators of damage. Elevations in plasma creatine kinase (CK) activity following eccentric exercise have been demonstrated in numerous studies in both humans (Newham *et al.*, 1983a; Byrnes *et al.*, 1985; *et al.*, 1986; Clarkson *et al.*, 1987; Newham *et al.*, 1987; Clarkson and Tremblay, 1988; Maughan *et al.*, 1989; Ebbeling and Clarkson, 1990; Manfredi *et al.*, 1991; Westerlind *et al.*, 1992; Balnave and Thompson, 1993; Westerlind *et al.*, 1994; Gleeson *et al.*, 1995a; Gleeson *et al.*, 1995b; Mair *et al.*, 1995; Nosaka and Clarkson, 1995; Sorichter *et al.*, 1995; Eston *et al.*, 1996a; Pizza *et al.*, 1996; Saxton and Donnelly, 1996; Brown *et al.*, 1997a; Brown *et al.*, 1997b; Nosaka and Clarkson, 1997; Child *et al.*, 1998; Hortobágyi *et al.*, 1998; Eston and Peters, 1999) and animals (Armstrong *et al.*, 1983; Schwane and Armstrong, 1983; Van Der Meulen *et al.*, 1991). Elevations in other muscle enzymes such as lactate dehydrogenase (LDH) and glutamic oxaloacetic transaminase (GOT) have also been demonstrated in both humans (Nosaka and Clarkson, 1995; Brown *et al.*, 1997b) and animals (Highman and Altland, 1963; Armstrong *et al.*, 1983; Van Der Meulen *et al.*, 1991). Additionally, elevated levels of muscle proteins such as myoglobin (Byrnes *et al.*, 1985; Balnave and Thompson, 1993; Pizza *et al.*, 1996) and myosin heavy chain fragments (Mair *et al.*, 1995) have been measured in humans following eccentric exercise.

Serum or plasma CK activity has been shown to peak one to two days following downhill running, returning to baseline within three to seven days (Armstrong *et al.*, 1983; Byrnes *et al.*, 1985; Maughan *et al.*, 1989; Balnave and

Thompson, 1993; Eston *et al.*, 1996a). In contrast, peak CK elevations occurred two to six days following eccentric contractions of the elbow flexors (Newham *et al.*, 1987; Clarkson and Tremblay, 1988; Ebbeling and Clarkson, 1990; Nosaka and Clarkson, 1995; Pizza *et al.*, 1996; Saxton and Donnelly, 1996; Nosaka and Clarkson, 1997) and knee extensors (Sorichter *et al.*, 1995; Eston *et al.*, 1996a; Brown *et al.*, 1997a; Brown *et al.*, 1997b; Child *et al.*, 1998; Hortobágyi *et al.*, 1998) returning to baseline within seven to 12 days. Serum myoglobin levels peaked and returned to baseline earlier than CK (Byrnes *et al.*, 1985; Balnave and Thompson, 1993; Pizza *et al.*, 1996).

1.2.7 Summary of Indices of Muscle Damage

Numerous measurements have been developed to directly or indirectly measure muscle damage following eccentric exercise. These indices of damage have been extensively studied in both humans and animals. However, the vast majority of data from humans is limited to the elbow flexors and knee extensors. It is not known if other muscle groups respond similarly.

1.3 NEURAL CONTROL OF MUSCLE FUNCTION

Voluntary and reflex neural control of muscle contractions is dependent on alterations in the number, type and firing rate of the activated motor units. For graded contractions, low threshold motor units comprising slow-twitch muscle fibres are recruited initially at relatively low firing rates. As contraction intensity increases to a maximum, the firing rates of previously active units increases and

higher threshold units comprising fast-twitch fibres are activated (Bigland and Lippold, 1954a; Moritano and Muro, 1987). This neural control of contraction can be measured from an electromyogram recorded from indwelling or surface electrodes. These techniques have been used to demonstrate significant differences in neural control between concentric and eccentric contractions (Enoka, 1996).

1.3.1 Surface Electromyography

Surface electromyography (EMG) refers to the measurement of electrical activity from electrodes placed on the skin covering the target muscle. The raw EMG signal (Fig. 1.1a) represents the sum of a large number of overlapping motor unit action potentials (Hagg, 1992). The amplitude of the raw EMG signal is typically quantified by integrating the area under the rectified signal for a given time constant (iEMG; Fig. 1.1b) (Komi *et al.*, 1987). The frequency content of the raw EMG signal can be estimated by applying a Fast Fourier Transform (FFT) algorithm (Hagg, 1992). The shape of the resultant frequency distribution (power spectrum) reflects the size, shape and frequency characteristics of the dominant action potentials (Fig. 1.1c). The two most common measures of EMG frequency are mean frequency (MPF) and median frequency (MF). MPF is calculated from a weighted sum of the individual points in the power spectrum while MF is the frequency that divides the power spectrum into two equal parts (Fig. 1.1c) (Hagg, 1992).

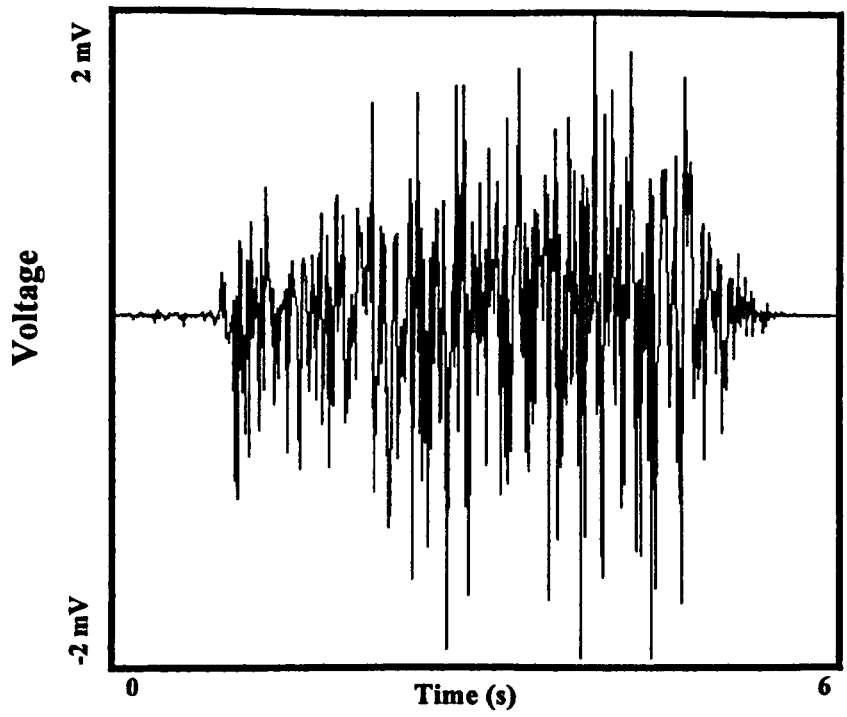

Figure 1.1a: Raw surface EMG signal from the semitendinosus muscle during a five second isometric contraction of the hamstrings at 45° of knee flexion.

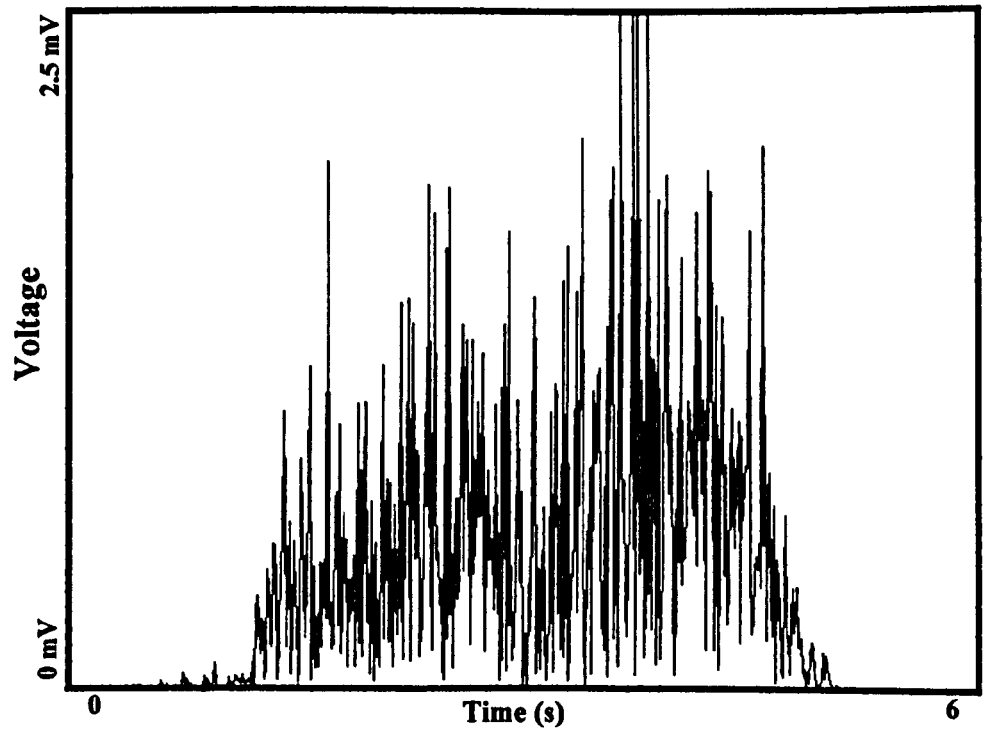

Figure 1.1b: Full-wave rectified surface EMG signal from the semitendinosus contraction shown in Figure 1.1a.

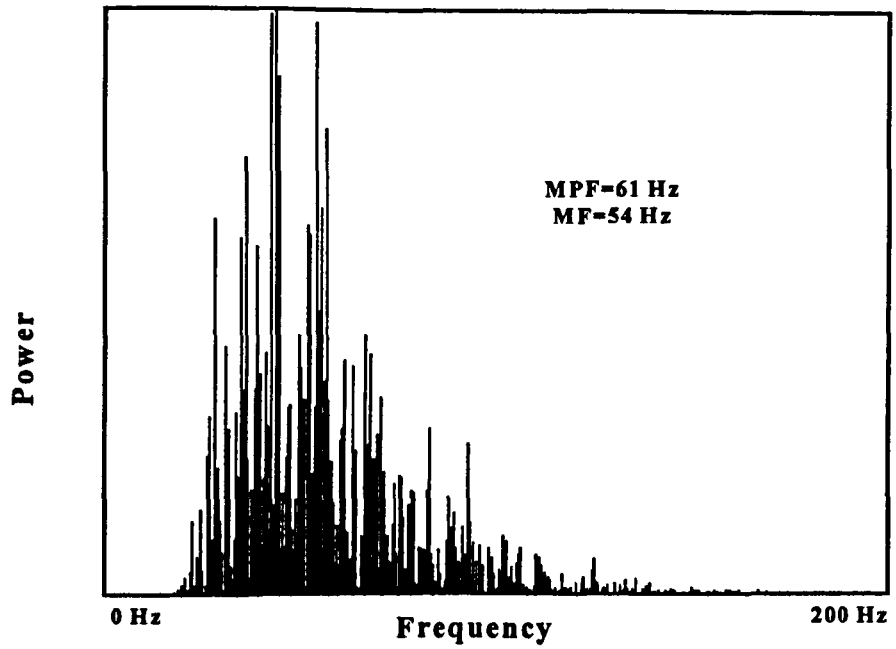

Figure 1.1c: Power spectrum from the raw EMG signal shown in Figure 1.1a. EMG signal was sampled from 0-500 Hz (0-200 Hz shown here).

1.3.2 Amplitude of the EMG Signal

EMG measurements can provide an indirect measure of the extent of motor unit activation and the type of motor units being recruited. EMG amplitude has been shown to be linearly related to the force generated by the muscle (Bigland and Lippold, 1954b; Adams *et al.*, 1992). The increase in iEMG with increasing contraction intensity primarily reflects additional recruitment of motor units with some contribution from an increasing discharge rate of active units. Numerous studies have demonstrated that EMG amplitude is approximately 50% lower for eccentric compared to concentric contractions at the same force level (Bigland and Lippold, 1954b; Komi *et al.*, 1987; Moritani *et al.*, 1988; Adams *et al.*, 1992; Potvin, 1997). These findings reflect reduced motor unit activation required for the more mechanically efficient eccentric contractions (Komi *et al.*, 1987).

Strength gains are generally associated with increased iEMG during the initial phase of strength training as motor unit recruitment improves (Sale, 1988). In the later phases, when hypertrophic effects predominate, iEMG changes are minimal. Greater initial increases in iEMG with eccentric compared to concentric strength training have been attributed to increased motor unit recruitment (Komi and Buskirk, 1978; Hortobágyi *et al.*, 1996a; Hortobágyi *et al.*, 1996b).

1.3.3 Frequency Content of the EMG Signal

MF primarily reflects muscle fibre conduction velocity (CV) (Kupa *et al.*, 1995; Kamen and Caldwell, 1996). Conduction velocity refers to the speed of propagation of the action potential along the muscle fibre (Kamen and Caldwell, 1996). Action potentials propagate rapidly along fast-twitch muscle fibres. Hence muscles with a high proportion of fast-twitch fibres tend to have higher MF (Kupa *et al.*, 1995). Other physiological factors such as muscle temperature and fatigue will decrease conduction velocity with concomitant affects on MF (Kamen and Caldwell, 1996). However, technical factors such as inter-electrode spacing and electrode configuration can affect the measurement of MF (Kamen and Caldwell, 1996). As the motor unit action potential travels along the muscle fibre it will de detected sequentially by each of the electrodes. Decreasing the inter-electrode distance will decrease the detection time between electrodes resulting in a higher measured frequency. Similarly, if the electrodes are not aligned in parallel to the underlying muscle fibres frequency measures will be inaccurate with respect to conduction velocity.

MF and MPF increased moderately with increasing isometric quadriceps contraction intensity from 40-100% MVC (Gerdle *et al.*, 1991). This effect is thought to be a function of recruitment of higher threshold motor units rather than an increase in discharge rate of active units (Solomonow *et al.*, 1990). MF and MPF typically decrease during repeated (Tesch *et al.*, 1990) or sustained contractions (Hagg, 1992) at a fixed intensity. Decreased frequency with sustained contractions provides an electromyographic indicator of muscle fatigue and is thought to primarily reflect decreasing conduction velocity (Moritani *et al.*, 1982; Hagg, 1992). Muscles with a higher proportion of fast-twitch fibres have a higher initial frequency but experience a greater decline in frequency with sustained or repeated contractions (Komi and Tesch, 1979; Moritani *et al.*, 1982; Kupa *et al.*, 1995).

1.4 MECHANICAL BEHAVIOUR OF SKELETAL MUSCLE

1.4.1 Mechanical Model of Skeletal Muscle

Tension generated by skeletal muscle has been modeled as two parallel components, a contractile component (CC) and a parallel elastic component (PEC), in series with the tendon-aponeurosis complex (Lieber, *et al.* 1992) (Fig. 1.2).

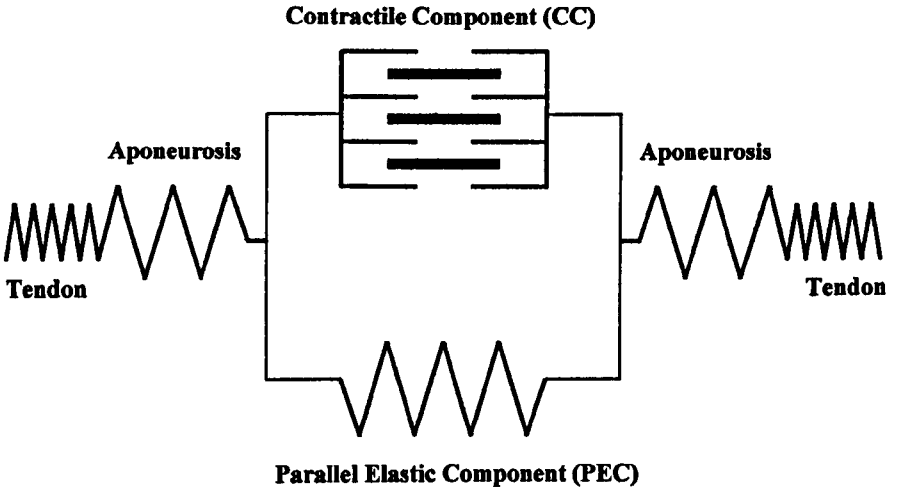

Figure 1.2: Mechanical model of skeletal muscle adapted from Lieber, *et al.* 1992.

The CC represents the active tension generated by the sarcomeres with contraction and the PEC represents the passive tension generated by the connective tissue elements in parallel with the muscle fibres. Tension generated

by the CC and PEC is transferred through the aponeurosis to the tendon and subsequently to the joint via the bone-tendon junction. A portion of the forces generated in either the CC, with contraction, or the PEC, with stretch, is absorbed by tendon and aponeurosis compliance (Griffiths, 1991, Lieber, *et al.* 1992). The tendon and aponeurosis are thought to act as mechanical buffers to CC or PEC force production (Griffiths, 1991).

The mechanical behaviour of skeletal muscle has been studied in both relaxed and contracted conditions. The change in tension with quick release (Pousson, *et al.* 1990) or quick stretch (Morgan, 1977) of isometrically contracted muscles has been referred to as active stiffness or stiffness in the series elastic component (SEC). These measures are thought to primarily reflect crossbridge stiffness with some contribution from tendon compliance (Morgan, 1977; Pousson, *et al.* 1990)). Eccentric strength training has been shown to increase stiffness in the SEC (Pousson, *et al.* 1990), an effect which was attributed to an increase in tendon stiffness.

The passive stiffness of skeletal muscle has been measured *in vitro* according to the length-tension relationship during passive elongation of the isolated muscle tendon units in rats (Kovanen, *et al.* 1984). Stiffness measurements were corrected for the cross-sectional area to give the elastic modulus. Differences in the elastic modulus between muscles were attributed to the collagen content of the connective tissue in parallel with the muscle fibres. Although passive stiffness in skeletal muscle is thought to reflect tension in the PEC, contributions from the aponeurosis cannot be excluded (Zuurbier, *et al.* 1994).

1.4.2 Viscoelastic Properties of Muscle *In Vivo*

The mechanical behaviour of relaxed skeletal muscle is a function of the tissue's viscoelastic properties. The term viscoelastic describes a tissue's loading response showing a combination of viscous and elastic properties (Fung, 1981). Viscoelasticity is classically described by mechanical models using linear springs (elasticity) in series or in parallel with dashpots (viscosity). In these models elastic deformation is instantaneous and proportional to load, and viscous deformation is proportional to the velocity of load application. Stiffness, hysteresis, stress relaxation and creep are viscoelastic properties of biological tissues.

Stiffness refers to the slope of the load-elongation curve during passive stretch. Passive muscle stiffness primarily reflects the tissue's elastic properties with increased viscous contribution at high rates of stretch. The load-elongation curve for unloading a passive stretch does not follow the same pattern as the loading response, with lower muscle tension at any given muscle length. This loss of energy during release of stretch is referred to as hysteresis and reflects the viscous component (Best *et al.*, 1994). In humans, passive stiffness and hysteresis have been quantified during passive stretch of skeletal muscle according to the relationship of change in ROM to change in torque, in the absence of EMG activity (Fig. 1.3) (Hufschmidt and Mauritz, 1985; Watts *et al.*, 1986; Weigner *et al.*, 1986; Gajdosik *et al.*, 1988; Gajdosik, 1991; Halbertsma *et al.*, 1994; Magnusson *et al.*, 1995; Halbertsma *et al.*, 1996; Klinge *et al.*, 1997; Magnusson *et al.*, 1997; McHugh *et al.*, 1998).

When a tissue under tensile stress is held at a fixed length the tensile stress will decrease over time (Fig. 1.4). This is referred to as stress relaxation

and reflects the viscous properties of the muscle (Fung, 1981). Stress relaxation has been demonstrated in vivo in human skeletal muscle during passive stretch held at a fixed ROM (McHugh *et al.*, 1991; Magnusson *et al.*, 1995a; Magnusson *et al.*, 1996). Creep refers to the elongation over time when a fixed tensile load is applied to a muscle. Creep has been demonstrated in rabbit skeletal muscle and also reflects the muscle's viscous properties (Taylor *et al.*, 1991).

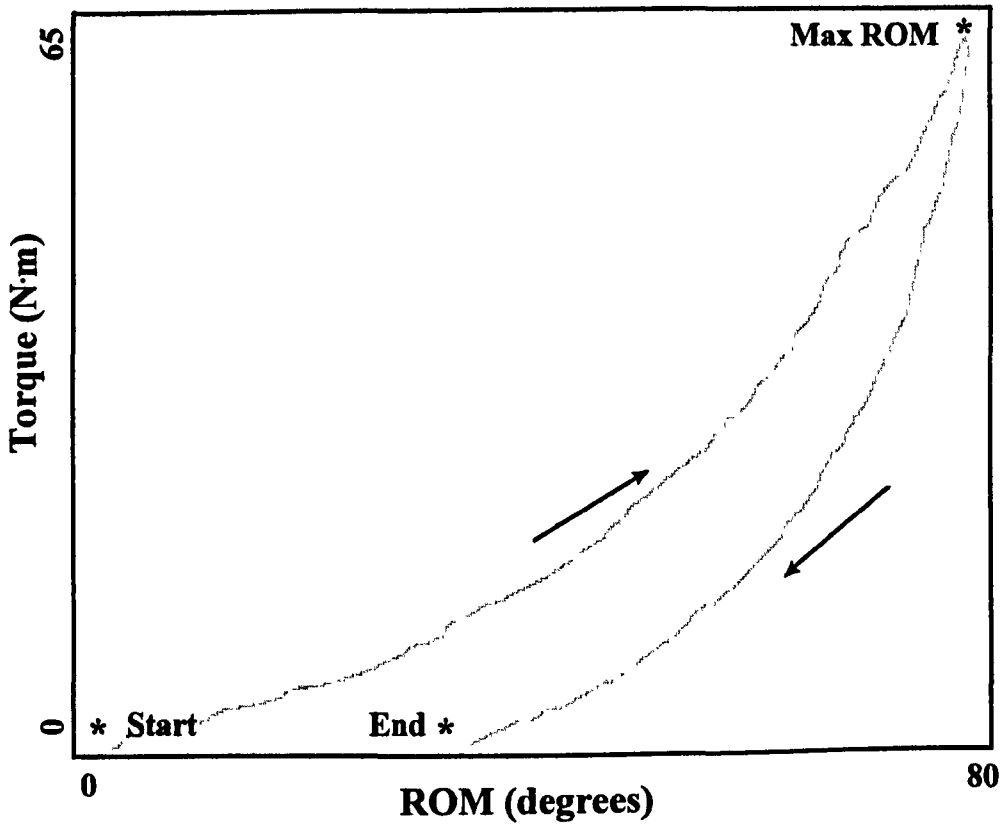

Figure 1.3: Torque/ROM curve depicting passive hamstring stiffness during a straight leg raise stretch. Torque is resistance to stretch continuously corrected for limb mass through the ROM. The arrows indicate raising and lowering the limb. The area between the two curves represents the energy lost during the stretch (hysteresis).

The viscoelastic properties of human muscle have been examined in response to exercise and training. Fatiguing concentric hamstring contractions resulted in a 20% reduction in passive muscle stiffness within one minute of the cessation of exercise, while stress relaxation was unaffected (Magnusson *et al.*,

1995b). In contrast, fatiguing eccentric contractions did not affect passive stiffness or stress relaxation. Isometric hamstring strength training (13 weeks) resulted in a 43% increase in strength and a 25% increase in passive stiffness, while stress relaxation was unaffected (Klinge *et al.*, 1997). The increase in stiffness was not counteracted by concurrent flexibility exercises. The observed effects were in part attributed to connective tissue adaptations within the muscle. These studies demonstrate changes in viscoelastic properties with acute and chronic muscle activity. However, few studies have specifically examined how differences in viscoelasticity effect muscle function.

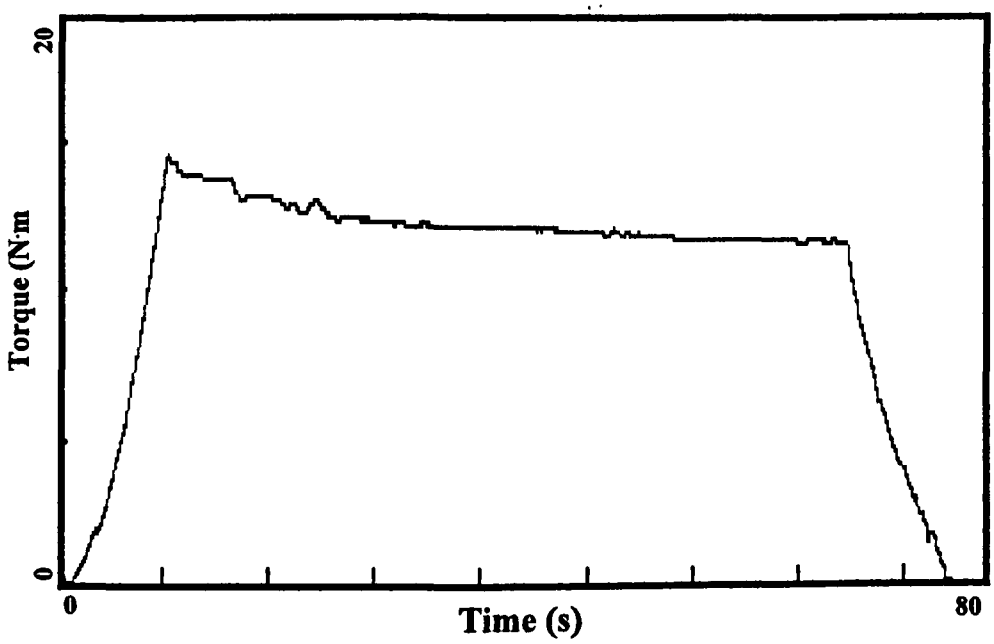

Figure 1.4: Passive hamstring tension during a straight leg raise stretch held at 60° for one minute. Peak torque corresponds with the stretch stopping at 60°. Decline in torque over the following minute represents stress relaxation. Tension returns to zero torque as the limb is lowered to zero ROM. A 19% decline in torque (stress relaxation) occurred during the one minute stretch at 60°.

CHAPTER 2

REVIEW OF LITERATURE

Portions of the content of this chapter appeared as a manuscript:
McHugh, M.P., Connolly, D.A.J., Eston, R.G., and Gleim, G.W. (1999). Exercise-induced muscle damage and potential mechanisms for the repeated bout effect. *Sports Medicine*, **27**: 157-170.

2.1 NEURAL FACTORS ASSOCIATED WITH MUSCLE DAMAGE

2.1.1 Neural Control of Eccentric Contractions

Electromyography (EMG) has been used extensively to identify specific characteristics of different contraction types (eccentric, concentric, isometric). The amplitude of the surface EMG signal has been used to indicate the extent of motor unit activation (Bigland and Lippold, 1954b; Komi and Buskirk 1972; Komi *et al.*, 1987; Moritani *et al.*, 1988; Adams *et al.*, 1992; Nakazawa *et al.*, 1993; Hortobagyi *et al.*, 1996a; Hortobagyi *et al.*, 1996b; Potvin 1997) while the frequency content of the signal has been used to indicate recruitment of different types of motor unit (Moritani *et al.*, 1988; Nakazawa *et al.*, 1993; Potvin 1997) (see section 1.3.1). EMG activity has also been studied extensively with respect to muscle fatigue (for review see Enoka and Stuart, 1992; Haag, 1992). In contrast, few studies have examined EMG activity during exercise that results in muscle damage. Komi and Viitasalo (1977) examined quadriceps EMG during maximal eccentric and concentric contractions in four subjects and subsequently documented changes in strength and serum CK activity. However, small sample size and lack of clear evidence of damage precluded any definitive conclusions. EMG activity has also been examined during contractions on the days following a bout of eccentric exercise (Komi and Viitasalo, 1977; Kroon and Naeije, 1991; Hasson *et al.*, 1993). Strength loss was associated with decreased activation (Hasson *et al.*, 1993) and greater activation was required for a given sub-maximal force production (Komi and Viitasalo, 1977). However, these data did not yield information on neural factors associated with the initiation of muscle damage.

It is well established that for a given force production, less motor unit activation is required for eccentric compared to concentric contractions (Bigland

and Lippold, 1954b; Komi *et al.*, 1987; Tesch *et al.*, 1990; Adams *et al.*, 1992; Potvin 1997). Surface EMG amplitudes for eccentric compared to concentric contractions have been shown to be 45-60% lower in the plantar flexors (Bigland and Lippold, 1954b), 35-50% lower in the knee extensors (Komi *et al.*, 1987; Tesch *et al.*, 1990), and 44-58% lower in the elbow flexors (Adams *et al.*, 1992; Potvin 1997).

In addition to less motor unit activation, some authors have suggested that fast-twitch motor units are selectively recruited during sub-maximal eccentric contractions, indicating reversal of normal recruitment (Enoka, 1996; Nardone *et al.*, 1989; Nardone and Schieppati, 1988). Nardone *et al.*, (1989) identified motor units according to their recruitment threshold during ramp isometric contractions of the plantar flexors. Subjects then performed reciprocal low intensity (<20% MVC) eccentric and concentric contractions. Interestingly, some motor units were activated during the eccentric portion that had been silent during both the concentric portion and the ramp isometric contractions. The amplitude of the action potentials from these units were consistent with fast-twitch motor units. Additionally, the soleus (predominantly slow-twitch) appeared to be inhibited during the eccentric contractions with a corresponding increase in gastrocnemius (predominantly fast-twitch) activation. These observations were taken to represent selective recruitment of fast-twitch motor units for sub-maximal eccentric contractions.

In contrast with the findings of Nardone and Schieppati (1988) and Nardone *et al.*, (1989) analysis of the frequency content of the surface EMG signal during sub-maximal eccentric contractions of the elbow flexors (Moritani *et al.*, 1988; Potvin, 1997) or maximal eccentric contractions of the quadriceps

(Tesch *et al.*, 1990) failed to demonstrate evidence for selective recruitment of fast-twitch motor units. In fact, Nakazawa *et al.*,(1993) demonstrated lower frequencies during sub-maximal eccentric contractions of the elbow flexors suggesting de-recruitment of fast-twitch motor units. However, as suggested by Potvin (1997) similar frequencies at lower activation levels may indicate preferential fast-twitch motor unit recruitment during eccentric contractions.

2.1.2 Eccentric Fatigue

Selective recruitment of fast-twitch motor units would be expected to increase the rate of fatigue. However, maximum eccentric contractions have been shown to be extremely fatigue-resistant despite high force production (Tesch *et al.*, 1990; Hortobágyi *et al.*, 1996c). Hortobágyi *et al.*, (1996c) demonstrated force decrements of 41% and 32% following 50 maximal isometric and concentric contractions of the plantar flexors but found no change in force following 50 eccentric contractions. Similarly Tesch *et al.*, (1990) demonstrated 34-47% fatigue following 96 maximal concentric contractions of the knee extensors with no fatigue following the same number of maximal eccentric contractions. Concentric fatigue was associated with a decrease in the mean power frequency (MPF) of the EMG signal with no change in MPF during eccentric contractions.

Hortobágyi *et al.*, (1996c) hypothesized that reduced fatigue with repeated eccentric contractions was due to recruitment of initially unrecruited motor units. This seems plausible given the possibility of reduced activation for maximal eccentric contractions (Webber and Kriellaars, 1997). Lower energy cost may also explain the fatigue resistance for eccentric contractions. Komi *et al.*, (1987)

demonstrated greater mechanical efficiency (ratio of output to input energy) for eccentric (85%) compared to concentric contractions (19%) of the knee extensors.

The lack of fatigue during repeated eccentric contractions in the knee extensors (Tesch *et al.*, 1990) and plantar flexors (Hortobágyi *et al.*, 1996c) contrasts with other studies which followed subjects on subsequent days for evidence of muscle damage. Isometric strength loss has been demonstrated immediately following eccentric contractions of the elbow flexors (Newham *et al.*, 1987; Clarkson and Tremblay, 1988; Newham *et al.*, 1988; Ebbeling and Clarkson, 1990; Cleak and Eston 1992b; Chleboun *et al.*, 1995; Saxton and Donnelly 1996; Nosaka and Clarkson 1997) and knee extensors (MacIntyre *et al.*, 1996; Brown *et al.*, 1997a; Brown *et al.*, 1997b; Child *et al.*, 1998). Eccentric and concentric isotonic strength loss has also been demonstrated immediately following 100 eccentric contractions of the knee extensors at 65% of MVC (Ploutz-Snyder *et al.*, 1996). Similarly, eccentric and concentric isokinetic strength loss has been demonstrated immediately following 100 maximal eccentric contractions of the knee extensors (Eston *et al.*, 1996). Although these studies demonstrate that fatigue can be induced with eccentric contractions, it is clear that eccentric contractions are less fatigable than either concentric or isometric contractions.

2.1.3 Selective Damage to Fast-Twitch Fibres

While the possibility of selective recruitment of fast-twitch motor units remains uncertain, it appears that fast-twitch fibres are more susceptible to damage during eccentric exercise (Fridén, 1984; Fridén *et al.*, 1983b; Lieber and Fridén, 1991; MacPherson *et al.*, 1996). Fridén et al (1983b) found myofibrillar disruption to be three times more prevalent in fast compared to slow twitch fibres three days following eccentric cycle ergometer exercise.

Muscle damage has been attributed to excessive stress on a small number of active fibres during eccentric contractions (Armstrong *et al.*, 1983; Moritani *et al.*, 1988). The extent of motor unit activation and the type of motor units recruited have not been studied with respect to exercise-induced muscle damage. It is possible that selective recruitment of a small number of fast-twitch motor units places excessive stress on the active fibres leading ultimately to damage. A neural recruitment pattern combining reduced motor unit activation, selective fast twitch fibre recruitment and fatigue resistance may predispose the muscle to injury.

2.2 MECHANICAL FACTORS ASSOCIATED WITH MUSCLE DAMAGE

2.2.1 The Role of Muscle Length

Muscle damage has been described as materials fatigue typical of ductile material subjected to cyclic tensile loading (Armstrong *et al.*, 1991; Warren *et al.*, 1993). Materials fatigue refers to structural failure due to *cumulative* tensile stress and is distinct from failure due to the application of a single stress that

exceeds the material's ultimate tensile strength. A ductile material under tensile stress experiences plastic deformation prior to failure. This is in contrast to a brittle material which fails without significant deformation (e.g. glass). Skeletal muscle is a ductile material and its behaviour during repeated eccentric contractions is consistent with materials fatigue (Warren *et al.*, 1993). Armstrong *et al.* (1991) have proposed that the passive elements in skeletal muscle experience excessive strain during eccentric contractions at muscle lengths on the "descending limb" of the length-tension curve. In this situation the ability to produce active tension is decreasing while passive tension is increasing.

Data from isolated whole muscle preparations in animals (Lieber and Fridén, 1993; Brooks *et al.*, 1995; Hunter and Faulkner, 1997) and voluntary contractions in humans (Newham *et al.*, 1988; Child *et al.*, 1998) have clearly shown that the length of the muscle during eccentric contractions appears to be a critical factor in determining the extent of damage. Lieber and Fridén (1993) demonstrated that damage to rabbit tibialis anterior muscles was a function of the length to which the muscle was elongated during stimulation rather than the magnitude of the contractile stimulus. Muscles actively strained 12.5% beyond resting length experienced a 40% decrease in maximum tetanic tension. Muscles strained 25% beyond resting length experienced a 60% decrease in tetanic tension. Newham *et al.* (1988) demonstrated that eccentric contractions of the elbow flexors performed at longer muscle lengths resulted in greater symptoms of muscle damage. On the following day, muscles that had exercised from 45° to full elbow extension (long) had 20% strength loss compared to 9% in the muscles exercised from full flexion to 60° (short). Two days following the initial exercise muscle tenderness was almost twice as high in the (long) group. Similar results

have recently been demonstrated in the knee extensors (Child *et al.*, 1998). Three days following 75 maximal eccentric contractions strength loss was 7% in subjects who had exercised at short muscle lengths and 29% in subjects who had exercised at long muscle lengths. Similar effects were seen in serum CK activity and muscle soreness. These studies (Newham *et al.*, 1988; Lieber and Fridén, 1993; Brooks *et al.*, 1995; Hunter and Faulkner, 1997; Child *et al.*, 1998) support the theory of disruption occurring on the "descending limb" of the length-tension curve.

2.2.2 Sarcomere Mechanics

The length-tension curve is determined by myofilament overlap which is a function of sarcomere length (Gordon *et al.*, 1966; Huxley, 1975). Sarcomere elongation during eccentric contractions is highly non-uniform with some sarcomeres maintaining length while others are stretched beyond the point of filament overlap (Huxley and Peachey, 1961; Flitney and Hirst, 1978; Morgan, 1990). This excessive stretch has been referred to as sarcomere "give" (Flitney and Hirst, 1978) or "popping" (Morgan, 1990). When a sarcomere is stretched beyond filament overlap ("popped"), a greater dependence is placed on the passive structures to maintain serial tension as the serial sarcomeres shorten (Morgan, 1990). Muscle damage is not a result of the actual "popping" (which is thought to occur with most eccentric contractions) but is thought to be due to the cyclic stress placed on the supporting passive structures by continued eccentric contractions following "popping". These elements are referred to as intermediate filaments and consist of the proteins desmin, vimentin and synemin (Waterman-Storer, 1991; Fridén and Lieber, 1992).

The intermediate filaments are responsible for maintaining the structural integrity of serial and parallel sarcomeres (Waterman-Storer, 1991; Fridén and Lieber, 1992; Patel and Lieber, 1997). Force transmission within skeletal muscle can be augmented by the intermediate filament system (Street, 1983; Patel and Lieber, 1997). Street (1983) demonstrated that the intermediate filament system provides a link to bypass damaged areas and maintain serial force production. While this may be beneficial for maintaining force production during eccentric exercise the ultimate effect may be to increase subsequent damage. When sarcomeres are stretched beyond myofilament overlap the intermediate filament system must bear the load of subsequent contractions. Repeated loading will result in mechanical failure of the intermediate filament system. Electron microscopic analysis of muscle damage shows significant disruption of the intermediate filaments characterized by Z bands streaming and loss of registration of Z bands in parallel myofibrils (Waterman-Storer, 1991; Fridén and Lieber, 1992).

2.2.3 The Role of Muscle Stiffness

A recent study examining the effect of fatigue and warm-up prior to a bout of eccentric exercise suggests that decreased muscle stiffness may be protective against muscle damage (Nosaka and Clarkson, 1997). In an initial experiment subjects performed 12 maximum eccentric contractions of the elbow flexors with each arm. In one arm the 12 eccentric contractions were preceded by 100 maximum concentric contractions. The concentric exercise resulted in a 20% decrease in isometric strength but did not affect eccentric force production which was similar between arms. In the arm exercising without prior concentric

exercise isometric strength loss was 40% one day later and 20% five days later. In the arm subjected to prior concentric exercise, isometric strength loss was only 25% one day later and had returned to baseline within five days. Other indices of muscle damage showed similar differences between arms during the five days following the respective exercise bouts. Paradoxically, these results suggested that whole muscle fatigue (induced concentrically) protected the muscle from damage. An additional experiment was performed to explain these effects. In this part of the study eccentric exercise was preceded by 100 concentric elbow flexions without resistance, to simulate warm-up exercise. Eccentric exercise preceded by warm-up resulted in less strength loss and minimal changes in plasma CK activity compared to the eccentric exercise without prior warm-up. These protective effects of prior concentric exercise (fatiguing and non-fatiguing) were attributed to decreased passive muscle stiffness. Although actual stiffness measurements were not made, the conclusions are supported by previous work demonstrating reduced passive stiffness following fatiguing concentric contractions (Magnusson *et al.*, 1995b) and repeated passive stretches (Magnusson *et al.*, 1996). However, it remains to be determined whether passive stiffness actually affects the initiation of muscle damage. Additionally, differences in passive muscle stiffness between individuals have not been examined with respect to muscle damage.

2.3 THE REPEATED BOUT EFFECT

Unfamiliar eccentric exercise frequently results in muscle damage, the symptoms of which include strength loss, pain, muscle tenderness and elevated plasma creatine kinase (see section 1.2). Following recovery, a repeated bout of the same exercise results in minimal symptoms of muscle damage and has been referred to as the "repeated bout effect" (Nosaka and Clarkson, 1995). This protective effect of prior exercise was first indicated by Highman and Altland (1963) and specifically attributed to eccentric contractions in later work (Schwane and Armstrong, 1983). The repeated bout effect has subsequently been demonstrated in human subjects and in animal models, with various types of activities using different muscle groups (Table 2.1). In humans the repeated bout effect has been demonstrated with downhill running or walking (Byrnes *et al.*, 1985; Pierrynowski *et al.*, 1987; Westerlind *et al.*, 1992; Balnave and Thompson, 1993; Westerlind *et al.*, 1994), eccentric contractions of the elbow flexors (Newham *et al.*, 1987; Clarkson and Tremblay, 1988; Ebbeling and Clarkson, 1990; Nosaka and Clarkson, 1995; Pizza *et al.*, 1996), eccentric contractions of the knee extensors (Golden and Dudley, 1992; Mair *et al.*, 1995; Eston *et al.*, 1996; Brown *et al.*, 1997a; Hortobágyi *et al.*, 1998) and eccentric hamstring contractions (Clarkson *et al.*, 1987). The repeated bout effect has also been demonstrated in rats with downhill walking (Highman and Altland, 1963), downhill running (Schwane and Armstrong, 1983) and stimulated eccentric contractions of isolated muscle-tendon units (Sacco and Jones, 1992).

Differences in electron micrographs of myofibrils, strength loss, pain and serum and plasma CK activity between bouts have provided evidence of the

repeated bout effect. In the following sections (2.3.1 to 2.3.4) references to values relating to symptoms of muscle damage are estimated from figures where the exact numbers are not provided in the text.

Table 2.1: Studies demonstrating the repeated bout effect.

SAMPLE	MUSCLE GROUP	EXERCISE MODE	DELAY BETWEEN BOUTS	PROPOSED MECHANISM	REFERENCE
12 men	elbow flexors	isotonic @ 80% MVC	3 & 6 days	neural adaptation	Nosaka and Clarkson, 1995
84 rats	soleus, vastus intermedius, triceps medialis	downhill running level running	3-8 days	strengthening of muscle tissue	Schwane and Armstrong, 1983
11 women 5 men	lower extremity muscles	downhill walking	1-8 wks	no mechanism discussed	Balnave and Thompson, 1993
18 women 6 men	knee extensors	max isokinetic	3 wks	improved ability to repair initial injury	Brown *et al.*, 1997a
11 women 11 men	lower extremity muscles	downhill running	3, 6 or 9 weeks	removal of weak fibres	Byrnes *et al.*, 1985
8 women	elbow flexors	max isotonic	2 wks	removal of weak fibres, strengthening cell membrane	Clarkson and Tremably, 1988
20 women	elbow flexors	max isotonic	5 or 14 days	strengthening connective tissue or cell membrane	Ebbeling and Clarkson, 1988
10 men	quadriceps	downhill running	2 wks	no mechanism discussed	Eston *et al.*, 1996
9 women	quadriceps	cycling	8 wks training	increased sarcomeres, intermediate filament remodelling	Fridén *et al.*, 1983a
15 men	quadriceps	cycling	4 & 8 wks training	reorganization of intermediate filament	Fridén, 1984
24 men	quadriceps	isotonic <85% MVC	3 wks	neural adaptations	Golden and Dudley, 1992
67 Rats	vastus intermedius	downhill running	1-3 wks	serial addition of sarcomeres	Lynn and Morgan, 1994
22 men	quadriceps	max isotonic	4 & 13 days	removal of weak fibres, connective tissue adaptation or neural adaptation	Mair *et al.*, 1994
5 women 3 men	elbow flexors	max isotonic	2 & 4 wks	connective tissue adaptation or removal of weak fibres	Newham *et al.*, 1987
9 men	lower extremity muscles	downhill running	4 days	tissue strengthening or neural adaptation	Pierrynowski *et al.*, 1987
10 men	elbow flexors	max isotonic	3 wks	reduced inflammation	Pizza *et al.*, 1996
Mice # not stated	tibialis anterior	maximal nerve stimulation	10,21,84 or 166 days	excludes possibility of neural adaptation	Sacco and Jones, 1992
3 women 3 men	lower extremity muscles	downhill running	2 wks	neural adaptation	Westerlind *et al.*, 1994
4 women 3 men	lower extremity muscles	downhill running	2 wks	no mechanism discussed	Westerlind *et al.*, 1992

2.3.1 Electron Micrographs Following a Repeated Bout

Reduced damage has been demonstrated in electron micrographs of biopsies taken from muscles following repeated bouts of eccentric exercise (Fridén *et al.*, 1983a; Fridén, 1984; Hortobágyi *et al.*, 1998). Hortobágyi *et al.* (1998) found evidence of damage in 65% of pixels in electron micrographs from vastus lateralis muscles two days following 100 eccentric knee extension contractions at 80% of eccentric MVC. Only 23% of pixels showed signs of damage two days following a repeated bout two weeks later. Similarly, Fridén *et al.* (1983b) found damage in 20% of micrographs from vastus lateralis biopsies three days following 30 minutes of eccentric cycling. Only 4% of micrographs showed damage following four weeks of eccentric cycling training (Fridén *et al.*, 1983a).

2.3.2 Strength Loss Following a Repeated Bout

Reduced strength loss has been demonstrated on the days following repeated bouts of eccentric contractions of the elbow flexors (Newham *et al.*, 1987; Ebbeling and Clarkson, 1990) and knee extensors (Golden and Dudley, 1992; Brown *et al.*, 1997a; Hortobágyi *et al.*, 1998). Isometric elbow flexion strength was reduced by 45% (Newham *et al.*, 1987) and 54% (Ebbeling and Clarkson, 1990) two days following an initial bout of isotonic eccentric contractions but only reduced by 14% and 13% respectively, two days following repeated bouts two weeks later. Similarly, isometric knee extension strength was reduced by 38% (Golden and Dudley, 1992), 42% (Brown *et al.*, 1997a) and 37% (Hortobágyi *et al.*, 1998) one to two days following an initial bout of isotonic eccentric contractions but only reduced by 14%, 25% and 4% respectively, one to

two days following repeated bouts two weeks later. Interestingly, strength loss immediately post exercise was similar between the initial and repeated bouts in both the elbow flexors (Newham *et al.*, 1987; Clarkson and Tremblay, 1988; Ebbeling and Clarkson, 1990) and knee extensors (Brown *et al.*, 1997a). This suggests that the strength loss evident immediately post-exercise primarily reflects muscle fatigue rather than damage.

Low frequency fatigue, a measure of contractile function in response to electrical stimulation at different frequencies (see 1.2.2), was less affected than strength by repeated bouts in the elbow flexors (Newham *et al.*, 1987) or the knee extensors (Brown *et al.*, 1997a). The ratio of force with low compared to high frequency stimulation was reduced by 37% two days following isotonic eccentric contractions of the elbow flexors and reduced by 23% two days following a repeated bout two weeks later (Newham *et al.*, 1987). Strength losses at the same times were 45% and 14% respectively.

2.3.3 Pain and Soreness Following a Repeated Bout

Reduced pain or soreness has been demonstrated following repeated bouts of eccentric contractions of the elbow flexors (Ebbeling and Clarkson, 1990; Pizza *et al.*, 1996) and knee extensors (Mair *et al.*, 1995; Brown *et al.*, 1997a; Hortobágyi *et al.*, 1998) and following downhill running (Byrnes *et al.*, 1985; Westerlind *et al.*, 1992; Westerlind *et al.*, 1994). Soreness was 5.5 (Ebbeling and Clarkson, 1990) and 5.6 (Pizza *et al.*, 1996) out of 10, two days following isotonic eccentric contractions of the elbow flexors but was only 2.8 and 2.1 respectively, two days following repeated bouts. Soreness was 8 (Mair *et al.*, 1995) and 5.2 (Hortobágyi *et al.*, 1998) out of 10 two days following isotonic

eccentric contractions of the knee extensors. Two days following repeated bouts soreness was completely absent (Mair *et al.*, 1995; Hortobágyi *et al.*, 1998). Similarly, soreness was 22 out of 60 one day following isotonic eccentric contractions of the knee extensors but only 14 out of 60 one day following a repeated bout three weeks later (Brown *et al.*, 1997a). One day following downhill running soreness was 6 (Byrnes *et al.*, 1985), 4.3 (Westerlind *et al.*, 1992) and 4.8 (Westerlind *et al.*, 1994) out of 10, but only 2, 1.2 and 1.8 respectively, one day following repeated bouts two weeks later.

2.3.4 Plasma Creatine Kinase Activity Following a Repeated Bout

Plasma creatine kinase (CK) activity has been studied following repeated bouts of downhill running (Byrnes *et al.*, 1985; Westerlind *et al.*, 1992; Westerlind *et al.*, 1994) and isotonic eccentric contractions of the elbow flexors (Newham *et al.*, 1987; Ebbeling and Clarkson, 1990; Pizza *et al.*, 1996), knee extensors (Mair *et al.*, 1995; Brown *et al.*, 1997a; Hortobágyi *et al.*, 1998) and knee flexors (Clarkson *et al.*, 1987). Serum CK activity was elevated by 200% (Byrnes *et al.*, 1985), 55% (Westerlind *et al.*, 1992) and 240% (Westerlind *et al.*, 1994) one day following an initial bout of downhill running, but only elevated by 40%, 13% and 65% respectively, one day following a repeated bout two weeks later. Approximately, 15 to 40 fold increases in plasma CK activity (Newham *et al.*, 1987) and serum CK (Ebbeling and Clarkson, 1990; Pizza *et al.*, 1996) were seen three to five days following isotonic eccentric contractions of the elbow flexors with no increase on any day following repeated bouts two weeks or three weeks later. Peak serum and plasma CK elevations following isotonic eccentric contractions of the knee extensors were 500% one day post-exercise (Mair *et al.*,

1995), 106% three days post-exercise (Brown *et al.*, 1997a) and 220% four days post (Hortobágyi *et al.*, 1998). No elevations in CK activity were seen following repeated bouts two weeks later (Mair *et al.*, 1995; Hortobágyi *et al.*, 1998) and only a 12.5% increase in CK activity was seen three days following a repeated bout three weeks later (Brown *et al.*, 1997a). Serum CK activity was elevated by 310% one day following isotonic eccentric contractions of the knee flexors but only elevated by 50% one day following a repeated bout one week later (Clarkson *et al.*, 1987).

2.4 MECHANISM OF THE REPEATED BOUT EFFECT

Many theories have been proposed to explain the repeated bout effect but a specific mechanism has not been identified. In general three categories of hypotheses have been proposed, which are neural, mechanical and cellular in origin (Fig. 2.1). Other theories include adaptations in excitation-contraction coupling (Warren *et al.*, 1993; Balnave and Allen, 1995) and reduced inflammatory response (Pizza *et al.*, 1996).

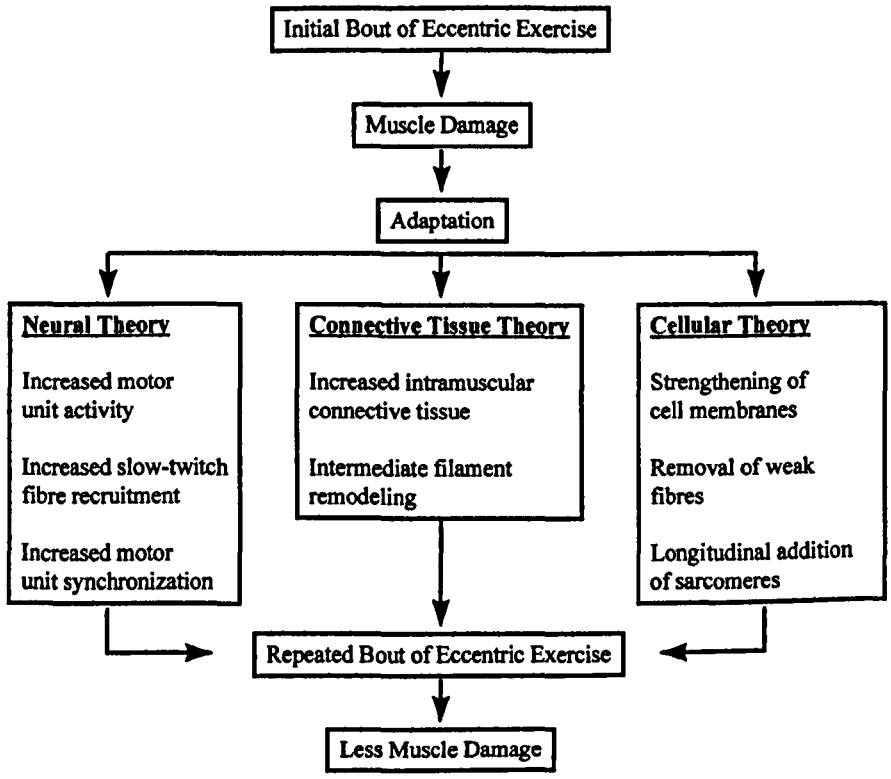

Figure 2.1: Possible mechanisms for the repeated bout effect.

2.4.1 Neural Theory

Several authors have discussed the possibility that there is a change in motor unit recruitment during the repeated bout, which limits the extent of damage (Pierrynowski *et al.*, 1987; Golden and Dudley, 1992; Mair *et al.*, 1994; Nosaka and Clarkson, 1995). Specifically, Golden and Dudley (1992) suggested that reduced motor unit activation associated with eccentric contractions "may provide the opportunity to 'learn' more efficient recruitment" for a repeated bout. Accordingly, Pierrynowski *et al.* (1987) suggested that "increased synchrony of motor unit firing" may reduce myofibrillar stresses during a repeated bout. Similarly, Nosaka and Clarkson (1995) suggested that the neural adaptation would "better distribute the workload among fibres."

Several studies point to the potential for neural adaptation. In strength training studies greater increases in integrated EMG activity (iEMG) have been demonstrated with purely eccentric compared to purely concentric training (Komi and Buskirk, 1972; Hortobágyi *et al.*, 1996a; Hortobágyi *et al.*, 1996b. Twelve weeks eccentric strength training of the knee extensors (in men) resulted in a 116% increase in strength with a 188% increase in iEMG compared to a 53% increase in strength and a 28% increase in iEMG with concentric strength training (Hortobágyi *et al.*, 1996b). A subsequent study by Hortobágyi *et al* (1996a) demonstrated similar neural adaptations, in women, with only six weeks of training. With eccentric training, strength increased by 42% while iEMG increased by 89%. With concentric training, strength increased by 36% and iEMG increased by 39%. Similarly, Komi and Buskirk (1972) found that seven weeks of eccentric strength training of the elbow flexors resulted in a 16% increase in eccentric strength associated with a 22% increase in iEMG, while

concentric strength training resulted in a 12 % increase in concentric strength associated with a 10% decrease in iEMG. Interestingly, the greatest increases in iEMG with eccentric training occurred at weeks two and three, the point at which the authors noted that the muscle soreness associated with the eccentric training had subsided. It was not clear whether the increase in iEMG was due to the repeated bouts (six maximum contractions, four days per week) or the initial bouts that caused muscle soreness. Despite strength improvements, force per iEMG was decreased in these studies, suggesting that eccentric strength training results in a decrease in force per motor unit activation. The fact that Komi and Buskirk (1972) noted the largest increase in iEMG at three weeks suggests the effect was not due to hypertrophy. This may represent a neural adaptation consistent with the theory of Nosaka and Clarkson (1995) whereby the workload for the repeated bouts is distributed over a greater number of active fibres.

Eccentric strength training also resulted in marked cross education to contralateral muscle groups (Weir *et al.*, 1995; Hortobágyi *et al.*, 1997). Hortobágyi *et al* (1997) demonstrated that twelve weeks of unilateral eccentric quadriceps training increased contralateral strength by 77% and iEMG by 54%. Concentric training increased contralateral strength by 30% and iEMG by 28%. Similarly, Weir *et al* (1995) demonstrated a 16% increase in eccentric strength and a 15% increase in isometric strength in the untrained limb following eight weeks of unilateral eccentric quadriceps training. These findings emphasize the ability of the central nervous system to adapt to eccentric exercise.

Indirect evidence of a neural adaptation with a repeated bout of eccentric exercise has been demonstrated in several studies (Clarkson and Tremblay, 1988; Mair *et al.*, 1994; Nosaka and Clarkson, 1995; Brown *et al.*, 1997a). In two

studies (Mair *et al.*, 1994; Nosaka and Clarkson, 1995) a repeated bout prior to full recovery did not exacerbate the symptoms, while in other studies (Clarkson and Tremblay, 1988; Brown *et al.*, 1997a) the initial bout did not have to cause appreciable damage to afford a protective effect. Nosaka and Clarkson (1995) had subjects repeat a bout of eccentric exercise after only three days when muscle soreness and plasma CK were significantly elevated. Decreases in soreness and plasma CK on the days following the repeated bout indicated that the protective effect was not dependent on full recovery. Similarly Mair *et al.* (1994) showed that a repeated bout of eccentric quadriceps exercise after four days did not further impair vertical jump or affect plasma CK on the following days. These effects may have been due to de-recruitment of motor units with injured fibres and increased activity in healthy motor units.

The initial bout of eccentric exercise does not have to cause appreciable damage to provide a protective effect (Clarkson and Tremblay, 1988; Brown *et al.*, 1997a). Clarkson and Tremblay (1988) had subjects perform 70 maximum eccentric contractions of the elbow flexors with one arm and 24 maximum contractions with the other arm. Two weeks later, the arm that had initially performed 24 contractions now performed 70 contractions. Following the initial bout, changes in strength, pain and muscle soreness were significantly lower in the arm that performed 24 contractions compared to the arm performing 70 contractions. Peak strength loss was 41% in the arm performing 70 contractions compared to 15% in the arm performing 24 contractions. When the arm that had initially performed 24 contractions performed 70 contractions two weeks later, strength loss was only 11%. Although the authors suggested that the protective effect may have been due to increased strength of the cell membrane or

surrounding connective tissue, a neural adaptation would also be a plausible explanation.

Brown et al (1997a) recently demonstrated results similar to Clarkson and Tremblay (1988). An initial bout of 10, 30 or 50 eccentric contractions of the knee extensors provided similar protection for a bout of 50 contractions three weeks later. Marked elevations in serum CK activity were found on the days following the initial bout of 30 and 50 repetitions. However, serum CK activity was not elevated following the initial bout of 10 repetitions. Three weeks later when all subjects performed a bout of 50 repetitions none of the groups demonstrated an increase in serum CK activity. Similar responses were seen for strength and soreness. While the initial bout of 10 repetitions did not cause appreciable damage it provided protection from a repeated bout which would have been expected to cause considerable muscle damage. Although not discussed, a neural adaptation to the initial exercise is a plausible explanation since the effects were not dependent on the occurrence of muscle damage. The number and intensity of contractions sufficient to provide a protective effect remains to be determined.

Data from electrical stimulation of rat tibialis anterior muscles has provided evidence against a neural adaptation (Sacco and Jones, 1992). In unconditioned muscles, stimulated force was 48% of the non-exercised control muscle three days after electrically stimulated eccentric contractions. In eccentrically preconditioned muscles, force was 80% of the control muscles three days following repeated bouts (10 or 21 days after the initial bout). The protection afforded to the preconditioned muscles could not be attributed to a neural adaptation since the exercise involved stimulated contractions. While

these results provide evidence of a peripheral component to the repeated bout effect, a concomitant neural adaptation may occur with voluntary contractions which results in less severe damage. Additionally, the 20% force loss in the preconditioned muscles suggests that the repeated bout still caused significant damage.

2.4.2 Mechanical Theory

Intermediate filament remodeling may also play a role in the repeated bout effect. In a study of eccentric cycle ergometry training, Fridén *et al.* (1983a) proposed that structural reorganization of the intermediate filament system could have prevented further damage. This explanation was offered because intermediate filament repair took 7-10 days and this corresponded with the duration of symptoms of muscle damage.

Newham *et al.* (1987) demonstrated a repeated bout effect following bouts of maximal eccentric contractions of the elbow flexors separated by two weeks. Pain and stiffness following the initial bout were attributed to shortening of the non-contractile connective tissue in parallel with the contractile elements. Adaptation in this intramuscular connective tissue was proposed as a possible mechanism for the decreased pain and stiffness following repeated bouts. This possibility was restated in subsequent studies (Clarkson and Tremblay, 1988, Ebbeling and Clarkson, 1990) but no additional supporting evidence was provided.

There is indirect evidence that increased intramuscular connective tissue can provide protection against muscle damage (Lapier *et al.*, 1995). Lapier et al (1995) increased intramuscular connective tissue of rat extensor digitorum longus

muscles by immobilizing the ankle joint for three weeks with the muscle in either a shortened or lengthened position. Muscles immobilized in the lengthened position had 63% more intramuscular connective tissue and 86% lower mass than contralateral control muscles. Muscles immobilized in the shortened position had 47% more intramuscular connective tissue and 21% lower mass than control muscles. Subsequent bouts of stimulated eccentric contractions resulted in 50% force loss in control muscles compared to 40% in muscles immobilized in the shortened position and 8% in muscles immobilized in the lengthened position. The protective effect was attributed to the ability of the increased connective tissue to dissipate myofibrillar stresses. The authors suggested that tissue repair following a damaging bout of eccentric exercise is characterized by a similar increase in intramuscular connective tissue thereby protecting against damage from repeated bouts. Alternatively, these findings with respect to immobilization could be interpreted as a cellular adaptation in the muscle tissue. The fact that the effect occurred primarily in the muscles immobilized in the lengthened position suggests that protection may in part have been due to longitudinal addition of sarcomeres (see section 2.4.3).

An increase in intramuscular connective tissue would be expected to result in increased muscle stiffness (Kovanen *et al.*, 1984). It is possible that the repair process results in a permanent increase in passive stiffness as a result of remodeling of the connective tissue as suggested by Lapier et al (1995). Passive stiffness of the elbow flexors has been measured following eccentric exercise (see section 1.2.5). Increased stiffness on the days following eccentric exercise has been attributed to soft tissue oedema (Chleboun *et al.*, 1995) while contractile resistance to painful passive extension has been discounted (Howell *et al.*, 1985).

However, stiffness has not been followed to the point when strength has fully recovered and long term changes in the mechanical properties of the connective tissues cannot be excluded.

In contrast, a recent study examining the effect of fatigue and warm-up prior to a bout of eccentric exercise (Nosaka and Clarkson, 1997) suggests that decreased muscle stiffness may be protective against muscle damage (see section 2.2.3). However, changes in passive muscle stiffness have not been examined with respect to the repeated bout effect.

2.4.3 Cellular Theory

Cellular adaptations explaining the repeated bout effect may occur at the level of the muscle fibre, the myofibril or the sarcomere itself. Proposed theories include strengthening of the cell membrane (Clarkson and Tremblay, 1988), removal of a pool of weak fibres or sarcomeres following the initial damage (Armstrong *et al.*, 1983; Byrnes *et al.*, 1985; Mair *et al.*, 1994) and longitudinal addition of sarcomeres (Fridén *et al.*, 1983a; Lynn and Morgan, 1994; Lynn *et al.*, 1998).

Clarkson and Tremblay (1988) suggested that strengthening of the cell membrane could be an alternative explanation to a connective tissue adaptation. Sarcolemmal disruption results in the loss of calcium homeostasis which initiates the cellular necrosis evident on electron micrographs (Armstrong *et al.*, 1991). Strengthening of the sarcolemma or the sarcoplasmic reticulum could prevent disruption during eccentric contractions thereby preventing the calcium influx and avoiding the subsequent cellular necrosis.

Injury following downhill running in rats was explained by Armstrong *et al.* (1983) as disruption of a pool of stress "susceptible" fibres. Accordingly, reduced injury following repeated bouts of downhill running (Byrnes *et al.*, 1985) and eccentric quadriceps exercise (Mair *et al.*, 1994) has been explained by the removal of the "susceptible" fibres following the initial injury. Removal of a pool of stress "susceptible" myofibres or sarcomeres, as opposed to whole fibres, would be more consistent with the electron micrographic evidence of damage. The initial bout may serve to identify and remove a select population of weak sarcomeres. The lack of further damage when the repeated bout occurs before full recovery supports such a theory (Mair *et al.*, 1994; Nosaka and Clarkson, 1995). However, a limitation to this theory is the fact that the initial bout does not have to cause appreciable damage in order to provide a protective effect (Schwane and Armstrong, 1983; Clarkson and Tremblay, 1988; Brown *et al.*, 1997a). If the weak sarcomeres are still intact and functional then they should be disrupted by the repeated bout and damage would be evident. This was clearly not the case in the studies by Schwane and Armstrong (1983), Clarkson and Tremblay (1988) and Brown *et al.* (1997a).

Since muscle damage can be explained in terms of sarcomere mechanics it is plausible that the repeated bout effect could be explained by an adaptation in sarcomere mechanics. Such a theory was proposed by Morgan (1990) whereby longitudinal addition of sarcomeres following an initial bout of eccentric exercise would reduce sarcomere strain for a given muscle excursion during a repeated bout. Reduced sarcomere strain would allow the myofilaments to maintain overlap, limit sarcomere "popping" and avoid the ensuing cellular disruption. The possibility that repair of muscle damage occurs by serial addition of

sarcomeres within a myofibril was previously discussed by Fridén *et al.* (1983a). Electron microscopic observations of biopsies from vastus lateralis muscles of women following eight weeks of eccentric cycle ergometry indicated lengthening of the myofibrils by addition of new sarcomeres. However, the authors failed to elaborate on their observations and did not provide any quantitative evidence of such an adaptation. More recently Lynn and Morgan (1994) tested Morgan's theory of longitudinal addition of sarcomeres by comparing the number of serial sarcomeres in rat vastus intermedius muscles following either incline or decline running. One week of training with downhill running resulted in an 8% increase in serial sarcomeres compared to a sedentary control group. Similar uphill training resulted in a 4% decrease in serial sarcomeres relative to control rats. These results directly support Morgan's original theory and provide a specific cellular mechanism for the repeated bout effect.

The plausibility of this theory depends on the time course for the cellular adaptation and the stimulus required to initiate the adaptation. As previously mentioned, human studies have demonstrated a repeated bout effect prior to full recovery from the initial bout (Mair *et al.*, 1994; Nosaka and Clarkson, 1995). All criterion measures indicated significant muscle damage three days following an initial bout of eccentric exercise yet a repeated bout at that time did not exacerbate the damage (Nosaka and Clarkson, 1995). In fact indices of muscle damage were reduced following the repeated bout. Morgan's theory may not be plausible in this instance since the sarcomeres were probably given inadequate time to regenerate. The data from Lynn *et al.* (1998) indicate that the addition of sarcomeres occurs within eight days in rats. Additionally, Morgan's theory predicts that the initial myofibrillar disruption is the stimulus for the addition of

sarcomeres. However, as stated before, the initial bout does not have to cause appreciable damage in order to provide a protective effect (Schwane and Armstrong, 1983; Clarkson and Tremblay, 1988; Brown *et al.*, 1997a).

The concept of sarcomere strain as the initial step in the initiation of muscle damage is supported by a shift in the length-tension curve to the right immediately following a series of eccentric contractions (Wood *et al.*, 1993). If longitudinal addition of sarcomeres occurs, one would expect the length-tension curve to be shifted to the right following repair. With more sarcomeres in series a greater muscle length would be required to reach optimal sarcomere length. However, the length-tension curve has been shown to return to normal within five hours in toad sartorius muscles (Wood *et al.*, 1993) and within two days in human triceps surae muscles (Jones *et al.*, 1997). In contrast, a rightward shift in the length-tension curve has recently been shown following five weeks of incline treadmill training in rats (Lynn *et al.*, 1998), however, such an effect has yet to be demonstrated following recovery from a single bout of eccentric exercise.

2.4.4 Other Theories

Force loss following eccentric contractions may not be entirely due to mechanical disruption. Impairment of calcium-mediated excitation-contraction (E-C) coupling has been shown to contribute to force loss following active stretches of isolated whole muscle (Warren *et al.*, 1993; Ingalls *et al.*, 1998) and single fibre preparations (Balnave and Allen, 1995) from mice. These results suggest impaired calcium release or sensitivity following myofibrillar disruption. An adaptation in E-C coupling may explain the reduced strength loss following a repeated bout. Strengthening of the sarcoplasmic reticulum, as suggested by

Clarkson and Tremblay (1988), may prevent impairment of E-C coupling with a repeated bout. However, studies demonstrating the repeated bout effect in humans do not directly support an adaptation related to E-C coupling. Impairment E-C coupling is greatest immediately post eccentric exercise, accounting for 75% of the reduction in force (Ingalls *et al.*, 1998) but strength loss immediately following eccentric exercise has been shown to be similar between repeated bouts (Newham *et al.*, 1987; Clarkson and Tremblay, 1988; Ebbeling and Clarkson, 1990; Balnave and Thompson, 1993; Brown *et al.*, 1997a). It was only on subsequent days that reduced strength loss is seen with a repeated bout. If the repeated bout effect was due to an adaptation in E-C coupling reduced strength loss should be seen immediately following the repeated bout as well as on subsequent days. This was clearly not the case in several studies (Newham *et al.*, 1987; Clarkson and Tremblay, 1988; Ebbeling and Clarkson, 1990; Balnave and Thompson, 1993; Brown *et al.*, 1997a). The role of E-C coupling in the repeated bout effect requires further examination.

Reduced muscle damage following a repeated bout has been attributed to a blunted inflammatory response (Pizza *et al.*, 1996). A decreased number of circulating neutrophils and monocytes was seen on the days subsequent to the repeated bout. It was not clear whether these effects reflected a blunted immune response to tissue damage, or the lack of tissue damage following the repeated bout. Gleeson *et al.* (1995a) have shown that the inflammatory response to an initial bout of eccentric exercise is primarily an acute reaction lasting only a couple of days despite continued tissue necrosis. An adaptation in this inflammatory response may explain the lack of further damage when the repeated bout is performed prior to recovery from the initial bout (Mair *et al.*, 1994;

Nosaka and Clarkson, 1995). The reduced inflammatory response may prevent proliferation of damage with subsequent eccentric contractions.

2.5 SUMMARY

Although a plethora of data exist on the neural basis of muscle fatigue (for review see Enoka and Stuart, 1992) very little data are available on the neural basis of muscle damage. Reduced motor unit activation during eccentric contractions has been implicated in the occurrence of muscle damage (Armstrong *et al.*, 1983; Moritani *et al.*, 1988) but has not been specifically studied. Additionally, recruitment patterns during eccentric contractions have not been examined with respect to the subsequent damage. The possibility of selective recruitment of fast twitch fibres for eccentric exercise remains controversial but may in part explain preferential damage to those fibres.

Despite several studies suggesting a neural adaptation to explain the repeated bout effect (Pierrynowski *et al.*, 1987; Golden and Dudley, 1992; Mair *et al.*, 1994; Nosaka and Clarkson, 1995) no studies have tested such an hypothesis. Training studies suggest that there is a disproportionate increase in motor unit activation relative to strength with eccentric training. Such an adaptation has not been examined following a single bout of eccentric exercise. Increased activation relative to strength would distribute contractile stresses among a greater number of active fibres and may reduce the potential for damage. Additionally, the possibility of a shift in motor unit recruitment with a repeated

bout has not been examined. A shift to recruitment of more slow-twitch motor units may spare fast-twitch fibres which appear to be more susceptible to damage.

Muscle damage has been described as mechanical failure of individual myofibrils subjected to cyclic tensile loading (Armstrong *et al.*, 1991; Warren *et al.*, 1993). Surprisingly the mechanical properties of muscle have not been examined in relation to muscle damage. Data from Lapier et al (1995) suggest that increased passive stiffness may be protective against muscle damage and may explain the repeated bout effect. In contrast, recent indirect evidence from Nosaka and Clarkson (1997) suggest that decreased passive stiffness may be protective. However, the specific effects of passive muscle stiffness on the initiation of muscle damage and the repeated bout effect have not been examined.

Four specific questions relating to these issues are addressed in the following chapters. Each chapter describes a separate study. However, the data described in each chapter were acquired during a single experimental protocol performed on one group of volunteers.

1. Is eccentric exercise resulting in symptoms of muscle damage associated with reduced motor unit activation and selective recruitment of fast-twitch motor units?

   (Chapter 3)
2. Does passive muscle stiffness affect symptoms of exercise-induced muscle damage following a bout of eccentric exercise?

   (Chapter 4)
3. Is the repeated bout effect associated with an increase in motor unit activation or a shift in the types of motor units recruited for eccentric exercise?

   (Chapter 5)

4. Is the repeated bout effect associated with concomitant changes in passive muscle stiffness?

   (Chapter 6)

CHAPTER 3

NEURAL FACTORS ASSOCIATED WITH EXERCISE RESULTING IN SYMPTOMS OF MUSCLE DAMAGE

Continued Over

The content of this chapter has appeared as an abstract:
McHugh, M.P., Connolly, D.A.J., Eston, R.G., Kremenic, I.J. and Gleim, G.W. (1998). Neural factors associated with exercise-induced muscle damage. *Medicine and Science in Sports and Exercise*, **30**, S2.

3.1 ABSTRACT

Surface electromyographic (EMG) signals were recorded from the hamstring muscles during six sets of 10 sub-maximal isokinetic (2.6 $rad \cdot s^{-1}$) eccentric (11 men, 9 women) or concentric (6 men, 4 women) contractions. Contraction intensity was 60% of maximum isometric knee flexion strength tested at 45° of flexion. EMG/torque increased by 31% during eccentric ($p<0.01$) but did not change during concentric exercise. Median frequency (MF) increased by 9% during eccentric ($p<0.01$) with no change during concentric exercise. EMG/torque for eccentric contractions was initially 53% of the concentric value and increased to 75% by Set 6 ($p<0.05$). MF was initially 16% higher for eccentric compared to concentric contractions and was 26% higher by set 6 ($p<0.01$). Eccentric exercise resulted in significant isometric strength loss ($p<0.01$), pain ($p<0.01$), muscle tenderness ($p<0.05$) and elevated plasma creatine kinase (CK) activity ($p<0.01$). Only plasma CK was affected by concentric exercise ($p<0.01$). In conclusion, eccentric exercise resulted in moderate symptoms of muscle damage which were mostly absent following concentric exercise. Lower EMG/torque and higher MF is consistent with selective recruitment of a small number of fast-twitch motor units during eccentric exercise. Increasing EMG/torque and MF during eccentric exercise indicated additional recruitment of fast-twitch motor units. Selective recruitment of fast-twitch motor units may, in part, explain previous studies which observed damage primarily in fast-twitch fibres.

3.2 INTRODUCTION

3.2.1 Neural Basis of Muscle Damage

Experiments using either, electrically stimulated contractions in animal models (Lieber and Fridén, 1991; MacPherson *et al.*, 1996), or voluntary exercise in humans (Clarkson *et al.*, 1992; Fridén *et al.*, 1983b; Fridén 1984), have consistently demonstrated that skeletal muscle is susceptible to damage with eccentric contractions. Specific neural characteristics of eccentric contractions may be involved in the initiation of damage. For example muscle damage has been attributed to excessive stress on a small number of active fibres during eccentric contractions (Armstrong *et al.*, 1983; Moritani *et al.*, 1988). It is well established that for a given force production, less motor unit activation is required for eccentric compared to concentric contractions (Bigland and Lippold, 1954b; Komi *et al.*, 1987; Tesch *et al.*, 1990; Adams *et al.*, 1992; Potvin 1997). Data from these studies, elicited from various muscle groups, have consistently shown motor unit activation to be 35-60% lower for eccentric contractions. However, the extent of motor unit activation and the type of motor units recruited have not been studied with respect to exercise resulting in muscle damage.

3.2.2 Motor Unit Recruitment for Eccentric Contractions

It has recently been suggested that there is a reversal of normal motor unit recruitment order for eccentric contractions (Enoka 1996). Nardone and Schieppati (1988) have demonstrated de-recruitment of the soleus (primarily slow-twitch) and facilitation of the gastrocnemius (primarily fast-twitch) for the eccentric phase of reciprocal sub-maximal concentric/eccentric contractions of

the plantarflexors. In a later study, Nardone *et al.* (1989) confirmed their previous findings and also demonstrated that some motor units were activated during the eccentric phase that had been silent during both the concentric phase, and the ramp isometric contractions. The amplitudes of the action potentials from these units were consistent with fast-twitch motor units. Similar results were demonstrated by Howell *et al.* (1995) in the first dorsal interosseous muscle. However, several studies (Moritani *et al.*, 1988; Nakazawa *et al.*, 1993; Potvin, 1997), using frequency analysis of the surface EMG signal during eccentric and concentric contractions, have failed to confirm these findings. In fact, Nakazawa *et al.* (1993) found lower mean power frequency (MPF) during sub-maximal eccentric compared to sub-maximal concentric contractions of the elbow flexors and suggested that this was consistent with de-recruitment of fast-twitch motor units. Other studies have shown no difference in MPF between sub-maximal eccentric and concentric contractions of the elbow flexors (Moritani *et al.*, 1988; Potvin, 1997). However, Potvin (1997) suggested that similar frequencies with less motor unit activation might indicate preferential fast-twitch motor unit recruitment during eccentric compared to concentric contractions.

3.2.3 EMG Techniques for Identifying Selective Recruitment

Limitations in EMG techniques may explain the disparity between studies using surface compared to intramuscular electrodes in detecting selective motor unit recruitment. The surface EMG signal represents the composite of the action potentials from the muscle fibres of the recruited motor units and the firing rates of those units (Kamen and Caldwell, 1996). The degree to which the frequency content of the EMG signal is affected by firing rate or recruitment is debated

(Hägg, 1992; Kamen and Caldwell, 1996). The action potentials of higher threshold motor units are larger and have higher conduction velocities resulting in higher MPF or median frequency (MF) (Hägg, 1992; Kamen and Caldwell, 1996). Firing rates probably affect the frequency content more at low contraction intensities than high intensities (Fuglsang-Frederiksen and Rønager, 1988; Hägg, 1992; Kamen and Caldwell, 1996). Additionally, in large limb muscles, contraction intensity is regulated by alterations in firing rates for low intensity contractions. However, motor unit recruitment primarily regulates contractions at higher intensities (DeLuca *et al.*, 1982; DeLuca, 1985; Kamen and Caldwell, 1996). Moritani *et al.* (1988), Nakazawa *et al.* (1993) and Potvin (1997) each examined the elbow flexors at relatively low intensity contractions ($\leq$30% MVC). A common misconception is that higher threshold motor units will have higher firing rates than lower threshold units at a given contraction intensity. However, it has been shown that higher threshold motor units actually have lower firing rates at a given sub-maximal contraction intensity and that at maximal intensities firing rates tend to converge to similar rates (DeLuca *et al.*, 1982; Erim *et al.*, 1996). Lower or similar firing rates in the biceps brachii during eccentric compared to concentric contractions at low intensities may have sufficiently affected the frequency content of the surface EMG signal to obscure detection of selective recruitment of higher threshold units. For example, Nardone *et al.* (1989) used fine wire electrodes to demonstrate selective recruitment of fast-twitch motor units during low intensity eccentric contractions (<20% MVC). However, the actual firing frequencies of these units were not different from the slow-twitch units active during concentric contractions.

Studies which have demonstrated selective fast-twitch motor unit recruitment for eccentric contractions by using intramuscular EMG techniques have also been limited in that they examined low intensity contractions only (Nardone and Schieppati, 1988; Nardone *et al.*, 1989; Howell *et al.*, 1995). With increasing contraction intensity it becomes increasingly difficult to identify individual motor units and therefore more difficult to identify selective recruitment. Techniques which attempt to decompose the EMG signal into individual motor unit action potential trains have been used to resolve this problem with high intensity isometric contractions (DeLuca *et al.*, 1985). However, selective recruitment at eccentric contraction intensities sufficient to cause muscle damage has not been specifically examined either by intramuscular or surface EMG techniques.

3.2.4 Selective Damage to Fast-Twitch Fibres

The apparent selective damage to fast-twitch fibres following eccentric exercise may, in part, be due to a selective recruitment phenomenon. Electron micrographs (Fridén *et al.*, 1983b; Fridén 1984) and histochemical analysis (Lieber and Fridén, 1991) of muscle fibres following eccentric contractions have shown damage primarily in fast-twitch fibres. Fridén (1984) found a 4:1 ratio of damage in fast-twitch compared to slow-twitch fibres from vastus lateralis biopsies taken three days following eccentric cycle ergometer exercise. Additionally, simulated eccentric contractions in isolated rat muscle fibres resulted in greater injury to fast-twitch fibres (MacPherson *et al.*, 1996).

Other studies have suggested that more damage occurs in slow-twitch fibres with eccentric exercise (Armstrong *et al.*, 1983; Mair *et al.*, 1992).

Histochemical staining of damaged fibres in rats following downhill running suggested that a greater number of slow-twitch fibres were affected but quantitative data were not provided (Armstrong *et al.*, 1983). Similarly, Mair *et al.* (1992) attributed increased MRI signal intensity in the deep portion of the vastus lateralis and elevations in plasma concentration of myosin heavy chain fragments of slow-twitch skeletal myosin as evidence of greater damage in slow-twitch fibres following isotonic eccentric contractions.

3.2.4 Purpose of the Study

Reduced motor unit activation associated with selective recruitment of fast-twitch motor units may, in part, explain the susceptibility of fast-twitch fibres to damage. Tesch *et al.*, (1990) demonstrated reduced activation despite higher torque during maximum eccentric compared to maximum concentric contractions of the quadriceps. In their study, MPF was initially similar between contraction types. MPF decreased with repeated concentric contractions. However, with repeated eccentric contractions MPF remained unchanged. These data suggest that fast-twitch motor units remained active during eccentric exercise but were presumably subject to fatigue during concentric exercise. However, the subjects were not followed to document any subsequent muscle damage. In fact, subjects were given several familiarisation sessions on each of four consecutive weeks prior to data collection. It is therefore possible that practice of eccentric contractions performed in these sessions may have afforded protection against muscle damage following the experimental session (Clarkson and Tremblay, 1988; Brown *et al.*, 1997).

The purpose of this study was to compare EMG activity between 1) a bout of eccentric exercise resulting in muscle damage and 2) a bout of concentric exercise at the same relative intensity. EMG activity was analysed for evidence of reduced activation and selective recruitment of fast-twitch motor units during eccentric exercise.

3.3 METHODS

3.3.1 Experimental Design

Thirty subjects (17 men, 13 women) volunteered with informed consent. The study was approved by the institutional review board of Lenox Hill Hospital (New York), where the data was collected. Descriptive data are shown in table 3.1. The subjects were without orthopaedic injury and had not been involved in any weight training in the preceding three months. Subjects were instructed not to take any pain medication during the course of the study and to refrain from physical exercise. Two separate groups were studied: 1) an experimental group (11 men, 9 women), which performed eccentric exercise and 2) a control group (6 men, 4 women), which performed concentric exercise. Subjects were healthcare professionals or students and were recruited by word of mouth. The initial 10 volunteers performed eccentric exercise, the next 10 volunteers performed concentric exercise and the last 10 volunteers performed eccentric exercise.

Table 3.1: Mean (±S.D.) for age height and body mass of the male and female subjects in the eccentric and concentric groups.

	Age (yrs)	Height (cm)	Body Mass (kg)
Men			
Eccentric n=11	29±6	181±7	80.0±9.5
Concentric n=6	33±7	178±7	90.9±23.3
Women			
Eccentric n=9	28±6	165±16	63.7±12.9
Concentric n=4	31±9	162±9	56.7±8.2

The response of the hamstring muscle group to sub-maximal isokinetic exercise was studied. The hamstring muscle group was chosen because its three major muscles (biceps femoris long head, semitendinosus and semimembranosus) are biarticular. Significant differences in the frequency content of the surface EMG signal have previously been demonstrated between uniarticulate and biarticulate quadriceps muscles (Gerdle *et al.*, 1991). Potential recruitment differences between uniarticular and biarticular synergists may obscure effects specific to contraction type.

On Day 0, prior to isokinetic exercise, baseline measures of isometric strength, pain, muscle tenderness and plasma creatine kinase activity (CK) were made. Immediately following isokinetic exercise isometric strength was re-tested. One, two and three days days following isokinetic exercise, isometric strength, pain, muscle tenderness and plasma CK activity were recorded. Measurements on consecutive days were made during the same period of the day (morning, afternoon or evening).

Subjects performed six sets of 10 sub-maximal isokinetic contractions (Biodex System 2, Shirley, NY) at 2.6 $rad \cdot s^{-1}$ (150 $deg \cdot s^{-1}$). Isokinetic exercise was chosen to control contraction speed between subjects. Based on data from

extensor digitorum muscles of mice (McCully and Faulkner, 1986) higher eccentric contraction speeds were expected to increase the resultant damage. The maximum speed available for eccentric contractions on this dynamometer was 2.6 rad·s^{-1}. The subjects were allowed to choose which limb to exercise. The intensity of contraction was set at 60% of maximum isometric strength (MVC) to control for relative work performed within and between groups (eccentric vs. concentric). Each set of 10 contractions was separated by one minute. The relatively low intensity for isokinetic exercise (60% isometric strength) was chosen to limit possible fatigue effects during concentric exercise. Removing the possible confounding effect of fatigue is important in comparing EMG activity between eccentric and concentric contractions. One set of 10 isotonic eccentric contractions of the elbow flexors has been shown to result in significant symptoms of muscle damage (Teague and Schwane, 1995).

3.3.2 Isometric Strength Measurements

Subjects were seated in an upright position with hips at approximately 1.6 rad (90°) of flexion. The thigh of the test limb was strapped to prevent hip flexion during testing. The knee joint was aligned with the axis of rotation of the dynamometer and the leg was secured to the dynamometer arm at the ankle. The knee joint was set at 0.8 rad (45°) of flexion and limb mass was recorded. The subjects were then instructed to maximally contract the knee flexors while visual feedback and consistent verbal encouragement were provided to ensure maximal effort (Baltzopolous *et al.*, 1991). Four 5 s contractions were performed with 10 s between efforts. Peak torque, mean torque and total area under the torque/time curve were recorded following correction for limb mass. These measurements

were made pre- and post-isokinetic exercise on Day 0, and subsequently on Days 1, 2 and 3.

3.3.3 Isokinetic Exercise

Isokinetic exercise was performed immediately following the baseline measure of isometric strength. With subjects in seated position, eccentric contractions were performed from 1.6 to 0 rad (90° to 0°) of knee flexion and concentric contractions were performed from 0 to 1.6 rad. The dynamometer arm was set to move through the selected range of motion at 2.6 $rad \cdot s^{-1}$ and subjects contracted with sufficient intensity to reach a visually displayed target. The arm returned to the starting position at the same speed while subjects remained relaxed. The area under the torque/time curves were recorded for each set following correction for limb mass. Isokinetic exercise was performed on Day 0 only.

3.3.4 EMG Measurements

During all isometric strength tests and isokinetic exercise EMG signals were recorded from surface electrodes placed over the biceps femoris (BF), semitendinosus (ST) and semimembranosus (SM) muscles. The skin was shaved, cleaned and abraded prior to application of 10 mm diameter Ag/AgCl electrodes on a 34 mm by 22 mm adhesive gel surface. For BF, a pair of electrodes were placed 3 cm apart (centre to centre), midway along a line between the ischial tuberosity and the fibular head. For ST, electrodes were placed midway along a line between the ischial tuberosity and the medial femoral condyle. For SM, electrodes were placed at the apex of the inverted "V" formed

by the distal portions of the biceps femoris and semitendinosus muscles. A ground electrode was placed on the patella.

The EMG signal was recorded by telemetry, bandpass filtered from 12 to 500 Hz and sampled at a rate of 1000 Hz with a common-mode rejection ratio of 135 dB (Noraxon, Scottsdale, AZ). For analysis of the amplitude of the EMG activity the raw EMG signal was full-wave rectified and integrated (iEMG). Since all eccentric and concentric contractions were performed at the same relative intensity for each of the six sets (60% of isometric MVC) EMG was expressed relative to torque. EMG/torque ($\mu V/N \cdot m$) was computed by dividing iEMG by the area under the torque-time curve for the corresponding time interval. Differences in iEMG between contraction types (isometric, isokinetic eccentric and isokinetic concentric) were also analysed relative to torque. EMG/torque was used because the primary purpose of the EMG amplitude measurement was to quantify the differences in activation relative to force between contraction types.

Fast Fourier transforms (FFT) were applied to the raw EMG signals from which the median frequency (MF) was computed. All computations were performed using the manufacturer supplied software (Noraxon, Scottsdale, AZ). At 2.6 $rad \cdot s^{-1}$ it should have taken 600 ms for the limb to move through 1.6 rad. However, the acceleration and deceleration phases meant that this motion took approximately 900 ms to complete. The number of data points analysed in an FFT must be a power of two (e.g. 16, 32, 64, ... 512, 1024 etc.) (Kamen and Caldwell, 1996). The length of the FFT was set at 4096 data points which at a sampling rate of 1000 Hz covered 4096 ms. Each contraction-relaxation phase lasted approximately 1800ms. A period of 4096 ms covered two contraction

phases and slightly over three relaxation phases. Although this length of FFT entered low power noise into the frequency calculations it avoided the problem of identifying when a contraction began and ended. Contraction length varied during the isokinetic contractions as subjects reacted to the dynamometer movement (Fig 3.1). During contractions muscle length was changing rapidly. The effect of muscle length on MF (Morimoto, 1986) necessitated analysing all contractile activity to avoid analysing activity at different lengths between contractions. Additionally, skinfold thickness was measured with calipers at the site of the BF electrode placement to assess possible high frequency filtering effects.

Changes in EMG activity (iEMG and MF) during the maximum isometric strength tests (Post, Day 1, 2, 3) were analysed as percent change from baseline. For these analyses iEMG was not expressed relative to torque since the intent was to determine if the ability to maximally activate was affected by symptoms of muscle damage.

3.3.5 Pain Measurement

Prior to isokinetic exercise and on each of the subsequent three days, subjects were asked to report their pain level. Subjects were specifically asked to report hamstring pain with activities of daily living such as walking, stepping and squatting. Pain ratings were recorded on a scale of 0="no discomfort" to 10="walking with a limp".

3.3.6 Muscle Tenderness Measurement

Muscle tenderness was evaluated by pressing an 18 mm diameter probe, attached to a load cell, into the respective muscles at the sites identified for electrode placement. The subjects were asked to report discomfort elicited by application of the probe. The signal from the load cell was recorded during each trial and was interrupted at the point of discomfort. The force at that point was computed and subtracted from 40 N to give a tenderness value (Newham *et al.*, 1983b). Any discomfort elicited at forces above 40 N was not included. One measurement was made for each muscle. The values from each muscle were summed for analysis.

3.3.7 Plasma Creatine Kinase Activity Measurement

Plasma CK activity was determined from a finger prick blood sample. The finger was warmed, cleaned with alcohol and dried. Following puncture the initial sample of blood was removed. A 30 µl sample was then collected in a capillary tube and immediately pipetted onto a test strip for analysis. CK activity was analyzed by a colorimetric assay procedure (Reflotron, Boehringer Mannheim, Indianapolis, IN.). The system uses a plasma separation principle incorporated in the reagent carrier on the test strip. CK measurements were not made on eight subjects in the eccentric group due to unavailability of the instrument. Based on the high inter-subject variability in plasma CK response values were subjected to logarithmic transformations for statistical analysis. This approach has been used previously for these measurements (Brown *et al.*, 1997a; Brown *et al.*, 1997b; Eston and Peters, 1999).

3.3.8 Statistical Analyses

Mixed-model ANOVA was used for combined within- and between-subjects analyses while repeat measures ANOVA was used for analyses performed independently on a group. Group (eccentric versus concentric) was the only between-subjects factor used in analyses. For analyses pertaining to isokinetic exercise (Day 0) within-subject independent variables were Set (1-6) and Muscle (BF, SM, ST). For analyses pertaining to measurements made over subsequent days the within-subject independent variable was Time (pre-, post, Day 1, Day 2, Day 3). Greenhouse-Geisser corrections (GG) were applied to significant ANOVAs which did not meet Mauchly's sphericity assumption. Probability values that have been corrected are denoted by the subscript $_{GG}$. All post-hoc pairwise comparisons were performed with Bonferroni corrections. Mean values in the text are reported with the standard deviation (S.D.) while mean values in figures are displayed with the standard error of the mean (S.E.M.).

3.4 RESULTS

Typical full-wave rectified EMG recordings for a subject in the eccentric group (Fig. 3.1a) and a subject in the concentric group (Fig. 3.1b) are shown for maximum isometric and sub-maximal eccentric contractions. For the subject in the eccentric group EMG/torque was 15 μV/N·m for isometric contractions, 4.9 μV/N m for eccentric Set 1, and 7.7 μV/N·m for Set 6. For the subject in the concentric group EMG/torque was 7.6 μV/N·m for isometric contractions, 6.1

μV/N m for eccentric Set 1, and 5.9 μV/N·m for Set 6. EMG/torque was considerably lower for eccentric compared to isometric contractions.

The EMG frequency spectrums from the raw EMG signals of selected contractions from the same subjects data shown in Fig. 3.1 are displayed in Fig. 3.2a (eccentric) and Fig. 3.2b (concentric).

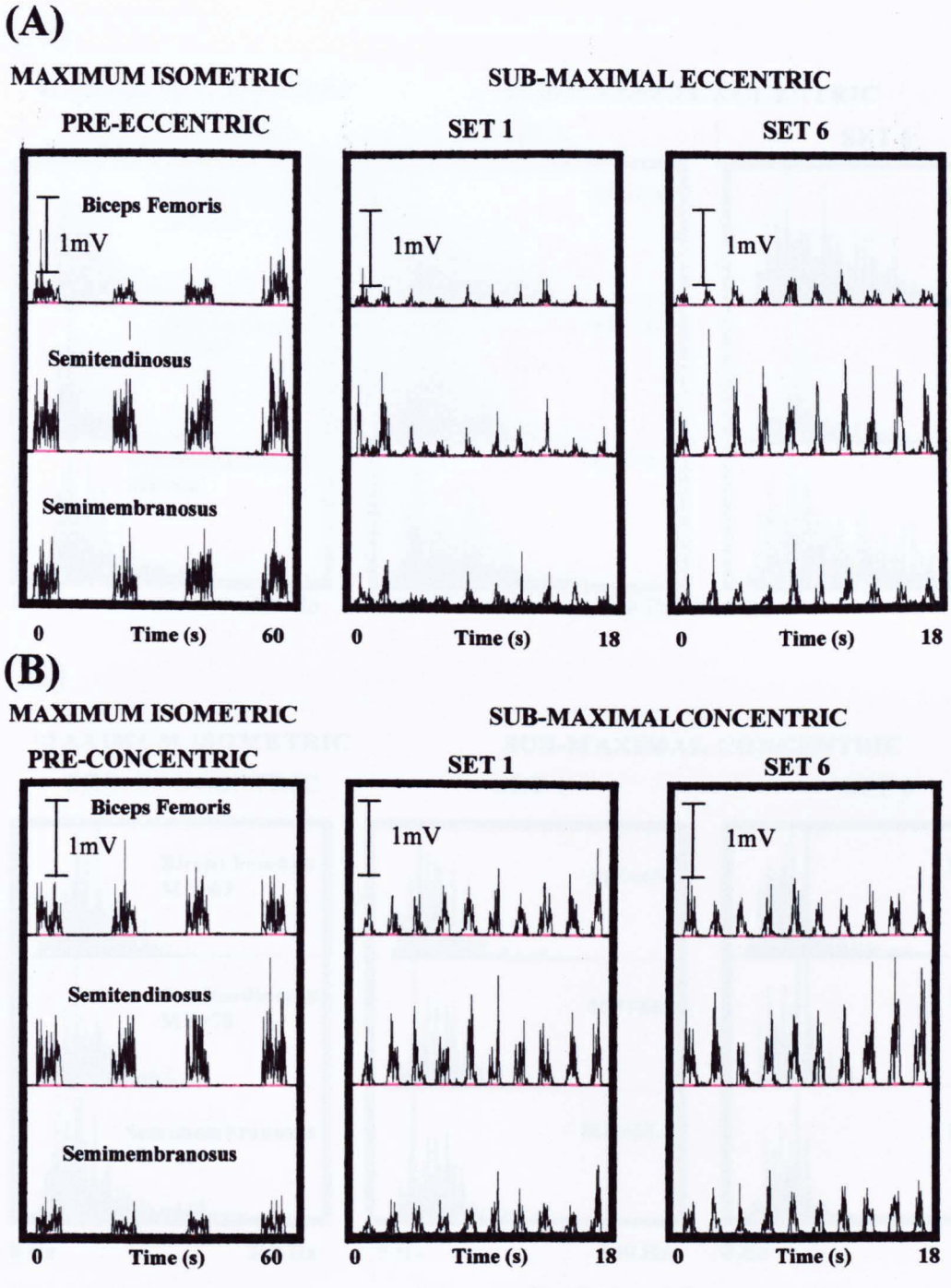

Figure 3.1: **(A)** Full-wave rectified EMG signals from one subject during four maximum isometric contractions followed by six sets of 10 eccentric isokinetic contractions at 60% of isometric strength (Set 1&6 are shown). Note the increase in magnitude of the EMG signal from Set 1 to Set 6. **(B)** Full-wave rectified EMG signals from one subject during four maximum isometric contractions followed by six sets of 10 concentric isokinetic contractions at 60% of isometric strength (Set 1&6 are shown). Note there is no increase in magnitude of the EMG signal from Set 1 to Set 6.

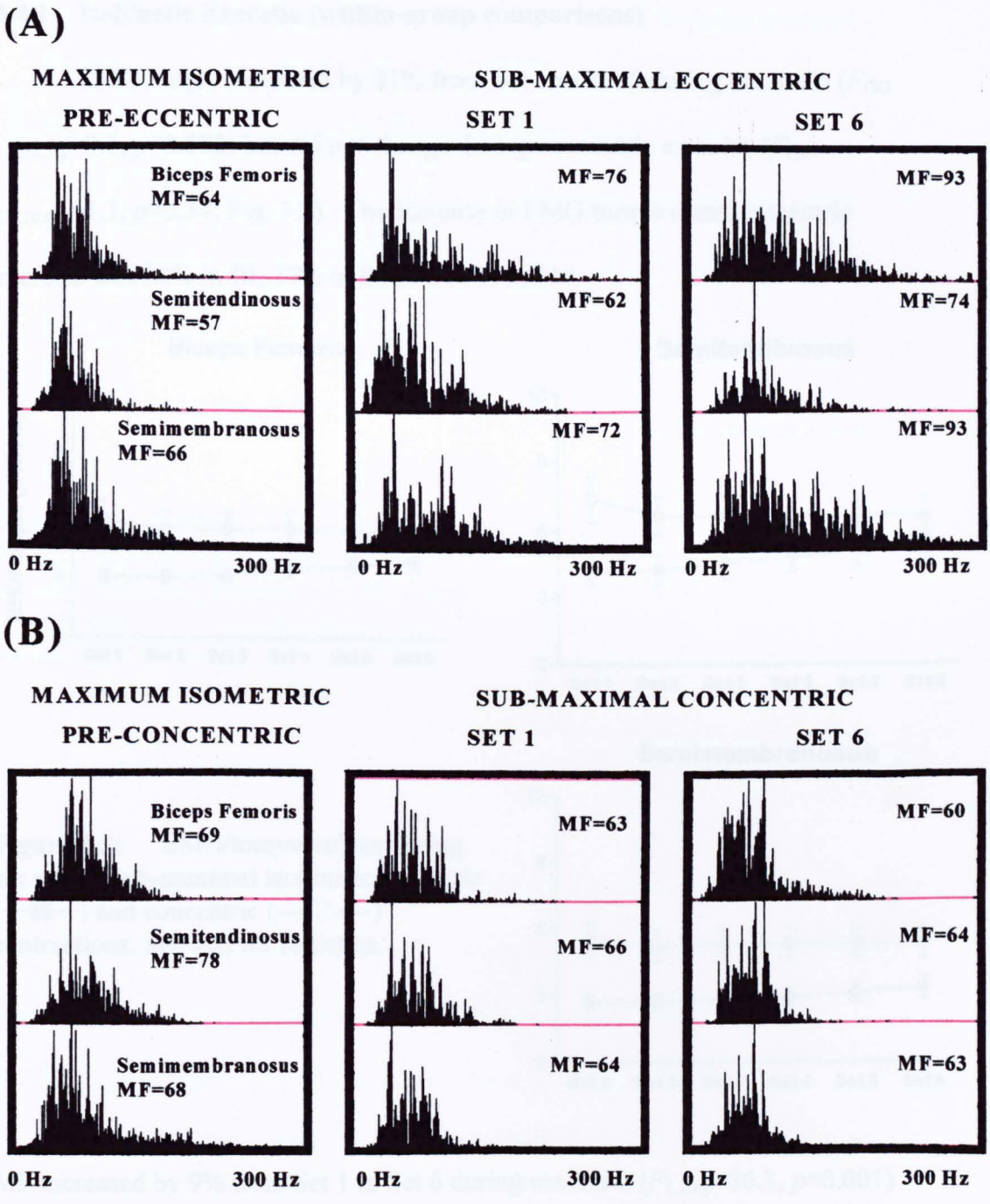

Figure 3.2: **(A)** Power spectrum of the raw EMG signal from one subject during selected maximum isometric contractions and sub-maximal eccentric isokinetic contractions. Note how the spectrum is wider for sub-maximal eccentric contractions compared to maximum isometric contractions. Additionally the spectrum broadens to the right from Set 1 to Set 6. **(B)** Power spectrum of the raw EMG signal from one subject during selected maximum isometric contractions and sub-maximal concentric isokinetic contractions. Note how the spectrum is similar between sub-maximal concentric contractions and maximum isometric contractions.

3.4.1 Isokinetic Exercise (within-group comparisons)

EMG/torque increased by 31% from Set 1 to Set 6 during eccentric ($F_{GG\ 1.6,30.5df}$=9.1, p=0.002) but did not change during concentric exercise ($F_{GG\ 1.2,10.6df}$=1.1, p=0.34; Fig. 3.3). The increase in EMG/torque during eccentric exercise was 28% in BI, 37% in ST and 25% in SM.

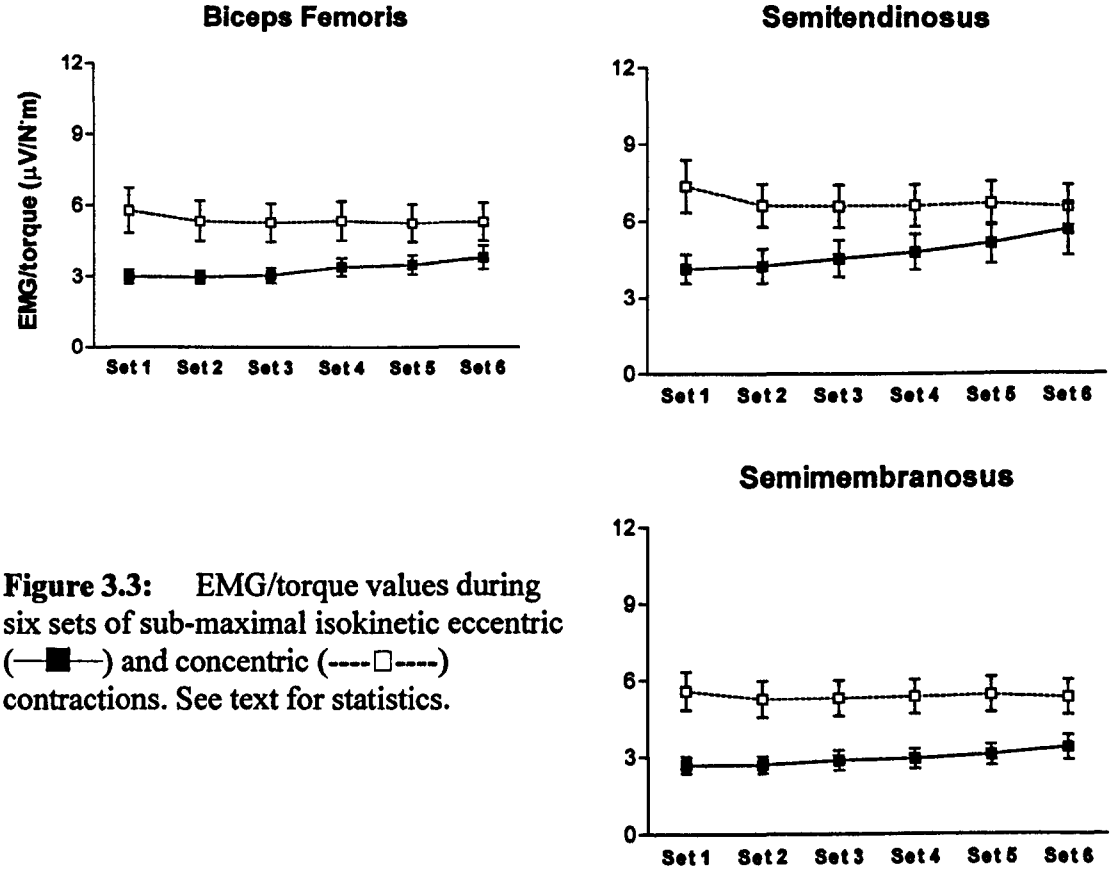

Figure 3.3: EMG/torque values during six sets of sub-maximal isokinetic eccentric (—■—) and concentric (----□----) contractions. See text for statistics.

MF increased by 9% from Set 1 to Set 6 during eccentric ($F_{1,19df}$=36.1, p<0.001) with no change during concentric exercise ($F_{1,9df}$=0.31, p=0.59; Fig. 3.4). The increase in MF during eccentric exercise was 13% in BI, 4% in ST and 9% in SM.

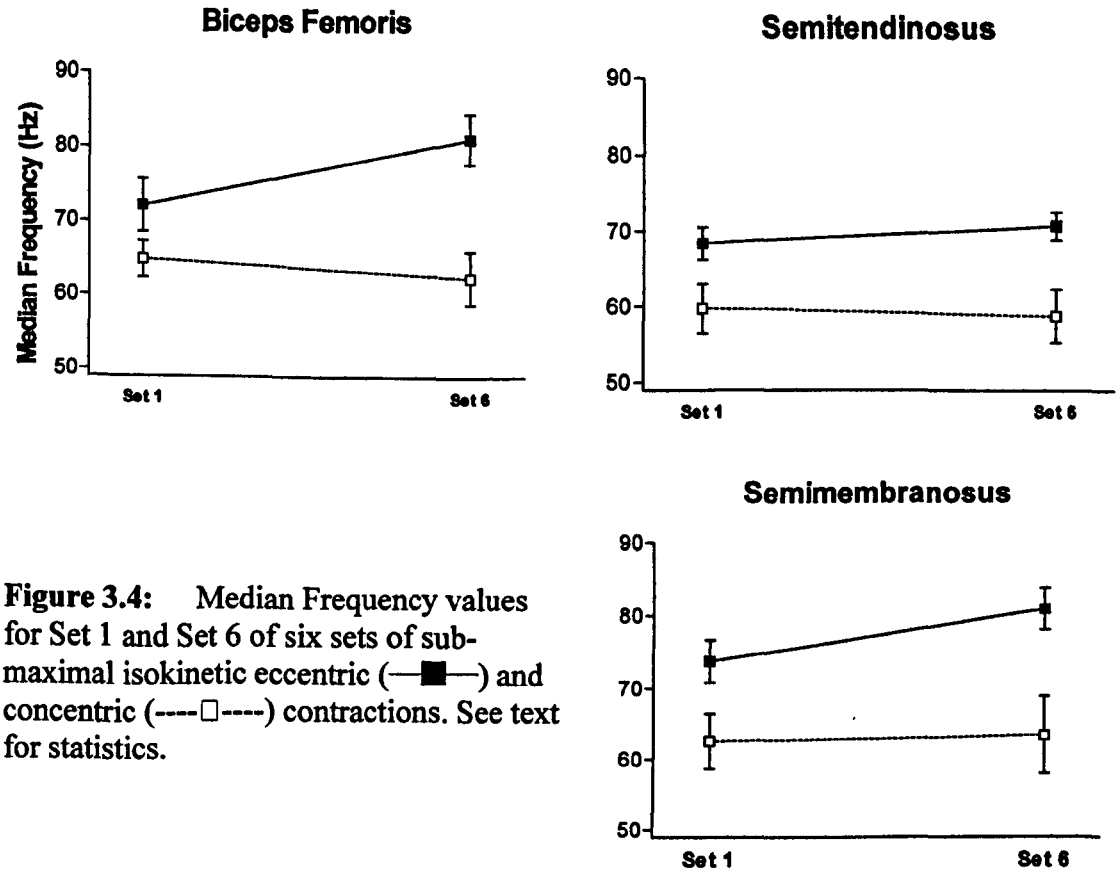

Figure 3.4: Median Frequency values for Set 1 and Set 6 of six sets of sub-maximal isokinetic eccentric (—■—) and concentric (----□----) contractions. See text for statistics.

3.4.2 Isokinetic Versus Isometric Contractions (within-group comparisons)

There were no differences in the values for EMG/torque during maximum isometric contractions from pre- to post-isokinetic exercise in either group. EMG/torque was lower for eccentric compared to isometric contractions ($F_{1,19df}$=30.2; p<0.001). EMG/torque for eccentric contractions was initially 41% of isometric, increasing to 51% by Set 6, with similar effects between muscles (Fig 3.5). EMG/torque for concentric contractions was also lower than isometric contractions ($F_{1,9df}$=13.5; p=0.005), initially 90% of isometric decreasing to 80% by Set 6.

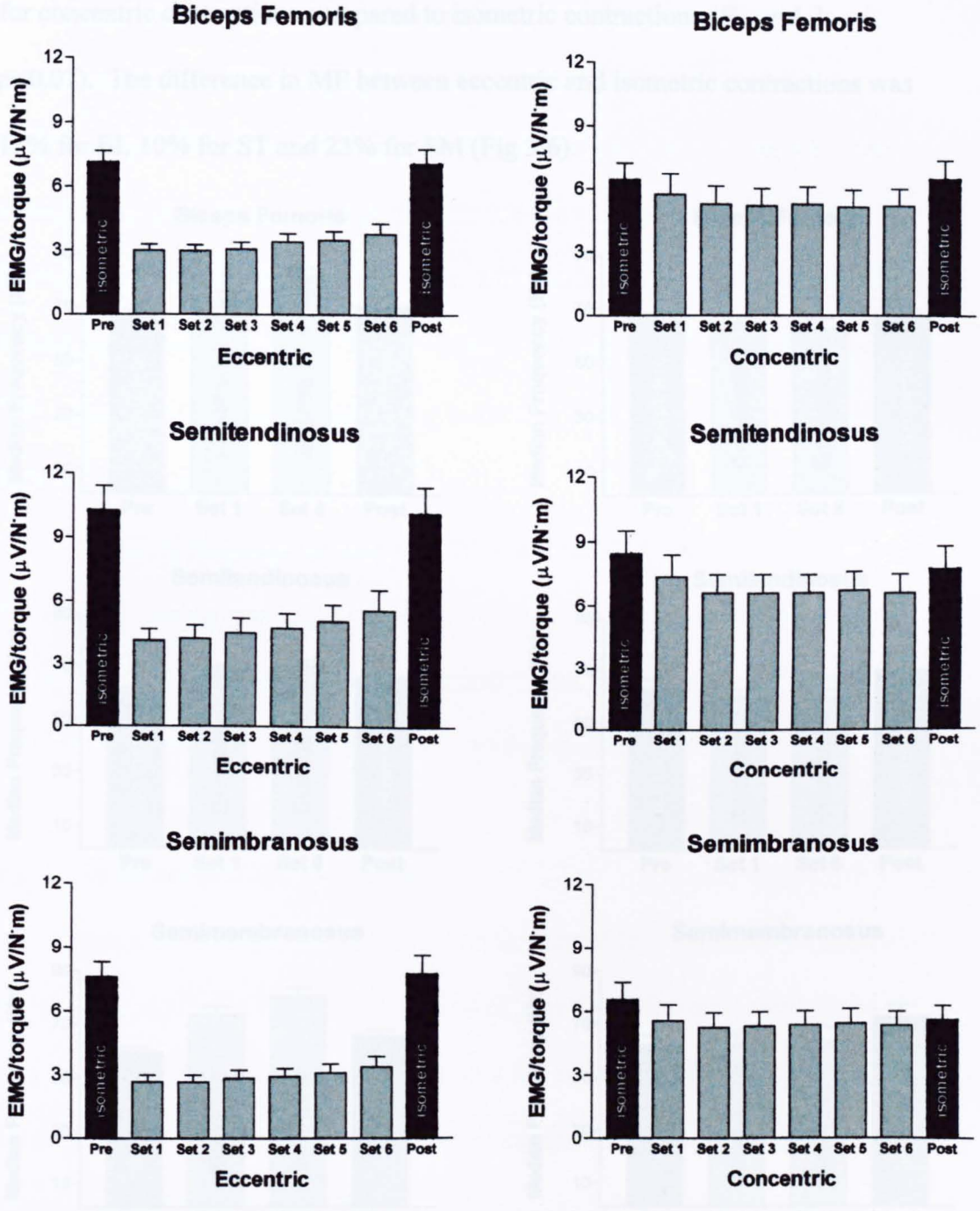

Figure 3.5: EMG/torque for maximum isometric contractions pre- and post-sub-maximal eccentric contractions (left side of figure) and concentric contractions (right side of figure). See text for statistics.

MF during isometric contractions increased from pre- to post-isokinetic exercise (10% eccentric $F_{1,19df}$=30.8; p<0.001; 11% concentric $F_{1,9df}$=11.9; p=0.005). MF was 15% higher ($F_{1,19df}$=37.3; p<0.001) for sub-maximal eccentric contractions compared to maximum isometric contractions. MF was not different

for concentric contractions compared to isometric contractions ($F_{1,9df}$=4.2; p<0.07). The difference in MF between eccentric and isometric contractions was 13% for BI, 10% for ST and 23% for SM (Fig 3.6).

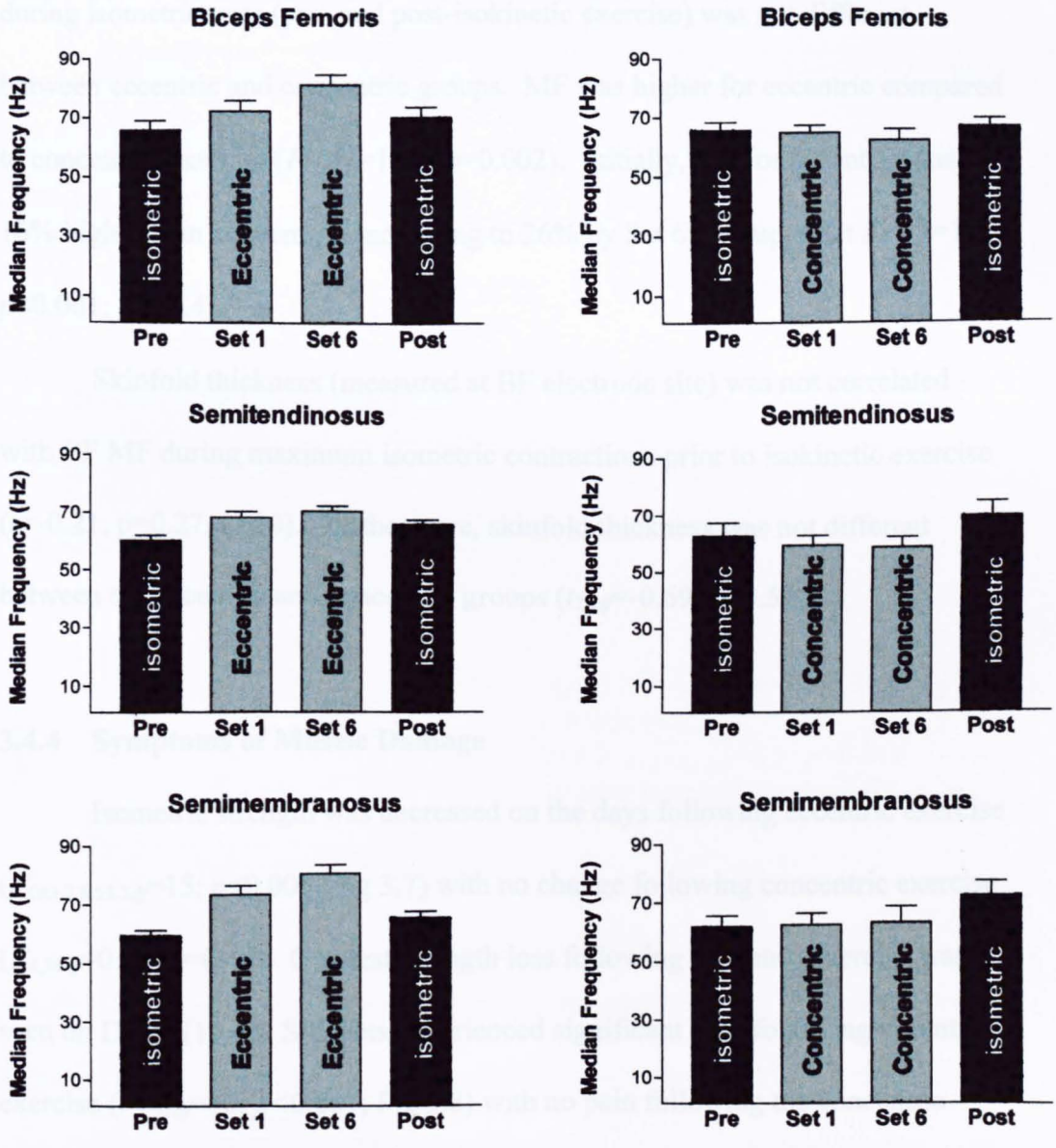

Figure 3.6: Median Frequency (MF) for maximum isometric contractions pre- and post-sub-maximal eccentric contractions (left side of figure) and sub-maximal concentric contractions (right side of figure). See text for statistics.

3.4.3 Eccentric vs. Concentric (between-group comparisons)

EMG/torque during isometric tests (pre- and post-isokinetic exercise) was not different between eccentric and concentric groups (Fig. 3.5). EMG/torque

was lower for eccentric compared to concentric exercise ($F_{1,28df}$=6.7; p=0.015). Initially, EMG/torque for eccentric exercise was 53% of concentric values, increasing to 75% by Set 6 (Group x Set $F_{GG\ 1.7,49df}$=5.1; p=0.013; Fig 3.3). MF during isometric tests (pre- and post-isokinetic exercise) was not different between eccentric and concentric groups. MF was higher for eccentric compared to concentric exercise ($F_{1,28df}$=11.2; p=0.002). Initially, MF for eccentric was 16% higher than concentric, increasing to 26% by Set 6 (Group x Set $F_{1,28df}$=15; p<0.001; Fig 3.4).

Skinfold thickness (measured at BF electrode site) was not correlated with BF MF during maximum isometric contractions prior to isokinetic exercise (r=-0.21, p=0.27, n=30). Furthermore, skinfold thickness was not different between the eccentric and concentric groups (t_{28df}=-0.69, p=0.50).

3.4.4 Symptoms of Muscle Damage

Isometric strength was decreased on the days following eccentric exercise ($F_{GG\ 2.8,53.2df}$=15; p<0.001; Fig 3.7) with no change following concentric exercise ($F_{4,36df}$=0.94; p=0.45). Greatest strength loss following eccentric exercise was seen on Day 1 (11%). Subjects experienced significant pain following eccentric exercise ($F_{3,57df}$=16; p<0.001; Fig 3.8) with no pain following the concentric exercise protocol ($F_{3,27df}$=1.9; p=0.16).

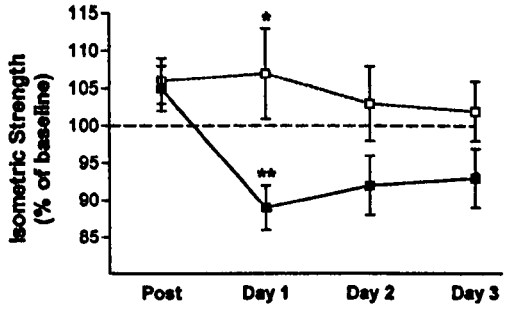

Figure 3.7: Strength loss following eccentric (-■-) and concentric (-□-) exercise. Group x Time $F_{4,112df}$=3.0; p=0.01.
* Eccentric lower than Concentric p<0.05;
** Lower than baseline p<0.01.

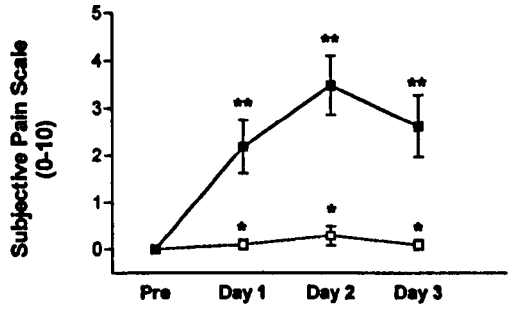

Figure 3.8: Pain following eccentric (-■-) and concentric (-□-) exercise. Group x Time $F_{GG\ 2.3,64.5df}$=9.0; p<0.001.
*Eccentric greater than Concentric p<0.01;
** Lower than baseline p<0.01.

Subjects also experienced significant muscle tenderness following eccentric exercise ($F_{GG\ 2.1,41.1df}$=4.3; p=0.018; Fig 3.9) with no tenderness experienced by any subject following concentric exercise.

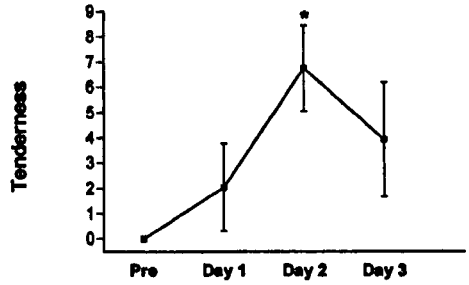

Figure 3.9: Tenderness following eccentric exercise.
Time $F_{GG\ 2.1,41.1df}$=4.3; p=0.018.
* greater than baseline p<0.05.

Plasma CK activity was increased following eccentric ($F_{3,33df}$=5.9; p=0.006) and concentric exercise ($F_{GG\ 1.5,13.7df}$=10; p=0.003). Absolute (Fig 3.10a) and percent (Fig 3.10b) increases in log CK activity were not different between eccentric and concentric groups.

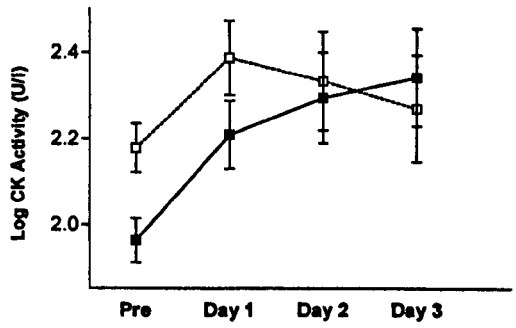

Figure 3.10a: Plasma CK activity (log values) following eccentric (-■-) and concentric (-□-) exercise. $F_{GG\ 1.9,38.9df}$=2.4; p=0.11

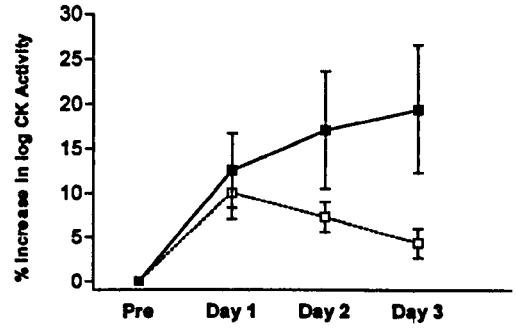

Figure 3.10b: Plasma CK activity (percent increase in log values) following eccentric (-■-) and concentric (-□-) exercise. Group x Time $F_{GG\ 1.9,38.9df}$=2.4; p=0.11.

3.4.5 EMG Activity During Isometric Tests Following Isokinetic Exercise

The iEMG values during isometric tests on Day 1,2 and 3 were not different from iEMG values during the isometric test recorded pre-isokinetic exercise (Fig. 3.11). Similarly, MF during isometric tests on Day 1,2 and 3 was not different from MF during the isometric test pre-isokinetic exercise (Fig. 3.12). However, both groups showed an increase in MF following isokinetic exercise (Time effect $F_{GG\ 2.5,70.5df}$=4.3; p=0.011). MF was increased during the isometric tests immediately following isokinetic exercise in both groups (eccentric $p<0.01$; concentric $p<0.05$).

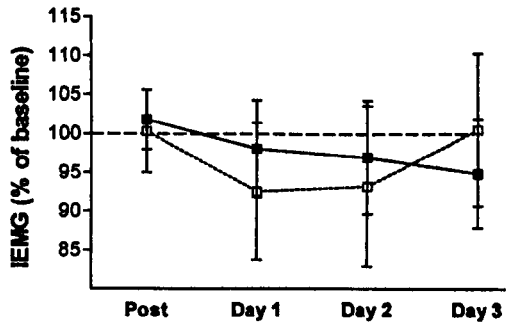

Figure 3.11: Change in iEMG following eccentric (—■—) or concentric (--□--) exercise grouped for all three muscles and expressed as a percentage of baseline (----). Group x Time $F_{GG\ 2.5,69.2df}$=0.36; p=0.74

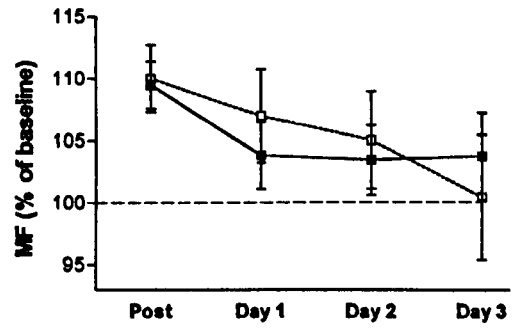

Figure 3.12: Change in MF following eccentric (—■—) or concentric (--□--) exercise grouped for all three muscles and expressed as a percentage of baseline (----). Group x Time $F_{GG\ 2.5,70.5df}$=0.47; p=0.67.

3.5 DISCUSSION

Numerous clinical investigations have demonstrated the propensity for exercise-induced muscle damage following eccentric exercise (see section 1.2). Similarly, numerous basic science studies have identified neural patterns specific to eccentric contractions (see Enoka, 1996 for review). However, the neural characteristics of an exercise bout resulting in muscle damage have not been specifically examined. In the present study, surface EMG analysis indicated differences in both motor unit activation and recruitment order, between eccentric and concentric contractions. Symptoms of muscle damage were evident following eccentric exercise, but were mostly absent following similar intensity concentric exercise. The results suggest that the muscle damage could, in part, be attributed to specific neural control of eccentric contractions.

3.5.1 Summary of EMG Activity

EMG/torque for sub-maximal eccentric contractions was 41-51% (set 1-6) of maximum isometric contractions. By contrast, EMG/torque for sub-maximal concentric contractions was 90-80% (set 1-6) of maximum isometric contractions. When directly comparing groups, EMG/torque for eccentric contractions was initially (set 1) 53% of concentric, increasing to 75% by set 6. These initial between-group values are very similar to published within-group comparisons for the plantarflexors (45-60%) (Bigland and Lippold, 1954b), knee extensors (35-50%) (Komi *et al.*, 1987; Tesch *et al.*, 1990) and elbow flexors (44-58%) (Adams *et al.*, 1992; Potvin 1997).

Despite an overall lower activation, MF was 15% higher for sub-maximal eccentric compared to maximum isometric contractions, whereas MF was 8% lower for concentric compared to isometric contractions. During isokinetic exercise, MF for eccentric contractions was 15%-26% higher than concentric values. Previous studies involving low intensity contractions of the elbow flexors (≤30% MVC compared to 60% in the present study) observed no difference (Moritani *et al.*, 1988; Potvin, 1997) or lower (Nakazawa *et al.*, 1993) frequency during eccentric compared to concentric contractions. It is possible that the hamstrings behave differently from the elbow flexors during eccentric exercise. However, the conflicting findings may also be explained by differences in contraction intensity. At low intensity contractions it may be difficult to distinguish selective recruitment patterns with surface EMG analysis (see section 3.2.3).

In the study by Tesch et al. (1990) subjects performed three sets of 32 maximum isokinetic quadriceps contractions at 3.1 $rad \cdot s^{-1}$. For eccentric contractions, initial EMG/torque was 50% of concentric, with no difference in MPF. With repeated concentric contractions EMG/torque increased and MPF decreased, while both measures were unaffected by repeated eccentric contractions. In the present study, during sub-maximal eccentric hamstring contractions the initial EMG/torque was 53% of the observed value for concentric contractions, while the initial MF was 16% higher than that observed for concentric contractions. With repeated hamstring contractions, EMG/torque and MF increased during eccentric exercise, but both were unaffected by concentric exercise. The common finding in both studies was higher frequencies for eccentric contractions over the duration of the exercise bouts.

3.5.2 Evidence of Selective Recruitment of Fast-Twitch Motor Units

The primary finding in the present study was the marked differences in MF between maximum isometric and sub-maximal eccentric contractions within subjects and the marked difference in MF between sub-maximal eccentric and concentric contractions between groups. With respect to between group differences it is important to emphasize that MF was not different between groups during maximum isometric contractions.

An increase in MF with increasing contraction intensity is thought to indicate recruitment of higher threshold motor units rather than an increase in firing frequency of previously active units (Solomonow *et al.*,1990; Hägg, 1992). In cat gastrocnemius muscles, Solomonow *et al.* (1990) demonstrated that MF increased in response to increased stimulation intensity at a fixed frequency (40 Hz). By contrast, when stimulation intensity was held constant (maximal) and frequency was increased (5-40 Hz), MF did not change. In the present study, comparatively higher initial MF and lower EMG/torque for sub-maximal eccentric contractions, indicates selective recruitment of a small number of fast-twitch motor units. With repeated eccentric contractions, concomitant increases in EMG/torque and MF suggests additional recruitment of fast-twitch motor units.

While these conclusions seem plausible there are technical and biological factors related to surface EMG which may provide alternative explanations for (1) the increase in MF during eccentric exercise and (2) the difference in MF between sub-maximal eccentric and maximum isometric contractions. Alternative explanations for the increase in MF during eccentric exercise include

increasing muscle temperature, decrease in synchronisation, increased firing rates, faster conduction velocities with muscle fibre swelling or a change in electrode orientation with repetitive movement.

a) Eccentric exercise is known to increase muscle temperatures more than concentric exercise (Nadel *et al.*, 1972). Such an effect may explain increased MF in the present study. However, although lowering muscle temperature has been shown to decrease MF no change in MF was evident with muscle warming (Holewijn and Heus, 1992). The effect of increased muscle temperature on MF has not been examined with respect to eccentric contractions.

b) During voluntary contractions motor unit action potentials are asynchronous or stochastic in nature (Hägg, 1992; Merletti *et al.*, 1992). Motor unit synchronisation refers to the tendency for two or more motor units to fire nearly simultaneously more often than might be expected by chance alone (Nordstrom *et al.*, 1990; Kamen and Caldwell, 1996) and may in part explain the decrease in frequency with sustained contraction (Hägg, 1992). It is possible, though unlikely, that an opposite effect could occur with repeated eccentric contractions. Since fewer motor units are activated for eccentric contractions the recruitment of units may become increasingly random causing a wider frequency spectrum.

c) An obvious explanation for the increase in MF would be an increase in the firing rates of motor units of all types. However, as previously stated, MF appears to be relativley insensitive to changes in firing frequencies at high contraction intensities (Solomonow *et al.*, 1990).

d) Eccentric exercise may result in increasing conduction velocities independent of motor unit type. For example an increase in fibre diameter secondary to swelling would theoretically increase conduction velocity and MF (Kamen and Caldwell, 1996). However, evidence for such a theory is lacking.

e) The fact that subjects were sitting on the electrodes during hamstring contractions may have changed the orientation of the electrodes with respect to the motor point of the respective muscles. For example MF would increase if the electrodes moved closer together or became oriented more in line with the muscle fibres (Kamen and Caldwell, 1996). However, it is difficult to explain why such a change in electrode orientation would have occured with eccentric contractions but not concentric contractions given that all other conditions were the same.

Alternative explanations for the difference in MF between maximum isometric contractions and sub-maximal eccentric contractions include differences in the muscle length at which peak activity occurred, greater synchrony with isometric contractions, movement artifact and ambient noise during eccentric contractions.

a) The shorter the muscle length, the larger the muscle fibre diameter, the faster the conduction velocity, the higher the MF (Morimoto, 1986). Isometric contractions were performed at an intermediate muscle length (0.8 rad) while eccentric contractions were performed from 1.6 to 0 rad. If peak EMG activity occurred before 0.8 rad it is possible that higher frequencies would have been recorded with eccentric contractions. However, it is more likely that peak EMG activity occurred later in the ROM given the speed of the dynamometer and the time required for the subject to initiate the eccentric contraction once the dynamometer arm began its upward movement.

b) It is possible that greater motor unit synchrony occurs with maximum isometric contractions compared to sub-maximal eccentric contractions. Such a difference may be related to the substantially lower level of activation for the eccentric contractions.

c) The length of the FFT was the same for computing the frequency spectrum for the isometric and eccentric contractions despite contrasting contraction times. The FFT covered 4.096 s which included most of the activity in a 5 s isometric contraction. In contrast, 4.096 s covered two eccentric contractions and therefore computations included low power movement artifact and ambient noise during the relaxation phases. While it is unclear whether such noise would increase the low or high frequency component of the spectrum it is important to note that similar noise would have been present during the computations for concentric contractions. However, MF for sub-maximal concentric contractions was actually slightly lower than for maximum isometric contractions.

Despite possible alternative explanations for higher initial MF and increased MF with repeated eccentric contractions selective recruitment of fast-twitch motor units remains an logical conclusion.

3.5.3 Evidence of Muscle Damage

Strength loss (peak day 1), pain (peak day 2), muscle tenderness (peak day 2) and elevated plasma CK activity (peak day 3) were clear symptoms of muscle damage following eccentric exercise. Conversely, concentric exercise did not affect strength, pain or muscle tenderness although there was a moderate increase in plasma CK activity (peak day 1). A similar increase in serum CK has

been reported one day following 6 sets of 10 maximum concentric isotonic contractions of the elbow flexors (Clarkson *et al.*, 1986). The changes in pain and plasma CK following eccentric exercise were comparable to changes in serum CK activity demonstrated by Clarkson *et al.* (1987) following 6 sets of 10 eccentric isotonic hamstring contractions at 100% of concentric strength.

The pattern of strength changes following eccentric hamstring exercise was somewhat different from studies using other muscle groups. Strength was not decreased immediately following eccentric or concentric exercise, suggesting that neither exercise bout resulted in fatigue. Although eccentric contractions have been shown to be fatigue resistant (Tesch *et al.*, 1990; Hortobágyi *et al.*, 1996c), most studies demonstrating muscle damage have shown strength loss immediately post-exercise (Cleak and Eston, 1992b; Nosaka and Clarkson, 1995; Teague and Schwane, 1995; Eston *et al.*, 1996). Nosaka and Clarkson (1995) demonstrated a 50% reduction in strength immediately following three sets of 10 eccentric isotonic contractions of the elbow flexors performed at 80% of isometric strength. Similarly, Teague and Schwane (1995) demonstrated 18% reduction in strength immediately post one set of 10 eccentric isotonic contractions of the elbow flexors at 60% of isometric strength. The disparity between these results and the present study may be attributed to differences in the muscle group studied or contraction type (slow isotonic versus fast isokinetic). It is not known to what extent the strength loss immediately following eccentric contractions reflects muscle damage, fatigue or other processes (see section 2.1.2)

Despite a significant reduction in strength, EMG activity (iEMG and MF) did not change during isometric strength tests on Day 1, 2 or 3. One day

following eccentric exercise, isometric strength was 89% of baseline (significant decrease) but iEMG and MF during the isometric tests were 98% and 104% of baseline, respectively (non-significant effects). This suggests that normal motor unit activation was maintained despite symptoms of muscle damage. Pain did not inhibit motor unit activation during maximum isometric contractions. This is in agreement with the findings of Saxton and Donnelly (1996) who demonstrated that strength loss following eccentric exercise was unaffected by superimposing supra-maximal stimulation during maximum voluntary isometric contractions.

3.5.4 Summary

In summary, iEMG (reflecting motor unit activation) and MF (reflecting recruitment patterns) were markedly different for eccentric compared to concentric hamstring contractions performed at the same relative intensity. The results suggest reduced motor unit activation and selective recruitment of fast-twitch motor units eccentric exercise. Furthermore, there was evidence of additional recruitment of fast-twitch motor units as eccentric exercise continued. Eccentric exercise resulted in moderate symptoms of muscle damage which were mostly absent following concentric exercise. These results are consistent with the hypothesis that muscle damage is due to high stress on a small number of active fibres during eccentric exercise. Selective recruitment of fast-twitch motor units may, in part, explain previous studies showing damage primarily in fast-twitch fibres.

CHAPTER 4

THE ROLE OF PASSIVE MUSCLE STIFFNESS ON THE SYMPTOMS OF EXERCISE-INDUCED MUSCLE DAMAGE

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4.1 ABSTRACT

Passive hamstring stiffness was measured during an instrumented straight leg raise (SLR) stretch in 20 subjects (11 men, 9 women) who were subsequently classified as "Stiff"(n=7), "Normal" (n=6) or "Compliant" (n=7). Passive stiffness was 78% higher in Stiff (36.2±8.7 $Nm \cdot rad^{-1}$) compared to Compliant subjects (20.3±4.8 $Nm \cdot rad^{-1}$). Subjects then performed six sets of 10 isokinetic (2.6 $rad \cdot s^{-1}$) sub-maximal eccentric contractions of the hamstring muscle group at 60% of isometric strength. Symptoms of muscle damage were documented by changes in isometric hamstring strength, pain, muscle tenderness and plasma creatine kinase (CK) activity on the following three days. Strength loss ($p<0.01$), pain ($p<0.05$), muscle tenderness ($p<0.05$) and elevations in plasma CK activity ($p<0.05$) were significantly greater in Stiff compared to Compliant subjects. Greater symptoms of muscle damage in subjects with stiffer hamstrings are consistent with the sarcomere strain theory of muscle damage. It is proposed that the strain imposed by active lengthening of stiff muscles is transferred from a rigid tendon-aponeurosis complex to the muscle fibres, resulting in myofibrillar strain. In compliant muscles the tendon-aponeurosis complex is able to absorb lengthening which thereby limits myofibrillar strain.

4.2 INTRODUCTION

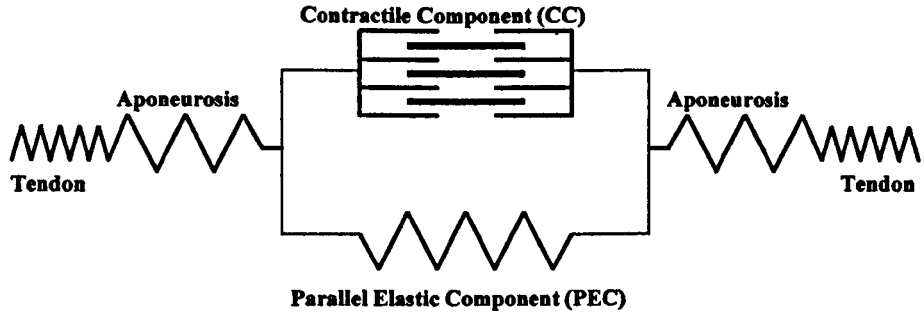
4.2.1 The Role of Muscle Length in Exercise-Induced Muscle Damage

Studies using simulated eccentric contractions in animal models (Lieber and Fridén, 1993; Brooks *et al.*, 1995; Hunter and Faulkner, 1997) and voluntary eccentric contractions in humans (Newham *et al.*, 1988; Child *et al.*, 1998) have demonstrated greater damage with contractions at long versus short muscle lengths (see section 2.2.1). Consistent with these findings, Armstrong *et al.* (1991) suggested that damage occurs at muscle lengths on the "descending limb" of the length-tension curve where myofilament overlap is compromised by sarcomere strain (see section 2.2.2). During eccentric contractions, sarcomere elongation is not uniform, with most sarcomeres maintaining length while others in series are stretched beyond the point of filament overlap (Huxley and Peachey, 1961; Flitney and Hirst, 1978; Morgan, 1990). Such excessive stretch has been referred to as sarcomere "give" (Flitney and Hirst, 1978) or "popping" (Morgan, 1990). Damage is thought to result from cyclic strain of "popped" sarcomeres which fail to recover filament interdigitation between cross-bridge cycles (Morgan, 1990; Jones *et al.*, 1997). Evidence in support of this theory was provided by studies which observed a shift to the right in the length-tension curve following damaging eccentric exercise (Wood *et al.*, 1993; Saxton *et al.*, 1996; Jones *et al.*, 1997). These effects were explained by shortening of intact sarcomeres secondary to strain of damaged serial sarcomeres. Greater lengthening of the muscle-tendon unit was required for the intact sarcomeres to reach optimal length.

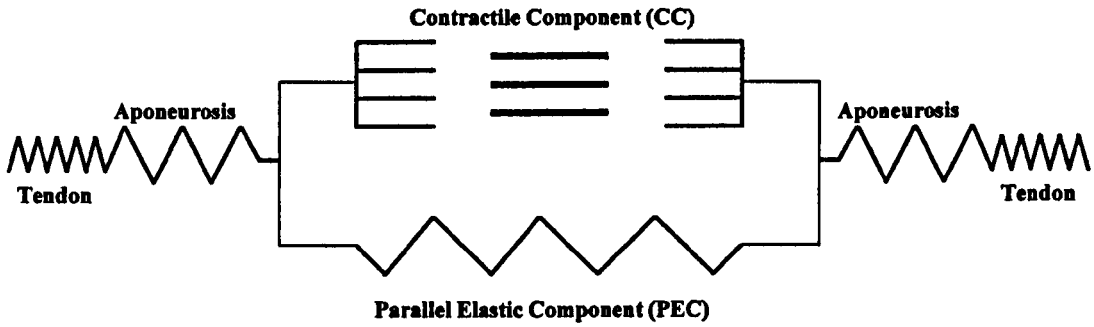
4.2.2 The Role of the Tendon and Aponeurosis in Muscle Function

Tendon-aponeurosis compliance plays an important role in maintaining myofilament overlap by reducing muscle fibre strain as the length of the muscle-tendon unit is increased (Griffiths, 1991; Legreneur *et al.*, 1992; Lieber *et al.*, 1992; Roberts *et al.*, 1996). Griffiths (1991) demonstrated muscle fibre shortening despite lengthening of the entire muscle-tendon unit during eccentric contractions of cat planarflexors. This effect was attributed to tendon compliance. Similar effects were demonstrated in turkeys during running. Muscles were shown to function relatively isometrically with the tendons primarily accounting for the lengthening and shortening of muscle tendon units (Roberts *et al.*, 1996). The ability of the tendon and aponeurosis to absorb lengthening imposed by eccentric contractions may play a role in the occurrence of sarcomere strain. A compliant tendon-aponeurosis complex may reduce sarcomere strain thereby avoiding myofibrillar disruption. The mechanical model of skeletal muscle (Lieber, *et al.*, 1992) described in section 1.4.1 can be used to illustrate the potential effect of tendon-aponeurosis compliance on sarcomere strain (Fig. 4.1). In the relaxed condition (Fig. 4.1a) the muscle is at resting length. In the muscle with a stiff tendon-aponeurosis complex (Fig. 4.1b) the lengthening imposed by eccentric contraction is transferred from a rigid tendon and aponeurosis to the sarcomere. In the muscle with a compliant tendon-aponeurosis complex (Fig. 4.1c) the lengthening imposed by eccentric contraction is absorbed by the tendon and aponeurosis allowing the sarcomeres to shorten.

(a) **Relaxed Muscle**



(b) **Eccentric Contraction**
Stiff Tendon-Aponeurosis Complex



(c) **Eccentric Contraction**
Compliant Tendon-Aponeurosis Complex

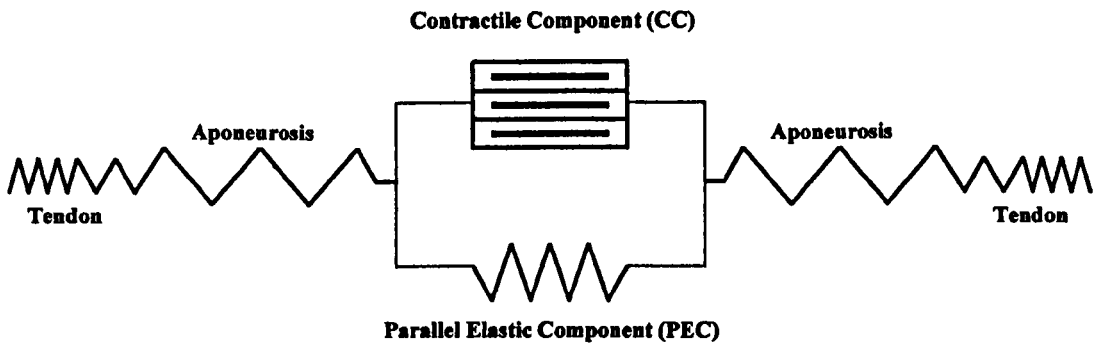


Figure 4.1: Mechanical muscle model of sarcomere strain versus sarcomere shortening during an eccentric contraction in a muscle with a stiff (b) versus a compliant (c) tendon-aponeurosis complex (see text for further description).

This model can be used to explain the findings of Griffiths (1991) who demonstrated muscle fibre shortening during eccentric contractions in cat muscles. However, it is important to note that sarcomere lengths are highly variably during eccentric contractions (Huxley and Peachey, 1961; Flitney and Hirst, 1978; Morgan, 1990). It is clear that not all sarcomeres will be strained beyond myofilament overlap as depicted in the middle figure. Additionally it is unlikely that any sarcomeres would achieve full overlap during eccentric contractions as depicted in Fig. 4.1c. The model serves to illustrate relative differences in sarcomere mechanics.

The relative differences in the behaviour of tendon, aponeurosis and muscle during both passive and active loading have been well described in animal models (Lieber *et al.*, 1992; Trestik and Lieber, 1993; Zuurbier *et al.*, 1994). During maximum isometric contractions of frog semitendinosus muscles, aponeurosis strain was 8% while tendon strain was 2% (Lieber *et al.*, 1992). When frog gastrocnemius muscles were passively strained until the tension was equivalent to maximum tetanic tension, tendon strain was 2%, aponeurosis strain was 3% and muscle strain was approximately 15% (Trestik and Lieber, 1993). However, strain was not distributed uniformly along the aponeurosis, with 6% strain in the most proximal region closest to the muscle fibres. Based on tendon-aponeurosis compliance, it was predicted that sarcomeres could shorten by 0.5μm at maximum active tension. During passive strain of frog semitendinosus muscles to a tension equivalent to maximum isometric contraction, tendon strain was 4% and aponeurosis strain was 8% (Trestik and Lieber, 1993). Zuurbier *et al.*, (1994) showed 14% aponeurosis strain in rat gastrocnemius muscles during

isometric contractions 5mm beyond optimal length. Again, strain was highest in the most proximal region closest to the muscle fibres.

4.2.3 Passive Stiffness of Human Skeletal Muscle

In humans, the extensibility of the relaxed muscle-tendon unit can be quantified by the relationship of joint torque to range of motion (ROM) during passive stretch (Hufschmidt and Mauritz, 1985; Watts *et al.*, 1986; Wiegner and Watts, 1986; Gajdosik *et al.*, 1990; Gajdosik, 1991; Halbertsma and Goeken, 1994; Magnusson *et al.*, 1995a; Magnusson *et al.*, 1996; McHugh *et al.*, 1998). The torque/ROM curve provides a measure of passive muscle stiffness and reflects the extensibility of the parallel elastic component (PEC) and the tendon-aponeurosis complex. The contractile component (CC) is thought to contribute negligible tension during passive elongation (Lieber *et al.*, 1992).

Passive hamstring stiffness has been shown to be inversely related to maximum straight leg raise (SLR) ROM ($r=-0.81$) (McHugh *et al.*, 1998) and toe touch flexibility ($r=-0.74$) (Magnusson *et al.*, 1997). In subjects with a mean maximum SLR ROM of 98° (1.7 rad), stiffness from 20°-50° accounted for 66% of the variability in maximum SLR ROM (McHugh *et al.*, 1998). These findings indicated that individuals with lower maximum ROM have greater muscle stiffness throughout the ROM and not just at the end of the ROM. Passive muscle stiffness in the functional ROM has not been studied with respect to muscle function in humans. The purpose of this study was to examine the effect of passive muscle stiffness on symptoms of exercise-induced muscle damage following a bout of eccentric exercise. It was hypothesized that, in a stiffer muscle, a greater proportion of the strain imposed by eccentric contraction is

transferred to the muscle fibre by a rigid tendon-aponeurosis complex, resulting in myofibrillar disruption and greater subsequent symptoms of damage.

4.3 METHODS

4.3.1 Experimental Design

The role of passive hamstring stiffness on symptoms of muscle damage was studied in the 20 subjects (11 men, 9 women) from the eccentric group described in Chapter 3 (section 3.3.1). Subjects characteristics are described in table 4.1.

Table 4.1: Mean (±S.D.) for age height and body mass of the male and female subjects.

	Age (yrs)	Height (cm)	Body Mass (kg)
Men (n=11)	28.7±5.6	180.6± 7.4	77.9±9.5
Women (n=9)	28.3±6.1	164.6±15.7	63.7±12.9

Subjects were without orthopaedic injury and had not been involved in any weight training in the preceding months. On the initial test day, passive hamstring stiffness was measured during an instrumented straight leg raise (SLR) stretch. A baseline measure of maximum isometric hamstring strength was then made. Subjects then performed six sets of 10 isokinetic eccentric hamstring contractions at 60% of maximum isometric strength. On the following three days maximum isometric strength, pain and muscle tenderness were assessed. Plasma creatine kinase (CK) activity was measured in 12 subjects at baseline and on each of the following three days. The protocols for isokinetic exercise (section 3.3.3), isometric strength tests (section 3.3.2), pain (section 3.3.5), muscle tenderness

(section 3.3.6) and plasma CK activity (section 3.3.7) are described in detail in Chapter 3.

4.3.2 Measurement of Passive Hamstring Stiffness

The instrumentation for measurement of passive hamstring stiffness has been described previously (McHugh *et al.*, 1998). In the present study a 60° (1.05 rad) SLR stretch was applied using an hydraulically powered motorised rotating frame aligned with the hip joint (Scientific Stretching, Nova Scotia, Canada) (Fig. 4.2).

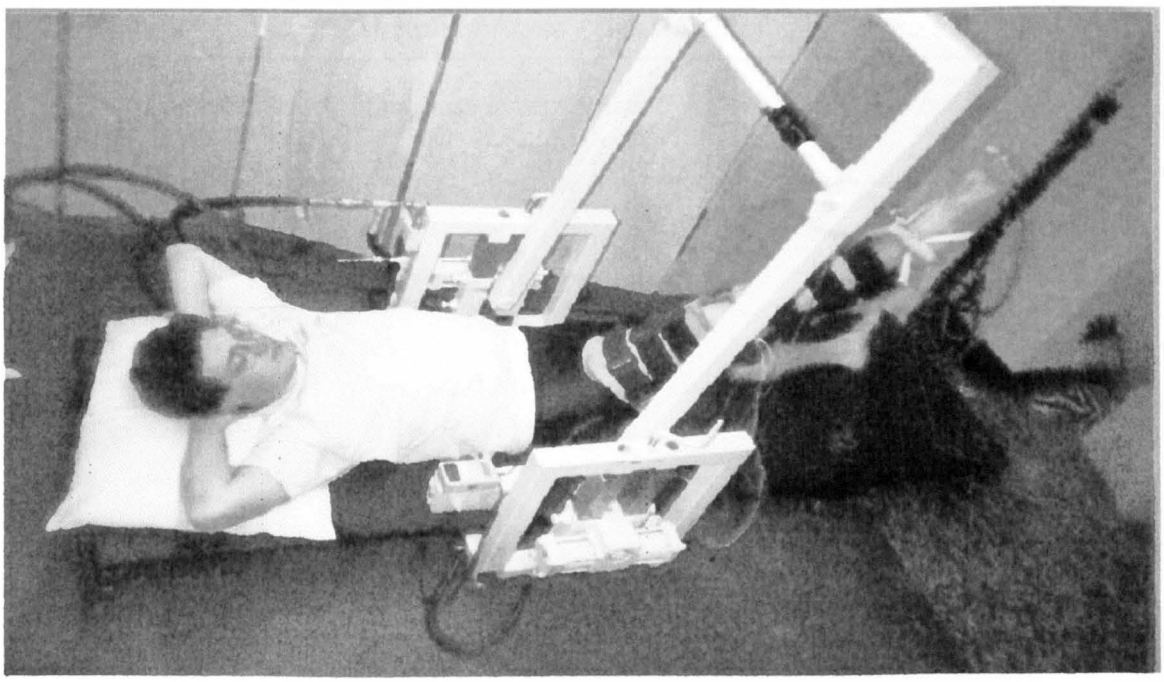


Figure 4.2: Instrumented SLR stretch on an hydraulically motorised moving frame. The frame is attached to the ankle by a chain in series with a load cell. An electrogoniometer is aligned with the hip and the knee is braced in extension.

The ankle was attached to the moving frame by a chain in series with a load cell (Kistler Instruments, Amherst, NY). The frame was adjustable to the subject's limb length to ensure that the direction of pull was at 90° to the limb during the stretch. During the stretch the knee was braced in full extension and

the contralateral limb was fixed to the table with a strap to limit pelvic rotation. Hip flexion ROM was recorded from an electrogoniometer (Penny & Giles, Gwent, U.K.) attached to the frame and aligned with the hip joint. Resistance to stretch recorded from the load cell was corrected for the limb mass as described previously (McHugh *et al.*, 1992; Halbertsma and Goeken, 1994; McHugh *et al.*, 1998). The force generated by the limb mass was measured at zero degrees SLR ROM which was defined as the point when the limb lost contact with the table at the initiation of the stretch procedure (Fig. 4.3). The measured forces during the stretch were corrected for limb force to provide a measure of resistance to stretch:

Resistance to Stretch = Measured Force - (cosine SLR ROM x Limb Force)

At 90° the contribution of limb mass is zero (cosine 90°=0) and all the measured force is passive tissue tension. At 0° (cosine 0°=1) passive tissue tension is regarded as zero.

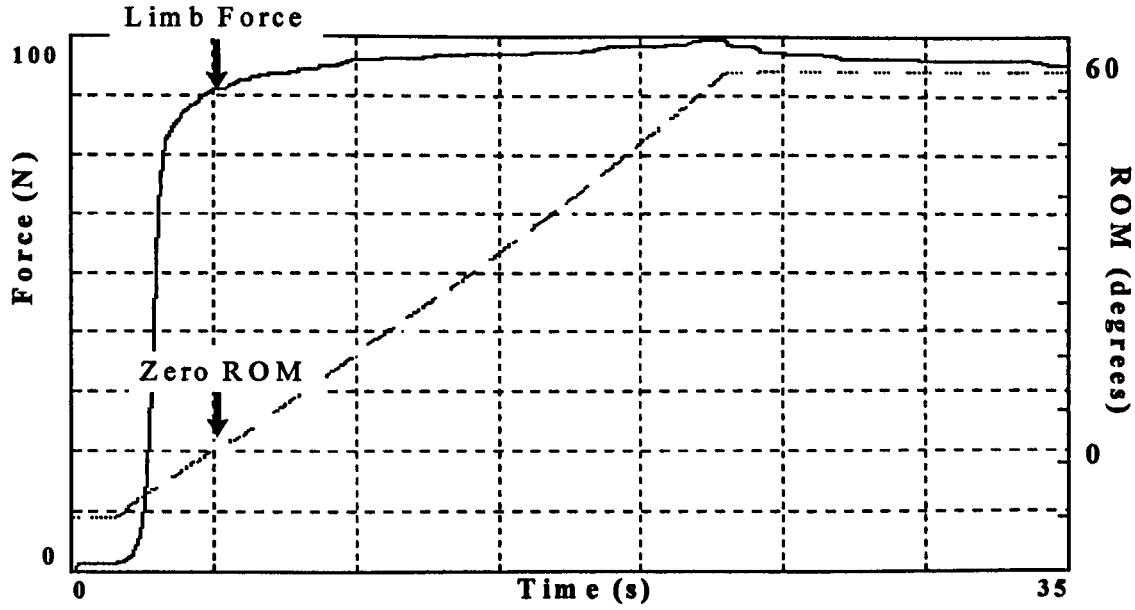


Figure 4.3: Limb mass correction for one subject during the SLR stretch. Limb force is defined as the point at which the rise in force plateaus as the limb is initially lifted from the table. The ROM at that point is designated zero and the limb is then raised to 60°.

Surface electromyographic (EMG) signals were recorded from pairs of Ag/AgCL surface electrodes placed over the rectus femoris, biceps femoris, semimembranosus and semitendinosus muscles. The skin was shaved, cleaned and abraded prior to electrode placement. Rectus femoris electrodes were placed 2 cm apart midway along a line between the anterior superior iliac spine and the superior pole of the patella. Electrode placements for the hamstring muscles and EMG instrumentation were described in detail in Chapter 3 (section 3.3.4). Rectus femoris activity was recorded to ensure the subject was not actively assisting the stretch. Hamstring EMG activity was recorded to ensure that contractile force production did not contribute to the measurement of passive stiffness.

Force measured during the instrumented SLR stretch was multiplied by the subject's limb length to provide a measurement of joint torque. Torque/ROM curves were calculated from 0 to 60° (0 to 1.05 rad) (Fig. 4.4). Passive stiffness was defined as the increase in torque from 20° to 50° (0.35 to 0.87 rad) in the absence of EMG activity. Previous studies have shown this region of the torque/ROM curve to be linear (Halbertsma and Goeken, 1994; McHugh *et al.*, 1998). The mean of two stiffness measurements was recorded. The two tests were separated by 60 s. Subjects were categorized into three groups ("Stiff", n=7; "Normal", n=6; "Compliant", n=7) based on the measurement of passive hamstring stiffness. Following the measurement of passive stiffness a goniometric measurement of maximum passive SLR ROM was made.

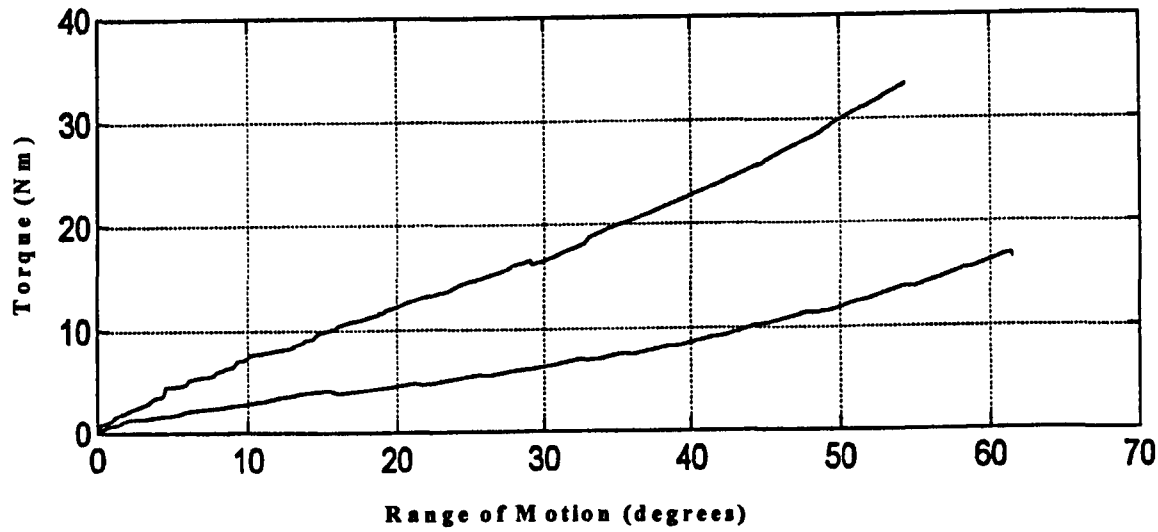


Figure 4.4: Torque/ROM curves for a subject in the "Stiff" group (top line) and a subject in the "Compliant" group (bottom line). The curves are relatively linear in the 20°-50° range.

4.3.3 Statistics

Mixed model analysis of covariance (ANCOVA) was used to test changes in isometric strength, pain and muscle tenderness between Stiff, Normal and Compliant subjects, with gender and body mass as control variables. Post-hoc tests with Bonferroni corrections were used to test specific differences between Stiff and Compliant subjects. The critical value of the *F* ratio was adjusted according to the Greenhouse-Geisser Epsilon correction procedure when Mauchly's sphericity assumption was not met. Degrees of freedom (*df*) which have been corrected by the Greenhouse-Geisser Epsilon are denoted by the subscript $_{GG}$. Based on the high inter-subject variability in plasma CK response, values were subjected to logarithmic transformations for statistical analysis. This approach has been used previously for these measurements (Brown *et al.*, 1997a; Brown *et al.*, 1997b; Eston and Peters, In Press). However, given the small uneven group sizes for plasma CK activity (Stiff n=4, Normal n=3, Compliant n=5) nonparametric statistics were used to analyse changes in log plasma CK activity. Differences in percent increase in log plasma CK activity between Stiff, Normal and Compliant subjects were analyzed by Kruskal-Wallis *H* tests. Where significant *H* values were found post-hoc Mann-Whitney *U* tests were used to compared Stiff and Compliant groups. Bonferroni corrections were applied to *p* values from Mann-Whitney *U* tests. Mean values in the text are reported with the standard deviation (S.D.) while mean values in figures are displayed with the standard error of the mean (S.E.M.).

4.4 RESULTS

4.4.1 Passive Hamstring Stiffness

The subjects were divided into three groups based on passive hamstring stiffness: Compliant (n=7; 6 women, 1 man) 20.3±4.8 $Nm \cdot rad^{-1}$; Normal (n=6; 2 women, 4 men) 27.1±1.0 $Nm \cdot rad^{-1}$; Stiff (n=7; 1 woman, 6 men) 36.2±8.7 $Nm \cdot rad^{-1}$ (Fig. 4.5). In one subject, rectus femoris activity was present during the first SLR stretch, but no activity was present during the second stretch. The stiffness value was computed from the second stretch only. All other subjects exhibited electrical silence in the rectus femoris and hamstring muscles from 0 to 0.87 rad during SLR stretches. There was no significant difference between the stiffness value for the first and second stretch across stiffness groups. Passive stiffness was significantly affected by gender (t_{18df}=3.0, p<0.01) and was correlated to body mass (r=0.76, p<0.01) . Therefore, the effect of passive muscle stiffness on symptoms of muscle damage was analyzed on means adjusted for gender and body mass (ANCOVA).

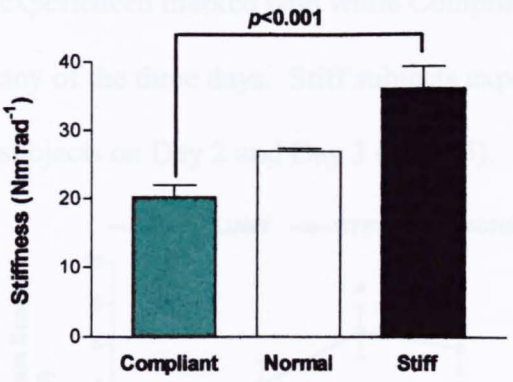


Figure 4.5: Passive hamstring stiffness during the instrumented SLR stretch for the Compliant, Normal and Stiff groups. Group effect $F_{2,17df}$=12.8, p<0.001.

4.4.2 Effect of Passive Hamstring Stiffness on the Symptoms of Muscle Damage

Strength loss following eccentric exercise was markedly different between stiffness groups (Group by Time $F_{8,60df}$=2.91, p=0.008; Fig. 4.6). Based on means adjusted for body mass and gender, subjects with stiff hamstrings experienced 22% strength loss on Day 1 and Day 2, and 31% strength loss on Day 3. By contrast subjects with compliant hamstrings did not experience any strength loss.

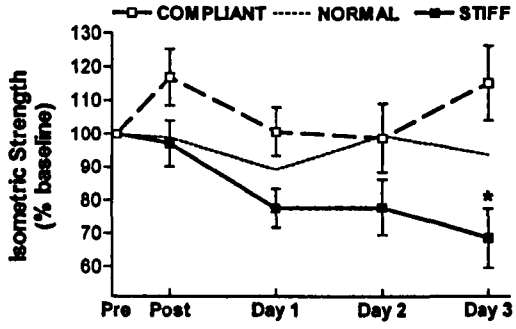


Figure 4.6: Isometric hamstring strength following eccentric exercise, expressed as a percent of baseline (pre-eccentric exercise). Means are adjusted for gender and body mass. Group (Compliant, Normal, Stiff) by Time (Pre, Post, Day 1, 2, 3) $F_{8,60df}$=2.91, p=0.008. * Stiff lower than Compliant p<0.05 (post-hoc comparison with Bonferroni corrections).

Pain experienced on the days following eccentric exercise was different between stiffness groups ($F_{2,15df}$=3.8, p=0.045; Fig. 4.7). Stiff subjects experienced marked pain while Compliant subject experienced minimal pain on any of the three days. Stiff subjects experienced greater pain than compliant subjects on Day 2 and Day 3 (p<0.05).

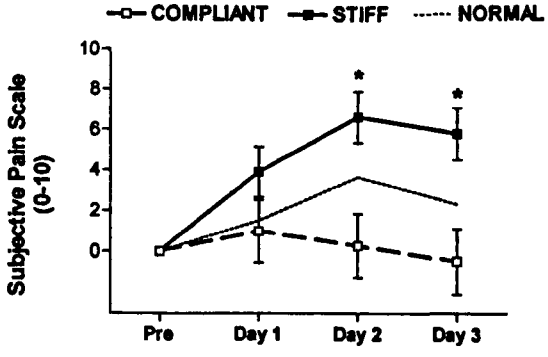


Figure 4.7: Pain following eccentric exercise. Pain values are arbitrary units on a scale of 0-10. Means are adjusted for gender and body mass. * Stiff greater than Compliant p<0.05 (post-hoc comparison with Bonferroni corrections).

Muscle tenderness following eccentric was different between stiffness groups (Group by Time $F_{GG\ 3.8,28.4df}$=2.81, p<0.05; Fig. 4.8). Compliant subjects did not experience any tenderness while Stiff subjects experienced tenderness on Day 2 and Day 3.

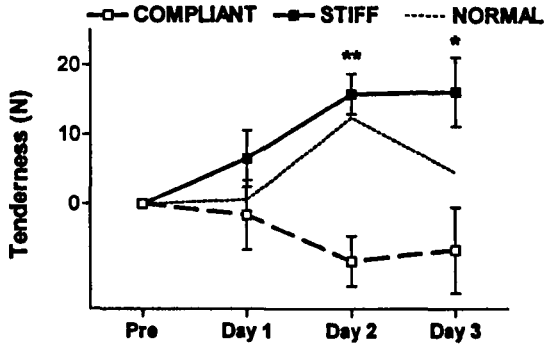


Figure 4.8: Changes in muscle tenderness following eccentric exercise. Tenderness values are summed for each muscle and means are adjusted for gender and body mass. Negative values are a function of the means adjustment and do not represent real values. Stiff greater than Compliant *p<0.05; **p<0.01 (post-hoc comparison with Bonferroni corrections).

Greater increases in log plasma CK activity were observed in the Stiff subjects (n=4) compared to Compliant subjects (n=5) (Fig. 4.9). Nonparametric tests showed a significantly greater increase in log plasma CK activity in the Stiff subjects on Day 1 (p<0.05). Similar results were seen if statistics were applied to absolute log CK values rather than percent increase in log CK (Day 1 H Test, p=0.027).

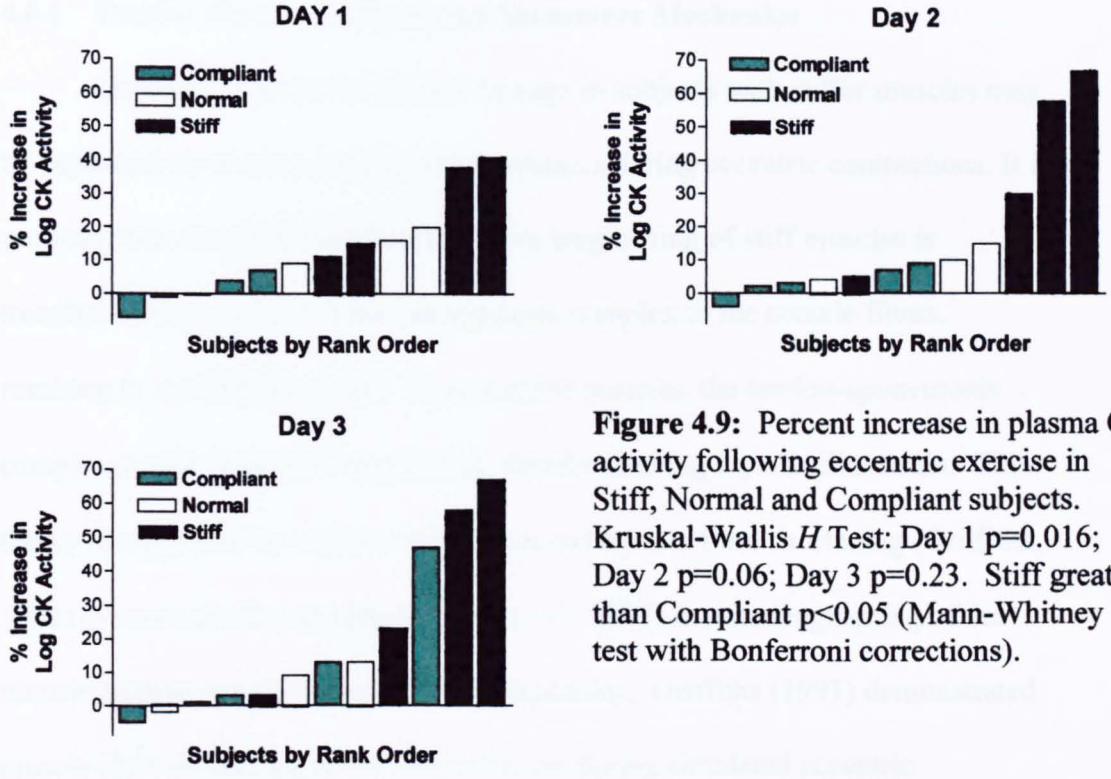


Figure 4.9: Percent increase in plasma CK activity following eccentric exercise in Stiff, Normal and Compliant subjects. Kruskal-Wallis *H* Test: Day 1 p=0.016; Day 2 p=0.06; Day 3 p=0.23. Stiff greater than Compliant p<0.05 (Mann-Whitney *U* test with Bonferroni corrections).

Symptoms of muscle damage were not different between male and female subjects: strength loss $F_{GG\ 1.5,27.6df}$=1.25, p=0.29; pain $F_{3,54df}$=2.81, p=0.21; tenderness $F_{GG\ 2.2,39.5df}$=0.97, p=0.39; plasma CK activity $F_{3,30df}$=1.15, p=0.35.

4.5 DISCUSSION

Strength loss, pain, muscle tenderness and elevated plasma CK activity are typical symptoms of muscle damage. In the present study these symptoms differed according to the subject's passive hamstring stiffness. Greater strength loss, pain, muscle tenderness and elevated plasma CK activity in Stiff compared to Compliant subjects suggests that subjects with stiffer hamstrings experienced greater muscle damage after exercising at the same relative intensity.

4.5.1 Passive Muscle Stiffness and Sarcomere Mechanics

Greater symptoms of muscle damage in subjects with stiffer muscles may be explained by tendon-aponeurosis mechanics during eccentric contractions. It is proposed that the strain imposed by active lengthening of stiff muscles is transferred from a rigid tendon-aponeurosis complex to the muscle fibres, resulting in myofibrillar strain. In compliant muscles, the tendon-aponeurosis complex is able to absorb lengthening, thereby limiting myofibrillar strain. This theory is supported by studies which observed muscle fibre shortening (Griffiths, 1991) or constant fibre length (Roberts *et al.*, 1997) despite lengthening of the muscle-tendon unit during eccentric contractions. Griffiths (1991) demonstrated muscle fibre shortening in cat plantarflexors during simulated eccentric contractions in isolated muscle-tendon units and during the eccentric phase of walking. These findings were attributed to lengthening in the tendon. It was suggested that tendons act as mechanical buffers to protect muscle fibres from abrupt length changes. Similarly, Roberts *et al.* (1997) demonstrated that tendon-aponeurosis compliance in turkey lateral gastrocnemius muscles allowed muscles fibres to maintain a relatively constant length during the stance phase of running, thus decreasing muscular work.

Previous studies in both humans and animals have clearly shown a strong association between exercising muscle length and muscle damage (Newham *et al.*, 1988; Lieber and Fridén, 1993; Brooks *et al.*, 1995; Hunter and Faulkner, 1997; Child *et al.*, 1998). These findings support the theory that muscle damage occurs with eccentric contractions on the descending limb of the length-tension curve when cross-bridge formation is compromised (Armstrong *et al.*, 1991). In

the present study all subjects performed eccentric contractions from 90° of knee flexion (short muscle length) to full extension (long muscle length). At 90° the hamstrings are on the ascending limb of the length-tension curve and sarcomeres have a limited capacity to shorten (Fig. 4.10). Close to full extension the hamstrings are on the descending limb of the length-tension curve and most sarcomeres are stretched beyond myofilament overlap.

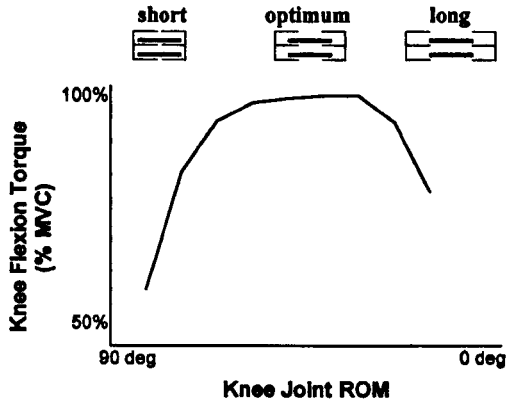


Figure 4.10: Length(ROM) -tension (torque) curve for knee flexion contractions adapted from Aagaard *et al.* (1999). On the ascending limb sarcomeres have a limited capacity to shorten ("short"). On the descending limb sarcomeres are stretched beyond myofilament overlap ("long"). This curve is qualitatively similar for isometric, eccentric and concentric contractions but varies with contraction speed.

It is possible that subjects with stiffer hamstrings had a smaller functional ROM. Maximum SLR ROM measured goniometrically was 93.7°±16.6° in subjects with compliant hamstrings and 72.4°±13.5° in subjects with stiff hamstrings (t_{11df}=2.5, p=0.027). At any given knee flexion angle, subjects with stiff hamstrings were probably further to the right on the length-tension curve than subjects with compliant hamstrings. Greater symptoms of muscle damage in these subjects could be attributed to performing eccentric contractions further on the descending limb of the length-tension curve.

4.5.2 Passive Muscle Stiffness, Fatigue and Muscle Damage

The results of the present study are indirectly supported by recent data which demonstrated that fatigue or warm-up lessens subsequent symptoms of muscle damage (Nosaka and Clarkson, 1997). In an initial experiment subjects performed 12 maximum eccentric contractions of the elbow flexors with each arm. In one arm the 12 eccentric contractions were preceded by 100 maximum concentric contractions. The concentric exercise resulted in a 20% decrease in isometric strength but did not affect eccentric force production which was similar between arms. In the arm exercising without prior concentric exercise isometric strength loss was 40% one day later and 20% five days later. In the arm subjected to prior concentric exercise, isometric strength loss was only 25% one day later and had returned to baseline within five days. Other indices of muscle damage showed similar differences between arms during the five days following the respective exercise bouts. Paradoxically, these results suggested that whole muscle fatigue (induced concentrically) protected the muscle from damage. The authors performed an additional experiment to help explain these effects. In the second part of the study the eccentric exercise was preceded by 100 concentric elbow flexions without resistance, to simulate warm-up exercise. The eccentric exercise preceded by warm-up resulted in significantly less strength loss and minimal changes in plasma CK activity compared to the eccentric exercise without prior warm-up. These protective effects of prior concentric exercise (fatiguing and non-fatiguing) were attributed to decreased passive muscle stiffness. Although actual stiffness measurements were not made, the conclusions are supported by previous work demonstrating reduced passive stiffness following 40 maximum concentric contractions (Magnusson *et al.*, 1995b). Concentric fatigue (19% decline in peak concentric torque) was

associated with a 28% decline in peak passive tension during stretch. A similar decrease in passive tension could have occurred following 100 concentric contractions in the study by Nosaka and Clarkson (1997) and may explain reduced damage following subsequent eccentric exercise.

4.5.3 Limitations

A theoretical explanation of the present results based on tendon-aponeurosis compliance assumes that the passive stiffness measurement reflects tendon-aponeurosis extensibility. It has been suggested that passive muscle stiffness primarily reflects the extensibility of the connective tissue elements in parallel with the muscle fibres (parallel elastic component; PEC) (Jewel and Wilkie, 1958; Latash and Zatsiorsky, 1993; McHugh *et al.*, 1998). However, aponeurosis strain does occur with lenghtening of isolated muscle-tendon units (Huijing and Ettema, 1988), with greatest strain in the region closest to the muscle fibres (Zuurbier *et al.*,1994). It is also reasonable to assume that, in a given muscle, PEC stiffness would be related to stiffness in the tendon-aponeurosis complex. Stiff subjects presumably had greater tendon-aponeurosis stiffness given that passive muscle stiffness was 78% higher than in Compliant subjects.

A further limitation of the present study was that stiffness groups differed in terms of gender and body mass. Stiff subjects (6 men, 1 woman) had 43% higher body mass and 78% higher stiffness than compliant subjects (1, man, 6 women). A large component of the between group differences in stiffness probably reflected differences in muscle cross-sectional area (CSA). However, it is unlikely that CSA accounted for the entire between group difference.

Furthermore, symptoms of muscle damage were not different between men and women. Analyses were controlled for both gender and body mass and indicated that the observed effects primarily reflected the role of stiffness.

4.5.4 Summary

The present study demonstrates a significant effect of passive muscle stiffness on the symptoms of muscle damage following eccentric exercise. Subjects with stiffer hamstrings experienced greater strength loss, more pain, greater muscle tenderness and greater elevations in plasma CK activity on the days following eccentric hamstring exercise. These effects are attributed to differences in sarcomere mechanics during eccentric contractions between stiff and compliant muscles. It is proposed that the strain imposed by active lengthening of a stiff muscle is transferred from a rigid tendon-aponeurosis complex to the muscle fibres, resulting in myofibrillar strain. In a more compliant muscle the tendon-aponeurosis complex can absorb lengthening imposed by eccentric contractions thereby reducing myofibrillar strain.

CHAPTER 5

NEURAL FACTORS ASSOCIATED WITH THE REPEATED BOUT EFFECT

Continued Over

5.5.6 Limitations

5.5.7 Summary

The content of this chapter has appeared as an abstract:
McHugh, M.P., Connolly, D.A.J., Eston, R.G., Kremenic, I.J. and Gleim, G.W. (1997). Neural adaptations to repeated bouts of eccentric and concentric exercise. *Proceedings of the 2nd Annual Congress of the European College of Sport Science*, Copenhagen Denmark 512-513.

5.1 ABSTRACT

The repeated bout effect refers to the protective effect provided by a single bout of eccentric exercise against muscle damage from a subsequent similar bout. The purpose of this study was to determine if the repeated bout was associated with either an increase in motor unit activation relative to force production or increased recruitment of slow-twitch motor units. Surface electromyographic (EMG) signals were recorded from the hamstring muscles during two bouts of sub-maximal isokinetic (2.6 $rad \cdot s^{-1}$) eccentric (11 men, 9 women) or concentric (6 men, 4 women) contractions separated by two weeks. EMG/torque and median frequency (MF) were analyzed. The initial bout of eccentric exercise resulted in strength loss, pain, muscle tenderness and elevated plasma creatine kinase (CK) activity on subsequent days while the repeated eccentric bout resulted in a strength gain, minimal pain, no muscle tenderness and minimal plasma CK elevation (Bout by Time effects $p<0.0001$, $p<0.0001$, $p<0.01$, $p<0.05$, respectively). Changes in strength, pain, tenderness and plasma CK activity were not different between bouts of concentric exercise. EMG/torque increased during both the initial and repeated bouts of eccentric exercise ($p<0.01$) but was not different between bouts. For concentric exercise EMG/torque was not different between bouts and did not change during the bouts. EMG/torque was lower for eccentric compared to concentric exercise. MF increased during both bouts of eccentric exercise with a greater increase in semitendinosus MF during the repeated bout. For concentric exercise MF was not different between bouts and did not change during either bout. MF was higher for eccentric compared to concentric exercise. Lower EMG/torque and higher MF for eccentric exercise are consistent with selective recruitment of a

small number of fast-twitch motor units. However, there was no evidence that the repeated bout effect was due to either an increase in motor unit activation relative to force production or a shift to recruitment of slow-twitch motor units. These data do not support a neural mechanism for the repeated bout effect.

5.2 INTRODUCTION

5.2.1 The Repeated Bout Effect

The repeated bout effect refers to the protective effect provided by a single bout of eccentric exercise against muscle damage from a subsequent eccentric bout (Nosaka and Clarkson, 1995). This effect has been consistently demonstrated with repeated bouts of isolated eccentric contractions of the elbow flexors and the knee extensors. Additionally, reduced damage has also been demonstrated with repeated bouts of downhill running, descending steps, downhill walking and eccentric cycling (see section 2.3). Protection appears to require eccentric exercise in the initial bout but is not specific to the type of eccentric exercise. Eston *et al.*, (1996) demonstrated that a bout of isokinetic eccentric contractions of the knee extensors provided some protection against damage following a subsequent downhill run. The protection was apparently limited to the pre-exercised quadriceps. Despite the number of studies demonstrating the repeated bout effect there is little consensus as to the mechanism (see section 2.4).

Several authors have suggested that a neural adaptation might explain the protective adaptation (Pierrynowski *et al.*, 1987; Golden and Dudley, 1992; Mair *et al.*, 1994; Nosaka and Clarkson, 1995). In general these studies have pointed to potential adaptations in motor unit behaviour during a repeated bout which might serve to limit the stress on the activated fibres. Approximately 50% less activation is required for eccentric compared to concentric contractions for a given contractile force. High stress on a small number of active fibres has been proposed as a mechanism of damage (Armstrong *et al.*, 1983; Moritani *et al.*,

1988). Accordingly, Nosaka and Clarkson (1995) suggested that the neural adaptation may "better distribute the workload among fibres." This could occur with (1) an increase in motor unit activation relative to force production as indicated by training studies, (2) a change in motor unit recruitment as proposed by Golden and Dudley (1992) or (3) increased synchrony of motor unit firing as suggested by Pierrynowski *et al.* (1987).

5.2.2 Neural Adaptations to Eccentric Training

Eccentric strength training studies have consistently demonstrated marked neural adaptations which were noticeably less with concentric or isometric training (Komi and Buskirk, 1972; Hortobágyi *et al.*, 1996a; Hortobágyi *et al.*, 1996b). The early weeks of eccentric strength training resulted in disproportionate increases in EMG amplitude which have been explained as increased motor unit recruitment. Eccentric EMG per unit force increased by approximately 20% with six weeks of eccentric quadriceps strength training (Hortobágyi *et al.*, 1996b). In contrast EMG per unit force did not change with six weeks of concentric training. Similar findings were previously demonstrated in the elbow flexors by Komi and Buskirk (1978) who noted that the greatest increases in EMG occurred after three weeks of training, the time at which symptoms of muscle damage had resolved. After three weeks of eccentric training, EMG per unit force had increased by 22% but had decreased by 10% with concentric training. While these adaptations to eccentric training indicate a decrease in neuromuscular efficiency, they suggest that contractile stresses are distributed among a greater number of active fibres. Such an adaptation to a single bout of eccentric exercise could explain the repeated bout effect.

5.2.3 Motor Unit Recruitment for Eccentric Exercise

Several authors have proposed that fast-twitch motor units are preferentially recruited for eccentric contractions (Nardone and Schieppati, 1988; Nardone *et al.*, 1989; Howell *et al.*, 1995; Enoka, 1996). The possibility of selective recruitment for exercise resulting in muscle damage is consistent with a neural theory of muscle damage whereby contractile stresses are distributed over a small number of active fast-twitch fibres (see Chapter 3). A change in motor unit recruitment with a repeated bout of eccentric exercise has not been examined specifically. A shift toward greater recruitment of slow-twitch motor units could reduce damage from a repeated bout of eccentric exercise. Slow-twitch fibres tend to have a smaller diameter thereby generating less tension per active fibre. A shift in recruitment to slow-twitch motor units would necessitate activating a greater number of motor units to match the force production. Such an adaptation would be consistent with the theory of distributing the workload over a greater number of active fibres (Nosaka and Clarkson, 1995). Additionally, fast-twitch fibres appear to be more susceptible to damage ((Fridén, 1984; Fridén *et al.*, 1983b; Lieber and Fridén, 1991; MacPherson *et al.*, 1996). While this may in part be attributable to selective recruitment, data from simulated eccentric contractions of isolated muscle fibres suggest that fast-twitch fibres are inherently weaker (MacPherson *et al.*, 1996). A shift in recruitment may serve to avoid stressing these susceptible fibres.

5.2.4 Motor Unit Synchronisation

Motor unit synchronisation refers to the tendency for two or more motor units to fire nearly simultaneously more often than might be expected by chance

alone (Nordstrom *et al.*, 1990; Kamen and Caldwell, 1996). The shift in the surface EMG frequency spectrum toward lower frequencies with muscle fatigue has been attributed, in part, to increased synchronisation, although other mechanisms predominate (Hägg, 1992). Hägg (1992) used the analogy of applause to describe the stochastic nature of motor unit firing rates and subsequent synchronisation. The clapping of each individual in an audience represents the firing of individual motor units. With continued applause the audience tends to synchronise clapping. Similarly, with sustained contraction, motor units tend to synchronise firing rates. Pierrynowski *et al.* (1987) suggested that increased synchronisation may explain the repeated bout effect, but an exact mechanism was not proposed. It is possible that increased synchronisation during a repeated bout of eccentric exercise would provide a more uniform distribution of contractile stress among the active fibres. However, this possibility has not been discussed before.

5.2.5 Detecting Neural Adaptations with Surface EMG

Surface EMG can provide some indication of the extent of motor unit activation and the type of motor units recruited (see sections 1.3; 2.1.1; 3.2.3; 3.5.2). The amplitude of the EMG signal relative to force production can be used to indicate changes in motor unit activation between repeated bouts of eccentric exercise. Changes in recruitment or synchronisation would be reflected by a shift in the EMG frequency spectrum. However, it would be difficult to differentiate between increased recruitment of slow twitch motor unit and increased synchronisation. Therefore, the purpose of this study was to compare surface

EMG (EMG/force and median frequency) between repeated bouts of eccentric exercise of sufficient intensity to demonstrate a repeated bout effect.

5.3 METHODS

5.3.1 Experimental Design

The same two groups from Chapter 3 (eccentric = 11 men, 9 women; concentric = 6 men, 4 women) repeated the bout of eccentric or concentric exercise two weeks following the initial bout. A between subjects model (eccentric group vs. concentric group) was chosen in preference to a within subjects model. In a within subjects model the experimental limb could perform eccentric exercise and the contralateral limb could perform concentric exercise. However, given the potential for cross-education with eccentric contractions (Hortobágyi *et al.*, 1997) a between subjects model was thought to be more appropriate. The experimental protocol described in Chapter 3 was performed twice. On the initial test day (Day 0) baseline measures of plasma creatine kinase (CK) activity and isometric strength were made prior to isokinetic exercise (eccentric or concentric). Subjects performed six sets of 10 contractions at a target intensity of 60% of isometric strength. Immediately after isokinetic exercise, isometric strength was re-tested. On the subsequent three days (Day 1,2,3) isometric strength, pain, muscle tenderness and plasma CK activity were measured. This four day protocol was repeated two weeks following Day 0 (11 days following Day 3) (Fig. 5.1). Measurements on consecutive days were made during the same period of the day (morning, afternoon or evening). Four subjects

entered the study each month, two in the initial week, two in the second week. The repeated bouts were performed on weeks three and four respectively. The initial 10 subjects performed eccentric exercise, the next 10 subjects performed concentric exercise and the final 10 subjects performed eccentric exercise.

Measurement procedures for isokinetic exercise, EMG activity, isometric strength, pain, muscle tenderness and plasma CK activity were described in sections 3.3.2 to 3.3.7. Plasma CK measurements were made on only 12 subjects in the eccentric group due to unavailability of the instrument. Plasma CK measurements were made on all 10 subjects in the concentric group.

Care was taken to follow the techniques exactly for the repeated bout. For EMG measurements a measuring tape and indelible ink were used to repeat exact anatomic positions for electrode placements on each occasion. The intensity for the repeated bout of isokinetic exercise was the same as the initial bout (60% of isometric strength prior to the initial bout not the repeated bout).

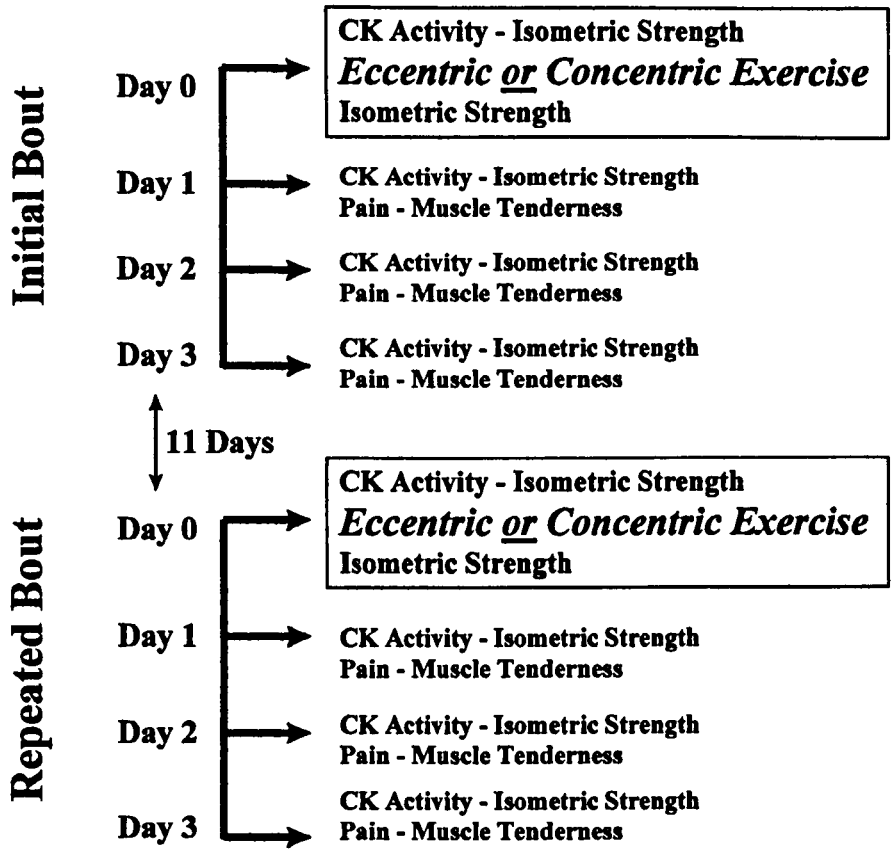


Figure 5.1: Experimental protocol for repeated bouts of isokinetic exercise (eccentric or concentric) and subsequent symptoms of muscle damage.

5.3.2 Statistical Analyses

Evidence of a repeated bout effect was tested by Group (eccentric or concentric) by Bout (initial vs. repeated) by Time (Pre, Post, Day 1,2,3) mixed model ANOVAs on each of four markers of muscle damage (isometric strength, pain, muscle tenderness, plasma CK activity). Within group repeat measures ANOVAs are also reported to elucidate the findings. Evidence of a neural adaptation was tested by Group by Bout by Set (Set 1 to Set 6) mixed model ANOVAs on EMG/torque and median frequency (MF). The critical value of the *F* ratio was adjusted according to the Greenhouse-Geisser Epsilon correction procedure when Mauchly's sphericity assumption was not met. Degrees of

freedom (*df*) which have been corrected by the Greenhouse-Geisser Epsilon are denoted by the subscript $_{GG}$. All post-hoc pairwise comparisons were performed with Bonferroni corrections for the number of comparisons made within a given ANOVA. Mean values in the text are reported with the standard deviation (S.D.) while mean values in figures are displayed with the standard error of the mean (S.E.M.).

5.4 RESULTS

5.4.1 Evidence of a Repeated Bout Effect

Changes in isometric strength (Fig. 5.2) clearly demonstrated a repeated bout effect with eccentric exercise (Bout by Time $F_{GG\ 2.4,45.1df}$=8.86, p<0.0001) but not with concentric exercise ($F_{4,36df}$=2.0, p=0.12). There was a significant decline in isometric strength following the initial bout of eccentric exercise (11% Day 1, 8% Day 2, 7% Day 3; Time effect $F_{GG\ 2.1,40.6df}$=8.0, p<0.001) and a significant increase in strength following the repeated bout (5% Day 1, 5% Day 2, 8% Day 3; Time effect $F_{GG\ 2.5,47.5df}$=3.8, p=0.022). There was no change in isometric strength following the initial or repeated bouts of concentric exercise.

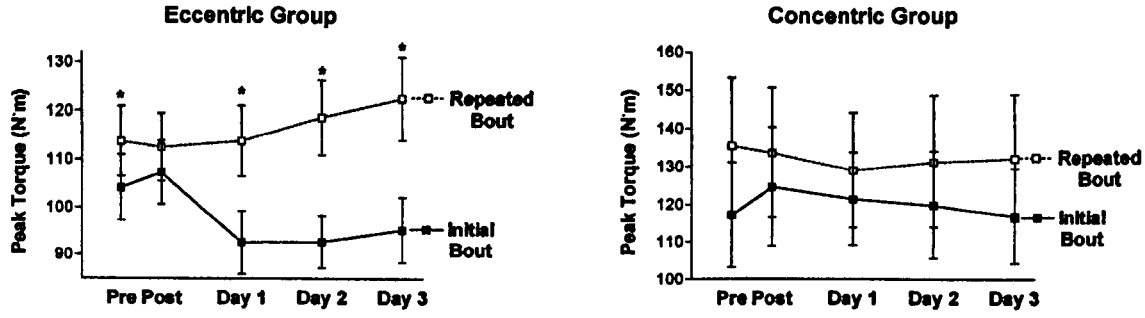


Figure 5.2: Changes in isometric strength (peak torque) following repeated bouts of eccentric or concentric exercise. * Initial bout less than repeated bout $p<0.01$ (see text for statistics).

Changes in pain (Fig. 5.3) clearly demonstrated a repeated bout effect with eccentric exercise (Bout by Time $F_{3,57df}=10.5$, $p<0.001$) but not with concentric exercise ($F_{3,27df}=1.9$, $p=0.16$). Subjects reported significant pain following the initial bout of eccentric exercise (Time effect $F_{3,57.6df}=16.0$, $p<0.001$) with minimal pain following the repeated bout ($F_{GG\ 1.3,24.3df}=3.4$, $p=0.07$). Minimal pain was reported following the initial bout of concentric exercise ($F_{3,27df}=1.9$, $p=0.16$) and no subjects reported any pain following the repeated bout.

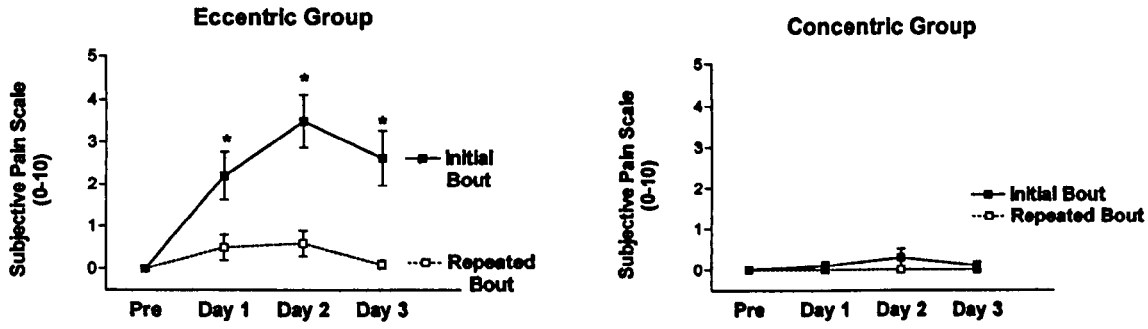


Figure 5.3: Pain following repeated bouts of eccentric or concentric exercise. * Initial bout greater than repeated bout $p<0.05$ (see text for statistics).

Changes in muscle tenderness (Fig. 5.4) clearly demonstrated a repeated bout effect with eccentric exercise (Bout by Time $F_{3,57df}=4.3$, $p=0.008$). Subjects

had significant tenderness following the initial bout of eccentric exercise (Time effect $F_{GG\ 2.2,41.1df}$=4.3, p=0.018) with minimal tenderness following the repeated bout ($F_{3,57df}$=1.8, p=0.16). No subject had any muscle tenderness following the initial or repeated bouts of concentric exercise.

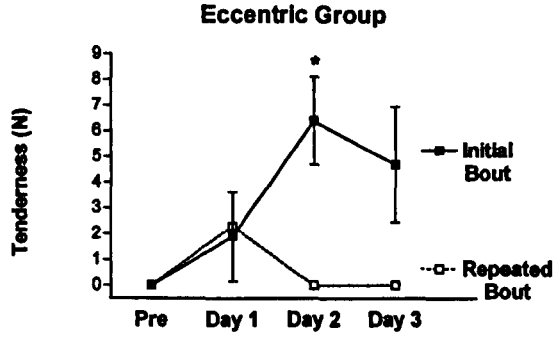


Figure 5.4: Muscle tenderness following repeated bouts of eccentric exercise. * Initial bout greater than repeated bout $p<0.01$ (see text for statistics).

Changes in plasma CK activity (Fig. 5.5) demonstrated a repeated bout effect with eccentric exercise (Bout by Time $F_{GG\ 1.7,17.0df}$=5.2, p=0.021) and not concentric exercise ($F_{3,27df}$=2.6, p=0.07). Elevations in plasma CK activity following the initial bout of eccentric exercise contrasted with minimal change in plasma CK following the repeated bout. Plasma CK measurements from one subject in the eccentric group were not included in the analysis because she had performed abdominal strengthening exercises on the day of the repeated bout. Subsequent abdominal soreness did not affect maximum isometric knee flexion strength tests.

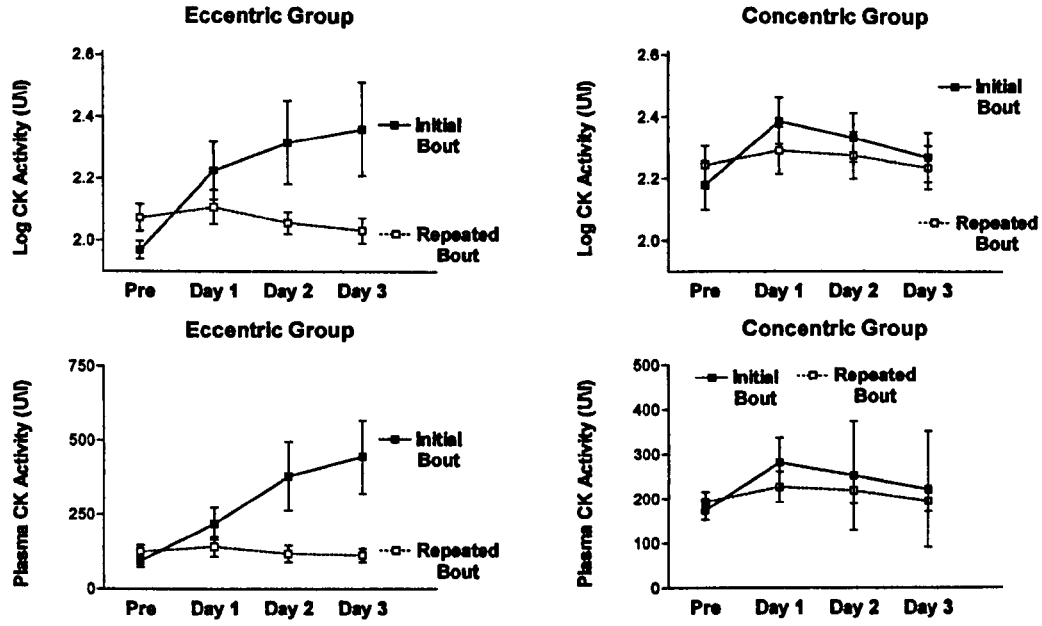


Figure 5.5: Changes in plasma CK activity following repeated bouts of eccentric or concentric exercise (log values top graphs, non-transformed values bottom graphs; see text for statistics).

5.4.2 EMG Activity During Repeated Bouts

Eccentric contraction intensity, expressed as a percentage of maximum isometric torque, was similar for the initial and repeated bouts (64.3±4.8% vs. 66.0±6.9%). Similarly, concentric contraction intensity was similar between the initial and repeated bouts of (58.3±2.0% vs. 58.0±3.2%). As expected EMG/torque was lower for eccentric exercise compared to the concentric exercise (Group effect $F_{1,28df}$=5.3, p=0.029). However, the difference diminished from Set 1 to 6 (Group by Set $F_{GG\ 1.7,47.2df}$=4.5, p=0.022). During eccentric exercise EMG/torque (Fig. 5.6) increased from Set 1 to Set 6 in both the initial bout (28% BI, 25% SM, 37% ST) and repeated bouts (21% BI, 14%SM, 16% ST) (Set effect $F_{GG\ 1.5,28.4df}$=7.2, p=0.006). The increase in EMG/torque was

not different between eccentric bouts (Bout by Set $F_{GG\ 3.0,57.3df}$=1.5, p=0.22). Overall EMG/torque was not different between eccentric bouts (Bout effect p=0.73). EMG/torque was not different between bouts of concentric exercise (Bout effect $F_{1,9df}$=1.0, p=0.34) and did not change during the initial or repeated bouts (Set effect $F_{GG\ 1.3,11.5df}$=1.1, p=0.34).

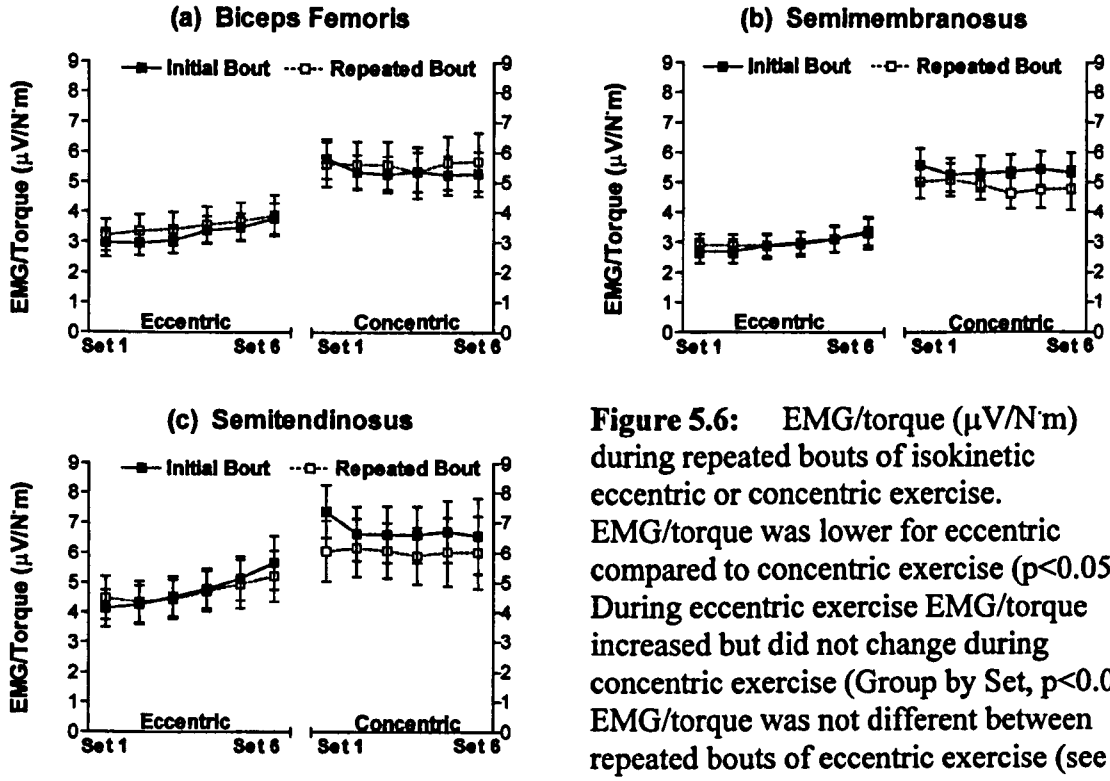


Figure 5.6: EMG/torque (μV/N·m) during repeated bouts of isokinetic eccentric or concentric exercise. EMG/torque was lower for eccentric compared to concentric exercise (p<0.05). During eccentric exercise EMG/torque increased but did not change during concentric exercise (Group by Set, p<0.05). EMG/torque was not different between repeated bouts of eccentric exercise (see text for details on statistics).

MF was higher for eccentric exercise compared to the concentric exercise during both the initial and repeated bouts (Group effect $F_{1,28df}$=13.9, p<0.001). MF (Fig. 5.7) increased from Set 1 to Set 6 in both the initial (BI 12.5%, p<0.01; SM 9.5%, p<0.01; ST 4.4%, p=0.3) and repeated bouts (BI 9.3%, SM 12.5%, ST 12.1%; all p<0.01) of eccentric exercise (Set effect $F_{1,19df}$=82.8, p<0.0001). By contrast, MF did not change during the initial ($F_{1,9df}$=0.31, p=0.59) or repeated bouts ($F_{1,9df}$=0.35, p=0.57) of concentric exercise. The difference in MF between

eccentric and concentric exercise increased from Set 1 to 6 (Group by Set $F_{1,28df}$=34.1, p<0.0001).

MF was not different between bouts of eccentric (Bout effect $F_{1,19df}$=0.13, p=0.73) or concentric exercise ($F_{1,9df}$=1.2, p=0.3). The increase in MF during eccentric exercise was not different between bouts (Bout by Set $F_{1,19df}$=1.02, p=0.33). However, individual muscles responded differently (Muscle by Bout by Set $F_{2,38df}$=3.2, p=0.05). A greater increase in ST MF from Set 1 to Set 6 was seen in the repeated bout (12.2% vs. 4.4%).

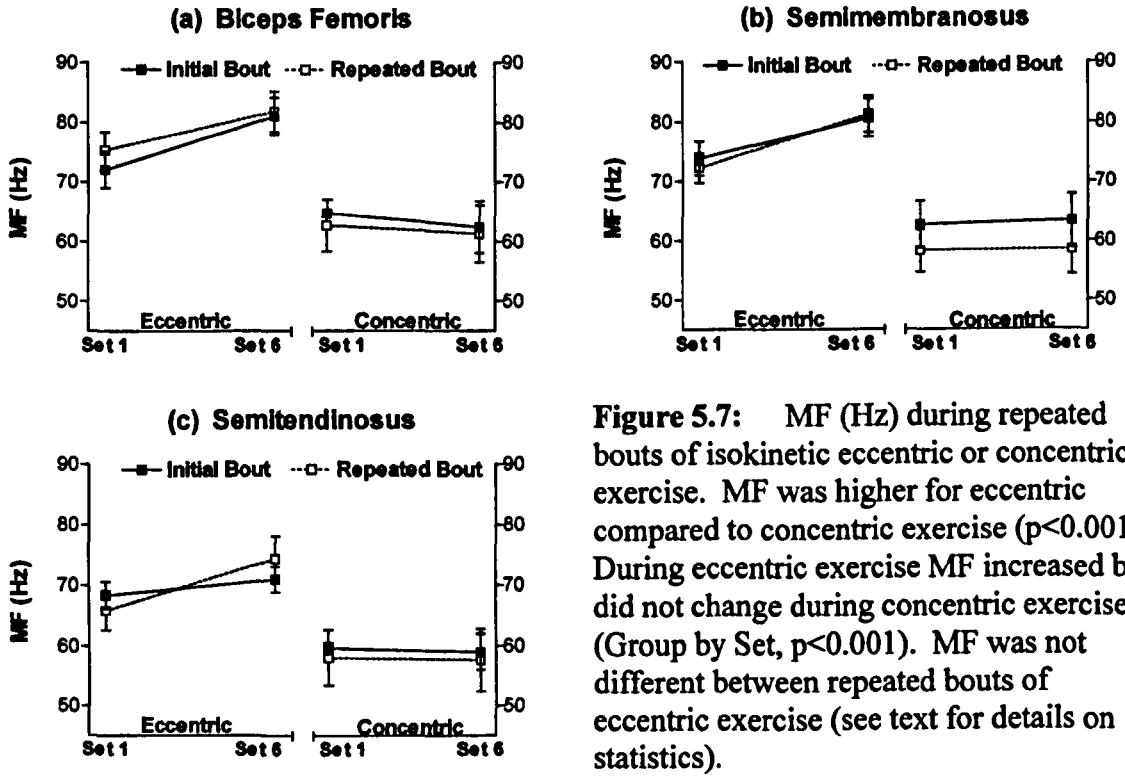


Figure 5.7: MF (Hz) during repeated bouts of isokinetic eccentric or concentric exercise. MF was higher for eccentric compared to concentric exercise (p<0.001). During eccentric exercise MF increased but did not change during concentric exercise (Group by Set, p<0.001). MF was not different between repeated bouts of eccentric exercise (see text for details on statistics).

5.4.3 EMG Activity During Isometric Strength Tests

Despite strength loss following the initial eccentric bout and strength gain following the repeated eccentric bout, iEMG during isometric strength tests did not change (Bout by Time $F_{GG\ 2.6,49df}$=0.34, p=0.77; Fig. 5.8). The iEMG during

isometric strength tests was also unaffected by concentric exercise (Bout by Time $F_{4,36df}$=2.0, p=0.15).

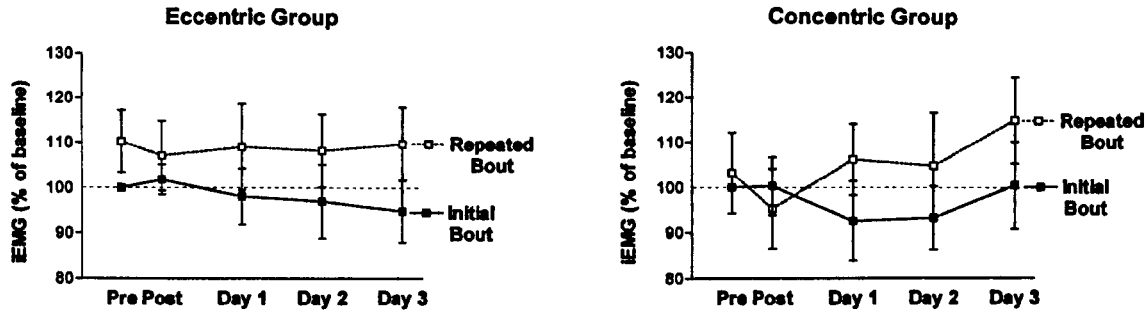


Figure 5.8: The iEMG (summed for all three muscles) during isometric strength tests (expressed as a percentage of values pre the initial bout) following repeated bouts of eccentric or concentric exercise. There was no significant difference over time between bouts or between groups (Group by Bout by Time $F_{GG\ 2.9,81.2df}$=0.33, p=0.8).

Despite strength loss following the initial bout of eccentric exercise and strength gain following the repeated bout, there was no difference in MF during the isometric strength tests (Bout by Time $F_{GG\ 2.5,46.7df}$=0.5, p=0.65; Fig. 5.9). However, MF was increased during isometric strength tests following eccentric exercise (Time effect $F_{GG\ 2.6,49.9df}$=4.3, p=0.012). MF was increased immediately following the initial (9.5%, p<0.01) and repeated bouts (7.9%, p<0.01) of eccentric exercise but returned to baseline on subsequent days. Similarly MF was increased during isometric strength tests following concentric exercise (Time effect $F_{4,36df}$=4.2, p=0.007). MF was increased immediately following the initial bout of concentric exercise (10%, p<0.05) but returned to baseline on subsequent days. Overall MF was lower during the isometric tests of the repeated bout of concentric exercise (Bout effect $F_{1,9df}$=16.8, p=0.003) despite the fact that strength was not different.

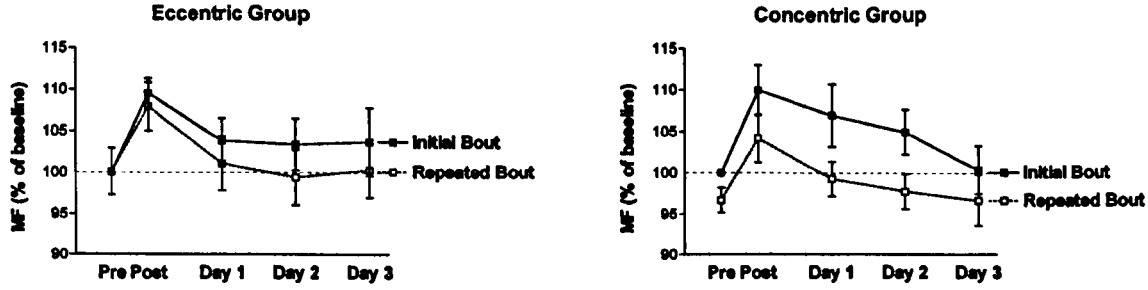


Figure 5.9: MF (summed for all three muscles) during isometric strength tests (expressed as a percentage of values pre the initial bout) following repeated bouts of eccentric or concentric exercise. Change in MF over time between bouts was not different between groups (Group by Bout by Time $F_{GG\ 2.8,77df}$=0.22, p=0.9).

There was a significant Group by Bout by Time interaction for EMG/torque during isometric strength tests ($F_{GG\ 2.8,79.1df}$=3.0, p=0.04). EMG/torque did not change during isometric tests on the days following the initial bout of eccentric exercise ($F_{GG\ 2.5,48.3df}$=1.4, p=0.25) but significantly decreased on the days following the repeated bout ($F_{4,76df}$=3.3, p=0.02). The 8% increase in strength by Day 3 following the repeated bout of eccentric exercise corresponded with a 10.3% decrease in EMG/torque (Fig.5.10). By contrast EMG/torque during the isometric strength tests did not change following the initial ($F_{4,36df}$=1.1, p=0.36) or repeated bouts ($F_{4,36df}$=2.4, p=0.06) of concentric exercise.

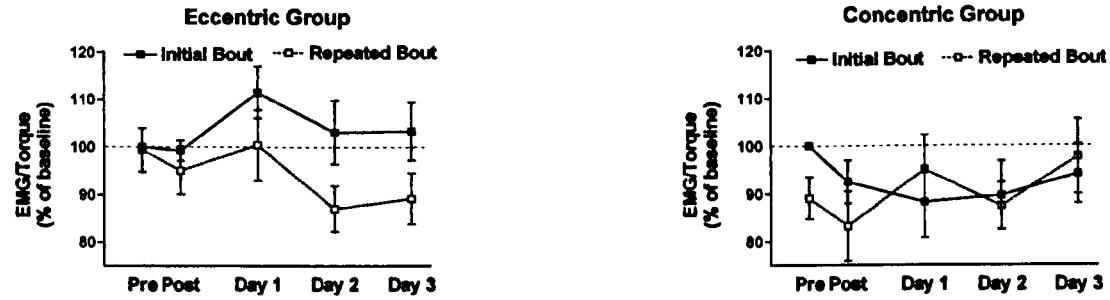


Figure 5.10: EMG/torque during isometric strength tests following repeated bouts of eccentric or concentric exercise. EMG/torque declined following the repeated bout of eccentric exercise (p=0.02) but did not change following the initial eccentric bout or either concentric bouts (Group by Bout by Time p=0.04; see text for details on statistics).

5.5 DISCUSSION

5.5.1 Repeated Bout Effect

All four indirect markers of muscle damage (strength loss, pain, muscle tenderness, plasma CK activity) showed clear evidence of a repeated bout effect with eccentric exercise. Strength loss following the initial bout of eccentric exercise was contrasted with strength gain following the repeated bout. On the three days following the repeated bout, isometric strength was 23%, 28%, and 29% higher, respectively, than on the equivalent days following the initial bout. Similarly subjects had marked pain following the initial bout of eccentric exercise with minimal pain following the repeated bout. Although muscle tenderness values were generally low following the initial bout of eccentric exercise, no tenderness was found in any subject on Day 2 or Day 3 following the repeated bout. Plasma CK activity was elevated following the initial bout of eccentric exercise but remained at baseline levels following the repeated bout. By contrast there was no evidence of a repeated bout effect with concentric exercise. Symptoms of muscle damage were mostly absent following the initial bout of concentric exercise and were not different following the repeated bout. In summary, these data indicate that even a relatively low intensity bout of eccentric exercise resulting in only moderate symptoms of muscle damage was sufficient to clearly demonstrate a repeated bout effect.

5.5.2 EMG/Torque and the Repeated Bout Effect

As discussed in Chapter 3 (sections 3.4, 3.5) the initial bout of sub-maximal eccentric contractions was characterised by lower EMG/torque and

higher MF relative to maximum isometric contractions (within subject comparisons) or sub-maximal concentric contractions (between group comparisons). Additionally, during the initial bout EMG/torque and MF increased with repeated eccentric contractions while neither changed with repeated concentric contractions.

Lower EMG/torque for eccentric exercise represents reduced motor unit activation. The occurrence of muscle damage with eccentric exercise has been attributed to high stress on a small number of active fibres (Armstrong *et al.*, 1983; Moritani *et al.*, 1988). In the present study it was proposed that the repeated bout effect could be due to an increase in activation relative to torque production thereby distributing contractile stresses among a greater number of active fibres. EMG amplitude has been shown to increase disproportionately to force with eccentric strength training but not concentric training (Komi and Buskirk, 1972; Hortobágyi *et al.*, 1996a; Hortobágyi *et al.*, 1996b). Komi and Buskirk (1972) saw approximately 20% greater elbow flexor EMG per unit force following three weeks of eccentric strength training compared to a 10% decrease with concentric training. In the present study there was no statistically significant change in EMG/torque between repeated bouts of eccentric contractions. EMG/torque was slightly higher (2.3%) for the repeated bout of eccentric exercise and marginally lower (5.8%) for the repeated bout of concentric exercise (neither change was significant and there was not a significant interaction).

The absolute increase in EMG/torque during the six sets of eccentric exercise was not different between bouts (Bout by Set $p=0.22$). However, the relative increases were less for each muscle in the repeated bout (Initial vs. Repeated Bout: BI 28% vs. 21%, SM 25% vs.14% ST 37% vs.16%). The mean

increase in EMG/torque (three muscles grouped) for the initial bout (29.7±30.4%) was greater ($F_{1,19df}$=4.7, p=0.04) than the increase for the repeated bout (16.6±25.0%). This finding is counter to the initial hypothesis that greater motor unit recruitment would be seen in the repeated bout. An increase in EMG amplitude during sustained sub-maximal contraction is generally attributed to recruitment of additional motor units secondary to fatigue (Enoka and Stuart, 1992). However, it is important to note that neither MVC (Fig. 5.2) nor EMG/torque during maximum isometric contractions were affected immediately following either the initial or repeated bouts of eccentric exercise. EMG/torque was 25.0±2.3 μV/N·m prior to and 25.1±2.7 μV/N·m immediately following the initial bout. Similarly, for the repeated bout values were 25.0±11.6 μV/N·m and 23.9±12.1 μV/N·m, respectively. This suggests that the increase in EMG/torque during eccentric exercise was not fatigue related. A 28% increase in EMG/torque during the initial eccentric bout was contrasted with no change in EMG/torque for a maximum isometric contraction immediately following the eccentric exercise. This indicates a specific neural control for eccentric exercise. It is possible that contractile mechanics were disrupted as sarcomeres were strained beyond myofilament overlap in accordance with the theory of Morgan (1990). If during the eccentric contractions strained sarcomeres failed to effectively re-interdigitate with repeated crossbridge cycling (as proposed by Morgan, 1990) aggregate force production would eventually decrease and greater activation would be required to maintain target force production. Such a theory is only plausible if myofilament interdigitation was restored with relaxation following the eccentric exercise before the maximum isometric contraction was performed. This is possible given that the isometric contractions were performed at a

relatively short muscle length and it took at least a minute to set up for the test. The smaller increase in EMG/torque in the repeated bout may reflect reduced sarcomere strain and subsequently less additional recruitment being required. However, it was not within the scope of this study to provide evidence for, or to test such a theory.

In general, the EMG/torque data do not point to a neural adaptation with respect to the repeated bout effect. There was no evidence of an increase in activation for the repeated bout consistent with a decrease in force per active fibre.

5.5.3 Median Frequency and the Repeated Bout Effect

It was proposed that a decrease in MF might occur between repeated bouts of eccentric exercise as a result of either decreased recruitment of fast-twitch motor units or increased motor unit synchronisation. However, the data provided no indication of such an adaptation. There was less than 1% difference between bouts in the mean MF for all three muscles combined. However, despite the overall relative consistency in MF response between the repeated bouts it was apparent that the ST muscle responded differently between bouts. The ST MF increased slightly during the initial bout of eccentric exercise (4.4%) but showed a marked increase during the repeated bout (12.1%). By contrast, ST EMG/torque increased by 37% in the initial bout but only by 16% in the repeated bout (Fig. 5.11). Additionally, MF was consistently 8-10% lower and iEMG 30-50% higher for ST compared to BI and SM (Fig. 5.6 and 5.7). The repeated bout in the ST was characterized by a greater increase in MF and a smaller increase in EMG/torque both of which were counter to the proposition of increased

recruitment of slow-twitch motor units. The significance of these changes in ST MF with respect to the repeated bout effect is unclear. A greater increase in MF could be due to higher muscle temperature, higher firing rates, greater muscle fibre swelling or more selective recruitment of fast-twitch motor units (see section 3.5.2) none of which would be consistent with reduced subsequent damage.

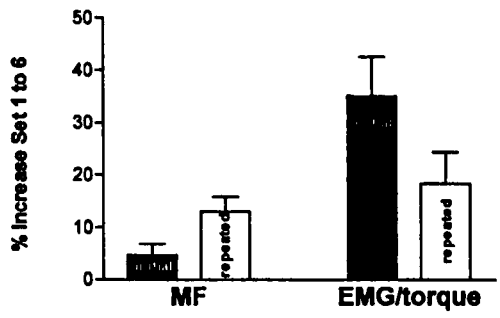


Figure 5.11: Percent increase in ST EMG/torque and MF from Set 1 to 6 during repeated bouts of eccentric exercise. Greater increase in MF for repeated bout (t_{19df}=2.3, p=0.04). Smaller increase in EMG/torque for repeated bout (t_{19df}=2.1, p=0.05). The repeated bout had opposite effects on MF compared to EMG/torque ($F_{1,19df}$=11.4, p=0.003).

In general the MF data suggest that both bouts of eccentric exercise were associated with selective recruitment of fast-twitch motor units and that recruitment patterns were mostly similar between bouts. These data argue against a neural adaptation explaining the repeated bout effect.

5.5.4 EMG Activity During Isometric Strength Tests

Despite decreased maximum isometric strength on the days following the initial bout of eccentric exercise iEMG and MF were unaffected. This indicates that motor unit activation was unaffected by the apparent muscle damage. Similarly iEMG and MF were unaffected on the days following the repeated bout of eccentric exercise despite a slight strength gain. Accordingly, EMG/torque was decreased during isometric tests following the repeated bout. This indicates a peripheral adaptation in contractile force production independent of neural activation. This adaptation was apparent following, but not prior to the repeated

eccentric bout. Therefore the change in EMG/torque cannot be directly attributed to the initial bout. However, since the concentric group did not show a similar change following either bout, the effect must be related to the repeated eccentric bout. A change in the mechanical properties of the muscle may have occurred, but it was not within the scope of this study to examine such an adaptation.

5.5.5 Reproducibility of EMG Measurements

It is difficult to determine the reproducibility of EMG activity during eccentric contractions because of the potential for inducing muscle damage with an initial bout and the potential for a neural adaptation between bouts. However, the reproducibility of EMG activity during concentric and isometric can be measured without such confounding factors. In the present study the control group performing concentric exercise provide some indication of measurement reproducibility. Additionally, since there was no apparent bout effect for eccentric contractions statistics on reproducibility can be calculated on both the eccentric and concentric groups. Based on previous data on repeated measures of surface EMG amplitude (Viitasalo and Komi, 1975) EMG/torque was surprisingly consistent between bouts in the present study. Viitasalo and Komi (1975) reported correlation coefficients of 0.8-0.86 for between day iEMG measures during sub-maximal isometric quadriceps contractions. Higher values are reported in the present study for EMG/torque (averaged across six sets of 10 contractions in each bout), despite the fact that high speed dynamic contractions were used and measurements were made two weeks apart. Linear regression of repeated bouts showed a high regression coefficient in both the eccentric (r=0.9)

and concentric groups (r=0.9). In both regressions the slopes were close to one and intercepts were close to zero (Fig. 5.12a, 5.12b).

Bland-Altman plots showed all but one value (95%) to be within two standard deviations (±2.1 μV/N·m) of the mean difference (-0.08 μV/N·m) in the eccentric group (Fig. 5.12c) and all values to be within two standard deviations (±2.1 μV/N m) of the mean difference (0.34 μV/N·m) in the concentric group (Fig. 5.12d). Bland and Altman (1986) suggested that 95% of the differences should be within two standard deviations of the mean as a standard for repeatability. Although the repeatability of EMG/torque for repeated bouts of eccentric and concentric contractions was the same in absolute terms (2.1μ V/N·m) only 90% of eccentric values, compared to 100% of concentric values, were within the accepted limits. Additionally, in relative terms repeatability was better for eccentric contractions since EMG/torque was approximately 2 μV/N·m higher for concentric exercise.

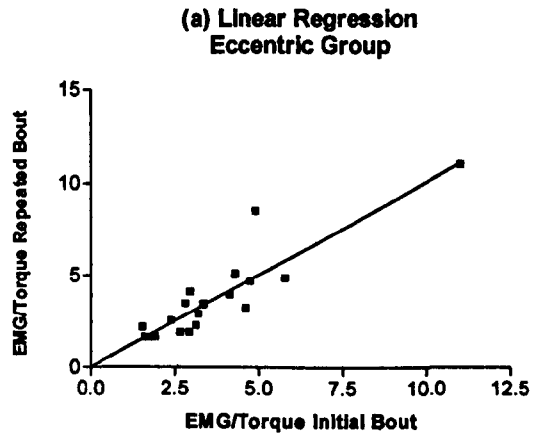


Figure 5.12a: Linear regression of EMG/torque from repeated bouts of eccentric contractions (r=0.9; p<0.001; y=1.02x+0.02).

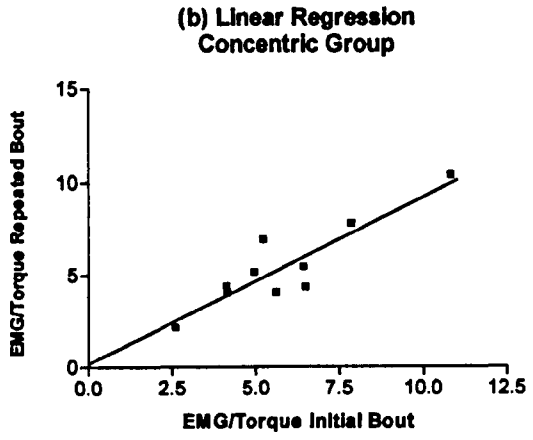


Figure 5.12b: Linear regression of EMG/torque from repeated bouts of concentric contractions (r=0.9; p<0.001; y=0.91x+0.18).

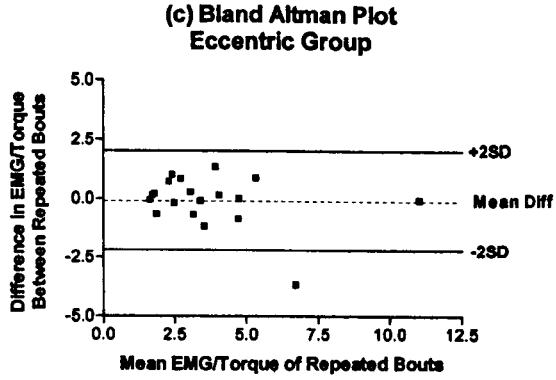


Figure 5.12c: Bland-Altman plot of the difference in EMG/torque between repeated bouts of eccentric contractions versus the mean EMG/torque for the two bouts. Coefficient of repeatability = 2.1μ V/N·m.

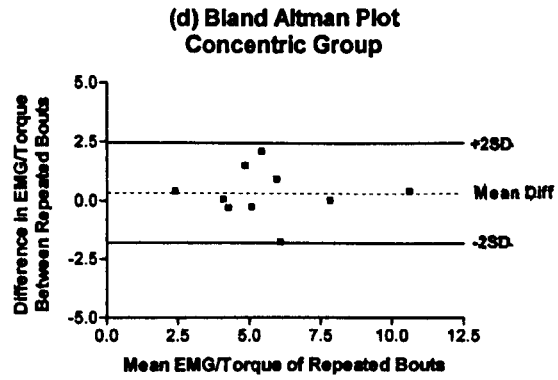


Figure 5.12d: Bland-Altman plot of the difference in EMG/torque between repeated bouts of concentric contractions versus the mean EMG/torque for the two bouts. Coefficient of repeatability = 2.1μ V/N·m.

With respect to the frequency content of the EMG signal, correlation coefficients of 0.8-0.76 were reported for between day MPF during sub-maximal isometric quadriceps contractions (Viitasalo and Komi, 1975). Slightly lower values were found in the present study for sub-maximal eccentric (0.7, Fig. 5.13a) and concentric (0.77, Fig. 5.13b) hamstring contractions. MF values in each bout were averaged between the three muscles and between Set 1 and Set 6. Bland-Altman plots showed all but two values (90%) to be within two standard deviations (±16.5 Hz) of the mean difference (-0.66 Hz) in the eccentric group (Fig. 5.13c) and all values to be within two standard deviations (±14.7 Hz) of the mean difference (2.6 Hz) in the concentric group (Fig. 5.13d).

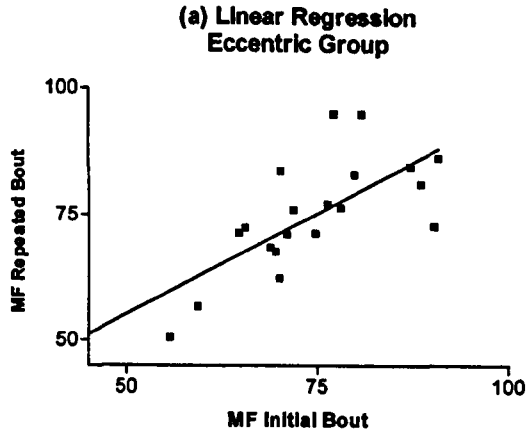


Figure 5.13a: Linear regression of MF from repeated bouts of eccentric contractions (r=0.7; p<0.001; y=0.81x+14.6).

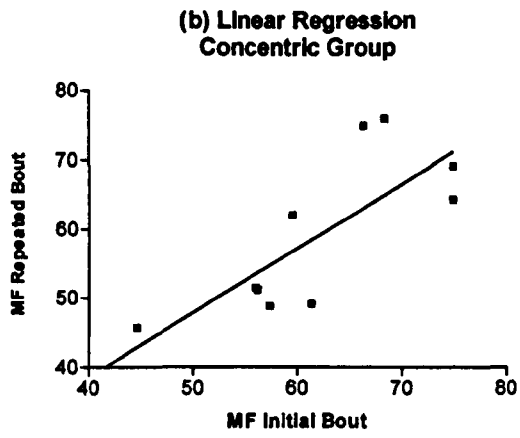


Figure 5.13b: Linear regression of MF from repeated bouts of concentric contractions (r=0.77; p<0.01; y=0.95x+0.34).

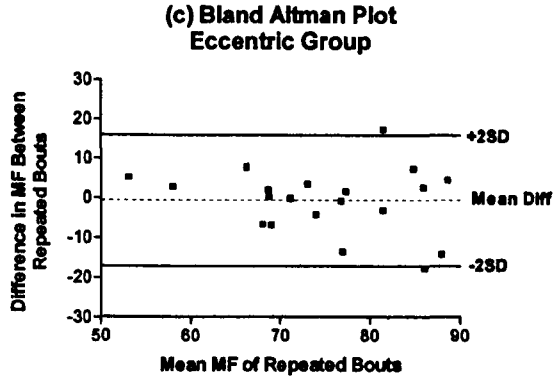


Figure 5.13c: Bland-Altman plot of the difference in MF between repeated bouts of eccentric contractions versus the mean MF for the two bouts. Coefficient of repeatability = 16.5 Hz.

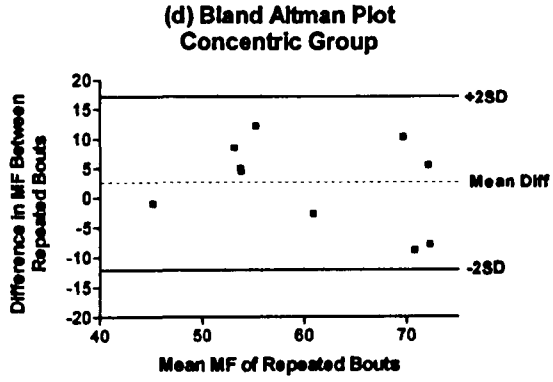


Figure 5.13d: Bland-Altman plot of the difference in MF between repeated bouts of concentric contractions versus the mean MF for the two bouts. Coefficient of repeatability = 14.7 Hz.

5.5.6 Limitations

The purpose of the present study was to determine whether the repeated bout effect could be explained by a neural adaptation. To test this hypothesis it was necessary to have: (1) an initial bout of eccentric exercise resulting in muscle damage; (2) a repeated bout of the same exercise resulting in markedly reduced

damage; (3) have a valid and reliable measurement of neural activity between the bouts of eccentric exercise.

Four commonly accepted markers of muscle damage were studied (isometric strength, pain, muscle tenderness and plasma CK activity). Although a repeated bout effect was evident with each marker of muscle damage it was apparent that the initial bout did not result in substantial damage. In particular muscle tenderness values and changes in plasma CK activity were lower than expected. However, the measurement technique for muscle tenderness may need modification for testing hamstring tenderness. The 40 N ceiling for determining tenderness may be too low for the hamstring muscles, which easily dispersed under the probe. As the probe was applied to the muscles the tissues dispersed considerably more than occurs with application to the quadriceps or biceps brachii. A higher maximum force may improve the sensitivity of the measurement in this muscle group.

The exercise intensity was set at 60% of isometric MVC to control for the relative work performed by the eccentric and concentric groups. If both groups had performed maximum contractions greater work would have been performed by the eccentric group given that eccentric strength is greater than concentric strength, especially at high velocities (Webber and Kriellaars, (1997). More importantly, the concentric group would probably have experienced considerable fatigue with the eccentric group experiencing minimal fatigue (Tesch *et al.*, 1990; Hortobágyi *et al.*, 1996c). This would have confused interpretation of EMG changes between groups. At 60% isometric MVC minimal fatigue effects were apparent in both groups based on EMG changes and isometric strength immediately post exercise. This allowed a direct comparison of changes in EMG

activity between the groups while avoiding fatigue effects and differences in exercise intensity.

The two main neural adaptations proposed were an increase in motor unit activation relative to force production and a shift toward greater recruitment of slow-twitch motor units. However, it is possible that surface EMG is not sensitive enough to detect such changes. In particular the ability to detect changes in motor unit recruitment by frequency analysis of surface EMG remains in question (see section 3.5.2). Although these are obvious limitations, both EMG/torque and MF measurements were surprisingly consistent between repeated bouts of eccentric exercise despite the fact that the subsequent symptoms of muscle damage were markedly different.

5.5.7 Summary

Changes in isometric strength, pain, muscle tenderness and plasma CK activity provided clear evidence of a repeated bout effect with eccentric exercise. However, based on measures of EMG/torque and MF, there was no evidence of a change in motor unit activation or recruitment associated with the repeated bout effect. Eccentric exercise was characterized by low EMG/torque and high MF in both bouts. Additionally, EMG/torque and MF increased during both bouts of eccentric exercise.

Lower EMG/torque and higher MF for sub-maximal eccentric contractions compared to maximum isometric (within subjects) or sub-maximal concentric contractions (between groups) is consistent with selective recruitment of a small number of fast-twitch motor units for eccentric exercise. However, there was no evidence that the repeated bout effect was due to either an increase

in activation or a shift to recruitment of slow-twitch motor units. These data suggest that the repeated bout effect may be due to non-neural mechanisms.

CHAPTER 6

VISCOELASTIC PROPERTIES OF SKELETAL MUSCLE FOLLOWING REPEATED BOUTS OF ECCENTRIC EXERCISE

6.1 ABSTRACT

The purpose of this study was to determine if the repeated bout effect was associated with any changes in the viscoelastic properties of skeletal muscle. Viscoelastic properties of the hamstring muscle group were measured in subjects following repeated bouts of unilateral eccentric (n=20) or concentric (n=10) exercise separated by two weeks. Hamstring muscle stiffness, estimated from torque/ROM curves during passive stretch, provided an indication of the elastic properties. Stress relaxation, measured as the percent decline in tension during a fixed stretch held for one minute, provided an indication of the viscous properties. Stiffness and stress relaxation were measured in both the experimental and non-experimental limb prior to both the initial and repeated bouts of eccentric or concentric exercise and on each of the subsequent three days. An intraclass correlation coefficient of 0.93 and a coefficient of variation of 7.5$\pm$3% for repeated measures of stiffness on the non-experimental limb indicated good reproducibility. An intraclass correlation coefficient of 0.61 and a coefficient of variation of 34.4$\pm$49.4% for repeated measures of stress relaxation on the non-experimental limb indicated poor reproducibility. The stress relaxation response was not different between subjects with stiff and compliant hamstrings. The initial bout of eccentric exercise resulted in strength loss, pain, muscle tenderness and elevated plasma CK activity on subsequent days while the repeated eccentric bout resulted in a strength gain, minimal pain, no muscle tenderness and minimal CK elevation (Bout by Time effects $p<0.0001$, $p<0.0001$, $p<0.01$, $p<0.05$, respectively). Changes in strength, pain, tenderness and plasma CK activity were not different between bouts of concentric exercise. Despite

symptoms of muscle damage following the initial bout of eccentric exercise passive muscle stiffness was not increased. There was no evidence of any change in stiffness or stress relaxation following either the initial or repeated bouts of eccentric or concentric exercise. Stiffness measurements remained within 5% of baseline throughout the duration of the study. The repeated bout effect could not be attributed to a change in the passive viscoelastic properties of the hamstring muscle group.

6.2 INTRODUCTION

6.2.1 Passive Muscle Stiffness Following Eccentric Exercise

Exercise-induced muscle damage is commonly described according to various symptoms which include strength loss, pain, muscle tenderness, stiffness, swelling and elevated circulating levels of specific muscle enzymes and proteins (see section 1.2). However, changes in stiffness have been examined in only one muscle group. Stiffness has been assessed in the elbow flexors by documenting changes in relaxed flexion angle (Clarkson and Tremblay, 1988; Ebbeling and Clarkson, 1990; Cleak and Eston, 1992b; Chleboun *et al.*, 1995; Saxton and Donnelly, 1996; Nosaka and Clarkson, 1997) or changes in torque/ROM curves (Howell *et al.*, 1993; Chleboun *et al.*, 1995). The maximum decrease in relaxed flexion angle (full extension is normally close to 180°) occurs two to four days following eccentric exercise, recovering by the fifth to tenth day. Immediately following three sets of between five and 15 sub-maximal eccentric contractions elbow flexor stiffness was increased by approximately 150%, remaining significantly elevated (>75%) for up to four days (Howell *et al.*, 1993). A later study using the same exercise protocol showed similar results with stiffness elevated by approximately 100% immediately post-exercise, peaking at 120% two days following exercise (Chleboun *et al.*, 1995). However, increases in stiffness were highly variable in both studies. It was unclear whether this was due to biological variability in response to eccentric exercise or measurement error.

Similar measures of stiffness have not been made in other muscle groups on the days following eccentric exercise. The increase in passive stiffness of the

elbow flexors (100-150%) immediately following three sets of 5-15 repetitions of sub-maximal eccentric contractions (Howell *et al.*, 1993; Chleboun *et al.*, 1995) contrasts with measurements of passive hamstring stiffness immediately following maximal eccentric contractions (Magnusson *et al.*, 1995b). Magnusson *et al.* (1995b) found no change in passive tension in the hamstrings immediately following 40 maximal eccentric hamstrings contractions. A test-retest correlation of 0.9 was reported for repeated measures with this stiffness measurement (Magnusson *et al.*, 1995b). However, subjects were not followed over subsequent days to monitor symptoms of muscle damage or changes in stiffness.

6.2.2 Passive Muscle Stiffness and the Repeated Bout Effect

Marked increases in relaxed elbow angle following an initial bout of eccentric exercise contrast with minimal changes following a repeated bout (Clarkson and Tremblay, 1988; Ebbeling and Clarkson, 1990). These findings are consistent with the repeated bout effect. However, passive muscle stiffness measurements have not been made following repeated bouts of eccentric exercise.

The increase in elbow flexor stiffness at terminal ROM is thought to be due to swelling (Howell *et al.*, 1993). However, the increase in stiffness in the mid-range has been attributed to connective tissue or cytoskeletal disruption (Howell *et al.*, 1993). Some authors have suggested that an adaptation in intramuscular connective tissue or the cytoskeleton following an initial bout of eccentric exercise protects the muscle from damage following a repeated bout (Fridén *et al.*, 1983a; Newham *et al.*, 1987; Clarkson and Tremblay, 1988,

Ebbeling and Clarkson, 1990; Lapier *et al.*, 1995). Lapier *et al.* (1995) increased intramuscular connective tissue of rat extensor digitorum longus muscles by immobilising the ankle joint for three weeks with the muscle in either a shortened or lengthened position. Muscles immobilised in the lengthened position had 63% more intramuscular connective tissue and 86% lower mass than contralateral control muscles. Muscles immobilised in the shortened position had 47% more intramuscular connective tissue and 21% lower mass than control muscles. Subsequent bouts of stimulated eccentric contractions resulted in 50% force loss in control muscles compared to 40% in muscles immobilised in the shortened position and 8% in muscles immobilised in the lengthened position. The protective effect was attributed to the ability of the increased connective tissue to dissipate myofibrillar stresses. The authors suggested that tissue repair following a damaging bout of eccentric exercise is characterised by a similar increase in intramuscular connective tissue thereby protecting against damage from repeated bouts. However, intramuscular connective tissue was expressed relative to muscle fibre area. The increase in intramuscular connective tissue may have been due to muscle fibre atrophy. An increase in intramuscular connective tissue would be expected to result in increased muscle stiffness (Kovanen *et al.*, 1984). Lapier *et al.* (1995) suggested that the repair process results in a permanent increase in passive stiffness as a result of remodelling of the connective tissue. However, the data presented in Chapter four indicated that greater passive muscle stiffness increased the severity of damage. Alternatively, the findings of Lapier *et al.* (1995) could be interpreted as a myofibrillar adaptation. The fact that the effect occurred primarily in the muscles immobilized in the lengthened position

suggests that protection may in part have been due to longitudinal addition of sarcomeres (see section 2.4.3).

6.2.3 Viscoelastic Stress Relaxation

Passive muscle stiffness primarily reflects the elastic properties of skeletal muscle. However, viscous properties are also important. Viscosity provides resistance to rapid change in muscle length and is thought to augment eccentric force production and reduce concentric force production (Martin *et al.*, 1996). Increased resistance to lengthening will increase eccentric force while increased resistance to shortening will decrease concentric force. However, a direct measure of muscle viscosity during dynamic contractions is difficult and estimates are made based on mathematical models (Martin *et al.*, 1996).

Stress relaxation can provide a direct measure of the viscous properties of relaxed skeletal muscle. When a viscoelastic material, such as muscle, is stretched and held at a fixed length the tension decreases over time and is referred to as stress relaxation. Stress relaxation has been demonstrated in isolated muscle preparations in rabbits (Taylor *et al.*, 1991) and *in vivo* in humans (McHugh *et al.*, 1991; Magnusson *et al.*, 1995a; Magnusson *et al.*, 1996). Passive tension during stretch is a function of the elastic and viscous properties. When a tissue is stretched and held at a given length elastic resistance remains constant while viscous resistance gradually decreases (stress relaxation). A greater stress relaxation response indicates greater viscous resistance to passive stretch. Stress relaxation has been shown to be unaffected by fatiguing concentric and eccentric contractions (Magnusson *et al.*, 1995b). However, changes in stress relaxation have not been examined on the days following

eccentric exercise. If increased stiffness associated with muscle damage is due to tissue oedema an increased stress relaxation response would be expected. Following a repeated bout of eccentric exercise which does not result in muscle damage no change in stress relaxation would be expected.

6.2.4 Purpose

The purpose of the present study was threefold:

1. Determine the reproducibility of measurements of passive muscle stiffness and stress relaxation.
2. Examine the relationship between passive muscle stiffness and stress relaxation.
3. Document changes in passive muscle stiffness and stress relaxation following repeated bouts of eccentric and concentric exercise.

6.3 METHODS

6.3.1 Experimental Design

The experimental design for the repeated bouts of eccentric (n=20) and concentric (n=10) exercise were described in Chapter 5 (section 5.3.1). However, reference to stiffness measurements were not made. On the initial test day, baseline measures of plasma creatine kinase (CK) activity, passive stiffness, stress relaxation and isometric strength were made prior to isokinetic exercise (eccentric or concentric). Subjects performed six sets of 10 contractions at a target intensity of 60% of isometric strength. Immediately after isokinetic

exercise isometric strength was re-tested. On the subsequent three days (Day 1,2,3) pain, plasma CK activity, muscle tenderness, passive stiffness, stress relaxation and isometric strength were measured. This four day protocol was repeated two weeks following the initial exercise day (11 days following Day 3). Measurement procedures for isokinetic exercise, isometric strength, pain, muscle tenderness and plasma CK activity were described in sections 3.3.2 to 3.3.7. Plasma CK measurements were made on only 12 subjects in the eccentric group due to unavailability of the instrument. Plasma CK measurements from one subject in the eccentric group were not included in the analysis because she had performed abdominal strengthening exercises on the day of the repeated bout. Subsequent abdominal soreness did not affect maximum isometric knee flexion strength tests. Plasma CK measurements were made on all 10 subjects in the concentric group.

6.3.2 Measurement of Passive Stiffness and Stress Relaxation

The measurement of passive stiffness was described in section 4.3.2, however, reference was not made to stress relaxation measurements. Stress relaxation was measured simultaneously with the stiffness measurement during the instrumented straight leg raise (SLR) stretch. The limb was raised to 60° (1.05 rad) at approximately $3°\cdot s^{-1}$ ($0.05\ rad\cdot s^{-1}$) and held at that angle for 60 s while force, ROM and EMG activity were continuously recorded. The torque/ROM relationship during the stretch was used to calculate passive stiffness (section 4.3.2). Stress relaxation with the stretch held at 60° was defined as the percent decline in force over the 60s. The mean of two stress relaxation measurements was recorded. However, tests which elicited EMG

activity in the hamstrings or rectus femoris were not included in analyses. Instrumented SLR stretches were applied to both the experimental limb and the contralateral non-experimental limb. Measurements on the non-experimental limb were used to determine the reproducibility of stiffness and stress relaxation measurements.

6.3.3 Statistics

Measurement reproducibility was assessed by computing intraclass correlation coefficients (ICC) and coefficients of variation (CV) on repeated measures of stiffness and stress relaxation on the non-experimental limb of all 30 subjects. ICC (3,1) was calculated using the equation (Portney and Watkins, 1993):

$$\frac{BMS - EMS}{BMS + (k-1)EMS}$$

where *BMS* is the between subjects mean square, *EMS* is the within subjects error mean square and k is the number of repeated measurements.

CV was defined as:

$$\frac{S.D.}{MEAN} \times 100$$

where *MEAN* is the mean of repeated measures on a given subject and *S.D.* is the standard deviation of those measures. The mean CV for the entire sample was determined.

The association between stiffness and stress relaxation was tested by separating subjects into tertiles based on the mean stiffness measurement from repeated measures on the non-experimental limb. The difference in stress relaxation between stiffness tertiles (Compliant, Normal, Stiff) was tested by

oneway ANOVA with post hoc comparisons using Bonferroni corrections. Changes in stiffness and stress relaxation following isokinetic exercise were tested by mixed model ANOVA: Group (Eccentric or Concentric) by Bout (Initial, Repeated) by Time (Pre, Post, Day 1, 2, 3). Mean values in the text are reported with the standard deviation (S.D.) while mean values in figures are displayed with the standard error of the mean (S.E.M.).

6.4 RESULTS

6.4.1 Reproducibility of Passive Stiffness and Stress Relaxation

Stiffness and stress relaxation values on the non-experimental limb for three subjects were not included in analyses because EMG activity was evident during one or more of the tests. The mean stiffness for the non-experimental limb was 26.1±9.0 $N{\cdot}m{\cdot}rad^{-1}$ with no systematic difference between repeated measures ($F_{GG\ 3.8,98.2df}$=1.4, p=0.24; Fig. 6.1a). The ICC value for repeated stiffness measures was 0.93 with 95% confidence intervals of 0.89 to 0.96. The mean intra-subject CV for repeated stiffness measurements was 7.5±3% with a range from 3.4% to 15.6%.

The mean stress relaxation for the non-experimental limb was 11.7±4.7 % with no systematic difference between repeated measures ($F_{GG\ 3.2,83.8df}$=0.95, p=0.42; Fig. 6.1b). The ICC value for repeated stress relaxation measures was 0.61 with 95% confidence intervals of 0.47 to 0.76. The mean intra-subject CV for repeated stress relaxation measurements was 33.7±50.9% with a range from 9.5% to 271%. However, the high CV for one subject (271%) was due to the fact

that this subject demonstrated a highly variable stress relaxation response with a low mean value (5%). The ICC value provides a better indication of reproducibility of stress relaxation.

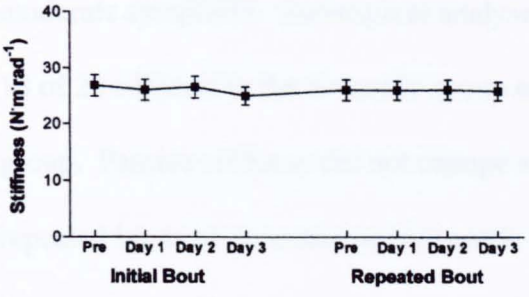


Figure 6.1.a: Stiffness values for the non-experimental limb of 27 subjects on each day of the study.

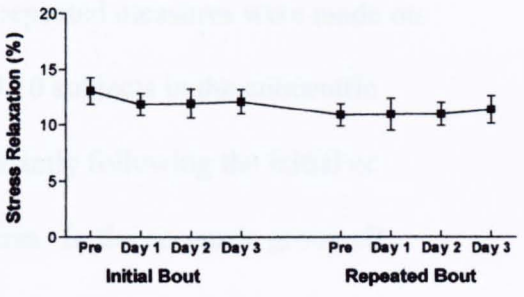


Figure 6.1.b: Stress relaxation values for the non-experimental limb of 27 subjects on each day of the study.

6.4.2 Association Between Passive Stiffness and Stress Relaxation

Stiffness measurements made on the non-experimental limb were averaged and subjects were split into tertiles (Compliant n=9, Normal n=9, Stiff n=9; Fig.6.2a). Stress relaxation on the non-experimental limb was not different between stiffness groups (Group effect $F_{2,24df}$=0.27, p=0.77; Fig. 6.2b).

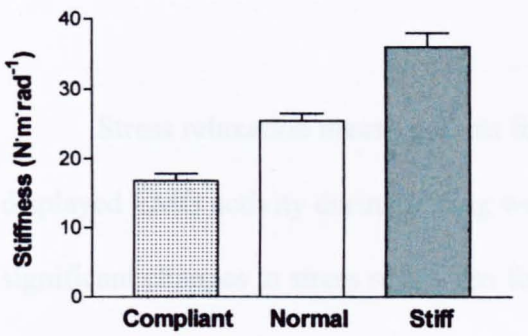


Figure 6.2a: Stiffness groups based on passive hamstring stiffness of the non-experimental limb (Group effect $F_{2,24df}$=44.4, p<0.001). Compliant < Normal < Stiff (p<0.001).

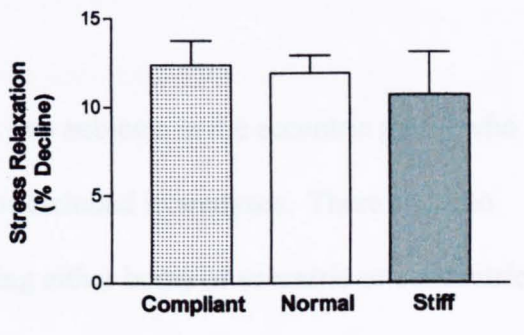


Figure 6.2b: Stress relaxation in the non-experimental limb across stiffness groups (Group effect $F_{2,24df}$=0.27, p=0.77).

6.4.3 Changes in Passive Stiffness and Stress Relaxation

EMG activity was evident during stretches of the experimental limb on the days following the initial bout in two subjects in the eccentric group. One of the subjects had minimal symptoms of muscle damage while the other had moderate symptoms. Subsequent analyses on repeated measures were made on 18 of 20 subjects in the eccentric group and all 10 subjects in the concentric group. Passive stiffness did not change significantly following the initial or repeated bouts of eccentric or concentric exercise. In the eccentric group all measures of stiffness were within 5% of baseline values (Fig 6.3).

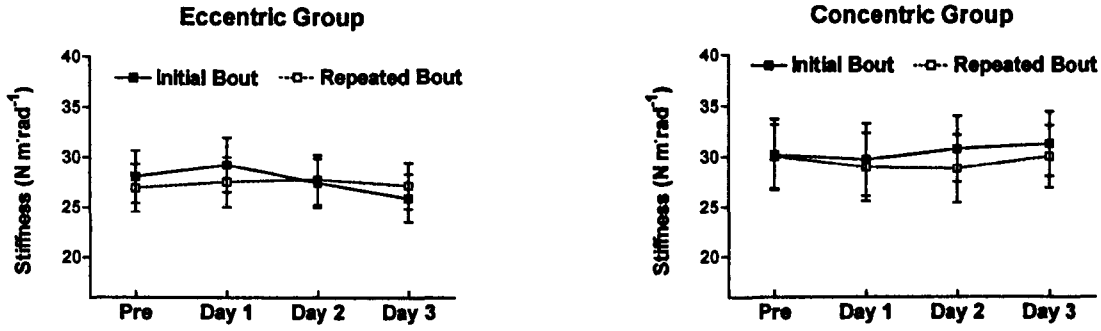


Figure 6.3: Passive hamstring stiffness following repeated bouts of eccentric and concentric exercise. Stiffness was not different between groups and did not change following either bouts of eccentric or concentric exercise (Group by Bout by Time $F_{GG\,2,51.3df}$=1.5, p=0.24).

Stress relaxation measurements for the two subjects in the eccentric group who displayed EMG activity during testing were not included in analyses. There were no significant changes in stress relaxation following either bouts of eccentric or concentric exercise (Fig. 6.4). Stress relaxation values were consistently higher in the eccentric group (13.2±3.6%) compared to the concentric group (8.9±3.6) (Group effect $F_{1,26df}$=9.0, p=0.006).

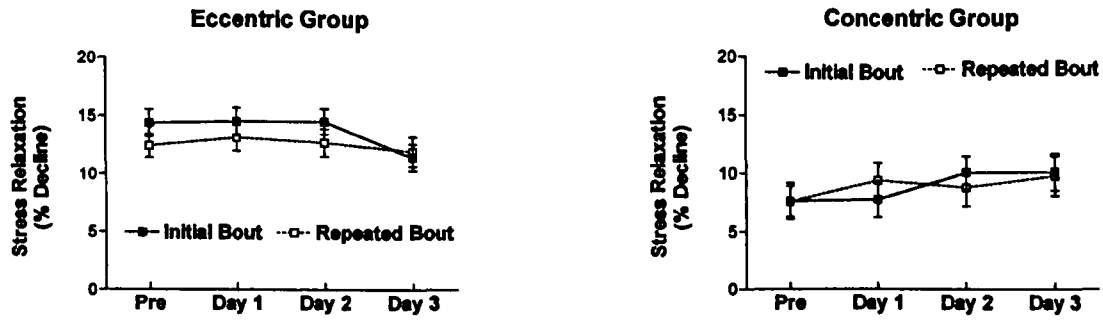


Figure 6.4: Stress relaxation following repeated bouts of eccentric and concentric exercise. Stress relaxation and did not change following either bouts of eccentric or concentric exercise (Group by Bout by Time $F_{GG\ 2.2,57.9df}$=1.01, p=0.38).

6.5 DISCUSSION

6.5.1 Reproducibility of Passive Stiffness and Stress Relaxation

Reproducibility of stiffness and stress relaxation measurements was assessed by both ICC and CV. Although there are no standard values for acceptable reproducibility, an ICC value of 0.93 and a CV value of 7.5% represent good reproducibility for stiffness measurements. By contrast an ICC value of 0.61 and a CV value of 33.7% represent poor reproducibility for stress relaxation measurements. Stiffness measurements were clearly more reproducible than stress relaxation measurements.

It was not possible to differentiate between measurement error and biological variability in assessing the reproducibility of stiffness and stress relaxation. Measurement error is affected by the sensitivity of the measuring instrument. The signal from the load cell was set to a sensitivity of approximately 0.4 N. The average force decline during the stress relaxation tests was approximately 3 N compared to a force increase from 20° to 50°during the

stretch of approximately 14 N. Measurement sensitivity (0.4 N) was on average 13% of the measured effect during stress relaxation tests compared to 3% during stiffness tests. The variability in stress relaxation could, in part, be explained by the small difference between measurement sensitivity and effect size.

6.5.2 Association Between Passive Stiffness and Stress Relaxation

The subjects were split into tertiles (Compliant, Normal, Stiff) based on the mean of eight stiffness measurements. Passive stiffness was 115% higher in Stiff compared to Compliant subjects. However, despite a marked difference in passive stiffness the average stress relaxation response was similar between groups. Stress relaxation was 12.4±4.2% in subjects with compliant hamstrings and 10.7±7.1% in subjects with stiff hamstrings. These data are in agreement with previous results comparing stress relaxation responses between stretches of different magnitudes (McHugh *et al.*, 1992). Stress relaxation was 14.4±8.5% during SLR stretches held at maximum ROM (90±23°) for 45 s compared to 13±8.9% for stretches held at a sub-maximal ROM (53±23°). This indicates that the decline in passive tension during the stretches held at 60° was proportional to the tension applied to the tissue.

Stress relaxation provides an indication of the viscous contribution to passive tension during stretch. The magnitude of the stress relaxation response to rapid small amplitude stretches has been used to identify specific viscous, viscoelastic and elastic characteristics of isolated rat muscle fibres (Mutungi and Ranatunga, 1996). A similar approach in the present study can provide an estimate of the viscous and elastic contributions to the passive stiffness measurement (Fig. 6.5). Force decline during the stress relaxation test represents

the viscous contribution to the peak force at 60°. At a fixed rate of stretch the viscous force is constant during the stretch. Therefore, a mean stress relaxation of 11.7±4.7% indicates that approximately 88.3% of the passive stiffness was due to the elastic component (Fig. 6.5). The data demonstrate that muscle stiffness measured during slow passive stretch primarily reflect the tissue's elastic properties with only a small contribution from the viscous component. However, at higher rates of stretch the viscous contribution is greatly increased (Mutungi and Ranatunga, 1996).

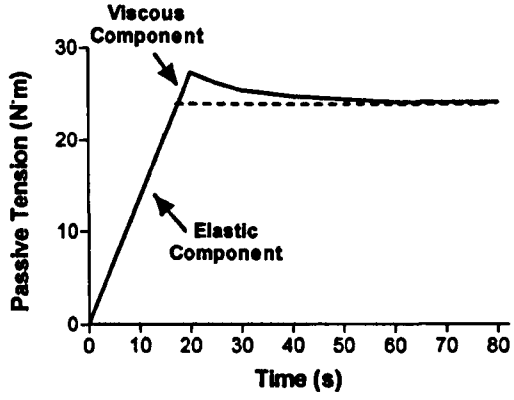


Figure 6.5a: Estimated elastic and viscous components to passive tension based on the average stress relaxation response.

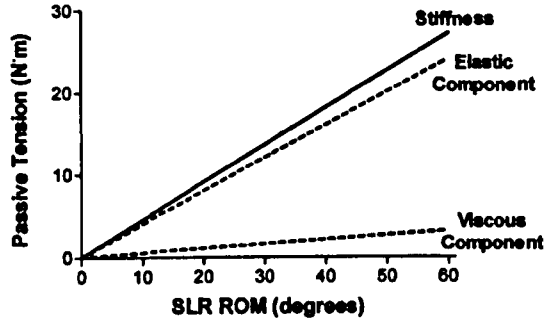


Figure 6.5b: Passive stiffness curves separated into elastic and viscous components based on the stress relaxation response shown in Fig. 6.5.a.

6.5.3 Stiffness and Stress Relaxation Following Eccentric Exercise

Previous studies that have attempted to evaluate stiffness following eccentric exercise have examined the elbow flexors (Clarkson and Tremblay, 1988; Ebbeling and Clarkson, 1990; Cleak and Eston, 1992b; Howell *et al.*, 1993; Chleboun *et al.*, 1995; Saxton and Donnelly, 1996; Nosaka and Clarkson, 1997). Only two studies (Howell *et al.*, 1993; Chleboun *et al.*, 1995) quantified elbow flexor stiffness according to the relationship between passive torque and ROM. Baseline elbow flexor stiffness was 0.73 $N{\cdot}m{\cdot}rad^{-1}$ (Chleboun *et al.*, 1995)

and 1.2 $N \cdot m \cdot rad^{-1}$ (Howell *et al.*, 1993) and was increased by approximately 120% and 140%, respectively, two days following eccentric exercise. In the present study baseline hamstring stiffness was much higher (27.9 $N \cdot m \cdot rad^{-1}$). Surprisingly, hamstring stiffness was not increased on the days following the initial bout of eccentric exercise. The lack of an increase in stiffness could have been due to: 1) the limited amount of damage induced by eccentric exercise; 2) an invalid or unreliable measurement of stiffness; or 3) anatomic and functional differences between the elbow flexors and the hamstrings.

1) Peak strength loss occurred on Day 1 and peak pain occurred on Day 2. Stiffness changes on those days were assessed in the subjects who experienced the greatest strength loss and pain. Ten subjects experienced strength loss of greater than 15% on Day 1. Stiffness in these subjects was increased by $5 \pm 7.6\%$. In the eight subjects who experienced the most pain ($\geq$5/10) on Day 2 stiffness was actually decreased by $4.1 \pm 14.9\%$. Elbow flexor strength loss was approximately 45% (Chleboun *et al.*, 1995) and 35% (Howell *et al.*, 1993) one day following the eccentric exercise and pain was approximately three out of four two days following eccentric exercise. Stiffness on those days was elevated by more that 100%. Although strength loss and pain were greater in the elbow flexors than the hamstrings it is unlikely that this difference could completely explain the contrasting effects on muscle stiffness.

2) The ability to detect a change in hamstring stiffness was dependent on whether the measurement technique actually measured stiffness of the hamstring muscle group and whether the measurement error was sufficiently small to detect a change. The SLR test is a standard goniometric

measurement of hamstring flexibility (Liemohn, 1993). Stiffness measured during the instrumented SLR test used in the present study has been shown to be highly correlated to an independent goniometric measure of maximum SLR ROM ($r=-0.81$, $p<0.0001$) (McHugh *et al.*, 1998). Although it is likely that other soft tissues surrounding the hip and knee joint contributed to the measured stiffness the measurement is thought to primarily reflect the extensibility of the hamstring muscle group.

The ability to detect a change in stiffness is dependent on the sensitivity of the instrument, the measurement error and the variability in the observed change between subjects. The sample size required to demonstrate a significant effect is dependent on the magnitude of the effect and the standard deviation of that effect and can be calculated according to the following equation (Kirkwood, 1988):

$$n = \frac{(u+v)^2 \sigma^2}{(\mu - \mu_0)^2}$$

where n = the sample size; u = the one-sided percentage point of the normal distribution corresponding to the chosen beta level (e.g. 80% power, beta = 0.2, u = 0.84); v = the percentage point of the normal distribution corresponding to the two-side alpha level (e.g. alpha = 0.05, v = 1.96); σ = the standard deviation (S.D.) of the effect; and μ-μ_0 = the effect size.

This equation can be manipulated to calculate the magnitude of effect size required for a given alpha and beta level assuming the same S.D. of effect:

$$effect\ size = \sqrt{\frac{(u+v)^2 \sigma^2}{n}}$$

In the present study repeated measures were made and the required alpha level must be corrected according to the number of comparisons being made. Baseline stiffness was compared to stiffness on each of the three days following the initial bout of eccentric exercise. The alpha level required for significance should be adjusted from 0.05 to 0.02. Given this adjusted alpha level and a beta level of 0.2 it was possible to detect a change in stiffness of 2.3 $N{\cdot}m{\cdot}rad^{-1}$ (8%) on Day 1, 2.9 $N{\cdot}m{\cdot}rad^{-1}$ (10%) on Day 2 and 4.3 $N{\cdot}m{\cdot}rad^{-1}$ (15%) on Day 3. These calculations suggest that there was sufficient power to detect a clinically significant increase in stiffness.

3) As has been discussed previously, the lack of an increase in stiffness in the hamstrings following eccentric exercise is in marked contrast to the dramatic increase in stiffness of the elbow flexors following eccentric exercise. It appears that neither measurement error nor the apparent magnitude of muscle damage can fully explain this disparity. The different responses may be related to anatomic and functional differences between the muscle groups. The biceps femoris, semitendinosus and semimembranosus muscles are biarticular muscles. By contrast the primary elbow flexor, the brachialis, is uniarticular while the secondary flexor, the biceps brachii, is biarticular. Elbow flexor muscle damage is typically induced in the non-dominant arm to limit functional impairment to the subject. In the presence of damage, elbow motion can be voluntarily reduced by substituting the dominant arm for required tasks. However, it is more difficult to limit normal hamstring function in the presence of damage since the hamstrings play an integral role in normal gait. A greater level of activity in the hamstrings compared to the

elbow flexors following eccentric exercise may have implications for the subsequent changes in stiffness.

Howell *et al.* (1993) demonstrated a 150% increase in elbow flexor stiffness immediately following eccentric exercise. As previously discussed, stiffness remained elevated on subsequent days. By contrast Magnusson *et al.* (1995b) found no change in passive hamstring tension immediately following 40 maximal eccentric hamstring contractions. These results support the present findings and suggest that there is an inherent difference between the response of the hamstrings and elbow flexors to eccentric exercise.

The lack of an effect on stress relaxation following eccentric exercise is consistent with the stiffness results. Since the stiffness measurement was a function of both elastic and viscous components (see section 6.5.2) a change in stress relaxation should be reflected by a change in stiffness. This was not the case in the present study.

To this point, changes in stiffness and stress relaxation have been discussed with regard to muscle damage and not in relation to the repeated bout effect. Although the analyses indicated no difference in stiffness and stress relaxation between the repeated bouts, the number of repeated measures may have obscured an effect. If the repeated bout effect was attributable to a change in viscoelasticity there should have been a significant difference in stiffness or stress relaxation between the measurements made immediately prior to the initial bout (pre-damage) compared to immediately prior to the repeated bout (post-

repair). However, there was no significant change in stiffness (p=0.42) or stress relaxation (p=0.35) between measurements made prior to the initial bout compared to prior to the repeated bout (Group by Bout ANOVA). In the eccentric group, stiffness was decreased by 1.2±3.1 $N{\cdot}m{\cdot}rad^{-1}$ (4%) compared to 0.2±2.8 $N{\cdot}m{\cdot}rad^{-1}$ (0.6%) in the concentric group. In the eccentric group, stress relaxation was decreased by 2.0±6.1% compared to 0.04±2.5% in the concentric group. The effect size required to demonstrate a significant difference between the eccentric and concentric groups can be calculated using the following equation (Kirkwood, 1988):

$$effect\ size = \sqrt{\frac{(u+v)^2\left(\sigma_1^2+\sigma_2^2\right)}{n}}$$

where n = the harmonic mean of the different sample sizes (eccentric=18; concentric=10):

$$n = harmonic\ mean = \frac{2 \cdot n_1 \cdot n_2}{n_1 + n_2}$$

For an alpha level of 0.05 and a beta level of 0.2 a decrease in stiffness of 3.7 $N{\cdot}m.rad^{-1}$ (13%) and a decrease in stress relaxation of 6% would be required in the eccentric group. These estimates suggest that there was probably sufficient power to detect a change in stiffness, but insufficient power to detect a change in stress relaxation given that the mean stress relaxation was 12%.

The data from Lapier *et al.* (1995) indicated that the repeated bout effect was attributable to an increase in intramuscular connective tissue (section 6.2.2). A significant increase in intramuscular connective tissue would be expected to increase passive muscle stiffness (Kovanen *et al.*, 1984). There was clearly no

such increase in stiffness in the present study. These data suggest that the repeated bout effect was not due to a change in the passive viscoelastic properties of the hamstring muscle group. However, an adaptation in the viscoelastic properties of active skeletal muscle cannot be ruled out. Pousson *et al.* (1990) demonstrated an increase in active stiffness of the elbow flexors following eccentric training. Active stiffness was measured by the quick-release technique. The effects were attributed to either increased tendon stiffness or increased cross-bridge stiffness. *In vivo* measurements of active muscle stiffness have not been studied with respect to eccentric exercise, muscle damage and the repeated bout effect. Further study in this area is warranted since cross-bridge stiffness would presumably have an impact on sarcomere popping during eccentric contractions. The non-uniformity of sarcomere length during eccentric contractions indicates that cross-bridges in some sarcomeres are more easily detached than in other sarcomeres in series. It is unclear whether this is due to variation in the rates of cross-bridge attachment and re-attachment or variation in the binding strength of individual cross-bridges. Greater active stiffness in slow-twitch motor units compared to fast-twitch units was attributed to slower rates of cross-bridge cycling in the slow-twitch units (Petit *et al.* 1990). With respect to the repeated bout effect, a decrease in the rate of cross-bridge cycling may protect sarcomeres from disruption during eccentric contractions.

6.5.4 Summary

In the present study the viscoelastic properties of the hamstring muscle group were measured following repeated bouts of eccentric exercise. Hamstring muscle stiffness, estimated from torque/ROM curves during passive stretch,

provided an indication of the muscles' elastic properties. Stress relaxation, measured as the percent decline in tension during a fixed stretch held for one minute, provided an indication of the muscles' viscous properties. The stiffness measurement had good reproducibility while the stress relaxation measurement had poor reproducibility. The stress relaxation response was similar between subjects with stiff and compliant hamstrings. It was estimated that 88% of the tension during slow passive stretch could be attributed to the elastic characteristics of skeletal muscle. A greater viscous component would be predicted with faster rates of stretch. Surprisingly, passive hamstring stiffness was not increased on the days following the initial bout of eccentric exercise despite symptoms of muscle damage. These findings were in marked contrast to changes in stiffness of the elbow flexors following eccentric exercise. This disparity may be explained by anatomic and functional differences between the muscle groups. Despite a clear repeated bout effect stiffness and stress relaxation measurements were similar between initial and repeated eccentric bouts. These findings indicate that the repeated bout effect was not attributable to a change in the passive viscoelastic properties of the hamstring muscle group.

CHAPTER 7

CONCLUSIONS

7.1 NEW CONTRIBUTIONS TO THE LITERATURE

7.1.1 Neural Control of Eccentric Contractions (Chapter 3)

The possibility that fast-twitch motor units are preferentially recruited for eccentric contractions was first suggested by Nardone and Schieppati (1988) who demonstrated de-recruitment of the soleus (primarily slow-twitch) and facilitation of the gastrocnemius (primarily fast-twitch) for the eccentric phase of reciprocal sub-maximal concentric/eccentric contractions of the plantarflexors. A subsequent study confirmed these findings and also demonstrated that some motor units were activated during the eccentric phase that had been silent during both the concentric phase, and ramp isometric contractions (Nardone *et al.*, 1989). The amplitudes of the action potentials from these units were consistent with fast-twitch motor units. These findings were recently confirmed by Howell *et al.* (1995) in the first dorsal interosseous muscle. All three studies (Nardone and Schieppati, 1988; Nardone *et al.*, 1989; Howell *et al.*, 1995) utilised fine wire intramuscular EMG recordings to identify the activation of individual motor units. However, with these techniques it becomes more difficult to identify newly recruited motor units as contraction intensity increases. Consequently, proposed selective recruitment was based on low intensity contractions (<25% MVC) (Nardone and Schieppati, 1988; Nardone *et al.*, 1989; Howell *et al.*, 1995).

Surface EMG techniques have also been used to study motor unit recruitment. An increase in mean frequency or median frequency with increasing contraction intensity is thought to indicate recruitment of higher threshold motor units (Solomonow *et al.*,1990; Gerdle *et al.*, 1991; Hägg, 1992). It follows that,

in non-fatigued muscle, differences in median frequency or mean frequency between contraction types at a given intensity would indicate differences in the type of motor unit recruited. Previous studies have failed to demonstrate higher frequencies during low intensity (<30% MVC) eccentric contractions compared to concentric contractions of the elbow flexors (Moritani *et al.*, 1988; Nakazawa *et al.*, 1993; Potvin, 1997). However, eccentric contractions require less motor unit activation than concentric contractions at the same intensity (Bigland and Lippold, 1954b; Komi *et al.*, 1987; Tesch *et al.*, 1990; Adams *et al.*, 1992; Potvin 1997). Similar frequencies despite reduced activation with may indicate preferential fast-twitch motor unit recruitment (Potvin, 1997).

Selective recruitment of fast-twitch motor units at eccentric contraction intensities sufficient to cause muscle damage has not been specifically examined either by intramuscular or surface EMG techniques. Surface EMG activity, recorded during eccentric (n=20) or concentric (n=10) hamstring contractions at 60% of maximum isometric strength, is described in Chapter three. Consistent with previous research, motor unit activation relative to force production was markedly lower for eccentric compared to isometric contractions. Despite lower activation, median frequency was 15% higher for sub-maximal eccentric compared to maximal isometric contractions. By contrast median frequency was 8% lower for sub-maximal concentric compared to isometric contractions. During eccentric exercise median frequency increased by 9% but did not change during concentric exercise. These results are consistent with selective recruitment of fast-twitch motor units for sub-maximal eccentric exercise.

As expected eccentric exercise resulted in symptoms of muscle damage while symptoms were mostly absent following concentric exercise. Several

studies have indicated that muscle damage occurs primarily in fast-twitch fibres (Fridén *et al.*, 1983b; Fridén 1984; Lieber and Fridén, 1991; MacPherson *et al.*, 1996), although, other studies have disputed these findings (Armstrong *et al.*, 1983; Mair *et al.*, 1992). Preferential damage to fast-twitch fibres may be due, in part, to selective recruitment of fast-twitch motor units for eccentric contractions. However, it was not within the scope of this research to examine the distribution of damage among fibre types.

7.1.2 Passive Muscle Stiffness and Symptoms of Muscle Damage (Chapter 4)

Passive muscle stiffness has been quantified according to the relationship of passive joint torque to joint range of motion (ROM) in the elbow flexors (Watts *et al.*, 1986; Wiegner and Watts, 1986; Howell *et al.*, 1993; Chleboun *et al.*, 1995), hamstrings (Gajdosik *et al.*, 1990; Gajdosik, 1991; Halbertsma and Goeken, 1994; Magnusson *et al.*, 1995a; Halbertsma *et al.*, 1996; Magnusson *et al.*, 1996; McHugh *et al.*, 1998) and plantarflexors (Hufschmidt and Mauritz, 1985). Increased passive muscle stiffness has been demonstrated in neurological disorders characterised by spasticity (Hufschmidt and Mauritz, 1985) and rigidity (Watts *et al.*, 1986). Increased passive muscle stiffness has also been demonstrated following eccentric exercise which caused symptoms of muscle damage (Howell *et al.*, 1993; Chleboun *et al.*, 1995). Passive stiffness in normal muscle varies considerably between individuals. The extent to which inter-individual differences in passive stiffness affect normal muscle function has not been specifically studied. Symptoms of muscle damage following eccentric hamstring contractions between subjects differing considerably in terms of

passive hamstring stiffness were described in Chapter four. Subjects with stiffer hamstrings experienced greater symptoms of muscle damage on the days following eccentric exercise. An exact mechanism for the apparent susceptibility of stiff muscles to damage was not immediately apparent. However, it is possible to explain these findings based on the sarcomere strain theory of muscle damage. Morgan (1990) proposed that damage occurs with cyclic strain of sarcomeres which have been strained beyond myofilament overlap. Consistent with this theory Armstrong *et al.* (1991) suggested that damage occurs with eccentric contractions on the descending limb of the length-tension curve. Greater damage in subjects with stiffer hamstrings could be explained by greater sarcomere strain during eccentric contractions in these subjects. It is proposed that the strain imposed by active lengthening of a stiff muscle is transferred from a rigid tendon-aponeurosis complex to the muscle fibres, resulting in myofibrillar strain. In a compliant muscle the tendon-aponeurosis complex can absorb lengthening imposed by eccentric contractions thereby reducing myofibrillar strain.

The apparent susceptibility of stiff muscles to damage with eccentric exercise is indirectly supported by data which indicated that warm-up prior to a bout of eccentric exercise decreased the subsequent symptoms of damage (Nosaka and Clarkson, 1997). Warm-up is thought to reduce passive muscle stiffness (Gleim and McHugh, 1998). Studies typically demonstrate a high inter-individual variability in symptoms of muscle damage following eccentric exercise. The present data suggest that this variability may, in part, be explained by differences in passive muscle stiffness. The possibility of reduced symptoms with warm-up warrants further investigation to determine if the protective effect is due to decreased stiffness.

7.1.3 Neural and Mechanical Mechanisms for the Repeated Bout Effect (Chapter 5 and Chapter 6)

Despite demonstrating only moderate symptoms of muscle damage following the initial bout of eccentric exercise a clear repeated bout effect was evident in each marker of muscle damage. Not only were symptoms mostly absent following the repeated bout, but subjects actually showed a slight increase in isometric strength over the three days following the repeated bout. However, there were no obvious neural or mechanical adaptations associated with the repeated bout effect.

Several authors have suggested that a neural adaptation might explain the protective adaptation (Pierrynowski *et al.*, 1987; Golden and Dudley, 1992; Mair *et al.*, 1994; Nosaka and Clarkson, 1995). In general these studies have pointed to potential adaptations in motor unit behaviour during a repeated bout which might serve to limit the stress on the activated fibres. Two potential neural adaptations were examined in Chapter five: 1) an increase in motor unit activation relative to torque production in the repeated bout; 2) increased recruitment of slow-twitch motor units during the repeated bout. Change in activation was tested by analysing EMG relative to torque production (EMG/torque). Change in recruitment was tested by analysing median frequency changes. The occurrence of muscle damage with eccentric exercise has been attributed to high stress on a small number of active fibres (Armstrong *et al.*, 1983; Moritani *et al.*, 1988). It was hypothesised that an increase in activation relative to force production would distribute the contractile stresses among a larger population of active fibres. Additionally, a shift to recruitment of more

slow twitch motor units might prevent damage to susceptible fast-twitch fibres. However, there was no evidence of such changes in either activation or recruitment during the repeated bout. EMG/torque and median frequency were surprisingly similar between the initial and repeated bouts.

Some authors have suggested that an adaptation in intramuscular connective tissue or the cytoskeleton following an initial bout of eccentric exercise protects the muscle from damage following a repeated bout (Fridén *et al.*, 1983a; Newham *et al.*, 1987; Clarkson and Tremblay, 1988, Ebbeling and Clarkson, 1990; Lapier *et al.*, 1995). Lapier *et al.* (1995) attributed the repeated bout effect to increased intramuscular connective tissue following repair of damage from the initial injury. An increase in intramuscular connective tissue would be expected to increase passive muscle stiffness (Kovanen *et al.*, 1984). The theory that increased stiffness would provide protection from a repeated bout of eccentric exercise is in direct contradiction to the results presented in Chapter three. Subjects with stiffer muscles experienced greater symptoms of muscle damage following an initial eccentric bout. Additionally, Nosaka and Clarkson (1998) proposed that warm-up prior to eccentric exercise decreases subsequent symptoms of damage by reducing passive muscle stiffness. Although increased passive muscle stiffness has been documented following eccentric exercise of the elbow flexors (Howell *et al.*, 1993; Chleboun *et al.*, 1995) these studies did not examine stiffness following a repeated bout. Passive stiffness following repeated bouts of eccentric exercise is described in Chapter six.

Surprisingly passive hamstring stiffness was not increased following the initial bout of eccentric exercise and remained very consistent across all measurements made during both the initial and repeated bouts. The lack of an

increase in stiffness following the initial bout is in direct contrast to the response of the elbow flexors following eccentric exercise (Howell *et al.*, 1993; Chleboun *et al.*, 1995). The different response may, in part, be explained by less damage in the hamstrings compared to the elbow flexors. However, the difference in the stiffness response to eccentric exercise may be attributed to inherent differences between the two muscle groups. The lack of a change in hamstring stiffness immediately following eccentric exercise is consistent with the results of Magnusson *et al.* (1995b) but is in contrast with a dramatic increase in stiffness of the elbow flexors (Howell *et al.*, 1993; Chleboun *et al.*, 1995). There was no indication of any change in stiffness between the initial and repeated bouts of eccentric exercise. All stiffness measurements were within 5% of baseline which suggests that the repeated bout effect was not due to a change in the passive mechanical properties of the muscle.

7.2 STUDY LIMITATIONS

7.2.1 Quantification of EMG activity

The amplitude of the EMG signal was quantified by integrating the unsmoothed, full-wave rectified signal for all activity of all contractions to provide a measure of iEMG in μV·s. The integrated EMG measure was divided by the integration of the corresponding torque curves to provide a measure of EMG/torque in μV/N·m. The advantage of this technique was that all EMG activity and torque production for the duration of activation through the full range of motion was accounted for. Alternatively, the peak amplitude of each

contraction could have been quantified from the root mean square (RMS) of the raw signal (Moritani and Muro, 1987) or the linear envelope of the signal (Kamen and Caldwell, 1996) to provide a measure of EMG in μV. This value could have been expressed relative to the peak torque for that contraction. A third possibility would have been to smooth the rectified signal prior to integration of discrete intervals of approximately 50-100 ms (Kamen and Caldwell, 1996). These alternative techniques would have provided quantification of the peak EMG activity for a given contraction as opposed to including all EMG activity associated with the entire contraction. The raw EMG activity of one subject for the first and sixth sets from the initial bout of eccentric exercise was analysed by the RMS technique for comparison to iEMG values (Fig. 7.1). The RMS values were averaged over 50 ms intervals to provide a smoothed signal. Peak RMS values were averaged for each contraction across the three muscles and divided by the average torque associated with those contractions. This provided a measure of EMG/torque based on peak values for comparison to the values calculated from the whole contraction. The techniques yielded comparable values despite the different methods of calculation.

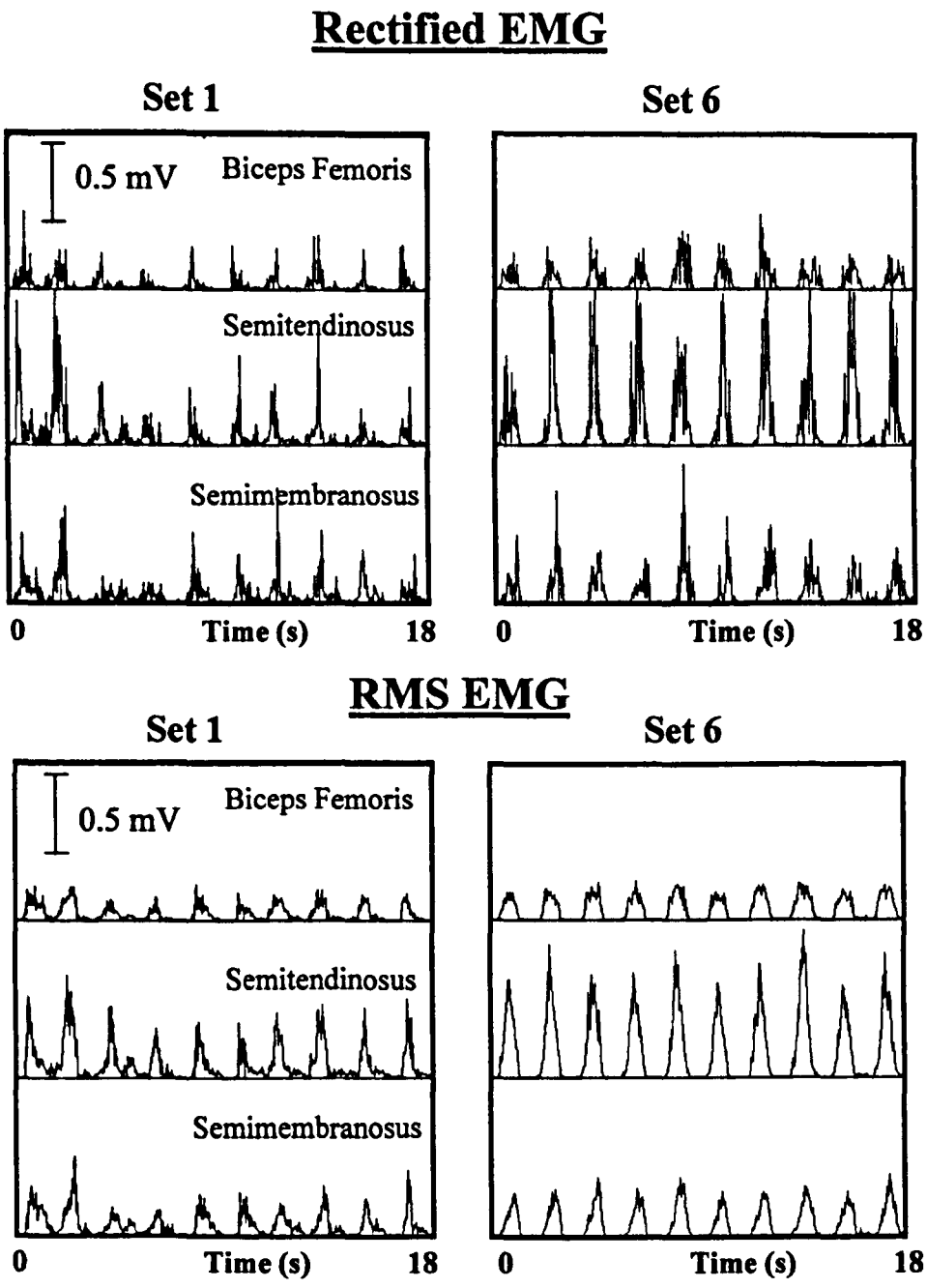


Figure 7.1: Comparison of full-wave rectified EMG (top two graphs) with smoothed RMS EMG (bottom two graphs) for the first and sixth sets of eccentric hamstring contractions of one subject (this subject's data was also shown in Fig. 3.1). EMG/torque calculated by integration of the rectified EMG signal and integration of the associated torque curves was 4.9 μV/N·m for Set 1 and 7.7 μV/N·m for Set 2. EMG/torque calculated by averaging peak RMS and peak torque values was 4.7 μV/N·m for Set 1 and 6.6 μV/N·m for Set 6.

EMG frequency analysis was performed by applying fast Fourier transforms (FFT) to the raw EMG signal. The number of data points entered in an FFT must be a power of two (e.g. 16, 32, 64 etc.). Given the stochastic nature of EMG signals a minimum of 512 data points are typically analysed (Kamen and Caldwell, 1996). At a sampling rate of 1000 Hz 512 data points covers 512 ms. All isometric contractions lasted 5 s while isokinetic contractions lasted approximately 1 s. For isometric contractions 4096 data points were analysed covering 4.096 s of the 5 s contractions. A common problem with frequency analysis of dynamic contractions is that muscle length is continually changing and conduction velocity decreases with increasing muscle length (Morimoto, 1986). Median frequency primarily reflects conduction velocity (Hägg, 1992). Therefore FFTs performed on EMG activity at longer muscle lengths will yield lower median frequencies. A difference in median frequency between two isokinetic contractions could be due to sampling activity at different muscle lengths. For this reason all EMG activity associated with a given contraction was included in the FFTs. This ensured analyses over the full ROM of the contraction. FFT length was set at 4096 points to be consistent with isometric analyses and included two consecutive isokinetic contractions. The inclusion of periods of inactivity between contractions adds noise to analyses compared to isometric contractions. Considering the relatively high contraction intensity the noise between contractions presumably contributed minimally to the total power. Additionally, the effect of the noise would have been similar for concentric and eccentric contractions. However, median frequency for eccentric contractions was markedly higher than for isometric contractions while concentric median

frequency was not different from isometric contractions. It appears that the inclusion of low level noise in frequency calculations was not sufficient to adversely affect the analyses.

7.2.2 EMG Measurement Error

Although it was possible to detect differences in EMG/torque and median frequency between contraction types no differences were seen in these variables between the initial and repeated bouts of eccentric or concentric exercise. It was concluded that the repeated bout effect was not due to neural adaptations. However, measurement error was not quantified for either EMG/torque or median frequency and the possibility of a type two error was not rejected. The ability to determine EMG measurement error for repeated bouts of eccentric exercise is confounded by the fact that a single bout tends to cause damage while a repeated bout does not. Comparison of bouts involving a small number of eccentric contractions in a large number of subjects might overcome this problem.

Based on the proposed theories (section 5.2) an increase in EMG/torque and a decrease in median frequency was expected. The negative findings could be due to a combination of limited sample size and measurement error. It is possible to estimate if the sample size was sufficient to demonstrate a physiologically relevant effect. EMG/torque at baseline was 8.4 $\mu V/N \cdot m$ for isometric contractions (prior to initial bout) and 3.3 $\mu V/N \cdot m$ for eccentric contractions (Set 1 of initial bout). EMG/torque increased to 4.3 $\mu V/N \cdot m$ during eccentric contractions but did not change for the isometric contraction immediately following the eccentric bout. Short-term eccentric training has been

shown to increase EMG/force by approximately 20% (Komi and Buskirk, 1972; Hortobágyi *et al.*, 1996b). Komi and Buskirk (1972) noted that this change was evident at the time that subjects no longer experienced the symptoms of muscle damage. A 20% increase in EMG/torque between the initial and repeated bouts of eccentric contractions would represent an average increase of 0.76 μV/N·m. The mean observed difference was 0.08±1.05 μV/N·m. Based on the standard deviation (S.D.) of this effect (1.05 μV/N·m) and an expected effect of 0.76 μV/N·m the sample size required for an alpha level of 0.05 and a beta level of 0.2 can be calculated using the following equation initially described in section 6.5.3 (Kirkwood, 1988):

$$n = \frac{(u+v)^2\sigma^2}{(\mu-\mu_0)^2}$$

where n = the sample size; u = the one-sided percentage point of the normal distribution corresponding to the chosen beta level (e.g. 80% power, beta = 0.2, u = 0.84); v = the percentage point of the normal distribution corresponding to the two-side alpha level (e.g. alpha = 0.05, v = 1.96); σ = the S.D. of the effect; and μ-μ_0 = the effect size.

Based on this calculation a sample size of 15 would have been sufficient to demonstrate a 20% increase in EMG/torque between repeated bouts of eccentric exercise. The negative findings with respect to EMG/torque were probably not due to a type two error.

Previous research has not examined changes in EMG frequency with either repeated bouts of eccentric exercise or eccentric strength training. The increase in frequency with increasing contraction intensity has been attributed to

increased recruitment of fast-twitch motor units (Gerdle *et al.*, 1991). Quadriceps mean power frequency increased by approximately 5-8 Hz with increasing isometric contraction intensity from 40% MVC to maximum. This increase in median frequency with increasing contraction intensity was low compared to the increase in EMG amplitude (Gerdle *et al.*, 1991). In the present study median frequency was approximately 10 Hz higher for sub-maximal eccentric compared to maximal isometric contractions. A 5 Hz decrease in median frequency between the initial and repeated bouts of eccentric contractions would represent a physiologically significant change. Median frequency was 0.7±8.3 Hz. higher for the repeated bout. Based on this S.D. and a predicted effect of 5 Hz a sample size of 22 would be required for an alpha level of 0.05 at a beta level of 0.2. There were 20 subjects in the eccentric group in the present study. However, since there was a minimal difference in median frequency between the initial and repeated bouts it is likely that a type two error was not committed.

7.2.3 Interpretation of EMG activity

The EMG amplitude and frequency measurements were intended to provide an indication of differences in motor unit activation and recruitment between contraction types and between repeated bouts. The findings with respect to EMG/torque are very consistent with previous studies showing lower activation for eccentric compared to concentric contractions (Bigland and Lippold, 1954b; Komi *et al.*, 1987; Tesch *et al.*, 1990; Adams *et al.*, 1992; Potvin 1997). It follows that an increase in EMG/torque during eccentric contractions at a fixed intensity reflected activation of additional motor units. The findings with respect to median frequency are not supported by previous studies. Higher

median frequency for eccentric contractions and increased median frequency during eccentric exercise were attributed to selective recruitment of fast-twitch motor units. However, many biological and technical factors can affect the frequency content of the EMG signal (Kamen and Caldwell, 1996). These factors were discussed in detail in section 3.5.2. Biological factors include motor unit firing rates, muscle temperature and motor unit synchronisation. Technical factors include electrode orientation, muscle length, movement artefact and ambient noise. Higher median frequency for eccentric contractions could be explained in biological terms by higher firing rates in motor units regardless of type, higher muscle temperatures or decreased synchronisation. However, such mechanisms were unlikely in the present study (see section 3.5.2). Technical factors such as placing electrodes closer together or recording EMG activity at a shorter muscle length would tend to increase median frequency. However, there is no reason to believe that such factors were involved in the observed effects in the present study.

7.2.4 Quantification of Passive Hamstring Stiffness

The term stiffness was used to describe the increase in passive torque from 20°-50° during the instrumented straight leg raise stretch. It was assumed that the relationship was linear between these points. Therefore, torque measurements between 20° and 50° were not included in the analyses. Although the slopes of the torque/ROM curves always appeared linear in this region, a calculation of stiffness from the linear regression of all points would have provided a more accurate measurement. However, it can be seen in Figure 7.2 that the margin of error is quite small for fitting a line between the two points. It

has been shown that as stretches approach a subject's maximum tolerable ROM the torque/ROM curve becomes non-linear (Howell *et al.*, 1993; Chleboun *et al.*, 1995; McHugh *et al.*, 1998). In the present study stiffness was measured in a common ROM to allow comparison between individuals. Although there was no apparent increase in hamstring stiffness following eccentric exercise, it is possible that stiffness was increased at terminal ROM. However, previous studies (Howell *et al.*, 1993; Chleboun *et al.*, 1995) have demonstrated increased elbow flexor stiffness early in the ROM.

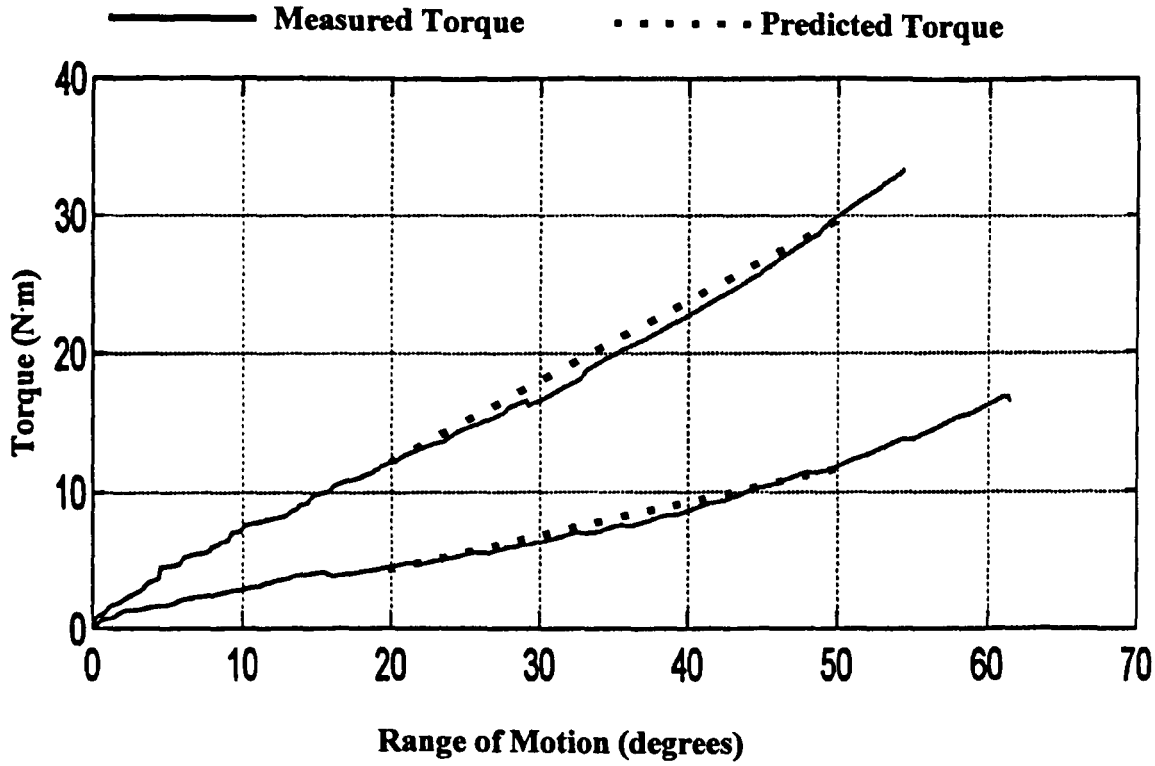


Figure 7.2: Passive torque measured during the instrumented straight leg raise stretch in two subjects differing considerably in terms of stiffness. The dotted line is a straight line from 20°-50°. It is apparent that torque increases linearly from 20°-50° in both subjects.

7.2.5 Symptoms of Muscle Damage

The purpose of this research was to examine neural and mechanical factors associated with exercise-induced muscle damage and the repeated bout effect. Therefore, the primary objective was to demonstrate exercise-induced muscle damage following an initial bout of eccentric exercise and reduced damage following a repeated bout. Unfortunately, a direct measure of muscle damage, such as electron microscopic analysis of myofibrils from muscle biopsies, was not made. Four indirect markers of muscle damage were used: isometric strength, pain, muscle tenderness and elevated plasma creatine kinase

(CK) activity. However, the difficulty in quantifying damage from electron micrographs makes this measurement more useful from a qualitative perspective. Faulkner *et al.* (1993) suggested that strength loss may be a better quantitative measure of muscle damage.

The changes in strength, pain, muscle tenderness and plasma CK activity indicated less damage than other studies using a similar exercise intensity and a similar or lower number of repetitions (Nosaka and Clarkson, 1995; Teague and Schwane, 1995). The disparity between these results and the present study may be attributed to differences in the muscle group studied (hamstring versus elbow flexors) or contraction type (slow isotonic versus fast isokinetic). Interestingly one study which documented serum CK activity and pain following six sets of 10 eccentric isotonic hamstring contractions at 100% of concentric strength showed similar results to the present study (Clarkson *et al.*, 1987). However, it is difficult to compare the symptoms of muscle damage between numerous studies which differ with respect to the muscle group studied, the number and intensity of contractions, the speed of contraction and the number of subjects studied.

The magnitude and pattern of strength loss in the present study was different from most previous studies. For example, Teague and Schwane (1995) demonstrated a 22% decrease in isometric elbow flexor strength one day following one set of 10 isotonic eccentric contractions at 60% of isometric strength. In the present study an 11% decrease in isometric hamstring strength was seen one day following six sets of 10 isokinetic eccentric contractions at 60% of isometric strength. Additionally, strength loss was decreased by 18% immediately following the eccentric elbow flexion contractions (Teague and Schwane, 1995). In contrast, hamstring strength was actually 5% higher

immediately following eccentric exercise. The lack of a decline in strength immediately following eccentric hamstring exercise contrasts with numerous previous studies which demonstrated dramatic strength loss (20-50%) immediately following eccentric contractions of the elbow flexors (Newham *et al.*, 1987; Clarkson and Tremblay, 1988; Newham *et al.*, 1988; Ebbeling and Clarkson, 1990; Cleak and Eston, 1992; Chleboun *et al.*, 1995; Nosaka and Clarkson, 1995, Teague and Schwane, 1995; Nosaka and Clarkson, 1997) and knee extensors (Brown *et al.*, 1997a; Brown *et al.*, 1997b). However, Hortobágyi *et al.* (1998) demonstrated only a 10% decline in isometric knee extension strength immediately following 10 sets of 10 isotonic eccentric contractions at 80% of eccentric MVC with 30% strength loss two days later.

It is unlikely that strength loss immediately following eccentric exercise is indicative of muscle damage. Several studies demonstrating a repeated bout effect showed that strength loss immediately following the repeated bout was similar to strength loss immediately following the initial bout despite marked decreases in all symptoms of muscle damage on subsequent days (Newham *et al.*, 1987; Clarkson and Tremblay, 1988; Ebbeling and Clarkson, 1990; Nosaka and Clarkson, 1995; Brown *et al.*, 1997a). Based on these studies the lack of a decrease in isometric hamstring strength immediately following the initial bout of eccentric exercise cannot be attributed to a failure to initiate muscle damage.

It was apparent that the muscle tenderness values for the hamstrings following the initial bout were significantly lower than values reported for the quadriceps (Newham *et al.*, 1983b; Eston *et al.*, 1996; Baker *et al.*, 1997) or elbow flexors (Newham *et al.*, 1988; Cleak and Eston, 1992). The measurement technique has not been validated for the hamstring muscle group. The ability of

the hamstring tissue to disperse as the pressure was applied may have affected measurement sensitivity. A ceiling force of greater than 40 N may have been appropriate. By documenting the force required to elicit discomfort prior to eccentric exercise a ceiling force could have been set for determining tenderness on the days following eccentric exercise. Despite these limitations it was clear that the initial bout resulted in muscle tenderness while no tenderness was found following the repeated bout. However, a valid comparison to tenderness values from studies examining different muscle groups cannot be made.

Although plasma CK activity was increased following the initial bout of eccentric exercise the response was not significantly different from that following concentric exercise. This can be explained by high measurement variability, small sample size and limited amount of damage. Unfortunately plasma CK measurements were only made on 12 subjects in the eccentric group and the measurement variability was extremely high in this group. Despite these limitations changes in plasma CK activity demonstrated a repeated bout effect with eccentric exercise but not concentric exercise.

In general while the symptoms of muscle damage indicated only moderate damage following the initial bout a clear repeated bout effect was evident in each of the four markers of damage. It terms of identifying a specific mechanism for the repeated bout effect it might have been beneficial to have induced greater damage with the initial bout. However, the advantage of the present experimental design was that subjects in both the eccentric and concentric group performed the same relative amount of work and the results were not subject to fatigue effects.

7.3 FUTURE DIRECTIONS

7.3.1 Selective Recruitment of Fast-Twitch Motor Units

Future studies are required to expand on the possibility of selective recruitment of fast-twitch motor units for eccentric contractions. Such studies should test: 1) various muscle groups; 2) a range of contraction intensities; 3) differences between contraction modes (isotonic or isokinetic); 4) different contraction velocities and 5) different measurement techniques (e.g. surface and indwelling EMG).

7.3.2 Selective Damage to Fast-Twitch Fibres

Although some studies have suggested that fast-twitch fibres are more susceptible to damage (Fridén *et al.*, 1983b; Fridén 1984; Lieber and Fridén, 1991; MacPherson *et al.*, 1996) other studies have suggested otherwise (Armstrong *et al.*, 1983; Mair *et al.*, 1992). These studies differed in terms of muscle group tested, animal model versus human volunteers, and only some of these studies (Fridén *et al.*, 1983b; Lieber and Fridén, 1991; MacPherson *et al.*, 1996) were designed to specifically address possible differences in damage between fibre types. A possible association between selective recruitment of fast-twitch motor units for eccentric exercise and selective damage to fast-twitch fibres has not been tested. An association could be inferred by demonstrating selective fast-twitch motor unit recruitment for a bout of eccentric exercise and subsequently demonstrating selective damage to fast-twitch fibres in the eccentrically exercised muscles.

7.3.3 Passive Muscle Stiffness and Exercise-Induced Muscle Damage

The association between passive muscle stiffness and subsequent symptoms of muscle damage demonstrated in the hamstring muscle group warrants further examination in other muscle groups. Furthermore, greater symptoms of muscle damage in subjects with stiff hamstrings were attributed to greater sarcomere strain. This may have been due to stiffer subjects having a smaller functional ROM (see section 4.5.1). Support for this theory could be provided by examining the length-tension relationship for maximum contractions through the ROM. Peak torque would be expected to occur earlier in the ROM (shorter muscle length) in subjects with stiffer muscles. Additionally, decline in torque beyond optimal length would be expected to be much more rapid in subjects with stiffer muscles.

The lack of an increase in hamstring stiffness following the initial bout of eccentric exercise is in contrast to findings in the elbow flexors (Howell *et al.*, 1993; Chleboun *et al.*, 1995). It was not clear whether this difference was due to differences in the magnitude of muscle damage or inherent differences between the muscle groups. These issues could be resolved by documenting changes in hamstring stiffness following a more intense bout of eccentric exercise resulting in greater symptoms of muscle damage. Additionally, examining stiffness changes in other muscle groups following eccentric exercise would establish if muscle specific effects occur.

The apparent susceptibility of stiffer muscles to muscle damage raises the clinical question of what can be done to reduce the potential for damage. It is not

known if reducing muscle stiffness with warm-up or stretching will, in fact, reduce subsequent muscle damage. Further work in this area could yield important practical information for athletes.

7.3.4 Alternative Mechanisms for the Repeated Bout Effect

The adaptations proposed to explain the repeated bout effect were categorised as neural, mechanical and cellular (see section 2.4). Specific neural and mechanical adaptations were examined in the present research, while cellular adaptations were not addressed. However, it may be possible to explain the repeated bout effect acutely by a blunted inflammatory response (Pizza *et al.*, 1996) (see section 2.4.4) and chronically by longitudinal addition of sarcomeres (Fridén *et al.*, 1983a; Lynn and Morgan, 1994; Lynn *et al.*, 1988) (see section 2.4.3). However, these possibilities have not been extensively tested and warrant further study.

Although the present data suggest that the repeated bout effect was not due to a change in the passive mechanical properties of skeletal muscle an adaptation in the mechanical properties of the active contractile component may have occurred. Pousson *et al.* (1990) demonstrated an increase in active stiffness of the elbow flexors following eccentric training. The effects were attributed to either increased tendon stiffness or increased cross-bridge stiffness. However, tendon compliance has been shown to be important during eccentric contractions (Griffiths, 1991; Roberts *et al.*, 1997). An increase in tendon stiffness would compromise normal eccentric muscle function. On the other hand an increase in cross-bridge stiffness could decrease sarcomere strain for a given load by transferring strain to the tendon and aponeurosis. Such an adaptation with the

repeated bout effect is attractive since the initial step in the damage process has been related to sarcomere strain (Morgan, 1990). The non-uniformity of sarcomere length during eccentric contraction is thought to result in strain of some sarcomeres while others in series remain intact (Huxley and Peachey, 1961; Flitney and Hirst, 1978; Morgan, 1990). An increase in cross-bridge stiffness of these "weak" sarcomeres may reduce sarcomere length non-uniformity during subsequent eccentric contractions. A decrease in the rate of cross-bridge cycling could effectively increase the stiffness of weak sarcomeres (Petit *et al.* 1990). The possibility that reduced damage with a repeated bout of eccentric exercise results from a more uniform distribution of sarcomere lengths during eccentric contractions has not been previously proposed. Difficulties in quantifying sarcomere lengths from a representative sample of fibres during eccentric contractions may preclude testing such a theory. However, several techniques are available for indirectly measuring cross-bridge stiffness. Quick-release (Pousson *et al.*, 1990) and quick-stretch (Morgan, 1977) techniques, or the damped oscillation technique (Wilson, *et al.*, 1994) can be used to estimate cross-bridge stiffness in either humans during voluntary contractions or in isolated muscles preparations from animals. While research in this area has been extensive the possible relationship to muscle damage and the repeated bout effect has not been addressed.

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