Measurements of Muscle Pain, Force Matching Ability and Muscle Adaptation after Eccentric Exercise

Nivan Sargara Weerakkody

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Department of Physiology

Monash University

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Declaration

I declare that all work presented in this thesis is my own, and to the best of my knowledge, it contains no material previously published or written by any other person, except where due reference or acknowledgment is made in the text. No part of this thesis has been submitted for the award of any other degree or diploma in any university or institution.

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Nivan S. Weerakkody

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Publications

Reviewed Papers

Whitehead, N.P., Weerakkody, N.S., Gregory, J.E., Morgan, D.L. and Proske, U. (2001). Changes in passive tension of muscle in humans and animals after eccentric exercise. *Journal of Physiology* **533,** 593-604.

Weerakkody, N.S., Whitehead, N.P., Gregory, J.E. & Proske, U. (2001). Large-fibre mechanoreceptors contribute to muscle soreness after eccentric exercise. *Journal of Pain* **2,** 209-219.

Weerakkody, N.S., Percival, P., Morgan, D.L., Gregory, J.E. & Proske, U. (2003). Matching different levels of isometric torque in elbow flexor muscles after eccentric exercise. *Experimental Brain Research* **149,** 141-150.

Weerakkody, N.S., Percival, P., Canny, B.J., Morgan, D.L., & Proske, U. (2003). Force matching at the elbow joint is disturbed by muscle soreness. *Somatosensory and Motor Research* **20,** 27-32.

Proske, U., Weerakkody, N.S., Percival, P., Morgan, D.L., Gregory, J.E. & Canny, B.J. (2003). Force matching errors after eccentric exercise attributed to muscle soreness. *Clinical and Experimental Pharmacology and Physiology* **30,** 576-579.

Weerakkody, N. S., Percival, P., Hickey, M., Morgan, D.L., Gregory, J.E. & Proske, U. (2003). Effects of compression and vibration on muscle pain after eccentric exercise and following injection of hypertonic saline. *Pain* **105,** 425-435.

Conference Abstracts

Whitehead. N.P., Weerakkody, N., Gregory, J.E. & Proske, U. (2000). Input from muscle spindles contributes to muscle soreness after eccentric exercise. *Proceedings of the 5th Annual Congress of the European College of Sport Science*, Jyväskylä, Finland, 19-23 July 2000, 784.

Weerakkody,N., Whitehead, N.P., Gregory,J.E. & Proske, U. (2000). Origins of delayed onset muscle soreness. *Proceedings of the Australian Physiological & Pharmacological Society*. 31(2): 93.

Weerakkody, N.S., Whitehead, N.P., Hickey, M.W., Gregory, J.E. & Proske, U. (2001). Origins of delayed onset of muscle soreness. *Proceedings of the Movement & Sensation International Symposium*, Cairns, Australia, 3-6 September, 6.

Proske U, Weerakkody, N. S., Percival, P., Gregory, J. E. & Morgan, D. L. (2002). Tendon organs signal the damage from eccentric exercise. *Satellite Symposium of the 3rd Forum of European Neurosciences: Motor Control and Proprioception*, Paris, 9-12 July, 2.

Gregory, J.E., Hesse, C.W., Morgan, D.L., Percival, P., Walsh, L., Weerakkody, N.S. & Proske, U. (2003). Are muscle receptors responsible for disturbance of proprioception after eccentric exercise? In:Understanding the nerve cells and leading to human behaviour. Editor: N. Gantchev (In press).

Summary

A number of aspects concerning the effects of eccentric exercise on muscle properties, muscle soreness and proprioception were investigated in this thesis.

It was hypothesised that delayed onset muscle soreness (DOMS) is mediated, at least in part, by non-nociceptive muscle mechanoreceptor afferents. The results provided support for this hypothesis. It was shown that applying vibration with controlled, painful indentation into the exercised muscles resulted in a significant increase in pain. Since only mechanoreceptors are responsive to vibration at such high frequencies this was attributed to primary endings from muscle spindles. In addition, pain thresholds were seen to increase after blocking afferent impulses from large-diameter nerve fibres. Also the effects of vibration, acting to exacerbate the pain were absent during the block of large-diameter nerve fibres. Moreover, it was hypothesised that if nociceptors were in a sensitised state in DOMS, their stimulation with hypertonic saline should produce more pain, since the two stimuli would be expected to summate. However, hypertonic saline was found not to produce more pain, thus it was proposed that the neural basis of DOMS is not just associated with nociceptor sensitization, at least in its simplest form and involves a separate mechanism.

The effects of eccentric exercise on the sense of muscle force was investigated in a force matching experiment. It was found that the presence of fatigue and muscle damage after eccentric exercise led to matching errors that were apparent at all levels of applied force. The patterns of these results were consistent with the view that subjects were not matching levels of torque as signaled by peripheral feedback, but rather, they were matching a centrally-mediated 'sensation of effort'. However, after the effects of fatigue and muscle damage were controlled for, errors still persisted, suggesting that factors other than fatigue and damage were contributing to the mismatch. Using EMG as an indirect measure of the level of neural drive to muscles, it was observed that eccentric exercise led to a larger than proportional increase in EMG for a given level of torque. This disturbance of the EMG : torque relation was not observed after concentric exercise, suggesting that it was a manifestation of muscle damage. Therefore, it was possible that the altered EMG : torque relation contributed to matching errors. In addition, the presence of DOMS was also suggested to contribute to the matching errors

that continued from 24 hours onward after the exercise at a time when the EMG : torque relationship had returned to pre-exercise levels.

The effects of pain on the sense of force was further examined, by injecting hypertonic saline into elbow flexors to see if this produced persistent force matching errors. The size of the matching errors observed was closely correlated with the level of pain, highlighting the fact that it was unlikely that other factors such as distraction were involved in generating the errors. The additional observation was made that painful heating of the skin over the contracting muscle produced a similar pattern of errors, although these were somewhat smaller. This suggested that the effect was not specific to muscle nociceptors. Hence it was argued that DOMS could also be contributing to the disturbance of the sense of force at times post-exercise when other factors were no longer contributing.

A final series of experiments asked the question, does an athletes lifestyle influence their susceptibility to eccentric damage. It was hypothesised that athletes who are concentrically trained have muscle angle-torque curves with optimum length for maximum force generation occurring at shorter muscle lengths than untrained individuals. It was found that the optimum angle for torque, for the quadriceps of a group of 8 trained cyclists lay in a direction of shorter muscle length, compared to a group of 12 untrained individuals. However for the hamstrings, optimums were not significantly different. To explain this result it was argued that during cycling the hamstrings are utilized considerably less than quadriceps and that in cyclists the quadriceps was more susceptible to damage from eccentric exercise that in untrained individuals. Such matters are of importance for triathletes.

Abbreviations

cm	centimeter
DOMS	delayed onset muscle soreness
EMG	electromyogram
Hz	hertz
kg	killograms
mA	milliamp
min	minute
ml	millilitre
mm	millimetre
ms	millisecond
mV	millivolt
MVC	maximum voluntary contraction
N	newton
Nm	newton-metre
°	degree
s	second
S.E.M.	standard error of the mean
µm	micrometre

TABLE OF CONTENTS

CHAPTER ONE

General Introduction

This thesis is concerned with the effects of eccentric exercise on muscle properties, muscle soreness and proprioception. There are a number of changes in muscle after such exercise, including its level of maximum isometric force, the length-tension relation and passive mechanical properties. In addition there is development of delayed onset muscle soreness (DOMS), which accompanies the muscle damage from eccentric exercise. The mechanism of DOMS has been studied here in detail and the implication of DOMS for motor control have been considered. Proprioception, in particular the sense of force, is known to be disturbed after eccentric exercise. This is studied in detail (Chapters 4 & 5) and a mechanism for the disturbance has been proposed. Finally, the well known training effect following regular eccentric or concentric exercise has been studied (Chapter 6). It is proposed that skeletal muscle may adapt to some regimes of exercise in ways that leave it vulnerable to injury from eccentric exercise. All of this is of importance in a wider context. It provides comment on the design of athlete training programs, the treatment of DOMS and the appropriateness of various training programs following sport injuries. In short, much of the work is relevant to exercise physiology and sport science.

Skeletal Muscle

Structure

Skeletal muscle is made up of bundles of multinucleated muscle fibres (cells) bound together by connective tissue. Each muscle fibre consists of sarcoplasm (cytoplasm) enclosed by the sarcolemma (plasma membrane) (Fig 1.1). The sarcoplasm contains the cellular contents, such as proteins, organelles and myofibrils; but most of the space is occupied by myofibrils.

Myofibrils are numerous threadlike structures arranged in highly organized configurations, that contain contractile proteins. Each myofibril comprises bundles of thousands of repeating units, known as sarcomeres: the basic force generating units of muscle contraction. These repeating units are arranged in series along the entire length

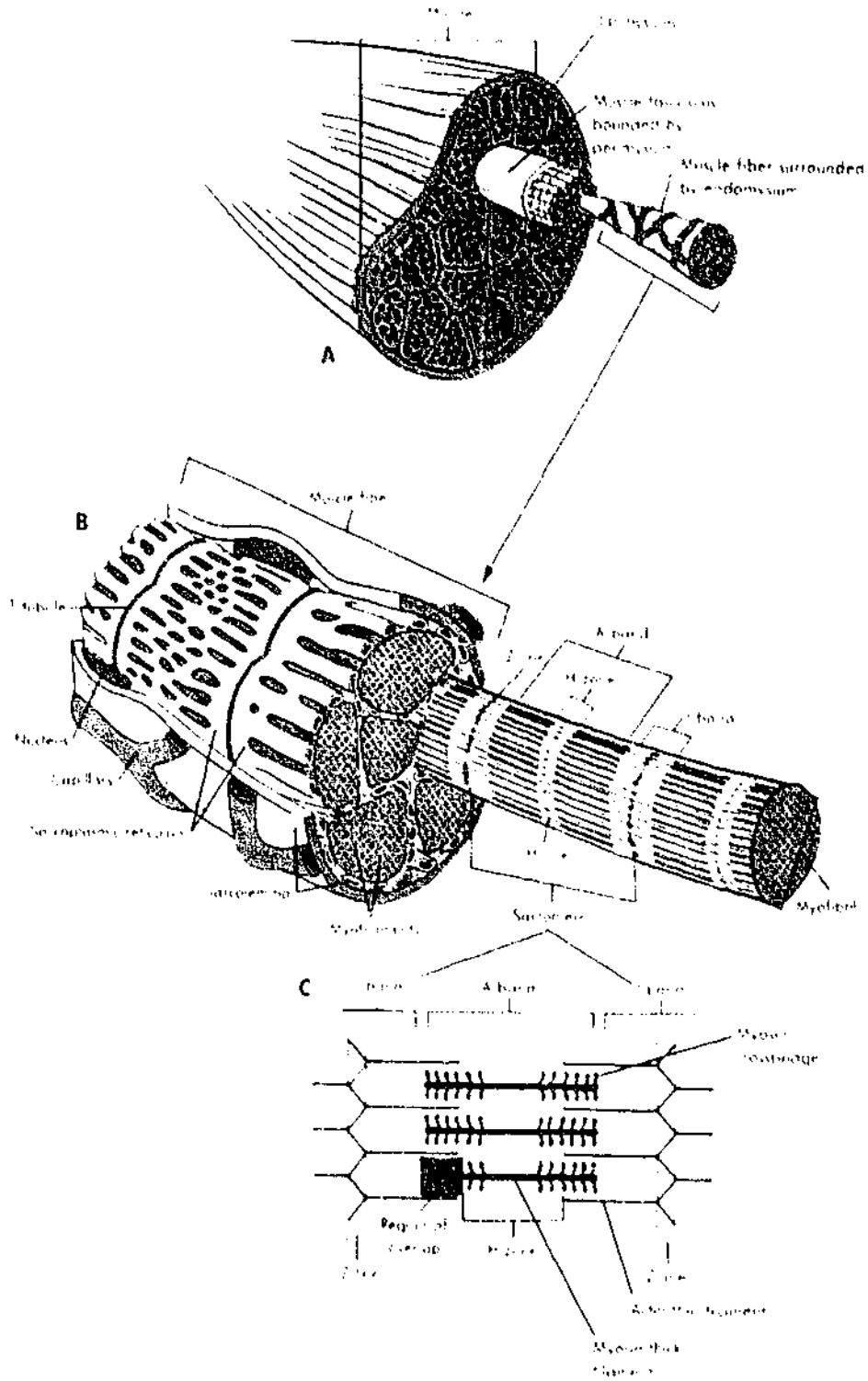


Figure 1.1

Diagrammatic representation of the composition of skeletal muscle and the connective tissue layers. *(A)* Shows the whole muscle covered in epimysium, and individual muscle fascicles surrounded by perimysium. *(B)* Shows the striation pattern of the myofibrils in an individual muscle fibre that is wrapped in a thin, elastic membrane (sarcolemma) and separated from adjacent fibres by endomysium. *(C)* Shows a sarcomere and the arrangement of thick and thin filaments. From Moffett, Moffett & Schauf (1990).

of the myofibril and consist of overlapping thick (myosin) and thin (actin) filaments. The alternating arrangement of these two protein filaments gives skeletal muscle its striated appearance, visible on microscopic examination as alternating light and dark regions.

The dark regions, or A-bands, are regions of the sarcomere that contain myosin filaments. The ends of the sarcomere, where the thin filaments are not overlapped by thick filaments, are the light regions, or I-bands, which continue into adjacent sarcomeres. Sarcomeres also have a central H-zone, in which the thick filaments are not overlapped by thin filaments. The junctions between sarcomeres is marked by a disk-like Z-line, to which the thin filaments are anchored. Elastic filaments, the gap filaments (titin and nebulin) attach the ends of the thick filaments to the Z-lines and keep adjacent thick filaments in register.

Contraction

The process of muscular contraction is best described by the 'Sliding Filament Theory' which was provided by two independent studies (Huxley & Hanson, 1954; Huxley & Niedergerke, 1954). The observations from these studies led to the theory that the thick and thin filaments slide past each other when a muscle contracts or lengthens, without the filaments themselves changing length.

This process is proposed to occur via the interactions between the actin filaments and the myosin heads, which draw the actin filaments toward the sarcomere's centre as a result of the rotation of their heads, thereby shortening the sarcomere as the Z-discs come closer together. This pulling of actin over the myosin molecule results in muscle shortening and the generation of force (Powers & Howley, 1997).

Excitation-contraction coupling

Excitation-contraction (E-C) coupling refers to the process of events by which an impulse (action potential) in the muscle membrane leads to muscle shortening as a result of cross-bridge activity.

The sequence of events starts at the neuromuscular junction. Action potentials arriving at the terminals of the motoneuron axon cause an influx of calcium ions that stimulates the

release of the neurotransmitter, acetylcholine (ACh). After crossing the synaptic cleft, ACh molecules combine with receptors (AChRs) on the sarcolemma, leading to conductance changes that result in the movement of sodium and potassium ions across the membrane. This results in depolarisation of the post-synaptic membrane, allowing an action potential to be set up across the sarcolemma.

The action potential propagates along the sarcolemma, with excitation spreading into the muscle via systems of membranous channels, the transverse tubules (T-tubules) and the sarcoplasmic reticulum (SR) (Fig 1.1). The SR has specialised calcium storage compartments, the terminal cisternae. The depolarisation of the SR causes the release of calcium from the terminal cisternae into the sarcoplasm, in the vicinity of the myofilaments that make up the force generating units of a muscle.

This increased intracellular calcium concentration is the trigger for muscle contraction. The calcium binds to troponin an actin regulatory protein, and this allows myosin cross-bridges to interact with actin molecules. This triggers cross-bridge cycling (for a review see Moffet, Moffet and Schauf, 1990).

Classification of motor units and muscle fibres

A motor unit is the basic element in motor control, consisting of a motoneuron and all the skeletal muscle fibres that it innervates. At optimum muscle length, the magnitude of tension that a motor unit can generate from a single action potential is primarily a function of the number of the muscle fibres innervated by an individual motoneuron (Feinstein *et al.*, 1955; Edstrom & Kugelberg, 1968).

Similarly, skeletal muscle is composed of three types of muscle fibres; type I (slow oxidative), type IIa (fast glycolytic) and type IIb (fast oxidative) (Brooke & Kaiser, 1970). The three fibre types differ mainly in their rate of ATP hydrolysis and hence shortening velocities. This classification of fibres also applies to motor units, as all the fibres in any one motor unit are the same fibre type. Accordingly, motor units are normally classified as slow (S), fast-fatigable (FF) or fast fatigue-resistant (FR), depending on what type of muscle fibres they have.

Types of contraction

The term 'contraction' has been used to describe a muscle in its active, force producing state. Depending on the nature of the work that is being performed, our muscles can undergo three types of muscle contraction; isometric, concentric and eccentric.

During activation, the cycling cross-bridges generate force and try to shorten the muscle. However, if the external forces on the muscle equals the isometric force-producing capability of the muscle, the result will be a static muscle action, where muscle length remains constant. In such 'isometric contractions' there is no movement about the related joints.

If the isometric force-generating capacity of the muscle exceeds the external force the muscle will shorten, which is referred to as concentric contraction. During concentric contractions actin filaments are pulled towards the centre of sarcomeres, allowing the muscle to shorten during contraction. Conversely, if the load to be moved or resistance exceeds the isometric force capability of the muscle, the muscle is forcibly lengthened, which is called an eccentric contraction. During eccentric contractions filaments slide away from the centre of sarcomeres, due to the lengthening of the muscle during the contraction. In contrast to isometric contraction, concentric and eccentric contractions are dynamic, where muscle length and joint angles change as the body part moves, and where muscles are performing external work or work is performed on the muscle.

All three types of contraction are regularly used in everyday activities. Isometric contractions are important in maintaining posture when we sit or stand, and also in maintenance of grip force in hand muscles while grasping objects. Concentric contractions predominate in activities such as cycling, swimming, walking uphill and lifting objects. Eccentric contractions are involved in activities where our muscles exert a braking action to control body motion, such as in skiing, horse riding and downhill walking.

The type of muscle contraction being used may affect motor unit recruitment. Although motor units during a graded voluntary contraction are recruited according to the 'size principle', with small motor units being recruited before larger motor units, exceptions

to the rule have been observed. Nardone, Romano & Schieppati (1989) observed that during voluntary eccentric contractions of the human triceps surae muscles, the high-threshold (fast) motoneurones were selectively recruited, while the low-threshold (slow) ones were derecruited. It was suggested that eccentric action was a skilled movement and that central command may be responsible for the specific recruitment of the high-threshold motor units.

Another interesting characteristic of eccentric contractions is that despite producing higher forces than concentric and isometric contractions, the net metabolic cost is considerably less (Asmussen, 1953; Curtin & Davies, 1973). To explain such findings Curtin and Davies (1973) suggested that during active lengthening of the muscle, some cross-bridges are forcibly detached without the breakdown of any ATP. More recently, studies have shown that during eccentric contraction, there is a much slower rate of ATP hydrolysis than during concentric contraction, and the proposed effect of stretch is to slow the rate of ATP splitting (Infante *et al.*, 1964; Rall, 1985; Woledge *et al.*, 1985).

In addition, prolonged bouts of eccentric exercise are known to lead to muscle soreness and to other characteristic signs of damage such as disruption of mechanical properties, histology and biochemistry of the muscle.

The active length-tension relationship

The sliding filament theory of muscle contraction predicts that uniformly distributed cross-bridges act as independent force generators. The amount of tension generated during a contraction is proportional to the amount of filament overlap, and therefore the number of cross-bridge attachments. This underlies a fundamental mechanical property of muscle, known as the active length-tension relationship.

Ramsay and Street (1940) first demonstrated the length-tension relationship for a single muscle fibre, where the amount of active tension or force, generated from an isometrically contracting muscle depends on the length of the muscle. Gordon, Huxley and Julian (1966) related this observation to the behavior of a single sarcomere, by recording the tension generated during isometric contractions of isolated frog muscle fibres over a wide range of sarcomere lengths. They realized the tension in a sarcomere

is determined by the degree of overlap between thick and thin filaments, which is directly related to individual sarcomere length (Fig 1.2A). It was evident there existed an ideal or optimum length (L_o) where maximum tension could be generated. This length is 2.0-2.25 μm in frog muscle. At L_o the overlap between thick and thin filaments is such that all myosin heads form cross-bridges with actin. At sarcomere lengths shorter than the L_o, termed the ascending limb, thick filaments collide with Z-lines and the thin filaments from opposite ends of the sarcomere overlap with each other, reducing the number of interacting cross-bridges. As the fibre is stretched beyond the L_o, tension declines in direct proportion to the amount of overlap between thick and thin filaments, until tension is reduced to zero because there is no longer any overlap between filaments. This region is called the descending limb of the length-tension relationship. The fact that the tension is directly proportional to the amount of filament overlap over this region is also of great theoretical importance since it provided the main evidence for the sliding filament hypothesis (refer to Morgan and Allen, 1999).

The general shape of the length-tension relationship for frog muscle is the same as for mammalian muscle. However, the entire relationship is shifted to longer sarcomere lengths, with optimum lengths ranging from 2.5 to 3.1 μm for various mammalian muscles (Close, 1972). The major reason for this difference is that, in mammals, thin filaments are longer than in amphibian muscle (Page & Huxley, 1963).

The force-velocity relationship

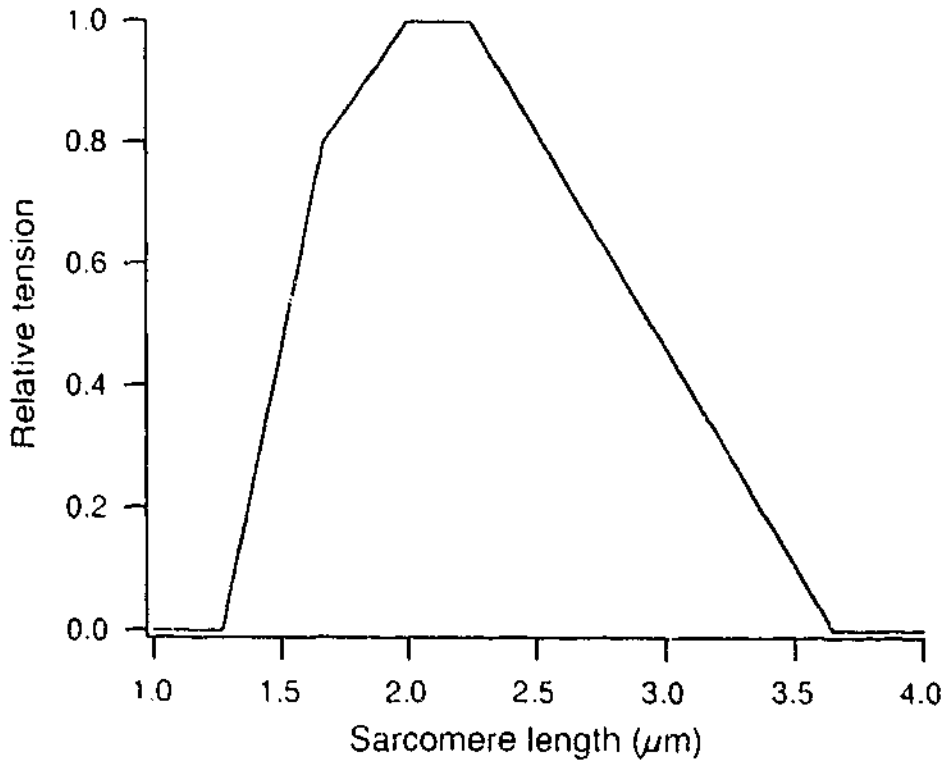
Another influence contributing to force output from a muscle is the dependence on the speed at which the muscle shortens or lengthens (Fenn & Marsh, 1935; Hill, 1938). This underlies another fundamental characteristic of the contractile properties of the skeletal muscle, known as the force-velocity relationship (Fig 1.2B). When a load is small, active force output is correspondingly small, but the speed of shortening is high. Conversely, if the load is large, the muscle generates more force but the shortening speed is slower.

During shortening (concentric) contractions, Hill (1938) showed that the relationship between active force and velocity was approximately hyperbolic, with zero velocity at maximum isometric tension (P_o), and maximum shortening velocity (V_{max}) at zero force.

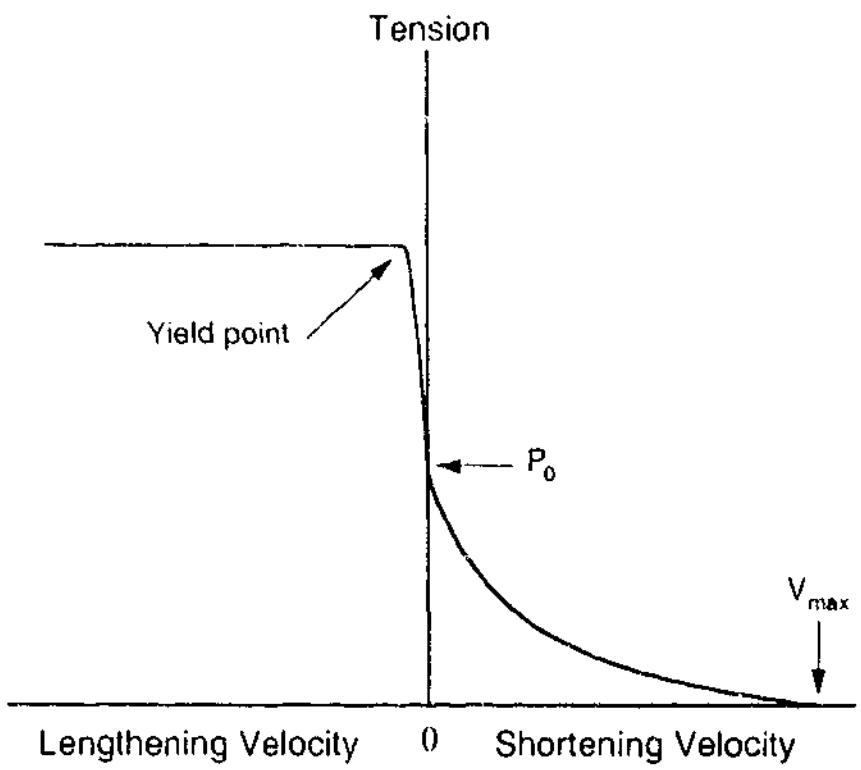
Figure 1.2

(A) The length-tension relationship of a sarcomere as shown for a single frog fibre as determined by Gordon, Julian and Huxley (1966). *(B)* The force-velocity of a frog muscle, shown for both shortening (Hill, 1938) and lengthening (Katz, 1939) velocities. The maximum isometric tension (P_o), maximum shortening velocity (V_{max}) and yield point of tension during lengthening are indicated (arrows).

A


1.0
0.8
0.6
0.4
0.2
0.0
Relative tension
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Sarcomere length (μm)

B


Tension
Yield point
P_0
V_{max}
Lengthening Velocity
0
Shortening Velocity

Hence, as the speed of shortening increases, the tension the muscle can develop decreases. This is thought to be due to the cycling cross-bridges having less time to attach to actin binding sites during the movement. This also means that there is less time for any cross-bridges which are opposing the motion to be able to detach. The V_{max} of a muscle has been shown to reflect the rate of cross-bridge cycling, which is determined by myofibrillar ATPase activity of the muscle (Barany, 1967).

For lengthening (eccentric) contractions, Katz (1939) showed when the applied force exceeded the isometric capability of the muscle, it would lengthen slowly as tension increased steeply until a 'yield tension' was reached. At the yield tension, tension reaches a plateau level where faster lengthening does not result in more tension; i.e. the tension becomes independent of velocity. The initial steep increase in force is though to reflect a greater force per attached cross-bridge. This is thought to be due to the initial high resistance to stretch from the elastic linkages of the cross-bridges, which has been termed the 'short-range stiffness' (Rack & Westbury, 1974; Flitney & Hirst, 1978). Beyond the yield point it is believed that the actin and myosin filaments are pulled past each other too rapidly for cross-bridges to be able to form to resist the load.

The passive length-tension relationship

The tension generated when a non-activated muscle is at rest or undergoing movement is known as the passive tension of the muscle. Passive properties play many important roles in everyday activities. For instance, when an agonist muscle contracts and shortens to rotate a joint, the inactive, antagonist muscle is passively stretched. Upon relaxation, both muscles return to their original length, one, the agonists, by passive stretch and the other, antagonist, by passive shortening.

The length-tension relationship of a passive muscle is approximately exponential with negligible tension at short muscle lengths, and a steeply rising tension at long lengths (Close, 1972). Passive tension in unexercised muscle is thought to be generated by a combination of connective tissue elements (Banas & Zetlin, 1938) and elastic filaments such as titin (Funatsu *et al.*, 1990; Granzier *et al.*, 2000). Titin is thought to be at least partly responsible for maintaining alignment of the myofilaments. Stable, non-cycling

cross-bridges, requiring resting levels of calcium, have also been proposed to be involved in the generation of passive tension (Hill, 1968).

Eccentric Exercise

During forms of demanding exercise, we have all experienced pain and weakness as we become exhausted. Such symptoms quickly disappear after the end of the exercise, and are associated with muscle fatigue. However, with eccentric exercise, where our muscles are active while being lengthened, we experience muscle weakness and fatigue immediately after exercise, but also our exercised muscles feel stiff and sore the following day.

The first experimental evidence to distinguish between delayed muscle soreness and soreness during exercise was published by Hough in 1902. In subjects performing both eccentric and concentric contractions of their middle finger, Hough observed that delayed soreness was experienced in untrained individuals who had not performed this activity for several months. Subsequent studies established that this delayed muscle soreness occurred after exercise involving eccentric exercise, but not concentric exercise (Talag, 1973; Davies & White, 1981; Armstrong, 1984; Newham *et al.*, 1988). There is now an abundance of experimental evidence to show that DOMS and the associated muscle stiffness and weakness are manifestations of muscle damage caused by eccentric contractions.

Support for this theory comes from histological evidence of structural damage to muscle from eccentric exercise, showing the initial damage occurs to the contractile apparatus at the ultrastructural level (Friden *et al.*, 1983b; Armstrong *et al.*, 1991; Wood *et al.*, 1993). Such morphological studies have shown specifically that sections of human biopsy samples, examined under electron microscope, had Z-lines which were broadened, streaming (that is, had wavy appearance) and disrupted. Adjacent sarcomeres were often hypercontracted or structurally disorganized, with the distribution of damage being random throughout the muscle sample and possibly restricted to one half sarcomere in a myofibril. The amount of histological damage has been shown to increase progressively with the number of eccentric contractions (Hesselink *et al.*, 1996). Such

structural damage to sarcomere architecture however, does not occur after concentric exercise (Armstrong *et al.*, 1983).

Mechanisms for damage from eccentric exercise

The damage process following eccentric contractions can be considered in stages (Armstrong, 1984). The 'initial stage' is associated with the events causing structural damage to fibres. The 'secondary stage' refers to the subsequent events, where disrupted fibres continue to degenerate, affecting other cellular structures. The final stage, the 'regeneration stage', is when the fibres are restored to normal function.

Initial events associated with muscle damage

It is known from the force-velocity relationship that during eccentric contractions the force generated can be almost twice that observed in isometric or concentric contractions (Katz, 1939). This led various researchers to propose that the structural damage to sarcomeres observed after eccentric exercise was caused by the high tensions (Armstrong *et al.*, 1983; Newham *et al.*, 1983; Friden *et al.*, 1984; McCully & Faulkner, 1985). Consequently, this extra tension could cause mechanical disruption to ultrastructural elements within the muscle fibres, or to connective tissue in series with the contractile elements.

However, many studies have shown that sub-maximal eccentric contractions can cause muscle damage and DOMS. Ultra-structural changes and soreness were observed to occur when the elbow flexors performed a number of repetitions lowering a weight that generated 60%, 50% or even 20% of maximum voluntary contraction (Jones & Newham, 1985; Teague & Schwane, 1995; Brockett *et al.*, 1997). Similarly it has been reported that damage occurred following eccentric contractions but not concentric or isometric contractions, even though the peak force level was the same in each case (85% P_o) (McCully & Faulkner, 1986). Therefore, based on such findings it was evident factors other than high peak forces were causing the initial damage from eccentric contractions.

Accordingly, other studies have shown length dependence of the amount of damage produced by eccentric contractions. A greater amount of injury occurred when eccentric

exercise was performed at longer muscle lengths than at short muscle lengths, even though lower levels of tension were produced at the longer lengths (Newham *et al.*, 1988; Jones *et al.*, 1989; Lieber & Friden, 1993). This suggested that muscle damage is more a length dependent, rather than a tension dependent phenomenon.

Morgan's hypothesis

Morgan (1990) proposed a hypothesis to explain the initial event leading to muscle damage from eccentric contractions. Morgan's theory provides an explanation for why muscle soreness and damage are more length dependent, rather than tension dependent, phenomena.

The theory was based on some earlier observations (Julian & Morgan, 1979) that indicated muscle fibres could develop substantial sarcomere non-uniformities consistent with random variations in strength along their length. Hence, the hypothesis used the assumption that the sarcomeres within a myofibril will always have some variation in their strengths due to inhomogeneities in lengths and cross-sectional areas. This was suggested to cause different sarcomeres to lengthen at different rates during a lengthening contraction.

During an eccentric contraction, tension would rise until the weakest sarcomere reached the yield point of its force-velocity relationship, causing it to lengthen more rapidly than other sarcomeres without increasing tension. If the weakest sarcomere was on the descending limb of its length-tension relationship, its ability to generate active tension would be reduced with further stretch, and so it would be unable to maintain the existing fibre tension at any velocity. Consequently, the sarcomere will continue to lengthen uncontrollably to beyond filament overlap, until eventually rising passive tension prevents further lengthening (Morgan, 1990). At this point, the sarcomere will not generate active force and will simply add a passive elastic element in series with the still functioning sarcomeres, leading to increased series compliance in the muscle. This process is hypothesized to then repeat itself for the next weakest sarcomere. Hence, in this manner rapid lengthening of active muscle at long lengths is proposed to occur virtually instantaneously, in a highly non-uniform way by uncontrolled over-extension ('popping') of sarcomeres, one at a time, from weakest to strongest. This would

continue until the stretch ended or tension in the fibre was reduced (Morgan, 1990). Hence, the descending limb is a region of potential instability for muscle fibres.

Various studies utilizing electron microscopy have offered direct support for Morgan's hypothesis. In toad sartorius muscles that had undergone 50 eccentric contractions, Jones et al. (1997) observed several scattered regions of over-extended sarcomeres, which were surrounded by apparently normal sarcomeres. Talbot and Morgan (1996) found that if a muscle was fixed while still contracting at the end of a single eccentric contraction, on the descending limb of its length-tension relationship, the number of overextended sarcomeres found in muscle fibers could account for more than half of the imposed stretch.

More recent support for the non-uniform lengthening of sarcomeres during eccentric contraction was provided by a study investigating the phenomena of 'permanent extra tension', in which maintained isometric tension at the end of an active stretch remains higher than the equivalent isometric tension at the same length (Morgan *et al.*, 2000). Permanent extra tension was found to occur only on the plateau or descending limb of the length tension relationship, becoming greater at longer lengths. This result was understood as being due to non-uniform lengthening of sarcomeres during the stretch, so that most of the length change was taken up by a few 'popped' sarcomeres while the majority of sarcomeres lengthened little.

After the muscle relaxes after a single eccentric contraction, most of the overextended sarcomeres would re-interdigitate, resuming normal function (Morgan, 1990). However, a few would not, instead remaining disrupted, with myofilament arrangement unable to realign into a normal pattern. Such regions of disruption are the foci of areas of damage. Hence with repeated eccentric contractions, it is these areas that are more susceptible to further damage, and additional damage spreads from here. Subsequent fibre necrosis and then death to some populations of 'stress-susceptible' fibres have also been shown (Armstrong *et al.*, 1983).

Spread of damage to cytoskeletal structures and membrane

The overextension of sarcomeres during eccentric contractions provides an explanation for the observed spread of damage to cytoskeletal structures within the muscle, such as titin and the intermediate filaments desmin, vimentin and synemin. As discussed earlier, when a sarcomere becomes overextended to a length beyond myofilament overlap, the rising tension borne by titin causes it to yield (Wang *et al.*, 1993). Friden & Lieber (1998) showed a regular straining of titin in some sarcomeres scattered throughout the fibre of a rabbit digitorum longus muscle.

Similarly, a recent study showed that in gene knockout mice, muscles lacking desmin were less vulnerable to injury than normal muscles with desmin (Sam *et al.*, 2000). This suggests that desmin played a direct role in the damage process. Because desmin mechanically links adjacent myofibrils at the Z-line, overextension of a sarcomere would put extra strain on desmin, which, in turn, could transmit the disruption of sarcomeres across a fibre from one to adjacent myofibrils.

In addition it has been suggested that the observed damage to the muscle membrane also stems from sarcomere disruption (Allen, 2001). Specifically, it was proposed that non-uniform lengthening of sarcomeres might strain adjacent T-tubules, causing them to rupture if the shear force became too great. Immediately after eccentric exercise in rats, disorganization of T-tubules was evident throughout the muscle fibres (Takekura *et al.*, 2001).

Secondary events associated with muscle damage

Following the initial mechanical disruption to sarcomeres, and the subsequent ultrastructural filament and membrane damage, the next stage in the damage process is the loss of calcium homeostasis (Armstrong 1991). There has been direct evidence (Balnave *et al.*, 1997; Lynch *et al.*, 1997; Ingalls *et al.*, 1998) and indirect (Duan *et al.*, 1990; Friden & Lieber, 1998) for an increase in resting calcium concentration soon after a period of eccentric contractions. The membrane damage allows Ca^{2+} to enter the fibre down its electrochemical gradient. If the disruption is minor, the entering Ca^{2+} may be adequately handled by ATPase pumps that expel Ca^{2+} from the cytoplasm. However, if the damage is sufficient, Ca^{2+} influx into the fibre overrides its expulsion, leading to an

uncontrolled increase in intracellular Ca^{2+} levels. This Ca^{2+} overload is then hypothesized to activate a number of Ca^{2+}-dependent enzymes that trigger proteolytic and phospholytic pathways (Belcastro, 1993). Such enzymes are suggested to further degrade the contractile filaments, cytoskeleton and muscle membrane (Armstrong *et al.*, 1991; Waterman-Storer, 1991). Progressive deterioration of the damaged fibres triggers an accumulation of neutrophils and macrophages (Armstrong *et al.*, 1983) initiating the 'inflammatory response'.

The presence of high calcium in the muscle fibre is also thought to lead to the development of localized regions of contracture, that is, areas of hypercontracted sarcomeres. Such segmented regions of hypercontraction have been observed in rabbit muscle (Friden & Lieber, 1998). Similarly, signs of contracture 'clots' have been shown in rat muscles after eccentric contraction (Ogilvie *et al.*, 1988). Warren et al. (1995), using calcium fluorescence techniques, found that these hypercontracted regions of muscle fibres contained elevated resting calcium concentrations.

The consequence of contracture, however, is not clearly understood. Its development could serve to accelerate or slow the progression of the damage process to the muscle. On one hand, the force generated by contracture may cause further disruption and damage to sarcomeres and membranes in adjacent regions. Conversely, it has been suggested that the contracture clots prevent the spread of calcium along the fibre, serving as barriers to seal off the damage area, protecting the unaffected 'healthy' sarcomeres from the degradation process (Carpenter & Karpati, 1989). Such a protective effect may be aided by the formation of a demarcating membrane on each side of a 'swollen' region, acting to 'wall off' the injury site (Carpenter & Karpati, 1989).

Regeneration of damaged muscle fibres

Following the inflammatory process, the final stage in the damage process is regeneration of previously injured regions of the muscle fibre. During this stage, satellite cells migrate to the injury site, where they become precursors for the regeneration of muscle fibres (Carlson, 1973). The phase commonly begins 4 to 5 days after the injury (McCully & Faulkner, 1985; Armstrong *et al.*, 1991). However, in both animal

(Armstrong *et al.*, 1983) and human (Friden *et al.*, 1983a; Jones *et al.*, 1986) studies, signs of regeneration have been shown to occur as early as 72 hours.

Signs of muscle damage

The degree of muscle damage experienced varies in different people. This is possibly due to differences in lifestyle or previous training, as well as age (Jones *et al.*, 1986). Nonetheless, the amount of muscle damage that has occurred can be accurately measured by observing the various characteristic signs of damage that eccentric exercise causes. These signs include the shift of optimum length for tension generation, the drop in force production and the rise of passive tension in the muscle.

The shift of optimum length for tension generation

One of the most important signs of damage from eccentric exercise is the shift to the right, that is, in the direction of longer muscle lengths, of the length-tension curve of the muscle. It is important because it is an immediate sign after the exercise and the amount of shift gives a quantitative indication of the amount of damage. The length dependence of tension is of immense practical importance as it determines the joint angle for optimum torque.

The shift has been observed in amphibian muscle (Wood *et al.*, 1993; Morgan *et al.*, 1996; Talbot & Morgan, 1998) and rat muscle fibres (Yeung *et al.*, 2002). Angle-torque curves of human triceps surae muscles have also shown the typical shift immediately following eccentric contractions (Jones *et al.*, 1997).

Various hypotheses have been put forward to explain the shift, but the only one consistent with all the observations is the proposition of Morgan (1990), suggesting that eccentric contractions overextend some, weaker, sarcomeres and on muscle relaxation a few of these become disrupted. The presence of disrupted, overextended and non-functioning sarcomeres, in series with still functioning sarcomeres leads to an increase in the passive series compliance of the muscle, shifting length-tension curve in the direction of longer muscle lengths. The muscle has to be stretched to longer lengths to produce the same myofilament overlap and consequent force level in still functional

sarcomeres. In this way, the shift in optimum length would provide a measure of the average number of disrupted sarcomeres in myofibrils after a series of eccentric contractions.

Importantly the shift in optimum length for tension generation has been shown to reverse rapidly to control values over the next 2 days post-eccentric exercise in humans (Jones *et al.*, 1997; Whitehead *et al.*, 1998), or as early as 5 hours after exercise in toad sartorius muscle (Jones *et al.*, 1997). This reversal of the shift has been suggested to be due to the re-interdigitation of overextended sarcomeres (Talbot & Morgan, 1996). However, the reversal could also be explained, for some fibres, by small regions of disrupted sarcomeres progressing into larger damaged regions until the fibre is no longer able to contract, and so is not contributing to the length-tension relationship (Morgan & Allen, 1999).

Drop in force production

Following eccentric exercise there is an immediate drop in force output. The force generation of a muscle depends on its cross-sectional area, that is, the number of sarcomeres in parallel. Normally, the drop is greatest immediately after the exercise, slowly recovering over several days or weeks for both humans (Jones *et al.*, 1997; Whitehead *et al.*, 1998) and animals (McCully & Faulkner, 1985; Wood *et al.*, 1993) (Lynn *et al.*, 1998). The size of tension drop after eccentric exercise has been reported as being as low as 10% (Brockett *et al.*, 1997), to as high as 90% (Warren *et al.*, 1999). Such varying extents of force deficit and lengths of time for which it persists are due to factors relating type and intensity of the exercise, such as the number of contractions, the stretch amplitude, and the length and range over which the contractions were carried out (Talbot & Morgan, 1998). However, when comparing different types of exercise with equivalent work loads, the force loss after eccentric exercise is greater and persists longer than after concentric or isometric exercise (Talag, 1973; Davies & White, 1981; Brockett *et al.*, 1997). There is still debate over whether the decline in force production is due entirely to the effects of fatigue, muscle damage or both. It seems likely that it may be a combination of both.

Fatigue is associated with all forms of intense muscle activity, whether it be concentric, isometric or eccentric exercise. Newham et al. (1983) found that a decrease in force generation occurred with 'long lasting fatigue'. Long lasting fatigue is thought to occur at the level of E-C coupling. The mechanism causing the E-C coupling failure has been proposed to involve structural and morphological damage to the membrane systems, in particular the T-tubules (Takekura *et al.*, 2001). As much as 75% of the initial drop in muscle force has been suggested to be due to E-C coupling failure (Warren *et al.*, 2001). This theory has come from studies on mouse muscles showing that the drop in tension from eccentric exercise can be recovered with caffeine contractures (Warren *et al.*, 1993; Balnave & Allen, 1995). As caffeine can bypass the normal E-C coupling process, as it acts directly on the SR to release Ca^{2+}, it was proposed the failure of the E-C coupling was the main cause of the force drop. However, there does seem to be a species difference for the effect of caffeine. After eccentric contractions in amphibian muscle, a reduced tension after eccentric contraction could not be recovered by inducing calcium release (Morgan *et al.*, 1996).

If a more intense eccentric contraction protocol on the muscle was used, with more and longer stretches, some tension deficit could not be recovered with caffeine (Balnave & Allen, 1995). Similarly, Brooks and Faulkner (1996) observed that damage from eccentric exercise can be seen even after a single contraction, provided the stretch is of sufficient magnitude. Such results suggest that there is a fatigue-independent component to the drop in force after eccentric exercise. Hence, structural damage to muscle fibres has also been proposed to contribute to the drop in tension.

However, in any case, the tension drop is not the most reliable indicator of muscle damage. Hesselink et al. (1996) found a poor correlation between muscle damage evaluated histologically and the measured drop in force. Similarly, it has been shown in human subjects that myofibrillar damage can be present after eccentric exercise without a significant decrease in maximum force production (Hortobagyi *et al.*, 1998). As such, using force drop as a measure of muscle damage immediately after exercise is complicated because of the presence of metabolic fatigue, and because the shift in the length-tension curve means that the optimum length for peak tension is no longer the same.

On a side issue, it is often suggested that the force drop is due to the pain and soreness preventing subjects from generating a maximum voluntary contraction. However, this seems unlikely, as the time course for the drop in force is not directly related to the time course for development of muscle soreness. Various studies have shown that no pain is experienced immediately after exercise, despite a significant reduction in force generation (Cleak & Eston, 1992; Balnave & Allen, 1995). In addition, muscle damaged by eccentric exercise has been shown to be fully recruited during both electrical stimulation and maximum voluntary contractions (Clarkson & Tremblay, 1988; Jones *et al.*, 1989). Therefore, motivational factors cannot be primarily responsible for the reduced ability to generate force.

Rise in passive tension

A common symptom experienced after eccentric exercise is the well-known increase in passive tension and stiffness of the resting muscle (Jones *et al.*, 1987; Howell *et al.*, 1993; Chleboun *et al.*, 1998). Passive torque of human triceps surae has been shown to increase 40% above control values, and was sustained, remaining over 20% for 4 days (Whitehead *et al.*, 2001). Similar trends have been observed in human elbow flexor muscles, being represented as a more flexed resting elbow angle (Clarkson & Tremblay, 1988; Cleak & Eston, 1992; Brockett *et al.*, 1997, Chleboum *et al.*, 1998).

Various mechanisms have been put forward for the rise in passive tension after eccentric exercise. The lack of accompanying electromyographic (EMG) activity points to changes in the intrinsic properties of the muscle as a result of damage from eccentric contractions (Howell *et al.*, 1985; Howell *et al.*, 1993; Chleboun *et al.*, 1998). Jones et al. (1987) suggested the increase was due to damage and shortening of parallel, connective tissue structures. It has also been suggested that damage-induced swelling within the muscle compartment is the cause, by stretching connective tissue surrounding muscle fibres, increasing the tension in the passive structures for a given muscle length (Howell *et al.*, 1993). However, other factors must be involved, as increases in passive stiffness at intermediate lengths, measured immediately after eccentric exercise, cannot be attributed to muscle swelling, since this does not become significant until 48 hours post-exercise (Chleboun *et al.*, 1998).

An alternative, yet unexamined explanation for the rise in passive tension is provided by Morgan's hypothesis of exercise-induced disruption of sarcomeres, leading to strain and tearing of membranous structures, causing uncontrolled Ca^{2+} influx into the fibres. This increase in intracellular Ca^{2+} could initiate the development of a contracture in some localized segments of the fibre, hence increasing passive tension. This would be active tension generated in the absence of EMG. As previously described, studies on both rats (Ogilvie *et al.*, 1988) and rabbits (Friden & Lieber, 1998) have reported the presence of such hypercontracted sarcomeres following eccentric contractions. Thus, it is plausible that damaged fibres can continue to contract and generate tension, without any accompanying electrical activity in the muscle, for a period of several days.

Muscle Pain

Several chapters in this thesis are concerned with muscle soreness and its mechanism. Before discussing the origin of exercise induced muscle pain, it is necessary to comment on the subject of pain sensation and its neural basis in a broader context.

The sensation of pain is a complex phenomena that has been of interest to humans for centuries. Early in history, theories surrounding pain were highly abstract. Ancient Greek philosophers such as Aristotle (384-322 BC) thought the heart was the seat of the mind where pain originated. In the latter part of the nineteenth century debate still remained over the nature of pain among proponents of the 3 major viewpoints: pain as an emotion; pain as an intensive concomitant of other sensations; pain as a particular sensation (Dallenbach, 1939). It was not until knowledge of anatomy increased, particularly of the central nervous system, that a better understanding of the mechanism behind the sensation of pain began to develop, which tended to support the last theory. Charles Bell (1774-1842) contributed to enlightenment about both the spinal roots and the function of sensory neurons. Work by Sherrington (1906) added insight that pain was ordinarily produced when tissue was damaged and argued that the receptors for pain could be considered detectors of tissue damage or of the physical threat of damage. It was through such work that the picture of pain receptors (nociceptors) become clearer.

Nociceptors are primary afferent neurons signaling the presence of tissue-damaging stimuli or the existence of tissue damage. Their activity produces adverse and protective reactions including the sensation of pain. Nociceptors may also be modified after nearby injury so that they become more sensitive, thus aiding in the defense of the injured site (Walters, 1994). Because of their importance for human pain, nociceptors have received intense study.

Most of our knowledge regarding pain physiology is based on studies of the cutaneous receptors. Cutaneous nociceptors can be classified using several different criteria, and include either C fibres (unmyelinated) or Aδ fibres (myelinated), and their type of response, for example, quickly adapting versus slowly adapting (Campbell & Meyer, 1996). In addition, nociceptors more often than not, respond to multiple stimulus modalities including mechanical, heat, chemical and to some extent cold stimuli. In these situations, where responses to three stimulus modalities are demonstrated, the afferents are labelled polymodal nociceptors. Consequently, the classification and identification of cutaneous nociceptor afferents is based on the adequate stimuli that most readily activate the afferent and the conduction velocity (Campbell & Meyer, 1996).

In contrast, although muscular pain is so common, relatively little is known about its neural basis. Similarly, little is known about the activation of visceral, joint and musculoskeletal nociceptors even though clinically treated pain often originates in these structures (Mense, 1993). This relative lack of knowledge regarding transmission of nociceptive information from the musculoskeletal system may be due to the complexity of factors which can influence muscle sensation, including inputs from muscle, joint and skin receptors as well as central nervous processing (Shepherd, 1994). The other problem is difficulty of access. Properties of cutaneous nociceptors can be studied by direct application of stimuli to the skin surface. This is not possible with muscle. As a result much more is known about cutaneous pain.

Nonetheless, Group III (myelinated) and Group IV (unmyelinated) muscle nociceptors are known to transmit the sensation of pain in skeletal muscle (Armstrong, 1984). However, unlike cutaneous receptors, the classification for muscle afferents is based on

the diameter of the fibres. Although Group III and IV fibres are mainly considered to have a nociceptive function, some do not. Some Group III afferents that are thought to be stretch and contraction sensitive may not be nociceptive, but ergoreceptors which give rise to increases in heart rate, respiration and blood pressure after exercise (reviewed by McCloskey & Mitchell, 1972). Similarly Group IV units with predominantly nociceptive as well as ergoreceptive characteristics have been found (Kniffki *et al.*, 1978). Group IV fibres are assumed to end exclusively in free nerve endings, whereas Group III fibres end in free nerve endings and, in addition, sometimes in encapsulated terminals (e.g., paciniform corpuscles) (reviewed by Graven-Nielsen & Mense, 2001). The typical location of free nerve endings in skeletal muscle is in the wall of arterioles and the surrounding connective tissue adjacent to muscle fibres (Stacey, 1969).

Nociceptors, unlike most other receptors, may be activated by chemical stimuli. They are also easily sensitised by chemical stimuli; that is, after a conditioning stimulus, they exhibit larger responses to the same stimulus. Specifically, sensitisation is characterized by a lowered threshold, an increase in the suprathreshold response, and a development of spontaneous activity (Treede *et al.*, 1992). In addition to exogenous chemicals like mustard oil and capsaicin, several inflammatory mediators excite nociceptors and cause pain. Histamine, 5-hydroxytryptamine (5-HT), and potassium ions have all been shown experimentally to have sensitising effects (Fock & Mense, 1976; Birrell *et al.*, 1991; Handwerker *et al.*, 1991). Bradykinin is known to have a sensitising action, as well as an excitatory effect. Its threshold concentration for excitation of polymodal nociceptors is the lowest among inflammatory mediators (Mizumura, 1997). In addition, prostaglandin type E_2 (PGE_2) enhance the sensitising action of bradykinin on nociceptors (Chahl & Iggo, 1977), as well as causing sensitisation itself. All such mediators are released from damaged cells, blood plasma and inflammatory cells, during mechanical lesions or inflammation. In addition, contrary to former belief, the sensitisation of a nociceptor is not an unspecific process (eg., because of mechanical damage to a ending), but it is the result of the binding of the aforementioned endogenous agents to highly specific receptor molecules in the membrane of the receptive ending (reviewed by Graven-Nielsen & Mense, 2001).

Exercise induced muscle pain

At one time or another we have all endured pain or soreness associated with exercise. No matter how fit or active an individual may be, pain from exercise is almost inescapable. Excluding muscular injuries and disorders, there are three types of pain related to exercise that affect skeletal muscle. Firstly there is the pain experienced during or immediately following exercise, that is considered to result from a combination of factors including lactic acid, ions, proteins, and hormones, although no single factor has ever been clearly identified as responsible. Secondly there is the pain induced by muscle cramps. Muscle cramps which are intense contractions elicited by motoneuron hyperexcitability (Miles & Clarkson, 1994), are commonly assumed to result from fluid electrolyte imbalance. However, many studies disagree, and the issue remains highly controversial (Miles & Clarkson, 1994). Lastly there is DOMS that develops 24-48 hours after strenuous exercise biased toward eccentric contractions. A prolonged strength loss and various other signs of muscle damage also accompany DOMS. It is certainly the most debilitating form of muscle pain.

Delayed onset muscle soreness (DOMS)

People who have muscles with DOMS are usually described as being stiff or tender, sensitive to palpation and stretch, with reduced mobility and flexibility (Armstrong, 1984). DOMS can be experienced as early as 8 to 10 hours after eccentric exercise, reaches a peak intensity between 24 and 48 hours and is usually gone by a week (Byrnes *et al.*, 1985; Jones *et al.*, 1987; Cleak & Eston, 1992; Howell *et al.*, 1993; Brockett *et al.*, 2001). The pain is initially most evident at the muscle-tendon junction, and thereafter spreads throughout the entire muscle (MacIntyre *et al.*, 1995). An interesting characteristic of DOMS is that it is only experienced when the muscle is stretched, contracted or palpated, all mechanical stimuli that are normally not painful. This makes DOMS a form of allodynia, where normally innocuous stimuli becomes painful. There is typically no persistent pain in completely relaxed muscles with DOMS. Therefore DOMS is sometimes referred to strictly speaking as 'tenderness', not soreness. In addition, DOMS is also not evenly distributed over the eccentrically exercised muscle, but instead, random localised areas of sore 'hot spots' are evident (Newham *et al.*, 1983). These hot spots presumably correspond to regions where there are damaged muscle fibres.

Origins of DOMS

It has been suggested that DOMS is transmitted mainly by nociceptors with axons in the Group IV range, as the activity of this group is known to evoke the type of dull, generalized pain that is commonly associated with delayed soreness (Armstrong, 1984). Hence, considering the fact that Group IV receptors can respond to mechanical, chemical and noxious stimuli (polymodal nociceptors), any of these stimuli may be involved in producing the pain after eccentric exercise (Ebbeling & Clarkson, 1989). It has often been suggested that the link between muscle damage and DOMS is provided by the inflammatory process (Smith, 1991).

Inflammation

Acute inflammation is a generalized response of the body to any kind of tissue injury, and is the first step in the healing process. It involves an influx of white blood cells (monocytes and neutrophils) into the site of injury (Smith, 1991). The hypothesis that DOMS is associated with the inflammatory response to muscle damage is supported by various studies (Hellsten et al., 1997; Smith, 1991; Tibell, 1995). Malm et al. (1999) found eccentric exercise increased the numbers of monocytes and neutrophils, which ultimately help to produce chemical inflammatory mediators. As mentioned earlier mediators such as PGE_2, histamine, acetylcholine, bradykinin, and potassium activate and sensitise group III and IV nociceptors, thus producing a state of increased sensitivity (Smith, 1991).

However, a role for inflammation in triggering DOMS is far from being universally accepted. Discrepancies between the intensity of soreness and peak inflammatory changes are clear, as peak pain is experienced between 1-2 days post-exercise, while the cellular response of inflammation reaches its peak 3-4 days post-exercise (Newham *et al*, 1988). Furthermore, the failure of prostaglandin-inhibiting drugs like aspirin to alleviate DOMS (Kuipers *et al.*, 1985; Donnelly *et al.*, 1988), as well as results showing no significant increase in white blood cell numbers after eccentric exercise, despite DOMS being experienced (Bobbert *et al.*, 1986), leave uncertainty as to the precise role of inflammation.

Nonetheless, the fact that DOMS is only evoked by mechanical means leads to the postulation that mechanical stimulation of nociceptors, which have already been sensitized, is the main cause of DOMS. In a sensitised state, nociceptors can respond to normally non-noxious mechanical stimuli, thus signalling pain. A role for 'silent' nociceptors that cannot be activated by physiological stimuli in uninjured tissue, but can be sensitised during inflammation, also must not be ignored (Michaelis et al., 1996).

Swelling

Swelling is the result of increased permeability of small blood vessels which allows protein-rich exudate to escape from capillaries and accumulate in the tissue of damaged areas (Smith, 1991). Swelling is minimal immediately post-exercise, and then slowly increases, peaking after 3-4 days (Chleboun *et al.*, 1998). Considering that swelling is a reliable sign of inflammation, the combination of increased intramuscular tissue pressure (IMP) and sensitization from substances such as PGE_2 have frequently been proposed to produce the sensation of DOMS (Peeze Binkhorst *et al.*, 1989). Hence, movement or palpation is thought to exacerbate small increases in pressure on top of the already elevated tissue pressure, thus providing a mechanical stimulus for nociceptors that have been sensitized (Smith, 1991). However, inflammation and swelling have been shown to have different time-courses (Newham *et al.*, 1988). Similarly, it was found that after eccentric exercise although resting IMP increased, the magnitude of IMP was not an accurate indication of the level of soreness experienced (Crenshaw *et al.*, 1994). Hence, such relationships with DOMS still remain unclear.

Alternative theories

Other theories for DOMS involve factors including the accumulation of metabolic waste products, insufficient ATP production and lowering of pH (Byrnes & Clarkson, 1986). However, such theories are weak as it is evident that although eccentric exercise causes damage, it is a metabolically efficient form of exercise, as the metabolic energy cost and the eletromyographic activity per unit tension are significantly less than during concentric exercise. This is because active muscle fibres generate more tension when stretched than when shortening (Armstrong, 1984).

Direct stimulation of pain nerve endings by the elevated temperatures that occur during exercise, including eccentric exercise (Nadel *et al.*, 1972), has also been proposed to lead to DOMS (Kumazawa & Mizumura, 1977). However, damage has also been observed in a single fibre at 4°C (Morgan *et al.*, 1996). Muscle contracture has also been suggested to contribute to DOMS. However, as previously mentioned, no increase in EMG has been reported in muscles with contracture and stiffness after eccentric exercise (Jones *et al.*, 1987).

Sequence of events leading to DOMS

Using the most accepted propositions and hypotheses, a sequence of events leading to muscle damage and necrosis, from the time of eccentric exercise to the onset of DOMS, can be constructed. As mentioned above, the initial event immediately following eccentric exercise is believed to involve the over-extension and disruption of sarcomeres. This damage can spread to neighboring myofibrils that until then have managed to stay intact. Damage to the connective tissue components is proposed to be involved in this lateral transmission of sarcomeric disorganization (Morgan & Allen, 1999). Armstrong et al. (1991) propose that this damage eventually reaches the sarcoplasmic reticulum and sarcolemma causing it to 'tear', leading to the disruption of the normal permeability barrier provided by the cell membrane and basal lamina. This allows Ca^{2+} to enter the fibre down its electrochemical gradient, leading to an uncontrolled increase in intracellular Ca^{2+} levels. This Ca^{2+} overload is then hypothesised to activate a number of Ca^{2+}-dependent proteolytic and phospholytic pathways, as well as leading to the development of a regions of contracture in the damaged fibres (contraction clot). During this so-called autogenic phase, structural and contractile proteins and membrane phospholipids are degraded. These active processes occur at the same time as the phagocytic phase, in which inflammatory processes take place along with the invasion by macrophages and other phagocytic cells involved in the removal of damaged tissue and regeneration of muscle fibre. The final phase is regeneration, which is evident from 4-6 days post-exercise (Armstrong *et al.*, 1991). The sensation of pain is typically believed to be due to the breakdown of the injured tissue and production of oedema caused by the initial muscle damage, where the breakdown products sensitise nociceptors.

Non-nociceptive mechanoreceptor involvement in DOMS

The heightened sensitivity in tissue surrounding an injury site is referred to as hyperalgesia. Specifically it is defined as a heightened sensation of pain to a painful stimulus. Although it remains to be demonstrated, DOMS is likely to be associated with mechanical hyperalgesia. As mentioned earlier, it is currently thought that the hyperalgesia and allodynia associated with DOMS is generated by the local release of chemical substances, such as prostaglandins, which sensitise polymodal nociceptors served by small diameter Group III (2-6μm diameter) and Group IV (0.2-1.2μm diameter) fibres. The hypothesis is that sensitisation is sufficient to make normally non-painful stimuli such as stretch, contraction or palpation able to generate pain. Specifically, it is believed the mechanical nociceptors no longer respond just to noxious stimuli but to innocuous mechanical stimuli as well.

However, most sensitised nociceptors develop background activity and therefore generate pain continuously (Mense, 1993). That is not the case with DOMS. Therefore, an alternative, although more controversial explanation is that non-nociceptive mechanoreceptors, not normally associated with pain are able, in some way, to access the pain pathway to the brain following the muscle damage from eccentric exercise. These larger myelinated afferents including the Group Ia (12-20μm diameter) and Group II (6-12μm diameter) afferents, supply muscle spindles within skeletal muscle.

Muscle spindles are stretch receptors, which consist of a small bundle of modified muscle fibres called intrafusal fibres. There are actually two kinds of intrafusal fibres classified as bag or chain fibres, based on histochemical, morphological and functional differences. Around the central regions of each fibre are wrapped the prominent annulospiral 'primary endings' of the group Ia afferent fibres. To one side of the primary endings, are the 'secondary endings' of the smaller group II afferent fibres, which make sprays of terminals terminating predominantly on chain fibres. All intrafusal fibres receive a motor innervation from γ- or fusimotor neurons.

Stretching the muscle stretches spindles which, in turn, leads to the opening of the spiral endings and the initiation of impulses (Proske, 1997). Specifically, the response of primary endings to passive lengthening of a relatively large amplitude is non-linear and

is characterised by an initial burst at the onset of stretch and high sensitivity to stretch velocity or dynamic sensitivity. For very small muscle stretches the primary endings respond linearly. The secondary endings do not have the same dynamic sensitivity as primary endings and are less sensitive to vibration (Brown *et al.*, 1967a). Their firing rate is more regular and linearly related to muscle length, making them well suited to provide information about absolute muscle length.

The properties of muscle spindles allow them to function in diverse tasks. For example impulses from muscle spindles have long been considered important for proprioception, the conscious perception of limb position and movement. Specifically, it has been inferred that primary endings of spindles signal both position and movement of our limbs while secondary endings signal position only. Muscle spindles also play a prominent role in motor learning, locomotion, and the control of precision movements. This is possible through Ia fibres branching within the spinal cord synapsing with α-motor neurons. In addition, Ia impulses of spindles provide the afferent limb of the phasic stretch reflex, or tendon jerk. The work presented in Chapter 2 and 3 of this thesis explores another possible aspect of spindle afferent function; specifically, to assess whether the generation of DOMS involves input from the non-nociceptive Group I and II afferents.

Proprioception

As mentioned above proprioception is the conscious perception of limb position and movement, which encompasses a group of sensations. First, sensations exist for timing muscle contractions. Second, there is the traditional sensation of position and movement of limbs and trunk. Thirdly are the sensations related to muscle force, including effort, tension, heaviness and stiffness.

Although certain receptors contribute to specific sensations more than others, it is generally agreed that signals provided by joint, skin and muscle receptors all make contributions to these sensations. Specialized joint receptors include Ruffini endings, that respond when a stimulus is present without much decrease in its firing rate (slowly adapting), and Pacinian and smaller paciniform endings, which respond with a sudden burst at the onset of a stimulus (rapidly adapting) (Proske *et al.*, 1988). Their

distribution is non-uniform within a joint. Cutaneous receptors embedded in a multi-layered 'glove' covering the skeleton are well placed to signal movement of the underlying skeleton (Gandevia, 1996). However, it is thought their capacity to generate perceived signals of joint position and movements is less well developed than for the other proprioceptive afferents. In contrast, as mentioned earlier, muscle spindles are thought to be the main muscle receptor contributing to this sensation, while, Golgi tendon organs are thought to be the principal intramuscular receptors that signal muscle force.

Sense of force

In many activities, whether it be picking up a carton of milk or pushing down the accelerator pedal in a car, we are constantly required to judge the magnitude of muscular contractions. Several mechanisms are involved in helping us judge the weight of objects or strength of our contractions. In the absence of any muscle contraction, the pressure of the object on the skin will excite cutaneous receptors and possibly some local joint and muscle receptors (Gandevia, 1996). The contraction itself may also provide cues. However, the basis of our sense of force and heaviness during muscular contraction has been the subject of much debate. The main issue is whether the perception of force is derived primarily from peripheral sensations originating in muscles, skin and joints or from centrally generated sensations arising from signals accompanying the motor command (McCloskey *et al.*, 1974).

The perception of force derived from peripheral sensations is often described as a 'sense of tension'. The sense of tension is thought to be derived chiefly from receptors in muscle and tendon, which are presumed to signal intramuscular tension and therefore provide a measure of the actual force exerted by the muscle (Roland & Ladegaard-Pedersen, 1977). Most of our skeletal muscles contain two prominent types of muscle receptors, the muscle spindle and tendon organ. After some earlier suggestions to the contrary, it is now believed that both muscle spindles and Golgi tendon organs have projections to the cerebral cortex and signals from both, therefore, potentially have access to consciousness (Landgren & Silfvenius, 1969; McIntyre *et al.*, 1984). Indeed, it was shown in 1972 by Goodwin, McCloskey and Matthews that signals from muscle spindles were concerned with the sense of position and movement of our limbs.

However, although muscle spindles function primarily in this way, it has been proposed that Ia muscle spindle afferents are also capable of signaling intramuscular tension (Cafarelli, 1988).

Like muscle spindles, tendon organs are stretch receptors. Over 90% of tendon organs are located at the musculotendinous junctions, with the remainder in the tendon itself (Barker, 1974). Therefore, tendon organs are uniquely placed to signal muscle force. The tendon organ consists of encapsulated bundles of collagen strands on which the nerve endings of large diameter (Ib) axons lie (Proske, 1981). On average, every motor unit in a muscle is connected to one or more tendon organs, ensuring that tension from all parts of the muscle is signaled faithfully to the central nervous system (CNS) (Houk & Henneman, 1967). Hence it has been suggested that the signal relevant to the CNS is the ensemble of tendon organ outputs, which is used to calculate mean muscle force (Jami, 1992; Gandevia *et al.*, 1996).

However, the situation for the sense of tension is complicated by the fact that it is associated with a closely related sense, the 'sense of effort' or heaviness. Effort is defined here as a centrally-mediated sensation assumed to arise from the voluntary motor command. It is based on the notion that together with the efferent command sent to the motoneuron pools, concomitant internal neural correlates (corollary discharges) are transmitted to sensory centres (Jones & Hunter, 1983a). These corollary discharges presumably reflect the magnitude of the voluntary motor command (Jones & Hunter, 1983a). The central origin of the relevant motor command signal for force and heaviness judgments has been little studied, although some evidence points to a role for the sensorimotor cortex (Gandevia, 1982).

Effects of exercise on the sense of force

It is well known that most forms of exercise lead to a reduced tension output from muscle due to fatigue. Furthermore, it is also well known that the ability of human subjects to make judgments of force deteriorates as a result of muscle fatigue (Gandevia & McCloskey, 1978). A variety of psychophysical methods have been used in order to explain the basis of these changes. For example, one method employed is the ratio scaling procedure that require subjects to assign numbers to forces in proportion to the

perceived magnitude of the force (Gescheider, 1985). However, the most widely used is the contralateral limb-matching method (McCloskey *et al.*, 1974), in which participants are required to generate a specified level of force by contracting the muscles of the reference limb in the presence of external feedback, and to match the subjective magnitude of this force using the muscles of the contralateral matching limb, without the assistance of feedback.

When individuals are required to reproduce the force applied during constant sustained fatiguing isometric contractions, by generating brief matching contractions using the contralateral limb, there is a progressive and linear increase in the perceived matching force, with the rate of increase dependent on the amplitude of the constant force being exerted (McCloskey *et al.*, 1974; Gandevia & McCloskey, 1978; Cafarelli & Layton-Wood, 1986). Such observations suggest that subjects base their judgments of force on the signal related to the motor command sent to the muscle, or some variable related to it, rather than on the force actually generated by the muscle. The overestimation of force results from efferent signals of comparable magnitude being transmitted to fatigued and unfatigued muscles, the latter having greater force generating capacity (Jones, 1995). Recordings of surface EMG further support this interpretation. As surface EMG reflects the number of active motor units and their discharge rate, it has been employed as an indirect measure of the level of neural drive directed to muscles during force estimation. Jones and Hunter (1983b) found a linear relation between the reference arm EMG and the perceived force, which suggests that the overestimation of the force was due to the increase in the excitatory input to the fatiguing muscle, again indicating that a sense of effort is used in the judgement of force.

Although such results favour the sense of effort, a contribution from intramuscular receptors to force perception cannot be discounted. Indeed, there is little doubt that in certain circumstances, a sense of force or tension can be generated independently of a sense of effort. The tonic vibration reflex (TVR) is the reflex response of a muscle to local vibration. The pathway is thought to be purely spinal and the reflex tension generated is not accompanied by any sense of effort; yet the force produced by a TVR can be accurately matched with a voluntary contraction of the other limb (McCloskey *et al.*, 1974). Furthermore, it is claimed that if subjects are appropriately instructed, they

can judge levels of intramuscular tension, independently of the effort required (McCloskey *et al.*, 1974; Roland & Ladegaard-Pedersen, 1977).

A dual role of sense of tension and sense of effort, where the mechanisms are complimentary rather than exclusive, is also possible. It has been noted that peripheral signals from the muscle must be used if the effectiveness of a contraction is to be judged (Gandevia & McCloskey, 1978), that is that the force produced was sufficient for the task. McCloskey et al. (1983) hypothesized that the matching of an efference copy (internal command-related signals) and proprioception re-afference (sensory discharges set up purely as consequences of motor signals) might facilitate generation of kinaesthetic perception and suggested that the sense of effort need not be a purely central mechanism. Accordingly, sensory inputs signalling the level of intramuscular force could be used to scale or calibrate the perceived motor commands. While such a calibration need only be intermittent in conditions where the state of the contractile elements, and thus the relationship between the motor command and the force output, remains relatively constant, it must necessarily become more frequent whenever there are rapid changes in the state of the muscle, such as those which occur during fatiguing contractions (Carson *et al.*, 2002). Thus, in the absence of experience, or visual input, it may be that the sense of tension and sense of effort co-exist. It is of interest to ascertain under what conditions each assumes the predominant role.

Adaptation to Eccentric Exercise

Although a single bout of eccentric exercise induces muscle damage and DOMS, it has been frequently observed that after a repeated bout of eccentric exercise a week later, the signs of muscle damage are much reduced, as is the soreness experienced. Evidently muscles are able to adapt to eccentric exercise.

This phenomenon, often referred to as a 'training effect' or a 'repeated bout effect' was first reported by Hough (1902). A repeated bout of the same exercise, performed 2-9 weeks later, leads to reductions in the damage indicators, and a much faster recovery of muscle function (Byrnes *et al.*, 1985; Newham *et al.*, 1987; Clarkson & Tremblay, 1988; Nosaka *et al.*, 1991; Golden & Dudley, 1992; Mair *et al.*, 1995). In addition, it has been

shown that a training effect from a single bout, for most indicators of damage, can last at least 6 weeks (Byrnes & Clarkson, 1986; Nosaka *et al.*, 1991), although generally not more than 12 weeks (Jones & Newham, 1985).

Although there have been significant amounts of research into the repeated bout effect of eccentric exercise, argument on the mechanism causing the effect still remains. A number of theories have been proposed. One of the earliest was that the damaged 'stress-susceptible' fibres were subsequently degraded and removed, so that a repeat exercise bout resulted in less damage and soreness (Armstrong *et al.*, 1983). Support comes from Foley et al. (1999) who reported a repeated bout effect in human biceps muscles that was attributed to a reduction in muscle mass from the removal of a small population of vulnerable fibres damaged after the first bout. However, other studies showed a repeated bout effect when there was only minimal muscle damage after the initial bout. If stress susceptible fibres were not sufficiently damaged from the first bout, and were not completely degraded and removed, then it may have been anticipated that they would be damaged after subsequent bouts, but this was also not consistent with findings (Schwane & Armstrong, 1983; Clarkson & Tremblay, 1988; Brown *et al.*, 1997a; Nosaka *et al.*, 2001).

Another theory is that neural mechanisms are involved in muscle damage and adaptation, particularly since fewer motor units are activated during eccentric contractions compared to concentric contractions, as shown from EMG recordings (Moritani *et al.*, 1988; Adams *et al.*, 1992). It has been proposed that the repeated bout effect is a result of a change in motor unit recruitment (Golden & Dudley, 1992) so that the workload during eccentric contractions is distributed more evenly among fibres (Nosaka & Clarkson, 1995). Evidence for neural adaptation comes predominantly from strength studies. Hortobagyi et al. (1996) showed a 42% increase in eccentric contraction torque after eccentric training of the knee extensors, which was associated with an 89% increase in integrated EMG (iEMG). In comparison concentric training improved concentric contraction torque by 36% and increased iEMG by 39%. In addition, contralateral unexercised muscles showed strength improvements of 30% in eccentric torque (Hortobagyi *et al.*, 1996; Hortobagyi *et al.*, 1997). This apparent 'cross education', further reflects a nervous system adaptation to eccentric exercise.

However, although these studies show a link between increased neural activity and increased strength, they do not show that increased neural activity reduces the susceptibility to damage. Uncertainty with neural adaptation as a cause for the repeated bout effect has been raised, as electrical stimulation of rat tibialis anterior muscles can induce a repeated bout effect (Sacco & Jones, 1992). After the first bout, force in the eccentrically exercised muscles was 48% of the non-exercised control muscles, 3 days after the exercise. After the repeat bout, the force in the eccentrically pre-conditioned muscles was 80% of the control value, 3 days after the repeat bout (Sacco & Jones, 1992). As the eccentric exercise involved identical activation patterns each time, the protection effect cannot be credited completely to neural adaptation.

The adaptation of parallel connective tissue elements has also been considered as a mechanism. It is likely that intermediate filaments, that are responsible for maintaining the structural integrity of the sarcomeres, and other passive structures such as the sarcolemma and sarcoplasmic reticulum, are damaged by eccentric contractions (Armstrong *et al.*, 1991). If remodelling of intermediate filaments and strengthening of the sarcolemma occurred as an adaptive process, it could potentially reduce damage from repeated bouts. Support for this theory comes from Lapier et al. (1995) who reported a 63% increase in intramuscular connective tissue in rat extensor digitorum longus muscles that had been immobilised at long muscle lengths, with an 86 % decrease in mass compared to non-immobilised control muscles. However, this study did not test for any changes in muscle fibre structure. Therefore as the changes occurred intramuscularly, it is possible that the adaptation could have occurred in the muscle fibres themselves.

Another theory for the mechanism of the adaptation to muscle damage from eccentric contractions is that there is an increase in the number of sarcomeres in series in muscle fibres (Morgan, 1990). It has been known for some time, through immobilisation experiments, that muscles can adapt to changes in their functional length by increasing or decreasing the number of sarcomeres in muscle fibres (Tabary *et al.*, 1972; Williams & Goldspink, 1973). Plaster casting the hindlimb of mice, to immobilise the soleus muscles in a lengthened position, led to an increase in sarcomere number of about 20% within one week, followed by a rapid reversal when immobilisation ceased (Williams &

Goldspink, 1978). Later studies showed that not only did sarcomere number increase or decrease relative to the immobilised position of the muscle, but the changes were reflected in the muscle length-tension relationship. Immobilisation in a lengthened position, where there was an increase in sarcomere number of about 16%, was accompanied by a shift in the optimum length for maximum tension generation to longer muscle lengths (Williams & Goldspink, 1978).

Having more sarcomeres in series would mean that at a particular fibre length, average sarcomere length would be shorter (Morgan, 1990). This was indeed observed in muscles immobilised at longer lengths, where an increase in sarcomere number was accompanied by reduction in average sarcomere length for a set muscle length (Williams & Goldspink, 1978). More sarcomeres at shorter lengths holds benefits as less of the working range of the muscle would have to include the descending limb of the length-tension relationship (Proske & Morgan, 2001).

Direct support for the sarcomere addition theory comes from experiments where rats were run for a week on an inclined or declined treadmill, after which the sarcomere numbers of the vastus intermedius muscles were then determined from fibre length and sarcomere length of representative fibres (Lynn & Morgan, 1994). It was found that on average, there were 11% more sarcomeres in muscles from animals that ran downhill, compared to those that had run uphill. In addition to the results showing that the length-tension relationship shifted after immobilisation (Williams & Goldspink, 1978), similar shifts have been observed after eccentric training. Angle-torque curves were determined for vastus intermedius in rats that ran on a declined or inclined treadmill. The decline trained group had a larger number of sarcomeres and the optimum knee angle for maximum torque occurred at a smaller angle, representative of a longer muscle length. The optimum for the incline trained group occurred at a larger knee angle, indicative of a shorter muscle length. In addition, it was found that if inclined and declined rats were subject to an acute series of eccentric contractions, the decline trained group showed a smaller immediate shift in optimum angle for torque compared to the incline trained group, indicating less damage had occurred. This shows that if a muscle's working range covers less of the descending limb of the length-tension curve it offers protection against damage from eccentric exercise (Lynn *et al.*, 1998).

The time course being supposedly not fast enough is the main doubt about an adaptation mechanism involving sarcomere addition into the muscle (McHugh *et al.*, 1999). If muscle fibres are going to be remodelled after eccentric damage, the process must be rapid enough to be substantially complete by the end of a week following the injury. There is good evidence that it can be. Immobilizing a muscle in the lengthened position with a plaster cast has been shown to lead to an increase in sarcomere number in muscle fibres within 5 days (Williams & Goldspink, 1973). This increase was rapidly reversible. In addition, signs of fibre regeneration in human muscle have been reported as early as 72 hours after damage from eccentric exercise, with reappearance of 'normal' fibre structures 6 days after exercise (Friden *et al.*, 1983b; Jones *et al.*, 1986). Hence, although it is poorly understood how, at the cellular level, the adaptation of sarcomere numbers takes place, such results suggest the speed of the process is sufficient to account for the adaptation observed after eccentric exercise.

Aims and Objectives of the Thesis

This thesis will focus on increasing the understanding and knowledge of eccentric exercise and its related effects. Specifically, the aims and objectives of this thesis are as follows:

1. To determine the neural origins of the pain and tenderness associated with DOMS. In particular, to assess to what extent DOMS might be mediated by non-nociceptive muscle mechanoreceptor afferents; or whether DOMS is transmitted solely by sensitized muscle nociceptor endings supplied by Group III and Group IV afferents.

2. To use hypertonic saline as an experimental model of muscle pain, to further explore the theory that large fibre mechanoreceptors have a role in DOMS.

3. To investigate the effects of eccentric exercise on our sense of muscle force. In particular to explore the effect of force matching ability of elbow flexor muscles at different %MVC force levels after eccentric exercise of one arm. Subsequently to determine whether our 'sense of tension' or 'sense of effort' is disturbed.

4. To investigate whether there is a pain mediated reduction in motor performance. Specifically to test the effects of muscle soreness produced by hypertonic saline and skin soreness produced from a heated probe on a force matching task. Consequently to use these findings to make inferences on DOMS and its potential effects on motor control.

5. To illustrate in human hamstring and quadriceps muscles, the typical length-tension properties of concentrically trained individuals compared to untrained individuals. It was predicted that the muscles of concentrically trained subjects would show adaptation changes and have a reduced sarcomere number. This would be represented with their optimum length for maximum torque generation occurring at a shorter muscle length.

CHAPTER TWO

Pain in response to mechanical stimulation of a muscle with DOMS

INTRODUCTION

In the past few years there has been considerable interest in the neurophysiological mechanisms of hyperalgesia and allodynia that follow various types of tissue injury and inflammation. These conditions are characterized by alterations of pain perception that include an enhanced response to normally noxious stimuli (hyperalgesia) and an abnormal pain sensitivity to previously non-painful stimuli (allodynia) (Merskey & Bogduk, 1994). Both hyperalgesia and allodynia are thought to provide protective mechanisms to the organism, preventing the individual from stimulating an injured area and in so doing helping the healing process.

Specifically, early works on the sensory consequences of cutaneous injury (Lewis, 1942; Hardy, 1950) led to the definition of two types of hyperalgesia that differ in their location relative to the injury site. The region adjacent to the area of injury is referred to as the region of 'primary hyperalgesia', where low intensity mechanical stimuli and warmth evoke pain and painful mechanical and heat stimuli cause more severe pain. A larger area of undamaged skin surrounding the injury shows a sensitivity to non-noxious mechanical stimuli and hyperalgesia but only a small change in heat pain threshold (Kilo *et al.*, 1994). This is referred to as the region of 'secondary hyperalgesia'.

The current accepted view is that primary hyperalgesia can be accounted for by the marked sensitization of nociceptor afferents (Meyer & Campbell, 1981; Treede *et al.*, 1992). An additional mechanism that may account for primary hyperalgesia, specifically to mechanical stimuli, is the expansion of the receptive field by means of the axon reflex. If the receptive field size of a primary afferent increased, any given stimulus would recruit more afferents, leading to an increased sensory response from the injury site.

Similarly, for the situation regarding secondary hyperalgesia two separate mechanisms have been proposed to account for this spread of hyperalgesia. Firstly, Lewis (1942)

suggested a peripheral mechanism of an axon reflex. This involves an initiation of action potentials from activated receptors within the injury site that spread along branching nerve fibres and propagate antidromically to invade peripheral nerve terminals located adjacent to the injured site, causing the release of substances that sensitise other nociceptors in uninjured areas. Alternatively, a central mechanism was proposed by Hardy (1950) involving a spreading of sensitisation within the spinal cord. Experiments showing that proximal nerve block prior to injection of capsaicin prevented the development of secondary hyperalgesia seems to support this view (LaMotte *et al.*, 1991). Specifically it is believed that there is an alteration in the processing of afferent inputs that include large-fibre mechanoreceptors which normally evoke non-painful tactile sensations (allodynia) (Torebjork *et al.*, 1992). It is known that after cutaneous injury, the sensitisation of spinothalamic tract neurons is involved in this hyperalgesia, with an enhanced excitability within the dorsal horn of the spinal cord of neurones thought to be mediating the response.

Most of the understanding of hyperalgesia has been gained through cutaneous studies. By contrast studies exploring the mechanisms of muscle hyperalgesia have been limited by the difficulty of access and lack of animal models that permit the use of behavioral, biochemical, pharmacological, and molecular biological approaches. Although the pathophysiological basis of muscle hyperalgesia is unclear, like cutaneous hyperalgesia, it is thought that the activation of both peripheral and central nociceptive mechanisms are responsible (Dubner, 1995; Mense, 1995). The most accepted mechanism proposed for the origin of muscle hyperalgesia is the activation of nociceptors by inflammatory mediators such as bradykinin, serotonin and histamine (Fock & Mense, 1976).

An animal model of muscle hyperalgesia recently developed is a carrageenan-induced myositis. Myositis is an inflammatory muscle disease, and provides an example of hyperalgesia. Different forms of myositis (eg., polymyositis dermatomyositis, myositis due to infections) are all associated with subjective symptoms of weakness, spontaneous pain and tenderness paresthesias (Mense, 1993). In the experimental model, a few hours after carrageenan injection the muscle exhibits all the signs of myositis (hyperemia, edema, infiltration of leukocytes). Evidence exists that carrageenan sensitises group III and IV muscle afferents, lowering their threshold to mechanical activation and increasing

background activity (Berberich *et al.*, 1988). Furthermore, carrageenan evokes local inflammation, thought to be associated with the release of neuropeptides such as substance P and other agents from nociceptive afferent units (Mense, 1993).

The origins of DOMS could have a similar basis to that proposed for carrageenan-evoked muscle hyperalgesia, as DOMS also is thought to involve an inflammatory component, but in this case where an inflammatory reaction is caused by muscle damage. However, because DOMS is a deep-tissue pain, it is not known whether there are regions of primary and secondary hyperalgesia. It is, in fact, not known whether there is any hyperalgesia at all associated with DOMS. Given that there is no chronic pain, the mechanism for DOMS therefore is likely to be different than for other kinds of muscle injury.

As outlined in the previous chapter, although it remains a point of controversy, there is evidence in support of the idea that the primary event during an eccentric contraction which ultimately leads to DOMS, is mechanical (Morgan & Allen, 1999). Repeated eccentric contractions produce sarcomere disruptions and membrane damage. The damage and raised Ca^{2+} triggers tissue breakdown and a local inflammatory response (Smith, 1991) that sensitises nociceptors (Mense, 1996b). Therefore, one plausible mechanism for DOMS is that it is the result of sensitisation of nociceptors to the point where innocuous stimuli, like stretch and contraction, are able to excite them.

A rather different explanation is that, like the secondary hyperalgesia seen in the skin, the generation of DOMS is based, at least in part, on mechanisms operating within the central nervous system and involves input from large-fibre mechanoreceptors (Hardy, 1950).

Although it is known that Group III and IV nociceptors transmit the sensations of pain in skeletal muscle, most sensitised nociceptors develop background activity and therefore generate pain continuously when sensitised (Mense, 1993). That is not the case with DOMS. This lead to the alternative explanation that following the muscle damage from eccentric exercise non-nociceptive mechanoreceptors, not normally associated with pain, are able, in some way, to access the pain pathway to the brain. These larger myelinated

afferents, including the Group Ia, Ib and Group II afferents, supply muscle spindles and tendon organs within skeletal muscle. The receptors served by these afferents are very sensitive to mechanical stimuli such as stretch or palpation.

Although there is evidence that the soreness from eccentric exercise is the result of damage to muscle fibres, and a comprehensive theory to account for the damaging effects of eccentric exercise has been proposed, the pain mechanism for DOMS remains less clear. In this current study the question of the mechanisms involved in the generation of DOMS is re-examined. Specifically, evidence is sought for receptor sensitisation and the possibility that primary endings of muscle spindles are involved in DOMS. The working hypothesis is that DOMS can be modeled by a secondary hyperalgesia as proposed by Hardy (1950). This possibility was explored by means of experiments using muscle vibration and nerve block.

Vibration

The possible role of muscle mechanoreceptors served by large diameter nerve fibres in DOMS was explored by using muscle vibration. Group III and IV fibres have long refractory periods, and are therefore unable to generate high frequency responses to vibration. Specifically, it is known that C mechanoreceptors are incapable of following an oscillating stimulus at frequencies above 1Hz and do not consistently alter their peak discharge frequency when skin indentation is varied over a range of rates (Bessou *et al.*, 1971). Conversely, Ia fibres are very sensitive to vibration over a wide range of frequencies. High frequency vibration of the tendon of a relaxed muscle preferentially excites group Ia afferents. Studies in cat muscle spindles have shown primary endings can be driven to discharge one impulse for each cycle of vibration over a frequency range of 100-500 Hz (Brown *et al.*, 1967b). In humans, in the range used to evoke kinaesthetic effects 10-120 Hz, primary endings have also been shown to respond to each vibration cycle (Roll & Vedal, 1982). Here the most effective frequency appears to be 80Hz (Roll *et al.*, 1989).

In contrast to the vibration sensitivity of Ia fibres, evidence suggests that Group II fibres are responsive only to vibration at lower frequencies. McCloskey (1973) found that low frequency vibration (less than 30 Hz) can induce errors of position without inducing

illusions of movement. He used this fact to argue that primary endings signal both position and movement of our limbs while secondary endings signal only position. The low frequency sensitivity of human Group II fibres is supported by observations that secondary endings of cat spindles do not respond to high frequency vibration (Brown *et al.*, 1967b). The Ib afferents of the tendon organs are also insensitive to vibration unless the muscle is contracting and generating force (Brown *et al.*, 1967b). By taking all of these different responses into account, vibration can be used as a selective stimulus to activate the afferents of muscle spindles. Therefore, it can be used to assist in answering the question of whether such fibres are involved in the generation of DOMS.

In considering responses to vibration the possibility was considered that vibration might actually reduce DOMS, since in other types of pain, mechanical stimulation such as vibration is commonly associated with pain relief. In a study with patients suffering acute or chronic musculoskeletal pain, sixty-nine per cent reported a reduction of pain during vibratory stimulation (Lundeberg *et al.*, 1984). In most patients the best pain reducing effect was obtained when the vibratory stimulus was applied with moderate pressure (Lundeberg *et al.*, 1984). Similarly, acupuncture is a widely practiced therapeutic intervention in a myriad of ailments such as headache, low back pain and osteoarthritis that utilizes mechanical stimulation to induce pain relief. Acupuncture involves stimulation of sites on the skin by a variety of techniques, the most common of which employs penetration of the skin by thin, metallic needles, which are manipulated manually or used to deliver electrical stimulation. In the light of these observations, it was decided to examine the effect of vibration on unexercised muscle as well as muscle with symptoms of DOMS. In addition experiments were conducted to reveal the time course of DOMS and its response to vibration, as this might help to reveal the underlying mechanisms responsible.

Nerve block

To further explore the possibility that spindle afferents contributed to DOMS compression block of nerve impulse conduction was used. Compression blocks are known to be more effective for afferent than efferent fibres (Moddel *et al.*, 1977) and the impulse conduction block progresses according to fibre size, with large myelinated afferents being affected first, followed by small myelinated afferents and lastly by

unmyelinated afferents (Torebjork & Hallin, 1973; Mackenzie *et al.*, 1975). The fundamental mechanism underlying a compression block is still yet to be fully resolved. Nonetheless, one major cause of the functional loss during nerve compression is undoubtedly the purely mechanical deformation of axonal membrane caused by pressure gradients in and around the nerve (Fern & Harrison, 1994). Thus during relatively severe compression, the nodes of Ranvier of myelinated fibres become displaced away from the compressed region (Ochoa *et al.*, 1972). In addition to deformation, the compressed nerve also becomes ischaemic as local blood flow becomes interrupted. However, uncertainty exists regarding the relative contributions of nerve deformation and ischaemia during a compression block. Fern and Harrison (1994) believe that the critical factor is the force of compression. Conduction block produced by lower forces is generated by local ischaemia in the nerve, while under more severe compression, deformation becomes more important. Presumably deformation of the membrane leads to its depolarization and that, in turn, generates the block as the sodium carrier becomes inactivated.

Here a compression block of the sciatic nerve was used to explore the possible contribution by large-diameter muscle afferents to DOMS. To assess progress of the block the H (Hoffman) reflex was monitored in the triceps surae muscle group. The H-reflex is an electrically evoked monosynaptic reflex, following the pathway of the tendon jerk reflex. The reflex is thought to be elicited largely by Group I fibres, although a later contribution from Group II fibres cannot be excluded.

In addition, the effect of vibration was studied while a nerve block was in place. This was done to further test the hypothesis that any effect on DOMS during local vibration of the muscle was the result of the vibratory stimulus exciting large, mechanosensitive afferents in the muscle. If this was so, vibration, as a painful stimulus, should become ineffective during a large nerve fibre block.

METHODS

Subjects

The experiments were carried out on a total of 35 healthy adults (mean age, 23 years), with some subjects taking part in more than one specific experiment. Anyone undergoing regular training exercise or who had some physical disability was excluded from the study. Subjects gave their written consent to participate in the study, which had been approved by the local human ethics committee and conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

The eccentric exercise

The exercise was carried out by all subjects. The triceps surae of one limb was exercised eccentrically by asking subjects to step backwards downhill on an inclined (~13°) moving treadmill for 1 hour. The treadmill speed was adjusted so that subjects carried out about 30 steps per minute. Subjects were asked to step backwards using a toe-to-heel action with their exercising leg (Fig 2.1). It meant that when the foot bore the weight of the body, triceps underwent an eccentric contraction. Subjects were also asked to lock the knee of the exercised leg while performing the eccentric contraction, and to step back with a consistently equal stride length. The other leg was brought alongside and placed flat on the treadmill so that its triceps did not undergo an eccentric contraction. In fact, it contracted very little and since it did not become sore it could act as a control (Jones *et al.*, 1997). The treadmill speed had been adjusted so that once the step had been completed, the subject would be carried back to the top of the treadmill ready for the next step backwards. Early on, it was evident that the effects of the exercise in lightly-built subjects were less severe because they weighed less when compared to heavier individuals. To ensure sufficient load on the exercising muscles subjects carried a weight belt with 5-10 kg of weight. Details of the method have been described previously (Jones *et al.*, 1997).

Having completed the bout, subjects were tested in an experiment 2 days later. The tests were performed on the second day after exercise because at this time DOMS reaches its peak (Jones *et al.*, 1997).

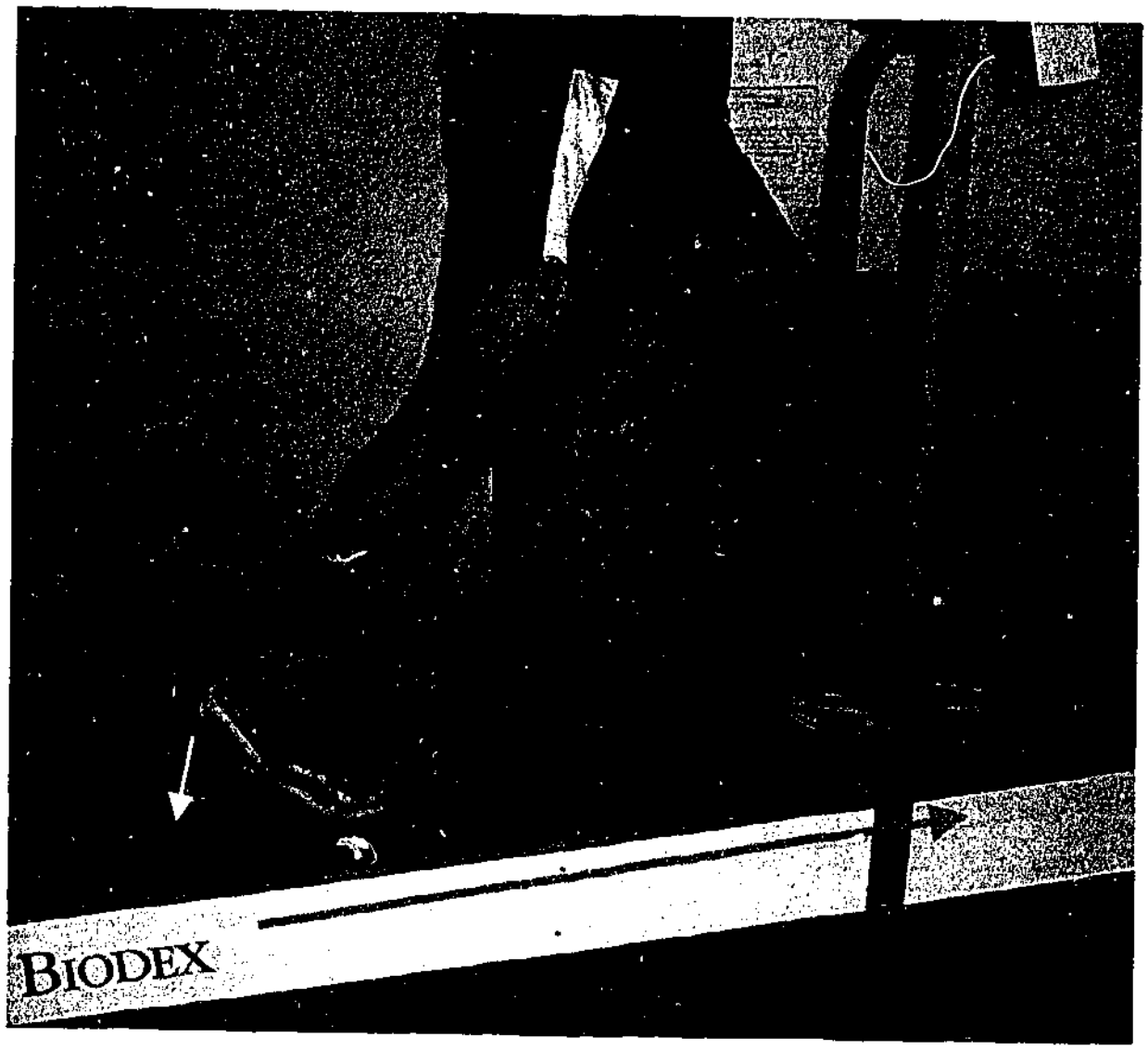


Figure 2.1

A subject performing eccentric exercise of their triceps surae muscle by walking backward on a treadmill inclined at 15°. Note the yellow arrow highlights the toe-to-heel action of the foot. The red arrow indicates the direction of the treadmill.

Skin anaesthesia

In making measurements of muscle soreness it was important to be sure that some of the sensation had not been generated by receptors in the overlying skin. Therefore, for three subjects who were subsequently tested in the other experiments, sore areas were mapped and then a region of overlying skin, 2 cm in diameter, was treated with local anaesthetic cream (EMLA, lignocaine/prilocaine 5%, Astra Pharmaceuticals) for two hours. After treatment, the region of affected skin had become insensitive to touch and pin prick stimulation. Stronger mechanical stimulation evoked some cutaneous sensation but this seemed to be coming from skin immediately adjacent to the treated area which became dimpled by the stimulus. Measurements of threshold for pain were made with a compression gauge (see next section) before and after anaesthesia of the skin.

Mapping tender areas

For 7 subjects (mean age, 22 years), the exercised muscles were also mapped in detail for tender areas. They were asked to lie prone on a mattress. In the prone position the ankle was plantar flexed so that the mapping was done on a rather short muscle. In the majority of experiments testing was carried out using a compression gauge equipped with a disc-shaped 2.5 cm diameter probe. In two experiments, where the tender areas were mapped in more detail, a 1.5 cm diameter probe tip was used. The curved surface of the muscle was represented, in two dimensions, as a rectangle subdivided into 1.5 cm squares. An outline of the sore calf was drawn and divided into a 1.5 cm or 2.5 cm square grid. Tenderness threshold was measured within each square made by the intersecting grid lines. For this a dial compression gauge was used with an attached 1.5 cm or 2.5 cm diameter plunger. This was slowly pressed onto the muscle and the gauge reading recorded when the subject first sensed the onset of pain. Tenderness threshold values were obtained for all parts of the muscle and a site identified, suitably placed for vibration testing, where threshold was particularly low.

Vibration experiment

This group of experiments was carried out on 24 subjects, 18 male and 6 female.

Seven subjects (mean age, 20 years) took part in the first experiment. Subjects were asked to lie on their side so that the tender area was within reach of a 1 cm diameter probe tip. The probe was connected via a shaft to a custom designed moving-coil

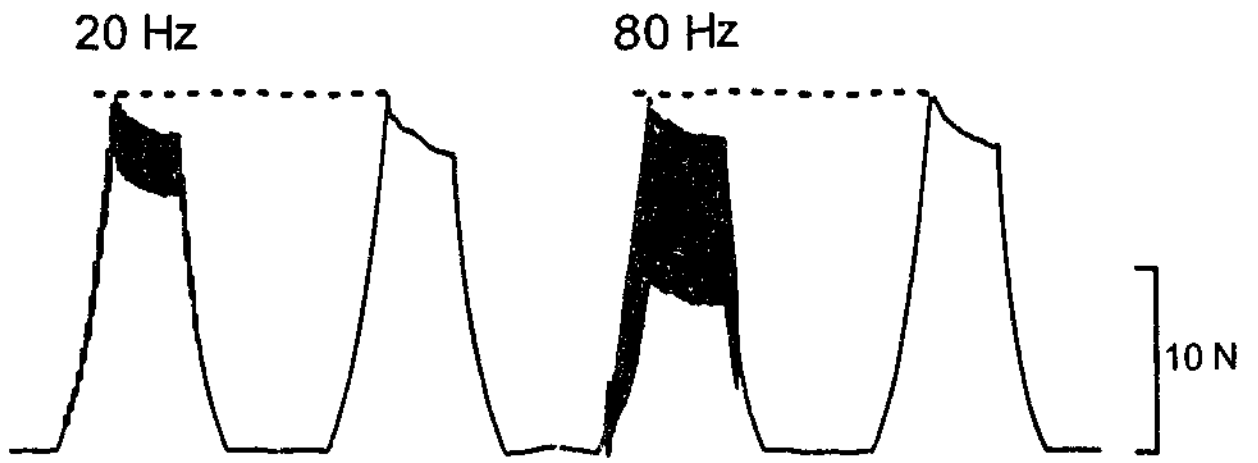
electromagnetic actuator (Department of Physiology, Monash University) supplied with position feedback from an LVDT (linear variable differential transformer) displacement transducer. This provided an output voltage, which could be calibrated to measure the extent of compression of the muscle. Inserted between the tip and the shaft of the actuator was a force transducer. It consisted of 4 foil strain gauges, which formed a Wheatstone bridge circuit. Strain gauge output was amplified and displayed along with displacement on a computer. A gating circuit was used to control the duration of compression.

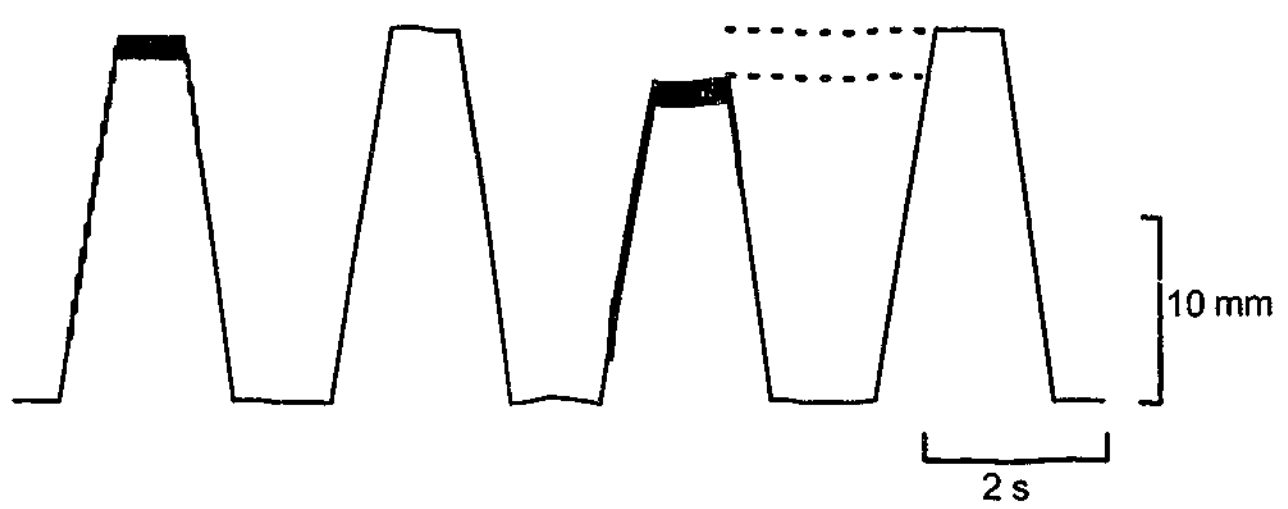
Signals from both tension and length transducers were processed by a commercial analog to digital converter (Maclab/8s, AD Instruments, Australia) and recorded using the program 'Chart' (AD Instruments, Australia) running on a Macintosh G4 computer (Apple, U.S.A.). Data was then analyzed using the software Igor Pro (Wavemetrics, Ore, U.S.A).

For each set of measurements, first the movement was applied, typically a 20 mm indentation at a rate of 30 mm s^{-1}. After trying different parameters this was found to be the most satisfactory. The subject was asked whether the prod was being applied at the centre of the tender area and, if not, minor adjustments were made to the position of the leg and the placement of the actuator. When a satisfactory position had been obtained, the leg was held fixed in that position by supports placed on each side. The subject was also asked, from this point on, to move as little as possible. Several trial indentations of the muscle were carried out to ensure that reproducible pain ratings were obtained during each trial. Here it was important to leave sufficient time between trials, typically 1 minute, to prevent the possibility of receptor desensitisation. The amplitude of the prod, the force applied and the amplitude of the superimposed vibration were altered slightly from subject to subject, depending on how tender they were. The peak force generated during each prod was kept the same by adjusting the peak displacement of the probe (Fig 2.2). Force rather than displacement was kept constant on the basis of cutaneous studies that standardized for force. The underlying rationale for this was that the stimulus evoking pain was not the movement as such, but the level of compression force. The force, displacement and amplitude of vibration used, varied from one experiment to another, but were maintained constant within any one experiment. Forces applied with

Figure 2.2

Example of force (top panel) and skin displacement (bottom panel) data. Although not shown to scale, each test was separated from the next by a 1-minute rest period. The records from left to right, show tension and length during compression combined with 20Hz vibration, compression without vibration, with vibration at 80Hz, and a repeat without vibration. Notice that with 80Hz vibration, the amplitude of displacement had to be reduced to allow matching of peak compression forces with and without vibration (dotted lines).


20 Hz
80 Hz
10 N


10 mm
2 s

the actuator lay in the range 15-40 N. The amplitude of the displacement was 20-24 mm, when not accompanied by vibration, or with 20 Hz vibration. When 80 Hz was used, amplitude had to be reduced to 14-18 mm to allow matching of peak forces with and without vibration (Fig 2.2). Vibration amplitude lay in the range 0.8-1.4 mm.

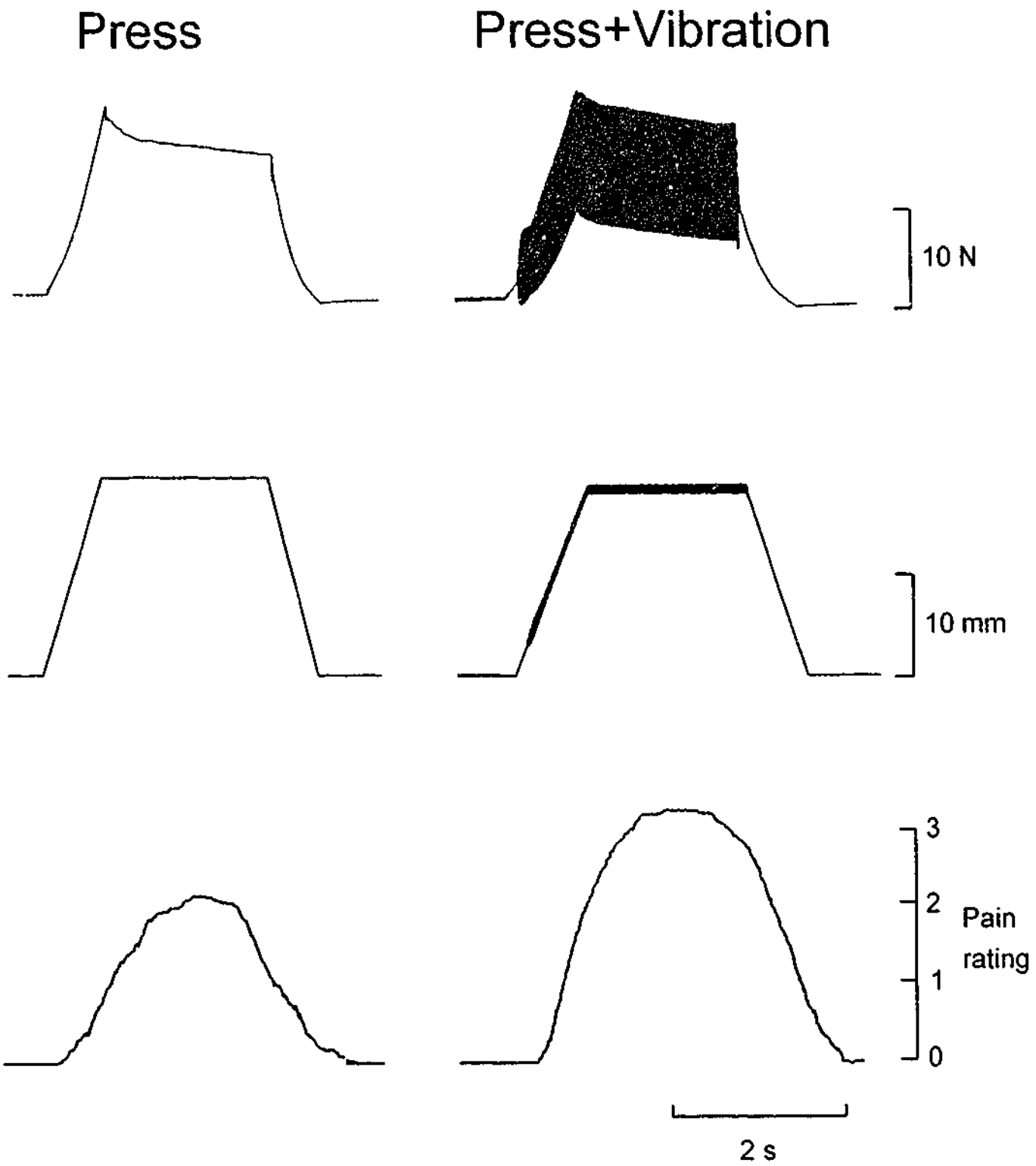
Subjects were asked to rate pain on a scale of 0-5, 0 being no pain at all and 5 being intolerably intense pain. They were asked to rate the pain in steps of 0.5. Trials of compression without the vibration were alternated with trials which included vibration. Each trial was separated from the next by a 1 minute interval. For each subject a total of 20 trials was recorded, 10 with vibration at 20Hz and 10 without, followed by 20 trials, with and without vibration, at 80 Hz. This was a sufficient number for each subject, given the reproducibility of subjects' scores and their ability to concentrate on the experiment without becoming tired.

In the second experiment another 6 subjects (mean age, 21 years) took part. This time measurements were made at the sore spot, defined here as having a threshold of <15N to applied pressure, a less-sore area (threshold 20-30 N) and in a comparable area of the unexercised leg. The pressure applied by the actuator was adjusted so that pain ratings at the sore spot and at the other sites were roughly comparable. Subjects were asked to report the intensity of the pain with and without vibration which in this case was at a frequency of 80 Hz only. Here a visual-analog scale was used where subjects recorded the level of pain by turning a dial with a scale of 0-10. A value of 0 was no pain and a value of 10 was almost unbearable pain. The dial was connected to a potentiometer that was connected to the MacLab/8s (ADInstruments, Australia), allowing the measurements to be recorded. Subjects were instructed to move the dial as they experienced the pain from the stimulus (Fig 2.3). There was no obvious difference in the pain ratings or thresholds between male and females. Therefore males and females were analyzed together.

The effect of vibration for a range of different frequencies in muscles with DOMS was also investigated in 5 subjects (mean age, 24 years) over the range 20-120Hz, measuring changes in threshold. After finding a tender area, several compressions with vibration were carried out, starting at a low compression force. The subject was instructed to report

Figure 2.3

Example of perceived pain in triceps surae of a subject at 48 hours after eccentric exercise, in response to local compression and compression plus 80Hz vibration of a tender area. Shown are compression force (top traces), actuator displacement (middle traces), and pain ratings from the visual-analog scale (bottom traces).


Press
Press+Vibration
10 N
10 mm
3
2
1
0
Pain
rating
2 s

whether 'yes' the stimulus evoked a painful sensation or 'no' it did not. For each stimulus the level of compression force was progressively increased by increasing the displacement of the electromagnetic actuator. This was done until the subject first reported that the stimulus became painful. The compression force for this stimulus was recorded as the pain threshold. Then compression force was backed off and the process repeated for 5 trials. Once the trials were complete the process was repeated at the next frequency of vibration. The frequencies used were 20, 40, 60, 80, 100 and 120 Hz. The vibration amplitude was maintained relatively constant for every frequency (0.8-1.2 mm).

For 6 subjects (mean age, 22 years) the time course of DOMS was explored. As described before, subjects were asked to report the intensity of the pain with and without vibration at a frequency of 80 Hz, on a visual-analog scale. However, unlike the other experiments, measurements for both the exercised and control leg were carried out before the eccentric exercise and immediately after, as well as at 4, 6, 8, 24, 48, 72, 96 and 120 hours after the exercise. This involved making measurements at comparable sites on the exercised and unexercised leg. Before DOMS had decreased, sites on the lateral or medial surfaces of the triceps surae (the two gastrocnemius muscles) were tested. These were chosen as past experience had indicated that they were likely to include the sorest areas. Once soreness had started to develop in the exercised leg, tender areas were mapped, and sore spots were used for further measurements, while an equivalent spot on the control leg was used.

Nerve block experiment

This was carried out on a total of 13 subjects (mean age, 20 years), 10 male and 3 female, and included a number of subjects on whom the vibration measurements had been made beforehand. Subjects sat on a wooden support, which was fitted to the adjustable chair. The chair was attached to a steel frame, and this supported two wooden footplates via a rotatable axle (Fig 2.4). The axle was aligned with the axis of rotation of the ankle joint. The subjects were seated on a chair and both feet strapped to a footplate with Velcro® straps. For each subject, the horizontal position of the chair was adjusted so that when the footplates were locked into a set position, the angle subtended between the shin and footplates was approximately 90°. The angle of the footplate, and thus the ankle angle, could be altered in increments by rotating the axle with a handle, which could be locked

in place with a metal pin. Torque about the ankle joint was measured by strain gauges glued to the axle. The gauges were aligned at 45° to the axis, along the planes of maximum principle stress during twisting, as the foot pushed into the footplate. Output from the strain gauges was sent to a MacLab/8s (ADInstruments, Australia) where it was amplified and converted from an analogue to digital signal, before being recorded on 'Chart' (ADInstruments, Australia) running on a Macintosh 6100 computer (Apple USA).

A stimulating cathode was strapped to the subjects popliteal fossa and an anode plate coated with electrode jelly was taped to the top of the knee above the patella (Fig 2.4). A 0.2 ms duration shock of 20-40 mA, delivered across the knee, stimulated the tibial nerve. Stimulus strength was adjusted to elicit a maximal peak-to-peak H-reflex (mV), the monosynaptic reflex evoked by Ia afferents of muscle spindles. The size of the H-reflex was approximately half of the maximal motor response. Sometimes it was necessary to adjust the position of the stimulating cathode before a satisfactory response had been obtained. The cathode was firmly strapped in place with Velcro® straps once an optimal position had been reached. For details see Wood, Gregory & Proske (1996). The H-reflex was monitored throughout the compression block as an indication of impulse transmission in Group Ia fibres.

Stimulating conditions were adjusted so that the H-reflex was accompanied by a small direct (M) response. This provided a convenient control for electrode movement. The triceps surae EMG was recorded with Ag-AgCl adhesive electrodes (3M Red Dot). Reflexes were displayed on an oscilloscope and recorded on a Macintosh 6100 computer (Apple, U.S.A) and a MacLab/8s (ADInstruments, Australia) system running 'Chart' software (ADInstruments, Australia). Nerve stimulation was triggered by a MacLab, while the timing of each impulse was set by a sequencer (Pulsemaster A300, World Precision Instruments, USA). This in turn was connected to a stimulator (Digitimer DS7, England) which had a maximum pulse width of 0.2ms, designed for experimentation on humans.

A differential compression block of the sciatic nerve was achieved by placing a wooden bar 6 cm high and 2 cm wide under the thigh of the exercised leg, just distal to the ischial

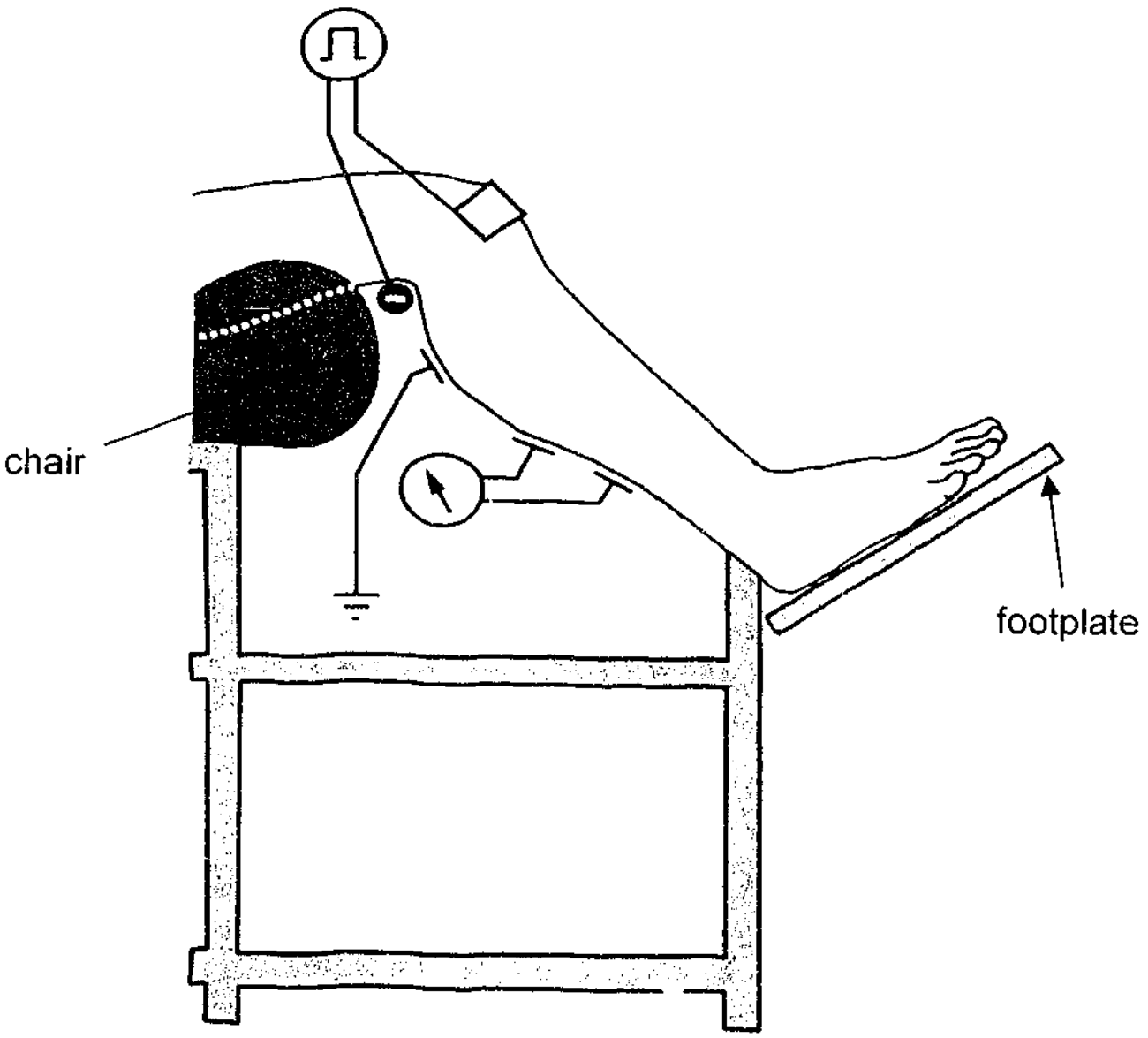


Figure 2.4

Diagram of the experimental arrangement showing the relative position of the leg and recording electrodes when studying the triceps surae H-reflex during nerve block experiment. A ground electrode was positioned between the knee and the two distally located recording electrodes. The ball cathode (-) and the plate anode (+) stimulating electrodes are shown in the positions they occupy during the experiment. Active torque was measured with strain gauges attached to the axle, which was coincident with the ankle joint. (Modified from Wood, 1997)

tuberosity (Garland, 1991). The sciatic nerve descends between the greater trochanter and the ischial tuberosity, along the back of the thigh, where proximal to the knee it divides into the tibial and common peroneal nerves. Throughout the test the H-reflex was evoked every 30s. Subjects were asked to lean towards the blocking side so that their full weight bore down on the bar. Once the subject was settled and the wooden bar was in place, reflex amplitude remained reasonably constant for the period before onset of the block.

Pain threshold for a sore region of the muscle was measured using the compression gauge, with a circular plunger of 2.5 cm in diameter. As compression force was increased, subjects indicated when they first experienced pain, and the force level indicated on the compression gauge was recorded: the greater the soreness the lower the tenderness threshold. The sorest areas were selected for the experiment, corresponding to regions on triceps where the force needed to induce soreness was at its lowest. In non-painful regions of the exercised muscle and all parts of the control muscle the gauge could be pushed in to its maximum extent (50N) without subjects reporting pain. Care was taken to apply compression at the same speed each time, since speed of compression could influence threshold values.

For most subjects it usually took about 15-20 minutes to achieve a block sufficient to abolish the H-reflex. Once the block was complete, the area of desensitised skin was mapped by asking subjects where they were able to detect a light touch stimulus. A large fibre block will include large cutaneous afferents in the Aα range. It was expected that fibres in the medial sural cutaneous nerve and the lateral cutaneous nerve of the calf would be blocked, as shown in figure 2.5, shaded in blue, as well as afferents from triceps surae. Care was taken to measure latencies to heat and cold stimuli within the boundary of the anaesthetised area which, experience dictated, was always on the lateral side, overlying the tibialis anterior muscle. Skin of the inside of the leg and overlying triceps surae is supplied by the saphenous nerve which, as part of the femoral nerve, had not been blocked.

It was assumed that the pressure block of the sciatic nerve acted preferentially on large nerve fibres, regardless of their skin or muscle origins (Garland, 1991). This includes

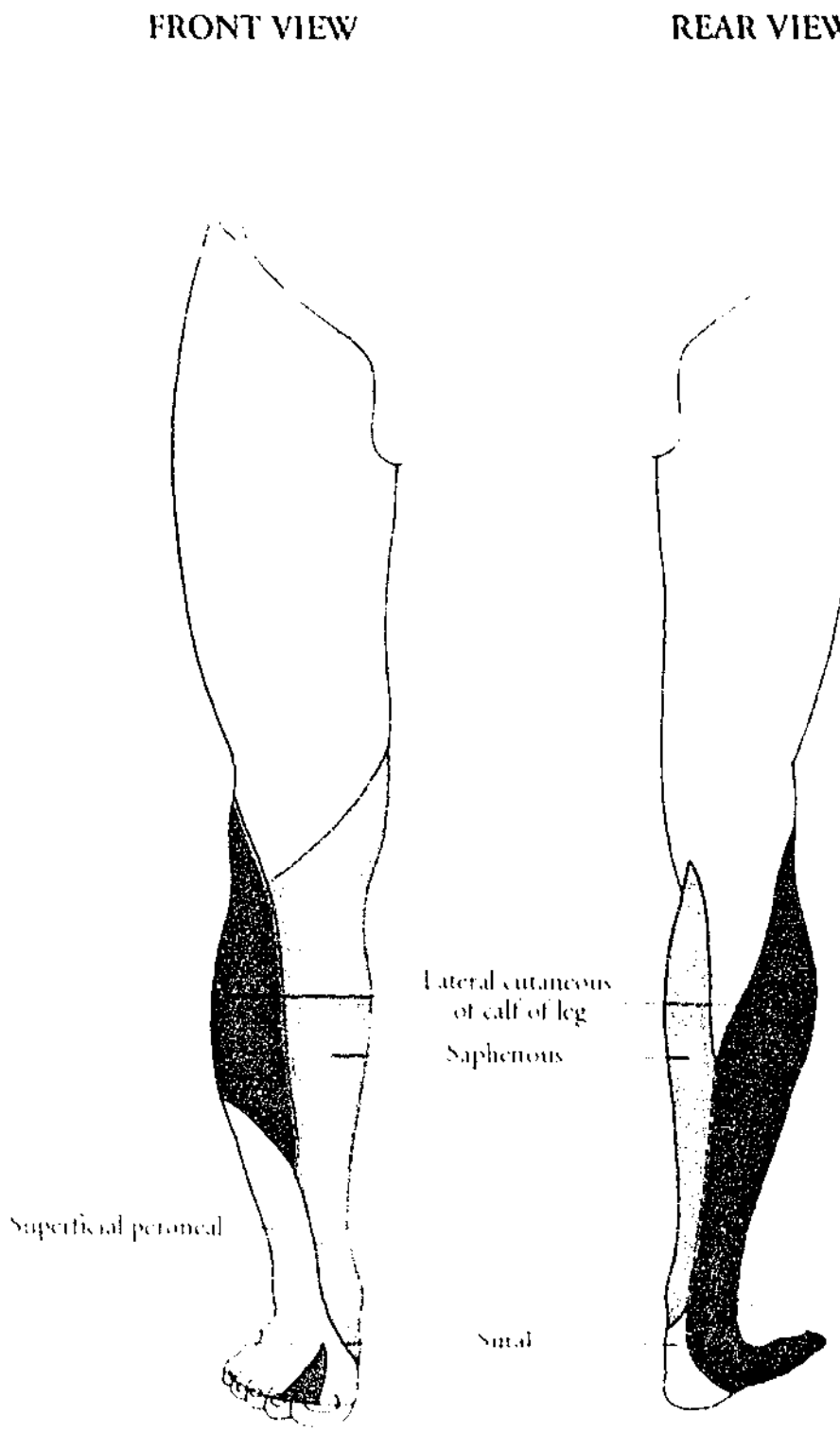


Figure 2.5

Diagram showing the skin immediately overlying triceps (blue) that is innervated by posterior and lateral cutaneous nerves which will be blocked. The pink region is innervated by the saphenous nerve, a branch of the femoral nerve which was not involved in the block..

Group Ia and Ib (12-20μm diameter) and Group II (6-12μm diameter) afferents, supplying muscle spindles and tendon organs. The receptors served by these afferents fibres are very sensitive to stretch and palpation. Conversely, small diameter afferent fibres in the Group III (2-6 μm diameter) and Group IV range (0.2-1.2μm diameter) are known to be sensitive to sensations of heat or cold. Specifically, Group IV muscle receptors have been reported to have a thermal threshold in the noxious range (above 43°C or below 20°C) (Kumazawa & Mizumura, 1977), while many cold receptors have group III axons (Iggo, 1969). Because of the difference in properties between large and small fibres, to ensure only large fibres were involved in the block, the conduction of small afferents was checked using warm and cold stimuli applied to the skin. Heat latency measurements and the sensation of cold were monitored to test the conduction of Group III and IV afferents during the compression block. Hence, as these receptors in the muscle could not be accessed directly, responses of cutaneous receptors (Aδ and C fibre nociceptors) were used to determine whether the block included any small fibres.

Latency to a painfully warm stimulus (50°C) was measured with a 2.5 cm diameter probe. Contact with the skin closed a circuit and started a digital timer on the stimulator, enabling accurate latencies to be measured. The probe was applied with light pressure to an area of skin adjacent to triceps, which was likely to become insensitive to touch during the block. The cold stimulus applied to the skin was a stainless steel bar with 2.5 cm circular diameter tip that had been immersed in ice. Subjects were asked to state whether or not they felt the cold stimulus. Control measurements of pain threshold, latency to painful heat and cold sensation were made 3-4 times before the block was applied. Throughout the block, similar measurements were carried out, initially at 5 minute intervals, and then every 2 minutes after the block began to take effect.

During the period of the block the subject was asked at regular intervals to try to contract their triceps muscle. Any active torque and the triceps EMG were recorded during each contraction. This was done to test the integrity of α motoneurons, the axons of which are 5-16 μm in diameter.

Nerve block and vibration

This experiment was carried out on a total of 6 male subjects (mean age, 23 years). A differential compression block of the sciatic nerve was achieved as described above. As before, the H-reflex and latencies to hot stimuli and cold stimulation were monitored. Now, however, soreness ratings from muscle compression and compression plus 80Hz vibration were measured, before, during and after the block.

As described before, subjects were seated in a chair and the foot on the exercised side was strapped to a footplate. While seated, tender areas of the triceps surae were found with a compression gauge. The probe tip of the moving-coil electromagnetic actuator was then maneuvered to be within reach of the most tender area. Several trial indentations of the muscle were carried out to ensure that reproducible pain ratings were obtained during each trial. Pain was measured using a 0-10 visual analog scale.

The H-reflex was then evoked by stimulating the tibial nerve. Throughout the experiment, the H-reflex was evoked every 60 s, while during the time between each reflex, compressions with and without 80 Hz vibration were applied to the tender area, with subjects reporting the level of pain on the visual analog scale. In addition, latency to a painfully warm stimulus (50°C) was measured.

After 5 trials of compression with and without vibration were completed, the differential compression block of the sciatic nerve was begun by placing a wooden bar under the thigh of the exercised leg, just distal to the ischial tuberosity. Once a complete block of the H-reflex was achieved, another 5 trials of mechanical stimulation were completed. Measurements of H-reflex, pain rating, probe force, probe amplitude and latency for painful heat were recorded throughout the session.

The reflexes, pain ratings and signals from both force and length transducers were processed as earlier mentioned by using MacLab/8s and recorded on 'Chart' software. Data was analyzed using the software Igor Pro (Wavemetrics, Ore, U.S.A). Nerve stimulation was triggered by MacLab, the same way as before (see Nerve block).

Responses to painful stimuli

In order to be able to make a direct comparison of pain levels in a muscle with DOMS and an unexercised muscle, a mechanical stimulus was devised which was strong enough to produce mild discomfort in an unexercised muscle. Three male subjects took part in this experiment (mean age, 21 years), which involved the muscle belly being gripped with a force of 50 N between the ends of a pair of large calipers, held together by a spring. The caliper ends were broad and blunt, covered with taped cloth to prevent any painful stimulation of the skin. Each arm of the calipers had a skin contact area of 0.7 cm^2. When the calipers were applied to skin overlying bony structures, no soreness resulted, when applied to gastrocnemius they evoked a dull sensation of deep pain.

The testing procedure was to keep the calipers open and to place them so that the ends just touched the skin overlying the muscle. Then the spring was released, leading to rapid compression of the muscle, without pinching the skin. As soon as subjects reported pain, typically within 1-2 seconds, the calipers were reopened. Measurements of the intensity of the pain evoked by muscle compression were made in a sensitive area of the exercised muscle, and in the corresponding area of the unexercised muscle of the control leg. The pain was measured on a visual analog scale (0-10 scale). Marks were drawn on the skin to make sure that the calipers were always placed in the same location for each trial. Ten trials were performed.

Measurements of pain

A general note is that for some experiments pain thresholds were measured, while for others the intensity of pain to a mechanical stimulus was measured. The preference for either measurement was based on the ease with which the method could be applied for a particular experiment. The two methods gave similar results.

Statistical analysis

In each experiment, individual measurements from all subjects were pooled. Values are given as means, plus or minus standard errors of the mean, calculated from the pooled data, unless otherwise stated. ANOVA testing was used to determine significance levels. A three factor repeated measures ANOVA was performed to test for significant differences in pain ratings. The factors used were subject, trial and stimulus, that is,

whether vibration was present or not. A two factor repeated measures ANOVA was also performed to test for significant changes in H-reflex, tenderness threshold, and latency for painful heat stimulation throughout the compression block procedure. The factors used here were time (before, during or after the block) and subject. Where an ANOVA was significant ($p<0.05$), an LSD (least significant difference) *post hoc* test was applied. In addition, a pooled t-test was used to determine the significance level between pain thresholds before and after skin anaesthesia. The analysis program used was Data Desk (Ithaca, N.Y., U.S.A.).

RESULTS

Skin anaesthesia

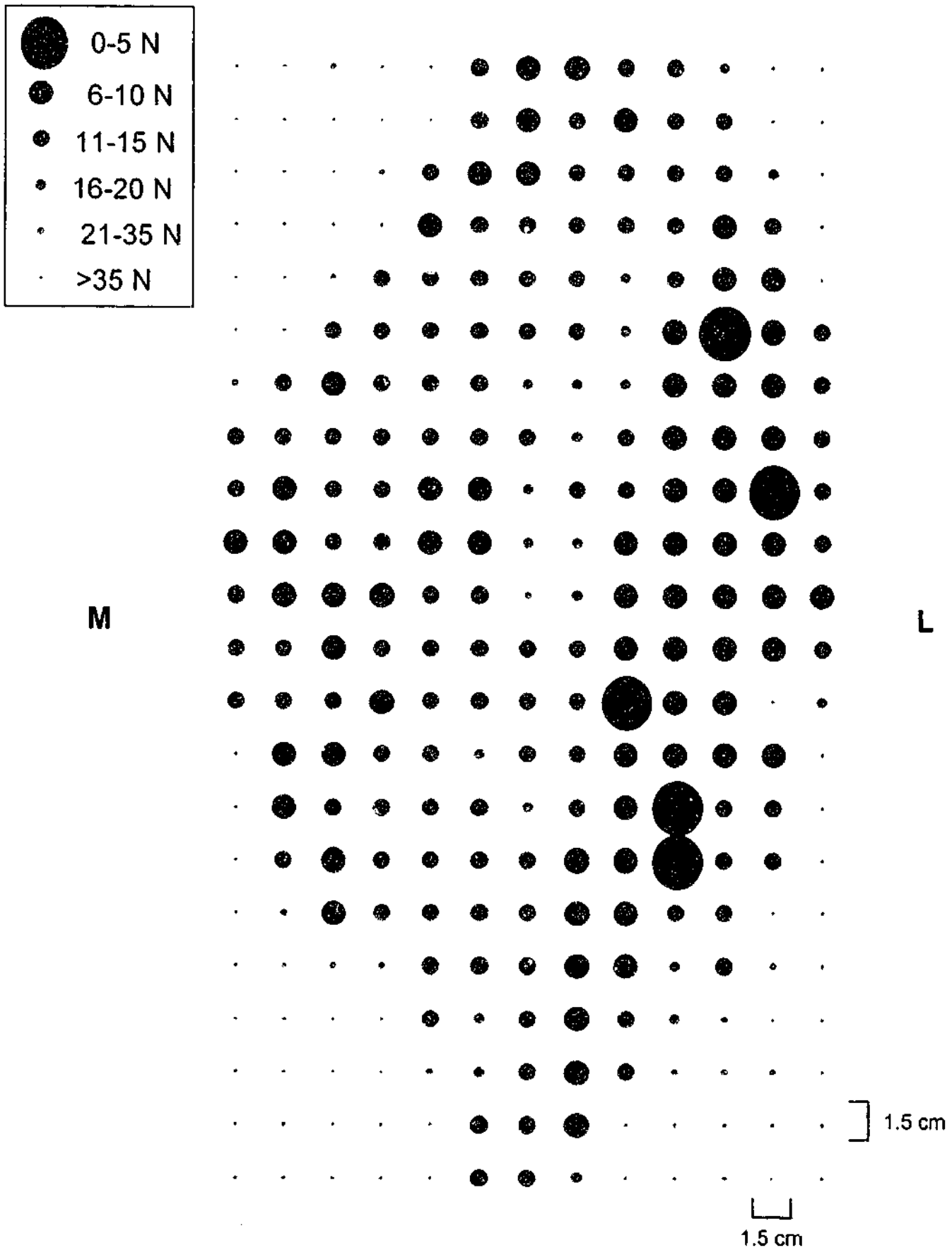
In practice, subjects insisted that the pain was deep and was coming from the muscle. Nevertheless, to control for any skin sensations, or skin-mediated reflex effects, before the compression gauge measurements, for 3 subjects the skin overlying the stimulated area was treated for 2 hours with anaesthetic cream (EMLA). By this time the region of treated skin had become insensitive to tactile and pin prick stimulation. Values for pain thresholds with skin anesthesia (12.2N ± 1.4) were not significantly different from values when skin sensation was intact (9.7N ± 1.3).

Mapping the tender areas

Subjects lay prone with the ankle plantarflexed, which made the muscle short. However, stretching the muscle increased the distribution of pain. In non-painful regions of the exercised muscle and in all parts of the muscle of the other non-exercised leg the gauge could be pushed in to a maximum extent (35 N force) without the stimulus becoming painful. In a subject in whom DOMS had developed, it was clear from the distribution of pain thresholds, that some parts of the exercised muscle were very much more tender than others (Fig 2.6). Areas 3 cm apart could differ in threshold by as much as 30 N. It suggested that the foci of damage underlying the soreness were discrete and separated by regions of muscle that were not sore. All subjects tested showed discontinuous distributions of thresholds across the muscle. However, there was no discernible pattern in the distribution of sore areas. In the example shown in figure 2.6 for a subject at 48 hours there is a suggestion that for this subject the lateral gastrocnemius had become

Figure 2.6

Distribution of areas of sensitivity to local pressure in one subject's triceps surae of the right leg that had undergone eccentric exercise 48 hours earlier. The surface of the muscle accessible for the tenderness testing by the probe has been shown. Its surface has been subdivided into a 1.5 x 1.5 cm grid. Medial boundary (M) to the left, lateral (L) boundary to the right. Pain threshold was measured by applying a calibrated compression gauge with 1.5 cm diameter plunger in each square of the grid. Thresholds have been represented as circles, the size of the circle, proportional to the measured threshold range, given in Newtons at the top of the figure. The larger the dot, the lower the threshold. Similar measurements were carried out on the left, unexercised leg and all pain thresholds were >35 N.


0-5 N
6-10 N
11-15 N
16-20 N
21-35 N
>35 N
M
L
1.5 cm
1.5 cm

sorer than medial gastrocnemius. This was not a consistent finding. The precise distribution of thresholds was unique for each subject, as is evident from the example for another subject shown in figure 2.7 (48 hrs). There was no suggestion that one part of the muscle like, for example, the long aponeuroses, was more prone to soreness than another (Newham *et al.*, 1983). However, it was our impression that the two gastrocnemius muscles were more sensitive to touch than soleus, although most of soleus lies below the gastrocnemii and only a limited region of the distal portion could be accessed directly through the skin.

In addition, it was observed that the soreness for most subjects peaked 48 hours after the exercise as reported by Jones et al., 1997. In the example shown, it is evident from mapping a subject's triceps at various times that there was little or no soreness immediately after the exercise or at 4 or 6 hours later (Fig 2.7). The first true signs of DOMS were present at 8 hours, with a reduction in threshold to 11-15 N developing. While the first very tender areas (0-5 N) were evident at 24 hours, soreness reached its maximum at 48 hours.

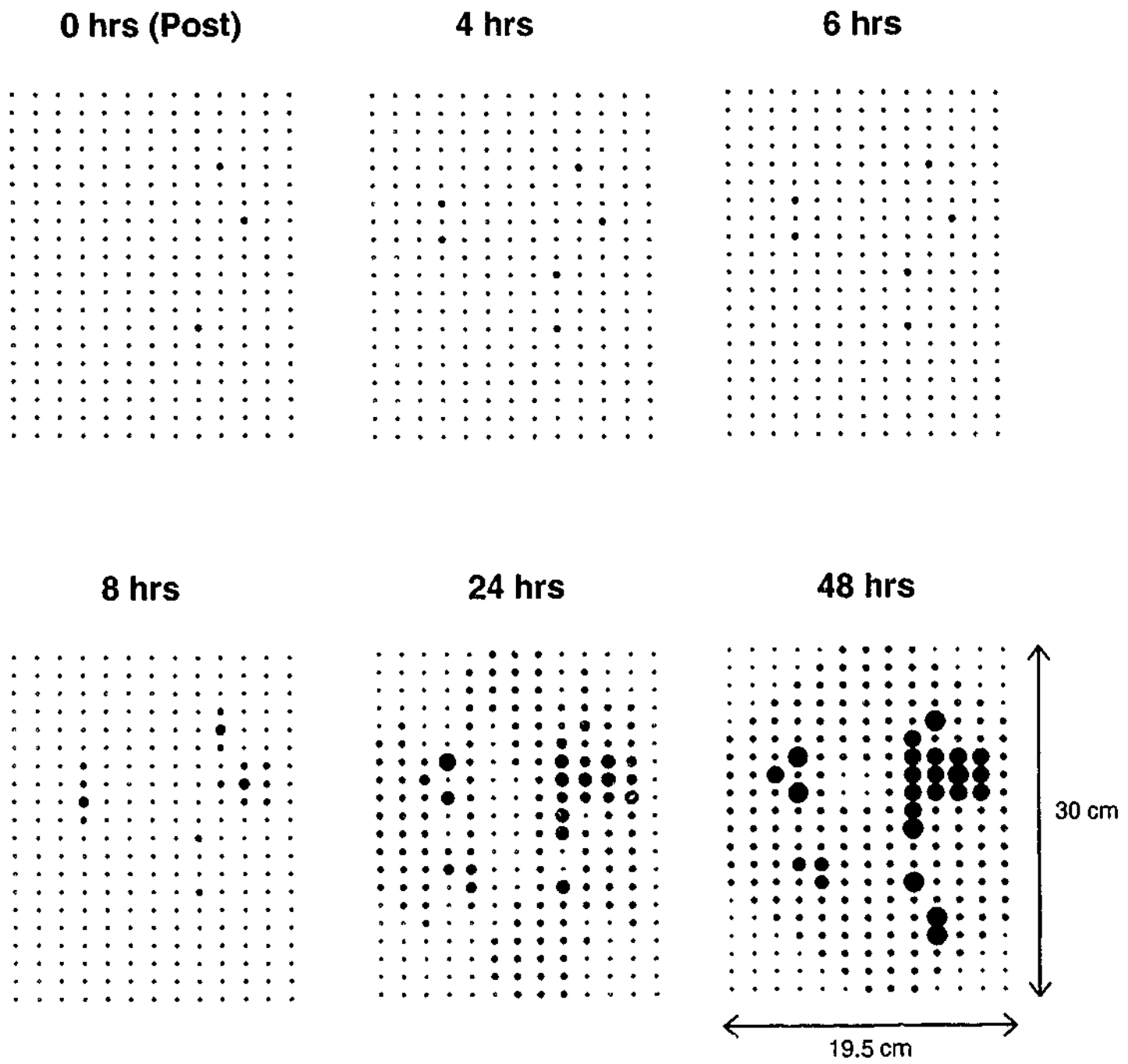
Responses to painful stimuli

The question was posed, was DOMS associated with any hyperalgesia? To test this idea, mildly painful stimuli were applied to a tender area of the muscle to determine whether subjects reported a heightened sensation of pain. Pain rating for the unexercised control leg in 3 subjects lay in the range 2.0-2.8 on a scale of 0 to10 with a mean of 2.66 ±0.16. For an equivalent region of the exercised muscle, values lay in the range 7.0-8.0 with a mean of 7.29 ±0.25, two days after exercise. This difference was significant ($p<0.05$). In addition subjects reported that the rate of onset of pain appeared to be more rapid when the calipers were applied to the exercised muscle.

The response to compression of different strengths was also investigated on 5 subjects. The force that produced a specific pain rating was different for each subject. Most likely this was because the level of muscle damage and soreness experienced by each subject was different, as was their specific perception and tolerance of pain. Nonetheless, as expected, as the force of compression increased so did the pain experienced by the subject (Fig 2.8). The slope of the relationship was found to be 0.18, with an r^2 of 0.69.

Figure 2.7

Distribution of areas of sensitivity to local pressure in one subject's triceps surae of the right leg at various times after eccentric exercise: immediately (0), 4, 6, 8, 24 and 48 hours after. The muscle surface has been subdivided into a 1.5 x 1.5 cm grid. Medial boundary to the left, lateral boundary to the right. Pain threshold was measured by applying a calibrated compression gauge with 1.5 cm diameter plunger in each square of the grid. Symbols as in Figure 2.6.


0 hrs (Post)
4 hrs
6 hrs
8 hrs
24 hrs
48 hrs
30 cm
19.5 cm
0-5N
6-10N
11-15N
16-20N
21-35 N
> 35 N

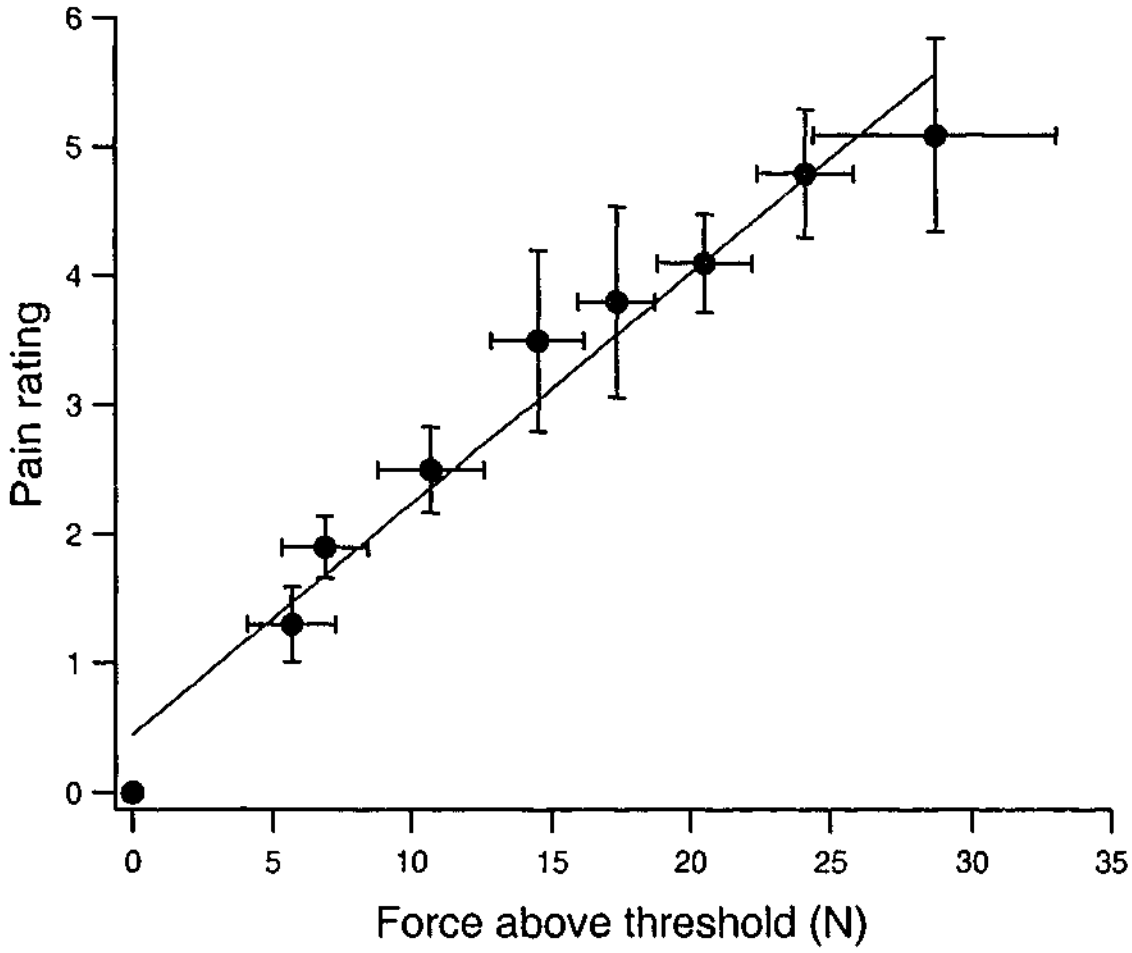


Figure 2.8

Plot of pain plotted against force above threshold (N). All values are means (± S.E.M.) for five subjects. A regression line has been drawn through the data.

Muscle vibration

In preliminary experiments, the exercised muscle surface was explored with a hand-held vibrator. It was apparent that at a sensitive spot the vibrator had to be pressed into the muscle much less than a non-vibrating probe for the subject to report pain. In other words, vibration, by itself, could initiate pain. Provided muscle length was kept short, vibration, like the measurements with a compression gauge, revealed discrete areas of tenderness, surrounded by less sore areas. Indeed, a vibrating probe represented a very effective tool for mapping sensitive areas. However, if muscle length was increased by passively dorsiflexing the foot, the area from which pain could be evoked by vibration increased dramatically. Presumably the longer, stiffer muscle fibres transmitted the vibratory stimulus more effectively. The point emphasized that whatever neural structures were being vibrated, they lay deep within the muscle and the stimulus was reaching them via vibrating muscle fibres.

The possibility was considered that responses of vibration receptors such as Pacinian or Paciniform corpuscles lying deep in the tissue and associated with bony structures were responsible for DOMS (Hunt & McIntyre, 1960). Vibrating bony structures adjacent to the muscle at the knee and ankle did not produce any pain; nor did vibration of the Achilles tendon.

Vibration with different frequencies

Once a conveniently located site of tenderness had been identified, it was marked and a supported electromagnetic vibrator brought up to the muscle so that its probe tip just contacted the skin. Subjects were asked to rate the degree of soreness in response to a prod of the muscle. Every second prod was accompanied by vibration of the probe tip at 20 Hz or 80 Hz (Fig 2.2).

Subjects were quite consistent in their scoring. For example, for pressure without vibration one subject reported scores of 2.5-3.5 (mean 2.95 ±0.09). For pressure with 80 Hz vibration the range was 3.5-4.5 (mean 3.85 ±0.11). It meant that 10 trials for each series were sufficient, without risking subjects losing their concentration.

At a sore spot subjects rated soreness as 2.57 (±0.08), increasing to 2.92 (±0.08) with 20 Hz vibration, and 2.65 (±0.09) increasing to 3.54 (±0.09) with 80 Hz vibration (Fig 2.9). The difference between the prod without vibration and with vibration for both 20 Hz and 80 Hz was significant ($p<0.05$). Subjects consistently reported that with vibration the intensity of the perceived soreness was greater than without vibration, especially at vibration-onset, but that it then began to fade. It was also noted that three subjects visibly winced when the vibration was applied. Such a response was never seen with compressions without vibration.

An example of one subject's response to different frequencies of vibration is shown in figure 2.10. For this subject the force required to produce pain was lowest when the frequency was at 60 Hz. The overall pain threshold response to different frequencies of vibration is shown in figure 2.11, with values being expressed relative to the pain threshold at 80 Hz, as thresholds varied considerably from subject to subject depending on how sore they became. The pain threshold was found to be highest at 20 Hz and it decreased to a minimum at 80 Hz (Fig 2.11). However, there was no significant difference between the threshold measured at 60 and 80 Hz. At the higher frequencies of 100 and 120 Hz the pain thresholds began to rise significantly again. It should be noted that the transmission through the muscle at different frequencies may not be the same.

Comparison of exercised and unexercised muscle

The experiment of comparing soreness with and without vibration was extended to include less sore regions adjacent to the sore spots on the exercised muscle and measurements on the unexercised leg. Sore spots were defined as having a compression threshold of <15N, less sore areas a threshold of 20-30N. By comparison the unexercised muscle required >35N compression force before it caused any discomfort. Once these areas were located, the electromagnetic actuator was brought up to apply 80Hz vibration. When moving away from a sore spot the force of the actuator needed to be increased for subjects to be able to report levels of pain that were approximately comparable to those they had experienced at a sensitive spot. For the exercised leg it meant only a small increase in force but for the unexercised leg, force had to be nearly doubled (Fig 2.12). Although for the exercised leg, vibration of a sore spot and less sore spot both increased perceived soreness, on the unexercised side it reduced it (Fig 2.13).

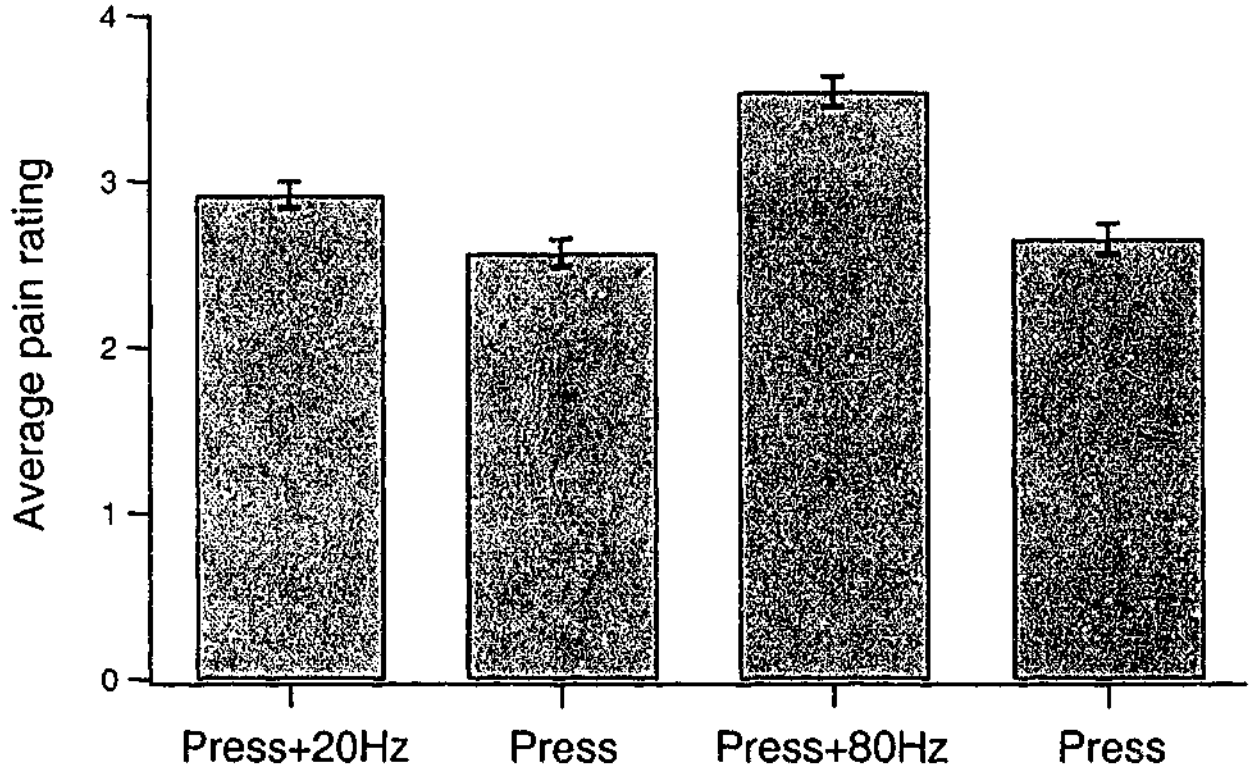
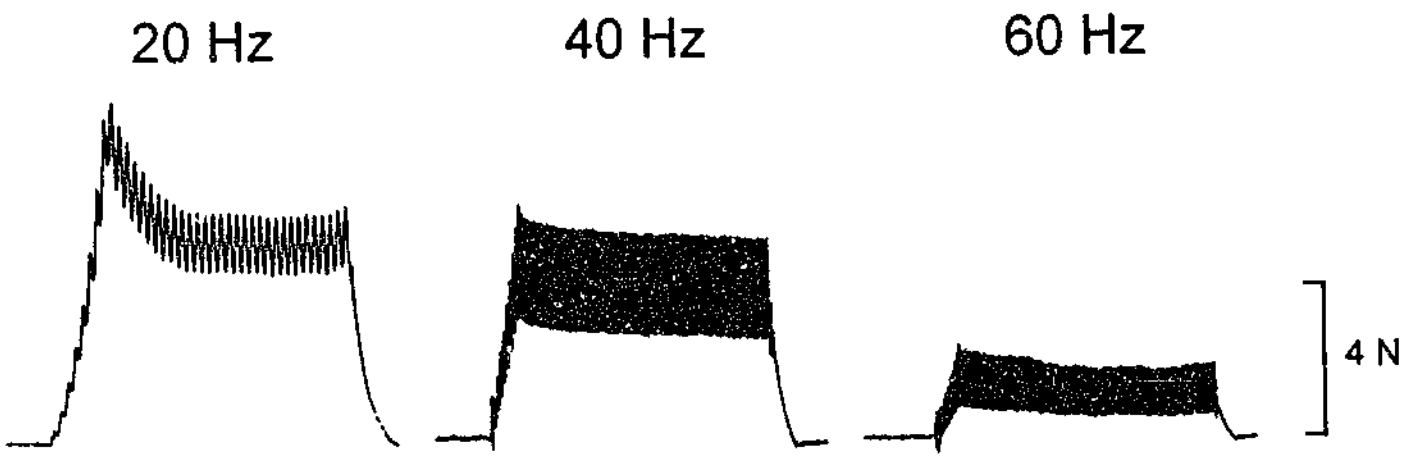


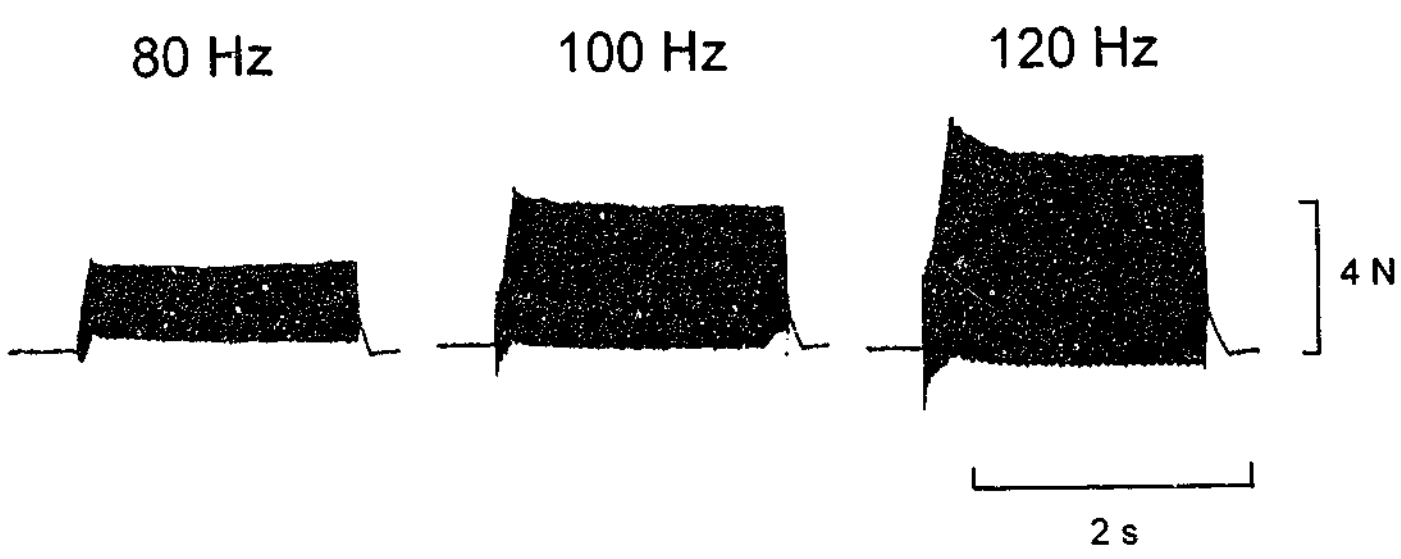
Figure 2.9

Histogram distribution of mean pain ratings (±SEM) in response to compression of the muscle without vibration (Press 1, Press 2) and with 20 Hz or 80 Hz vibration (Press +20 Hz; Press + 80 Hz). A rating of 0 was no pain, a rating of 5 was intolerably intense pain. Subjects were encouraged to rate pain in steps of 0.5.

Figure 2.10

Force profiles registered by the strain gauge on the stimulator during locally applied pressure with superimposed vibration at different frequencies. The traces are all at a subject's pain threshold. Here the stimulator probe was initially pressed into the muscle with a steady force of 20 N and only forces above that level have been shown.


20 Hz
40 Hz
60 Hz
4 N


80 Hz
100 Hz
120 Hz
4 N
2 s

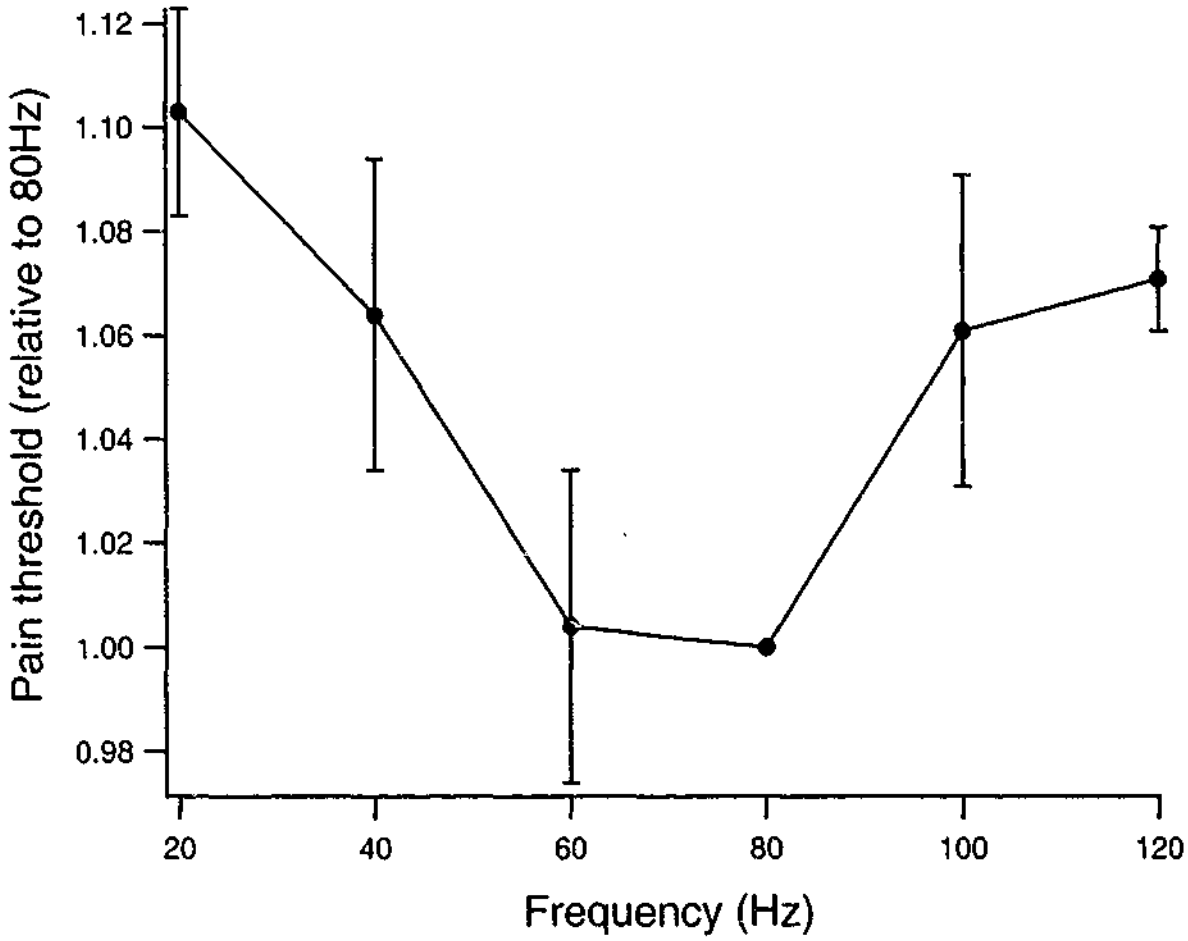
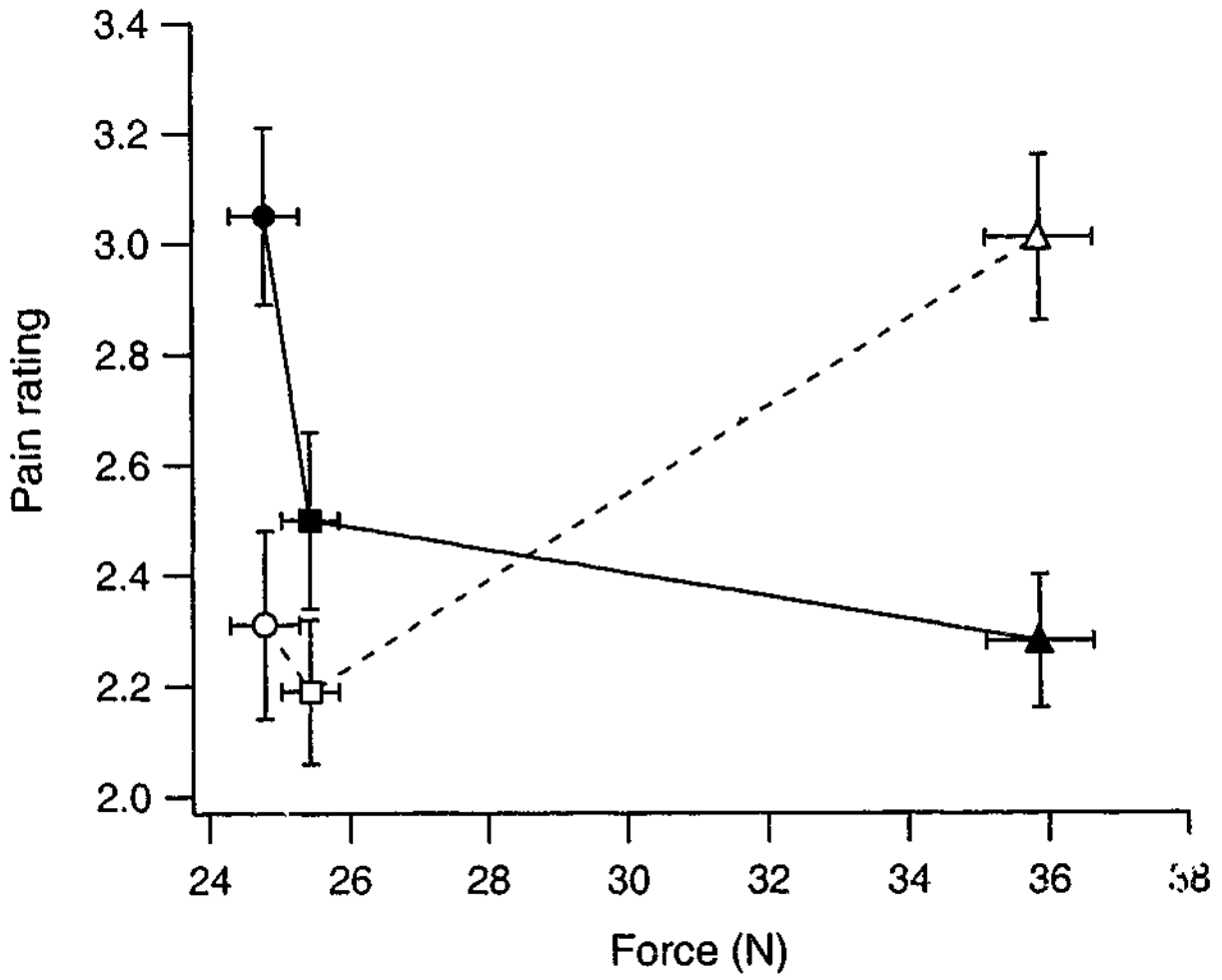


Figure 2.11

Mean pain thresholds (±S.E.M.) in response to vibration at various frequencies. Thresholds have been expressed relative to the threshold measured at 80 Hz. Thresholds at 60 Hz and at 80 Hz were not significantly different. Thresholds at other frequencies were significantly higher.

Figure 2.12

Pooled data from 6 subjects experiencing DOMS in one leg showing mean (±S.E.M.) soreness ratings and levels of applied force in response to compression with (filled symbols) and without vibration (open symbols). Measurements were made at two locations in the exercised muscle and at comparable places in the unexercised muscle. Circles, sore spot, squares moderately sore spot, triangles, comparable area in unexercised muscle. The lines joining the points are for clarity of presentation and are not meant to imply values between the points. Force values for a given level of soreness were lowest at a sensitive spot and here vibration produced the largest increase in soreness. In the unexercised muscle vibration reduced the perceived level of soreness.


Pain rating
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
24
26
28
30
32
34
36
Force (N)

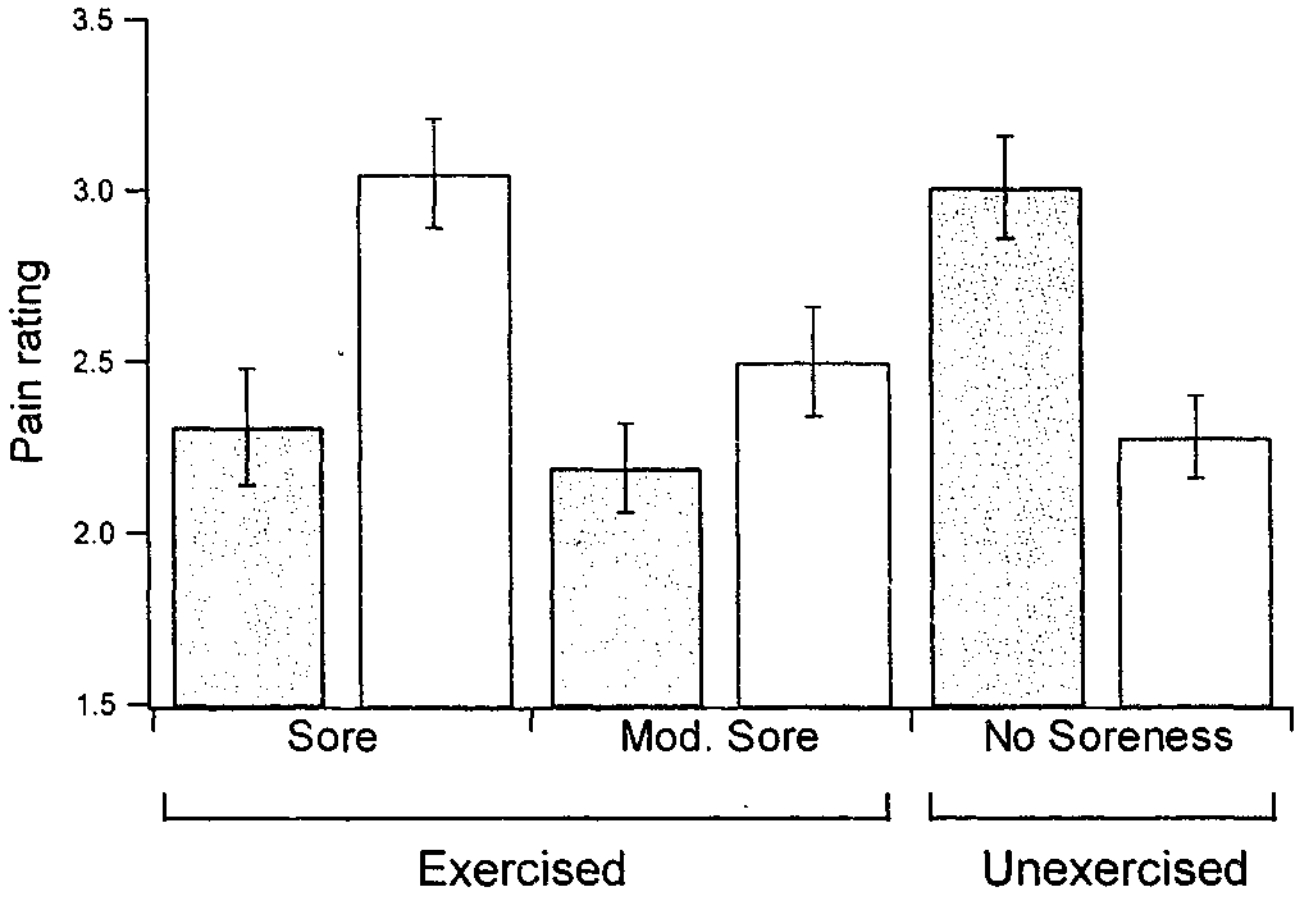


Figure 2.13

Histogram distribution of mean pain ratings (±SEM) in response to compression of the muscle with (red bars) and without (blue bars) 80 Hz vibration. A visual analogue scale of 0-10 was used to rate pain. Measurements were made at the sore spot, defined here as having a threshold of <15N to applied pressure (Sore), a less moderately sore area with a approximate threshold of 20 30 N (Mod. Sore) and in a comparable area of the unexercised leg (No Soreness).

Time course

The finding of a change in the response to vibration in the presence of DOMS led to the question, when exactly, after a muscle has been subjected to eccentric exercise, does the reversal in the response to vibration, from reducing to increasing pain, take place? Responses to mechanical indentation with and without 80Hz vibration of an eccentrically exercised muscle were, therefore, measured at various times after the exercise.

In the unexercised muscle and the muscle of the experimental leg pre-exercise, mechanical indentation had to reach compression forces in excess of 35N before subjects reported any discomfort. As described before, when vibration was superimposed on the movement subjects reported less soreness.

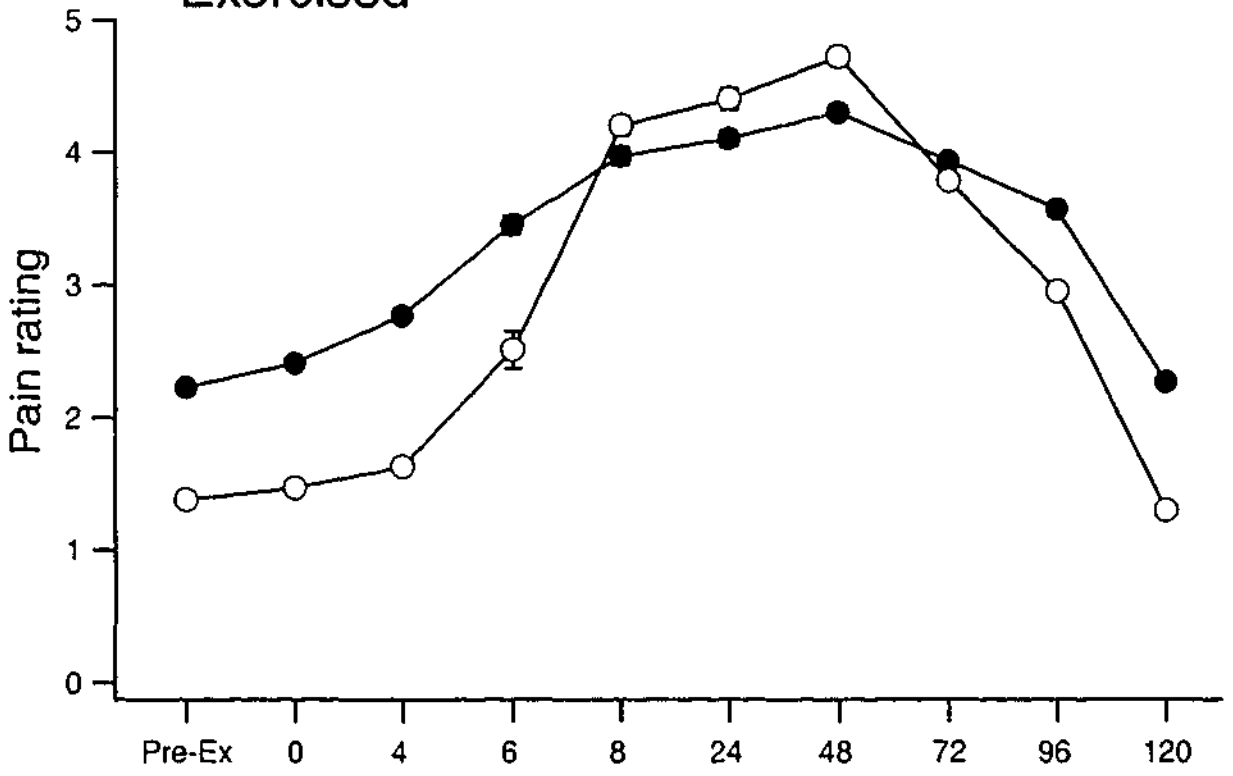
Four hours following the eccentric exercise there was a small but significant rise in soreness in response to muscle compression and to compression plus vibration compared to control values (Fig 2.14A). Pain from both vibration and compression followed a similar pattern over time, peaking at 48 hours and returning to pre-exercise levels by 120 hours.

Immediately after the exercise, for all subjects, vibration significantly reduced soreness by the same amount as in the control muscle, but the responses were both slightly higher. By 4-6 hours post-exercise vibration still reduced the soreness. The first time when sites could be identified where pain threshold had fallen to 11-15N was at 8 hours post-exercise for most subjects (Fig 2.7). At this time, soreness ratings with and without vibration were no longer significantly different (Fig 2.14A). At 24 hours, for most subjects, vibration of a sore spot had begun to increase the perceived soreness significantly above that from compression alone (Fig 2.14A). The increase in soreness from vibration was greatest at 48 hours (4.7 ±0.07). Also at this time for most subjects, soreness threshold was at its lowest (Fig 2.7). By 72 hours pain from vibration and compression were no longer significantly different. At 120 hours, soreness had returned to control values with vibration again significantly reducing soreness. In contrast, for the unexercised control muscle, vibration significantly reduced soreness throughout the test period (Fig 2.14B). So the cross-over points for compression and vibration were at 8 and

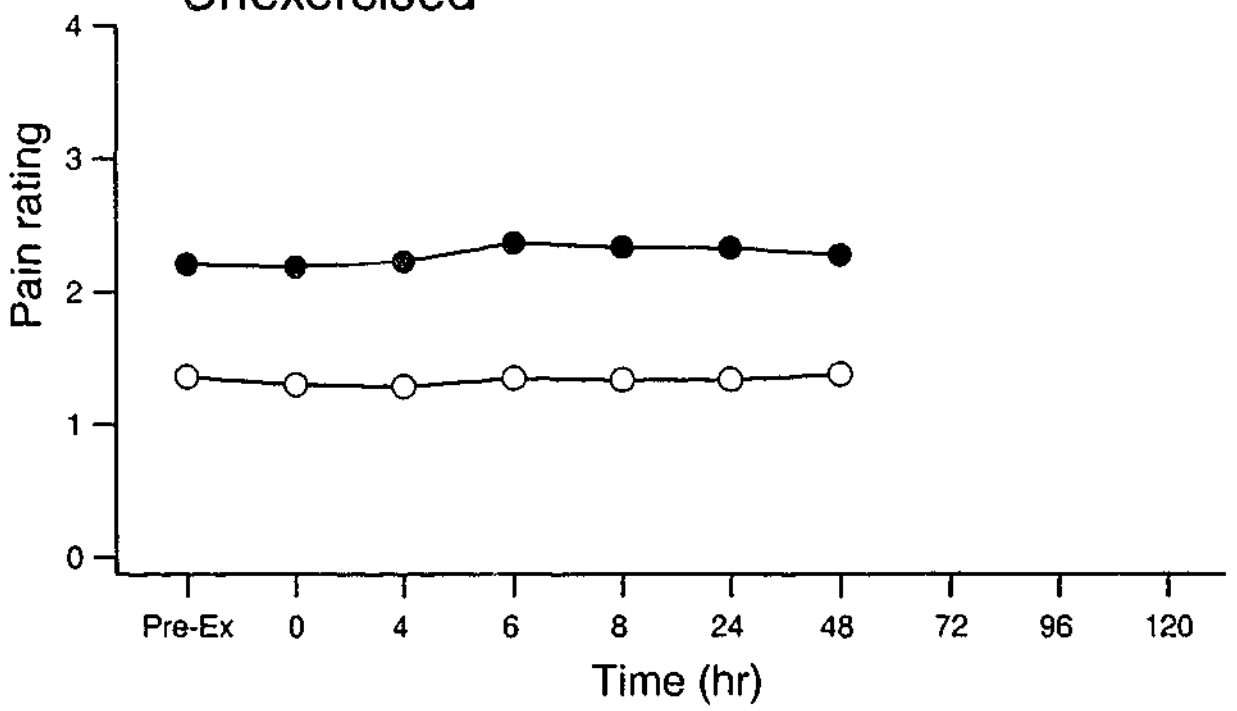
Figure 2.14

Pain ratings given by subjects in response to muscle compression (filled circles) and compression combined with vibration (open circles). All values are means (±S.E.M.). At the start of the experiment (Pre-Ex) compressions were applied that generated pain levels of 2-3. From then on, for the rest of the experiment, compression forces were kept at that level. *(A)* Mechanical stimulation of the eccentrically exercised muscle. *(B)* Mechanical stimulation of the unexercised muscle.

A


Exercised
Pain rating
5
4
3
2
1
0
Pre-Ex
0
4
6
8
24
48
72
96
120

B


Unexercised
Pain rating
4
3
2
1
0
Pre-Ex
0
4
6
8
24
48
72
96
120
Time (hr)

at 72 hours. An important conclusion from this experiment was that reversal of the effect of vibration required a significant increase in pain from compression. Initially the soreness in response to compression had increased but vibration still reduced it. A change in vibration response seemed to correlate with a soreness ratings of about 4.

Nerve block

For 11 of the 13 subjects that took part, an effective block of large afferents was achieved. For the other 2 subjects conduction was not blocked, presumably because the wooden bar had not been placed correctly. The sensitive spot used for the vibration measurements was also used to test the effect of a nerve block, provided it was suitably placed. Threshold values to muscle compression in the tender area before the block lay in the range 15-20N. Thresholds in unaffected parts of the muscle were >35 N.

The H-reflex

In figure 2.15A, the trace in blue is a typical record of EMG from the muscle, in response to an electrical shock delivered at the popliteal fossa. The first deflection, which occurs almost immediately, is the stimulus artifact. The second elevation that occurs about 5-10 ms later is the direct response or 'M-wave' that is caused by the direct activation of α motoneuron axons. The third elevation that occurs 30-40ms after stimulation is the H-reflex itself. Figure 2.15B shows a circuit diagram of the H-reflex. The afferent impulses travel up to the spinal cord where they synapse monosynaptically with α motoneurons supplying the same muscle.

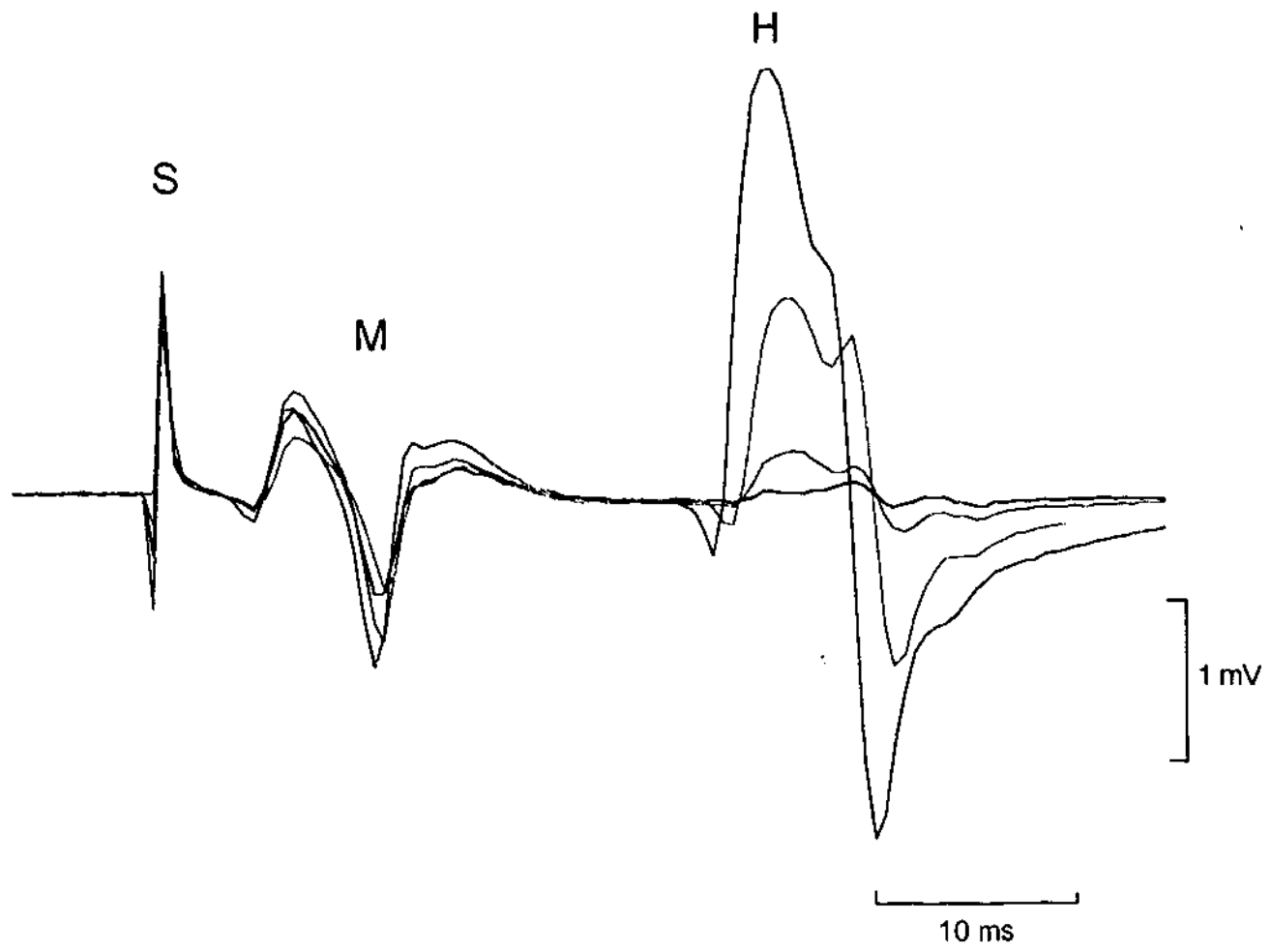
The other superimposed traces in figure 2.15A (red, green and black) show the decline in the H-reflex amplitude over time during the compression block. Note that the M-wave remains relatively unchanged, indicating that the stimulating electrode had not moved significantly from its position and there were no other changes in stimulating conditions during this time. The H-reflex responses were not averaged, because of the rapid changes of amplitude during block onset. Furthermore a maximum reflex was used with, relatively small amplitude fluctuations.

Data from one experiment is shown in figure 2.16. The amplitude of the H-reflex began to fall about 13 minutes after onset of pressure on the sciatic nerve and the reflex was

Figure 2.15

(A) H-reflex recorded as surface EMG from triceps surae in response to electrical stimulation of the tibial nerve at the popliteal fossa. A nerve block was effected by compression of the sciatic nerve at the thigh. As the block took effect, reflex (H) amplitude fell while stimulus artifact (S) and direct muscle response (M) remained essentially unchanged. Blue trace, signal before the block, red and green traces, declining signal as the block began to take effect (15-19 min), **black** trace, signal when H-reflex was fully blocked (20 min). ***(B)*** Circuit diagram of the spinal pathway for the H-reflex. Primary spindles within muscle spindles give rise to Ia afferent fibres (green) that travel toward the spinal cord. In the spinal cord the Ia fibres form excitatory synapses on the homonymous motoneurons whose axons (purple) descend back toward the muscle to form excitatory synapses with the skeletal fibres.

A



B

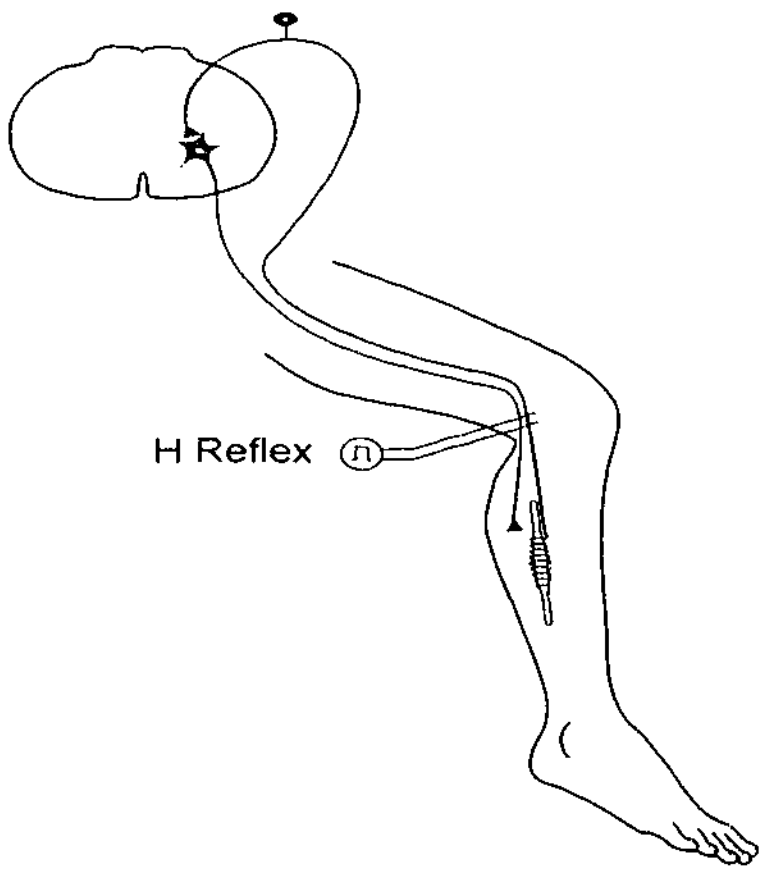
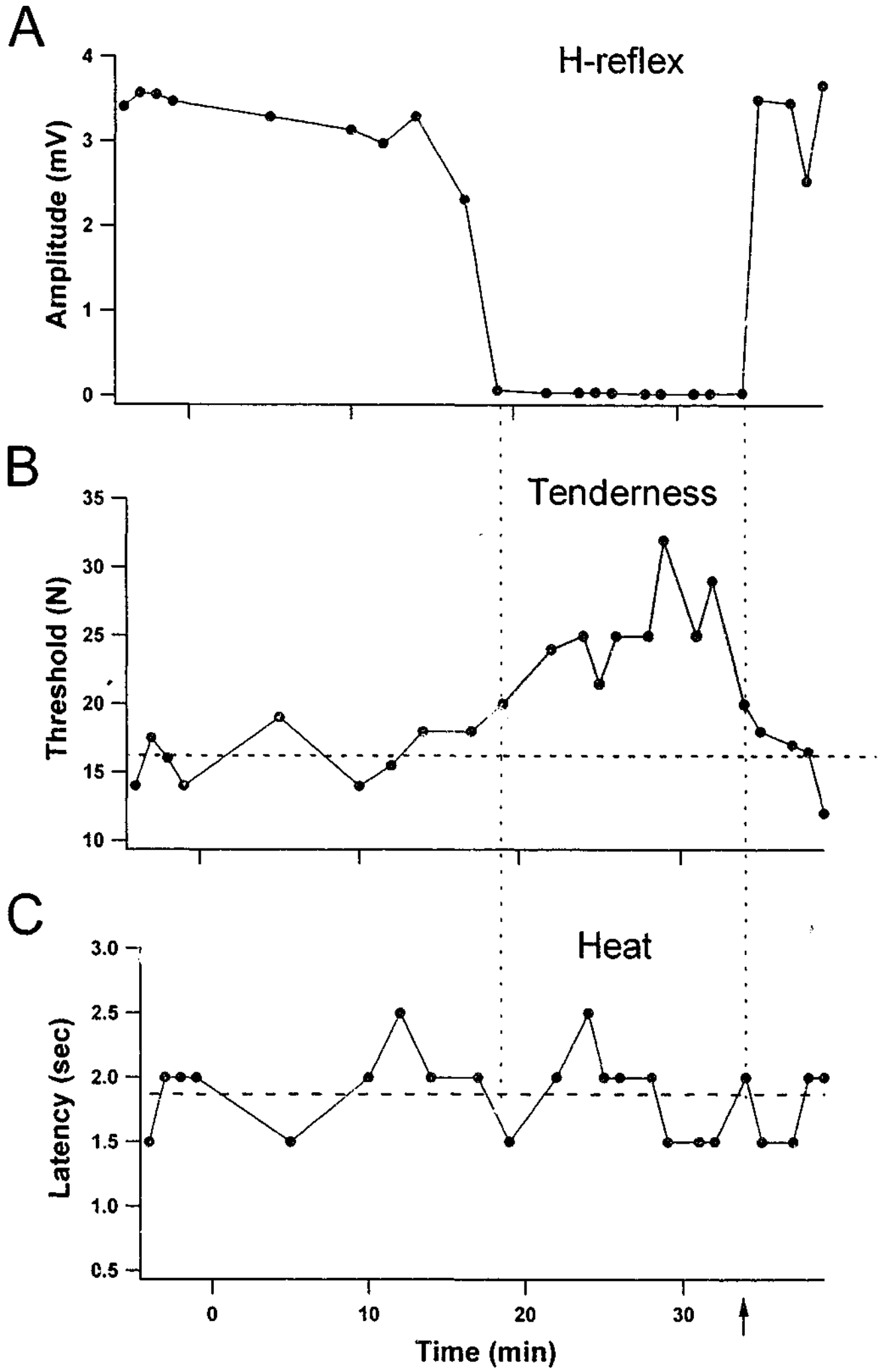


Figure 2.16

Examples of measurements of H-reflex, tenderness threshold and latency to pain for one subject, before, during and after a pressure block of the sciatic nerve by placing a wooden bar under the thigh of the exercised leg, just distal to the ischial tuberosity. The block was initiated at 0 min and was removed at 34 min (arrow). *(A)* The size of the H (Hoffman) reflex, measured as mV of recorded surface EMG from triceps surae, over the 40 minutes of recording before, during and after the block. *(B)* The tenderness threshold in response to compression of a sensitive region of the muscle, exercised 48 hours earlier. *(C)* The latency of pain to a hot (50 °C) probe applied to the region of skin adjacent to the muscle and innervated by a branch of the sciatic nerve. The dashed horizontal lines indicate mean control thresholds and latencies, the vertical dotted lines, the duration of the block. Notice that during onset of the block the H-reflex disappears within 2-3 trials and after removal of the block it recovers rapidly. Changes in tenderness ratings are more gradual. There was no change in painful heat latency.


A
H-reflex
Amplitude (mV)
4
3
2
1
0
B
Tenderness
Threshold (N)
35
30
25
20
15
10
C
Heat
Latency (sec)
3.0
2.5
2.0
1.5
1.0
0.5
0
10
20
30
Time (min)

fully blocked within 3 minutes of that time (i.e., 16 minutes after placement of the wooden bar). The bar was left in place for a further 15 minutes, and after it was removed from under the thigh, the reflex recovered very rapidly, within 1-2 trials. Subjects at times reported that recovery from the block was accompanied by some paresthesia in the lower leg. Paresthesia has been attributed directly to a disturbance in peripheral afferent fibres (Burke & Applegate, 1989). During the block there was an increase in pain threshold to a mechanical prod, with values rising from about 17N to 30N. However, this increase was not as abrupt as the H-reflex decline. Subjects described the pain as becoming less localized, and more diffuse over the muscle. An important and unexpected result was that pain was not abolished by the nerve block. The pooled data showed a significant increase in pain threshold, from a control value of 16.2N (±0.6) to 27.4N (±1.6), during the block ($p<0.05$) and recovery of normal thresholds (18.1N ±0.6) after the block (Fig 2.17B).

Sensations of Heat and Cold

The latency for painful heat sensation remained unchanged (Fig 2.16C). There was, in fact, a small increase in latency for warm stimuli in four subjects but for the pooled values from the 11 subjects, but this was found to be not significant (Fig 2.17C). There was also found to be no change in the sense of cold.

Motor Functions

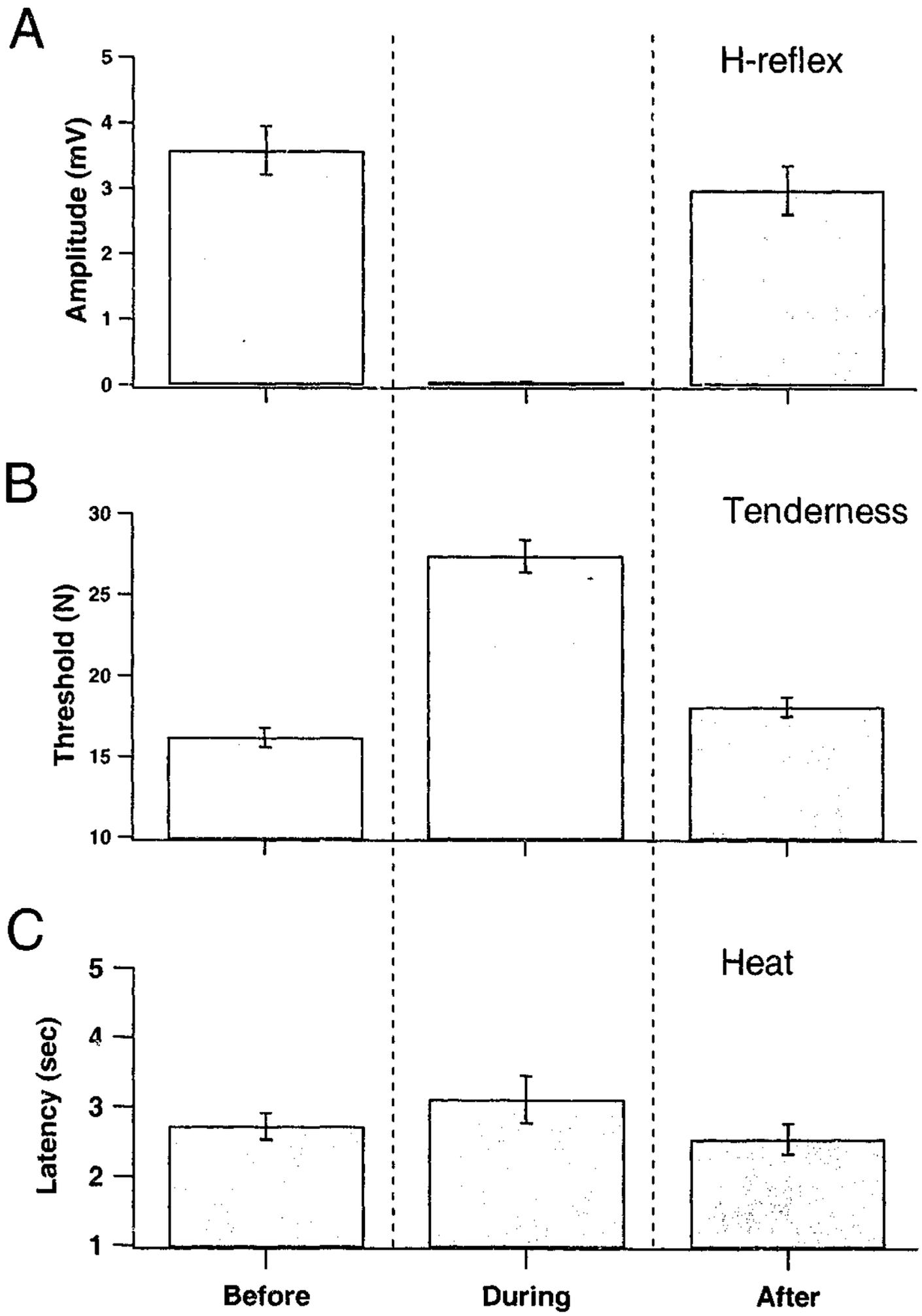
Throughout the experiment each subject was asked to attempt to contract their triceps. Torque and EMG recordings both showed reductions in amplitude during the block, suggesting conduction failure in some, but not all, motoneurones during the period of H-reflex block. This supported the view that only large myelinated fibres had been blocked since some skeletomotor neurones supplying triceps were obviously still conducting through the region of the block. A similar result was obtained with all but two of the eleven subjects. In these two, there was a complete motor nerve block.

Nerve block and vibration

Six subjects participated in this experiment. As above, during the block, a small, but non-significant increase of latency to heat was found, while subjects continued to report the ability to detect cold stimuli. Again voluntary contractions during the nerve block were

Figure 2.17

Pooled measurements from 11 subjects of *(A)* H-reflex amplitude, *(B)* tenderness threshold and *(C)* latency to painful heat before, during and after a pressure block of the sciatic nerve. All values are given as means (± SEM) averaged over the period of recording, typically 20 minutes before onset of the block, 10 minutes during the block and 10 minutes after recovery from the block.


A
H-reflex
Amplitude (mV)
5
4
3
2
1
0
B
Tenderness
Threshold (N)
30
25
20
15
10
C
Heat
Latency (sec)
5
4
3
2
1
Before
During
After

weakened but not fully abolished, suggesting that motor axons had not become fully blocked.

Before the onset of the nerve block, compression combined with vibration gave a higher pain rating of 3.79 ± 0.2 than compression without vibration, 3.15 ± 0.1 (Fig 2.18). This difference was found to be significant ($p<0.05$). Once the block had become effective, soreness ratings with and without vibration both fell to 2.15 ±0.2 and 2.11 ±0.2 respectively, and were no longer significantly different. After full recovery from the block, muscle compression with vibration again gave a significantly higher pain rating of 2.93 ±0.3, compared to compression without, 2.35 ± 0.2. Although soreness ratings increased again after the block, it is evident that they did not return to same level. This could have been due to the extended duration of the test, with subject's overall perception of pain shifting slightly. Alternatively, as subjects were required to move their position a little to allow the removal of the block, the probe may have shifted slightly from the sore spot. Nonetheless, this result suggests that in a muscle with DOMS, any differences in the effects of compression and vibration disappear during a large nerve fibre block.

DISCUSSION

DOMS is characterized by a mild soreness to mechanical stimuli such as stretch, contraction or palpation. Soreness onset is prompt with no persistence at the end of stimulation. The regions of soreness are not distributed uniformly throughout the muscle, but rather there are areas of heightened sensitivity which can be found in any part of the muscle (Fig 2.6&2.7) (Newham *et al.*, 1983). The central question was whether DOMS resulted from a heightened sensitivity of muscle nociceptors to mechanical stimuli or whether large diameter afferents were involved, as occurs in the region of secondary hyperalgesia surrounding a cutaneous injury site (Torebjork *et al.*, 1992). The results of this series of experiments are presented in support of the latter view, that muscle mechanoreceptors served by large diameter, Group I, nerve fibres play a role in DOMS. However, the involvement of muscle nociceptors cannot be discounted. It is likely that both mechanisms play a role.

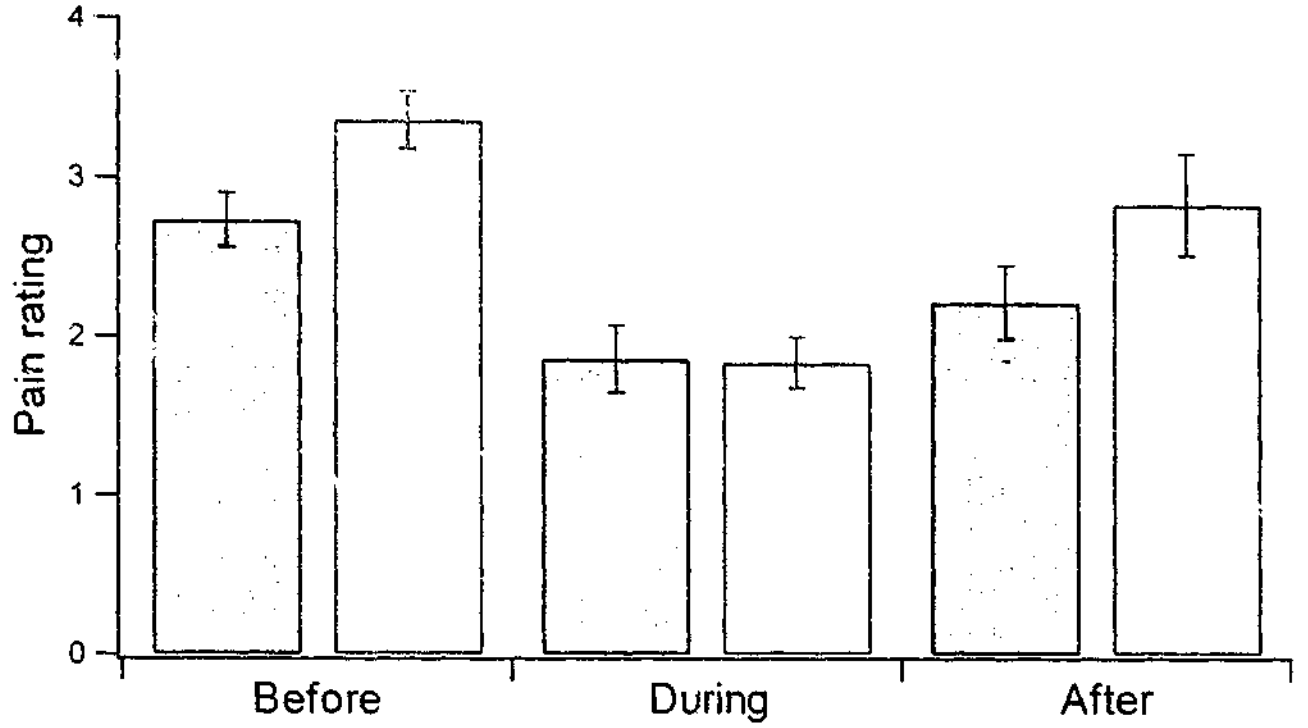


Figure 2.18

Pain ratings in response to local compression(blue bars) and pressure plus 80 Hz vibration (red bars) before, during and after the large-fibre nerve block. All values are means (±S.E.M.).

Vibration responses

In view of the heightened mechanical sensitivity of a muscle with DOMS, vibration stimulation of the muscle was used to ask the question: was this sensitivity simply an expression of the sensitisation process of Group III and IV afferents or did the mechanoreceptors served by large axons make a contribution?

Vibration applied to the skin has been found to relieve pain in both experimental (Pertovaara, 1979; Ekblom & Hansson, 1982; Bini *et al.*, 1984) and clinical investigations (Lundeberg, 1984; Lundeberg *et al.*, 1984). Therefore the finding that in an unexercised muscle any pain from strong pressure was reduced by vibration (Fig 2.12&2.13), was not unexpected. However, in eccentrically exercised muscles suffering DOMS, pressing on the muscle in a tender area produced mild soreness which became more intense during vibration, an exactly opposite effect (Fig 2.9). From the specific properties of different afferents to vibration, such a result is consistent with the notion that large-fibre mechanoreceptors are involved in the generation of DOMS.

Small afferents, and particularly unmyelinated afferents, typically do not respond to high-frequency vibration because of the duration of axonal refractory periods. The dynamic properties of the cutaneous C mechanoreceptors (corresponding to Group IV fibres) are such that they are of little value in signaling rapid changes in mechanical stimuli. The long time constant required, its easy exaggeration by fatigue, and the occurrence of an after-discharge make these receptors relatively insensitive to rapid stimulus changes (Bessou *et al.*, 1971). C mechanoreceptors are incapable of following an oscillating stimulus at frequencies above 1 Hz and do not consistently alter their peak to peak discharge frequency when the rate of skin indentation is varied over a wide range (Bessou *et al.*, 1971). Similarly, like most Group IV afferents, many of the Group III afferents also lack a sensitivity to small degrees of muscle stretch, such as would be produced by vibration ((Iggo, 1961; Franz & Mense, 1975; Kumazawa & Mizumura, 1977; Kaufman *et al.*, 1982).

In contrast, large fiber mechanoreceptors have been shown to be very much responsive to vibration stimulation. In humans the optimum frequency at which mechanoreceptors respond is 80 Hz. This has been demonstrated through proprioceptive illusions caused by

tendon vibration, that showed muscle spindle primary endings (Ia) had a maximal sensitivity (one-to-one response) at a frequency of 80 Hz (Roll *et al.*, 1989). The illusion of movement increased from 10 to 70-80 Hz, and was shown to decrease from 80 to 120 Hz (Roll & Vedal, 1982). For any given muscle length, primary endings can generally be driven to higher rates than secondary endings (Burke *et al.*, 1976). The secondary endings of spindles will not respond much above 30 Hz (Hagbarth & Eklund, 1966; Brown *et al.*, 1967b) while tendon organs remain unresponsive at all frequencies unless the muscle is contracting (Brown *et al.*, 1967b). That leaves only the occasional paciniform corpuscle, served by a Group III axon. In cats, they have been shown to follow a vibrating stimulus to 300 Hz or more (Bessou *et al.*, 1971). However, paciniform corpuscles are sparsely distributed in muscle where they occur mostly at the musculotendinous junctions (Barker, 1974). On the contrary, from mapping tender muscles, it is obvious DOMS spreads over the majority of the muscle. Therefore, most likely there are too few of these in the muscle to be able to account for DOMS (Barker, 1962). In addition, pacinian corpuscles associated with structures such as the interosseous membrane (Hunt & McIntyre, 1960; Barker, 1962) do not seem to be involved since vibration of bony structures in the lower leg evoked no sensations of pain. Interestingly, several attempts at evoking pain by vibrating the Achilles tendon in subjects experiencing DOMS were unsuccessful, presumably because the relevant spindles were not excited sufficiently strongly.

Applying 20 Hz vibration to the muscle is likely to excite secondary endings of spindles (Bianconi & van der Meulen, 1963). Since a small, but significant, increase in pain rating was reported by subjects during 20 Hz vibration (Fig 2.9), the possibility of a contribution to DOMS from secondary endings cannot be excluded. More importantly, the further increase in perceived pain when vibration was increased from 20-80 Hz further specifically points to the involvement of the primary endings of muscle spindles.

Although primary endings of muscle spindles are likely to be the only mechanoreceptors in a passive muscle that respond to vibration at 80 Hz, it must be noted that the properties of sensitised nociceptors to vibration are still unknown. Therefore, it is possible that nociceptors become vibration sensitive when sensitized. However, there are still details to suggest that for DOMS, sensitised nociceptors play at most only a limited role. To

begin with, mechanosensory Group III afferents, which are one of the most likely type to be involved in DOMS, do not show sensitisation (Graven-Nielsen & Mense, 2001). Free nerve endings in muscle classified as low-threshold mechanoreceptors, which probably mediate non-painful pressure sensations, are not sensitized by endogenous algesic agents (Mense & Meyer, 1988). Moreover, the coincidence of the human spindle optimum frequency (80 Hz) and DOMS effects, appears to support large fibre mechanoreceptor, rather than nociceptor involvement. The pain threshold was found to be lowest at 60-80 Hz (Fig 2.11). It seems unlikely, but not impossible, that sensitized nociceptors have the same frequency response as spindles. If nociceptors were to become sensitive to vibration, they would have been expected to follow a vibrating stimulus at the low frequencies, while possibly increasing in response as the frequency increased. Hence, rather than the observed inverse bell-shaped curve (Fig 2.11), a rising linear or exponential relationship may have been anticipated.

A possibility that cannot be excluded is involvement of 'silent' nociceptors (Michaelis et al., 1996). Silent nociceptors by definition, cannot be detected in uninjured tissue, since they have no spontaneous activity and do not respond to physiological stimulation of the tissue. However, they do become activate during an inflammation. It could be that the vibration enhancement of pain is due to recruitment of these previously silent nociceptors.

Considering the time course of the response to vibration, it is clear that the mechanism responsible for the effects of vibration take time to develop after the exercise. After the exercise, for compression both with and without vibration, the pain increased, but from 4 hours onward the rate of increase was higher with the vibration (Fig 2.14A). From our results it seems that the events occurring within the muscle, or at higher centres, which cause the switch from reducing to exacerbating pain from vibration, occur around 6-8 hours post-exercise. This obviously relates to the time-course of development of DOMS itself. The cross-over of vibration back to reducing pain occurred at 72 hours. The reversals at 8 and 72 hours both seemed to occur at a pain rating of about 4. Only when pain ratings were above 4 did vibration exacerbate pain. This may suggest that DOMS has to intensify to a certain level before evoking a switch.

It has been noted that a period of 8 hours is about the time required for the onset of the inflammation response after eccentric exercise (Armstrong *et al.*, 1991). This suggests two possible interpretations of the results. The first is that inflammation, which occurs in damaged muscle, had sufficiently sensitised nociceptors to the point where they had become vibration sensitive. As the change in vibration response appeared to be gradual, occurring within 4 hours, from 4 to 8 hours post-exercise, a gradual sensitization of nociceptors could have occurred. Alternatively, central neural mechanisms associated with the onset of inflammation may lead to alterations, at the spinal level, in the processing of mechanosensory and nociceptive inputs.

In addition, it is evident there are two stages in the time course of the changes: firstly the drop in compression threshold and then later the reversal of the vibration response (Fig 2.14A). This suggests that there are likely to be two separate mechanisms operating. It favors a theory where initially the stimulation threshold of nociceptors is reduced by inflammation, while later, non-nociceptor mechanoreceptors gain access to the pain pathway, allowing muscles to show a vigorous response to vibration.

Other receptors

As highlighted above, it is not known whether sensitised muscle nociceptors are able to respond to vibration, although they are known to respond to non-noxious mechanical stimulation (Mense & Meyer, 1988). Receptors served by unmyelinated axons are unlikely to show vibration responses (Bessou *et al.*, 1971). While evidence points to Group III nociceptors also lacking a sensitivity to vibration, other non-nociceptive receptors served by Group III afferents may be involved. Skeletal muscle contains a group of mechanoreceptors served by Group III axons which are not stretch-sensitive but respond to local pressure (Paintal, 1960; Bessou & Laporte, 1961; Kumazawa & Mizumura, 1977). In addition, non-nociceptive Group III afferents that are stretch and contraction sensitive (ergoreceptors) are involved in circulatory and respiratory adjustments during activity (Mense, 1996a), while a considerable proportion of Group IV units are activated by muscular contractions (Kniffki *et al.*, 1978). It is not known whether these receptors respond to vibration. But, it is conceivable that they, too, are involved in the generation of DOMS.

There are observations from animal experiments which suggest that the responses of sensitised muscle Group III nociceptors are not consistent with the characteristics of DOMS (Berberich *et al.*, 1988). The compound carrageenan was injected into cat hind limb muscles. Within 1-2 hours this evoked a powerful inflammatory response in the muscle, associated with the release of 5-HT, histamine, bradykinin and prostaglandins. These substances stimulate or sensitise muscle nociceptors. The main effect of the inflammation on muscle Group III nociceptors was that they developed resting activity. The discharges consisted of single or grouped impulses at irregular intervals (Berberich *et al.*, 1988). Interestingly, their mechanical thresholds did not change. The finding led the authors to conclude that this group of receptors might be associated with the spontaneous pain and dysesthesias associated with myositis, an inflammatory disease of the muscle, at least to the extent that carrageenan-evoked inflammation is a realistic model of the disease. The symptoms associated with myositis are quite unlike those of DOMS. With DOMS there is no pain in the resting subject. However, it has been suggested that the spontaneous activity of sensitized nociceptors may not elicit subjective sensations because single impulses, at least in cutaneous nociceptive fibres have been reported not to reach consciousness (Torebjork, 1985). Therefore, nociceptors may also be able to access the pain pathway involved for DOMS.

Nerve block

Compression block is a well-established and non-invasive technique, which has been used extensively as a differential block of large diameter nerve fibres (Garland, 1991; Price *et al.*, 1991; Davis, 1998). The block progresses according to fibre size, with large myelinated afferents being affected first, followed by small myelinated afferents and lastly by unmyelinated afferents (Torebjork & Hallin, 1973; Mackenzie *et al.*, 1975). Therefore, according to the size principle of the block, Ia fibres should have been blocked first followed by α-motoneurons, and then if kept for long enough, Group III and IV fibres supplying nociceptors.

The H-reflex typically disappeared concurrently with the rise in pain threshold. This occurred at a time when subjects were still able to contract their muscle voluntarily, demonstrating that the reflex block was not due to α fibre blockage, but rather Group Ia fibre blockage. However, the contraction was usually weaker, suggesting some blockage

of motor axons. Nonetheless, it makes it unlikely that at this stage significant numbers of small-diameter nerve fibres were blocked.

In support of this view are the findings that the latency for the sense of painful heating of the skin remained unchanged during the block, and the sense of cold also remained intact. These sensations were tested to ensure the conduction integrity of the nociceptive afferents that belong to the thinly-myelinated Aδ fibre and the unmyelinated C fibre groups. This was done to indirectly monitor the conduction of muscle nociceptors, Group III fibres (corresponding to Aδ fibres) and Group IV fibres (corresponding to C fibres), which could not be measured directly without invasive methods. In the primate, the most commonly studied C fiber nocicieptor is the polymodal nociceptor, which responds to mechanical as well as heat stimuli. Studies have shown that blocking of C fibre responses is accompanied by a decrease in the experience of burning pain (Torebjork & Hallin, 1973). Responses to cold stimuli or chemical stimuli may also be evident, in which case the term polymodal nociceptor is appropriate. Some C nociceptors may respond preferentially to decreases in skin temperature. Their cooling response has been shown to be proportional to the rate at which the skin is cooled and there is little or no activity with slow cooling (Bessou *et al.*, 1971). The majority of Aδ fibre nociceptors have been classified as high-threshold mechanoreceptors. Unlike C fibres which all respond similarly to heat and mechanical stimuli, Aδ fibres are classified into two groups. Type 1 Aδ fibres have relatively high threshold and exhibit a slowly increasing response to heat stimuli of long duration with an onset latency of several seconds (Treede *et al.*, 1992). They may be responsible for continuous pain caused by heat stimuli of several seconds duration. Type 2 Aδ fibres have low thresholds and account for 'first pain' sensation, responding to the onset of a heat stimulus within a few milliseconds (Treede *et al.*, 1992). Some Aδ mechanoreceptors also respond to cooling, but their thermally evoked discharge varies from one unit to another and rarely exceeds 5% of the maximal discharge frequency elicited by mechanical stimulation (Bessou *et al.*, 1971).

Although, pain threshold increased with the blockage of Group Ia fibres, subjects still reported a distinct pain threshold in response to muscle compression during the block. However, several reported that they were not sure whether the discomfort was any greater than that experienced from pressing the probe into an unaffected part of the

muscle. They declared that the sensations had become rather vague. This point needs to be explored in future experiments, but the balance of our data suggests that some component of DOMS persists after a Group I fibre block. If correct, it would imply that DOMS is generated as a result of combined inputs from nociceptors and large mechanoreceptor afferents.

It could be argued that at the point of disappearance of the reflex the Group I fibres had not been blocked, but that their conduction had just been slowed in the region of the block. This would lead to dispersion of impulses and less summation of excitatory post-synaptic potentials, so that motoneuron firing threshold would not be reached. However, evidence of a partial large fibre block was indicated by the weakened voluntary contraction. The simplest interpretation of the accompanying rise in pain threshold was that it was due to withdrawal of input from large afferents. In any case, whether partial or complete block, there was a significant effect on pain thresholds. If DOMS was mediated only by small afferents there should have been no change.

There is some support from the work of others for the conclusion about the role of large muscle afferents in DOMS. Barlas et al. (2000) showed that there was a significant increase in mechanical pain threshold in elbow flexor muscles with DOMS, when the conduction in large myelinated fibres was blocked using the differential ischaemic block technique. The authors concluded that DOMS was mediated, at least in part, by alterations in central processing of non-noxious afferent information from large diameter nerve fibres.

Nerve block and vibration

Here the question was posed, did pain evoked by vibration persist after a nerve block. The results showed that vibration, which increased the pain from compression, became ineffective during a large nerve fibre block (Fig 2.18). This finding further supports the view that the increase in soreness experienced by subjects with DOMS during local vibration was the result of the vibratory stimulus exciting large mechanosensitive afferents in the muscle.

Although, during the conduction block of large fibres, vibration induced no more soreness than compression alone, both stimuli together still produced significant amounts of pain. This again indicates that DOMS persists after a Group I fibre block. Therefore, other mechanisms related to nociceptor activity are likely to contribute to the perceived soreness. Hence, once again, the balance of the data suggests that DOMS is generated as a result of the combined inputs from nociceptors and large mechanoreceptor afferents, but the increase in pain experienced in response to vibration is attributable to large afferents.

Mechanism

The results from these series of experiments support the suggestion that large diameter myelinated afferent nerve fibres are able to access the pain pathway that contributes to DOMS. But, if so, how are these afferents able to access the pain pathway?

The principal process that offers mechanoreceptor access to the pain pathway is central sensitization. Modulation in central pathways is triggered by peripheral nociceptor input and results in enhanced responsiveness of pain transmission neurons, which either outlasts the initiating input or requires a low-level peripheral drive to maintain it (Woolf & Salter, 2000). Most excitatory input to pain pathway neurons is subthreshold, and increased gain results in recruitment of these inputs to the output of neurons, causing them to fire to normally ineffective inputs. These changes constitute central sensitization and are responsible for pain produced by low-threshold afferent inputs and the spread of hypersensitivity to regions beyond injured tissue.

Specifically, it is known from cutaneous studies that secondary hyperalgesia involves the sensitisation of spinothalamic tract neurons (STT) within the dorsal horn of the spinal cord. Evidence for this is shown in the significant increase in responses of STT neurons after cutaneous stimulation from capsaicin. The time course of changes in the discharge rate of STT neurons parallels the time course in the magnitude estimates of pain by humans. Hence, it was concluded that capsaisin mediated the response. Furthermore, a proximal nerve block prior to the intradermal injection of capsaicin prevents the occurrence of any secondary hyperalgesia after recovery from the anesthetic 1-3 hours later (LaMotte *et al.*, 1991). LaMotte et al. (1991) proposed chemonociceptor afferents,

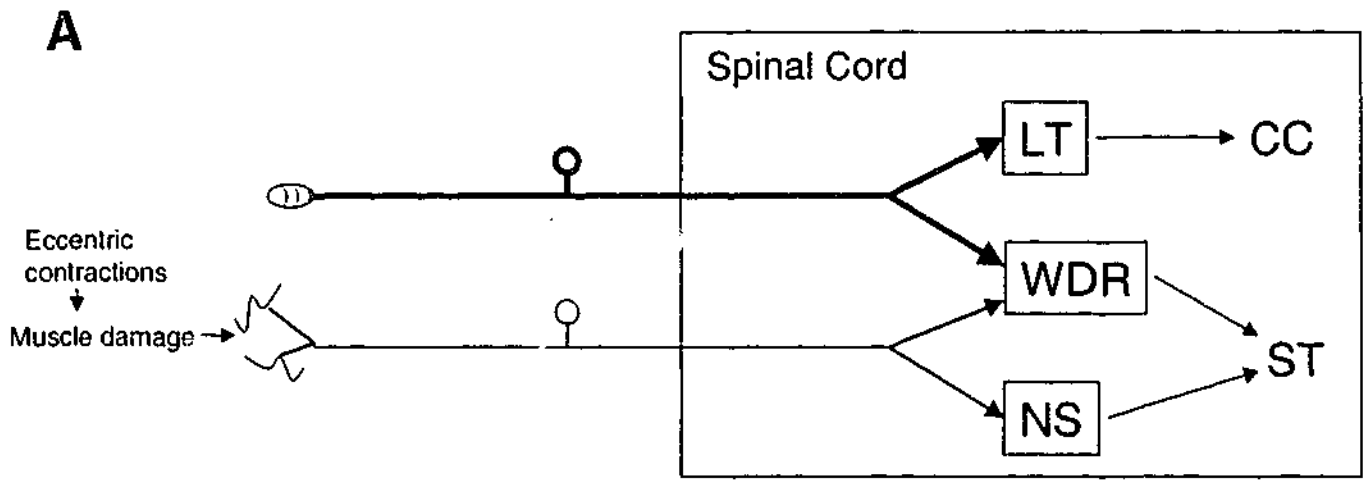
which directly respond to capsaicin, terminate in the dorsal horn on interneurones that also receive input from cutaneous low-threshold mechanoreceptors with myelinated axons. In response to neuromodulator released by activated nociceptive afferents, these interneurones become sensitized, i.e. they develop lowered thresholds and enhanced suprathreshold responses to normal evoked activity from the low-threshold and nociceptive afferents. The sensitization is initially dependent on continual input from the chemonociceptive afferents, but subsequently may persist to varying degrees without input. Hence, in this way, previously non-painful mechanical stimulation would now induce pain.

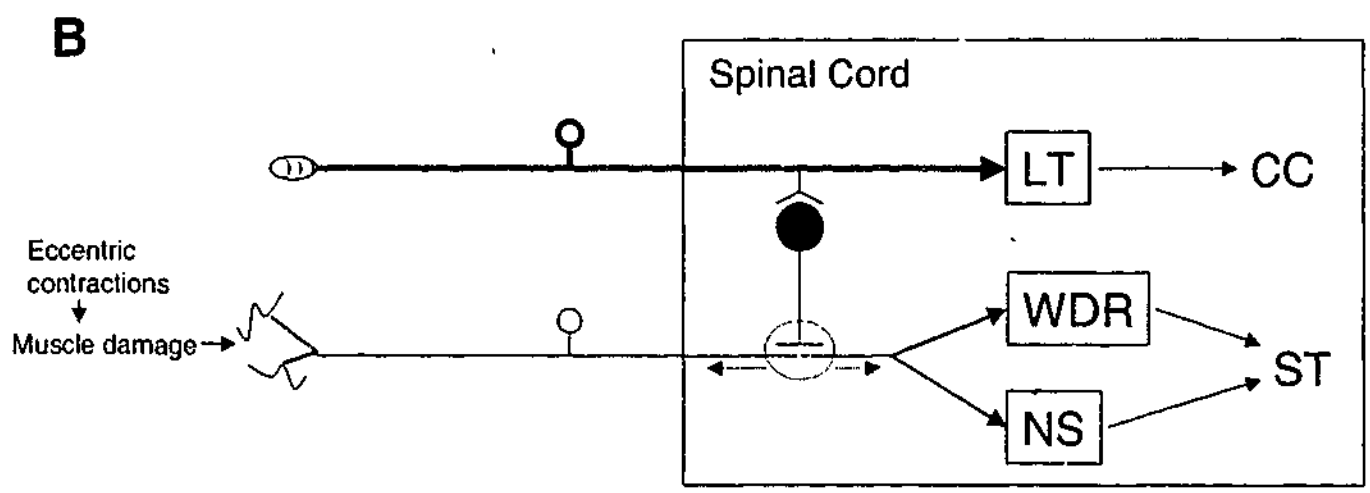
This pain pathway could also account for mechanoreceptor involvement in DOMS. It has been known for some time that in superficial layers of the dorsal horn of the spinal cord there are relay cells in the principal pain pathway, the spinothalamic tract, which receive nociceptor inputs from muscle (Craig & Kniffki, 1985). Within the spinothalamic tract most cells are nociceptor specific cells (NS) activated by nociceptors (Cervero & Laird, 1996). However, a proportion of cells (20%) were found to have thresholds in the innocuous range so they could respond to inputs from both nociceptive and non-nociceptive receptors. Cells responding to a range of inputs have been called wide dynamic range (WDR) cells (Price & Dubner, 1977). So one mechanism by which spindle afferents could access the pain pathway is by acting through the WDR cells (Fig 2.19A). It has also been proposed recently that the nociceptor sensitisation process associated with tissue injury leads to a raised excitability in postulated presynaptic inhibitory interneurones between large diameter mechanoreceptor afferents and nociceptor afferents (Cervero & Laird, 1996). These interneurones could include WDR cells. Interestingly, studies of cells in the dorsal horn are confounded by the presence of many cells with proprioceptive inputs, cells which have been thought not to be involved in the processing of pain information, but to be part of the Clarke's column – dorsal spinocerebellar tract system (Mense, 1993). It would now be interesting to reinvestigate dorsal horn neurons, seeking evidence, specifically, for cells which receive proprioceptive as well as noxious inputs.

The analgesic effects of vibration have been suggested to be mediated at the spinal level by depressing responses of nociceptive neurons and/or by excitatory responses of non-

Figure 2.19

Diagrammatic representation of the proposed mechanisms by which mechanoreceptors might access the pain pathway after eccentric exercise. Two types of afferent fibres are illustrated: large diameter, supplying mechanoreceptors and fine fibres supplying nociceptors. Key: LT = Low threshold cells, WDR = Wide dynamic range cells, NS = Nociceptor specific cells, CC = Clarke's column, ST = Spinothalamic tract. *(A)* In response to muscle damage, sensitised nociceptors activate WDR cells. Mechanoreceptors that activate LT second order neurons with proprioceptive inputs may also have subthreshold connections to WDR cells. When these cells become sensitized from nociceptive inputs, the LT connections reach threshold to provide access to the ST. ***(B)*** It is postulated that reduction of pain from mechanoreceptor stimulation is the normal situation is by pre-synaptic inhibition (black neuron). When as a result of increased nociceptive activity the inhibitory interneuron is sensitised mechanoreceptor input generate nociceptive inputs via a dorsal root reflex to activate both WDR and NS neurons. From Cervero & Laird (1996).


A
Spinal Cord
LT
CC
WDR
ST
NS
Eccentric
contractions
Muscle damage


B
Spinal Cord
LT
CC
WDR
ST
NS
Eccentric
contractions
Muscle damage

nociceptive neurons (Salter & Henry, 1990). Salter & Henry et al. (1990) found all WDR cells responding to non-nociceptive neurons were excited, whereas depressant responses were shown only by nociceptive specific neurons. Moreover, in normal, uninjured muscle, input from large afferents has been shown to inhibit nociceptive input by presynaptic inhibition, the basis of the 'gate control' theory of pain (Melzack & Wall, 1965). This may underlie the mechanism of how the activation of mechanoreceptors can reduce pain, as occurs when we 'rub a spot to make it better' (Julius & Basbaum, 2001). Such a mechanism would account for our finding that in unexercised muscles, painfully strong pressure becomes less painful during vibration, whereby an increase in mechanoreceptor input acts to gate out some of the nociceptor input.

It is postulated muscle injury and increased nociceptive activity sensitises spinal inhibitory interneurones. As a result mechanoreceptor input are able to generate nociceptive inputs via a dorsal root reflex (DRR) to activate both WDR and NS neurons (Fig 2.19B) (Cervero & Laird, 1996). The DRR would evoke localized flares in areas of secondary hyperalgesia (via antidromic activation of nociceptors), but would also be conducted forward activating second order neurons normally driven by nociceptors (Fig 2.19B). This in turn, would lead to the generation of pain by the activation of low threshold mechanoreceptors. However, the present data provides no information on which of the two mechanisms might be involved in DOMS, and it is not known what the trigger for DOMS is. Presumably at some point there is sufficient nociceptive input produced by the muscle injury to initiate the central sensitisation process. It is known that sensitisation of cutaneous nociceptors requires a low level of maintained activity (Woolf & Salter, 2000).

The trigger for the sensitization of STT neurons after eccentric exercise could involve the nociceptive mechanisms of spinal glutamate receptors. Specifically, the activation of spinal N-methyl-d-aspartate (NMDA) receptors, a subtype of glutamate receptors, are thought to be the key mechanism in the induction of central sensitisation and in particular the increase in mechanosensitivity (Neugebauer *et al.*, 1993). NMDA receptors have been shown to mediate sensitization phenomena in spinal neurons following peripheral insult (Woolf & Thompson, 1991). Blockade of NMDA receptors by D-APV or a decrease in the number of thalamic NMDA receptors reduced acute thermal and

mechanical hyperalgesia by carrageenan injection (Kolhekar *et al.*, 1997). Therefore, NMDA has been implicated in many states of central hypersensitivity including mechanisms underlying hyperalgesia in models of acute and chronic nociception. In addition, it has been shown that the activation of the peripheral NMDA receptors is involved in both induction and maintenance of persistent firing of the dorsal horn WDR neurons induced by bee venom injection (Chen *et al.*, 1999). Topical application of prostaglandin E_2 to the spinal cord enhances the responses of nociceptive neurons to the ionophoretic application of NMDA, suggesting that inflammation induced central sensitization may involve an interaction with prostaglandins and NMDA receptors (Vasquez *et al.*, 2001). Therefore, DOMS could involve similar mechanisms, by which muscle damage evokes an inflammatory response which leads to NMDA receptor activation, triggering WDR cells, allowing the mechanoreceptors access to the pain pathway.

An alternative or, perhaps, supplementary hypothesis for a mechanism for DOMS is that the muscle damage from eccentric exercise triggers an inflammatory response, and substances such as prostaglandins released by the inflammation sensitise nociceptors to the point where they respond to non-noxious mechanical stimulation, but without generating tonic activity and therefore chronic pain. Such a suggestion is possible, as a considerable percentage (more than 40%) of Group III and IV receptors have not been found to develop a background discharge, even though the whole muscle is infiltrated with the carrageenan suspension (Berberich *et al.*, 1988). This hypothesis is supported by recent studies showing anti-inflammatory drugs such as ibuprofen (Tokmakidis *et al.*, 2003) and ketoprofen (Cannavino *et al.*, 2003) are effective in reducing muscle soreness after eccentric exercise. However, such findings remain controversial, with other studies giving the opposite result. The administration, after exercise, of non-steroidal anti-inflammatory drugs which block the enzyme cyclooxygenase that produces prostaglandins, was found not to lead to significant reductions in DOMS after eccentric exercise (Croisier *et al.*, 1996; Bourgeois *et al.*, 1999). This would suggest that if a sensitisation process is involved in DOMS, its pharmacology is different from that of sensitised cutaneous nociceptors. Hence, its evident more research into the understanding of the inflammatory role in DOMS is required.

CHAPTER THREE

Effects of eccentric exercise and mechanical stimulation on muscle pain following injection of hypertonic saline

INTRODUCTION

As mentioned earlier the process of nociceptor sensitisation is the currently accepted hypothesis used to explain the features of DOMS. It is thought that breakdown of the damaged muscles fibres, in association with elevated intracellular Ca^{2+} levels generates a local inflammatory response and sensitisation of nociceptors by the tissue breakdown products (MacIntyre, Reid & McKenzie, 1995; Smith, 1991). This process may also cause hyperexcitability of dorsal horn neurons, prolonged neuronal discharge, increased responses to noxious stimuli and expansion of receptive fields (Hoheisel, Koch & Mense, 1994; Hoheisel et al., 1993).

In view of the characteristic features which distinguish DOMS from other forms of pain, in Chapter 2 it was proposed that DOMS may be generated, not just by sensitised nociceptors, served by Group III and IV axons, but include a component attributable to the large-fibre mechanoreceptors in the muscle, the muscle spindles and tendon organs (Weerakkody et al., 2001). Thus DOMS might be thought of as having a component resembling the secondary hyperalgesia and allodynia observed following skin lesions (Hardy, 1950).

Reported here are the results from a number of additional studies of DOMS, seeking further support for the view that there is a role for large fibre mechanoreceptors in DOMS. In this set of experiments hypertonic saline was used as an experimental model of muscle pain. In human studies, intramuscular injections of algesic substances, such as capsaicin, bradykinin, serotonin, potassium chloride, levoascorbic acid and hypertonic saline have all been used to experimentally induce muscle pain (Graven-Nielsen & Mense, 2001). Intramuscular injection of hypertonic saline has been used for some time as a useful model for studying and characterizing the sensory and motor effects involved in muscle pain (Iggo, 1961; Kellgren, 1938). It is convenient because the quality of the pain it induces is comparable to acute clinical muscle pain (Kellgren, 1938; Svensson et

al., 1995) and it shows localized and referred characteristics, allowing pain to be isolated to one specific muscle. The pain intensity and its duration have been shown to correlate with the concentration of saline injected (Jensen & Norup, 1992).

Although saline-induced muscle pain has been used in a number of studies, being first introduced during the middle of last century (Kellgren, 1938), the underlying mechanisms responsible for the pain are still not fully understood. Factors involved may include chemical mediators of an inflammatory process, an increasing intramuscular pressure (Allen & Barnes, 1986), and local muscle spasms (DeVries, 1966). However these have all been subsequently ruled out. Recent work has suggested that it is most likely that hypertonic saline produces its effects by increasing extracellular sodium concentrations leading to depolarisation of excitable membranes and glutamate release from activated nociceptors, resulting in the generation of action potentials which could mediate the pain sensation (Graven-Nielsen et al., 1997a). A longer lasting but delayed increase in intramuscular potassium concentration was also observed after hypertonic saline injection (Graven-Nielsen et al., 1997b). Therefore it was suggested increases in potassium concentrations reflected local damage of muscle cells and also may play a secondary role in the generation of pain.

Nonetheless, it has been well established for a long time that in animals muscle nociceptors respond to hypertonic sodium chloride (Iggo, 1961). Hypertonic saline led to excitation of mainly unmyelinated group III and IV afferents (Laursen et al., 1999; Paintal, 1960). The saline-induced pain sensation is typically delayed. This delay could be due to the diffusion process of saline from the needle to sites of the nociceptors. Stretch receptors have been shown to be much less responsive to saline (Kumazawa & Mizumura, 1977).

Because of the relatively selective excitation of unmyelinated afferents by hypertonic saline injections, saline injection has been used to investigate the nociceptor sensitisation hypothesis. Firstly, hypertonic saline was injected into muscles with DOMS. It was argued that if DOMS resulted from sensitisation of nociceptors, pain from saline, a stimulus believed to act by depolarisation of nociceptor nerve endings, should summate with DOMS, producing a larger pain response than the saline alone.

The combined effects of saline injections and mechanical stimulation were also examined. As, there is evidence that hypertonic saline preferentially excites small muscle afferents, and it is believed these afferents do not respond to vibration, the rationale underlying this experiment was to confirm the effects of vibration on saline-mediated pain.

In addition, the effect of a nerve block was examined on the intensity of pain from saline. Here the underlying rationale was that if saline pain was mediated largely by small muscle afferents, its intensity should remain unaffected by a large nerve fibre block.

METHODS

Subjects

A total of 18 subjects (mean age, 22 years) took part in these experiments. Informed consent was obtained from each subject. All experiments were approved by the Monash University Committee for Ethics in Human Experimentation and conformed with the 1975 Helsinki declaration.

Eccentric exercise

As outlined in Chapter 2, the triceps surae of one limb was exercised eccentrically by stepping backwards on an inclined (~13°) moving treadmill for 1 hour. Subjects typically carried out 30 steps per minute over about one hour (see Jones et al., 1997). Having completed the exercise bout, subjects were tested 48 hours later. The test was performed on the second day after exercise because at this time DOMS reaches its peak (Jones et al., 1997).

Saline injection experiment

This experiment was carried out on a total of 6 subjects, 4 male and 2 female (mean age, 24 years). A volume of 0.2 ml saline was injected into medial or lateral gastrocnemius of each leg. Subjects were asked to rate the perceived soreness that was produced by each injection using a visual-analog scale, where they turned a dial with a scale of 0-10 over

300° of rotation. The dial was connected to a potentiometer that was connected to the MacLab/8s (ADInstruments, Australia), allowing the measurements to be continuously recorded. Measurements were recorded using 'Chart' (AD Instruments, Australia) running on a Macintosh G4 computer (Apple, U.S.A.). Subjects were instructed to move the dial as they experienced the pain from the stimulus, tracking it as it developed and subsided. Measurements of pain continued to be recorded until subjects were sure the pain had subsided.

Subjects lay chest down on a cushioned surface, with their triceps surae in a slightly platerflexed position. They received a hypertonic (5%) saline injection into the muscle of both legs before and 48 hours after the eccentric exercise of one leg. When subjects were tested after the exercise, the exercised muscle was first mapped for tender areas and the saline injected into the centre of a tender region. The equivalent spot on the other, unexercised, muscle was used for the control injections. Injections were made with a 25-gauge needle. In addition, after the exercise, subjects also received injections of isotonic (0.9%) saline into both legs. The rationale was that a control was needed for possible mechanical effects from the volume of fluid injected into the tender muscle. Injections were given in a random order and subjects were unaware of the composition of a particular solution. For 3 subjects injections of 0.9% saline were also given before the exercise. The injection process was carried out in two stages. First the needle was pushed through the skin to a depth of 2-3 cm and then held there for 10-20 seconds before saline was injected. It allowed subjects to distinguish between pain from the needle prick and pain from the injected saline.

Saline injection and vibration

Five subjects, 4 male and 1 female (mean age, 20 years) took part in this experiment.

As described in the previous chapter, the probe tip of a moving-coil electromagnetic actuator was used to apply compression pulses to the muscle with and without 80 Hz vibration superimposed. The electromagnetic actuator was supplied with position feedback from an LVDT (linear variable differential transformer) displacement transducer. Inserted between the tip and the shaft of the actuator was a force transducer. Strain gauge output was amplified and displayed along with displacement on a computer.

A gating circuit was used to control the duration of compression. The amplitude of the prod, the force applied and the amplitude of the superimposed vibration were altered slightly from subject to subject, depending on how tender they were. The peak force generated during each prod was kept the same by adjusting the displacement of the probe. The force, displacement and amplitude of vibration used, varied from one experiment to another, but were maintained constant within any one experiment. Forces applied with the actuator lay in the range 15-40 N. When 80 Hz was used, amplitude had to be reduced to 14-18 mm to allow matching of peak forces with and without vibration. Vibration amplitude lay in the range 0.8-1.4 mm. Signals from both tension and length transducers were processed by a commercial analog to digital converter and recorded. Data was then analyzed using the software Igor Pro (Wavemetrics, Ore, U.S.A).

Subjects were asked to lie on their side so that the tender area was within reach of the 1 cm diameter probe tip. Firstly, measurements were made of responses to compression with and without vibration at a sensitive spot on the eccentrically exercised muscle and a comparable site on the unexercised leg. Then at the same spot 0.2 ml of sterile 5% sodium chloride was injected into the muscle to a depth of 2–3 cm. After placing a small band-aid over the injection site, the mechanical actuator was re-positioned over the sensitive spot and further measurements were made as the pain from the injection began to manifest itself. Before injection, 10 control measurements with and 10 without vibration were made, each at 50 s intervals, to minimize any desensitization effects. Intervals between measurements made after the injections were reduced to 40 s, in an attempt to obtain as many values as possible during the soreness from the saline, while still leaving enough time for any effect of desensitization to be minimal. Subjects rated the perceived overall soreness that they experienced from the combination of hypertonic saline and each mechanical stimulation on a visual-analog scale as described before.

Three of the five subjects were also tested with 0.2 ml of isotonic (0.9%) saline. For these subjects two areas of equivalent tenderness in the muscle were selected. One site was subjected to compression and vibration, which were then repeated after isotonic saline was injected. The same procedure was followed at the other site after hypertonic saline was injected. The two doses of saline were given blindly and subjects did not know which injection they were about to receive.

To confirm that subject's reports of pain and the effects of vibration were not mediated by skin afferents, for 3 subjects the region of skin overlying the muscle, 2 cm in diameter, was treated with a local anaesthetic cream (EMLA, lignocaine / prilocaine 5%, Astra Pharmaceuticals) for two hours before the injection. Following treatment the skin had become insensitive to touch and a pin prick. Injections of hypertonic saline were then carried out and pain ratings compared with those from subjects with untreated skin.

Saline injection and nerve block

Five male subjects (mean age, 20 years) took part in this experiment. Unlike in the other experiments, these subjects did not undergo eccentric exercise.

Subjects sat on a wooden support that was fitted to an adjustable chair. The chair was attached to a steel frame, and this supported two footplates. This allowed measurements of torque about the ankle joint. While seated, a volume of 0.2 ml sterile 5% hypertonic saline was injected into the triceps surae. On a 0-10 visual analog scale subjects reported the progression and intensity of the pain. After the pain had subsided a differential compression block of the sciatic nerve was achieved by placing a wooden bar 6 cm high and 2 cm wide under the thigh, as described in the previous chapter. A H-reflex was elicited every 30 s, by applying a 0.2 ms duration shock of 20 – 40 mA amplitude to the electrode which stimulated the tibial nerve. A stimulating cathode was strapped to the popliteal fossa and an anode plate was taped to the top of the knee. The size of the reflex, measured as EMG, was approximately half of the maximal motor response. Reflexes were displayed on an oscilloscope and recorded in 'Chart' (ADInstruments, Australia) running on a Macintosh 6100 computer (Apple USA). To ensure that smaller nerve fibres in the Aδ and C fibre range were not included in the block responses to hot and cold stimuli were monitored (see Chapter 2). Measurements of latency to painfully warm (50°C) stimuli, delivered with a probe 2.5 cm in diameter were made at regular intervals. Cold sensations were produced by a round metal bar, 2.5 cm in diameter that had been immersed in ice.

Once the H-reflex was completely blocked, as evidenced by disappearance of the reflex EMG, hypertonic 5% saline was injected again into the muscle. Once more subjects reporting the level of pain on a visual analog scale.

Statistical analysis

In each experiment, individual measurements from all subjects were pooled. Values are given as means, plus or minus standard errors of the means, calculated from the pooled data, unless otherwise stated. ANOVA testing was used to determine significance levels. A three factor repeated measures ANOVA was performed to test for significant differences in pain ratings. The factors were time (before or after exercise), leg (exercised or unexercised) and subject. Where an ANOVA was significant ($p<0.05$), an LSD (least significant difference) *post hoc* test was applied. Also a four factor repeated measures ANOVA was used to test for significant differences in pain increases from mechanical stimulation in the presence of hypertonic saline pain. The factors used were leg (exercised or unexercised), stimulus (compression or vibration), subject and trial. A two factor repeated measures ANOVA was also performed to test for significant changes in H-reflex, tenderness threshold, and latency for painful heat stimulation throughout the compression block procedure. The factors used here were time (before, during or after the block) and subject.

In addition a pooled t-test was used to determine the significance level between pain ratings before and during large nerve fibre compression block. The statistical analysis program used was Data Desk (Ithaca, N.Y., U.S.A.).

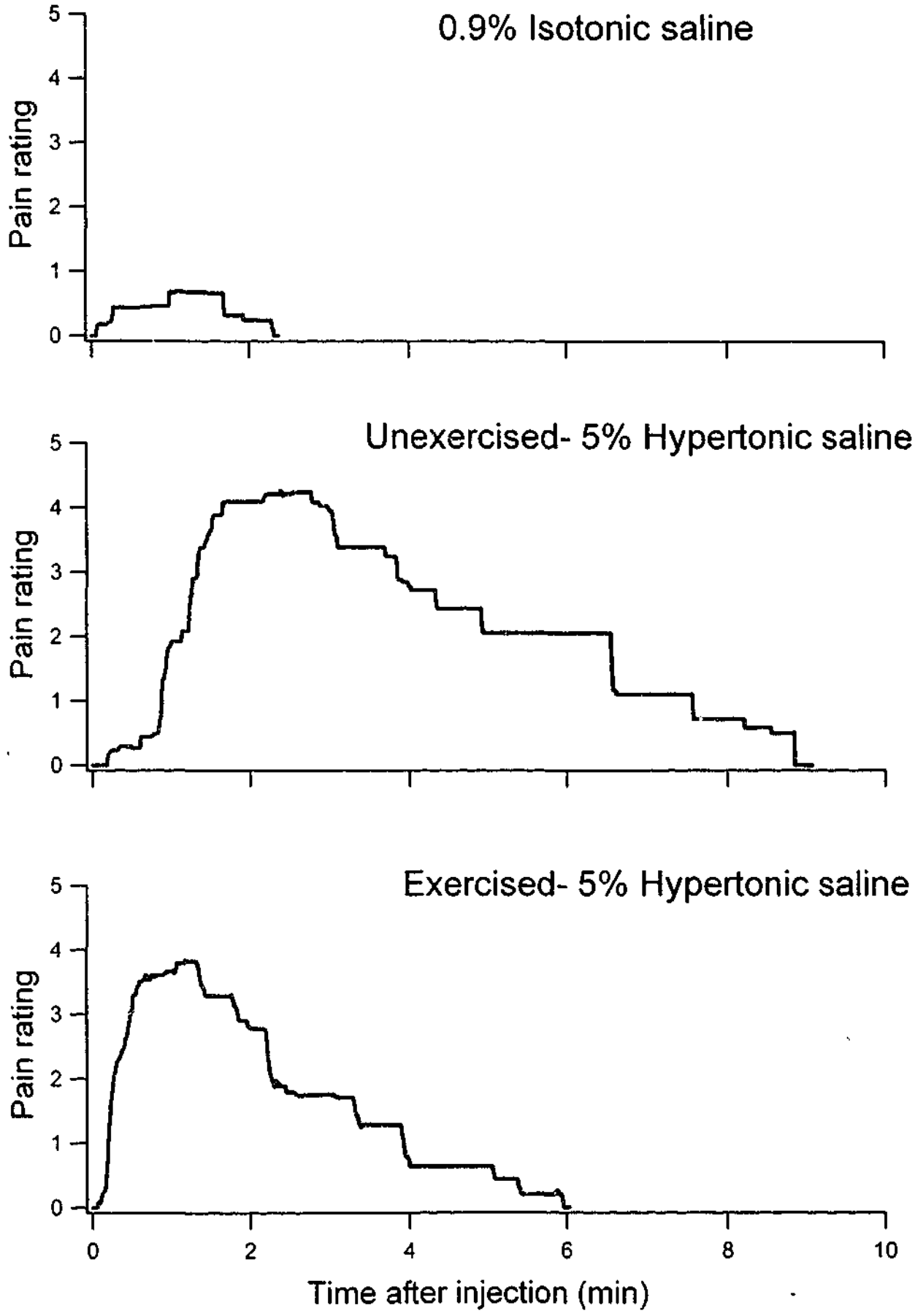
RESULTS

Saline injection experiment

An example of a subject's pain rating in response to saline injection is shown in figure 3.1. For this subject the 0.9% isotonic saline injection produced some slight soreness, with a rating of about 0.7, which lasted only briefly. It should be noted that some subjects indicated that the 0.9% injection produced no soreness at all. Overall, before and after the exercise, injection of 0.9% saline into both the exercised and control

Figure 3.1

A subject's pain rating response over time to saline injections. ***Top Panel:*** 0.9% isotonic saline injection into unexercised muscle. ***Middle Panel:*** 5% hypertonic saline injection into unexercised muscle. ***Bottom Panel:*** 5% hypertonic saline injection into eccentrically exercised muscle with DOMS.


0.9% Isotonic saline
Unexercised- 5% Hypertonic saline
Exercised- 5% Hypertonic saline
Pain rating
0
1
2
3
4
5
0
2
4
6
8
10
Time after injection (min)

muscles produced very little soreness, giving a mean rating of 0.63 (± 0.22). Conversely, injecting 5% hypertonic saline before the exercise produced much more soreness that peaked in about 2-3 minutes and then gradually subsided until it had completely gone by approximately 9 minutes (Fig 3.1). Similarly, in the exercised muscle the peak pain produced by hypertonic saline was much higher, but for this subject the peak was reached faster and the duration was about 3 minutes less. All subjects rated the decline in pain as a step-wise process; perceiving no change for a period, and then sensing a small decline, followed again by a period of constant soreness (Fig 3.1). Most subjects described the sensation as a mild, dull or aching soreness.

Before the exercise mean soreness ratings for the experimental and control legs were 4.0 (±0.7) and 3.7 (±0.7) respectively (Fig 3.2). There was no significant difference in pain rating between the legs. Injecting 5% saline into the sore region of the exercised muscle 48 hours after exercise gave a mean rating of 3.2 (± 0.7) (Fig 3.2). This compared with a mean value of 3.1 (± 0.4) for the unexercised muscle (Fig 3.2). This difference was also not significant.

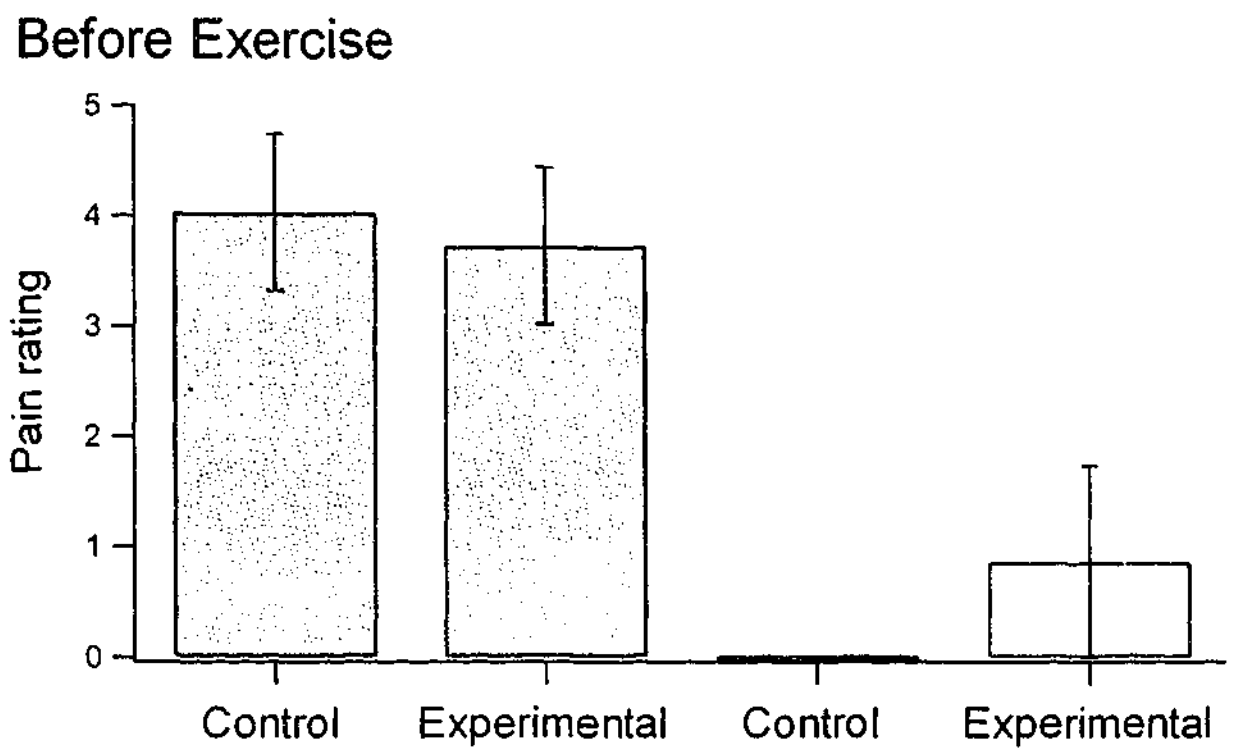
A point noted in passing was that some subjects claimed that the pricking sensation, as the needle penetrated the skin, was more intense at a sore spot in the exercised muscle. However, all subjects were able to clearly distinguish between the initial sharp needle prick as it penetrated the skin and the subsequent sensations arising from the injected saline 10-20s later. For two subjects pain ratings to 5% saline injections were measured after the skin had been rendered anesthetic by treatment with anesthetic cream (EMLA). Soreness levels after the skin had been anaesthetized were not significantly different from values when the skin was fully sentient ($p < 0.05$).

Saline injection and vibration

Before injection of saline into an unexercised muscle, measurements were made of levels of soreness in response to compression of the muscle, with and without vibration. The force of the actuator was adjusted to produce slight but measurable discomfort. In the unexercised muscle the superimposed vibration significantly decreased the soreness, while in the exercised muscle vibration significantly increased pain, which is consistent with the findings in Chapter 2 ($p<0.05$) (Fig 3.3).

Figure 3.2

Mean pain ratings (±S.E.M.) from 6 subjects from their experimental (exercised) and control (unexercised) muscle to 5% hypertonic (blue) and 0.9% isotonic (red) saline injections. ***Top Panel:*** Mean response before exercise. ***Bottom Panel:*** Mean response 48 hours after eccentric exercise.


Before Exercise
Pain rating
5
4
3
2
1
0
Control
Experimental
Control
Experimental

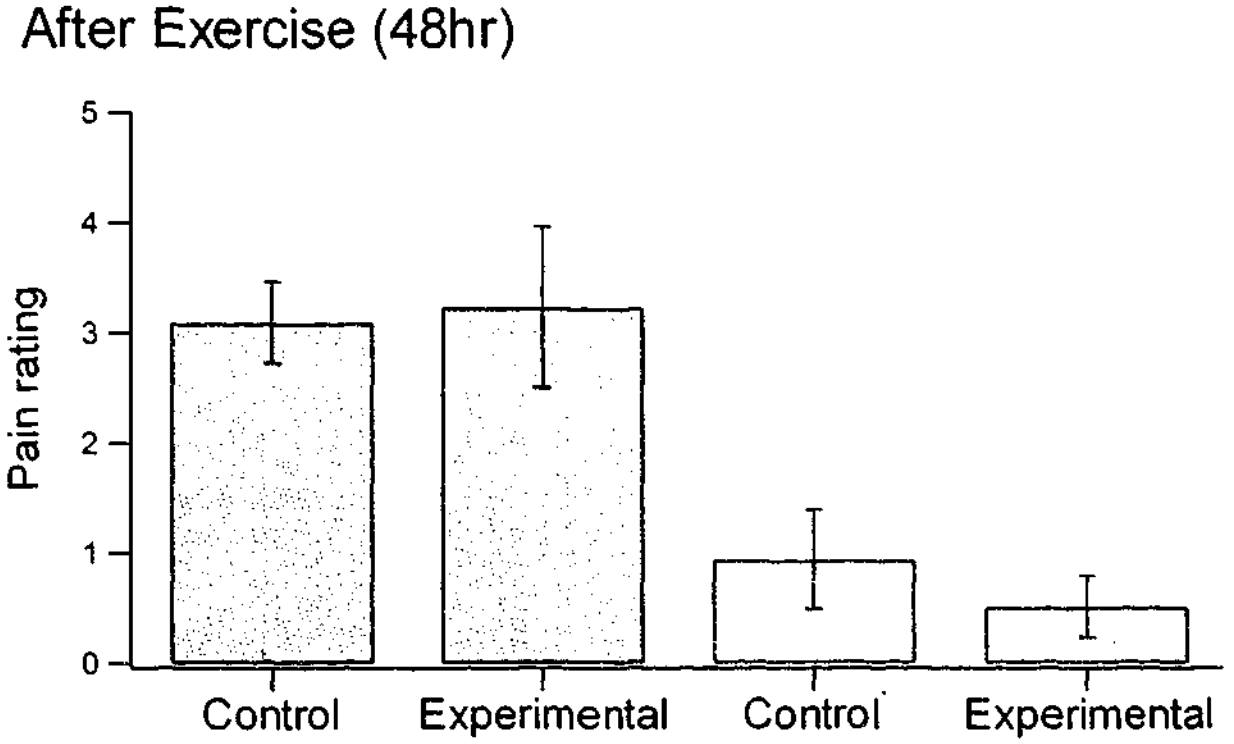
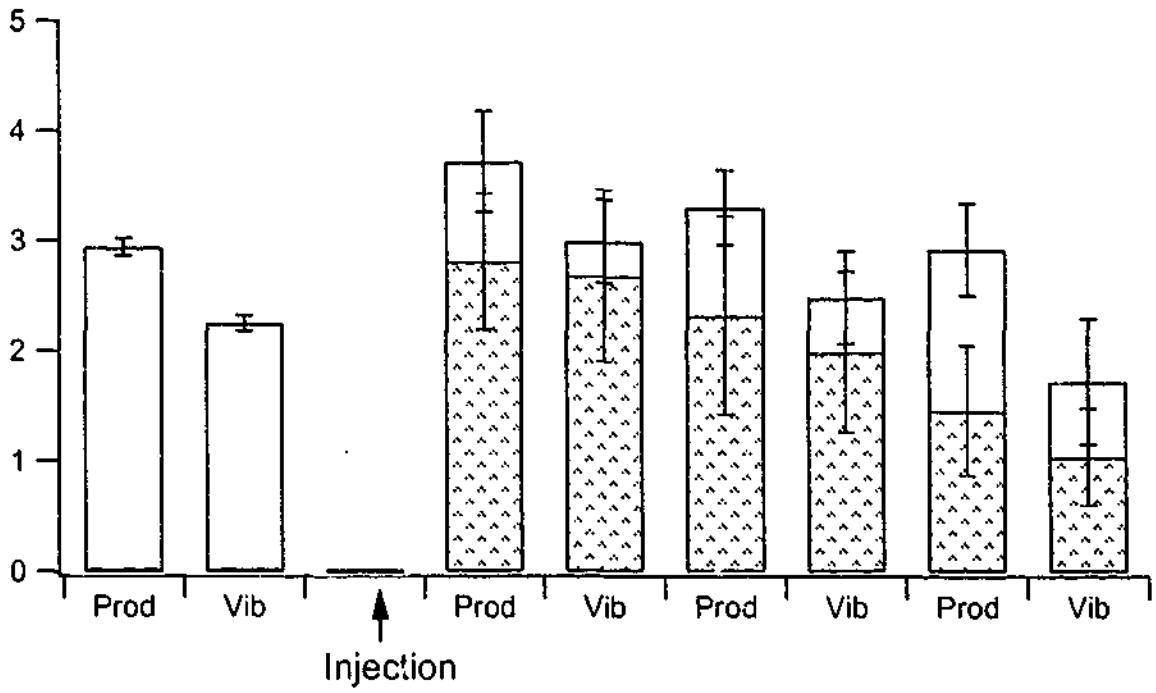

After Exercise (48hr)
Pain rating
5
4
3
2
1
0
Control
Experimental
Control
Experimental

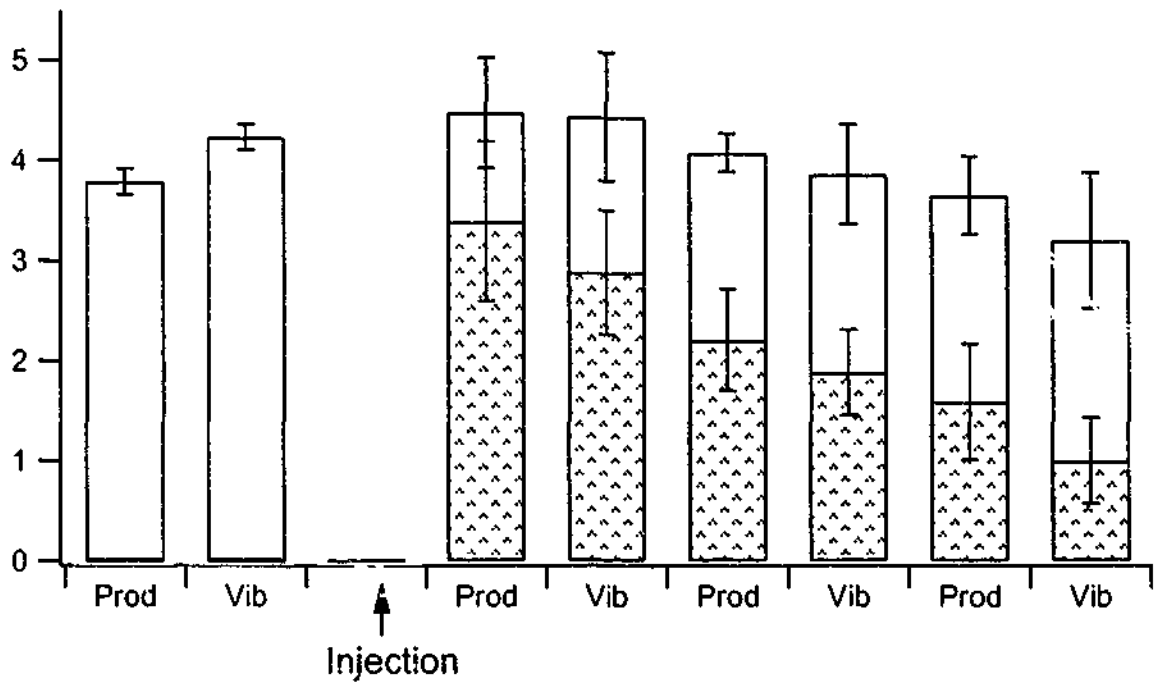
Figure 3.3

Mean pain ratings (±S.E.M.) from 5 subjects in response to muscle compression and vibration before and after injection of hypertonic (5%) saline. ***Top Panel:*** Unexercised leg. ***Bottom Panel:*** Exercised leg. First two columns in each panel, ratings before saline injection. The stippled areas in the subsequent columns represent saline pain, the open portions compression (Prod) or compression plus vibration (Vib). Each subsequent measurement was made 40sec apart.

Unexercised



Exercised



To ensure that measurements with mechanical stimulation during saline injections were made during a significant level of pain, only values were used that were made in the presence of 25%, or more, of the peak saline pain. Mechanical stimulation always increased soreness ratings above those reported from saline only. However, in the unexercised muscle, compression alone applied in the presence of saline pain increased the reported soreness more than compression superimposed with vibration (Fig 3.3). The opposite was true for the exercised DOMS muscle, with vibration in the presence of saline pain increasing the pain more so than compression alone (Fig 3.3). The difference in the unexercised muscle between the extra pain produced by compression alone and compression with vibration was significant ($p<0.05$). However in the exercised muscle this difference was not significant.

Since the mechanical stimulation was applied at various times after the onset of pain from saline, it was being measured during a changing baseline. It was found that at the peak of saline pain, the increments in soreness rating produced by mechanical stimulation were smaller than when saline pain had begun to subside (Fig 3.3). This suggests some kind of saturation effect. The first pair of measurements for the 5 subjects made close to the peak of saline pain in an unexercised muscle led to an average increase of 32% in soreness ratings from compression (Fig 3.3). The increase was only 11% when compression was combined with vibration, that is, it reduced soreness levels by 21%.

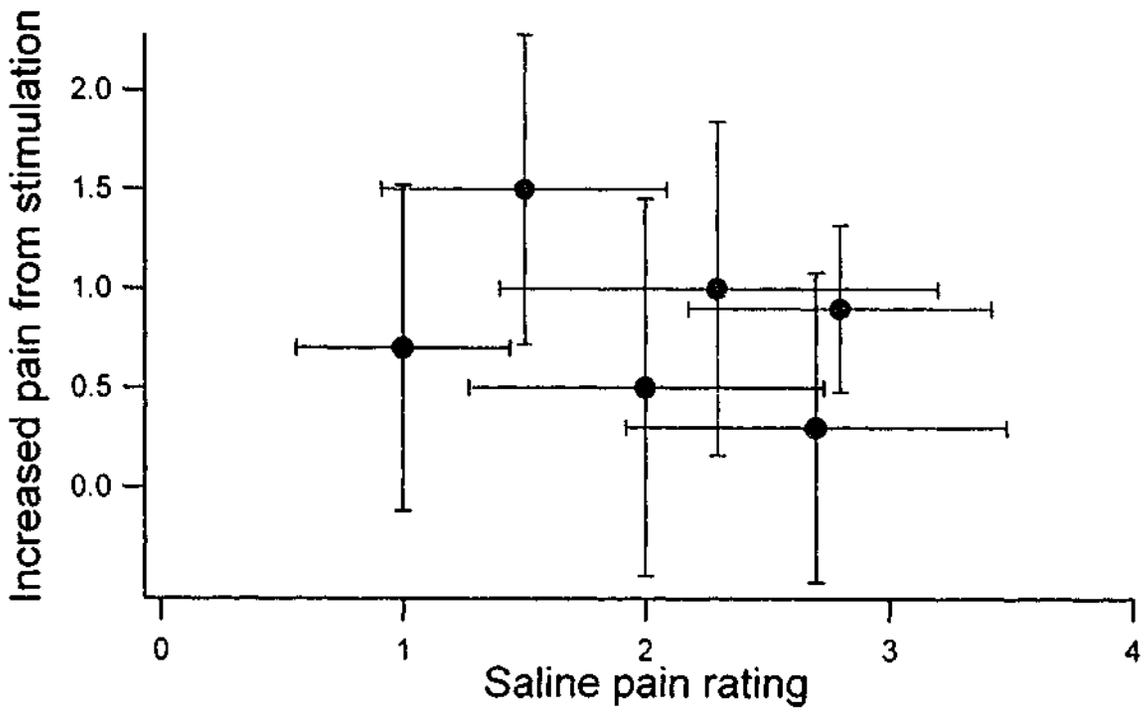
Larger differences emerged as the pain subsided (Fig 3.3). The mean reduction in soreness from vibration across the three pairs of measurements was 24.1% (± 5.1). When the experiment was repeated in a muscle exhibiting the symptoms of DOMS, compression at the peak of saline pain increased soreness by an average of 32% while compression plus vibration increased soreness further, by a total amount of 54%, that is, vibration increased soreness from compression by an additional 22%. For the three pairs of measurements, pain increases from vibration were 44.1% (± 23.4).

The effects of saline pain on the incremental increase in pain from mechanical stimulation can be seen more clearly in figure 3.4. The extra increments in soreness rating were found to be significantly dependent on the existing saline pain level ($p<0.05$). As the saline pain increased the extra increments in pain rating from the mechanical

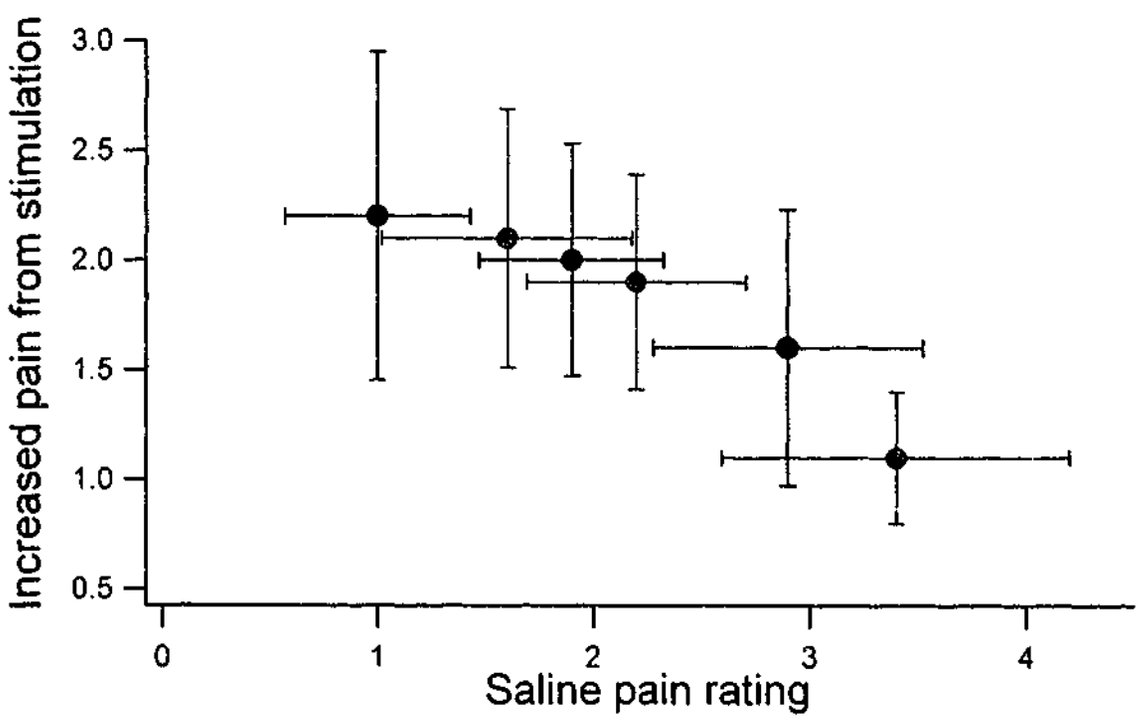
Figure 3.4

Mean incremental increase in pain from mechanical stimulation (±S.E.M.) plotted against mean saline pain rating (±S.E.M.). ***Top Panel:*** Unexercised leg. ***Bottom Panel:*** Exercised leg. Shown are the mechanical stimulations expressed as compression (red) or compression plus vibration (blue).

Unexercised



Exercised



stimulation decreased for both exercised and unexercised muscles. In the unexercised muscle the r^2 for the compression plus vibration and compression were 0.62 and 0.68 respectively. In the exercised muscle the r^2 were 0.22 and 0.67 respectively.

For 3 subjects with DOMS, as well as receiving an injection of 5% saline at a sensitive spot, a second injection of 0.9% isotonic saline was given at another tender site. A second injection of isotonic saline was given into their unexercised muscle. None of the subjects reported any sustained soreness from the isotonic saline. Pooled pain ratings showed that while compression and vibration had their expected effects, these were not significantly different before and after injection of isotonic saline.

Saline and nerve block

First, pain ratings from hypertonic saline injection were measured before the block. When the large-fibre block was complete, as evidenced by disappearance of the H-reflex, another saline injection was given and the soreness rating scored again. The scatter of pain ratings for each subject shows that there was a distinct trend for pain ratings before the block to be higher than during the block, although for 2 subjects the difference was very small (Fig 3.5A). The overall pain rating from hypertonic saline injection before the block gave a mean of 4.26 (± 0.3) (Fig 3.5B). The mean value measured with the block in place was 3.5 (± 0.3) (Fig 3.5B). This difference in pain ratings was significant ($p<0.05$).

In addition, during the block, the pooled data suggested a small, but non-significant, increase of latency to heat. It was also observed that although contractions during the nerve block were weakened they were not fully abolished, suggesting that motor axons had not become fully blocked.

DISCUSSION

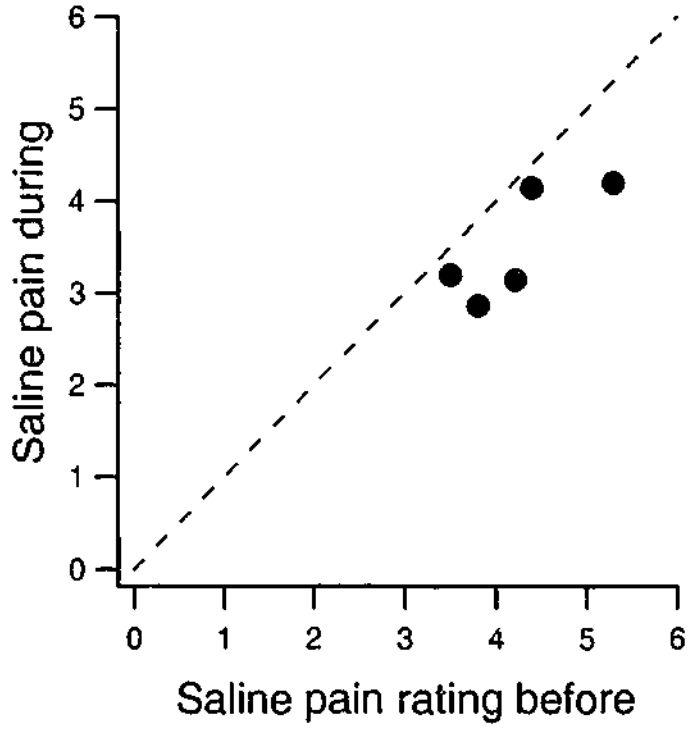
Saline injections

As mentioned earlier, hypertonic saline excites a majority of small diameter afferents, with minimal effects on muscle spindle afferents (Paintal, 1960; Iggo, 1961). Experiments on the gastrocnemius muscle of the dog (Kumazawa & Mizumura, 1977)

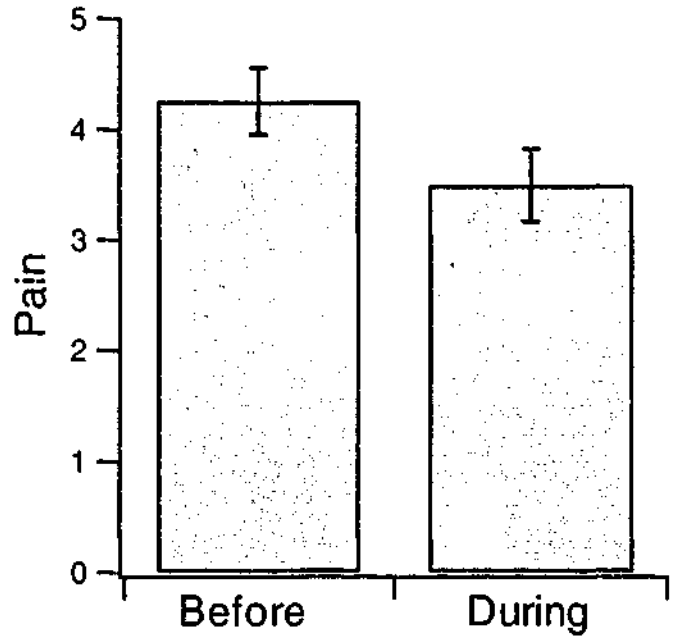
Figure 3.5

(A) Each subject's mean saline pain rating during the nerve block plotted against mean saline pain rating after the nerve block. *(B)* Histogram representation of mean pain rating (±S.E.M.) for 5 subjects before and during the nerve block.

A


Saline pain during
Saline pain rating before
0
1
2
3
4
5
6

B


Pain
Before
During
0
1
2
3
4
5

indicated that close arterial injection or topical application of sodium chloride excited 89% of units with Aδ and C fibre conduction velocities, presumed to be polymodal nociceptors. Only 19% of stretch receptor afferents responded. Such findings suggest some degree of selectivity in the action of sodium chloride. While conduction velocity of the stretch receptor afferents was not given, by these authors responses from muscle spindles and tendon organs would be expected to be limited because of the presence of a capsule surrounding the sensory endings which would act as a diffusion barrier. Hence, hypertonic saline's relatively selective action on small fibres made it a suitable model for studying DOMS.

Currently DOMS is largely accepted as being due to the sensitisation of nociceptors, through the inflammatory response. The injection of hypertonic saline into the muscle causes high extracellular Na^+ concentrations that stimulate nociceptors directly, which does not normally occur in muscle tissue (Graven-Nielsen et al., 1997b; Thunberg et al., 2002). Hence, if nociceptors were in a sensitised state from DOMS, hypertonic saline stimulation of nociceptors would be expected to produce a larger response, to produce more pain.

However, the findings showed that the soreness evoked by hypertonic saline was not significantly different in muscles of subjects experiencing DOMS compared with unexercised muscles (Fig 3.2). These observations are not consistent with the nociceptor sensitisation hypothesis for DOMS, at least in its simplest form. Effectively, this result suggests that the origin of pain associated with DOMS is different to the pain associated with injections of hypertonic saline.

It might be argued that in the subjects tested here, nociceptors were in a temporally desensitised state. But that does not fit the observations. There was no evidence of systematic changes in measured pain thresholds to local muscle pressure throughout the half-hour, or so, over which subjects lay prone while test solutions were injected into their muscles.

It also could be argued that the phenomenon of diffuse noxious inhibitory control (DNIC) may have played a role. DNIC is an electrophysiological phenomenon in

animals in which the activity in convergent dorsal horn multireceptive neurons is inhibited by noxious stimulation applied to various sites on the body (Le Bars, Dickenson & Besson, 1979). In humans the effect is associated with reduced subjective pain ratings or increased pain thresholds when painful conditioning stimuli are applied to widespread areas of the body (Talbot et al., 1987). Therefore, it is conceivable that a hypertonic saline injection into a DOMS muscle acted to inhibit the overall pain, so preventing an additive effect of pain in the DOMS muscle, compared to pain in the unexercised muscle. However, recent work has shown that saline–induced muscle pain has an inhibitory effect on perception of painful electrical stimulation only when it was injected into an extra-segmental location, anatomically separated from the stimulation (Watanabe, Svensson & Arendt-Nielsen, 1999). In contrast, pain induced in the same location as the stimulus did not cause an inhibitory effect (Watanabe et al., 1999). Therefore, it is unlikely that in the present experiments DNIC was a factor, as saline was injected directly into the site of the muscle with DOMS.

Although hypertonic saline does not have direct effects on spindle afferents, recent studies have suggested that it may have indirect effects. Small volumes of hypertonic saline have been shown to modulate movement-related responses from jaw muscle spindles (Ro & Capra, 2001). Similarly in cats saline injections were shown to induce large, statistically significant changes in muscle spindle afferent activity (Thunberg et al., 2002). The most frequent result was an increase in mean rate of discharge together with a reduction in depth of modulation, the type of changes usually associated with increased static fusimotor drive (Thunberg et al., 2002). It has been suggested that group III and IV muscle afferents could constitute the afferent branch of the reflex pathway involved in the altered muscle spindle afferent activity. A number of studies have shown by direct recording or by recordings from muscle spindle afferents, that group III and IV muscle afferents reflexly excite fusimotor neurons (Ellaway, Murphy & Tripathi, 1982).

Therefore if noxious stimulation of muscle nociceptors can activate a chain of neuronal responses that ultimately influences muscle spindle afferent discharge, the possibility that muscle spindles may be involved in generating the hypertonic saline pain should be considered. However such a mechanism is not consistent with the results. If hypertonic saline produced a reflex effect from muscle spindles, one would expect, first the

production of an initial pain phase caused by the direct action of small afferents, and then a second phase of muscle pain induced by reflex action. However, no such biphasic increase in pain was observed, although the time course may be too slow to show such a biphasic change (Fig 3.1). Nonetheless, in unexercised muscles, if hypertonic saline had an indirect action on spindles one would have anticipated a similar, but weakened form of vibration effect, whereby saline would cause less pain, compared to that produced in exercised muscles with DOMS. However, hypertonic saline produced pain levels that were not significantly different between the unexercised and exercised muscles. Therefore, it is unclear whether indirect effects of hypertonic saline on spindles have played a role.

The specificity of hypertonic saline action is further complicated by recent observations that saline has varied effects in different muscle groups. Most of the work using hypertonic saline has been carried out on large muscles such as biceps brachii and triceps surae. More recent experiments on the hand muscle adductor pollicis brevis revealed some problems with the technique (Taylor, Proske & Gandevia, unpublished observation). Hypertonic saline was observed to evoke local muscle fasciculation, presumably a direct action of high levels of extracellular Na^+ on motor axons. Such motor unit activity could engage muscle spindles to produce central effects which would complicate the findings further. Although, the experiments here were carried out in the triceps surae where no such activity is observed, in future experiments surface EMG should be carefully monitored during and following the period of the injection to detect such direct effects.

In future experiments alternate algesic substances rather than hypertonic saline could be used. It has been demonstrated recently that isotonic saline heated to 48°C, when injected into a muscle acts as a noxious stimulus (Graven-Nielsen, Arendt-Nielsen & Mense, 2002). The muscle pain induced by hypertonic saline and by isotonic saline at 48°C is presumably mediated by two different mechanisms. Hypertonic saline-induced muscle pain is most probably due to a non-specific excitation of nociceptors whereas the thermal effects are probably mediated by heat-sensitive nociceptors (Hertel, Howaldt & Mense, 1976; Mense & Prabhakar, 1986) similar to those present in the skin. Therefore, this method may be preferable to the use of hypertonic saline for modeling muscle pain as it

would avoid muscle fasciculation and possibly potential reflex effects. Furthermore the argument for differences between the neural mechanisms for DOMS and other forms of muscle pain would be strengthened if it could be shown that there was no summative effect between the effects of DOMS and hot isotonic saline.

Saline and vibration

When the injected site was stimulated mechanically, using compression or compression plus vibration, there was a non-linear summation of perceived pain levels. At the peak of saline pain there were only modest further increases in perceived pain from mechanical stimulation, while as the saline pain declined the pain response to the mechanical stimulation grew proportionally. A saturation effect seemed to be operating, limiting the total level of pain subjects could experience with these kinds of stimuli. It remains unclear whether the saturation is simply due to the value of the total pain or to its constituents , mechanical pain and chemical pain.

In both the exercised and unexercised muscle, compression and vibration increased the level of pain above that produced from saline alone. However, in an unexercised muscle, for all levels of saline pain, vibration reduced the soreness from compression. As saline pain wore off, and mechanical pain increased, the effect of vibration also increased. This increase may have been the result of less saturation. By contrast, in exercised muscles vibration produced a small non-significant increase in pain above that from compression alone. This compares with a significant increase in pain from vibration when measured in the absence of saline pain (Fig 2.2).

The finding that, after hypertonic saline injection, in an unexercised muscle, vibration reduced the pain from compression, the opposite from the effect of vibration in muscles with DOMS, suggests that DOMS and the pain associated with hypertonic saline are caused by different mechanisms. Although they both may involve endogenous mediators that sensitise nociceptors, the nociceptive information from saline injection appears to be processed differently from that for DOMS. Hence, once again this suggests that in DOMS, mechanisms other than nociceptor sensitization are involved. Although a contribution from the sensitization of silent nociceptors or the induction of central

sensitization in DOMS cannot be discounted, a component from large fibre involvement seems increasingly likely.

As explained in the previous chapter, an area of uncertainty in the vibration experiment was that although it was known that nociceptors do not respond to vibration, it is unknown whether they do so in a sensitized state. Hence, a situation with DOMS where sensitized nociceptors become vibration sensitive, could not be ruled out. However, the results of this chapter suggest that nociceptors when activated by saline are not vibration sensitive. This finding points to the likelihood of a neural mechanism for DOMS, that is different. Perhaps the onset of inflammation alters processing of mechanosensory and nociceptive information at the spinal cord level.

Saline and nerve block

In the previous chapter it was shown that a large-fibre block of the sciatic nerve led to a significant increase in pain threshold from DOMS. This supported the view that there was a large-fibre contribution to DOMS. Here a further experiment using the nerve block technique was carried out.

It has been argued here that the nociceptive pathways for saline pain and DOMS are different. As hypertonic saline pain is thought to be mediated largely by small muscle afferents, its intensity would be expected to remain unaffected by a large nerve fibre block. However, it was found that across all the subjects the intensity of pain produced by saline significantly decreased by an average of 19% during the nerve block. This result was unexpected and is at odds with our previous results.

It could be argued that the compression block not only blocked the conduction of large fibres, but also a significant proportion of small fibres, hence decreasing the pain from hypertonic saline. However, such a progression of the block is doubtful, as the integrity of conduction in small afferents was monitored indirectly with hot and cold stimuli. The latency of painful heat stimuli did not significantly change. Alternately, it may be that hypertonic saline is not completely specific for small fibres (Kumazawa & Mizumura, 1977).

Therefore, it could be concluded that the pain associated with hypertonic saline acts in part through large fibres. As previously mentioned, various studies attest to the indirect effects of hypertonic saline on spindles (Capra & Ro, 2000; Ro & Capra, 2001; Thunberg et al., 2002). Its been suggested that stimulation of the muscle nociceptors may activate a chain of neuronal responses that will ultimately influence muscle spindle discharge (Ro & Capra, 2001). Therefore, the block of large fibres would prevent reflex responses of spindles, and block any access that large fibres may have to the pain pathway. Thus, although this result was unexpected, it does not rule out the possibility that a proportion of large fibre are involved in the generation of DOMS.

Recently Matre et al. (1998) showed that muscle pain in the ankle muscles from hypertonic saline increases the stretch reflex, but not the H-reflex. A potential interpretation was that muscle pain increases dynamic sensitivity of the muscle spindles during active or passive stretch, but not the excitability of the alpha motoneurons. Therefore, as hypertonic saline was injected into the relaxed muscle in my experiment, it could be reasoned that a increase in muscle spindle discharge does not play a significant role in the initiation of the muscle pain associated with saline.

In addition, it could be argued that an adaptation process inhibited the possible level of pain from the second hypertonic saline injection into the same muscle. Tegeder et al. (2002) found that following the injections of hypertonic saline into the biceps muscle there was a decline in pain intensity during the second series of injections suggesting that some sort of adaptation occurs in this muscle pain model. In animal studies, recordings from muscle afferents have shown that the firing rate after a second infusion of hypertonic saline was decreased as compared to the first (Paintal, 1960). Although the mechanism for such a suppression is unknown, it has been suggested it could be due to an increase in K^+ levels which may result in the elevation of the resting potential of the nociceptor (Markowitz, Bilotto & Kim, 1991) and the axon membrane (Orchardson, 1978).

CHAPTER FOUR

Matching different levels of isometric torque in elbow flexor muscles after eccentric exercise

INTRODUCTION

As we have all experienced, our ability to make judgments of muscle force worsens after strenuous exercise. Fatigue that reduces the tension output from muscles is thought to be a contributing factor (Gandevia & McCloskey, 1978). However, whether it's a disturbance of predominantly the 'sense of tension' or 'sense of effort' is still under debate.

After eccentric exercise the fall in tension is attributed both to fatigue and to damage to muscle fibres. Eccentric exercise is particularly interesting because it leads to DOMS. In a recently reported experiment (Brockett et al., 1997), subjects' elbow flexors of one arm were subjected to concentric exercise while elbow flexors of the other arm were given eccentric exercise. In a subsequent tension matching task, subjects were found to systematically undershoot the target tension with their eccentrically exercised arm. As there was no significant drop in MVC, it was suggested the errors were not likely to be due to a central mechanism. Rather, it was proposed that the matching errors might be the result of a disturbance of the sense of tension, resulting from damage to tendon organs. Contractures in the muscle fibres damaged by eccentric contractions would cause tendon organ output of the muscle to rise because of the higher resting tension, leading to the observed tension mismatch. This conclusion, however was contrary to that arrived at by others (Carson et al., 2002; Saxton et al., 1995).

Since that time it has been shown in other experiments, that although after eccentric exercise whole-muscle passive tension increased, the ability of tendon organs to accurately signal changes in muscle tension was not disturbed (Gregory et al., 2002). The above findings led us to reassess the earlier results of Brockett et al. (1997) and to repeat the experiments, but asking subjects to match a range of tensions. A more severe eccentric exercise was also used as only small drops in MVCs were observed by Brockett et al. (1997), possibly indicating that only minimal muscle damage was induced.

During a weak voluntary contraction by elbow flexors against a spring, subjects can be instructed to maintain either the tension constant or maintain the angular position of the elbow. Under these conditions, very small changes in active force or position were detectable (Colebatch & McCloskey, 1987). It was suggested that the afferents upon which subject's judgments were based did not arise from cutaneous receptors within the forearm but most probably arose from receptors in the elbow flexor muscles. In addition, given the difficulty in achieving maintenance of constant force or position by a subject with complete deafferentation (Gandevia, 1996) a contributory role from the motor command was thought to be unlikely. It was reasoned that for weak voluntary contractions that more precision may be required to achieve a match, and that therefore the influence, if any, of a sense of tension, might emerge more clearly. Hence, a range of matching tensions were used going low as 2% MVC to high as 30% MVC.

Based on the assumption that the EMG recorded from the contracting muscle was an indication of the level of motoneuron activation and therefore the centrally generated effort (Gandevia, 2001), EMG was also recorded to see whether the observed torque matching errors were accompanied by any mismatch in the EMG. Furthermore, any changes to the EMG:force relation were studied after eccentric and concentric exercise.

Support for the idea of using the surface EMG as an estimate of the descending motor command comes from a study in which the force generating capacity of a muscle was varied by changing its length and asking subjects to match the forces in two corresponding muscles at different lengths (Cafarelli & Bigland-Ritchie, 1979). The matching function changed depending on whether the reference or matching muscle had the greater force generating capacity. However, force sensation remained constant whenever the activation, as measured by EMG, of the two muscles was the same. Those results were interpreted as providing support for a centrally mediated sensation of force (Cafarelli & Bigland-Ritchie, 1979). Moreover they suggested the surface EMG could be considered a measure of the excitatory input to a muscle. However, factors such as spinal reflexes could undoubtedly alter the activity in motoneurons and therefore the

EMG, independent of centrally generated effort. Therefore such a measure of EMG is an approximation of effort and may not be completely accurate.

Although the slope of the resulting force-matching was proportional to the ratio of the MVCs of the two muscle groups, the relation between the EMGs recorded from the two muscles was not influenced by muscle length, which suggests that when the excitatory inputs to the muscles were the same, the sense of effort associated with each contraction was similar.

To further investigate the predominance of effort in force matching tasks, but without the complicating effects of muscle damage and fatigue, we also repeated the experiments of Cafarelli and Bigland-Ritchie (1979) using changes in the muscle's torque-angle relation to alter levels of torque.

METHODS

Subjects

A total of 26 subjects took part in these studies (mean age, 22 years). Subjects were required to have no existing musculoskeletal abnormalities, or be involved in any regular exercise program. All subjects gave informed consent and the experiments were approved by the Monash University Committee for Ethics in Human Experimentation.

Matching tension after eccentric exercise

Apparatus

The testing equipment consisted of two padded boards hinged along an axis of rotation coincident with the elbow joint (Fig 4.1). The boards were locked in the vertical position by a pair of horizontal aluminium shafts which were each attached to a proving ring with strain gauges cemented on the inside and the outside and connected in a full-bridge configuration (Brockett et al., 1997). Subjects were seated with their forearms strapped to the boards using Velcro® strapping. Extra padding was put around their forearms to minimize cutaneous sensation from skin and for added comfort. A screen prevented the

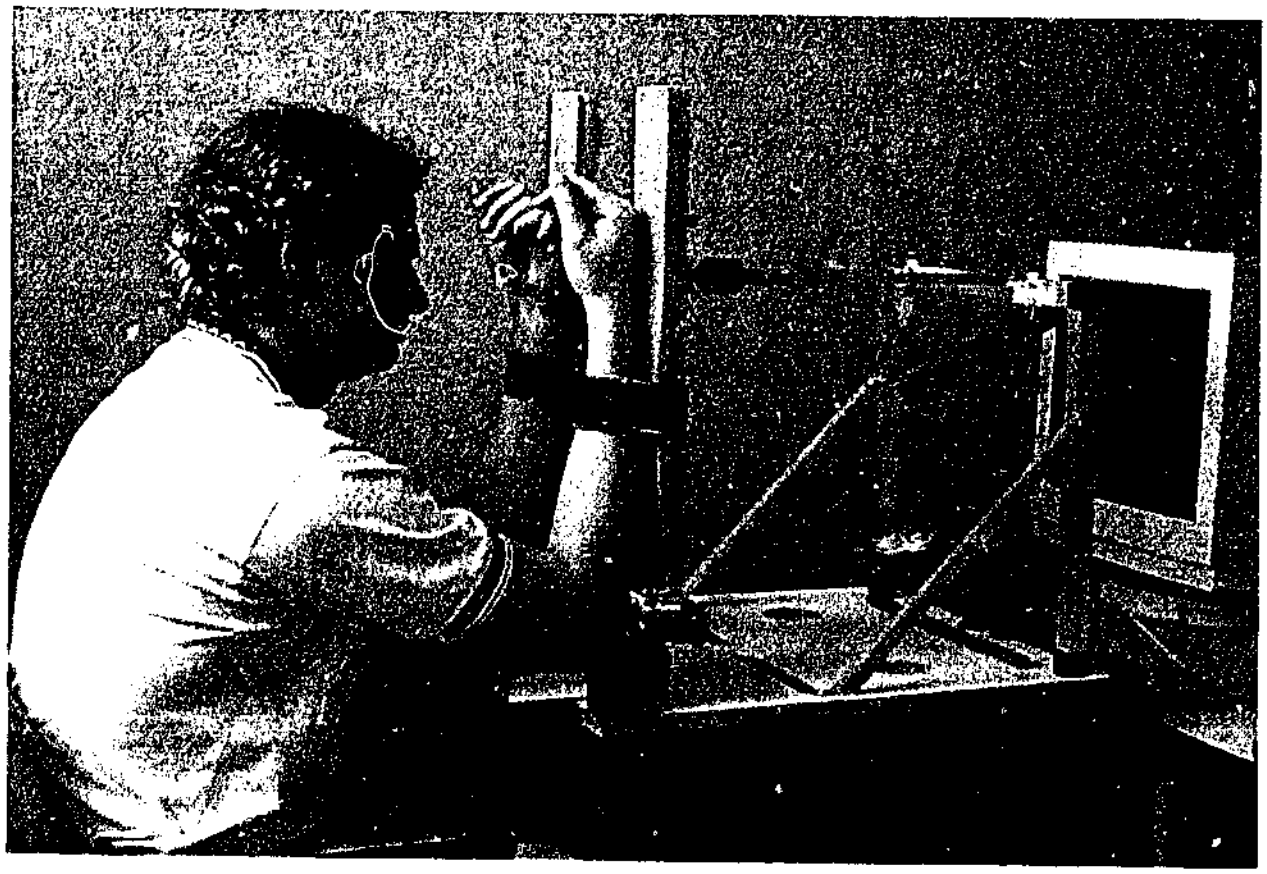


Figure 4.1

A subject performing the force matching task on the testing equipment. Strain gauges measured the amount of torque generated by the elbow flexors of each arm. A visual display of the target torque needed to be matched by the reference arm was shown on the computer screen. Note the screen that prevents subjects from seeing their arms was removed in the picture so that the testing equipment could be seen.

subjects from being able to see their arms but they were able to see a computer monitor which provided visual feedback of the torque level generated by the reference arm.

The torque traces were acquired into Igor Pro (Wavemetrics, Ore, U.S.A) running on a Macintosh 6100 Computer (Apple, U.S.A.) for subsequent analysis.

Matching Tension

Eleven subjects, 8 males and 3 females (mean age, 23 years), took part in this study. Subjects were seated and the height of the chair adjusted in relation to the padded board so that the elbow angle was ≈90 degrees, with the forearm upright. Once subjects' forearms were secured to the vertical boards, the maximum voluntary contraction (MVC) of subjects elbow flexor muscle, the biceps brachii, was measured. Three MVCs of 5 seconds duration were carried out by each arm separately before each test session. The MVCs were averaged for each arm, and this value was taken as the MVC for that session.

Subjects were asked to generate, under visual feedback, a given target level of force measured as torque in elbow flexors of one arm, designated the reference arm. Once subjects had satisfactorily achieved the reference torque for ≈2 seconds, they were asked to match it with the other, indicator arm, whose torque output was not visually displayed. Subjects were instructed to say 'yes' when they believed the torques in the arms were matched, at which point the recording was stopped. For each arm the average torque they generated over the last 0.7 s before recording was stopped was taken as the value for the match. Throughout the series, subjects were asked to match what they perceived to be the torque generated in the reference elbow flexors and not the effort required to reach that tension.

Five different target torque levels were used, 2%, 5%, 10%, 20% and 30% MVC of the reference arm. Six subjects performed the torque matching task at 2%, 5% and 10% MVC. Two of these and another five new subjects carried out matches at 10%, 20% and 30% MVC.

A total of 10 trials was performed by a subject at each torque level with the right and left

arms alternately acting as reference. To avoid any biasing effects, the 10 trials at each target were split into two sets of five. This created six sets (two for each target) of five trials, the order of which was randomised to avoid any systematic effects from fatigue. Immediately after the exercise and at 2, 24, 48, 72 and 96 hours later, measurements of MVC were made and subjects repeated the matching tasks.

Additional measurements

At the start of each test session, relaxed elbow angle was measured using a goniometer, with the subjects standing upright, their relaxed arms at their side (Fig 4.2). A decrease in angle represented a more flexed elbow. Pain thresholds were also measured using a compression gauge with a plunger diameter of 1.5 cm. This was used to assess the amount of DOMS the subject experienced. Subjects were asked to report as soon as they felt any pain during muscle compression by the gauge. Thresholds were measured at two sites, the musculo-tendinous region and the muscle belly, to receive an approximate idea of the distribution of the pain.

The exercise

One arm was randomly chosen to undergo eccentric exercise. The other arm acted as a control and did not exercise. This detail differs from Brockett et al. (1997), as they concentrically exercised the other arm. However, here it was decided a true control was required. The subject performed eccentric contractions of the elbow flexors using a Biodex® (Biodex Medical Systems, Inc., U.S.A.) isokinetic dynamometer which forcibly lengthened the elbow flexors as they contracted against it at a constant velocity (Fig 4.3). Isokinetic contractions were used because they could be maintained more easily, while also reducing the risk of injury.

Subjects were seated in the Biodex® with their arm flexed to approximately 60 degrees and holding the handle of the arm attachment (Fig 4.3). Subjects were required to resist elbow extension generated by the Biodex® which was set to yield at 30% of the subject's MVC. Brockett et al. (1997) used 20% MVC, therefore it was thought 30% MVC would be sufficiently more severe. Movement velocity was set at $60°s^{-1}$. This velocity was chosen as it appeared ideal, enabling subjects to remain in control, without discomfort. Subjects were instructed to exert just enough force to marginally slow the movement, but

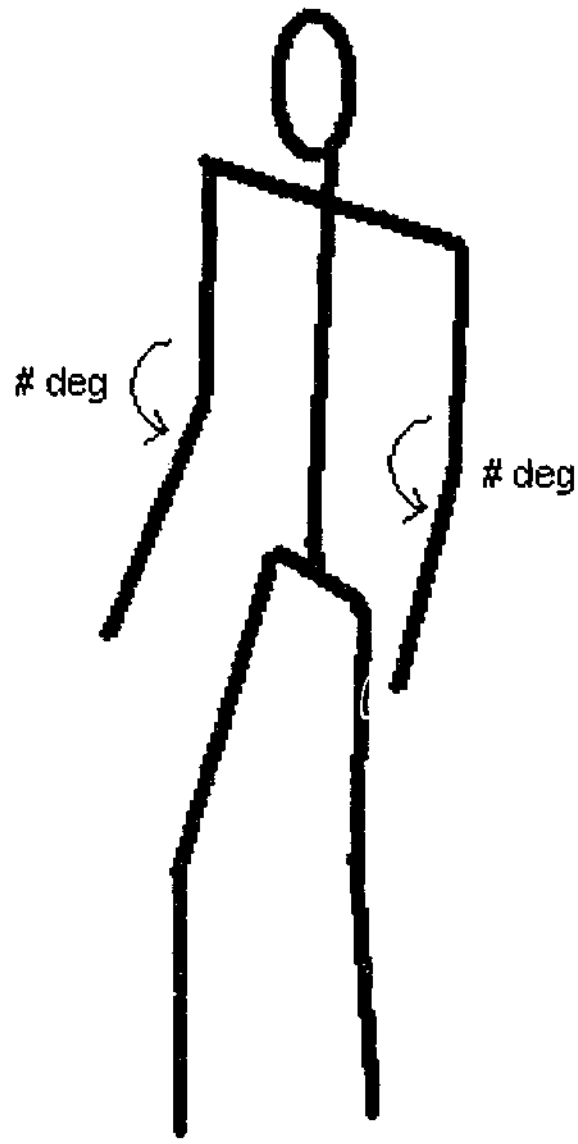


Figure 4.2

Diagrammatic representation of the relaxed elbow angles that were measured with subjects standing upright. Landmarks were drawn on the forearm and upper arm so that placement of the goniometer was consistent for each session.

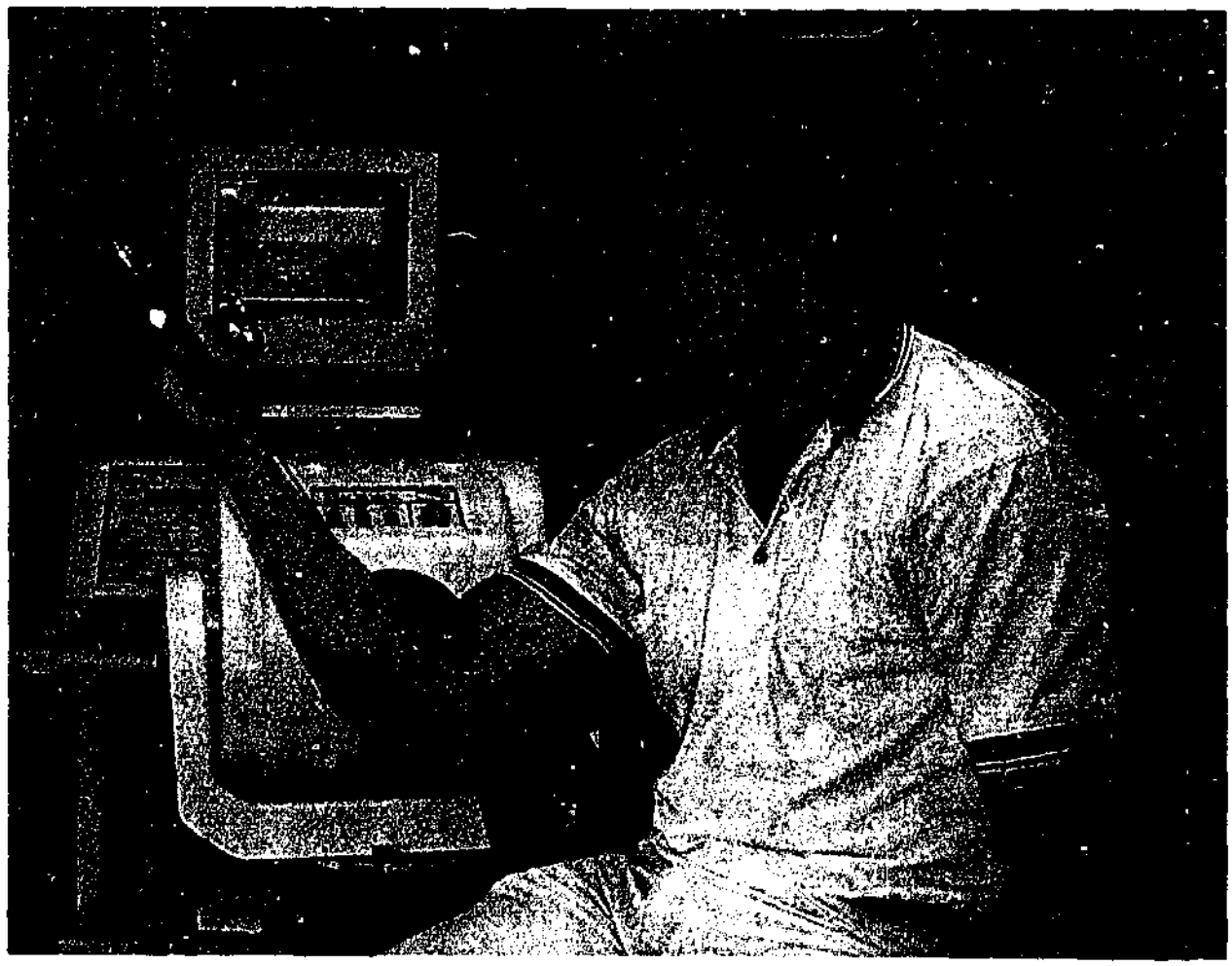


Figure 4.3

A subject performing eccentric contractions of their elbow flexors on the Biodex® isokinetic dynamometer. The subject is comfortably seated with their arm supported in position. The subject grips to the arm attachment that is adjusted to rotate coincident with the axis of rotation at the elbow joint.

not to the point where the machine stopped. When the subject's arm reached full extension the subject was instructed to relax their arm, which was returned to its starting position by the operator. The process was then repeated. The subject was constantly encouraged to maintain torque levels throughout each contraction. An exercise session consisted of bouts made up of 5 sets of 10 contractions with 30 seconds of rest between each set. Each subject performed 2, 3 or 4 bouts separated by 5 minutes of rest. The number of bouts performed was determined by subjects' levels of fitness and their ability to maintain torque levels during each contraction. Some subjects tired more readily than others. The relationship between raw torque and angle signals from the dynamometer, recorded for one individual during the eccentric contractions can be seen in figure 4.4. This subject eccentrically contracted their biceps muscle over approximately 100°, where 0° represented approximately full extension. An increased angle represented a flexed elbow, and so a shorter biceps muscle. The torque during 5 repetitions of eccentric contractions can also be seen in figure 4.4. Between each lengthening contraction, the muscle is at rest as the arm is returned to full flexion by the operator. The goal for each repetition was to have subjects keep their contraction as smooth as possible whilst contracting through their full range of movement.

EMG after eccentric exercise

Procedure

Six male subjects took part in the study, (mean age, 24 years). Only one arm was required for this experiment. Once the subject's forearm was secured to a padded board, locked in the 90° position, the MVC was measured. This MVC value was recorded and used to derive %MVC target levels.

With the aid of visual feedback, subjects were asked to develop levels of torque representing 2, 5, 10, 20, 30 and 50 %MVC. Once subjects had satisfactorily achieved the target for ≈2 seconds, they were asked to relax. The process was then repeated with another torque level. A total of thirty trials was carried out with five trials for each %MVC level. The order in which the 30 trials were carried out was randomised to control for any effects of fatigue.

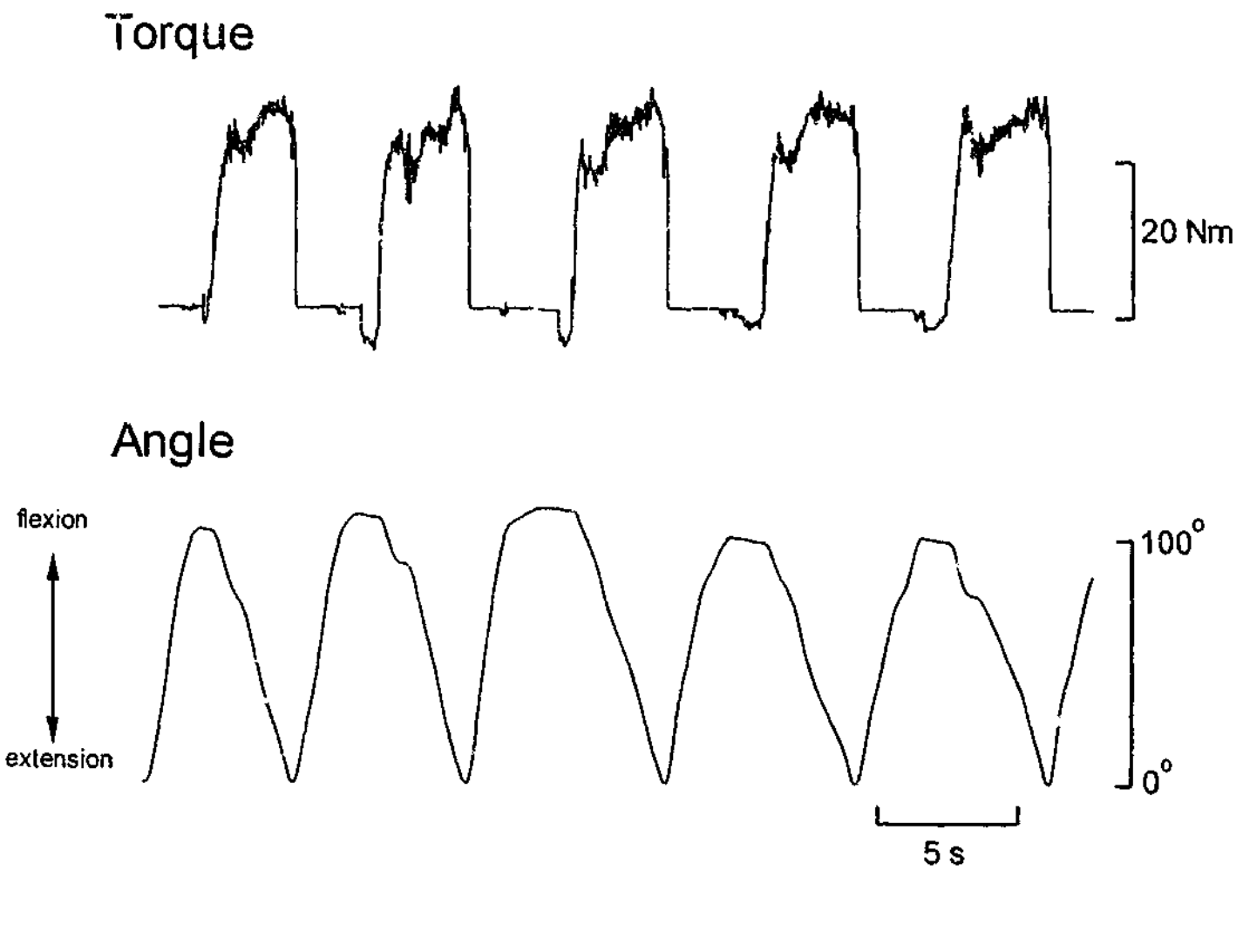


Figure 4.4

Torque (top panel) and angle (bottom panel) data from one subject's eccentric exercise bout on the Biodex® dynamometer. In the section shown there are 5 repetitions of eccentric contractions An angle of 0° represents full extension of the biceps muscle.

EMG was recorded throughout the test using 20mm diameter Ag/AgCl adhesive surface electrodes (3M 'Red Dot' paediatric electrodes, 3M HealthCare, Borken, Germany). The skin was shaved, cleaned and abraded before application of the electrodes longitudinally over the belly of the biceps brachii muscle. A pair of electrodes was placed approximately 20 mm apart, and a reference was placed on the dorsal surface of the wrist.

The EMG signal was acquired using 'Chart' software v3.6.3/s (ADInstruments, Australia), running on an Apple PowerBook using MacLab/8s (ADInstruments, Australia) with MacLab bioamps. The MacLab bioamps were set to apply a 10 Hz high pass filter and a 500 Hz low pass filter, with a 50 Hz notch filter. The EMG signal was sampled at 1000 Hz. The signal was full-wave rectified, and the relevant sections averaged (Fig 4.5). These sections were a three-second period of recording where the torque was held constant on the target and a one-second period of baseline signal. The baseline mean rectified EMG was subtracted from the active EMG in order to remove amplifier and electrode noise.

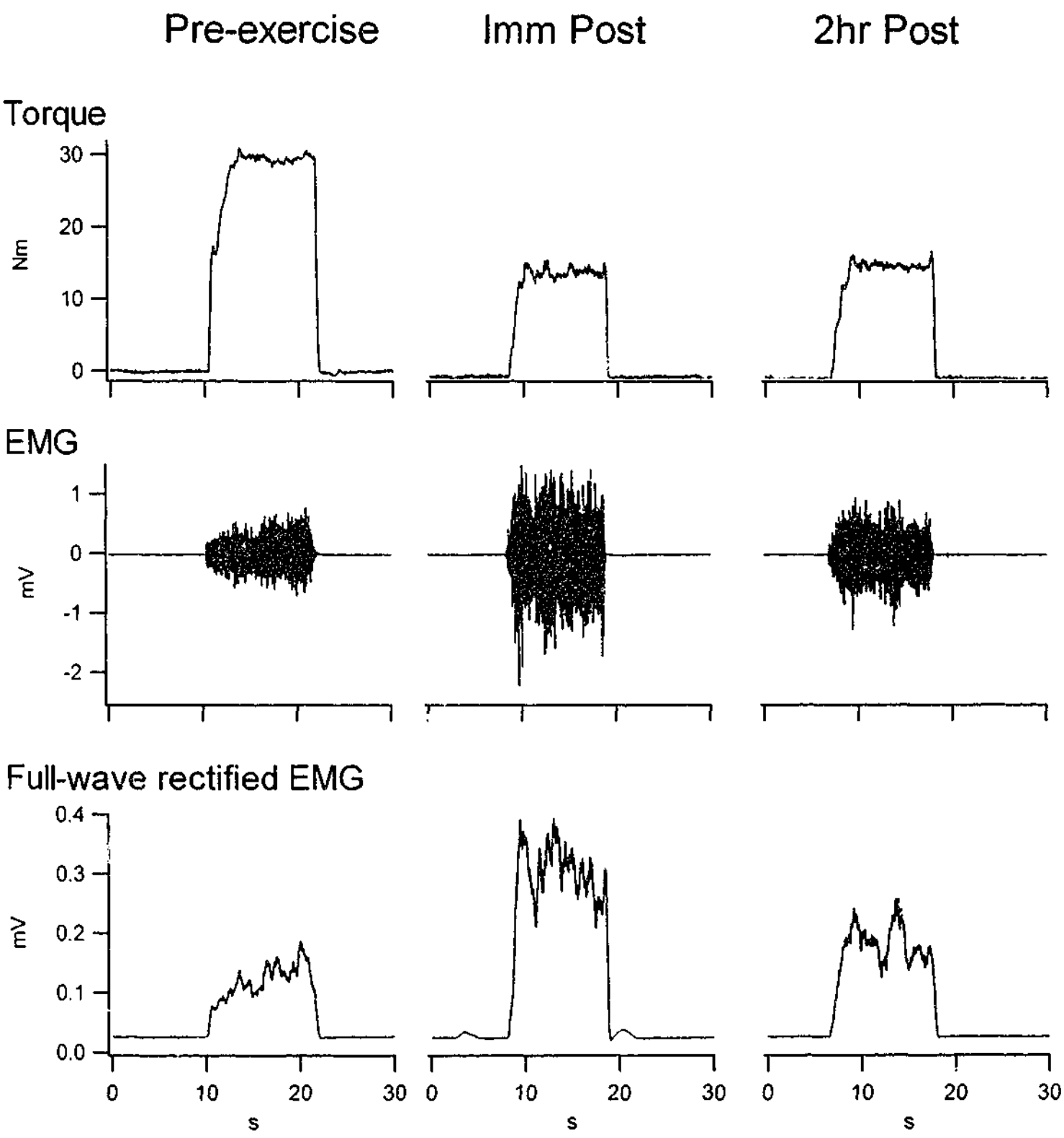
Exercise

The same group of 6 subjects carried out both eccentric and concentric exercise but with the exercise sessions separated by about a week. Each subject carried out the concentric exercise first since the period to full recovery is only a few hours. Subjects carried out the eccentric contractions on the Biodex® as described previously. They also performed the concentric exercise on the Biodex®. Here subjects were asked to flex their elbow, starting from an angle of 60° and to continue the movement until the arm was fully flexed. At that point they were asked to relax while the arm was re-extended for the next contraction. An exercise session consisted of bouts made up of 5 sets of 10 contractions with 30 seconds of rest between each set. Each subject performed 3-4 bouts separated by 5 minutes of rest. Subjects found the concentric exercise much more exhausting than eccentric exercise and not everyone was able to complete 4 bouts.

Immediately after the exercise and at 2, 24, 48, and 96 hours later, measurements of EMG were made at the different torque levels.

Figure 4.5

Example of torque (top panel), raw EMG (middle panel) and full-wave rectified EMG (bottom panel) data for one subject matching 30% MVC target tension at three different times: Pre-exercise, immediately post-exercise and 2hr post-exercise.


Pre-exercise
Imm Post
2hr Post
Torque
30
20
10
0
Nm
EMG
1
0
-1
-2
mV
Full-wave rectified EMG
0.4
0.3
0.2
0.1
0.0
mV
0
10
20
30
s

Matching torques at different elbow angles

Procedure

Eight subjects, 7 male and 1 female, took part in this experiment (mean age, 24 years). The testing equipment consisted of two padded boards as described previously. The indicator arm was locked in the vertical (90°) position, while the reference arm could be locked in place at various degrees of flexion (30°, 60°, 90°, 110°, 120°) (Fig 4.6). With the reference arm locked at one of the test angles, its maximum torque was measured. Subjects were then asked to develop a specific level of torque (%MVC) in the reference arm, and were given a visual display of the target to help them maintain it. Once subjects had satisfactorily achieved the reference torque for ≈2 seconds, they were asked to match it with the indicator arm flexed to 90°, without visual feedback. Subjects were instructed to say 'yes' when they believed they had achieved an accurate match with the two arms, at which point the tensions were recorded. Two different target levels were used, 5% and 20% MVC, measured for the reference arm at each angle. A total of 5 trials was performed at each torque level. This was then repeated, with the reference arm moved to five different angles of flexion (30°, 60°, 90°, 110°, 120°). EMG was also recorded throughout the test using surface electrodes, as described previously.

Statistical analysis

Data was stored on a Macintosh computer (Apple, Cupertino, California) and analysed using the program Igor Pro (Wavemetrics, Ore, U.S.A) and Excel (Microsoft, Redmond, WA). The statistical analysis program used was Data Desk (Data Description, Ithaca, NY). All results listed in the text figures are expressed as the mean, plus or minus standard errors of the mean (± SEM) , calculated from the pooled data.

For the matching tension after eccentric exercise experiment, three factor ANOVA tests were performed to test the significance of the changes in tenderness, relaxed arm angle and MVC. The factors used for tenderness were site (muscle-tendon junction, muscle belly), time and subject For relaxed arm angle and MVC the factors were arm, time and subject. Arm and time were classified as fixed factors, while subject was defined as being a random factor. This was based on the fact that, although the arms were paired, there were likely to be differences between them.

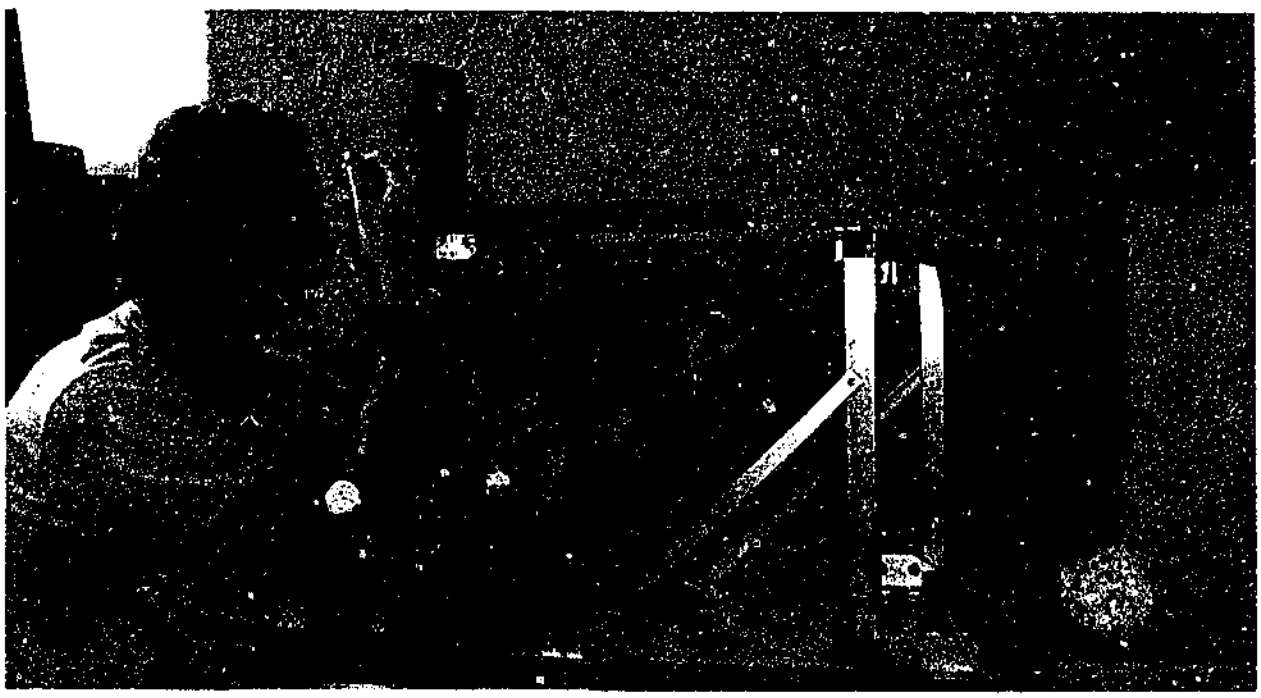


Figure 4.6

A subject performing the force matching task on the testing equipment with the indicator arm locked in the vertical (90°) position, while the reference arm is locked in at 60°.

Because the strength of subjects varied, all matching torque values were normalised relative to the subject's pre-exercise MVC. A three factor ANOVA was performed to test the significance of errors in matching torques produced by the indicator arm compared to the reference arm, for different torque levels. The factors tested were target torque, time since the eccentric exercise bouts and subject. Where an ANOVA was significant ($p<0.05$), an LSD (least significant difference) *post hoc* test was used to look for significant differences at different times.

The EMG for each subject was normalised with respect to the EMG of their pre-exercise MVC (see results). A three-factor ANOVA was performed to test for significant changes in the normalised EMG. The factors used were time, target (% MVC) and subject. A two-factor ANOVA was performed to test for significant differences in normalised EMG over time for a particular % target with subject as a random factor.

For the matching torques at different elbow angles experiment, a three-factor ANOVA was performed to test for significance in errors in torque produced by the indicator arm compared to the reference. The factors used were angle, target (%MVC) and subject. Where an ANOVA was significant ($p<0.05$), an LSD *post hoc* test was used to look for significant differences in error at 90^{o} compared to other angles.

Regression tests were done to analyse the relationship between error and MVC drop. A *chi squared* test was performed to assess the significant difference for this relationship between the conditions: after eccentric exercise and after changing elbow angle.

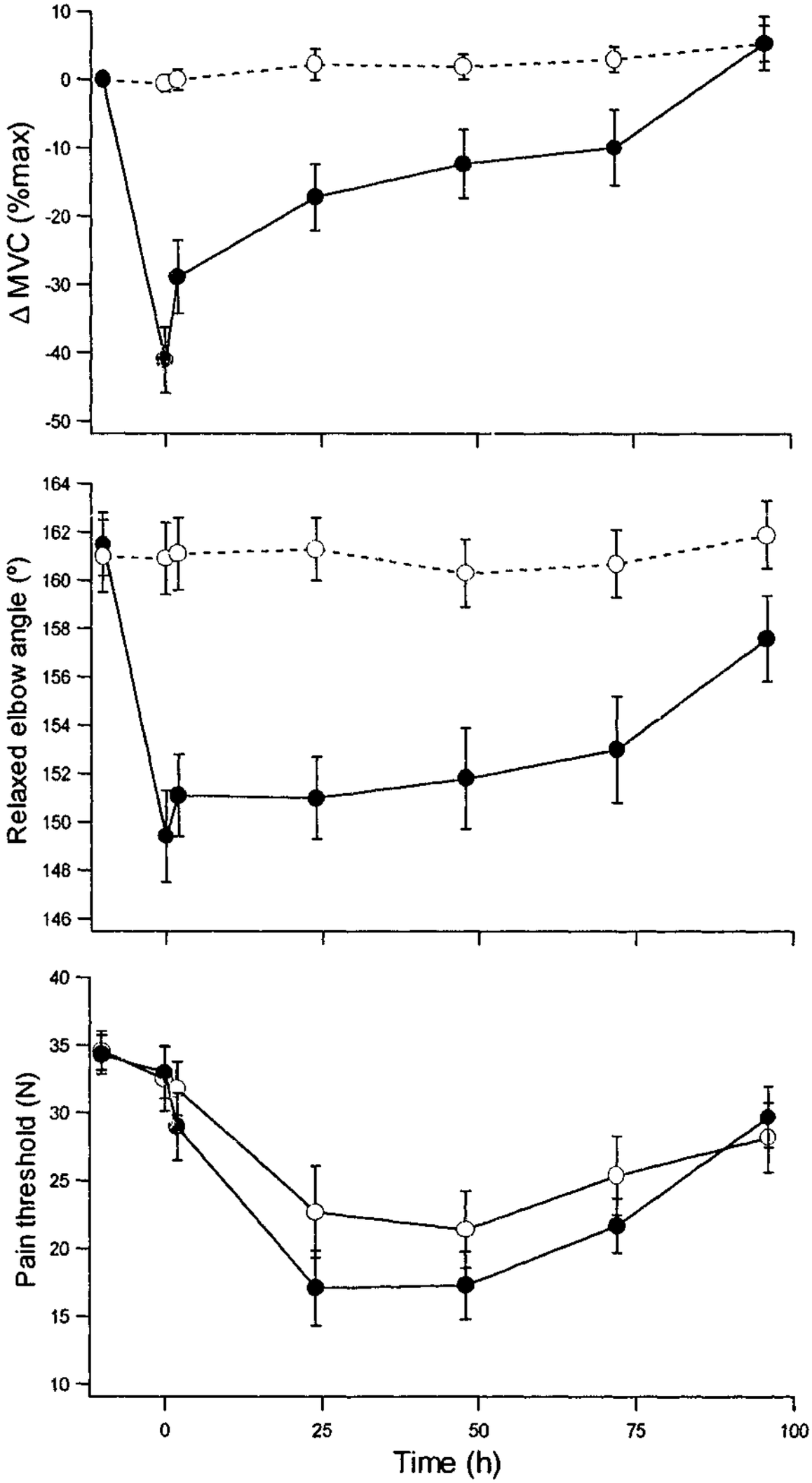
RESULTS

Muscle properties after eccentric exercise

For the unexercised arm, MVC values remained close to control levels throughout the period of measurement. For the exercised arm, MVC fell immediately after the exercise by about 40% and then gradually recovered, reaching control levels after 4 days (Fig 4.7).

Figure 4.7

Top Panel: Maximum voluntary contraction, expressed as a percent of the pre-exercise value, immediately after the exercise and over the subsequent four-day period for the unexercised arm (open circles) and the arm which had undergone eccentric exercise (filled circles). All values are given as means ± S.E.M. for 11 subjects. ***Middle Panel:*** Angle subtended at the elbow in a standing subject with the relaxed arm hanging by their side. Values (means ± S.E.M.) given for both the unexercised (open circles) and exercised arm (filled circles). ***Bottom Panel:*** Level of soreness in the exercised muscle. Pain threshold, in Newtons, was measured with a compression gauge over the 4 days after eccentric exercise. Values (means ± S.E.M.) were measured over the muscle belly (filled circles) and the muscle-tendon region (open circles).


10
0
-10
-20
-30
-40
-50
Δ MVC (%max)
164
162
160
158
156
154
152
150
148
146
Relaxed elbow angle (°)
40
35
30
25
20
15
10
Pain threshold (N)
0
25
50
75
100
Time (h)

Eccentric exercise is known to lead to muscle damage. For elbow flexors, a sign that some damage has occurred in the exercised muscles is the adoption of a more flexed than normal elbow in a standing subject with the relaxed arm hanging by their side (Jones, Newham & Clarkson, 1987). Here it was found, immediately after the exercise, that the elbow angle was more flexed by about 10°, and this gradually returned towards control values over the 4 days of measurements (Fig 4.7).

Measurements of pain thresholds to local compression of the muscle showed a significant fall in threshold ($p<0.01$), by 24 hours after the exercise (Fig 4.7). This is the well known, delayed onset muscle soreness (DOMS). From pain threshold measurements, it was evident the level of soreness was greater in the muscle belly than in the muscle-tendon junction region, although there was no significant difference between the two. Soreness was gone by the end of the 4 days.

Matching errors

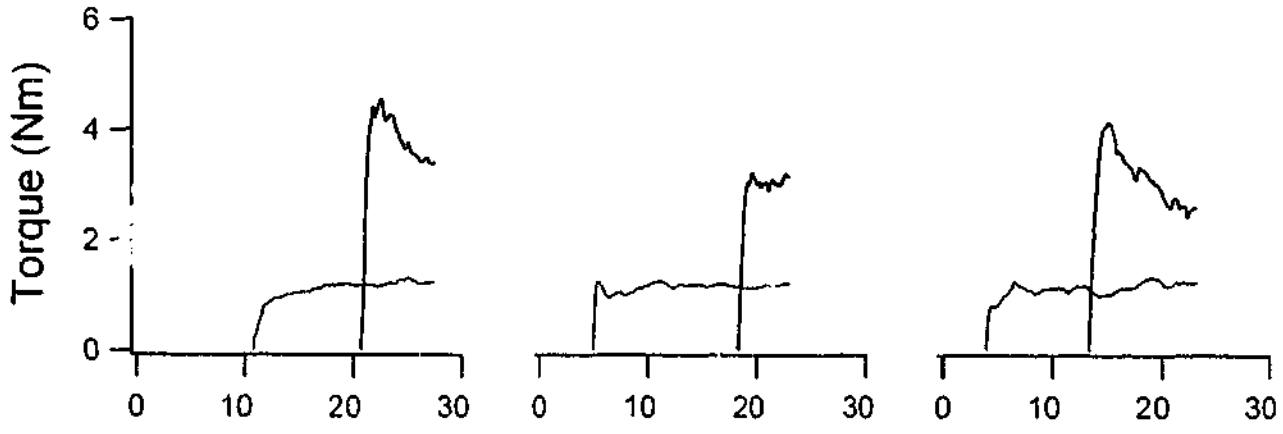
We carried out matching experiments for a range of target torques down to 2% MVC (see Methods). We reasoned that, for the lower levels, more precision would be required to achieve a match, and that here the influence, if any, of peripheral sensory feedback, might emerge more clearly. In practice, even before any arm was exercised subjects all found 2% difficult to match, frequently reporting that it was difficult to tell if they were generating any torque at all. As shown in the three examples of raw records for one subject matching 2% MVC targets, the indicator arm often overestimated the force of the reference arm for control pre-exercise matches (Fig 4.8). This produced % errors that were very large as the target was so low. However, 5% MVC targets were seemingly much easier to match (Fig 4.8). Therefore, 5% was the lowest target included in the analysis. Subjects quickly learned to generate a given level of torque with the reference arm, making use of the visual feedback. For the pre-exercise measurements, subjects varied in their ability to achieve an accurate match with their indicator arm. However, they tended to be consistent in the direction and magnitude of errors throughout a series of trials.

Figure 4.9 shows the raw records for one subject matching the 20% MVC target force, when their left arm was exercised and acted as the indicator, and their right arm was

Figure 4.8

Examples of torque records of a subject matching 2% MVC targets (top panel) and 5% MVC targets (bottom panel), before either arm had undergone eccentric exercise. In the 3 trials shown for the 2% MVC match the indicator arm (blue) overestimates the force of the reference arm (red). For the 5% MVC target the subject was able to match torque levels much more accurately. Note that when matching the 5% MVC this subject intentionally wiggled both arms momentarily to try to gauge the force. Not all subjects used this approach.

2% MVC


Torque (Nm)
6
4
2
0
0 10 20 30
0 10 20 30
0 10 20 30
Time (s)

5% MVC

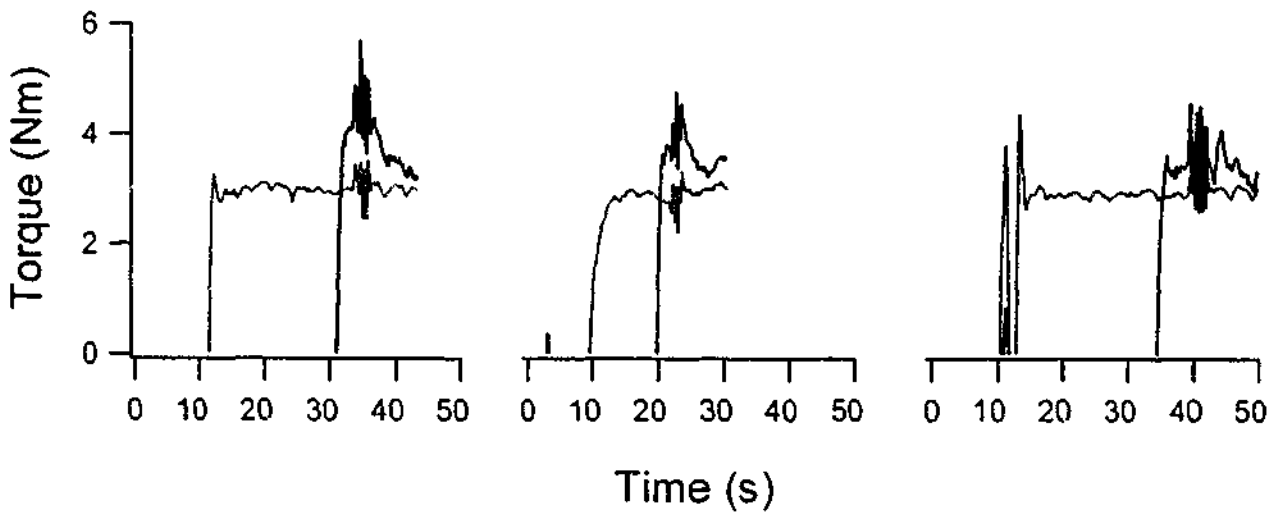
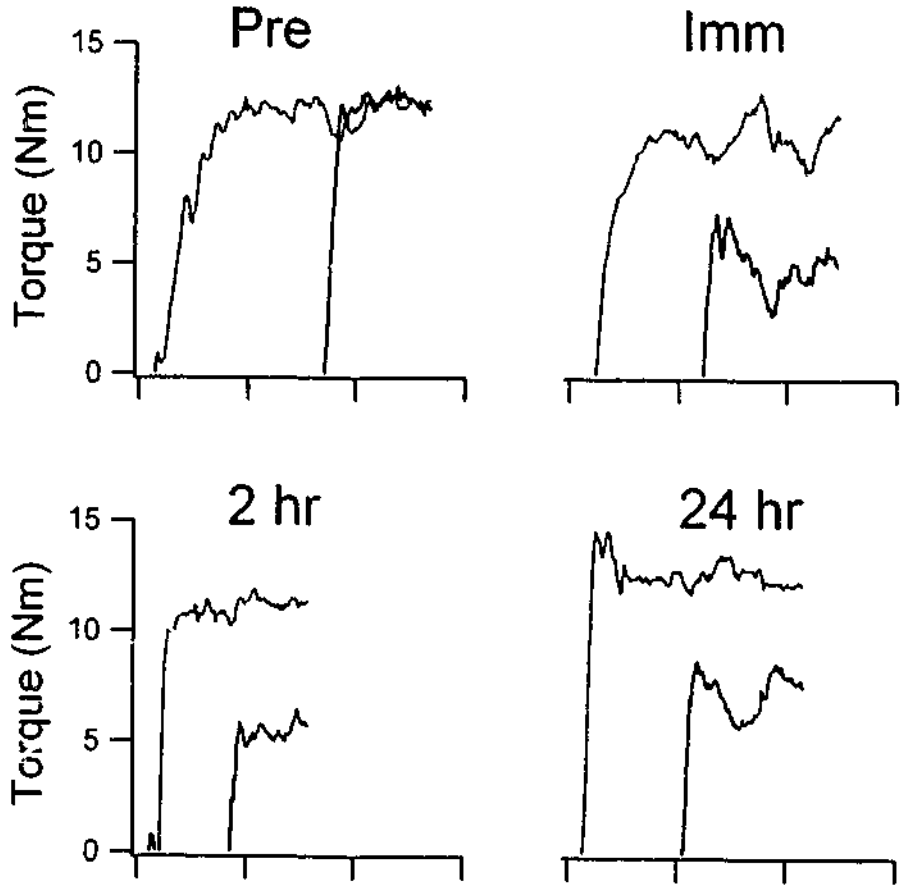

Torque (Nm)
6
4
2
0
0 10 20 30 40 50
0 10 20 30 40 50
0 10 20 30 40 50
Time (s)

Figure 4.9

Examples of torque records of a subject's unexercised reference arm (red) matching 20% MVC targets of the exercised indicator arm (blue) at different times: pre-exercise (Pre), immediately post-exercise (Imm) and 2, 24, 48 72 and 96 hours after the exercise. Immediately after the indicator arm was exercised, it underestimated the tension that was produced by the reference. Matching errors remained but slowly recovered until by 96 hours errors were minimal and back to pre-exercise levels.


Pre
Imm
2 hr
24 hr
48 hr
72 hr
96 hr
Torque (Nm)
Time (s)
0
5
10
15
0
10
20
30

unexercised and acted as the reference. Before the eccentric exercise it is evident this subject was able to match forces very closely. The reference arm is able to generate a relatively constant tension with the assistance of the visual feedback. Once the subject has reached the target, it can be seen the indicator arm was able to match the reference with precision. Immediately after the indicator arm was exercised, it underestimated the tension that was produced by the reference by approximately 20N (remembering the average tensions generated over the last 0.7 s before the recording was stopped, were taken as the values for the match). For the 2 and 24 hour measurements the underestimation of force persisted. By 48 and 72 hours errors were becoming smaller, in the range of approximately 5-7 N. By 96 hours errors were back to control levels. Note the time it took to match the indicator to the reference before the subject was satisfied with each match varied from trial to trial, however for most subjects no more than 15sec was usually necessary.

Subjects were regularly reminded to match the level of torque generated in the reference arm, not the effort involved and to try to ignore other cues, such as pressure on the skin of the forearm. In practice, whether or not subjects received these instructions made little difference to the measurement (c.f. McCloskey et al., 1974). Subjects instinctively matched what they felt and that appeared to be the effort required to generate a given torque.

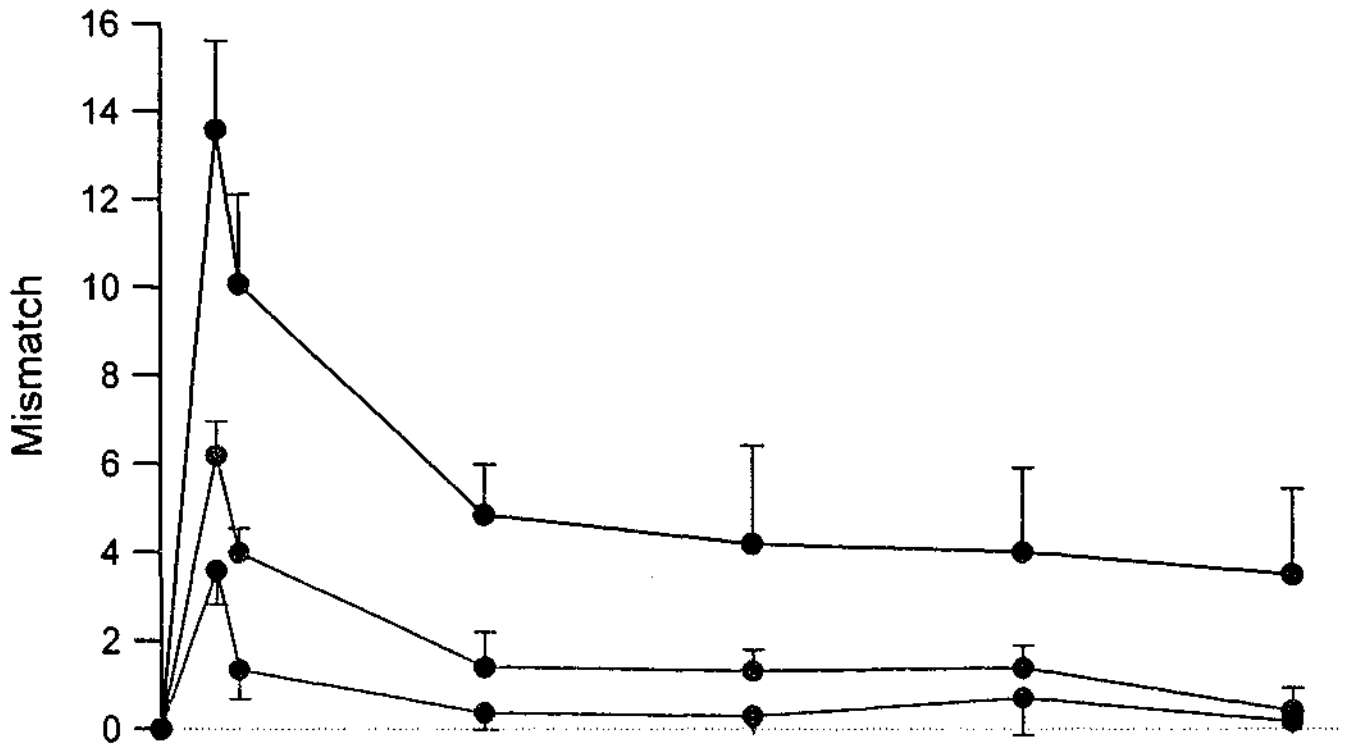
Errors increased dramatically after the exercise. As different subjects have different strengths, figure 4.10 shows the errors expressed relative to pre-exercise MVC. When the exercised arm was the reference, the unexercised arm indicated torques well above the reference level. When the unexercised arm was the reference, the reverse trend was apparent, the exercised arm indicating torques below those being generated by the reference arm. That is, the matching errors were similar in amplitude but of opposite sign, depending on which arm was used as the reference (Fig 4.10). There was an overall significant difference in the value of the mismatch depending on whether the left or right arm, that is the exercised or unexercised arm, acted as reference (ANOVA). The effect was most obvious with a target of 30% MVC, but when scaled proportionately, the same trend was apparent for the smaller targets.

Figure 4.10

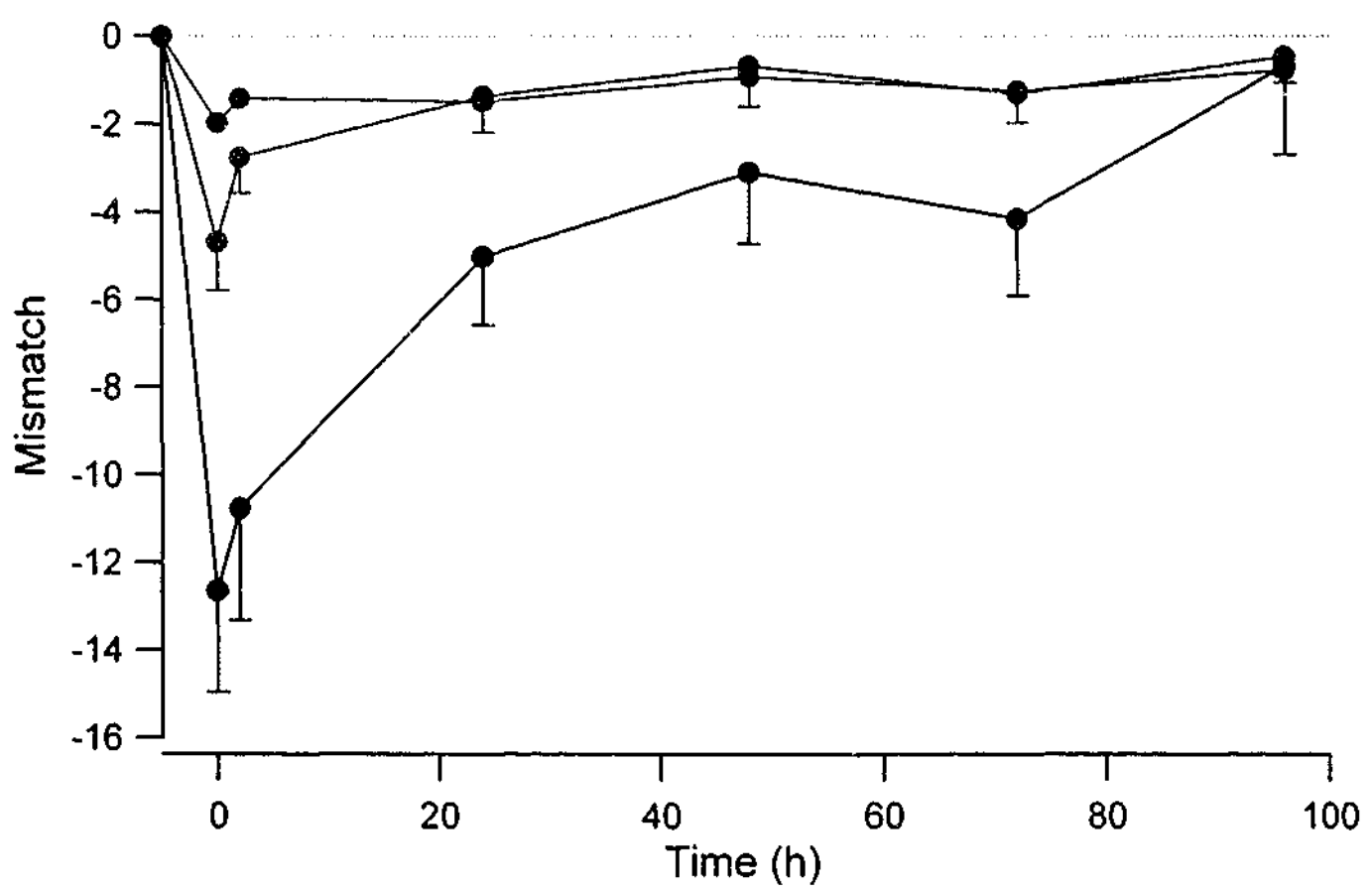
Top Panel: The reference arm is the exercised arm, the indicator has not been exercised. Mismatches in tension are the torque generated by the indicator arm minus the reference after the torque of each arm is calculated as a % of its own pre-exercise MVC (Pre MVC's). Matching accuracy was measured at various times after the exercise over a 4-day period. Target tensions were 5% (green), 10% (red) and 30% (blue) of the reference MVC. Values are given as means ± S.E.M. n=6 for 5%, n=11 for 10% and n=7 for 30%. ***Bottom Panel:*** Mismatches when the reference arm was unexercised, the indicator exercised. Errors (means ± S.E.M.) given as above. In both panels the dotted line indicates zero error.

% Pre MVC's

Exercised Reference



Unexercised Reference



After the reference arm had been exercised, errors were significantly greater than the pre-exercise errors for all four targets. Accuracy returned to the pre-exercise levels at 24 hours for the 5 %MVC target and at 96 hours for the 10 and 20 %MVC targets. Although the errors for the 30 %MVC target remained significantly elevated at 96 hours, the size of errors was significantly less from 24 hours onwards. After the indicator arm had been exercised, the errors were again significantly greater than the pre-exercise errors for all targets up to the 72 hour session. Errors had returned to pre-exercise levels for all targets at the 96 hour session (Fig 4.10). Note that the 20% target level has been left out of figure 4.10 for clarity.

The working hypothesis for this study was that when subjects were asked to match a given level of torque, they were actually matching the perceived effort. If a muscle was fatigued and damaged by eccentric exercise, the tension it would generate for a given level of effort would be lower. To test this idea, the torque produced by an exercised arm was expressed relative to its new MVC, measured post-exercise, rather than the pre-exercise MVC. This significantly made matching errors smaller in value (Fig 4.11). However, when the exercised arm was the reference, the error was still significantly increased from the pre-exercise value at the 0 and 24 hour sessions for the 5 %MVC target and at the 0 and 2 hour measurements for the 10 and 20 %MVC targets. For the 30 %MVC target the error had increased significantly immediately and at 2 hours after the exercise. At 24 hours it was not significant. The error then increased slightly to again be significantly different from the pre-exercise value for the remainder of the sessions. When the unexercised arm was the reference, errors were significantly increased only at 2 hours for the 5% target, 0 hours for the 10% and 20% targets and up to 24 hours for the 30% target.

The result suggested that for some torque levels and early after the exercise there were components of the errors that could not be accounted for by normalising for the reduction in MVC. There was no significant difference in the magnitude of matching errors whether it was the left arm or right arm that was the exercised one (ANOVA).

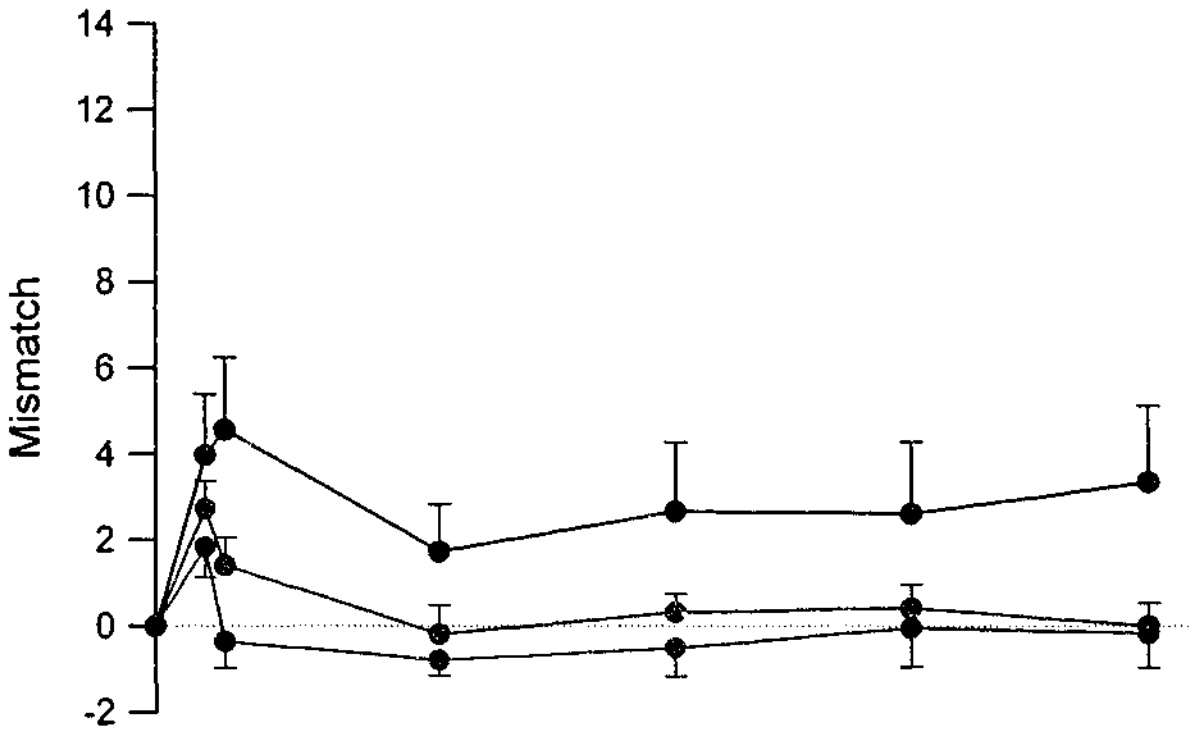
For all targets, errors that represented underestimates were compressed into the range between zero and the target whereas overestimates could potentially range from the

Figure 4.11

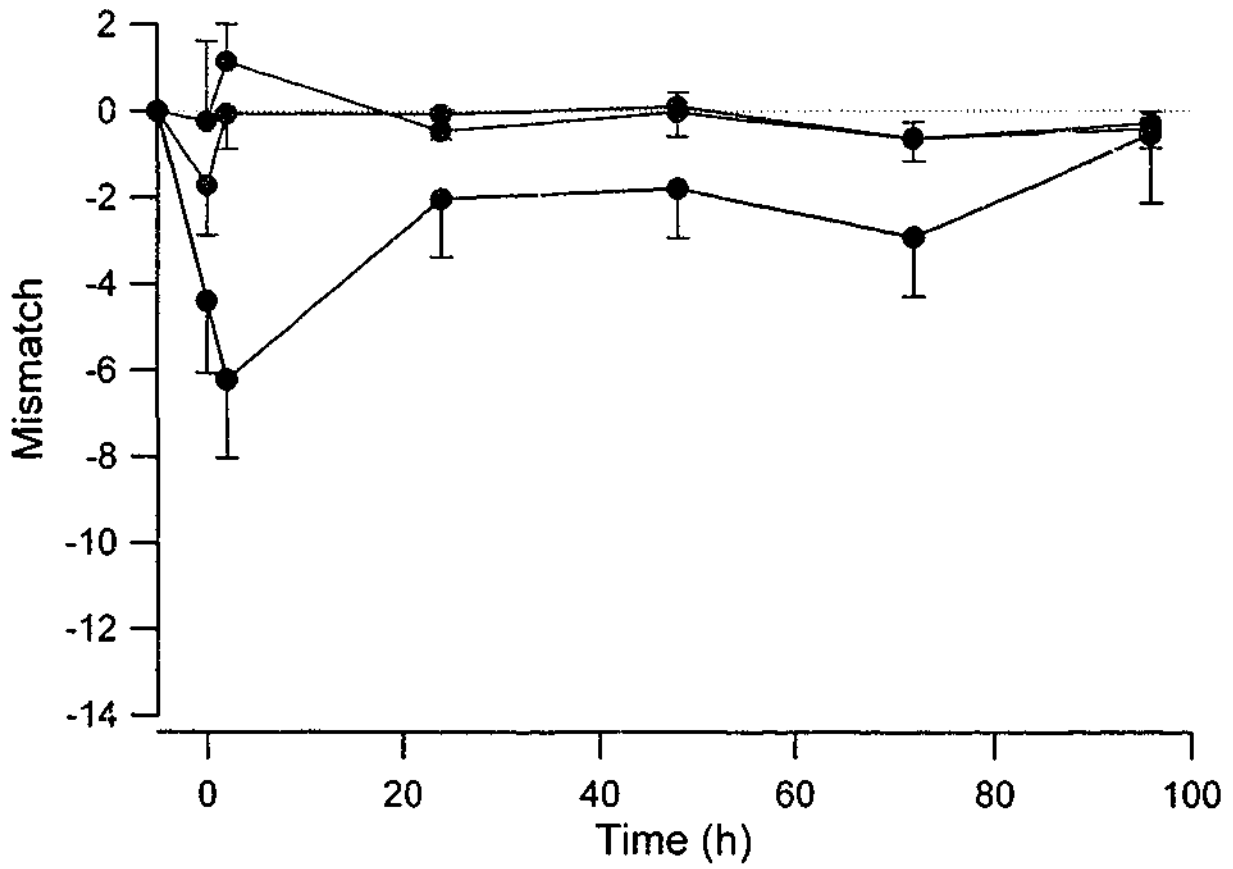
Top Panel: The exercised arm was the reference, the unexercised arm the indicator. Mismatches in tension are the torque generated by the indicator arm minus the reference after the torque of each arm is calculated as a % of its own MVC recorded at the start of each session (Post MVC's). Measurements were made at various times post-exercise over a 4-day period. All values, means (± S.E.M.) n=7 for 5%, n=12 for 10% and n=7 for 30%. Symbols as for Figure 4.10. ***Bottom Panel:*** Unexercised arm was the reference, exercised arm the indicator. Dashed lines in both panels indicate zero errors.

% Post MVC's

Exercised Reference



Unexercised Reference



target to infinity. To take this into account, the data was re-plotted by calculating the ratio of indicator:reference torque and taking the logarithm of it (Fig 4.12). It meant that values were distributed more evenly, particularly for the low forces and allowed a direct comparison of errors for different targets. It is based on the assumption that damage to muscle fibres is distributed evenly over the measured range of target forces. The result yielded no significant difference in matching performance for different target levels. As the muscle recovered, and the MVC drop became smaller, errors were smaller (Fig 4.12). Regression lines fitted to the data had an r^2 of 0.53 with the exercised arm as the reference and an r^2 of 0.34 with the unexercised arm as the reference. The r^2 became larger (i.e. the relationship between drop in MVC and error became stronger) for higher target torques.

EMG after eccentric exercise

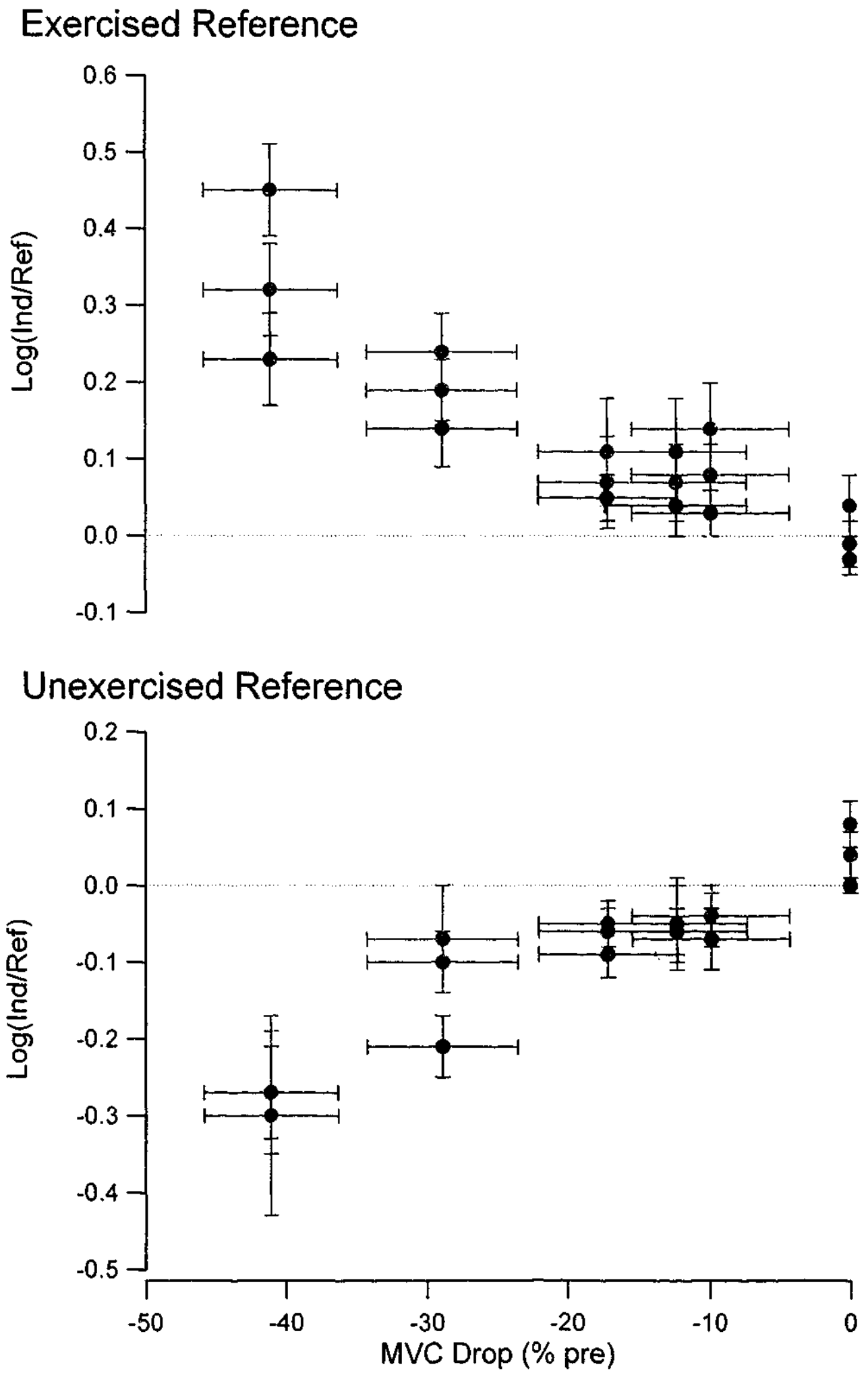
The working hypothesis was that subjects were matching levels of effort, not tension. However, even after having taken the effects of depressed maximum voluntary torque production into account, for some torque levels, especially immediately after the exercise and at 2 hours, significant errors were still present. This suggested that additional factors were contributing to the errors.

If subjects were matching effort, a simple measure of that effort would be the neural drive to the muscle. In order to obtain an estimate of the neural drive, EMG levels were measured before and after exercise. To be able to distinguish between changes in EMG from fatigue and changes as a result of muscle damage, measurements were made after both concentric and eccentric exercise. It was argued that after concentric exercise only changes from fatigue would be present while the combined effects of fatigue and damage would be present after eccentric exercise.

After the concentric contractions, torque for an MVC had fallen by an average of about 26%. After eccentric contractions, MVC torque had fallen by 28%. This higher drop in MVC was expected after eccentric exercise as it involves both fatigue and muscle damage, although the degree of MVC drop after eccentric exercise was not as high as expected.

Figure 4.12

The tension matched by the indicator arm, for a level set by the reference arm, was measured and expressed as a fraction of the reference value. The log of this ratio was plotted over the 7-day period against the fall in MVC for the exercised arm, expressed as a % of its pre-exercise value. ***Top Panel:*** The exercised arm was the reference, the unexercised arm the indicator. ***Bottom Panel:*** The unexercised arm was the reference, the exercised arm the indicator. Symbols as in Figure 4.10. All values given as means (± S.E.M.)


Exercised Reference
Log(Ind/Ref)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
Unexercised Reference
Log(Ind/Ref)
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
-0.5
-50
-40
-30
-20
-10
0
MVC Drop (% pre)

The EMG recorded at MVC was not significantly different after the two exercise protocols. Inspection of the EMG at different levels of absolute torque showed that it had increased after both concentric and eccentric exercise as expected (solid lines in Fig 4.13A&B) and this persisted for up to 48 hours for the eccentric exercise and for 24 hours for the concentric exercise.

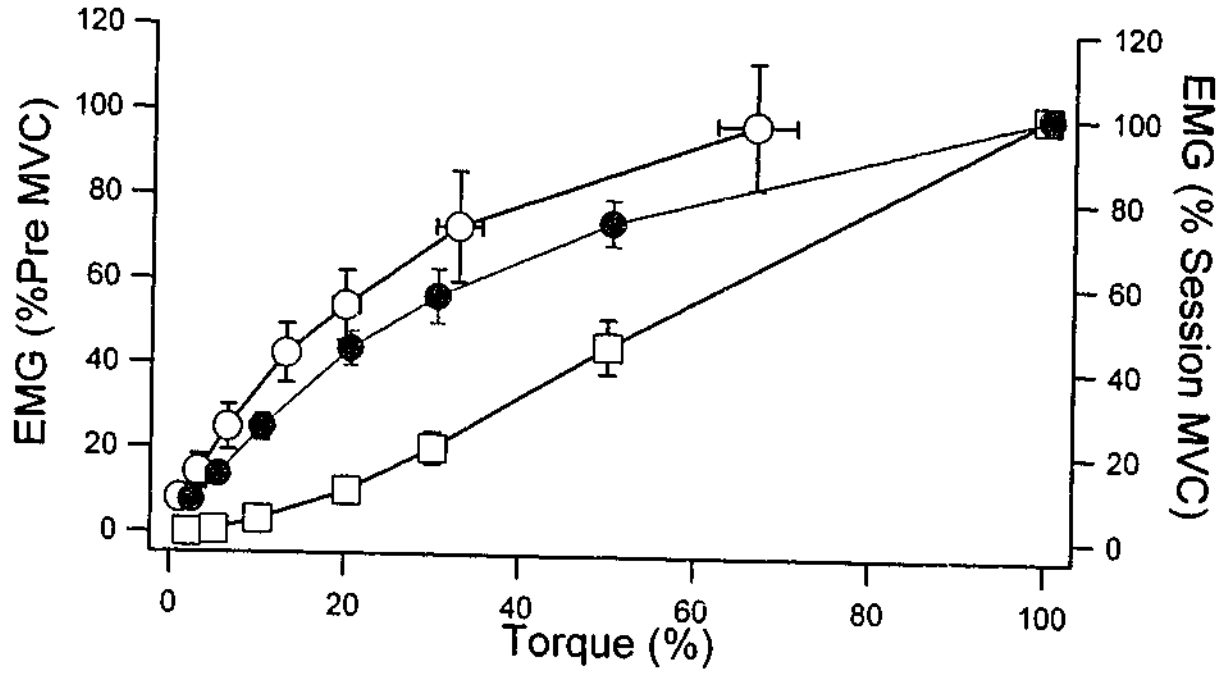
Since torque had fallen as a result of the exercise, the EMGs were measured at targets that were calculated as percentages of the MVC torque recorded at the beginning of each testing session, that is, taking into account the effects of fatigue and damage. Raw EMG measurements of one individual's 30% MVC tension matching task is shown in figure 4.5. Immediately after the eccentric exercise, despite a large drop in force that meant the absolute force required to be matched was less, the raw EMG record increased and remained elevated 2 hours post-exercise.

However, it must be noted this result was not common to all subjects. The average values for all subjects, for the EMG recorded from a sub-maximal %MVC level, did not differ from pre-exercise. These variations were assumed to be the result of variations caused by factors such as differences in electrode positioning. To take this into account, EMG was expressed as a percent of the EMG recorded during the MVCs performed at the beginning of each testing session (post-exercise MVCs). In figures 4.13A and B are shown the relation between EMG and torque, before and immediately after eccentric and concentric exercise respectively, normalised to their pre-exercise MVC values (open symbols) and normalised with respect to the post-exercise MVC values (filled red circle symbols). The relationship before the exercise (square symbols) was curvilinear with a less-than-proportional increase in EMG with target torque for small torques, as previously described by Woods and Bigland-Ritchie (1983). After concentric exercise the EMG increased similarly across the recorded torque range so that when the EMG and torque were normalised to the session MVC values this relation remained essentially unchanged (Fig 4.13B). After eccentric exercise, on the other hand, the EMG increased more than proportionately with target torque for the lower torques (Fig 4.13A). This difference was greatest immediately after the exercise and it then fell back towards control levels, within 48 hours (Fig 4.13C).

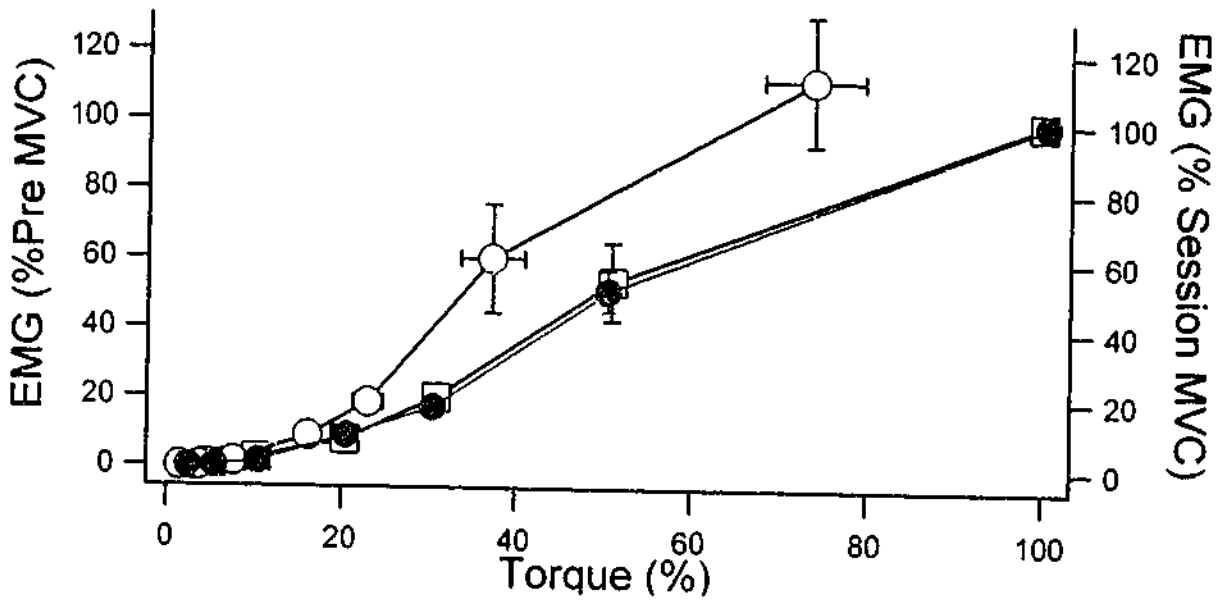
Figure 4.13

(A) Plot of the full-wave rectified EMG before and immediately after eccentric exercise. The open symbols are the EMG expressed as a % of the EMG during a pre-exercise MVC, plotted against torque expressed as a % of its pre-exercise MVC value. The filled red symbols are the immediately post-exercise EMG normalised with respect to the EMG during a post-exercise MVC, plotted against the torque expressed as a % of the post-exercise MVC value. Squares, pre-exercise values; circles, immediately post-exercise values. *(B)* Plot of the integrated, rectified EMG before and immediately after concentric exercise. Symbols as for (A). *(C)* Plot of the % change in EMG at various times after a period of exercise for a target tension of 30% MVC. Circles, values after eccentric exercise, squares, values after concentric exercise. In all panels values are given as means (± S.E.M.)

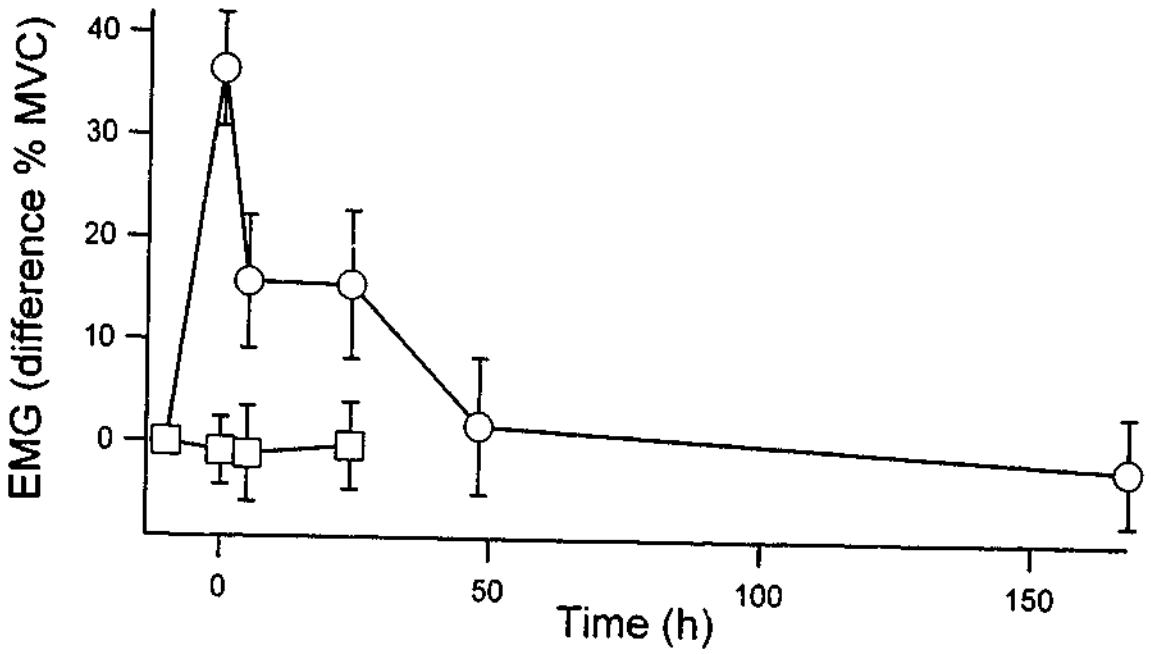
A


EMG (%Pre MVC)
EMG (% Session MVC)
Torque (%)
0
20
40
60
80
100
120

B


EMG (%Pre MVC)
EMG (% Session MVC)
Torque (%)
0
20
40
60
80
100
120

C


EMG (difference % MVC)
Time (h)
0
10
20
30
40
50
100
150

Matching torques at different elbow angles

The observations up to this point were consistent with the view that in the torque matching experiment, errors were due to fatigue, damage and an increase in neural drive for a given fraction of muscle activation to compensate. To model this situation, while avoiding the complicating effects of fatigue, a further experiment was carried out which took advantage of the muscle's length-tension relation (Cafarelli & Bigland-Ritchie, 1979). Here the rationale was that the effort required to generate an MVC remains the same, whatever the muscle length, yet the torque generated will follow the muscle's angle-torque relationship. So in this experiment both arms remained unfatigued. The reference arm was moved to different angles, representing positions on its elbow flexor angle-torque curve. The indicator remained in one position, somewhere near the muscle's optimum length for active torque generation. The prediction was that if subjects were matching effort, the indicator would overestimate the level of torque when the reference was at lengths below or above its optimum.

First the angle-torque relationship for elbow flexors of the reference arm was determined (Fig 4.14A). The optimum angle for all but one subject was approximately 90° (his was 110°). At other angles torque fell, to a mean of 78% of maximum at 120° and 73% at 30°.

To take into account the differences in strengths between subjects, errors were expressed as a percentage of the MVC measured at the optimum length. For matches using both 5% and 20% MVC as targets, as predicted, torque levels were overestimated when the reference muscle was at lengths longer or shorter than its optimum (Fig 4.14B). This change in error with angle was significant ($p<0.05$). The errors increased more with the 20% target, although differences between targets were not significant. The error at 60°, however, was not as large as expected, and was about the same as the error at 90°, even though the average MVC at 60° had fallen to 87% of that at 90°.

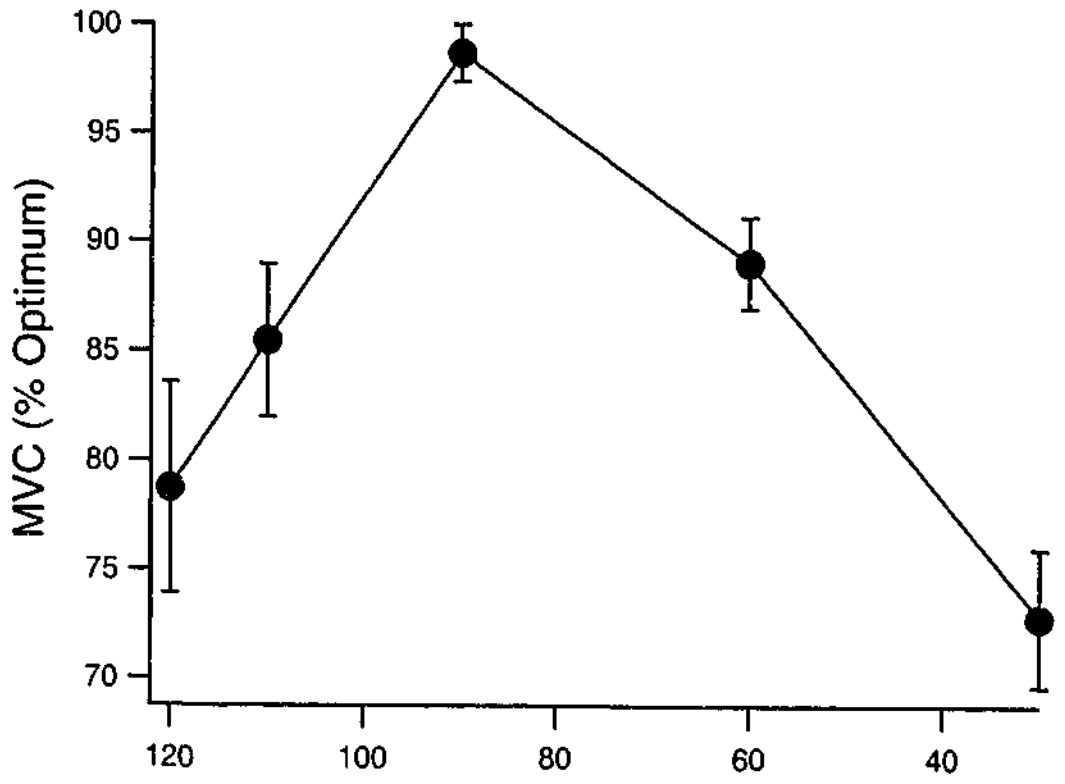
DISCUSSION

In the earlier tension-matching experiment Brockett et al. (1997) had preferred an explanation based on disturbed peripheral feedback since the exercise had led to only

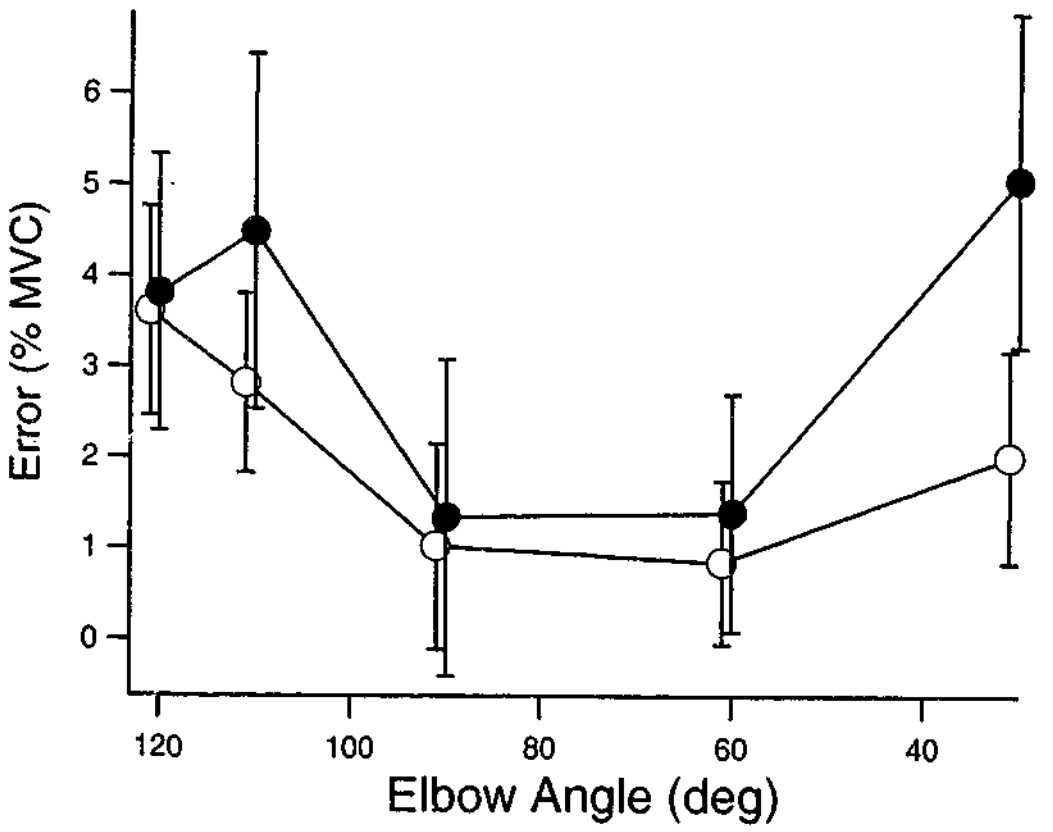
Figure 4.14

(A) Length-tension curve for elbow flexors from eight subjects. A larger angle represents a less flexed elbow. Tensions are MVCs and are expressed as % optimum MVC. *(B)* Matching errors for two different target tensions, 5% (open circles) and 20% (filled circles) of the reference elbow flexor MVC at a particular elbow angle. When the reference arm was at angles greater or less than the optimum, the indicator arm, which always remained at 90°, generated higher tensions than those produced by the reference. All values are means (± S.E.M.) for 8 subjects.

A


MVC (% Optimum)
100
95
90
85
80
75
70
120
100
80
60
40

B


Error (% MVC)
6
5
4
3
2
1
0
120
100
80
60
40
Elbow Angle (deg)

small falls in isometric tension, yet the matching errors were comparatively large. Since that time, in a series of animal experiments it was demonstrated that eccentric exercise produced significant changes in the mechanical properties of the exercised muscle, but without impairing its tendon organs' tension signaling ability (Gregory et al., 2002). Therefore, the earlier experiments were repeated using more severe exercise and tested matching ability over a range of torques.

It was clear that with this more severe exercise protocol that significant amounts of muscle damage were produced since in the period immediately after the eccentric exercise, the level of force generated during a MVC was about 60% of that produced prior to the exercise (Fig 4.7). At 2 hours the MVC had recovered slightly to be about 70% of that produced prior to the exercise, while a residual deficit of approximately 15% was evident from 24 to 72 hours. Indirect evidence of muscle damage was also provided by a significant decrease in relaxed elbow angle, which became more acute by 12° (Fig 4.7). This increased flexion at the elbow is believed to be the result of a rise in passive tension in the damaged muscle from contracture (Chleboun et al., 1998; Whitehead et al., 2001). This is supported by the change in relaxed elbow angle over time, which closely followed the trend observed for the increase in passive tension after eccentric exercise (Whitehead et al., 2001). In addition, by 24 hours pain threshold had also fallen by 17 N (Fig 4.7). The findings of this, more comprehensive, study are consistent with the view that subjects are not matching levels of torque as signaled by peripheral feedback. Rather, they are more nearly matching levels of effort. That conclusion is consistent with other reports on tension-matching errors after eccentric exercise (Carson et al., 2002; Saxton et al., 1995).

The accuracy of force estimation was significantly altered by eccentric exercise (Fig 4.10). When subjects performed the eccentric exercise and were subsequently required to reproduce a target level set by the unexercised reference, with a matching contraction of the exercised contralateral indicator limb, the level of force applied was significantly below that required. When the exercised arm acted as the reference errors were in the opposite direction, the unexercised indicator applying higher forces than required. This tendency to make matching errors was apparent at all levels of applied force. There was no suggestion that a different mechanism was operating at lower forces, provided the

errors were appropriately scaled (Fig 4.12). The pattern of these results was consistent with the idea that subjects were estimating levels of force on the basis of the sense of effort.

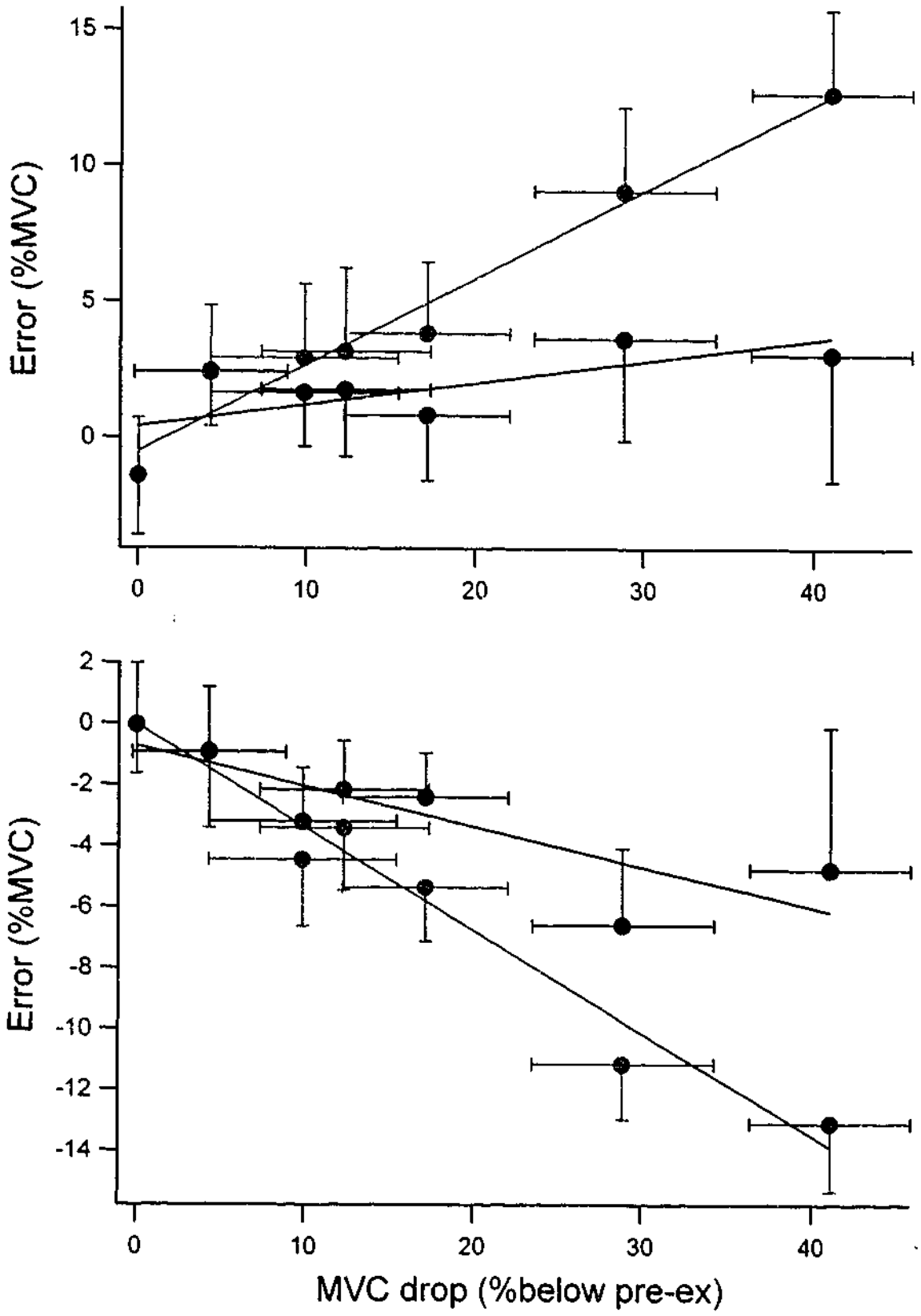
It has been known for a long time that sensations of effort and of heaviness are generated centrally. Since eccentric exercise leads to damage and fatigue in muscle fibres (Proske & Morgan, 2001), subjects would be expected to make torque matching errors after the exercise if they used their sense of effort to achieve the match. That is, the effort:tension relation would be altered so that the exercised arm developed less torque for a given level of effort to match the torque generated by the unexercised arm (Fig 4.10). Also, torque errors would be in a direction depending on whether the exercised arm was the reference or the indicator, as was found in this study. If subjects had been matching on the basis of a sense of tension, they should have been able to detect changes in torque as a result of fatigue and damage, and achieve the same level of matching accuracy before and after the exercise.

After expressing errors in terms of the maximum force that could be generated by that arm at the time (post MVCs), the result was that matching error was significantly reduced (Fig 4.11). However, unlike the findings from Carson et al. (2002), errors were not completely abolished. Rather, components of errors remained and these could not be accounted for by normalising for the reduction in MVC. Hence, this result suggests that factors other than fatigue and damage are contributing to the mismatch. This difference between my results and those of Carson et al. 2002 may be due to the exercise regime that was used. In the experiments conducted here a larger number of contractions was used (on average 100 repetitions more), which would have led to more severe muscle damage. This is consistent with the difference in the drop in MVC, which was larger by 10% in this study.

The persistent error even after taking into account the effects of fatigue and damage can be clearly seen in figure 4.15 that shows matching errors (%MVC) for 30% MVC targets plotted against drop in MVC (% below pre-exercise). Moreover, it is evident the drop in MVC was proportional to the matching errors (%Pre MVC), with the r^2 equalling 0.65 and 0.56 for when the exercised and unexercised were the references respectively. The

Figure 4.15

Matching errors (%MVC) for 30% MVC targets plotted against drop in MVC (% below pre-exercise). Shown are the errors expressed as %Pre MVC (red) and %Post MVC (blue). All values are means (± S.E.M.). Regression lines have been drawn through the data. ***Top panel:*** Errors when the exercised arm was the reference, the unexercised arm the indicator. ***Bottom panel:*** Errors when the reference arm was unexercised, the indicator exercised.


Error (%MVC)
15
10
5
0
0
10
20
30
40
2
0
-2
-4
-6
-8
-10
-12
-14
Error (%MVC)
0
10
20
30
40
MVC drop (%below pre-ex)

slope of the relationship for errors expressed as %Pre MVC had a gradient of 0.37 and 0.3 for when the exercised and unexercised were the reference respectively. When the errors were adjusted to %Post MVCs the gradients were lower, 0.16 and 0.2 respectively. Therefore its clear errors associated with fatigue and damage were much more dependent on the change in MVC, than errors that were not.

Furthermore, it is evident from figure 4.15 that a unit of MVC drop does not produce a one-to-one proportional increase in error. It may have been expected that a drop in the force producing capacity of a muscle would lead to a one-to-one proportional increase in effort to compensate, thereby leading to one-to-one proportional force matching errors. However, instead a change in MVC is associated with only a fractional change in effort. A 10% decrease in MVC produced approximately a 3.35% increase in error. Therefore, a given force drop is equivalent to a lower fractional (0.35) change in the voluntary motor command response.

Based on the assumption that the level of EMG recorded from the contracting muscle was an indication of the level of motoneuron activation and therefore the centrally generated effort (Gandevia, 2001), we recorded EMG to see whether the observed torque matching errors were accompanied by any mismatch in the EMG. The EMG signals at MVC were, on average, unchanged after the exercise. This was anticipated, since if fibres were maximally recruited during an MVC, the EMG amplitude would be expected to be maximal.

It may be argued that neuromuscular block could have been expected to reduce the EMG response. Neuromuscular block is attributable to transmission failure at axonal branch points and at neuromuscular junctions (Enoka et al., 1989; Krnjevic & Miledi, 1958). The post synaptic membrane has also been suggested to become less sensitive to ACh (Krnjevic & Miledi, 1958). However, neuromuscular block is unlikely to have played a role in this experiment, as it occurs more often after long maximally sustained tetanic contractions. In voluntary contraction there is a progressive increase in the number of active motor units, and, over a certain range of tension, also a increase in frequency of discharge of each motor unit (Bigland & Lippold 1954). Units in normal muscle do not fire at frequencies much above 20-50 Hz, even for comparatively short periods (Bigland

& Lippold 1954). Its doubtful that the submaximal exercise bout used here required units to fire above such a rate. Neuromuscular failure is only likely to occur if the CNS maintains a discharge at a frequency above 50 Hz (Krnjevic & Miledi, 1958). Similarly, low stimulation rates used with distributed stimulation were shown to be very effective in avoiding the problem of neuromuscular failure (Wise et al., 2001). Moreover, an impact from neuromuscular block is most probably small, as after cessation of the contraction, the effects of the block rapidly dissipates (Wise et al., 2001).

It was expected that fatigue of the contractile machinery would reduce the torque for a given level of EMG post-exercise, but the proportional increase in EMG should remain unchanged. This is indeed what was found after concentric exercise. After EMG and torque were normalised to the session MVC, the pre and post-exercise plots were identical (Fig 4.13B). Increased extracellular potassium concentration in the contracting muscles is suggested to be the cause of the increase in EMG after exercise (Jorgensen et al., 1988). After eccentric exercise, although the EMG required to achieve a given torque again increased, the increase was not proportional for different targets and hence the relation between EMG and torque, normalised to their trial session MVC values, was altered. Before the exercise, and after concentric exercise, for a given fraction of maximal torque, the increase in EMG was less than proportional at lower torques (Woods & Bigland-Ritchie, 1983).

It has been suggested that this non-linear EMG/force relation over the lower force range may be attributed to selective recruitment of units at different distances from the recording electrodes (Woods & Bigland-Ritchie, 1983). In many animal muscles of fixed fibre type the motor units are not evenly distributed within the muscle. The fibres of low threshold units selectively recruited during low force contractions predominate in the deeper layers (Gonyea & Ericson, 1977), therefore their signals could be weakened when recording from the surface. After the eccentric exercise this reversed so that a given fraction of maximum torque was now accompanied by a larger than proportional increase in EMG at the lower torque levels (Fig 4.13A). At the higher torque levels the relationship slowly begins to converge.

Therefore there are at least two possible contributing mechanisms to the matching errors observed after exercising one arm. One is a depression of torque, caused by damage and fatigue leading to a changed relationship between effort and torque. The same effort would generate less torque. This is probably the most important factor since, by expressing the torques as a percent of post-exercise MVC, the errors were significantly reduced. However, as alluded to before, errors remained significant for matches made immediately after the exercise and at 2 hours. Subsequent errors were significant only for the 30% MVC target (Fig 4.11). In the second experiment, normalising EMG and torque to the MVC values recorded at the start of each testing session showed that after eccentric exercise additional errors could be present from a larger than proportional increase in submaximal EMG (Fig 4.13A). This effect, too, peaked immediately after the exercise, but had largely subsided by 48 hours (Fig 4.13C).

Why should eccentric exercise lead to a larger than proportional increase in EMG for a given level of torque? A similar observation was made by Carson et al. (2002). These authors made the additional observation that cortically-evoked potentials from magnetic brain stimulation were larger in the eccentrically exercised muscle, suggesting a rise in central excitability. Various mechanisms are possible.

When a muscle is damaged and fatigued, perhaps larger motor units are recruited earlier during a graded contraction, leading to generation of a proportionately bigger EMG. If so, then a similar trend might have been expected after concentric exercise, which was not the case. Yet both forms of exercise produced similar falls in torque. A further potential complication is the possible influence of motor unit synchronization associated with muscle tremor. Motor unit synchronization is quantified as the effect of common inputs on motor unit activity (Semmler et al., 2002). It has been demonstrated conclusively that the source of the contributors to normal physiological tremor is motor unit synchronization (Halliday et al., 1999).

Studies have found differences in the activation strategies used by the nervous system to different types of contractions. The strength of motor unit synchronization was shown to be approximately 50% greater during lengthening contractions, compared to shortening or postural (isometric) contractions (Semmler et al., 2002). Such findings suggest there is

an increase in common input to motoneurons during eccentric contractions. It has also been proposed that motor unit synchronization is more significant, not only after eccentric exercise, but also for low levels of activation (Lippold, Redfearn & Vuco, 1957).

There is evidence that when muscles perform eccentric contractions, there is a greater reliance on faster motor units, both within muscles and among synergist muscles (Howell et al., 1995; Nardone, Romano & Schieppati, 1989). A decreased EMG median frequency after eccentric contractions may also indicate selective damage of the fast twitch fibres in this type of exercise (Linnamo, Bottas & Komi, 2000). The presumed greater proportion of injury to the large motor units is in agreement with other studies that have provided morphological evidence of preferential damage of large Type II fibres (Friden & Lieber, 1992; Friden, Sjostrom & Ekblom, 1983b). Therefore, as motor unit recruitment with increasing force production normally progresses from slow (Type I fibres) to fast fatigue-resistant (Type IIa fibres) to fast fatigable (Type IIb fibres) units, this could provide another explanation. If eccentric exercise damages fast muscle fibres preferentially, at the higher torque levels more EMG would be required, leading to an alteration of the EMG : torque relation. This theory may explain the difference in EMG : torque relation found between eccentric and concentric exercise (Fig 4.13A&B). Although it would have to be assumed that damaged fibres could sustain firing or that undamaged fibres increase there firing.

It is known that after eccentric exercise there are a number of changes in a muscle's mechanical properties. One of these is a shift, in the direction of longer muscle lengths, of the muscle's optimum length for force generation. This is caused by some sarcomeres, disrupted by the eccentric contractions, being overstretched allowing the remaining active sarcomeres to assume a shorter length (refer to General Introduction). It means that after the eccentric exercise the EMG would have been recorded at a shorter sarcomere length in the active sarcomeres. At shorter lengths a higher activation rate would be required to achieve a given fraction of maximal force (Rack & Westbury, 1969). Thus the proportionately larger increase in EMG at low torque levels could be the result of subjects trying to reach the target level, but operating at an effectively shorter muscle length (Fig 4.14).

Such mechanisms do not alter the main working hypothesis, that is, when subjects are faced by a torque matching task, they match the effort more closely than the torque, since errors were greatest with uncorrected torques. To further investigate the role of effort in torque matching tasks, but without the complicating effects of muscle damage and fatigue, we repeated the experiments of Cafarelli & Bigland-Richie (1979), using changes in the muscle's angle-force relation to alter levels of torque, while keeping levels of activation (MVCs) constant (Fig 4.14). Although errors were not in direct proportion to the changes in torque (Fig 4.15), their distribution was consistent with a match of effort rather than of level of torque.

Based on the assumption that the size of the matching error depended on the change in MVC, matching errors at different elbow angles and after eccentric exercise, for 20% MVC target levels, were compared directly with the drops in MVC (Fig 4.16). The slope of the relationship for matching at different angles had a lower gradient (0.15), compared to the relation when matching after eccentric exercise (0.25). However, regression analysis showed that there was no significant difference between the two. Therefore, errors that were associated with fatigue and damage (matching after eccentric exercise) and due to changes in the length-tension relation (matching at different elbow angles) were similarly dependent on the change in MVC. This suggests that the means by which the MVC drop was effected had little influence on the matching errors. Changes in the force-generating capacity of the contractile elements of a muscle, whether it be adjusted by muscle length or fatigue and damage, require comparable increases in efferent signals when matching forces. This implies that the alterations in afferent discharge arising from damage to the muscle fibres do not act directly upon the neural mechanisms that mediate the sense of effort. If they did one would have expected muscle damage to cause larger errors for a given amount of MVC drop.

It remains to explain the result obtained by Brockett et al. (1997). In those experiments, although tension after the exercise, measured as % MVC, had fallen rather little, subjects did become sore by 24 hours, due to the onset of DOMS. It occurred to us that torque mismatches measured from 24 hours onwards could be due, in part, to the effects of the muscle soreness.

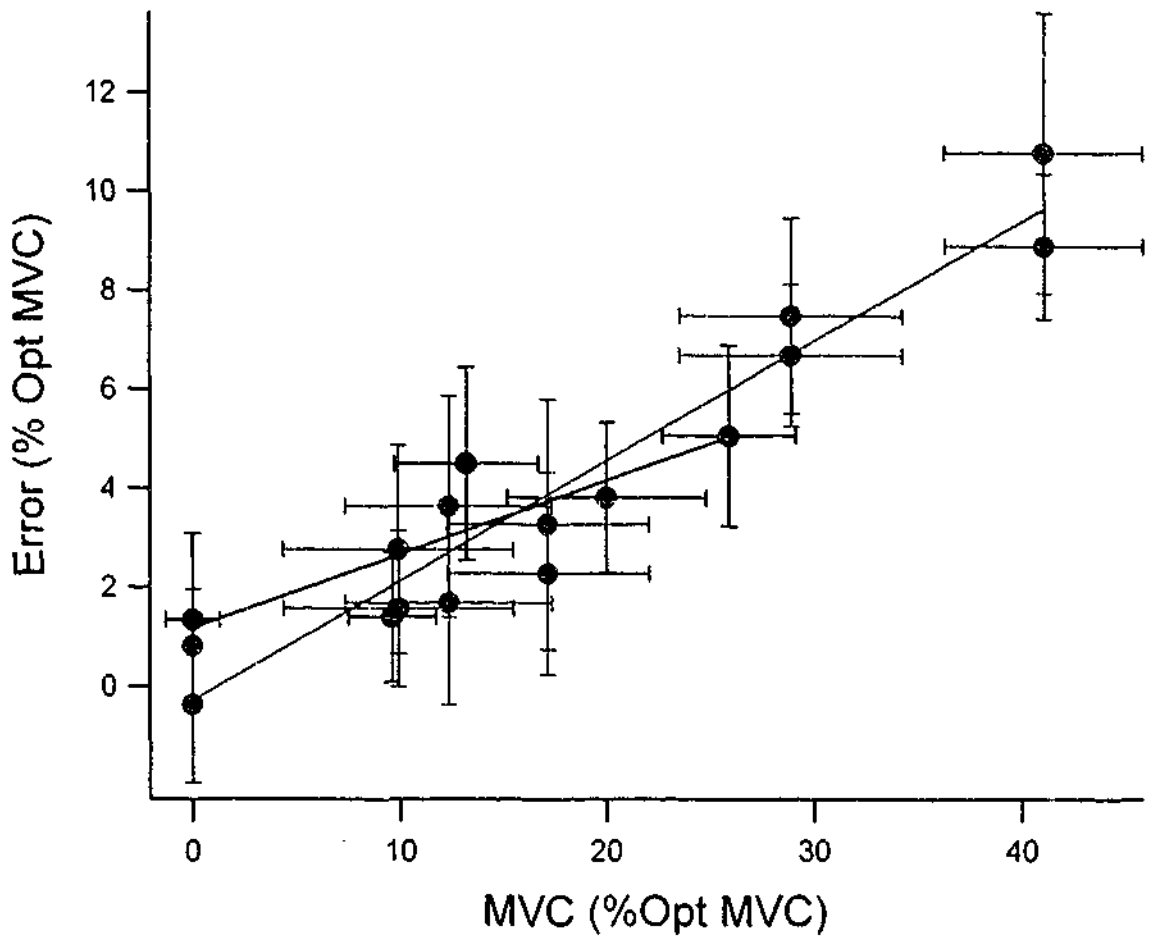


Figure 4.16

Matching errors (%MVC) for 20% MVC targets plotted against MVC drop in MVC (% Optimum MVC). Shown are the errors from eccentric exercise expressed as %Pre MVC (red) and the errors from matching at different angles expressed as %Optimum MVC (blue). All values are means (± S.E.M.). Regression lines have been drawn through the data.

The significant errors which persisted 24 hours onward after expressing errors relative to post MVCs (normalising for fatigue and damage), are unlikely to be due to changes in EMG since these had largely subsided by 48 hours. Therefore, since no other mechanism is available to account for such long term changes, an involvement of pain in the mismatch becomes a possibility. In addition, although muscle damage may be the major factor responsible for the sustained drop in MVC over the period 24-76 hours post exercise (Fig 4.7), a contribution from muscle soreness cannot be discounted.

Notice in figure 4.11 that when the exercised arm was the reference, for 30% MVC significant errors persisted for the whole period of measurement. When the unexercised arm was the reference, the persisting errors were smaller and not quite significant. If soreness was having an effect on matching performance, it might be expected to have a larger effect at higher torques since these generate more soreness (Weerakkody et al. 2001). Such an explanation may also account for the right : left difference. When the sore arm was the reference, the visual target ensured that it reached 30% MVC. This meant the reference arm would have to tolerate more pain to sustain the target, hence allowing pain to disrupt the central command, leading to larger errors. Conversely, when the sore arm was the indicator, it undershot the target, generating less torque and therefore less soreness. Consequently, in this case pain would be less of a contributing factor, leading to smaller errors. This may suggest there is a threshold effect, in that pain levels corresponding to 30%MVC are needed for an unexercised muscle before the pain effect becomes significant. Presumably if a sore indicator had to generate higher forces, those errors would become significant too.

Why should muscle soreness induce torque matching errors? It has recently been shown that motor-evoked potentials in finger muscles, in response to transcranial magnetic stimulation, were reduced if the muscles were made sore by injection of hypertonic saline (Le Pera et al., 2001). We have followed up this finding by measuring torque matching ability in subjects after the biceps of one arm had hypertonic saline injected into it (see Chapter 5). The level of soreness generated was comparable to that seen in DOMS (Weerakkody et al. 2001). Significant errors were generated, as a result of the soreness with a pattern entirely consistent with those reported in this study (Weerakkody et al. 2002).

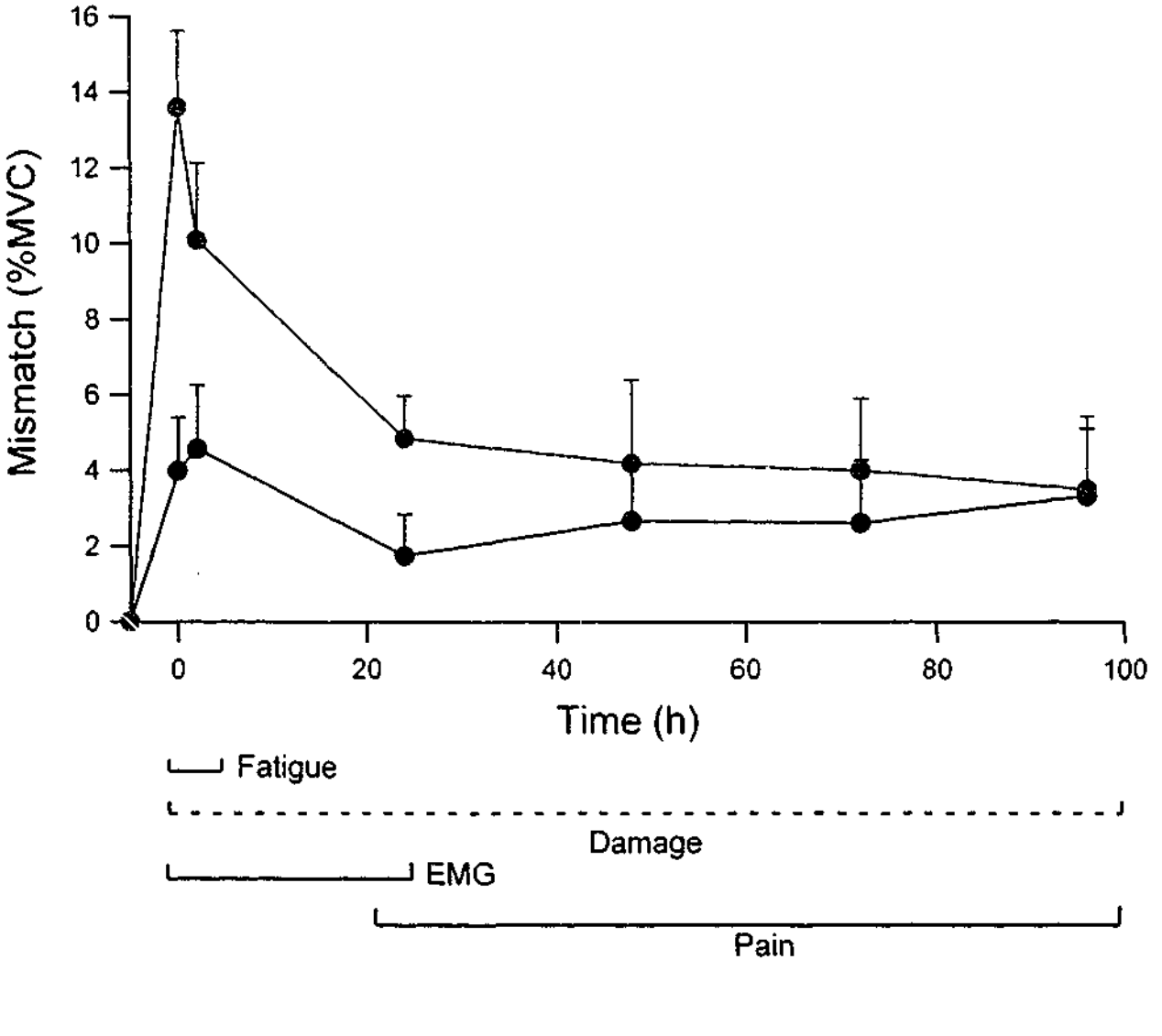
One possible theory of how soreness may effect central action is that in the presence of DOMS a given level of effort generates less than the expected motor output. There is some evidence in support of such a view. Small diameter muscle afferents transmitting nociceptive information, which are thought to be responsible for generating DOMS (see Chapter 5), are capable of reducing voluntary drive during sustained contractions (Gandevia et al., 1996), even though neither motoneurones nor motor cortical cells are subject to direct inhibition (Taylor et al., 2000). Such findings suggest that Group III and Group IV afferents may exert their effects upstream of the motor cortex. Recently, however experiments showed that during contraction of the soleus muscle, nociceptive stimulation produced inhibition of a test H-reflex (Rossi et al., 2003). Although such a result suggests Group III and Group IV stimulation may inhibit muscle afferents directly, the work of Taylor et al. (2000) provide evidence that they are not involved. Furthermore during contractions the changes in the descending drive to the motoneurons cannot be controlled for. Therefore this is a area of study that requires further investigation.

Also, MacIntyre et al. (1996) reported that the immediate fall in torque after eccentric exercise of the human quadriceps was followed by a secondary decline at 20-24 hours, when DOMS had set in. Such a bimodal fall in MVC was not found in this experiment. However, for the 30% MVC target errors did increase slightly from 24-48 hours (Fig 4.17). Furthermore, Newham et al. (1987) found by electrical stimulation that in the presence of DOMS, subjects were still able to fully activate their muscle despite any discomfort. Such experiments need to be confirmed and extended.

To conclude, it has been demonstrated that there are large errors in a torque matching task involving elbow flexors, after one arm was exercised eccentrically. The errors appear to be due to subjects using the level of effort required to reach a given torque as the matching cue. After eccentric exercise the effort : torque relationship is disturbed, as a result of damage and fatigue in muscle fibres. In addition there is a disturbance of the EMG : torque relation, not seen after concentric exercise. This change in EMG may have various possible causes. It may be due to a change in central, motoneuronal excitability, a change in the muscle length-tension relation, due to motor unit synchronisation or preferential Type II fibre damage. Perhaps all such mechanisms are operating. Finally,

Figure 4.17

Mismatches in tension at the 30% MVC target generated by the indicator arm minus the reference after the torque of each arm is calculated as Pre MVC's (red) and Post MVC's (blue). Shown are the various periods of times that the proposed factors affecting matching accuracy are thought to be acting after the exercise. All values are means (± S.E.M.).


16
14
12
10
8
6
4
2
0
Mismatch (%MVC)
0
20
40
60
80
100
Time (h)
Fatigue
Damage
EMG
Pain

persisting errors at 48 hours or longer may be due to the central action of the nociceptor input from the sore muscle (Fig 4.17).

CHAPTER FIVE

Force matching at the elbow joint is disturbed by muscle soreness

INTRODUCTION

As described earlier, delayed onset muscle soreness (DOMS) results from eccentric exercise, where the contracting muscle is forcibly lengthened. It causes damage in muscle fibres which leads to the soreness (Proske & Morgan, 2001). DOMS can be quite debilitating during movements and is therefore an important consideration for the design of athlete training programs. However, apart from the debilitating effects of the pain itself, DOMS may also have effects on motor control that are not consciously perceived.

An interesting finding from the previous chapter on force matching after eccentric exercise was that, even after expressing errors relative to post-exercise MVCs as a means of taking into account any fall in force due to damage and fatigue, significant errors persisted from 24 hours onward. As DOMS peaked at 24-48 hours post-exercise, it was hypothesised that any persisting mismatches from 24 hours onwards could be due, in part, to the effects of the muscle pain. There are findings in support of such a notion. It has been reported by MacIntyre et al. (1996) that the immediate fall in torque after eccentric exercise of human quadriceps is followed by a secondary decline at 20-24 hours, at a time when muscle soreness begins to manifest itself. However, MacIntyre et al. (1996) did not attribute the secondary fall to the effects of pain, but suggested instead it was the result of phagocytic activity causing further damage.

Pain in general is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or is described in terms of such damage (Merskey & Bogduk, 1986). It is a complex, multi-dimensional phenomenon that includes a wide variety of aspects. The interaction between pain and motor function is still not totally understood, but from everyday life it is clear that deep muscle pain affects our movements. The effect of muscle pain on motor control is typically seen as a reluctance to perform movements.

From the previous chapter, it was concluded the pattern of matching errors made were what was expected if the subjects were estimating levels of force on the basis of the 'sense of effort' rather than 'sense of tension'. Sensations of effort are defined as a centrally-mediated sensation, assumed to have arisen from the voluntary motor command. Evidence points to the sensorimotor cortex as the site of origin of the relevant motor command signal for force and heaviness judgments (Gandevia, 1982). Therefore, one way in which soreness could affect central action is that in the presence of DOMS a given level of effort generates less than the expected motor output. There is some evidence in support of such an idea. It has recently been reported that in the presence of muscle soreness induced experimentally by injection of hypertonic saline into a muscle, motor-evoked potentials (MEP) in the muscle, in response to magnetic brain stimulation of the contralateral motor cortex, are depressed below normal levels (Le Pera et al., 2001). This depression was specifically attributed to the pain quality, as MEP amplitude was not modified when non-painful stimulation was used (Le Pera et al., 2001). This finding raises the possibility that the debilitating effects of DOMS are due, in part, to reduced motor output resulting from a depressed motor cortex.

Although the data in the previous chapter showed that, when faced with a force matching task, subjects were using a sense of effort rather than a sense of tension, contributions to the matching process from peripherally originating sensations cannot be totally discounted. De-afferented subjects are seriously impaired at weight estimation (Cole and Sedwick 1992), showing the importance of afferent input either in directly providing sensory information about the weight or in providing a calibration signal for central mechanisms contributing to a sense of effort.

It may be that input from nociceptor afferents in some way disturbs the peripheral sensory component to the force matching process. Furthermore, it cannot be excluded that the muscle's tension sensors, the tendon organs, contribute information to the force matching process. Tendon organ pathways are also subject to influences from nociceptive inputs. Studies have indicated that Ib interneurons receive input from group III and IV muscle afferents (Kniffki *et al.*, 1981). Therefore, there is a possibility that the 'sense of tension' derived from peripheral sensations via Golgi tendon organs also may be affected by pain.

Muscle nociceptors may not be the only receptors with the effects on motor control. Studies focusing on painful stimulation of the skin have also found evidence to suggest a reduction in the excitability of the motor cortex during stimulation. Farina et al. (2001) induced tonic cutaneous pain in skin of the hand using the topical application of capsaicin (a pain producing chemical, extracted from hot chilli peppers), which activates mainly C-polymodal nociceptors. It produces a local burning pain lasting about 80 min (Kenins, 1982; Farina *et al.*, 2001). MEPs recorded from muscle in response to transcranial electrical stimulation were shown to be inhibited for 20-30 min after the application of capsaicin to the skin overlying the muscle; the inhibition commenced at onset of pain (Kenins, 1982; Farina *et al.*, 2001).

In an attempt to obtain further evidence for a pain mediated reduction in motor performance, it was decided to test the effects of muscle soreness produced by hypertonic saline on a force matching task. Subjects were required to match, with one arm, the elbow flexor force generated by the other in the presence of pain in the biceps of one arm (Brockett et al., 1997). In addition, to determine whether the effect of force matching performance was a generalised effect on nociceptive input or specific to input from the muscles, mildly painful stimulation of the skin overlying the muscle was also tried. The longer term goal of the experiments was to seek evidence in support of the idea that a component of the debilitating effects of DOMS was a central action of the nociceptive input which subjects were unaware of.

METHODS

Subjects

Five male subjects participated in the experiments in which saline was injected into the biceps brachii muscle. These five subjects plus a further three subjects, 2 male and 1 female, participated in the experiment using noxious stimulation of the skin (mean age, 29 years). All experiments were approved by the Monash University Standing Committee on Ethics in Research involving Humans.

Apparatus

The testing equipment consisted of two padded boards hinged along a horizontal axis of rotation coincident with the elbow joint (see Chapter 4). The boards were locked in the vertical position by a pair of horizontal aluminium shafts in series with strain gauges (see Chapter 4). Subjects were seated with their forearms strapped to the boards so that their elbows subtended an angle of approximately 90°. Extra padding was put around their forearms to minimize cutaneous sensation from skin. Subjects were able to see a computer monitor which provided visual feedback of the torque level generated by the reference arm but not by the indicator arm.

Matching tension

Once subjects' forearms were secured, their maximum voluntary contraction (MVC) was measured for elbow flexors of each arm. Subjects were then asked to generate, under visual feedback, a 30% MVC target level of force measured as torque in elbow flexors of one arm, designated the reference arm. Once subjects had reached the reference torque and maintained it for ≈2 seconds, they were asked to match it with the other, indicator arm, but without visual feedback. Once subjects believed the torques in the arms were matched, they were asked to maintain the match for a further ≈3 seconds. Torque values were averaged over the last second of each match. The torque traces were acquired into Igor Pro (Wavemetrics, Ore, U.S.A) running on a Macintosh 6100 Computer (Apple, U.S.A.) for subsequent analysis.

Each subject performed 5 trials before the saline injection and another 5 after the pain had subsided, to act as control measurements. A rest period of approximately 1 minute was given between trials to minimise effects of fatigue. However, during the pain from saline as many matching trials as possible were carried out, typically 6-8. These were averaged.

Inducing muscle pain

A volume of 0.2 ml of sterile hypertonic (5%) sodium chloride was injected into the belly of biceps to a depth of 2-3 cm. Pain onset was almost immediate and as soon as subjects felt the pain they began the matching experiment. During this time they indicated on a visual-analog scale of 0-10 the intensity of the pain. Pain from hypertonic saline

typically lay in the range 3-4 out of 10. Subjects were asked to rate pain levels repeatedly as they continued their matching trials.

Each subject received two injections of hypertonic saline, in separate testing sessions. One injection was into the reference arm and the other into the indicator arm. Control experiments were also carried out by determining matching performance after injection of 0.2 ml of isotonic (0.9%) sodium chloride.

Matching errors (Δ mismatch) were graphed from the calculated differences in the two torque values, expressed as a percent of the MVC for each arm, minus the pre-injection average.

Painful stimulation of skin

Painful heat stimuli were applied to the skin with a 2.5 cm diameter metal probe. Skin temperature was constantly monitored throughout stimulation. Mild pain, in the range 3-4 /10 was produced with a probe tip temperature of 48–50°C. In one series of experiments, torque-matching trials were carried out during painful stimulation of the skin overlying biceps, and in a second series, skin on a region of the lower forearm was stimulated.

MVC and control matches were measured as described previously. Then subjects repeated a match but with the hot probe sitting on the skin over biceps of either the reference or indicator arm or on skin of the forearm. The probe was applied to the medial side of the forearm near the wrist to avoid heating skin over brachioradialis. Once a match had been made, the probe was removed and subjects were rested. At this time a cool, moist cotton pad was placed on the skin to prevent persistent heating of the stimulated area. Five successive trials were carried out, each time with the probe moved slightly to avoid excessive heating of only one area of skin. At the end of the experimental series an additional set of control measurements was made with the unheated probe sitting on the skin.

Statistical analysis

An analysis of co-variance (ANCOVA) was carried out on the muscle pain data with pain entered as a continuous factor, the injected arm as a fixed factor and subject as a random factor. The cutaneous pain data was analysed by a three factor ANOVA with subject as a random factor and the painful arm and the trial (control or test) as fixed factors. Where there were significant ($p<0.05$) interactions between the stimulated arm and the trial, a further two factor ANOVA was carried out with an LSD (least significant difference) *post-hoc* test.

In addition, a pooled t-test was used to determine the significance level between MVCs before and after hypertonic saline injection. For each test, means and standard errors were calculated across all subjects. The statistical analysis program used was Data Desk (Data Description, Ithaca, NY).

RESULTS

Muscle pain

Subjects quickly learned to generate a maintained torque of 30% MVC with their reference arm, making use of the visual feedback provided. For the pre-treatment control measurements, subjects varied in their ability to achieve an accurate match with their indicator arm, but they tended to be consistent in the magnitude and direction of any matching errors.

The pain generated by the hypertonic saline had a diffuse, dull character and the pain appeared to arise from a large part of the muscle. Pain commenced approximately 20 seconds after the saline had left the syringe and persisted for 5–7 minutes. The average peak pain rating reported by subjects from the hypertonic saline injection was 3.8 ±0.35. Measurements of subjects MVCs were made before the saline injection and after the pain had subsided (Fig 5.1). The amplitudes of MVCs were found not to be significantly different, therefore indicating no long-term impairment of muscle function other than a small, insignificant amount of fatigue.

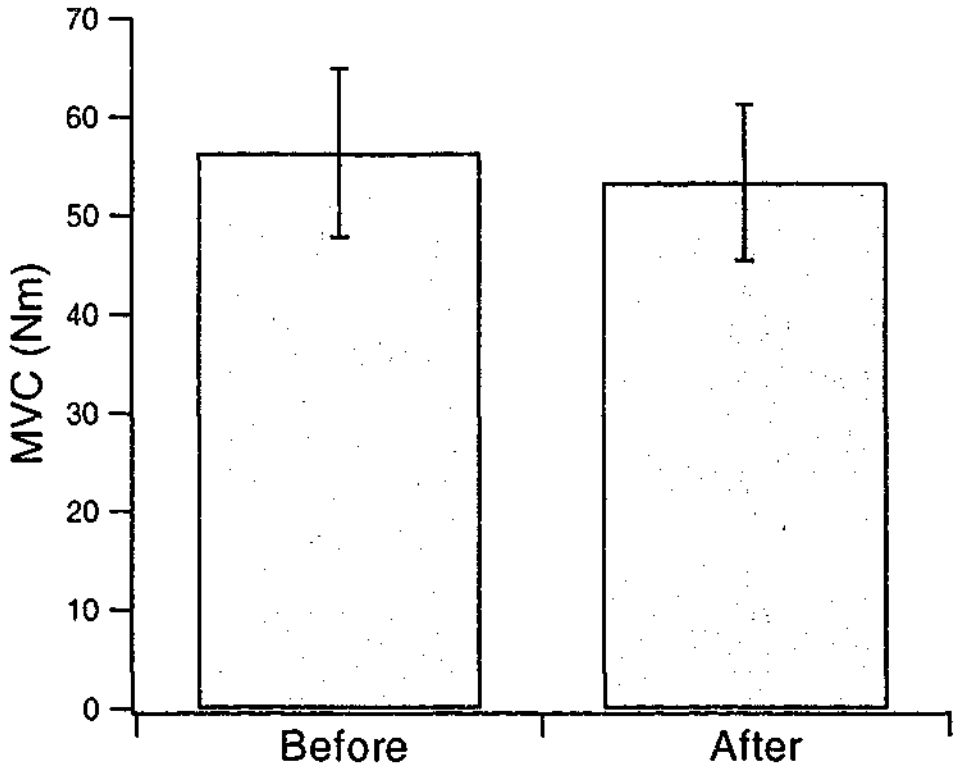


Figure 5.1

Histogram representation of mean MVC (±SEM), before and after injection of hypertonic saline. Values are given in torque (Nm).

During the pain, matching trials were carried out at 20–25 second intervals. It meant that the first four trials after pain onset were carried out in less than 2 minutes. As soon as pain had set in, matching errors increased dramatically. When the indicator biceps was sore, reference torques were underestimated, and when the reference biceps was sore reference torques were overestimated.

Figure 5.2 shows a typical trace of the raw torque matching data for one subject. This subject initially overshot with their indicator, slowly coming back towards the reference level, until they believed that the tensions in both arms were closely matched. For this specific subject the initial overshooting by the indicator could have been done deliberately as a means of finding the right matching range. However it should be noted that not all subjects used this method of force matching. When biceps of the reference arm was made sore, it can be seen that immediately after the pain had set in, the indicator arm overestimated the target torque by about 10 Nm. The amount by which the subject overestimated the reference torque became less as the pain began to subside, that is, apart for the initial transient until matching performance returned to near control levels. Errors were in the opposite direction when the indicator biceps was made sore. That is, the sore indicator arm tended to underestimate the torque generated by the reference arm.

Data for the same subject but for both arms is shown in figure 5.3. As well as errors being in opposite directions, depending on which muscle had been injected, the magnitude of the errors correlated reasonably well with the level of soreness perceived at the time of making the match.

For the same subject, when isotonic saline was injected into the reference arm, it induced a very small amount of pain with a rating of about 0.7 that was only short lived (Fig 5.4). There was little or no accompanying mismatch. The average peak pain rating reported from the isotonic saline injection was 0.3 ±0.2. For some subjects isotonic saline did not induce any pain at all.

The pooled data from all five subjects is shown in figure 5.5. Matching errors measured during the first five trials after saline injection, when pain was still present, were

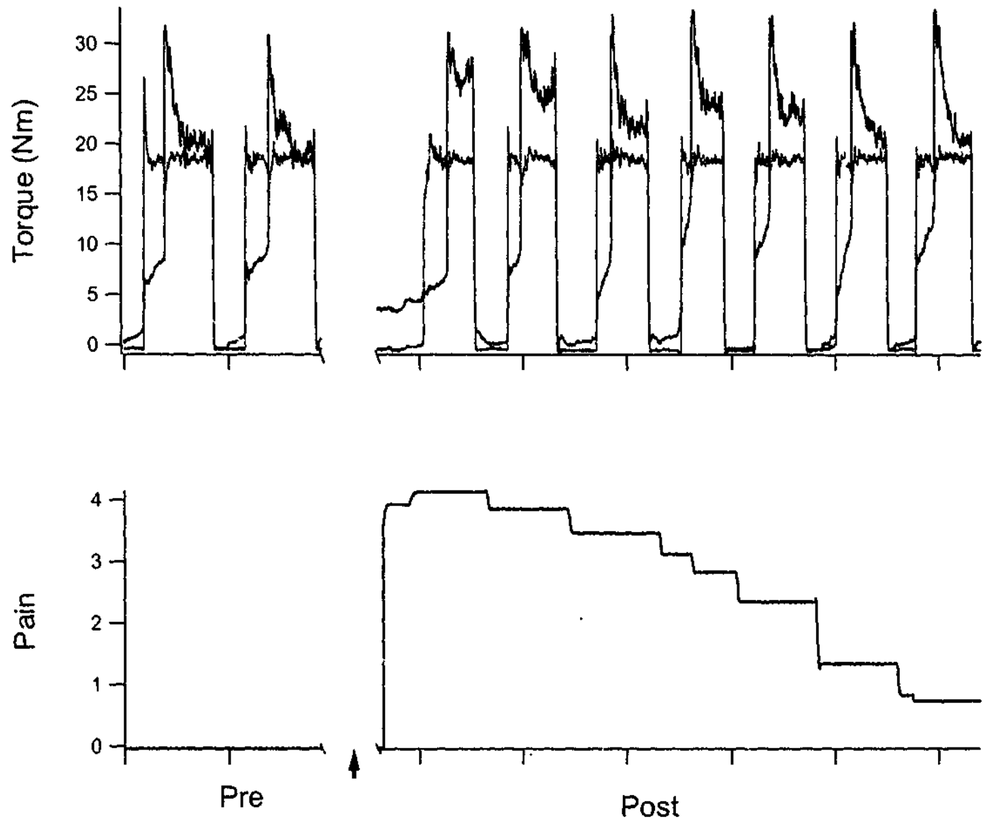


Figure 5.2

Records for one subject matching elbow torque, before and after injection of hypertonic saline into the biceps muscle of the reference arm. Two trials are shown before the injection, followed by a series of trials after the injection. ***Top panel:*** Torque traces. The torque (Nm) exerted by the indicator arm (blue) is matching the torque exerted by the reference arm (red). ***Bottom panel:*** The pain rating of the subject. Pain was rated on a scale of one to ten using a visual analog scale (see text).

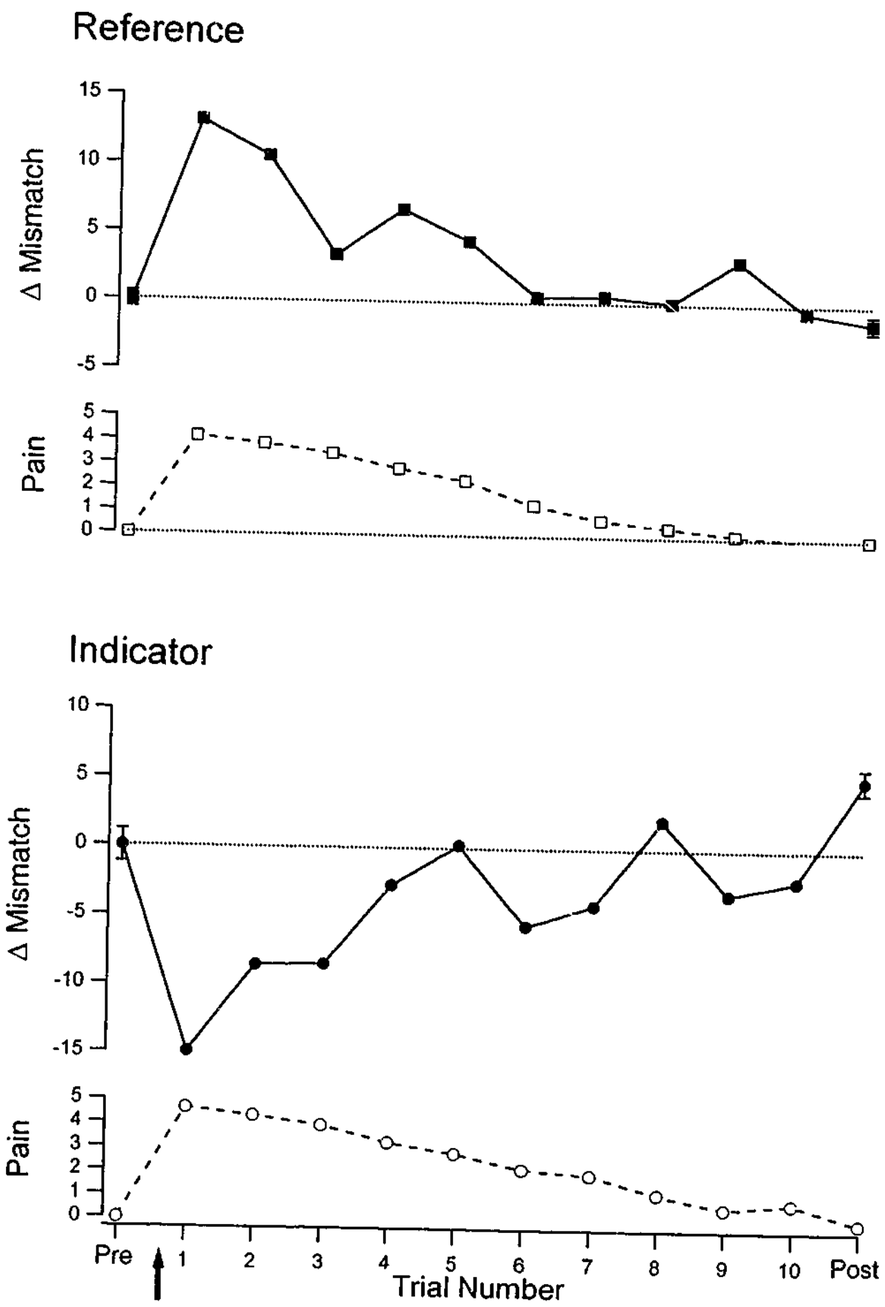


Figure 5.3

The %MVC mismatch error in the presence of pain. In each panel the torque matching difference (upper trace) was compared with the level of reported pain (lower trace). Squares reference biceps sore, circles indicator biceps sore. Matching errors (Δ mismatch) were calculated as the difference in the two torque values, expressed as a percent of the MVC for each arm, minus the average pre-injection difference. The arrow at the bottom indicates the point of injection of the hypertonic saline. The first value in each series is zero (± S.E.M.) of the five pre-injection controls. Subsequent values are individual measurements. The final value is the mean (± S.E.M.) of the five post-injection controls. ***Top panel:*** Hypertonic injection into the reference arm. ***Bottom panel:*** Hypertonic injection into the indicator arm.

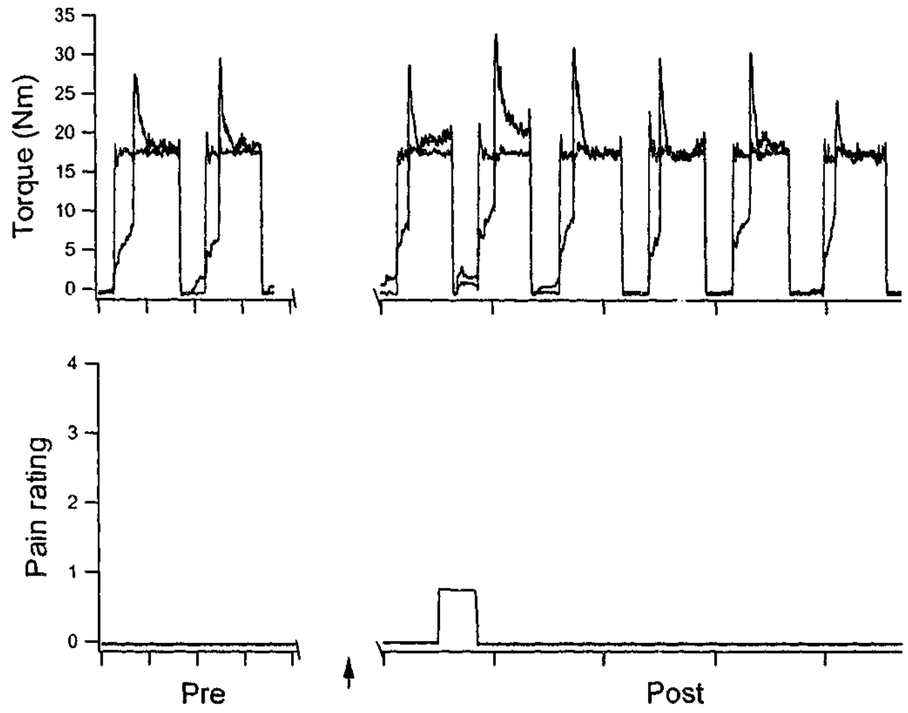


Figure 5.4

Records for one subject matching elbow torque, before and after injection of isotonic saline into the biceps muscle of the reference arm. Two trials are shown before the injection, followed by a series of trials after the injection. ***Top panel:*** Torque traces. The torque (Nm) exerted by the indicator arm (blue) is matching the torque exerted by the reference arm (red). ***Bottom panel:*** The pain rating of the subject. Pain was rated on a scale of one to ten using a visual analog scale (see text).

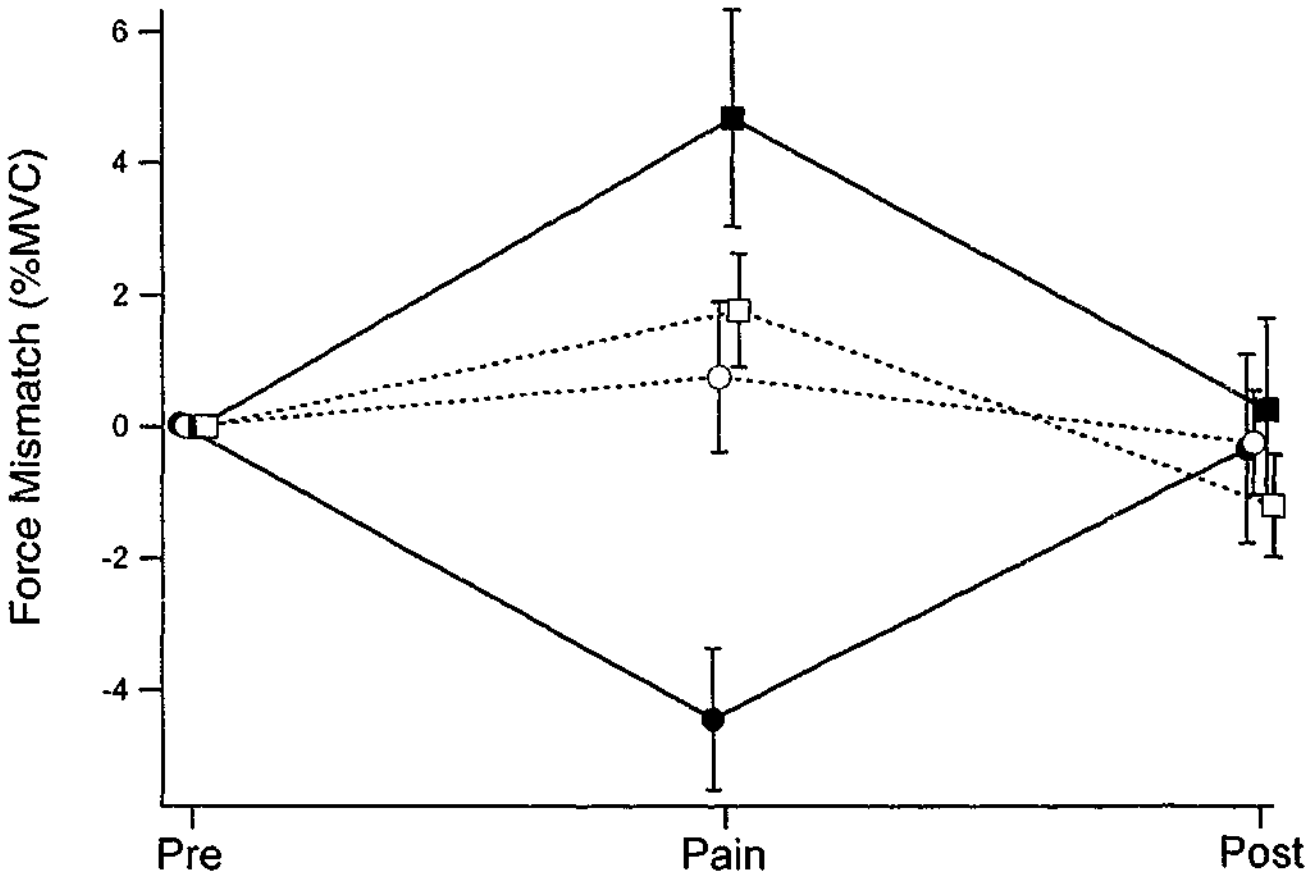


Figure 5.5

Mean mismatches adjusted to pre pain values. Torque mismatches before, during and after pain generation by injection of saline into biceps. Squares, values from injections into the muscle of the reference arm, circles, values from injection into the indicator. Filled symbols and solid line, hypertonic saline. Open symbols and dashed line, isotonic saline. Values are means (± S.E.M.) for 5 subjects.

significant and of opposite sign for the two arms. By contrast, injection of isotonic saline into the muscle did not lead to production of significant errors.

Injection of hypertonic saline into the muscle led to a rapid rise in pain, followed by a slow decline. It meant that each torque matching trial was carried out at a slightly different level of pain. The analysis of co-variance (ANCOVA) showed that pain accounted for a greater proportion of the experimental variation than the arm injected or the subject.

To demonstrate that the size of the matching error was correlated with the level of pain, measurements for the 5 subjects were sorted into bins of one unit of pain rating and averaged (Fig 5.6). A regression analysis using individual measurements rather than means showed that the relation was significant both for a sore indicator arm and a sore reference arm ($p<0.05$).

Painful stimulation of the skin

The question was posed, was the effect of pain on torque matching performance a generalised effect of nociceptive input, or was it specific to input from the muscles involved in the matching task. To test this idea, mildly painful heat was applied to skin overlying biceps during the matching trials, and in a second series of experiments, to skin of the same arm but remote from the region overlying the matching muscles.

It was found that painful stimulation of the skin did interfere with matching performance in a manner similar to that seen following the saline injections (Fig 5.7). The example of raw force matching data, for one subject, shows that during the trials with painful heat stimulation of the skin overlying the biceps of the reference arm, the indicator arm overestimates the target torque, by an average of approximately 5 Nm. While control matches before and after the pain were quite accurate.

The overall effect was found to be significant ($p<0.05$) although errors were smaller than produced by muscle pain (Fig 5.8). When a region of skin of the forearm near the wrist, and not directly overlying the contracting muscles, was stimulated, a small, but not significant increase in matching errors occurred (Fig 5.8). For 3 subjects measurements

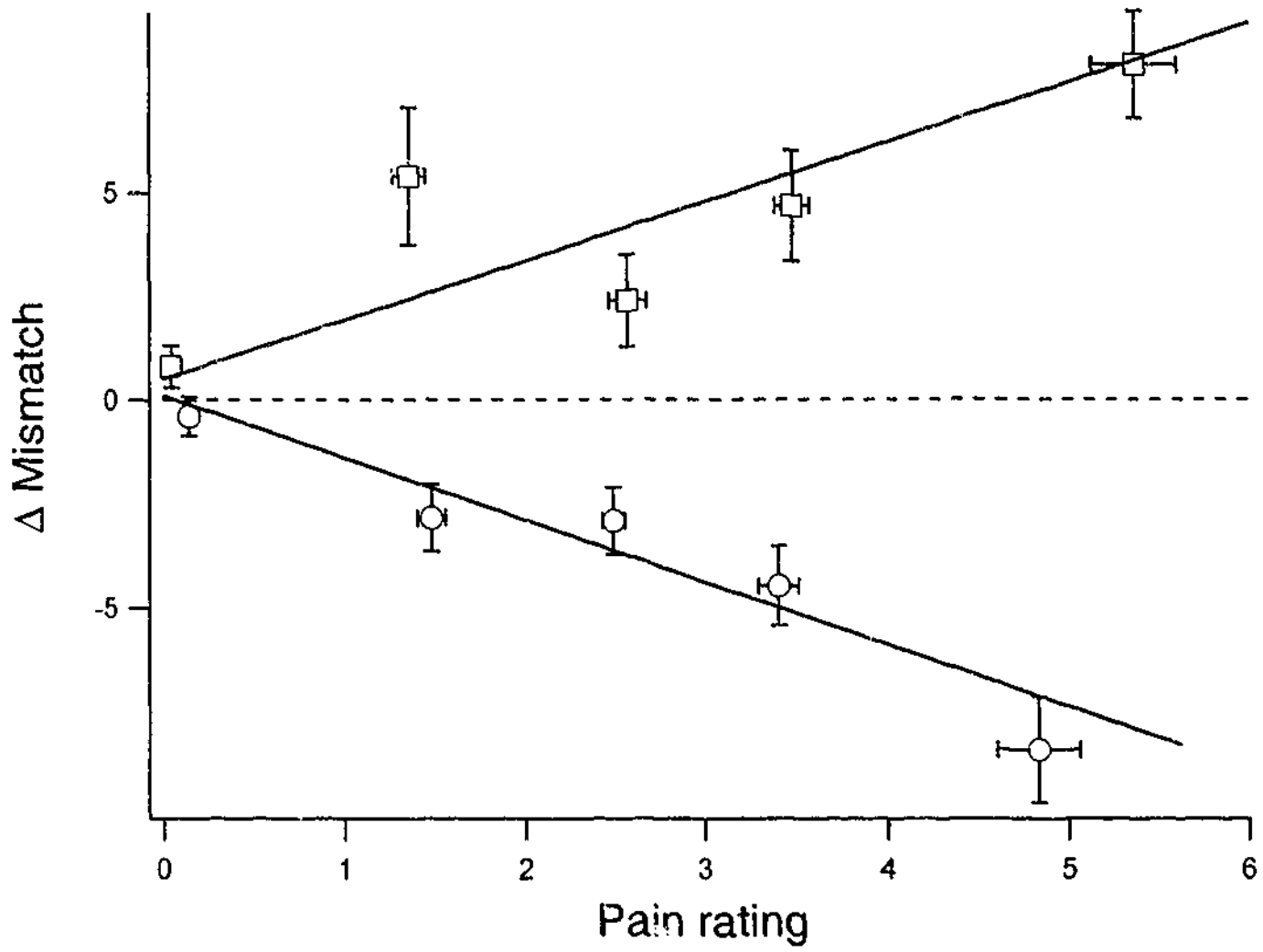


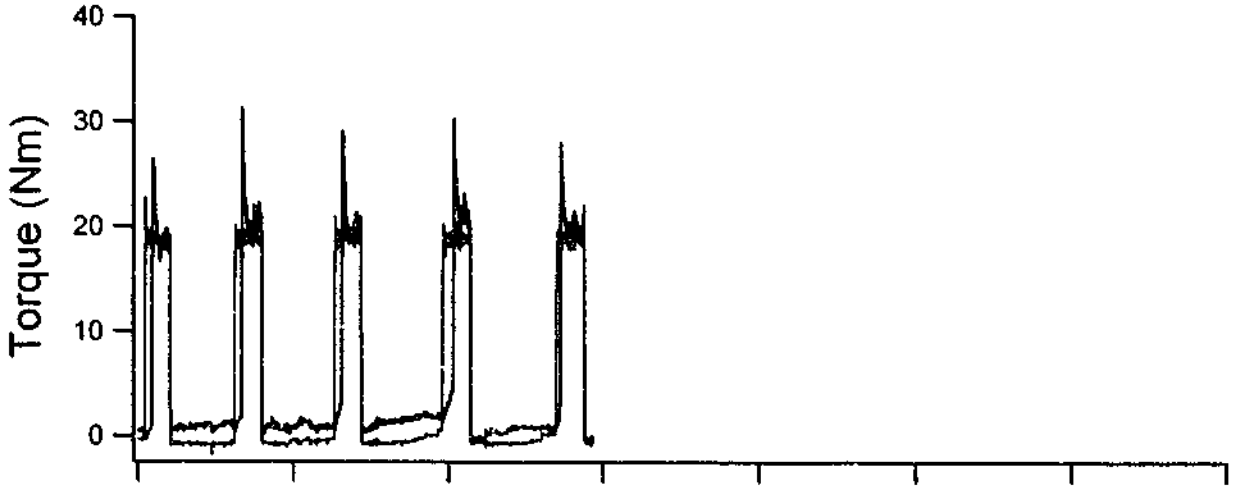
Figure 5.6

Plot of size of mismatch against level of pain. Values are given as means (± S.E.M.) after pooling of values in bins of 1 unit of pain over the range 0 - 6. Squares, values from injections into the muscle of the reference arm, circles, values from injection into the indicator. Regression lines have been drawn through the data.

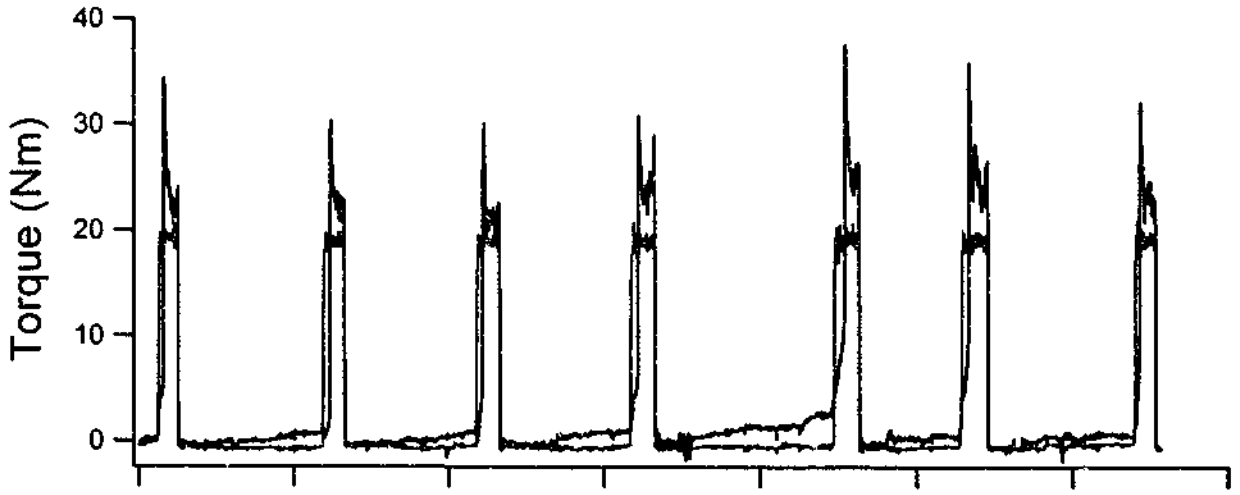
Figure 5.7

Records for one subject of matches of elbow torque, before, during and after application of heat stimulation of the skin overlying biceps skin of the reference arm. The torque (Nm) exerted by the reference arm (red) is being matched by the torque exerted by the indicator arm (blue).

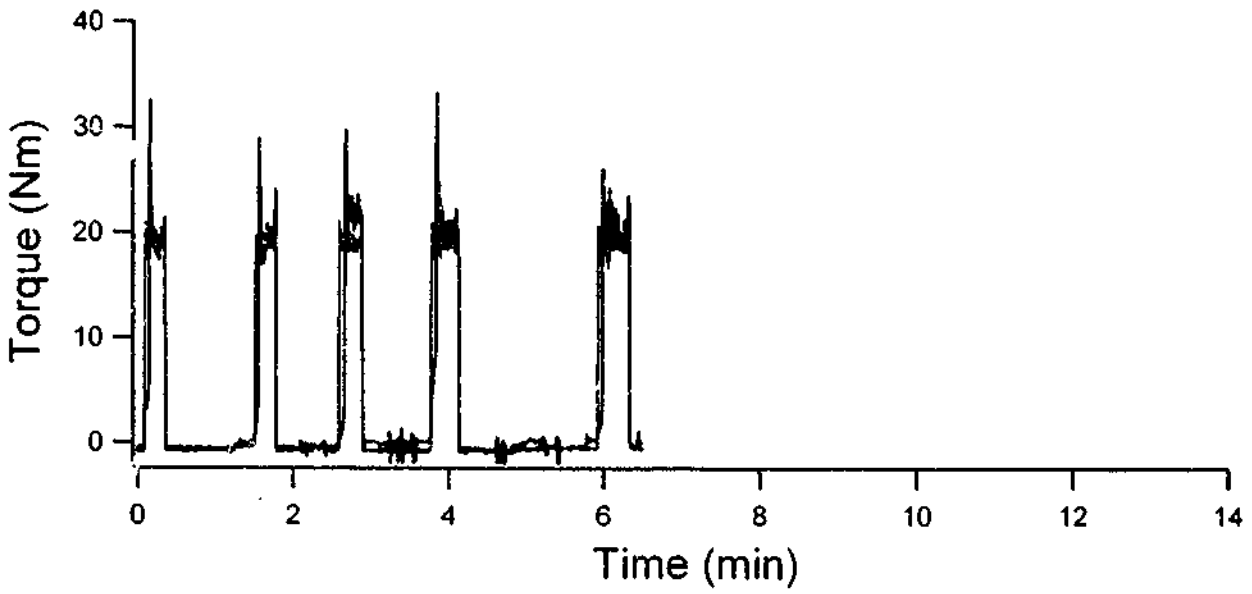
Before



During



After



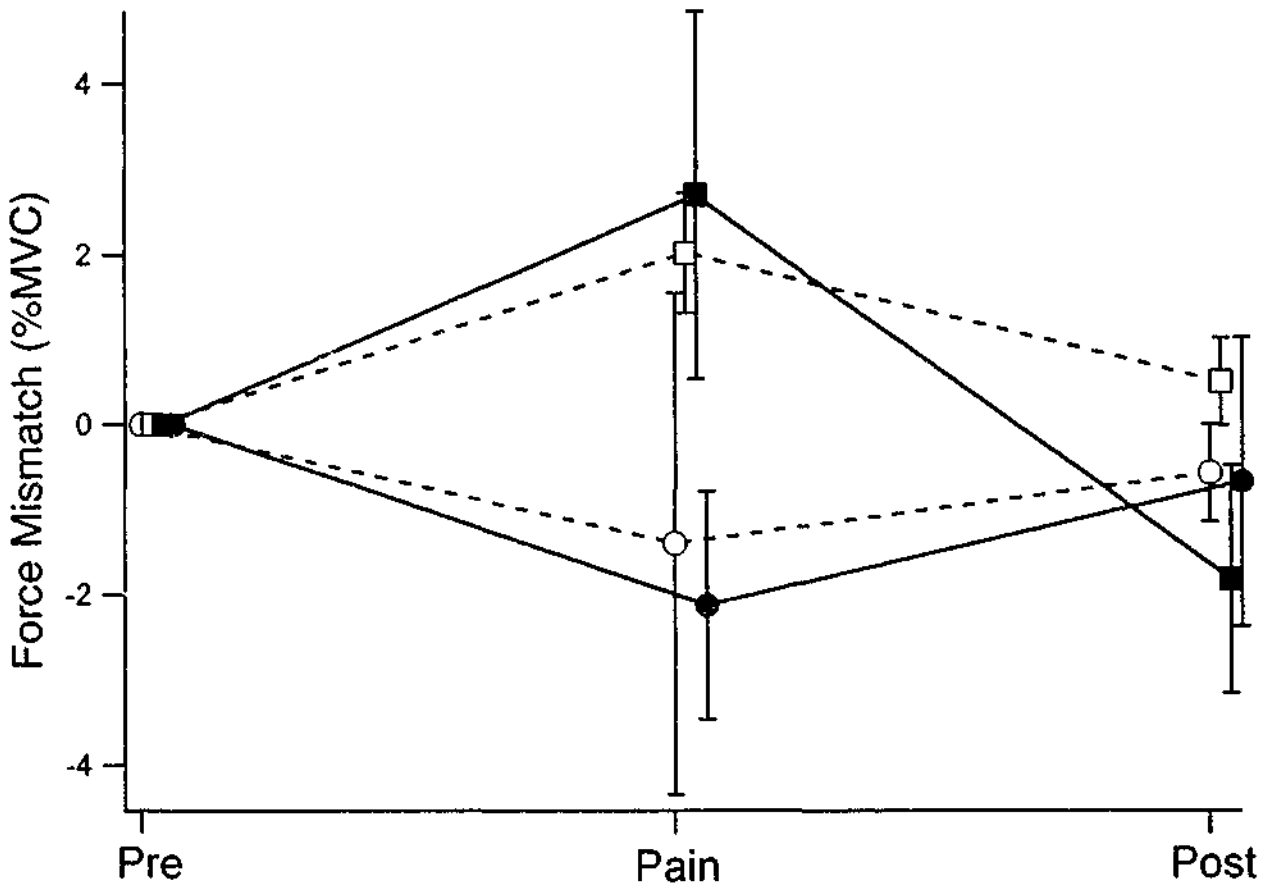


Figure 5.8

Mean mismatches adjusted to pre pain values. Torque mismatches before, during and after pain from heating the skin. Filled symbols, heating the skin over biceps (8 subjects), open symbols, heating the skin on the lower forearm (6 subjects). Squares, heating skin of the reference arm, circles, indicator arm. Values are means (± S.E.M.).

were also carried out during heating of skin of the calf. This had no effect at all. It was concluded that the effect on torque matching performance was greatest when the muscles involved in the task were made sore. There appeared to be a lesser effect from painful stimulation of skin overlying the muscle and no effect from stimulating remote skin.

DISCUSSION

The main finding of this study was that experimentally induced muscle soreness induced by means of hypertonic saline injections led subjects to make torque matching errors. When the contracting muscle of the indicator arm was sore, subjects tended to underestimate the target torque set by the reference arm, and when the reference arm was sore, they tended to overestimate it. That is, subjects believed that the level of torque being generated by the sore arm was more than it really was. Such errors were not present when isotonic saline was injected into the muscle.

It could be argued that the presence of pain simply distracted subjects from their matching task, leading to larger than normal errors. Perhaps subjects were simply 'favouring' their sore muscles? Several studies have shown that strong pain takes priority in subject's attention (Eccleston & Crombez, 1999). Luoto et al. (1996) demonstrated that pain or fear of pain decreased some components of motor performance in, for instance, a group of patients with chronic lower-back pain. However factors such as distraction and unintentional sparing of the muscle during contraction would not have been expected to lead to a close correlation between the size of the errors and the level of pain (Fig 5.6). Nor would simple reversal of the pattern of errors have been expected, depending on whether the reference or indicator arm was sore. Furthermore, in the study of Luoto et al. (1996) patients had been suffering chronic pain for many months, while here healthy subjects experienced mild pain for only a few minutes. The nature and origin of pain in DOMS is very different than for chronic lower back pain and the limitation for motor performance associated with lower back pain may be much greater than for DOMS, which subjects know will resolve itself within a few days. Therefore, it is unlikely soreness had a distracting effect in this experiment.

Painful heating of the skin over the contracting muscle produced a similar pattern of errors, although these were somewhat smaller (Fig 5.8). Therefore, the effect does not seem to be specific to muscle nociceptors. The injection of hypertonic saline in a muscle probably excites both non-nociceptive and nociceptive afferents within the Group III and IV range (Mense, 1993), while cutaneous heat pain mainly activates C-polymodal nociceptors (Bauman et al., 1991). Accordingly, the quality of the induced pain is different. Pain in the skin is usually described as sharp and burning, whereas muscle pain is often a deep aching sensation. Despite the fact that cutaneous and muscle pain are qualitatively different, suggesting that different neural mechanisms subserve these sensations, both appear to have effects on motor control. Yet, the effect does show some specificity since noxious heating of regions of skin remote from the contracting muscles did not lead to significant errors. This result further supports the view that it is unlikely that subjects were distracted by the pain or that errors were the result of non-specific reflexes. In addition, the errors were in a direction opposite to that predicted for a flexor reflex, so that a mechanism based on facilitation of flexor motoneurons through this pathway is also unlikely to be involved (Hagbarth, 1952).

The situation is a little similar to that reported by Bolanowski et al. (2001). They reported that heat-induced pain in the skin led to attenuation of tactile responsiveness. This was not thought to be due to attentional processes since subjects' ability to discriminate between different tactile stimuli remained unaffected.

The injection of hypertonic saline did not lead to long-term impairment of muscle function. Subjects were able to generate an MVC of comparable amplitude during the control series before and after the injections (Fig 5.1). Reference torques were generated without difficulty and subjects felt that they were able to carry out the matching task just as effectively before and during the muscle soreness. No subject reported any change in the amount of effort required to carry out the task in the presence of pain. They appeared to be quite unaware that during the pain their matching accuracy had fallen.

Mechanisms

The finding that noxious stimulation of both skin and muscle produced matching errors is consistent with observations in animal experiments. Studies of neurones in the dorsal

horn of the spinal cord, believed to be concerned with the central projection of nociceptive information, were rarely driven exclusively by slowly conducting afferents from muscle and many showed convergent input from skin (Hoheisel & Mense, 1990). Indeed, some cells identified as spinothalamic tract cells had convergent input from skin and deep structures (Craig & Kniffki, 1985).

The observations presented in this chapter provide no information about how the nociceptive input might interfere with motor control. The experiments of Le Pera et al. (2001) showed that following saline injection into finger muscles, at the peak of pain, transcranial magnetic stimulation of the left primary cortex produced MEPs of reduced amplitude, without any change in H-reflexes. Then a minute later, MEP amplitude fell further, accompanied by a fall in H-reflex amplitude. The findings led the authors to conclude that the primary effect of the noxious muscle input was at the level of the motor cortex, while there was some subsequent inhibition of spinal motoneurons, as indicated by the reduction of H-reflex size.

Le Pera et al. (2001) reported the onset of H-reflex depression at 2–4 minutes after saline injection. In the present experiments, by 2 minutes after pain onset, 4 matching trials had been completed. It was during these first trials, when pain levels were still high, that matching errors were at their largest. Assuming a similar time-course for saline pain in finger muscles and in biceps, these observations are not consistent with a primary effect by the nociceptive input on spinal motoneurone excitability. The close correlation, between the size of the errors and the level of pain (Fig 5.6) does suggest a single, underlying mechanism.

The sense of muscle pain is believed to be mediated by Gp III and Gp IV muscle afferents (Mense, 1996a). These become active during a contraction (Kaufman et al., 1983). It is known that during a sustained isometric contraction voluntary activation declines, despite the subject's continuing to exert a maximal effort. For a review see Gandevia (2001). It raises the possibility that excitability of the motor cortex is reduced as a result of the Gp III and IV activation from hypertonic saline. There is some evidence that suggests that Gp III and IV afferents exert their effects somewhere upstream of the motor cortex. The evidence is based on the observation that if after a fatiguing

contraction the muscle is kept ischaemic, with a pressure cuff, EMG responses to motor cortex stimulation recover but voluntary activation remains depressed, suggesting that neither cortical neurons nor spinal motoneurons are subject to direct inhibition (Taylor et al., 2000). It is known that Gp III and IV afferents are stimulated by ischaemia (Adreani & Kaufman, 1998). Additionally, it has been suggested that some small-diameter muscle afferents provide information about the disposition of the limbs including the degree of muscle stretch, muscle force and fatigue (Gandevia, 1996). Therefore, activity in Gp III and IV fibres could be responsible for the matching errors observed here.

Assuming that the noxious input from muscle produced by the hypertonic saline acts primarily at the level of the brain, the above discussion raises the question of where in the brain the nociceptor activity might exert its effects. The experiments of Le Pera et al. (2001) suggest a direct action on the motor cortex, while those of Taylor et al. (2000) propose an action on areas involved in driving the motor cortex. The experiments reported here provide no information about which of these alternatives is more likely.

Cutaneous pain has also been shown to produce inhibitory effects at the level of the motor cortex. MEPs recorded from muscle after transcranial electrical stimulation were shown to be inhibited for 20-30 min after the application of capsaicin to the skin overlying the muscle (Kenins, 1982; Farina *et al.*, 2001). Similarly painful CO_2 laser stimulation of the skin reduced the amplitude of EMG responses evoked by transcranial magnetic stimulation of the primary motor cortex, while non-painful CO_2 laser pulses did not produce any effect (Valeriani et al., 1999). All of this suggests a direct action on motor cortex excitability by the nociceptive afferents.

Positron emission tomography has shown that both noxious cutaneous and intramuscular stimulation cause regional cerebral blood flow increases in the secondary somatosensory cortex (SII) as well as in the inferior parietal lobule (Svensson et al., 1997). Such an overlap between cerebral processing of skin and muscle pain would be expected given the known convergence between nociceptor afferents from deep tissues and from skin onto WDR neurons in the spinal cord (Mense, 1993). The pain dependent inhibitory inputs in the primary motor cortex (MI) observed by Le Pera et al. (2001) have been suggested to be driven by the nociceptive input. The SII and MI areas have been

demonstrated to have many connections (Burton, 1986), so here the possible pathway remains uncertain.

The finding that smaller matching errors were produced from painful stimulation of the skin overlying the muscle compared to muscle pain, suggests that cutaneous pain has less effect at the motor cortex than deep muscle pain (Fig 5.5 and 5.8). This view is supported in other studies. The inhibitory effect of muscle pain at a spinal level was shown by a significant reduction of the excitability of spinal motoneurons, as demonstrated by a reduction in H-reflex amplitude (Le Pera et al., 2001). This does not seem to be so for capsaicin-evoked cutaneous pain (Farina et al., 2001; Le Pera et al., 2001). Moreover, it is known that an input via muscle afferents is more effective than cutaneous input in inducing prolonged changes in central excitability (Wall & Woolf, 1984). This was demonstrated by the observation that inhibition of the motor cortex after the induction of muscle pain persists even when the pain had receded (Le Pera et al., 2001). By contrast, during cutaneous pain, motor cortex excitability begins to recover while the pain is still present (Farina et al., 2003).

If muscle pain is interfering with motor control, one has to ask why might this be so? Apart from any differences in central processing of cutaneous and muscle pain the more intense pain during muscle stimulation may be responsible for such differences (Farina et al., 2003). Compared to cutaneous pain, muscle pain might be physiologically more relevant and meaningful, requiring a stronger and longer-lasting motor inhibition at both cortical and spinal levels, perhaps to prevent motor actions that might worsen the pain (Le Pera et al., 2001).

In this study, force matching errors correlated strongly with pain. No persistent errors were evident after the cessation of pain. This is different from the findings of Le Pera et al. (2001) who showed a prolonged effect. Such a difference may be due to the muscle groups that were used. Le Pera et al. (2001) injected hypertonic saline into abductor digiti, while here the injection was into biceps. Recent experiments on adductor pollicis brevis revealed hypertonic saline generated quite strong pain and evoked local muscle fasciculation, presumably by direct action of high levels of extracellular Na^+ on motor axons (Taylor, Proske and Gandevia, unpublished observation). Such motor unit activity

could engage spindle reflexes and central effects which could conceivably increase the level of pain. In addition, while in this experiment hypertonic saline had a diffuse, dull character and the pain appeared to arise from a large part of the biceps muscle, in a series of previous experiments (Weerakkody et al., 2001) injecting hypertonic saline into medial gastronemius, the pain seemed to arrive from only a small area, 1-2 cm in diameter, of the muscle. This may suggest nociceptors densities and their central representation differs between muscles. Therefore, saline injection into a smaller muscle group may induce a higher intensity of pain, producing more prolonged effects on the motor cortex. Such an idea is consistent with the peak visual analogue pain ratings that were found, which were lower in this study at 3.8 ±0.4, compared to 5.8 ±1.3 that was observed in the abductor digiti (Le Pera et al., 2001). There is also evidence that the central effects of nociceptive inputs from more distal muscles such as those of the hand are more potent than from the larger proximal muscles (Taylor, Proske and Gandevia, unpublished observation).

Muscle soreness after exercise

The currently accepted interpretation of matching errors made after eccentric exercise is that subjects do not match torque levels but the level of effort required to achieve a given torque (Saxton *et al.*, 1995; Carson *et al.*, 2002). If the torque for a given effort is below normal because of muscle fatigue or damage, it leads to matching errors. The additional consideration provided by the experiments reported from the previous chapter was that from 24 hours onwards, not only is muscle fibre damage a factor, but the sensation of DOMS may also be contributing to the matching errors. This is the inescapable conclusion, based on the fact that when effects of fatigue and damage were controlled for, small but significant errors persisted for up to four days.

Small diameter muscle afferents transmitting nociceptive input, which are thought to be responsible for DOMS, are capable of reducing voluntary drive during sustained contractions (Gandevia et al., 1996). Thus in the presence of DOMS, a given level of effort could generate less than the expected motor output. There is some evidence in support of such a view in the literature. It was reported by MacIntyre et al. (1996) that voluntary eccentric torque in human quadriceps declined after eccentric exercise with a

time-course that was bimodal, the second decline occurring at 20-24 hours, when DOMS had set in. This appeared to be present in women, but not in men (MacIntyre et al., 1996).

From the earlier findings in Chapters 2 and 3, it was concluded that large muscle afferents also contributed to DOMS. A proposed mechanism was that large muscle afferents were able to access the pain pathway at the level of the spinal cord. Therefore, if Ia or Ib fibres contribute to DOMS it is possible they may be responsible for some matching errors 24 hours post-eccentric exercise. That is, any Gp I activity generated by the matching contraction could have a central effect similar to that from pain. The fact that bradykinin, a well-known chemical substance involved in inflammation, has been shown to induce effects on muscle spindles in neck muscles of cats (Pedersen et al., 1997), provides the additional possibility that in response to inflammatory mediators accompanying DOMS, large fibre afferents could be responding to the pain to disturb motor control.

In addition, there is the complication of spindle reflex excitation which could be working to cause matching errors in response to pain. This mechanism could be taking place particularly after hypertonic saline injections. As described earlier, hypertonic saline preferentially activates Gp III and IV muscle afferents, with minimal effects on muscle spindle afferents (Paintal 1960). However, a number of studies have shown by direct recording or by recordings from muscle spindle afferents, that group III and IV muscle afferents reflexly excite fusimotor neurons (Ellaway *et al.*, 1982). Activation of muscle nociceptors with injections of hypertonic saline were found to significantly affect properties of jaw muscle spindles as a result of the action of central neural mechanism (Ro & Capra, 2001). Similarly saline injections were shown to induce large, statistically significant changes in cat gastrocnemius muscle spindle afferent activity (Thunberg et al., 2002). The activation of nociceptors may, therefore, induce pain and at the same time cause disturbances in proprioception and motor control via their effects on the γ-muscle spindle system (Johansson & Sojka, 1991; Johansson *et al.*, 1993).

However, uncertainty over DOMS and its effects on motor control does exist. Newham et al. (1987) found by means of electrical stimulation and twitch interpolation methods that in the presence of DOMS, subjects (5 female, 3 male) were still able to fully activate

their muscles despite any discomfort. In addition some studies have observed that muscle soreness has litile effect on motor performance. Kauranen et al. (2001) concluded DOMS had no effect on motor performance in the hand including simple reaction time, choice reaction time, speed of movement and coordination. However, the exercise used by these authors included a lot of concentric activity and was of low intensity, which therefore may not have produced as much damage and soreness as in my study. In addition, increases in plasma creatine kinase (CK) activity were used to justify their training session was of high enough intensity. However, CK activity does not appear to accurately reflect the level of structural damage to muscle fibres (Kuipers, 1994). The magnitude of increase in CK also can vary considerably between subjects (Balnave & Thompson, 1993). Therefore, such experiments on DOMS and motor control should be confirmed and extended.

From the above discussion its evident the real effect of DOMS on motor control still remains unresolved. To resolve this issue, in future experiments the next step would be to assess motor cortex excitability by using transcranial magnetic simulation or transcranial electrical simulation techniques, before, during and after DOMS is induced in a muscle. Alternatively, neuroimaging techniques such as positron emission tomography could be used to study the central pathways of DOMS in humans. Only by such methods can it be certain that DOMS causes reductions in motor output from a depressed motor cortex.

CHAPTER SIX

The length-tension properties of quadriceps and hamstring muscles of trained cyclists

INTRODUCTION

Up to this point the thesis has been concerned with DOMS and its mechanisms as well as with the effects of muscle pain on proprioception. However, it is a well-known experience that while a period of unaccustomed eccentric exercise makes our muscles sore, the same exercise a week later leaves us much less sore, this marking an adaptation or training effect. In this chapter subjects with different lifestyles, representing different training effects, will be compared, to study further the adaptation changes after eccentric exercise.

The adaptation process has been well established by studies showing that after an initial bout, if the eccentric exercise bout is repeated a week later, there is a reduction in stiffness and soreness and that there is a much faster recovery of muscle function (Byrnes et al., 1985; Clarkson & Tremblay, 1988; Golden & Dudley, 1992; Mair et al., 1995; Newham, Jones & Clarkson, 1987; Nosaka et al., 1991). Although this process has been known to occur for some time, the underlying mechanism remains controversial.

Although a number of theories have been proposed to account for the repeated bout effect (see General Introduction), the one that is of interest here is the hypothesis of Morgan (1990). Morgan suggested that the adaptation process involved the incorporation of more sarcomeres in series into the exercised muscle fibres. Having more sarcomeres in series without a change in fibre length means that at a particular length, average sarcomere length will be shorter. The advantage of having more sarcomeres at shorter lengths is that the length change during subsequent eccentric contractions brings fewer sarcomeres into a range where they become unstable and overextended, i.e., onto the descending limb of the length-tension relationship, a region of instability (Proske & Morgan, 2001). This is achieved by a shift of the muscle's length-tension relationship in the direction of longer lengths, as a result of the extra sarcomeres.

Support for this theory comes from studies showing that after a second bout of eccentric contractions there was a sustained shift in optimum muscle length toward longer lengths in the length-tension relationship that persists for several weeks (Brockett, Morgan & Proske, 2001; Jones et al., 1997; Lynn, Talbot & Morgan, 1998; Wood, Morgan & Proske, 1993). It is important to distinguish this sustained shift in optimum length, representing adaptation by increasing sarcomere number, from the initial short-lived shift resulting from muscle damage after an initial eccentric exercise bout. The initial shift is thought to be the result of the increase in series compliance from disrupted sarcomeres (Morgan, 1990). It rapidly reverses to control values over the next 2 days in humans (Jones et al., 1997; Whitehead et al., 1998). This quick reversal of the shift has been suggested to be due to the re-interdigitation of overextended sarcomeres (Talbot & Morgan, 1996), as well as several damaged fibres dying so they are no longer contributing to the compliance change.

Direct support for the sarcomere addition theory of adaptation is provided by the experiments of Lynn & Morgan (1994) who measured the fibre lengths and sarcomere lengths of fibres from fixed, acid digested muscles, by using laser light diffraction techniques. They showed that, after a week of training, muscle fibres of knee extensor muscles in rats, trained to run downhill (eccentric contractions) on a treadmill, contained significantly more sarcomeres than muscle fibres of sedentary animals and animals trained to run uphill (concentric contractions) over the same period. Furthermore, the uphill trained rats had fewer sarcomeres per fibre than the unexercised, sedentary rats, suggesting that exercise biased towards concentric contractions lead to a reduction in sarcomere number (Lynn & Morgan, 1994). Such findings suggest that the postulated adaptation process is bi-directional and could lead to either an increase or a decrease in sarcomere number depending on the nature of the exercise. Evidence of sarcomere reduction was shown years ago with studies of mouse soleus muscles immobilized in a shortened position showing a decrease in sarcomere number of about 20% (Williams & Goldspink, 1973). The active length-tension tension curve shifted in the direction of shorter muscle lengths, so that maximum tension was produced at a shorter muscle length (Williams & Goldspink, 1973).

Doing exercise that is predominantly concentric is advantageous for adapting a muscle to be able to generate significant levels of force at short muscle lengths. This adaptation could be achieved by reducing the number of sarcomeres in series in muscle fibres, therefore resulting in a shift of the length-tension relationship in the direction of shorter muscle lengths. It would also increase efficiency by reducing the mass of muscle required for a given level of force.

However, having fewer sarcomeres in series could make a muscle fibre more susceptible to damage from eccentric exercise. Support for this idea comes from the findings that decline-trained rats showed less signs of muscle damage (torques fell less and optimum angles shifted less) following a test series of eccentric contractions, compared to incline trained rats (Lynn & Morgan, 1994; Lynn et al., 1998). Similarly, it has been demonstrated that following concentric training, human subjects exhibit signs of greater muscle damage after an acute bout of eccentric exercise compared to those who undertake eccentric training prior to the test bout, or whose muscles remain unexercised (Whitehead et al., 1998). It was concluded that concentric training leads to adaptive changes in the muscle directed at optimizing muscle performance during the exercise, but in the process renders it increasingly susceptible to damage from eccentric exercise (Whitehead et al., 1998).

The aim of this study was to examine length-tension properties in human hamstring and quadriceps muscles, of subjects who regularly undergo concentric training and to compare this with untrained individuals. It was hypothesised that the muscles of regularly training cyclists (a predominantly concentric activity) would show such adaptive changes. Specifically, it was hypothesised that their optimum length for maximum force generation occurs at a shorter muscle length, than for untrained individuals, perhaps because fibres had fewer sarcomeres in series. Such a result would lend further support to Morgan's hypothesis, and demonstrate that adaptive changes of sarcomere reduction can be induced in regular training practices.

Angle-torque curves, reflecting the length-tension relationship, were constructed for the hamstring and quadriceps muscles. Length-tension curves for human muscle have customarily been made by using isometric measurements, where the muscle is fixed at a

particular length, then contracted voluntarily or stimulated electrically (McComas, 1996). This is then repeated at other muscle lengths (joint angles) to eventually construct a curve. An alternative method is to use isokinetic contractions. Isokinetic contractions are where a muscle contracts as the corresponding joint is rotated at constant velocity. In other words, it is a constant velocity movement for a given level of resistance. Such a resistance can be provided by an isokinetic dynamometer.

In this study the isokinetic method of measuring muscle length-tension property was chosen, because it would require a large number of maximum voluntary contractions (MVC) to generate an isometric angle-torque curve, and subjects would find this exhausting. The alternative to voluntary contractions, electrical stimulation of the motor nerve, would have been technically difficult and painful for the subjects, for these particular muscle groups. Electrical stimulation also brings with it complications arising from reflex responses (Collins, Burke & Gandevia, 2002).

Although an isokinetic test relies on voluntary effort, very good repeatability is achieved in motivated subjects (see review by Nitschke, 1992) with high levels of muscle activation (Babault et al., 2001). Factors that influence these measurements include subject related factors, such as age, weight, height, gender, limb dominance, athletic background and motivation. Testing conditions such as concentric or eccentric action, joint range of motion, test speed, rest intervals, type of subject feedback and number of repetitions, also affect measurements. Furthermore, pre-testing procedures such as warm-ups, joint start-position, limb stabilization, accuracy in aligning joint with axis of rotation of dynamometer, lever arm length, pre-load and gravity corrections as well as the method of data analysis can also affect the reliability of an isokinetic test (Keating & Matyas, 1996a; Keating & Matyas, 1996b; Nitschke, 1992; Wrigley & Grant, 1995).

The commonest protocol for isokinetically testing the knee joint, comprises subjects being seated upright in the dynamometer and performing concentric muscle activity consisting of 3-8 continuous repetitions of knee extension and flexion at an angular velocity between $60°s^{-1}$ and $240°s^{-1}$ (Brockett et al., 2001; Harding et al., 1988; Kannus & Beynnon, 1993). The isokinetic protocol for this study was modeled on the reports of

Harding et al. (1988) and Brockett et al. (2001), since both the protocol and analysis of repetition reliability were particularly well described for these studies.

METHOD

Subjects

The experiments were carried out on healthy males. The first group of subjects consisted of 8 trained cyclists who each regularly completed a minimum of 300 km on their bicycle each week (Mean age: 35.5 ±2.5 years, Mean weight: 75.8 ±2.2 kg). Cycling was required to be their only major activity. Cyclists who performed other types of training, such as regular running or weight training for their legs, were excluded from this study. The second group consisted of 12 subjects that participated in no regular training regime of any form (Mean age: 22.1 ±1.1 years, Mean weight: 77.8 ±2.3 kg). This second group acted as controls. Subjects gave their written consent for these experiments, which had been approved by the local human ethics committee and conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Isokinetic dynamometer

A Biodex® (Biodex Medical Systems, Inc., U.S.A) was used to test the angle-torque relationship of the knee flexors and extensors for the subjects (Fig 6.1).

For the purposes of this study, an angle-torque curve for a muscle about a joint relates to the torque produced when the muscle is voluntarily maximally activated during a constant velocity movement as a function of joint angle (which is indicative of muscle length). The optimum angle is the joint angle at which muscle torque is maximal.

The testing protocol was an average of 7 MVC isokinetic contractions at a velocity of $60°s^{-1}$. This velocity was used as it has been reported to produce the most reliable angle-torque curves in terms of reproducibility of the measurements (Brockett et al., 2001; Harding et al., 1988). Animal experiments comparing isokinetic measurements with isometric measurements confirmed that view (Brockett et al., 2003). At $60°s^{-1}$ velocity subjects were able to produce multiple maximum isokinetic contractions without

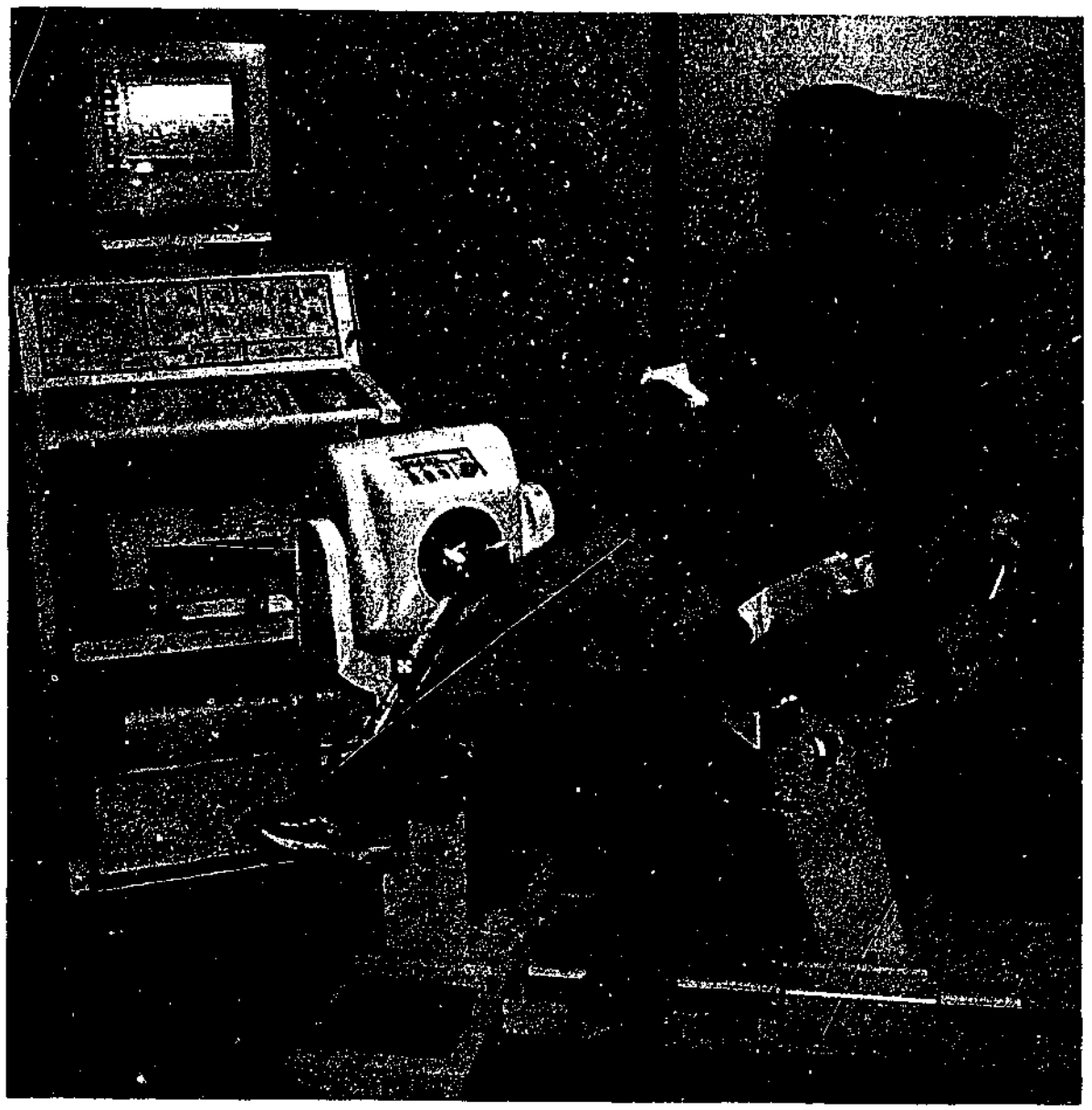


Figure 6.1

A subject performing isokinetic contractions on their quadriceps and hamstrings on the Biodex® isokinetic dynamometer. The subject is seated with their hips at approximately 90° flexion and their upper body secured with dual crossover straps, as well as a thigh strap over the test leg and a waist strap. Subjects gripped handles on the sides of the seat to assist in maintaining stable body posture.

discomfort. A gravity correction was included using the dynamometer software. During the test, subjects were seated with their hips at approximately 90° flexion and their upper bodies secured with dual crossover straps, as well as a thigh strap over the test leg and a waist strap (Fig 6.1). Each contraction was carried out over a knee angle range of approximately 100° from 70° to 170°. This range was found to be large enough to include the muscle's optimum lengths for force generation. Subjects also gripped handles on the sides of the seat to assist in maintaining stable body posture (Fig 6.1). Each leg was tested one at a time, with a suitable rest period between each test. For some subjects, the first test resulted in contractions that were not smooth, so a second test was performed. In an attempt to maintain motivation for extension and flexion MVC's, vocal encouragement from the experimenter was used with every subject.

Digital signals from the Biodex® were directly available through the system's software. All digital data was stored and analyzed using the program Igor Pro (Wavemetrics, Lake Oswego, OR).

Measurements

Optimum angle

A continuous recording was made of torque and knee angle over the time required for the seven repetitions, which was approximately 23 seconds. Torque values during flexion were digitized at a 100Hz sampling rate, then extracted and ordered according to knee angle. The data were then compressed using a decimation function, which averaged every 20 successive data points to calculate means and standard errors (SEM). The resulting plot of torque against angle allowed determination of optimum angle.

A curve was fitted to the decimated data points, using Igor Pro. The curve used a combination of two parabolae, one for the ascending limb of the curve up to the optimum and one for the descending limb (Brockett et al., 2003). The two parabolae had different curvatures but both had zero slope at the optimum angle and the values at optimum were equal. This curve fit was chosen because it gave a smooth fit to the data, and provided a convenient means of locating the optimum angle, for non-symmetrical curves. Only data points above 60% of peak torque were included in the analysis.

Peak active torque

For each session, the maximum torque produced, measured in Newton-metres (Nm), was determined at the peak of the fitted curve.

Width of curves

The width of the angle-torque relationship was taken to be the width of the fitted curve at 60% of peak torque.

Submaximal VO_2max test

To demonstrate differences in fitness between the trained cyclists and the controls, incremental exercise tests were performed on all the cyclists and on four of the twelve control subjects. This would also confirm that the cyclists chosen were suitably trained. The test was done using a Monark® cycle ergometer and by monitoring subject's heart rate (HR) using a Polar® heart rate monitor.

On the cycle ergometer, subjects were instructed to ride at a constant work rate of 60 watts. The HR was monitored, and recorded for 1 minute once it had stabilized. The work rate was then increased and the process repeated at 120, 180 and 240 watts (if required) until HR reached 120-150 bpm. From these measurements, an estimate of the maximal oxygen uptake (VO_2max) in litres per minute was obtained using the modified Åstrand-Ryhming nomogram (Astrand, 1960). Percentage body fat measurements were also performed using a BIM 4® Bioimpedance meter (UniQuest, St Lucia, QLD). This was done so that estimated VO_2max measurements could be adjusted for body mass and age (ml/kg/min), allowing for direct comparisons between subjects.

Statistical analysis

Means and plus or minus standard errors of the means (±SEM) across subjects were calculated for each measurement. A two factor repeated measures ANOVA was performed to test for significant differences in optimum angle, peak torque and VO_2max between the cyclist group and control group. Where an ANOVA was significant ($p<0.05$), an LSD (least significant difference) *post hoc* test was applied. The statistical analysis program used was Data Desk (Data Description, Ithaca, NY).

RESULTS

Typical examples of the relationship between raw torque and angle signals from the dynamometer, recorded for the left leg of one control subject and one trained cyclist subject can be seen in figure 6.2.

The control subject contracted quadriceps over a knee angle range of about 63° to 176°, where 90° represented the shin perpendicular to the thigh, that is, the lower leg hanging vertically. An increase in angle represented an extended knee, and so a shorter quadriceps muscle. The trained cyclist contracted over a narrower angle range of about 70° to 178° (Fig 6.2). Figure 6.2 also shows the muscle torque during 7 repetitions of knee extension and flexion. A positive torque represents quadriceps contraction, a negative torque, hamstring contraction. In the control subject the test sequence begins with a knee extension, producing an average peak torque of about 191 Nm (Fig 6.2). This is followed by knee flexion with an average peak torque of 86 Nm, from contracting hamstrings. This shows the hamstring peak torque was about half of that of the quadriceps (0.45) which is what would be expected from a healthy, but untrained individual. In contrast, for the trained cyclist, a quadriceps peak torque of approximately 215 Nm compared to 122 Nm for the hamstrings, giving a ratio of 0.57 (Fig 6.2). This is consistent with the idea that a trained athlete may have peak hamstring torque that is 60-70% of the quadriceps torque (Heiser et al., 1984; Orchard et al., 1997).

For each subject, knee flexion and extension torque and knee angle data were combined to construct angle-torque curves, then these curves were averaged using a computer generated decimation function as described in 'Methods'. Typical examples are shown in figure 6.3 and 6.4, which show a cyclist's left quadriceps and hamstring curves respectively. In these records, optimum angle was 114° and peak torque was 215 Nm for the quadriceps, and 129° and 122 Nm for the hamstrings.

The average optimum angle for torque generation for the quadriceps and hamstrings for each group can be seen in figure 6.5. For the quadriceps the average optimum angle was larger, representing a shorter muscle length, in the cyclist group compared to the control group, for both the right and left leg (Table 6.1A). These differences were found to be significant ($p<0.05$). For the hamstrings, the average optimum angle was also larger, but

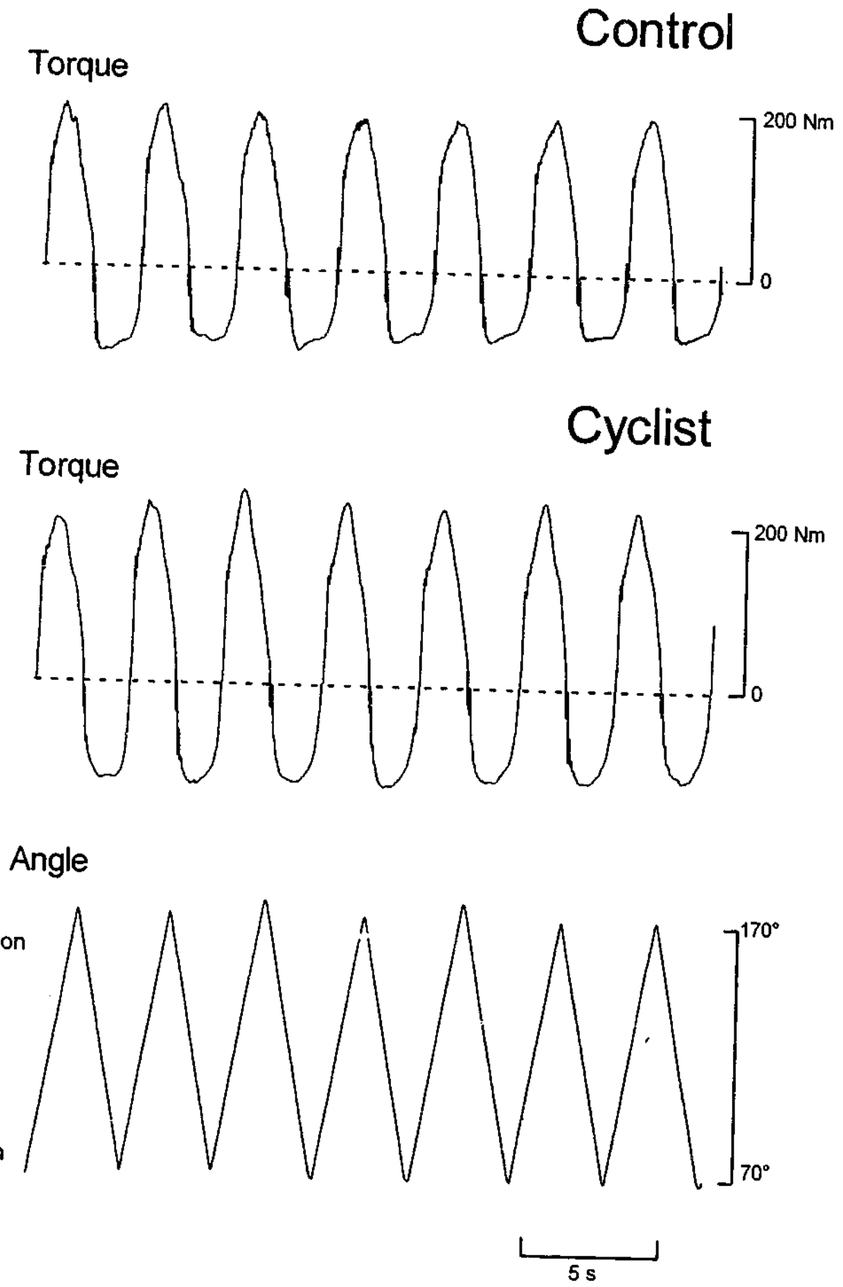
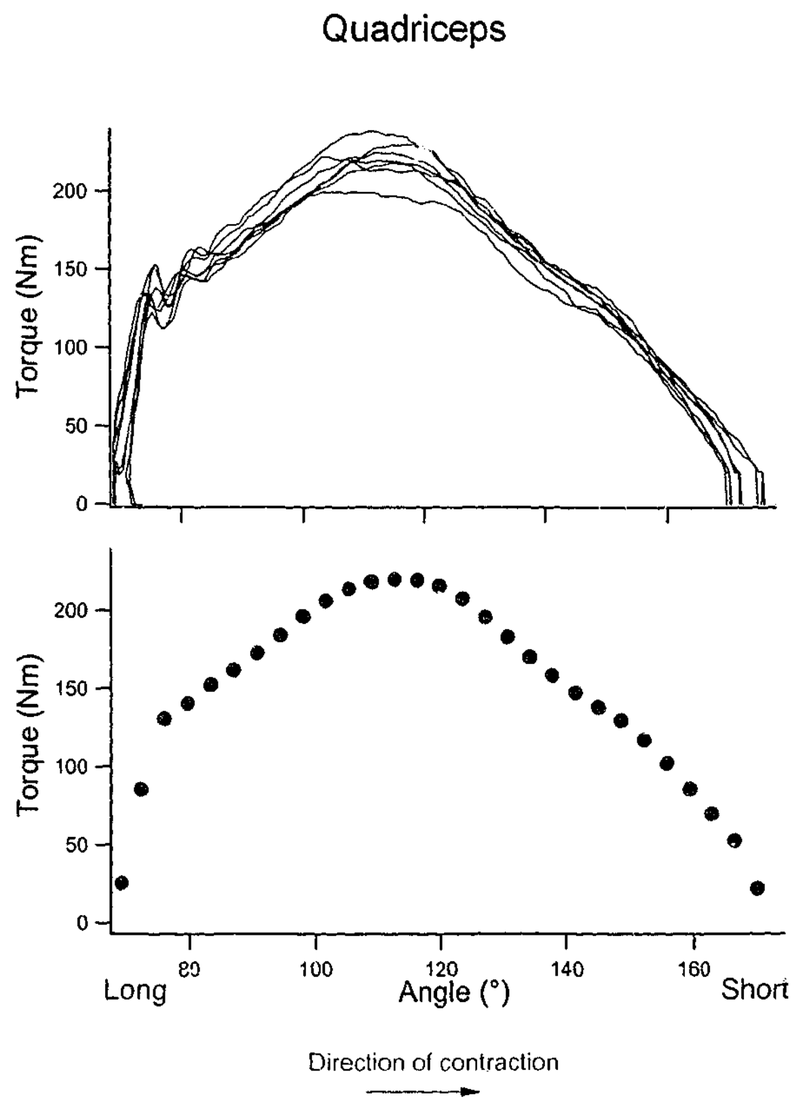


Figure 6.2

Top and Middle panels: Torque records from one control subject's and one cyclist's test on the Biodex® dynamometer respectively. For the test, 7 successive maximal voluntary isokinetic knee extension and flexion movements were performed at an angular velocity of $60°s^{-1}$. A positive torque represents quadriceps contraction, a negative torque, hamstring contraction. The dotted line at 0Nm represents the transition from knee extension to flexion. ***Bottom panel:*** Angle data for the cyclist's test. The test began with knee extension from an initial knee angle of approximately 70° to a final angle of 178°. An angle of 90° represents the lower leg hanging vertically.

Figure 6.3

Quadriceps angle-torque curves from one subject. Upper panel- torque records from seven knee extensions, plotted against knee angle. Lower panel- data have been decimated (average over every 20 continuous points). A two parabolae curve fit was set to the top 10% of the averaged values to determine the angle for peak torque.



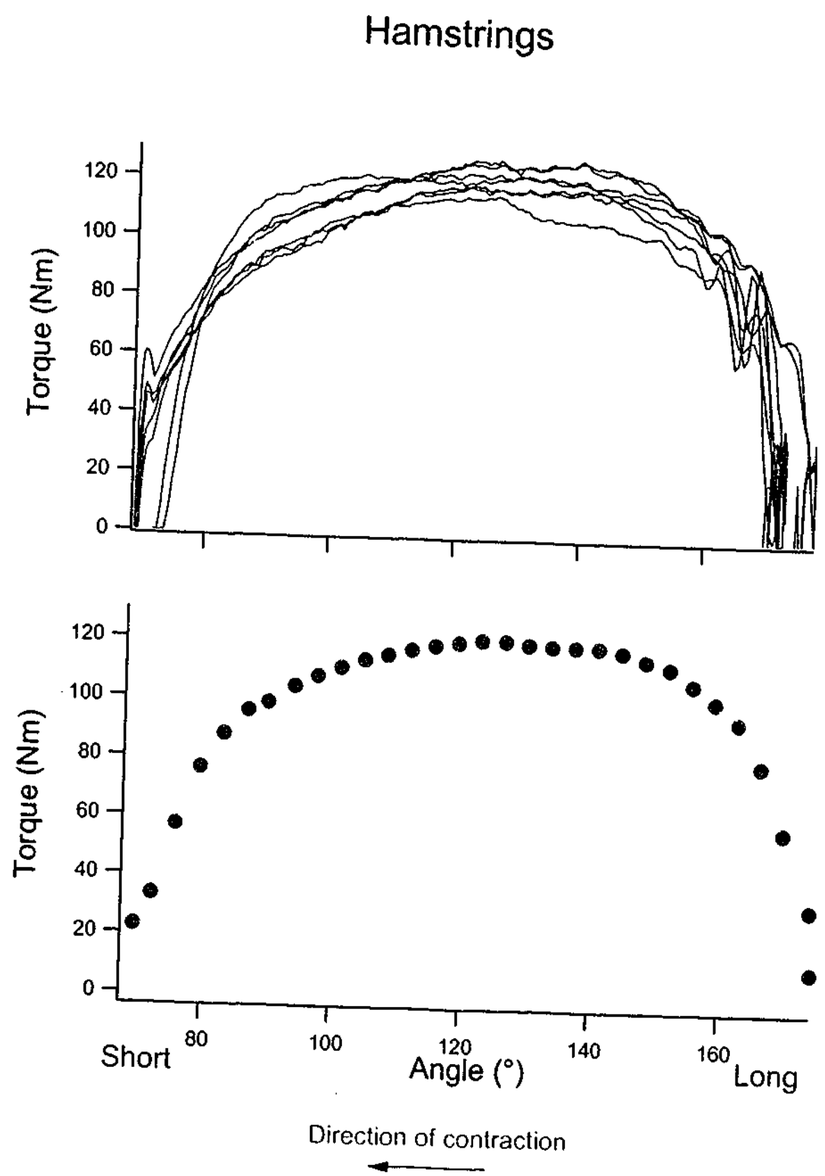


Figure 6.4

Hamstrings angle-torque curves from one subject. Upper panel- torque records from seven knee flexions, plotted against knee angle. Lower panel- data have been decimated (average over every 20 continuous points). A two parabolae curve fit was set to the top 10% of the averaged values to determine the angle for peak torque.

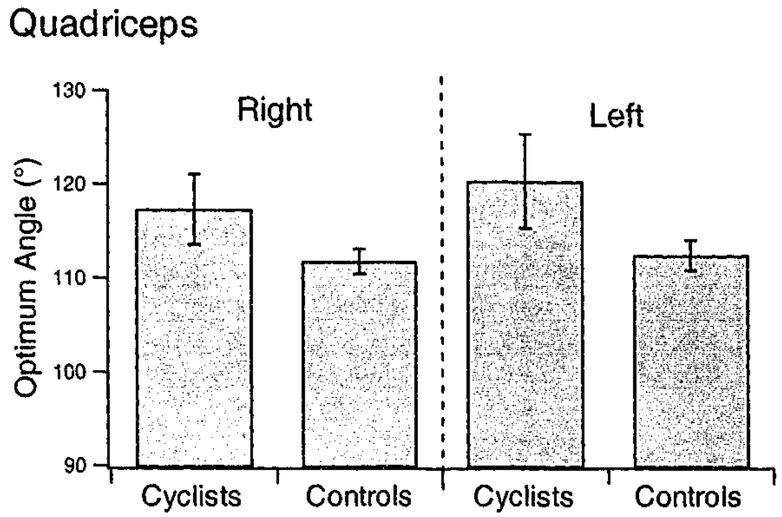


Figure 6.5

Histogram representation of mean optimum angle of quadriceps (top panel) and hamstrings (bottom panel) for the cyclist and control groups (± S.E.M.).

Table 6.1
Summary of the optimum angles, peak torques and the widths of angle-torque curves for the cyclist and control groups.

A

Average optimal angle (°)

	Quadriceps		Hamstrings	
	Cyclists	Controls	Cyclists	Controls
Right	117.3 ±3.8	111.8 ±1.3	149.9 ±4.1	138.4 ±3.4
Left	120.4 ±5.0	112.5 ±1.6	144.3 ±5.7	139.2 ±5.0

B

Average peak torque (Nm)

	Quadriceps		Hamstrings	
	Cyclists	Controls	Cyclists	Controls
Right	182.4 ±8.2	181.2 ±8.7	105.7±8.1	98.5 ±5.0
Left	164.4 ±18.3	182.9 ±7.1	92.8 ±9.4	94.8 ±3.7

C

Average width of angle-torque curve at 60% of peak torque (°)

	Quadriceps		Hamstrings	
	Cyclists	Controls	Cyclists	Controls
Right	73.3 ±2.3	72.3 ±2.4	75.7 ±3.5	79.22 ±2.7
Left	72.6 ±2.5	70.3 ±1.6	83.3 ±2.4	83.1 ±2.2

this time representing a longer muscle length, in both the right and left. However these differences were not significant.

The peak torque (in Nm) generated by each group can be seen in figure 6.6. The average peak torque generated by the quadriceps of the cyclist group was not significantly different to that generated by the control group for both the right and left legs (Table 6.1B). The peak torque generated by the hamstrings in the cyclist and control groups, was also not significantly different, for either the right or left legs.

If cyclists had fewer functional sarcomeres lying in series this would be expected to give their muscles a narrower length-tension curve, since a given change in sarcomere length would result in a smaller change in fibre length and hence muscle length. However, the average width of the angle-torque curves (in degrees) for quadriceps, determined at the 60% of maximum torque level, for the cyclist group was found not to be significantly different from those of the control group for both the right and left legs (Fig 6.7) (Table 6.1C). Similarly for the hamstring muscles the widths were also not significantly different for either the right or left sides (Fig 6.7).

The average VO_2 max (ml/kg/min) was higher for the cyclists than the control group (63.7 ±2.9 and 54.1 ±5.1 respectively) (Fig 6.8). The difference between the groups was found to be significant ($p<0.05$). This confirms that the cyclists that partook in the study were adequately trained and were significantly fitter than the members of the control group.

DISCUSSION

Cycling is a popular transportation, competitive sporting and recreational activity all over the world. It is an activity that requires predominantly concentric contractions from a variety of muscle groups. Although the gluteal group and the lower leg's soleus, gastrocnemius and anterior tibial muscles are all employed in cycling, for this study only the properties of the quadriceps and hamstring muscle groups were investigated. This was because ankle-torque curves for the quadriceps and hamstrings could be easily obtained from the dynamometer.

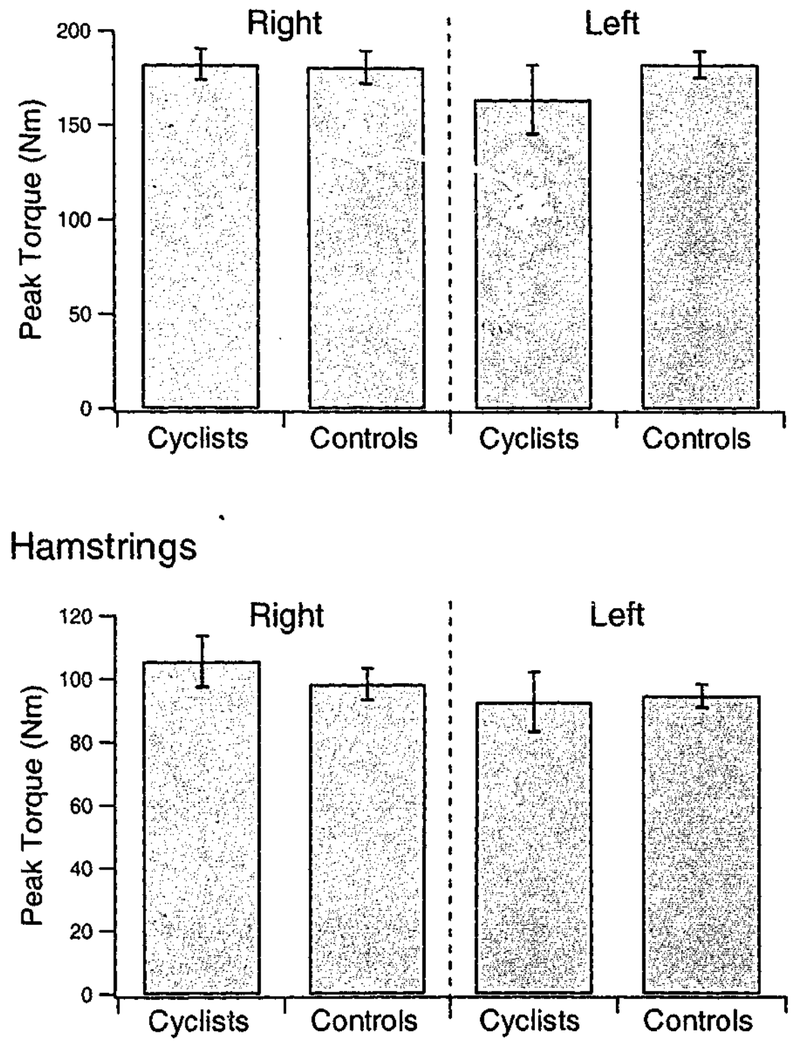


Figure 6.6

Histogram representation of mean peak torque of quadriceps (top panel) and hamstrings (bottom panel) for the cyclist and control groups (± S.E.M.).

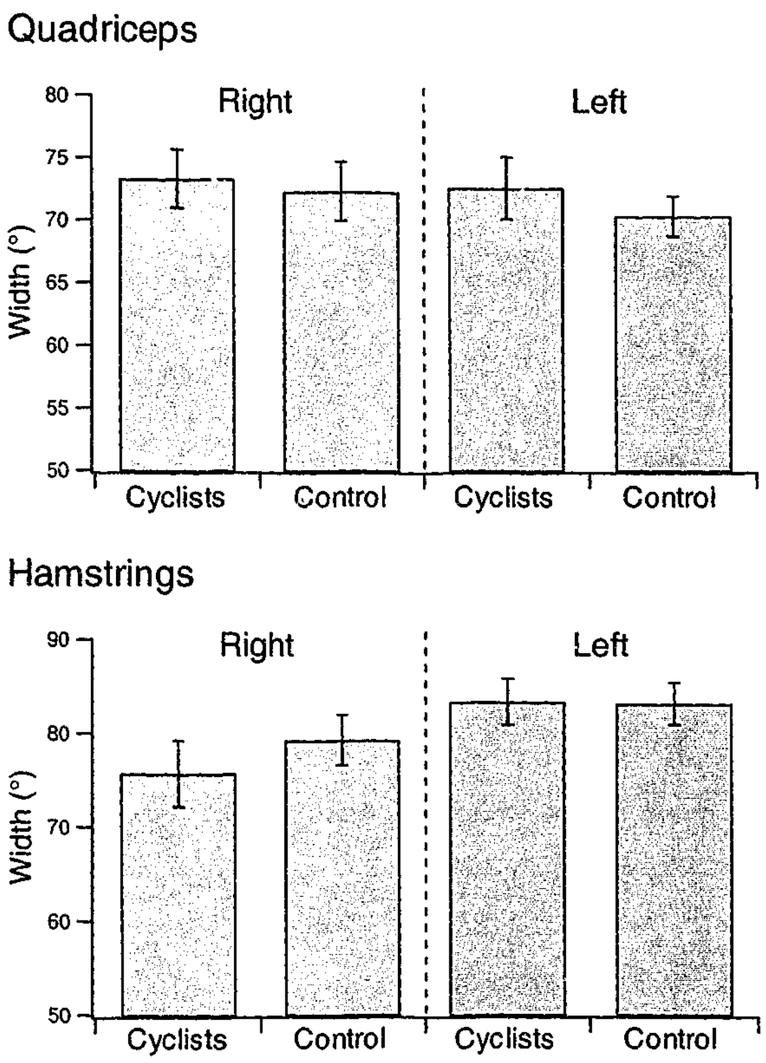


Figure 6.7

Histogram representation of the mean width of the angle-torque curves at 60% peak torque of quadriceps (top panel) and hamstrings (bottom panel) for the cyclist and control groups (± S.E.M.). Values of width are in degrees.

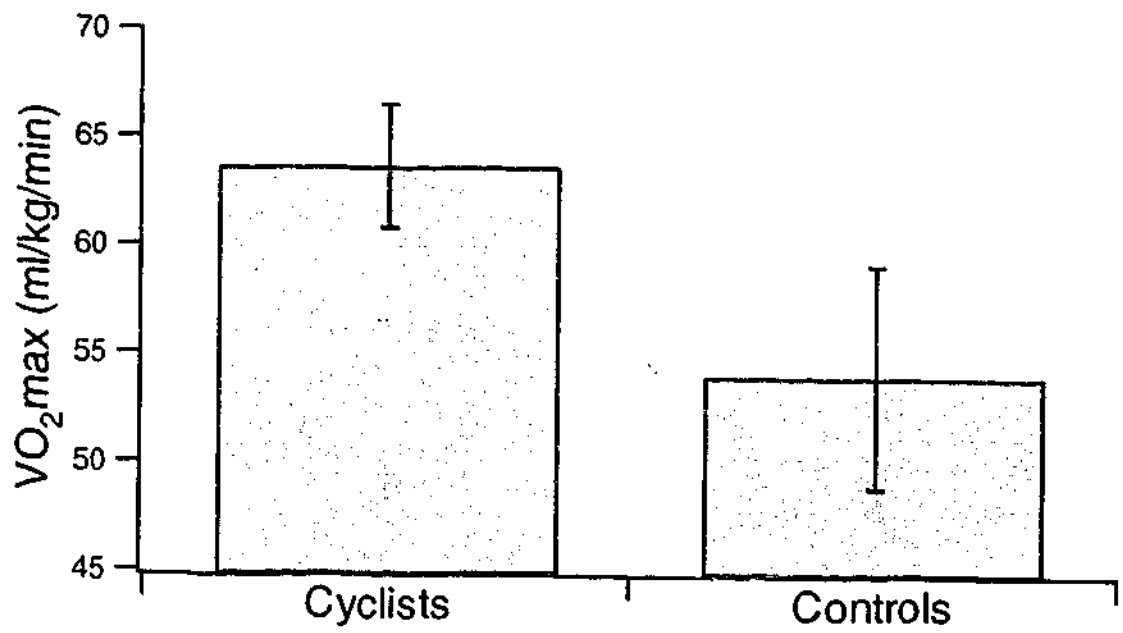


Figure 6.8

Histogram representation for the mean VO_2max (ml/kg/min) for the cyclist and control groups (± S.E.M.).

The cyclists used in the study were trained over long distances, therefore one may expect a training effect primarily in type I and type IIa motor units. It may be argue that the measurements of torque-angle curves that were made reflect a substantial contribution from fast-twitch (IIb) motor units, hence may not reflect the properties of those motor units that have been trained the most. However, any change in motor unit properties as a result of the training would not be expected to affect torque-angle curves since subjects were encouraged to generate maximal isokinetic contractions which recruited the whole muscle. Here it is assumed that changes in motor unit composition from training do not alter the whole muscle length-tension relation.

For the trained cyclists, the optimum angle for maximum torque generation represented a significantly shorter muscle length for the quadriceps muscle, when compared with the control group (Fig 6.5). Although direct analysis of sarcomore number was not made, it can be deduced from this finding that cyclists have fewer sarcomeres in series in their quadriceps. This is consistent with the hypothesis, that concentric training leads to an adaptive reduction in the number of sarcomeres in series in the muscle. Therefore, ultimately this result lends further support to Morgan's 'sarcomere addition' theory of adaptation from eccentric exercise.

However, for the hamstrings there was no significant difference in optimum angle between the cycling and control groups. Hamstring muscles are also known to undergo concentric contractions during cycling, therefore the question had to be asked, why did they not also show shorter optimums in the cyclist group? This can be better understood by considering more closely the biomechanics of cycling.

Cycling involves a repeated pattern of force application to the pedal-crank system of a bicycle. The pedaling action is a circular one with one complete circular motion called a pedal cycle (Sanner & O'Halloran, 2000). The pedal cycle has been considered to occur in two phases: a power or propulsion phase during which the forces are applied to the first 180° of crank rotation beginning at top center and ending at bottom center, and the recovery phase, usually considered to be the second 180° of movement from bottom center to top center (Sanner & O'Halloran, 2000) (Fig 6.9). Electromyographic (EMG) studies have been used to identify the specific muscle involvement during the power and

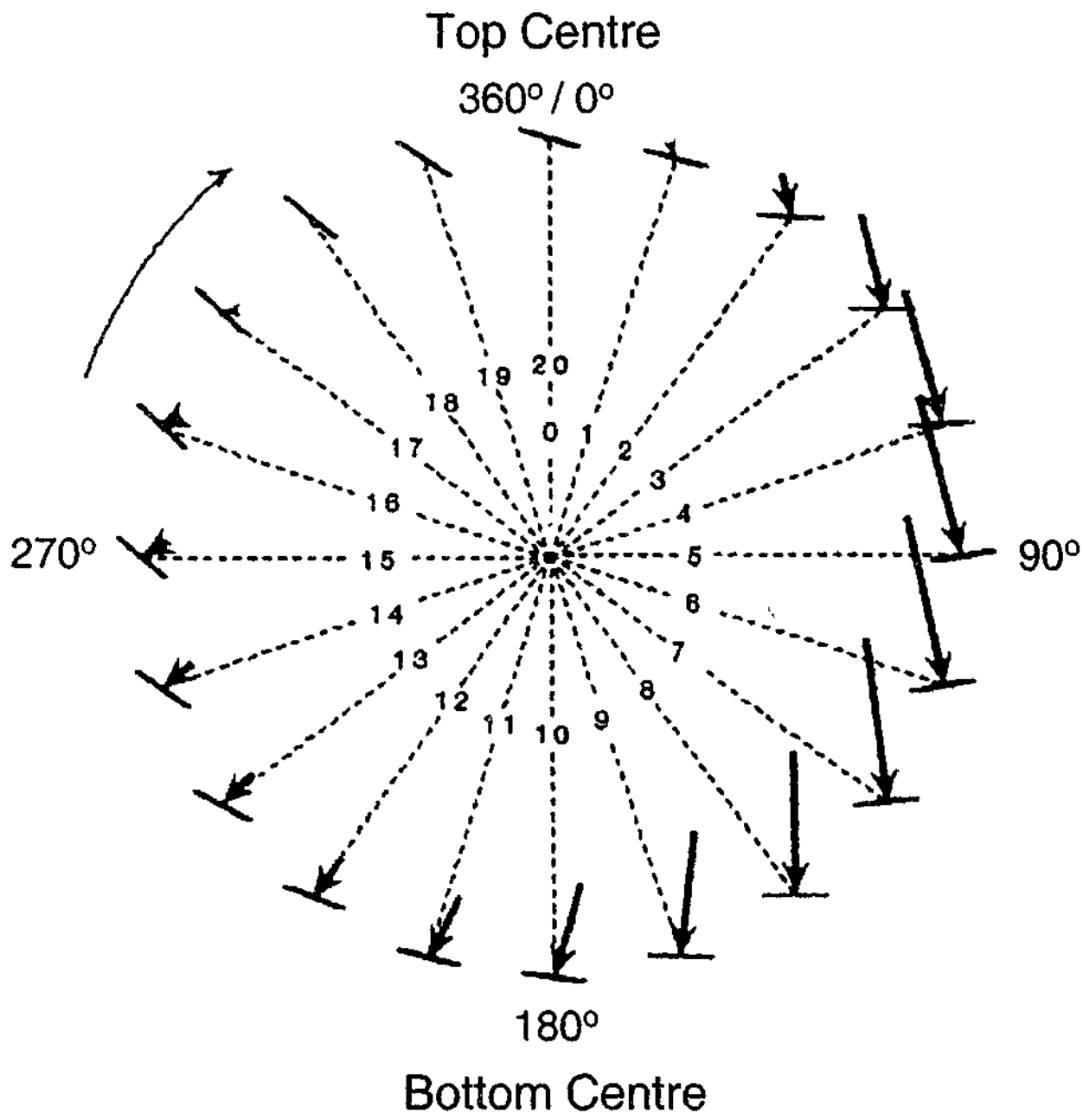


Figure 6.9

Diagrammatic representation of the pedal cycle. The radiating dotted lines represent the position of the crank at 20 positions of the crank cycle. The resultant force vectors that are applied to the pedals are shown by the bold arrows. The length of the arrow is proportional to the magnitude of the force, and its orientation shows the angle at which the force is applied. It is interesting to note the orientation of the force vector during the first half of the revolution and the absence of pull up forces in the second half. From Cavanagh & Sanderson (1986).

recovery phase of pedaling. For a review see Faria and Cavanagh (1978). The power stroke in cycling is produced predominantly by contraction of the quadriceps muscles, flexing the hip and extending the knee. The recovery phase primarily involves the hamstrings muscles serving to extend the hip and flex the knee.

More specifically from top centre to 90° the muscles of the quadriceps group are active (as well as the gluteus maximus and gastrocnemius muscles) (Jorge & Hull, 1986), with the vasti muscles exhibiting greatest activity between 340° and 100°. From 90° to 270°, activity is limited to the hamstrings (and gastrocnemius) (Jorge & Hull, 1986). The biceps femoris and semimembranosus are most active in the middle of the recovery phase (Faria, 1984). Active muscles from 270° to top centre include the rectus femoris (and tibialis anterior). Although different studies show some differences in the regions of activity of the quadriceps and hamstring muscles, they are reasonably consistent. The differences are thought to be due to different foot-to-pedal connections, different equipment and variability of the subjects.

The consequences of the circular action and changing orientation, and of inertia, are that the pedal forces vary continuously both in magnitude and orientation throughout the pedal cycle (Fig 6.9). The resultant force during the recovery phase is frequently applied in a direction opposite to that intended. The effective component of this force works against the rider, remembering that both legs are moving in synchrony, but 180° out of phase. That is, as the left leg is moving down in propulsion, the right leg is moving up in recovery. Figure 6.9 shows that propulsion occurs whenever the effective component of the applied force is positive and in the direction of pedaling. Also it is clear that the resultant force is effective beyond the first 180 degrees. Indeed, the goal during the recovery phase is to introduce as much positive effective force as possible, that is, to make the propulsive phase as long as possible. Negative peak torque represents lifting the foot during the recovery phase by the opposite pedal. Experienced cyclists do not pull up on the pedal during the upstroke (Faria, 1984). Rather, the larger peak torque observed during high power output carries the leg through the upstroke (Faria, 1984). Hence, as the desired goal in cycling is to make the propulsive phase (quadriceps contraction) as long as possible, trained cyclists maximize the work during this phase. Therefore, the knee extensor muscles are the most important group during cycling. The

total concentric positive mechanical work during standardized ergometer cycling was found to be 39% for the knee extensors (quadriceps), while only 10% for knee flexors (hamstrings) (Ericson, 1986). Similarly, in particular, the vastus medialis and vastus lateralis of the quadriceps group were found to be the most activated lower limb muscles during standardized ergometer cycling (Ericson, 1986). This greater concentric work completed by the quadriceps, compared to the hamstrings could ultimately account for why hamstring optimum angles of the cyclist group were not significantly different from the control group. Although the hamstrings of cyclists are concentrically trained, they may not be trained sufficiently to observe a shift in the length-tension relationship using the method that was used here with the dynamometer.

In a study by Lynn & Morgan (1998), angle-torque curves were measured isometrically. Following eccentric exercise training, the curves became wider and the optimum length for the contraction occurred at longer lengths, consistent with an increase in the number of sarcomeres in series in muscle fibres (Lynn et al., 1998). Although the absolute working range of the muscle remains relatively constant, the shift added with the broader length-tension relationship means that less of the muscle's working range is on the descending limb. Conversely, concentrically trained subjects were expected to have narrower length-tension curves. However, narrower curves for the cyclist group's quadriceps or hamstrings were not particularly obvious in this study (Fig 6.7). This could possibly be due to the fact that the curves measured for the quadriceps were the sum of the angle-torque curves from rectus femoris, vastus intermedius, vastus medialis and vastus lateralis. Similarly, the hamstring curves were the sum of the angle-torque curves from biceps femoris, semitendinosus and semimembranosus. As each of these muscles, as well as the different fibre types within the muscles, are not utilized equally during cycling, and so are likely to respond to the concentric training slightly differently, and therefore adapt differently, any change in the width of the overall quadriceps or hamstring curves from sarcomere reduction, would be difficult to detect. Furthermore, width measurements would have relied on the accuracy of torque measurements over the testing range, and it was evident that variability increased at the extreme knee angles (see Fig 6.3 and 6.4). It is suggested that the variability early in the contractions, over approximately the first 10^{o} of the knee flexion, is the result of different activation times

and patterns of motor unit recruitment. Likewise de-recruitment patterns at the end of a contraction may be slightly different from one contraction to the next.

The average peak torques for both the quadriceps and hamstrings for the cyclist group were found not to be significantly different from the control group (Fig 6.6). It may have been anticipated that cyclists would show higher peak torques. A possible explanation for this finding could be that the cyclists who took part in the study were road cyclists. Unlike sprint cyclists, they are not trained for power output, but for endurance, as is evidenced by their higher VO_2max (Fig 6.8). Therefore, maximum effort isokinetic contractions may not have entailed a higher average peak torque than a set of randomly chosen controls. In addition, the respective ages of the subject groups may have also been a factor. The cyclist group was, on average, about 13 years older than the control group. Therefore, the younger untrained group may have been able to generate as much force as the older trained group.

The cyclists who took part in this study had undergone years of regular training. As a result muscular adaptations such as hypertrophy, changes in capillary density, mitochondrial density, muscle enzyme content and myoglobin levels had probably occurred (Tesch, 1991). Neural adaptation may also contribute to a strength training effect. Changes in EMG recordings after short periods of strength training have been attributed to changes in motor unit recruitment patterns, involving lowering of threshold for activation of the large fast motor units, changing motor unit recruitment order, or increasing the rate of motor unit activation (Sale, 1991). Such changes would result in peak torque being reached earlier in a shortening contraction, thereby shifting the angle-torque curve in the direction of longer muscle lengths, which is in a direction opposite to that found here after concentric training. Therefore, this is not consistent with mechanisms such as strength training effects or neural adaptation effects causing a maintained shift of the optimum. Hence, this could lend support to other mechanism such as sarcomere reduction after concentric training. Alternatively neural adaptation may be a factor that counters a shift from a proposed adaptation of sarcomere reduction. Therefore, this maybe causing an underestimation of the true amount of shift.

In summary, this study has demonstrated that normal real life regular concentric training practices such as cycling, can induce measurable adaptive changes to the muscle, by a possible mechanism of removing the number of sarcomeres in series. This result has implications for athletes and their choice of training strategy, in particular athletes involved in sports which include both concentric and eccentric exercise, such as triathletes and marathon runners. Specific eccentric training may be considered as a regular component of their preparation. However, such athletes should try and achieve a balance in their training program which promotes enhancement of the muscle performance without rendering the muscle more susceptible to the damage of eccentric exercise.

CHAPTER SEVEN

General Discussion

This thesis has focused on exploring various aspects of eccentric exercise. The neural origins of the resulting soreness, DOMS, was investigated to determine to what extent it might be mediated by non-nociceptive muscle mechanoreceptor afferents. The effect of eccentric exercise and soreness on our proprioceptive sense of muscle force was also studied. It was observed that the disturbance in proprioception may be more complex than previously thought, with various factors possibly being involved. The length-tension properties of differently trained individuals was also examined to gain better insights into training effects and to determine how lifestyle might influence someone's susceptibility to the damage from eccentric exercise.

The origins of DOMS

The specific characteristics of DOMS which are unlike other forms of pain, lead to the question could altered processing of activity in large diameter afferents underlie the mechanical allodynia observed in DOMS? Barlas et al. (2000) found evidence for such a mechanism, showing a significant increase in mechanical pain threshold in elbow flexor muscles with DOMS, when the conduction in large myelinated fibres was blocked using a differential ischaemic block technique. The claim that non-nociceptive muscle mechanoreceptor afferents are involved in DOMS is important, because, if true, it would require modification of the traditional hypothesis that an increased sensitivity of nociceptor endings supplied by Group III and Group IV afferents was responsible.

The findings of Chapter 2 showed that in a subject with DOMS in the exercised muscle, applying vibration with controlled, painful indentation into the exercised muscles resulted in a significant increase in pain. This was attributed to primary endings from muscle spindles, since they are known to be the only mechanoreceptor responsive to vibration at such high frequencies (Brown, Engberg & Matthews, 1967a). Also, the lowest pain threshold for DOMS during vibration coincided with the human optimum response frequency of human spindles, 80 Hz (Roll, Vedel & Ribot, 1989). Although it is possible that nociceptors become vibration sensitive when sensitized, it seems unlikely

that they are able to acquire the same optimum frequency response as spindles. In addition, pain thresholds were seen to increase after blocking afferent impulses from large-diameter nerve fibres, where conduction was monitored by means of the H-reflex. At the same time latency to painful heat did not significantly change, nor was the sensation of cold affected, indicating indirectly that small diameter afferents were still conducting impulses through the block. Moreover the effects of vibration, acting to exacerbate the pain were absent during the block of large-diameter nerve fibres. All of these findings suggest that large diameter afferents from non-nociceptive muscle mechanoreceptor are involved in DOMS.

In Chapter 3 another approach was tried, using hypertonic saline as an experimental model of muscle pain, to further explore the possibility of large fibre mechanoreceptor involvement in DOMS. It was suggested that if nociceptors were in a sensitised state in DOMS, stimulation of such nociceptors with hypertonic saline should produce more pain, since that two stimuli would be expected to summate. However, the soreness evoked by hypertonic saline was not significantly different in muscles of subjects experiencing DOMS, compared with unexercised muscles. It was also found that, in unexercised muscles, compression plus vibration in the presence of saline pain decreased the amount of pain produced by compression alone. Vibration plus compression in the presence of saline pain in a muscle with DOMS tended to increase soreness levels. That is the effect of vibration on pain from compression followed the same pattern in the presence of soreness from hypertonic saline in both exercised and unexercised muscles. All of this suggested that DOMS and the pain associated with hypertonic saline were caused by different mechanisms. The balance of the data led to the conclusion that DOMS is not just associated with nociceptor sensitization, at least in its simplest form, and is likely to involve input from muscle mechanoreceptors.

The implication of this conclusion is wide-ranging, providing new insight into our understanding of muscle nociception and giving us a better appreciation of why DOMS can be so debilitating. Whenever we move a muscle with DOMS, it leads to pain. Perhaps this is some kind of protective mechanism to help facilitate the repair process following the muscle damage from eccentric exercise. However, since large afferent activity was not directly monitored in these studies, more direct evidence is required to

confirm the theory. The next step, in future experiments, would be to use animal models. The monitoring of spindle afferent activity during mechanical stimulation of muscles with DOMS compared with unexercised muscles might provide new information.

It was proposed that central mechanisms were responsible for the change in processing of mechanoreceptor information to give non-nociceptive mechanoreceptors access to the pain pathway responsible for DOMS. Specifically, central sensitisation manifested as increased spontaneous activity, hyperexcitability and increased receptive fields of central WDR neurons secondary to primary afferent hyperactivity (Willis et al., 1996), may be induced by muscle fibre damage from eccentric contraction. Exploring such a mechanism is an important area of enquiry in the future. Direct recording from dorsal horn neurons is necessary to test this theory. In future experiments, it would be interesting to study receptive field properties of dorsal horn neurons in animal models, seeking evidence, specifically, for cells which receive proprioceptive as well as noxious inputs, before and after a period of eccentric exercise.

Whenever observations involve conscious sensations it is difficult to obtain direct supporting evidence for a hypothesis. For DOMS, it may in the future be possible to obtain direct evidence of a large-fibre contribution using the techniques of microneurography and microstimulation in conscious human subjects, to stimulate identifiable spindle afferents to see if they produce pain, as has been done for secondary hyperalgesia and allodynia in skin nociception (Torebjork, Lundberg & LaMotte, 1992).

Although the vibration experiments (Chapter 2) point to muscle spindles as the most likely muscle afferents to be involved in DOMS, work showing an increased responsiveness of tendon organs to vibration after eccentric contractions means that they are potentially involved in DOMS as well (Gregory et al., 2002). Therefore, study of the specific kinds of afferents involved is another area for future investigations.

An issue that may require further confirmation is the extent to which cutaneous mechanoreceptors and nociceptors contribute to sensations of DOMS. In the present study, to ensure that muscle soreness was not generated by cutaneous receptors, the skin overlying the stimulated area was treated with anaesthetic cream (EMLA). Values for

pain thresholds with skin anesthesia were not significantly different from values when skin sensation was intact, hence cutaneous involvement was considered minimal. However, it has been suggested that the anaesthetic depth and efficiency of EMLA is highly influenced by application time, skin thickness and regional anatomical differences (Arendt-Nielsen & Bjerring, 1988; Arendt-Nielsen & Bjerring, 1989). This raises the possibility that a contribution from cutaneous mechanoreceptors may not have been entirely eliminated. Therefore, future experiments involving a combination of EMLA and subcutaneous lidocaine injection should be used on muscles with DOMS to determine whether there is a significant role for cutaneous receptors.

Lastly, the question must be posed; what is the significance of DOMS? Presumably the individual will try to minimise the pain by limiting use of the affected muscles during the period of repair following damage from eccentric exercise. It is known that within a week of a period of unaccustomed eccentric exercise and the accompanying muscle soreness, there is a remodeling of the muscle so that a second period of exercise leads to much less soreness (Jones et al., 1997). Nevertheless DOMS can have a seriously debilitating effect on athletes' performance. It means training programs must be devised to minimise DOMS by subjecting the individual to regular, mild eccentric exercise. The insight into the mechanism of DOMS put forward here may help to generate new therapeutic strategies to minimise the soreness whenever it arises. Perhaps, in the future, it will be possible to give drugs which target dorsal horn neurons and their transmission properties as a means of alleviating DOMS.

Effect of eccentric exercise on sense of force

In Chapter 4 it was shown that the accuracy of force estimation was significantly altered after eccentric exercise. The presence of fatigue and muscle damage after eccentric exercise, led to matching errors that were apparent at all levels of applied force. The patterns of these results were consistent with the view that subjects were not matching levels of torque as signaled by peripheral feedback, but rather, they were matching levels of effort. This finding was consistent with observations from previous studies on tension-matching errors after eccentric exercise (Carson, Riek & Shahbazpour, 2002; Saxton et al., 1995). It was also supported by the finding that identified tendon organs in

cat muscle did not show any changed responsiveness after a series of eccentric contractions, despite the evidence of damage in the muscle (Gregory et al., 2002).

However, after the effects of fatigue and muscle damage were controlled for by expressing errors to take into account the drop in MVC, small but significant errors persisted for up to four days. This suggested that factors other than fatigue and damage were contributing to the mismatch.

Using EMG as an indirect measure of the level of neural drive to muscles during force estimation, it was observed that eccentric exercise led to a larger than proportional increase in EMG for a given level of torque. This disturbance of the EMG : torque relation was not observed after concentric exercise, suggesting that it was a manifestation of muscle damage. Therefore, it was possible that the altered EMG : torque relation contributed to matching errors.

Various causes for this alteration of EMG : torque relation were proposed. Motor unit synchronization associated with muscle tremor could be a factor as findings have suggested that there is an increase in common input to motoneurons during eccentric contractions, compared to concentric contractions (Semmler et al., 2002). Also there is evidence that suggests muscles performing eccentric contractions rely more on faster motor units (Howell et al., 1995; Nardone, Romano & Schieppati, 1989). This has led to the theory that eccentric exercise may damage fast muscle fibres preferentially. It would mean that to achieve higher torque levels even more effort, and hence more EMG would be required. In addition, the shift, in the direction of longer muscle lengths, of the muscle's optimum length for force generation after eccentric exercise, could be a contributing factor. The EMG would be recorded at shorter sarcomere lengths in the active sarcomeres. Therefore, proportionately larger increases in EMG at low torque levels would result, as subjects attempt to reach the target level, while operating at effectively shorter muscle lengths. However, such theories require further investigation. In addition, the presence of DOMS was also suggested to contribute to the matching errors that continued from 24 hours onward after the exercise (this was investigated more formally in Chapter 5).

Additionally, the muscle's angle-torque relation was used to alter levels of torque, while keeping levels of activation (MVCs) constant (Cafarelli & Bigland-Richie, 1979). Although errors were not in direct proportion to the changes in torque, their distribution was consistent with a match of effort rather than of level of torque. Significantly, regression analysis showed that there was no significant difference in the relation between the change in MVC and matching errors when torque levels were altered either using the length-tension relation of by means of eccentric exercise. This suggested that changes in the force-generating capacity of a muscle, whether they are from adjustments of muscle length or from fatigue and damage, involve comparable changes in command signals during matching trials.

Future experiments should deal with the question, why is the 'sense of effort' used for force matching tasks, rather than the 'sense of tension', which various studies have shown to exist? Moreover, what is the purpose of having a sense of tension? Tendon organ signaling ability is not impaired by eccentric exercise, despite significant changes in the mechanical properties of the exercised muscle (Gregory et al., 2002), yet they still seem not to play a major role in force matching tasks.

Effect of pain on sense of force

In Chapter 5, hypertonic saline injection into a muscle was shown to produce persistent force matching errors. Importantly, the size of the matching errors was closely correlated with the level of pain, highlighting the fact that it was unlikely that other factors such as distraction were involved in generating the errors. Adding to this point was the observation that painful heating of the skin over the contracting muscle produced a similar pattern of errors, although these were somewhat smaller. This suggested that the effect was not specific to muscle nociceptors.

It was suggested that the effect of pain on motor performance might be acting at the level of the motor cortex. This proposal was based on the experiments of Le Pera et al. (2001) who showed that following saline injection into finger muscles, at the peak of pain, transcranial magnetic stimulation of the left primary cortex produced MEP's of reduced amplitude, without any change in H-reflexes. It raises the possibility that excitability of motor cortex is reduced as a result of the Gp III and IV activation from hypertonic saline.

This has wider implications. Does the pain following an injury lead to disturbed motor control, to the point where it has to be taken into account during rehabilitation?

It was proposed that DOMS could act on motor control in a similar way. However the observations presented in Chapter 5 provide no information about how the nociceptive or non-nociceptive inputs might interfere with motor control. This is certainly an important issue that warrants further investigation. Future experiments that assess motor cortex excitability by using transcranial magnetic simulation or transcranial electrical simulation techniques, before, during and after DOMS is induced in a muscle, would help to resolve this issue.

Adaptation

The question posed in Chapter 6 was, did muscles of concentrically trained individuals show adaptive changes in their muscles compared with untrained individuals? This issue is important as differences in length-tension relationships could indirectly lend support to Morgan's hypothesis involving the incorporation of more sarcomeres in series into muscle fibres after eccentric training.

Indeed the optimum angle for torque in the isokinetic curve, for the quadriceps of cyclists, was found to lie significantly in a direction of shorter muscle lengths. According to theory, this shift indicates fewer sarcomeres in series in the quadriceps muscles. Therefore it was concluded that an adaptive change by sarcomere reduction from concentric training had occurred. However, for the hamstrings such a result was not obtained. A review of cycling biomechanics suggested that the hamstring muscles were utilized considerably less than quadriceps, during cycling. Hence, it was suggested this could account for the hamstrings finding. That is, that one of the considerations for an adaptive change is frequency of use.

The implications of these results are that it is now clear that different lifestyles, representing different training regimes, can induce measurable adaptive changes to the muscle, by mechanisms including adjustment of the number of sarcomeres in series. Hence an individual's lifestyle seemingly can influence their susceptibility to the damage from eccentric exercise. Further testing of other sporting groups would be interesting in

this aspect. For example, testing of skiers, who predominantly undergo eccentric exercise would be interesting. If their length-tension relationships showed optimum angles that were in a direction of longer muscle lengths, this would lend more direct support to a sarcomere addition mechanism of adaptation after repeated bouts of eccentric exercise. It might then be fascinating to look at the performance of cyclists in skiing.

In a complementary study, Brockett et al. (2001) showed a component of the shift in the length-tension property of human hamstring muscles was sustained after eccentric exercise, indicating a training effect from which the muscle received protection against subsequent damage from eccentric exercise. Such results support Morgan's (1990) proposal that in-series sarcomere addition allows sarcomeres to operate at shorter lengths, so avoiding the descending limb of the length-tension curve, which is the region of instability for the sarcomere length distributions.

In addition, findings from my study have wider implications to muscle strain injuries that are common in many sports such as football and athletics. Specifically for hamstring injury, from clinical reports the common conclusion is that most occur as a result of eccentric contractions (Garrett, 1990; Kujala, Orava & Jarvinen, 1997; Stanton & Purdam, 1989). The initial damage in the muscle fibres that leads to muscle tears is believed to be disruption of sarcomeres in single fibres (Brockett, Morgan & Proske, 2001). Therefore, DOMS and gross muscle injury tears may have a common origin. This issue is of immense importance to all regular exercise enthusiasts, particularly athletes and their choice of training program. The inclusion of eccentric training strategies may offer protective effects for athletes who are at risk with a history of muscle strains. Furthermore, it may be beneficial to athletes involved in sports that include both concentric and eccentric exercise, such as triathletes and marathon runners. Such athletes should endeavor to achieve a training program that promotes enhancement of muscle performance without making the muscle more susceptible to the damage of eccentric exercise. Here the optimum angle for torque can be used as a diagnostic measure of susceptibility. Preventative strategies for individuals who are vulnerable could avoid injuries in the future. Hence the applications of the findings in this thesis have some clinical relevance.

REFERENCES

Adams, G. R., Duvoisin, M. R. & Dudley, G. A. (1992). Magnetic resonance imaging and electromyography as indexes of muscle function. *Journal of Applied Physiology* **73,** 1578-1583.

Adreani, C. M. & Kaufman, M. P. (1998). Effect of arterial occlusion on responses of group III and IV afferents to dynamic exercise. *Journal of Applied Physiology* **84,** 1827-1833.

Allen, D. G. (2001). Eccentric muscle damage: mechanisms of early reduction of force. *Acta Physiologica Scandinavica* **171,** 311-319.

Allen, M. J. & Barnes, M. R. (1986). Exercise pain in the lower leg. Chronic compartment syndrome and medial tibial syndrome. *Journal of Bone & Joint Surgery - British Volume* **68,** 818-823.

Arendt-Nielsen, L. & Bjerring, P. (1988). Laser-induced pain for evaluation of local analgesia: a comparison of topical application (EMLA) and local injection (lidocaine). *Anesthesia and Analgesia* **67,** 115-123.

Arendt-Nielsen, L. & Bjerring, P. (1989). The effect of topically applied anaesthetics (EMLA cream) on thresholds to thermode and argon laser stimulation. *Acta Anaesthesiologica Scandinavica* **33,** 469-473.

Armstrong, R. B. (1984). Mechanisms of exercise-induced delayed onset muscular soreness: a brief review. *Medicine and Science in Sports & Exercise* **16,** 529-538.

Armstrong, R. B., Ogilvie, R. W. & Schwane, J. A. (1983). Eccentric exercise-induced injury to rat skeletal muscle. *Journal of Applied Physiology* **54,** 80-93.

Armstrong, R. B., Warren, G. L. & Warren, J. A. (1991). Mechanisms of exercise-induced muscle fibre injury. *Sports Medicine* **12,** 184-207.

Asmussen, E. (1953). Positive and negative muscular work. *Acta Physiologica Scandinavica* **28,** 364-382.

Astrand, I. (1960). Aerobic work capacity in Men and Women with Special Reference to Age. *Acta Physiologica Scandinavica* **49,** 51.

Babault, N., Pousson, M., Ballay, Y. & Van Hoecke, J. (2001). Activation of human quadriceps femoris during isometric, concentric, and eccentric contractions. *Journal of Applied Physiology* **91,** 2628-34.

Balnave, C. D. & Allen, D. G. (1995). Intracellular calcium and force in single mouse muscle fibres following repeated contractions with stretch. *Journal of Physiology* **488,** 25-36.

Balnave, C. D., Davey, D. F. & Allen, D. G. (1997). Distribution of sarcomere length and intracellular calcium in mouse skeletal muscle following stretch-induced injury. *Journal of Physiology* **502,** 649-659.

Balnave C. D. & Thompson, M. W. (1993). Effect of training on eccentric exercise-induced muscle damage. *Journal of Applied Physiology* **75,** 1545-1551.

Banas, M. G. & Zetlin, A. (1938). The relation of isometric tension to length in skeletal muscle. *Journal of General Physiology* **12,** 403-420.

Barany, M. (1967). ATPase activity of myosin correlated with speed of muscle shortening. *Journal of General Physiology* **50,** Suppl:197-218.

Barker, D. (1962). The structure and distribution of muscle receptors. In *Symposium of Muscle Receptors*. ed. Barker, D., pp. 227-240. University Press, Hong Kong.

Barker, D. (1974). The morphology of muscle receptors. In *Handbook of Sensory Physiology*, vol. III, part 2. ed. Hunt, C. C., pp. 1-190. Springer, Berlin.

Barlas, P., Walsh, D. M., Baxter, G. D. & Allen, J. M. (2000). Delayed onset muscle soreness: effect of an ischaemic block upon mechanical allodynia in humans. *Pain* **87,** 221-225.

Bauman, T. K., Simone, D. A., Shain, C. N. & La Motte, R. H. (1991). Neurogenic hyperalgesia: The search for the primary cutaneous afferent fibres that contribute to capsaicin-induced pain and hyperalgesia. *Journal of Neurophysiology* **66,** 212-227.

Belcastro, A. N. (1993). Skeletal muscle calcium-activated neutral protease (calpain) with exercise. *Journal of Applied Physiology* **74,** 1381-1386.

Berberich, P., Hoheisel, U. & Mense, S. (1988). Effects of a carrageenan-induced myositis on the discharge properties of group III and IV muscle receptors in the cat. *Journal of Neurophysiology* **59,** 1395-1409.

Bessou, P., Burgess, P. R., Perl, E. R. & Taylor, C. B. (1971). Dynamic properties of mechanoreceptors with unmyelinated (C) fibers. *Journal of Neurophysiology* **34,** 116-131.

Bessou, P. & Laporte, Y. (1961). Étude des recepteurs musculaires innervé par les fibres afférentes du Groupe III (fibres myelinisées fines) chez le chat. *Archives Italiennes de Biologie* **99,** 270-292.

Bianconi, R. & van der Meulen, J. P. (1963). The response to vibration of the end organs of mammalian muscle spindles. *Journal of Neurophysiology* **26,** 177-190.

Bini, G., Cruccu, G., Hagbarth, K. E., Schady, W. & Torebjork, E. (1984). Analgesic effect of vibration and cooling on pain induced by intraneural electrical stimulation. *Pain* **18,** 239-248.

Birrell, G. J., McQueen, D. S., Iggo, A., Coleman, R. A. & Grubb, B. D. (1991). PGI2-induced activation and sensitization of articular mechanonociceptors. *Neuroscience Letters* **124,** 5-8.

Bobbert, M. F., Hollander, A. P. & Huijing, P. A. (1986). Factors in delayed onset muscular soreness of man. *Medicine and Science in Sports & Exercise* **18,** 75-81.

Bolanowski, S. J., Gescheider, G. A., Fontana, A. M., Niemiec, J. L. & Tromblay, J. L. (2001). The effects of heat-induced pain on the detectability, discriminability, and sensation magnitude of vibrotactile stimuli. *Somatosensory and Motor Research* **18,** 5-9.

Bourgeois, J., MacDougall, D., MacDonald, J. & Tarnopolsky, M. (1999). Naproxen does not alter indices of muscle damage in resistance-exercise trained men. *Medicine and Science in Sports & Exercise* **31,** 4-9.

Brockett, C. L., Morgan, D. L. & Proske, U. (2001). Human hamstring muscles adapt to eccentric exercise by changing optimum length. *Medicine and Science in Sports & Exercise* **33,** 783-790.

Brockett, C. L., Morgan, D. L. and Proske, U. (2003). Predicting hamstring stain injury in elite athletes. *Medicine & Science in Sports & Exercise* **(submitted).**

Brockett, C. L, Warren, N., Gregory, J. E., Morgan, D. L. & Proske, U. (1997). A comparison of the effects of concentric versus eccentric exercise on force and position sense at the human elbow joint. *Brain Research* **771,** 251-258.

Brooke, M. H. & Kaiser, K. K. (1970). Muscle fiber types: how many and what kind? *Archives of Neurology* **23,** 369-379.

Brooks, S. V. & Faulkner, J. A. (1996). The magnitude of the initial injury induced by stretches of maximally activated muscle fibres of mice and rats increases in old age. *Journal of Physiology* **497 (Pt 2),** 573-580.

Brown, M. C., Engberg, I. & Matthews, P. B. (1967a). Fusimotor stimulation and the dynamic sensitivity of the secondary ending of the muscle spindle. *Journal of Physiology* **189,** 545-550.

Brown, M. C., Engberg, I. & Matthews, P. B. (1967b). The relative sensitivity to vibration of muscle receptors of the cat. *Journal of Physiology (Lond)* **192,** 773-800.

Brown, S. J., Child, R. B., Day, S. H. & Donnelly, A. E. (1997). Exercise-induced skeletal muscle damage and adaptation following repeated bouts of eccentric muscle contractions. *Journal of Sports Sciences* **15,** 215-222.

Burke, D. & Applegate, C. (1989). Paraesthesiae and hypaesthesia following prolonged high-frequency stimulation of cutaneous afferents. *Brain* **112,** 913-929.

Burke, D., Hagbarth, K. E., Lofstedt, L. & Wallin, B. G. (1976). The responses of human muscle spindle endings to vibration of non-contracting muscles. *Journal of Physiology* **261,** 673-693.

Burton, H. (1986). Second Somatosensory cortex and related areas. In *Cerebral Cortex.* ed. Jones, E. G. & Peters, A., pp. 31-98. Plenum Press, Orlando, FL.

Byrnes, W. C. & Clarkson, P. M. (1986). Delayed onset muscle soreness and training. *Clinics in Sports Medicine* **5,** 605-614.

Byrnes, W. C., Clarkson, P. M., White, J. S., Hsieh, S. S., Frykman, P. N. & Maughan, R. J. (1985). Delayed onset muscle soreness following repeated bouts of downhill running. *Journal of Applied Physiology* **59,** 710-715.

Cafarelli, E. (1988). Force sensation in fresh and fatigued human skeletal muscle. *Exercise & Sport Sciences Reviews* **16,** 139-168.

Cafarelli, E. & Bigland-Ritchie, B. (1979). Sensation of static force in muscles of different length. *Experimental Neurology* **65,** 511-525.

Cafarelli, E. & Layton-Wood, J. (1986). Effect of vibration on force sensation in fatigued muscle. *Medicine & Science in Sports & Exercise* **18,** 516-521.

Campbell, J. N. & Meyer, R. A. (1996). Neurobiology of Nociceptors. ed. Belmonte, C. & Cervero, F., pp. 117. Oxford University Press, New York.

Cannavino, CR., Abrams, J., Palinkas, LA., Saglimbeni, A. & Bracker, MD. (2003). Efficacy of transdermal ketoprofen for delayed onset muscle soreness. *Clinics in Sports Medicine* **13,** 200-208.

Capra, N. F. & Ro, J. Y. (2000). Experimental muscle pain produces central modulation of proprioceptive signals arising from jaw muscle spindles. *Pain* **86,** 151-162.

Carlson, B. M. (1973). The regeneration of skeletal muscle. A review. *American Journal of Anatomy* **137,** 119-149.

Carpenter, S. & Karpati, G. (1989). Segmental necrosis and its demarcation in experimental micropuncture injury of skeletal muscle fibers. *Journal of Neuropathology and Experimental Neurology* **48,** 154-170.

Carson, R. G., Riek, S. & Shahbazpour, N. (2002). Central and peripheral mediation of human force sensation following eccentric or concentric contractions. *Journal of Physiology* **539,** 913-925.

Cavanagh, P. R. & Sanderson, D. J. H. K. B., Champaign, ch5. (1986). The biomechanics of cycling: Studies of the pedaling mechanics of elite pursuit riders. In *Science of Cycling*, pp. 103-109. Human Kinetics Books, Champaign.

Cervero, F. & Laird, J. M. (1996). Mechanisms of touch-evoked pain (allodynia): a new model. *Pain* **68,** 13-23.

Chahl, L. A. & Iggo, A. (1977). The effects of bradykinin and prostaglandin E1 on rat cutaneous afferent nerve activity. *British Journal of Pharmacology* **59,** 343-347.

Chen, J., Li, H., Luo, C., Li, Z. & Zheng, J. (1999). Involvement of peripheral NMDA and non-NMDA receptors in development of persistent firing of spinal wide-dynamic-range neurons induced by subcutaneous bee venom injection in the cat. *Brain Research* **844,** 98-105.

Chleboun, G. S., Howell, J. N., Conatser, R. R. & Giesey, J. J. (1998). Relationship between muscle swelling and stiffness after eccentric exercise. *Medicine and Science in Sports & Exercise* **30,** 529-535.

Clarkson, P. M. & Tremblay, I. (1988). Exercise-induced muscle damage, r' pair, and adaptation in humans. *Journal of Applied Physiology* **65,** 1-6.

Cleak, M. J. & Eston, R. G. (1992). Muscle soreness, swelling, stiffness and strength loss after intense eccentric exercise. *British Journal of Sports Medicine* **26,** 267-272.

Close, R. I. (1972). Dynamic properties of mammalian skeletal muscles. *Physiological Reviews* **52,** 129-197.

Colebatch, J. G. & McCloskey, D. I. (1987). Maintenance of constant arm position or force: reflex and volitional components in man. *Journal of Physiology* **386,** 247-261.

Collins, D. F., Burke, D. & Gandevia, S. C. (2002). Sustained contractions produced by plateau-like behaviour in human motoneurones. *Journal of Physiology* **538,** 289-301.

Craig, A. D. & Kniffki, K. D. (1985). Spinothalamic lumbosacral lamina I cells responsive to skin and muscle stimulation in the cat. *Journal of Physiology* **365,** 197-221.

Crenshaw, A. G., Thornell, L. E. & Friden, J. (1994). Intramuscular pressure, torque and swelling for the exercise-induced sore vastus lateralis muscle. *Acta Physiologica Scandinavica* **152,** 265-277.

Croisier, J. L., Camus, G., Deby-Dupont, G., Bertrand, F., Lhermerout, C., Crielaard, J. M., Juchmes-Ferir, A., Deby, C., Albert, A. & Lamy, M. (1996). Myocellular enzyme leakage, polymorphonuclear neutrophil activation and delayed onset muscle soreness induced by isokinetic eccentric exercise. *Archives of Physiology and Biochemistry* **104,** 322-329.

Curtin, N. A. & Davies, R. E. (1973). Chemical and mechanical changes during stretching of activated frog skeletal muscle. *Cold Spring Harbour Symposia on Quantitative Biology* **37,** 619-626.

Dallenbach, K. M. (1939). Pain: history and present status. *American Journal of Psychology* **52,** 331-347.

Davies, C. T. & White, M. J. (1981). Muscle weakness following eccentric work in man. *Pflugers Archiv European Journal of Physiology* **392,** 168-171.

Davis, K. D. (1998). Cold-induced pain and prickle in the glabrous and hairy skin. *Pain* **75,** 47-57.

DeVries, M. J. (1966). Quantitative electromyographic investigation of the spasm theory of muscle pain. *American Journal of Physiological Medicine* **45,** 119-134.

Donnelly, A. E., McCormick, K., Maughan, R. J., Whiting, P. H. & Clarkson, P. M. (1988). Effects of a non-steroidal anti-inflammatory drug on delayed onset muscle soreness and indices of damage. *British Journal of Sports Medicine* **22,** 35-38.

Duan, C., Delp, M. D., Hayes, D. A., Delp, P. D. & Armstrong, R. B. (1990). Rat skeleta! muscle mitochondrial [Ca2+] and injury from downhill walking. *Journal of Applied Physiology* **68,** 1241-1251.

Dubner, R. (1995). Hyperalgesia in respose to injury to cutaneous and deep tissues. In *Orofacial pain and temporomandibular disorders*. ed. Friction, J. & Dubner, R., pp. 61-71. Raven Press, New York.

Ebbeling, C. B. & Clarkson, P. M. (1989). Exercise-induced muscle damage and adaptation. *Sports Medicine* **7,** 207-234.

Eccleston, C. & Crombez, G. (1999). Pain demands attention: a cognitive-affective model of the interruptive function of pain. *Psychological Bulletin* **125,** 356-366.

Edstrom, L. & Kugelberg, E. (1968). Histochemical composition, distribution of fibres and fatiguability of single motor units. Anterior tibial muscle of the rat. *Journal of Neurology, Neurosurgery and Psychiatry* **31,** 424-433.

Ekblom, A. & Hansson, P. (1982). Effects of conditioning vibratory stimulation on pain threshold of the human tooth. *Acta Physiologica Scandinavica* **114,** 601-604.

Ellaway, P. H., Murphy, P. R. & Tripathi, A. (1982). Closely coupled excitation of gamma-motoneurones by group III Muscle afferents with low mechanical threshold in the cat. *Journal of Physiology* **331,** 481-498.

Enoka, R. M., Rankin, L. L., Stuart, D. G. & Volz, K. A. (1989). Fatigability of rat hindlimb muscle: associations between electromyogram and force during a fatigue test. *Journal of Physiology* **408,** 251-270.

Ericson, M. (1986). On the biomechanics of cycling. A study of joint and muscle load during exercise on the bicycle ergometer. *Scandinavian Journal of Rehabilitation Medicine Supplement* **16,** 1-43.

Faria, I. E. (1984). Applied physiology of cycling. *Sports Medicine* **1,** 187-204.

Faria, I. E. & Cavanagh, P. R. (1978). *The Physiology and Biomechanics of Cycling*. John Wiley and Sons, New York.

Farina, S., Tinazzi, M., Le Pera, D. & Valeriani, M. (2003). Pain-related modulation of the human motor cortex. *Neurological Research* **25,** 130-142.

Farina, S., Valeriani, M., Rosso, T., Aglioti, S., Tamburin, S., Fiaschi, A. & Tinazzi, M. (2001). Transient inhibition of the human motor cortex by capsaicin-induced pain. A study with transcranial magnetic stimulation. *Neuroscience Letters* **314,** 97-101.

Feinstein, B., Lindegard, B., Nyman, E. & Wohlfart, G. (1955). Morphologic studies of motor units in normal human muscles. *Acta Anatomica* **23,** 127-142.

Fenn, W. O. & Marsh, B. S. (1935). Muscle force at different speeds of shortening. *Journal of Physiology* **85,** 227-297.

Fern, R. & Harrison, P. J. (1994). The contribution of ischaemia and deformation to the conduction block generated by compression of the cat sciatic nerve. *Experimental Physiology* **79,** 583-592.

Flitney, F. W. & Hirst, D. G. (1978). Cross-bridge detachment and sarcomere 'give' during stretch of active frog's muscle. *Journal of Physiology* **276,** 449-465.

Fock, S. & Mense, S. (1976). Excitatory effects of 5-hydroxytryptamine, histamine and potassium ions on muscular group IV afferent units: a comparison with bradykinin. *Brain Research* **105,** 459-469.

Foley, J. M., Jayaraman, R. C., Prior, B. M., Pivarnik, J. M. & Meyer, R. A. (1999). MR measurements of muscle damage and adaptation after eccentric exercise. *Journal of Applied Physiology* **87,** 2311-2318.

Franz, M. & Mense, S. (1975). Muscle receptors with group IV afferent fibres responding to application of bradykinin. *Brain Research* **92,** 369-383.

Friden, J., Kjorell, U. & Thornell, L. E. (1984). Delayed muscle soreness and cytoskeletal alterations: an immunocytological study in man. *International Journal of Sports Medicine* **5,** 15-18.

Friden, J. & Lieber, R. L. (1992). Structural and mechanical basis of exercise-induced muscle injury. *Medicine and Science in Sports & Exercise* **24,** 521-530.

Friden, J. & Lieber, R. L. (1998). Segmental muscle fiber lesions after repetitive eccentric contractions. *Cell Tissue Research* **293,** 165-171.

Friden, J., Seger, J., Sjostrom, M. & Ekblom, B. (1983a). Adaptive response in human skeletal muscle subjected to prolonged eccentric training. *International Journal of Sports Medicine* **4,** 177-183.

Friden, J., Sjostrom, M. & Ekblom, B. (1983b). Myofibrillar damage following intense eccentric exercise in man. *International Journal of Sports Medicine* **4,** 170-176.

Funatsu, T., Higuchi, H. & Ishiwata, S. (1990). Elastic filaments in skeletal muscle revealed by selective removal of thin filaments with plasma gelsolin. *Journal of Cell Biology* **110,** 53-62.

Gandevia, S. C. (1982). The perception of motor commands or effort during muscular paralysis. *Brain* **105,** 151-159.

Gandevia, S. C. (2001). Spinal and supraspinal factors in human muscle fatigue. *Physiological Reviews* **81,** 1725-1789.

Gandevia, S. C., Allen, G. M., Butler, J. E. & Taylor, J. L. (1996). Supraspinal factors in human muscle fatigue: evidence for suboptimal output from the motor cortex. *Journal of Physiology* **490,** 529-536.

Gandevia, S. C. & McCloskey, D. I. (1978). Interpretation of perceived motor commands by reference to afferent signals. *Journal of Physiology* **283,** 193-199.

Gandevia, S. G. (1996). Kinesthesia: roles for afferent signals and motor commands. In *Handbook on Integration of Motor, Circulatory, Respiratory and Metabolic Control during Exercise*. ed. Rowell, L. B. & Shepherd, J. T., pp. 128-172. Am Physiol Soc, Bethesda, MD.

Garland, S. J. (1991). Role of small diameter afferents in reflex inhibition during human muscle fatigue. *Journal of Physiology (Lond)* **435,** 547-558.

Garrett, W. E., Jr. (1990). Muscle strain injuries: clinical and basic aspects. *Medicine and Science in Sports & Exercise* **22,** 436-443.

Gescheider, G. A. (1985). *Psychophysics: method, theory and application (2nd Ed.).* Hillsdale, NJ: Erlbaum.

Golden, C. L. & Dudley, G. A. (1992). Strength after bouts of eccentric or concentric actions. *Medicine and Science in Sports & Exercise* **24,** 926-933.

Gonyea, W. J. & Ericson, G. C. (1977). Morphological and histochemical organization of the flexor carpi radialis muscle in the cat. *American Journal of Anatomy* **148,** 329-344.

Goodwin, G. M., McCloskey, D. I. & Matthews, P. B. (1972). The contribution of muscle afferents to kinaesthesia shown by vibration induced illusions of movement and by the effects of paralysing joint afferents. *Brain* **95,** 705-748.

Gordan, A. M., Huxley, A. F. & Julian, F. J. (1966). The variation in isometric tension with sarcomere length in vertebrate muscle fibres. *Journal of Physiology* **184,** 170-192.

Granzier, H., Helmes, M., Cazorla, O., McNabb, M., Labeit, D., Wu, Y., Yamasaki, R., Redkar, A., Kellermayer, M., Labeit, S. & Trombitas, K. (2000). Mechanical properties of titin isoforms. *Advances in Experimental Medicine and Biology* **481,** 283-300.

Graven-Nielsen, T., Arendt-Nielsen, L. & Mense, S. (2002). Thermosensitivity of muscle: high-intensity thermal stimulation of muscle tissue induces muscle pain in humans. *Journal of Physiology* **540,** 647-656.

Graven-Nielsen, T., Arendt-Nielsen, L., Svensson, P. & Jensen, T. S. (1997a). Quantification of local and referred muscle pain in humans after sequential i.m. injections of hypertonic saline. *Pain* **69,** 111-117.

Graven-Nielsen, T., McArdle, A., Phoenix, J., Arendt-Nielsen, L., Jensen, T. S., Jackson, M. J. & Edwards, R. H. (1997b). In vivo model of muscle pain: quantification of intramuscular chemical, electrical, and pressure changes associated with saline-induced muscle pain in humans. *Pain* **69,** 137-143.

Graven-Nielsen, T. & Mense, S. (2001). The peripheral apparatus of muscle pain: evidence from animal and human studies. *Clinical Journal of Pain* **17,** 2-10.

Gregory, J. E., Brockett, C. L., Morgan, D. L., Whitehead, N. P. & Proske, U. (2002). Effect of eccentric muscle contractions on Golgi tendon organ responses to passive and active tension in the cat. *Journal of Physiology* **538,** 209-218.

Hagbarth, K. E. (1952). Excitatory and inhibitory skin areas for flexor and extensor motoneurones. *Acta Physiologica Scandinavica Suppl* **26,** 1-58.

Hagbarth, K. E. & Eklund, G. (1966). Motor effects of vibratory muscle stimuli in man. In *Nobel Symposium I Muscular Afferents and Motor Control*. ed. Granit., R. Almqvist & Wiksell, Stockholm, Sweden.

Halliday, D. M., Conway, B. A., Farmer, S. F. & Rosenberg, J. R. (1999). Load-independent contributions from motor-unit synchronization to human physiological tremor. *Journal of Neurophysiology* **82,** 664-675.

Handwerker, H. O., Forster, C. & Kirchhoff, C. (1991). Discharge patterns of human C-fibers induced by itching and burning stimuli. *Journal of Neurophysiology* **66,** 307-315.

Harding, B., Black, T., Bruulsema, A., Maxwell, B. & Stratford, P. (1988). Reliability of a reciprocal test protocol performed on the Kinetic Communicator: An isokinetic test of knee extensor and flexor strength. *Journal of Orthopaedic and Sports Physical Therapy* **10,** 218-223.

Hardy, J. D., Wolff, H.G. & Goodell, H. (1950). Experimental evidence on the nature of cutaneous hyperalgesia. *Journal of Clinical Investigation* **29,** 115-140.

Heiser, T. M., Weber, J., Sullivan, G., Clare, P. & Jacobs, R. R. (1984). Prophylaxis and management of hamstring muscle injuries in intercollegiate football players. *American Journal of Sports Medicine* **12,** 368-70.

Hellsten Y, Frandsen U, Orthenblad N, Sjodin B and Richter EA. (1997). Xanthine oxidase in human skeletal muscle following intense eccentric exercise: a role in inflammation. *Journal of Physiology* **498,** 239-348.

Hertel, H. C., Howaldt, B. & Mense, S. (1976). Responses of group IV and group III muscle afferents to thermal stimuli. *Brain Research* **113,** 201-205.

Hesselink, M. K., Kuipers, H., Geurten, P. & Van Straaten, H. (1996). Structural muscle damage and muscle strength after incremental number of isometric and forced lengthening contractions. *Journal of Muscle Research and Cell Motility* **17,** 335-341.

Hill, A. V. (1938). The heat of shortening and the dynamic constants of muscle. *Proceedings of Royal Society of London B* **126,** 135-195.

Hill, D. K. (1968). Tension due to interaction between the sliding filaments in resting striated muscle. The effect of stimulation. *Journal of Physiology* **199,** 637-684.

Hoheisel, U., Koch, K. & Mense, S. (1994). Functional reorganization in the rat dorsal horn during an experimental myositis. *Pain* **59,** 111-118.

Hoheisel, U. & Mense, S. (1990). Response behaviour of cat dorsal horn neurones receiving input from skeletal muscle and other deep somatic tissues. *Journal of Physiology* **426,** 265-280.

Hoheisel, U., Mense, S., Simons, D. G. & Yu, X. M. (1993). Appearance of new receptive fields in rat dorsal horn neurons following noxious stimulation of skeletal muscle: a model for referral of muscle pain? *Neuroscience Letters* **153,** 9-12.

Hortobagyi, T., Barrier, J., Beard, D., Braspennincx, J., Koens, P., Devita, P., Dempsey, L. & Lambert, J. (1996). Greater initial adaptations to submaximal muscle lengthening than maximal shortening. *Journal of Applied Physiology* **81,** 1677-1682.

Hortobagyi, T., Houmard, J., Fraser, D., Dudek, R., Lambert, J. & Tracy, J. (1998). Normal forces and myofibrillar disruption after repeated eccentric exercise. *Journal of Applied Physiology* **84,** 492-498.

Hortobagyi, T., Lambert, N. J. & Hill, J. P. (1997). Greater cross education following training with muscle lengthening than shortening. *Medicine and Science in Sports & Exercise* **29,** 107-112.

Hough, T. (1902). Ergographic studies in muscular soreness. *American Journal of Physiology* **7,** 76-92.

Houk, J. & Henneman, E. (1967). Responses of Golgi tendon organs to active contractions of the soleus muscle of the cat. *Journal of Neurophysiology* **30,** 466-481.

Howell, J. N., Chila, A. G., Ford, G., David, D. & Gates, T. (1985). An electromyographic study of elbow motion during postexercise muscle soreness. *Journal of Applied Physiology* **58,** 1713-1718.

Howell, J. N., Chleboun, G. & Conatser, R. (1993). Muscle stiffness, strength loss, swelling and soreness following exercise-induced injury in humans. *Journal of Physiology* **464,** 183-196.

Howell, J. N., Fuglevand, A. J., Walsh, M. L. & Bigland-Ritchie, B. (1995). Motor unit activity during isometric and concentric-eccentric contractions of the human first dorsal interosseus muscle. *Journal of Neurophysiology* **74,** 901-904.

Hunt, C. C. & McIntyre, A. K. (1960). Characteristics of responses from receptors from the flexor longus digitorum muscle and the adjoining interosseous region of the cat. *Journal of Physiology* **153,** 74-87.

Huxley, A. F. & Hanson, J. (1954). Changes in the cross-striations of muscle during contraction and stretch and their structual interpretation. *Nature* **173,** 973-976.

Huxley, A. F. & Niedergerke, R. (1954). Structural changes in muscle during conraction. *Nature* **173,** 971-973.

Iggo, A. (1961). Non-myelinated afferent fibres from mammalian skeletal muscle. *Journal of Physiology* **155,** 52-53P.

Iggo, A. (1969). Cutaneous thermoreceptors in primates and sub-primates. *Journal of Physiology* **200,** 403-430.

Infante, A. A., Klaupiks, D. & Davies, R. E. (1964). Adenosine triphosphate: Changes in muscles doing negative work. *Science* **144,** 1577-1578.

Ingalls, C. P., Warren, G. L., Williams, J. H., Ward, C. W. & Armstrong, R. B. (1998). E-C coupling failure in mouse EDL muscle after in vivo eccentric contractions. *Journal of Applied Physiology* **85,** 58-67.

Jami, L. (1992). Golgi tendon organs in mammalian skeletal muscle: functional properties and central actions. *Physiological Reviews* **72,** 623-666.

Jensen, K. & Norup, M. (1992). Experimental pain in human temporal muscle induced by hypertonic saline, potassium and acidity. *Cephalalgia* **12,** 101-106.

Johansson, H., Djupsjobacka, M. & Sjolander, P. (1993). Influences on the gamma-muscle spindle system from muscle afferents stimulated by KCl and lactic acid. *Neuroscience Research* **16,** 49-57.

Johansson, H. & Sojka, P. (1991). Pathophysiological mechanisms involved in genesis and spread of muscular tension in occupational muscle pain and in chronic musculoskeletal pain syndromes: a hypothesis. *Medical Hypotheses* **35,** 196-203.

Jones, C., Allen, T., Talbot, J., Morgan, D. L. & Proske, U. (1997). Changes in the mechanical properties of human and amphibian muscle after eccentric exercise. *European Journal of Applied Physiology and Occupational Physiology* **76,** 21-31.

Jones, D. A. & Newham, D. J. (1985). The effect of training on human muscle pain and damage. *Journal of Physiology* **365,** 76P.

Jones, D. A., Newham, D. J. & Clarkson, P. M. (1987). Skeletal muscle stiffness and pain following eccentric exercise of the elbow flexors. *Pain* **30,** 233-242.

Jones, D. A., Newham, D. J., Round, J. M. & Tolfree, S. E. (1986). Experimental human muscle damage: morphological changes in relation to other indices of damage. *Journal of Physiology* **375,** 435-448.

Jones, D. A., Newham, D. J. & Torgan, C. (1989). Mechanical influences on long-lasting human muscle fatigue and delayed- onset pain. *Journal of Physiology* **412,** 415-427.

Jones, L. A. (1995). The senses of effort and force during fatiguing contractions. *Advances in Experimental Medicine & Biology* **384,** 305-313.

Jones, L. A. & Hunter, I. W. (1983a). Effect of fatigue on force sensation. *Experimental Neurology* **81,** 640-650.

Jones, L. A. & Hunter, I. W. (1983b). Force and EMG correlates of constant effort contractions. *European Journal of Applied Physiology and Occupational Physiology* **51,** 75-83.

Jorge, M. & Hull, M. L. (1986). Analysis of EMG measurements during bicycle pedalling. *Journal of Biomechanics* **19,** 683-94.

Jorgensen, K., Fallentin, N., Krogh-Lund, C. & Jensen, B. (1988). Electromyography and fatigue during prolonged, low-level static contractions. *European Journal of Applied Physiology and Occupational Physiology* **57,** 316-321.

Julian, F. J. & Morgan, D. L. (1979). The effect on tension of non-uniform distribution of length changes applied to frog muscle fibres. *Journal of Physiology* **293,** 379-392.

Julius, D. & Basbaum, A. I. (2001). Molecular mechanisms of nociception. *Nature* **413,** 203-210.

Katz, B. (1939). The relation between force and speed in muscular contraction. *Journal of Physiology* **96,** 45-64.

Kannus, P. & Beynnon, B. (1993). Peak Torque occurrence in the range of motion during isokinetic extension and flexion of the knee. *International Journal of Sports Medicine* **14,** 422-426.

Kaufman, M. P., Iwamoto, G. A., Longhurst, J. C. & Mitchell, J. H. (1982). Effects of capsaicin and bradykinin on afferent fibers with ending in skeletal muscle. *Circulation Research* **50,** 133-139.

Kaufman, M. P., Longhurst, J. C., Rybicki, K. J., Wallach, J. H. & Mitchell, J. H. (1983). Effects of static muscular contraction on impulse activity of groups III and IV afferents in cats. *Journal of Applied Physiology* **55,** 105-112.

Kauranen, K., Siira, P. & Vanharanta, H. (2001). Delayed-onset muscle soreness and motor performance of the upper extremity. *European Journal of Applied Physiology* **84,** 302-309.

Keating, J. L. & Matyas, T. A. (1996a). The influence of subject and test design on dynamometric measurements of extremity muscles. *Physical Therapy* **76,** 866-89.

Keating, J. L. & Matyas, T. A. (1996b). Method-related variations in estimates of gravity correction values using electromechanical dynamometry: a knee extension study. *Journal of Orthopaedic and Sports Physical Therapy* **24,** 142-53.

Kellgren, J. H. (1938). Observations on referred pain arising from muscle. *Clinical Science* **3,** 175-190.

Kenins, P. (1982). Responses of single nerve fibres to capsaicin applied to the skin. *Neuroscience Letters* **29,** 83-88.

Kilo, S., Schmelz, M., Koltzenburg, M. & Handwerker, H. O. (1994). Different patterns of hyperalgesia induced by experimental inflammation in human skin. *Brain* **117 (Pt 2),** 385-396.

Kniffki, K. D., Mense, S. & Schmidt, R. F. (1978). Responses of group IV afferent units from skeletal muscle to stretch, contraction and chemical stimulation. *Experimental Brain Research* **31,** 511-522.

Kniffki, K. D., Schomburg, E. D. & Steffens, H. (1981). Synaptic effects from chemically activated fine muscle afferents upon alpha-motoneurones in decerebrate and spinal cats. *Brain Research* **206,** 361-370.

Kolhekar, R., Murphy, S. & Gebhart, G. F. (1997). Thalamic NMDA receptors modulate inflammation-produced hyperalgesia in the rat. *Pain* **71,** 31-40.

Krnjevic, K. & Miledi, R. (1958). Failure of neuromuscular propogation in rats. *Journal of Physiology* **140,** 440-461.

Kuipers, H., Keizer, H. A., Verstappen, F. T. & Costill, D. L. (1985). Influence of a prostaglandin-inhibiting drug on muscle soreness after eccentric work. *International Journal of Sports Medicine* **6,** 336-339.

Kujala, U. M., Orava, S. & Jarvinen, M. (1997). Hamstring injuries. Current trends in treatment and prevention. *Sports Medicine* **23,** 397-404.

Kumazawa, T. & Mizumura, K. (1977). Thin-fibre receptors responding to mechanical, chemical, and thermal stimulation in the skeletal muscle of the dog. *Journal of Physiology* **273,** 179-194.

LaMotte, R. H., Shain, C. N., Simone, D. A. & Tsai, E. F. (1991). Neurogenic hyperalgesia: psychophysical studies of underlying mechanisms. *Journal of Neurophysiology* **66,** 190-211.

Landgren, S. & Silfvenius, H. (1969). Projection to cerebral cortex of group I muscle afferents from the cat's hind limb. *Journal of Physiology* **200,** 353-372.

Lapier, T. K., Burton, H. W., Almon, R. & Cerny, F. (1995). Alterations in intramuscular connective tissue after limb casting affect contraction-induced muscle injury. *Journal of Applied Physiology* **78,** 1065-1069.

Laursen, R. J., Graven-Nielsen, T., Jensen, T. S. & Arendt-Nielsen, L. (1999). The effect of differential and complete nerve block on experimental muscle pain in humans. *Muscle Nerve* **22,** 1564-1570.

Le Bars, D., Dickenson, A. H. & Besson, J. M. (1979). Diffuse noxious inhibitory controls (DNIC). I. Effects on dorsal horn convergent neurones in the rat. *Pain* **6,** 283-304.

Le Pera, D., Graven-Nielsen, T., Valeriani, M., Oliviero, A., Di Lazzaro, V., Tonali, P. A. & Arendt-Nielsen, L. (2001). Inhibition of motor system excitability at cortical and spinal level by tonic muscle pain. *Clinical Neurophysiology* **112,** 1633-1641.

Lewis, T. (1942). *Pain*. MacMillan, New York.

Lieber, R. L. & Friden, J. (1993). Muscle damage is not a function of muscle force but active muscle strain. *Journal of Applied Physiology* **74,** 520-526.

Linnamo, V., Bottas, R. & Komi, P. V. (2000). Force and EMG power spectrum during and after eccentric and concentric fatigue. *Journal of Electromyography & Kinesiology* **10,** 293-300.

Lippold, O. C. J., Redfearn, J. W. T. & Vuco, J. (1957). The rhythmical activity of groups of motor units in the voluntary contraction of muscle. *Journal of Physiology* **137,** 1415-1447.

Lundeberg, T. (1984). Long-term results of vibratory stimulation as a pain relieving measure for chronic pain. *Pain* **20,** 13-23.

Lundeberg, T., Nordemar, R. & Ottoson, D. (1984). Pain alleviation by vibratory stimulation. *Pain* **20,** 25-44.

Luoto, S., Taimela, S., Hurri, H., Aalto, H., Pyykko, I. & Alaranta, H. (1996). Psychomotor speed and postural control in chronic low back pain patients A controlled follow-up study. *Spine* **21,** 2621-2627.

Lynch, G. S., Fary, C. J. & Williams, D. A. (1997). Quantitative measurement of resting skeletal muscle [Ca2+]i following acute and long-term downhill running exercise in mice. *Cell Calcium* **22,** 373-383.

Lynn, R. & Morgan, D. L. (1994). Decline running produces more sarcomeres in rat vastus intermedius muscle fibers than does incline running. *Journal of Applied Physiology* **77,** 1439-1444.

Lynn, R., Talbot, J. A. & Morgan, D. L. (1998). Differences in rat skeletal muscles after incline and decline running. *Journal of Applied Physiology* **85,** 98-104.

MacIntyre, D. L., Reid, W. D. & McKenzie, D. C. (1995). Delayed muscle soreness. The inflammatory response to muscle injury and its clinical implications. *Sports Medicine* **20,** 24-40.

MacIntyre, D. L., Reid, W. D, Lyster, D. M, Szasz, I. J. & McKenzie, D. C. (1996). Presence of WBC, decreased strength, and delayed soreness in muscle after eccentric exercise. *Journal of Applied Physiology* **80,** 1006-1013.

Mackenzie, R. A., Burke, D., Skuse, N. F. & Lethlean, A. K. (1975). Fibre function and perception during cutaneous nerve block. *Journal of Neurology, Neurosurgery & Psychiatry* **38,** 865-873.

Mair, J., Mayr, M., Muller, E., Koller, A., Haid, C., Artner-Dworzak, E., Calzolari, C., Larue, C. & Puschendorf, B. (1995). Rapid adaptation to eccentric exercise-induced muscle damage. *International Journal of Sports Medicine* **16,** 352-356.

Malm, C., Lenkei, R. & Sjodin, B. (1999). Effects of eccentric exercise on the immune system in men. *Journal of Applied Physiology* **86,** 461-468.

Markowitz, K., Bilotto, G. & Kim, S. (1991). Decreasing intradental nerve activity in the cat with potassium and divalent cations. *Archives of Oral Biology* **36,** 1-7.

Matre, D. A., Sinkjaer, T., Svensson, P. & Arendt-Nielsen, L. (1998). Experimental muscle pain increases the human stretch reflex. *Pain* **75,** 331-339.

McCloskey, D. I. (1981). Corollary discharges: motor commands and perception. In *Handbook of Physiology-The Nervous System II, Motor Control*. ed. Brooks, V. B., pp. 1415-1447. Amer. Physiol. Soc., Bethesda, Maryland.

McCloskey, D. I., Ebeling, P. & Goodwin, G. M. (1974). Estimation of weights and tensions and apparent involvement of a "sense of effort". *Experimental Neurology* **42,** 220-232.

McCloskey, D. I., Gandevia, S., Potter, E. K. & Colebatch, J. G. (1983). Muscle sense and effort: motor commands and judgments about muscular contractions. *Advances in Neurology* **39,** 151-167.

McCloskey, D. I. & Mitchell, J. H. (1972). Reflex cardiovascular and respiratory responses originating in exercising muscle. *Journal of Physiology* **224,** 173-186.

McComas, A. J. (1996). Skeletal Muscle: Form and function. In *Human Kinetics*, Illinois.

McCully, K. K. & Faulkner, J. A. (1985). Injury to skeletal muscle fibers of mice following lengthening contractions. *Journal of Applied Physiology* **59,** 119-126.

McCully, K. K. & Faulkner, J. A. (1986). Characteristics of lengthening contractions associated with injury to skeletal muscle fibers. *Journal of Applied Physiology* **61,** 293-299.

McHugh, M. P., Connolly, D. A., Eston, R. G. & Gleim, G. W. (1999). Exercise-induced muscle damage and potential mechanisms for the repeated bout effect. *Sports Medicine* **27,** 157-170.

McIntyre, A. K., Proske, U. & Rawson, J. A. (1984). Cortical projection of afferent information from tendon organs in the cat. *Journal of Physiology* **354,** 395-406.

Melzack, R. & Wall, P. D. (1965). Pain mechanisms: a new theory. *Science* **150,** 971-979.

Mense, S. (1993). Nociception from skeletal muscle in relation to clinical muscle pain. *Pain* **54,** 241-289.

Mense, S. (1995). Mechanisms of pain in hindlimb muscles: experimental findings and open questions. In *Temporomandibular disorders and related pain conditions.* ed. Sessle, B.J., Bryant, P.S. & Dionne, R.A., pp. 63-69.

Mense, S. (1996a). Group III and IV receptors in skeletal muscle: are they specific or polymodal? *Progress in Brain Research* **113,** 83-100.

Mense, S. (1996b). Nociceptors in skeletal muscle and their reaction to pathological tissue changes. In *Neurobiology of Nociceptors.* ed. Belmonte, C. & Cervero, F., pp. Ch.7. Oxford Press.

Mense, S. & Meyer, H. (1988). Bradykinin-induced modulation of the response behaviour of different types of feline group III and IV muscle receptors. *Journal of Physiology* **398,** 49-63.

Mense, S. & Prabhakar, N. R. (1986). Spinal termination of nociceptive afferent fibres from deep tissues in the cat. *Neuroscience Letters* **66,** 169-174.

Merskey, H. & Bogduk, N. (1994). *Classification of chronic pain.* IASP Press, Seattle.

Meyer, R. A. & Campbell, J. N. (1981). Myelinated nociceptive afferents account for the hyperalgesia that follows a burn to the hand. *Science* **213,** 1527-1529.

Miles, M. P. & Clarkson, P. M. (1994). Exercise-induced muscle pain, soreness, and cramps. *Journal of Sports Medicine & Physical Fitness* **34,** 203-216.

Mizumura, K. (1997). Peripheral mechanism of hyperalgesia--sensitization of nociceptors. *Nagoya Journal of Medical Science* **60,** 69-87.

Moddel, G., Best, B. & Ashby, P. (1977). Effect of differential nerve block on inhibition of the monosynaptic reflex by vibration in man. *Journal of Neurology, Neurosurgery & Psychiatry* **40,** 1066-1071.

Moffet, D., Moffet, S. & Scauf, C. (1990). *Human Physiology: Fondations and frontiers.* Mosby-Year Book Inc., St.Louis.

Morgan, D. L. (1990). New insights into the behavior of muscle during active lengthening. *Biophysical Journal* **57,** 209-221.

Morgan, D. L. & Allen, D. G. (1999). Early events in stretch-induced muscle damage. *Journal of Applied Physiology* **87,** 2007-2015.

Morgan, D. L., Claflin, D. R. & Julian, F. J. (1996). The effects of repeated active stretches on tension generation and myoplasmic calcium in frog single muscle fibres. *Journal of Physiology* **497,** 665-674.

Morgan, D. L., Whitehead, N. P., Wise, A. K., Gregory, J. E. & Proske, U. (2000). Tension changes in the cat soleus muscle following slow stretch or shortening of the contracting muscle. *Journal of Physiology* **522 Pt 3,** 503-513.

Moritani, T., Muramatsu, S. & Muro, M. (1988). Activity of motor units during concentric and eccentric contractions. *American Journal of Physical Medicine* **66,** 338-350.

Nadel, E. R., Bergh, U. & Saltin, B. (1972). Body temperatures during negative work exercise. *Journal of Applied Physiology* **33,** 553-558.

Nardone, A., Romano, C. & Schieppati, M. (1989). Selective recruitment of high-threshold human motor units during voluntary isotonic lengthening of active muscles. *Journal of Physiology* **409,** 451-471.

Neugebauer, V., Lucke, T. & Schaible, H. G. (1993). N-methyl-D-aspartate (NMDA) and non-NMDA receptor antagonists block the hyperexcitability of dorsal horn neurons during development of acute arthritis in rat's knee joint. *Journal of Neurophysiology* **70,** 1365-1377.

Newham, D. J., Jones, D. A. & Clarkson, P. M. (1987). Repeated high-force eccentric exercise: effects on muscle pain and damage. *Journal of Applied Physiology* **63,** 1381-1386.

Newham, D. J., Jones, D. A., Ghosh, G. & Aurora, P. (1988). Muscle fatigue and pain after eccentric contractions at long and short length. *Clinical Science (Lond)* **74,** 553-557.

Newham, D. J., Mills, K. R., Quigley, B. M. & Edwards, R. H. (1983). Pain and fatigue after concentric and eccentric muscle contractions. *Clinical Science (Lond)* **64,** 55-62.

Nitschke, J. E. (1992). Reliabilty of isokinetic torque measurements: A review of the literature. *Australian Journal of Physiotherapy* **38,** 125-134.

Nosaka, K. & Clarkson, P. M. (1995). Muscle damage following repeated bouts of high force eccentric exercise. *Medicine and Science in Sports & Exercise* **27,** 1263-1269.

Nosaka, K., Clarkson, P. M., McGuiggin, M. E. & Byrne, J. M. (1991). Time course of muscle adaptation after high force eccentric exercise. *European Journal of Applied Physiology and Occupational Physiology* **63,** 70-76.

Nosaka, K., Sakamoto, K., Newton, M. & Sacco, P. (2001). The repeated bout effect of reduced-load eccentric exercise on elbow flexor muscle damage. *European Journal of Applied Physiology* **85,** 34-40.

Ochoa, J., Fowler, T. J. & Gilliatt, R. W. (1972). Anatomical changes in peripheral nerves compressed by a pneumatic tourniquet. *Journal of Anatomy* **113,** 433-455.

Ogilvie, R. W., Armstrong, R. B., Baird, K. E. & Bottoms, C. L. (1988). Lesions in the rat soleus muscle following eccentrically biased exercise. *American Journal of Anatomy* **182,** 335-346.

Orchard, J., Marsden, J., Lord, S. & Garlick, D. (1997). Preseason hamstring muscle weakness associated with hamstring muscle injury in Australian footballers. *American Journal of Sports Medicine* **25,** 81-5.

Orchardson, R. (1978). The generation of nerve impulses in mammalian axons by changing the concentrations of the normal constituents of extracellular fluid. *Journal of Physiology* **275,** 177-189.

Page, S. & Huxley, H. E. (1963). Filament length in striated muscle. *Journal of Cell Biology* **19,** 369-390.

Paintal, A. S. (1960). Functional analysis of Group III afferent fibres of mammalian muscle. *Journal of Physiology* **152,** 250-270.

Pedersen, J., Sjolander, P., Wenngren, B. I. & Johansson, H. (1997). Increased intramuscular concentration of bradykinin increases the static fusimotor drive to muscle spindles in neck muscles of the cat. *Pain* **70,** 83-91.

Peeze Binkhorst, F. M., Kuipers, H., Heymans, J., Frederik, P. M., Slaaf, D. W., Tangelder, G. J. & Reneman, R. S. (1989). Exercise-induced focal skeletal

muscle fiber degeneration and capillary morphology. *Journal of Applied Physiology* **66,** 2857-2865.

Pertovaara, A. (1979). Modification of human pain threshold by specific tactile receptors. *Acta Physiologica Scandinavica* **107,** 339-341.

Powers, P. S. & Howley, E. T. (1997). *Exercise Physiology: theory and application to fitness and performance (3rd edition).* Brown publishers.

Price, D. D. & Dubner, R. (1977). Neurons that subserve the sensory-discriminative aspects of pain. *Pain* **3,** 307-338.

Price, D. E., Alani, S. M. & Wales, J. K. (1991). Effect of aldose reductase inhibition on resistance to ischemic conduction block in diabetic subjects. *Diabetes Care* **14,** 411-413.

Proske, U. (1981). The Golgi tendon organ. Properties of the receptor and reflex action of impulses arising from tendon organs. *International Review of Physiology* **25,** 127-171.

Proske, U. (1997). The mammalian muscle spindle. *News in Physiological Sciences* **12,** 37-42.

Proske, U. & Morgan, D. L. (2001). Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. *Journal of Physiology* **537,** 333-345.

Proske, U., Schaible, H. G. & Schmidt, R. F. (1988). Joint receptors and kinaesthesia. *Experimental Brain Research* **72,** 219-224.

Rack, P. M. & Westbury, D. R. (1969). The effects of length and stimulus rate on tension in the isometric cat soleus muscle. *Journal of Physiology* **204,** 443-460.

Rack, P. M. & Westbury, D. R. (1974). The short range stiffness of active mammalian muscle and its effect on mechanical properties. *Journal of Physiology* **240,** 331-350.

Rall, J. A. (1985). Energetic aspects of skeletal muscle contraction: implications of fiber types. *Exercise and Sport Sciences Reviews* **13,** 33-74.

Ramsay, R. W. & Street, S. F. (1940). The isometric length-tension diagram of isolated skeletal muscle fibres of the frog. *Journal of Cellular and Comparative Physiology* **15,** 11-34.

Ro, J. Y. & Capra, N. F. (2001). Modulation of jaw muscle spindle afferent activity following intramuscular injections with hypertonic saline. *Pain* **92,** 117-127.

Roland, P. E. & Ladegaard-Pedersen, H. (1977). A quantitative analysis of sensations of tension and of kinaesthesia in man. Evidence for a peripherally originating muscular sense and for a sense of effort. *Brain* **100,** 671-692.

Roll, J. P. & Vedal, J. P. (1982). Kinaesthetic role of muscle afferents in man, studies by tendon vibration and microneurography. *Experimental Brain Research* **47,** 177-190.

Roll, J. P., Vedel, J. P. & Ribot, E. (1989). Alteration of proprioceptive messages induced by tendon vibration in man: a microneurographic study. *Experimental Brain Research* **76,** 213-222.

Rossi, A., Mazzocchio, R. & Decchi, B. (2003). Effect of chemically activated fine muscle afferents on spinal recurrent inhibition in humans. *Clinical Neurophysiology* **114,** 279-287.

Sacco, P. & Jones, D. A. (1992). The protective effect of damaging eccentric exercise against repeated bouts of exercise in the mouse tibialis anterior muscle. *Experimental Physiology* **77,** 757-760.

Sale, D. G. (1991). Neural adaptation to strength training. In *Strength and Power in sport* (ed. P. V. Komi), pp. 249-265. Blackwell Scientific Publications, London.

Salter, M. W. & Henry, J. L. (1990). Differential responses of nociceptive vs. non-nociceptive spinal dorsal horn neurones to cutaneously applied vibration in the cat. *Pain* **40,** 311-322.

Sam, M., Shah, S., Friden, J., Milner, D. J., Capetanaki, Y. & Lieber, R. L. (2000). Desmin knockout muscles generate lower stress and are less vulnerable to injury compared with wild-type muscles. *American Journal of Physiology and Cell Physiology* **279,** C1116-1122.

Sanner, W. H. & O'Halloran, W. D. (2000). The biomechanics, etiology, and treatment of cycling injuries. *Journal of the American Podiatric Medical Association* **90,** 354-76.

Saxton, J. M., Clarkson, P. M., James, R., Miles, M., Westerfer, M., Clark, S. & Donnelly, A. E. (1995). Neuromuscular dysfunction following eccentric exercise. *Medicine and Science in Sports & Exercise* **27,** 1185-1193.

Schwane, J. A. & Armstrong, R. B. (1983). Effect of training on skeletal muscle injury from downhill running in rats. *Journal of Applied Physiology* **55,** 969-975.

Semmler, J. G., Kornatz, K. W., Dinenno, D. V., Zhou, S. & Enoka, R. M. (2002). Motor unit synchronisation is enhanced during slow lengthening contractions of a hand muscle. *Journal of Physiology* **545,** 681-695.

Shepherd, G. (1994). *Muscle sense and kinesthesia.* In: Neurobiology Oxford University Press, New York.

Sherrington, C. S. (1906). *The intergrative action of the nervous system*, New York.

Smith, L. L. (1991). Acute inflammation: the underlying mechanism in delayed onset muscle soreness? *Medicine and Science in Sports & Exercise* **23,** 542-551.

Stacey, M. J. (1969). Free nerve endings in skeletal muscle of the cat. *Journal of Anatomy* **105,** 231-254.

Stanton, P. & Purdam, C. (1989). Hamstring injuries in sprinting: The role of eccentric exercise. *Journal of Orthopaedic and Sports Physical Therapy* **10,** 343-349.

Svensson, P., Arendt-Nielsen, L., Nielsen, H. & Larsen, J. K. (1995). Effect of chronic and experimental jaw muscle pain on pain-pressure thresholds and stimulus-response curves. *Journal of Orofacial Pain* **9,** 347-356.

Svensson, P., Minoshima, S., Beydoun, A., Morrow, T. J. & Casey, K. L. (1997). Cerebral processing of acute skin and muscle pain in humans. *Journal of Neurophysiology* **78,** 450-460.

Tabary, J. C., Tabary, C., Tardieu, C., Tardieu, G. & Goldspink, G. (1972). Physiological and structural changes in the cat's soleus muscle due to immobilization at different lengths by plaster casts. *Journal of Physiology* **224,** 231-244.

Takekura, H., Fujinami, N., Nishizawa, T., Ogasawara, H. & Kasuga, N. (2001). Eccentric exercise-induced morphological changes in the membrane systems involved in excitation-contraction coupling in rat skeletal muscle. *Journal of Physiology* **533,** 571-583.

Talag, T. S. (1973). Residual muscular soreness as influenced by concentric, eccentric, and static contractions. *Research Quarterly* **44,** 458-469.

Talbot, J. A. & Morgan, D. L. (1996). Quantitative analysis of sarcomere non-uniformities in active muscle following a stretch. *Journal of Muscle Research and Cell Motility* **17,** 261-268.

Talbot, J. A. & Morgan, D. L. (1998). The effects of stretch parameters on eccentric exercise-induced damage to toad skeletal muscle. *Journal of Muscle Research and Cell Motility* **19,** 237-245.

Talbot, J. D., Duncan, G. H., Bushnell, M. C. & Boyer, M. (1987). Diffuse noxious inhibitory controls (DNICs): psychophysical evidence in man for intersegmental suppression of noxious heat perception by cold pressor pain. *Pain* **30,** 221-232.

Taylor, J. L., Petersen, N., Butler, J. E. & Gandevia, S. C. (2000). Ischaemia after exercise does not reduce responses of human motoneurones to cortical or corticospinal tract stimulation. *Journal of Physiology* **525 Pt 3,** 793-801.

Teague, B. N. & Schwane, J. A. (1995). Effect of intermittent eccentric contractions on symptoms of muscle microinjury. *Medicine and Science in Sports & Exercise* **27,** 1378-1384.

Tegeder, L., Zimmermann, J., Meller, S. T. & Geisslinger, G. (2002). Release of algesic substances in human experimental muscle pain. *Inflammation Research* **51,** 393-402.

Tesch, P. A. (1991). Short- and long-term histochemical and biochemical adaptations in muscle. In *Strength and power in sport* (ed. P. V. Komi), pp. 239-248. Blackwell Scientific Publications, London.

Thunberg, J., Ljubisavljevic, M., Djupsjobacka, M. & Johansson, H. (2002). Effects on the fusimotor-muscle spindle system induced by intramuscular injections of hypertonic saline. *Experimental Brain Research* **142,** 319-326.

Tiball JG. (1995). Inflammatory cell response to acute muscle injury. *Medicine and Science in Sports & Exercise* **27,** 1022-1032.

Tokmakidis, SP., Kokkinidis EA., Smilios, I & Douda, H. (2003). The effects of ibuprofen on delayed muscle soreness and muscular performance after eccentric exercise. *Journal of Strength & Conditioning Research* **17,** 53-59.

Torebjork, E. (1985). Nociceptor activation and pain. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **308,** 227-234.

Torebjork, H. E. & Hallin, R. G. (1973). Perceptual changes accompanying controlled preferential blocking of A and C fibre responses in intact human skin nerves. *Experimental Brain Research* **16,** 321-332.

Torebjork, H. E., Lundberg, L. E. & LaMotte, R. H. (1992). Central changes in processing of mechanoreceptive input in capsaicin-induced secondary hyperalgesia in humans. *Journal of Physiology (Lond)* **448,** 765-780.

Treede, R. D., Meyer, R. A., Raja, S. N. & Campbell, J. N. (1992). Peripheral and central mechanisms of cutaneous hyperalgesia. *Progress in Neurobiology* **38,** 397-421.

Valeriani, M., Restuccia, D., Di Lazzaro, V., Oliviero, A., Profice, P., Le Pera, D., Saturno, E. & Tonali, P. (1999). Inhibition of the human primary motor area by painful heat stimulation of the skin. *Clinical Neurophysiology* **110,** 1475-1480.

Vasquez, E., Bar, K. J., Ebersberger, A., Klein, B., Vanegas, H. & Schaible, H. G. (2001). Spinal prostaglandins are involved in the development but not the maintenance of inflammation-induced spinal hyperexcitability. *Journal of Neuroscience* **21,** 9001-9008.

Wall, P. D. & Woolf, C. J. (1984). Muscle but not cutaneous C-afferent input produces prolonged increases in the excitability of the flexion reflex in the rat. *Journal of Physiology* **356,** 443-458.

Walters, E. T. (1994). Injury-related behavior and neuronal plasticity: an evolutionary perspective on sensitization, hyperalgesia, and analgesia. *International Review of Neurobiology* **36,** 325-427.

Wang, K., McCarter, R., Wright, J., Beverly, J. & Ramirez-Mitchell, R. (1993). Viscoelasticity of the sarcomere matrix of skeletal muscles. The titin-myosin composite filament is a dual-stage molecular spring. *Biophysical Journal* **64,** 1161-1177.

Warren, G. L., Ingalls, C. P., Lowe, D. A. & Armstrong, R. B. (2001). Excitation-contraction uncoupling: major role in contraction-induced muscle injury. *Exercise and Sport Sciences Reviews* **29,** 82-87.

Warren, G. L., Ingalls, C. P., Shah, S. J. & Armstrong, R. B. (1999). Uncoupling of in vivo torque production from EMG in mouse muscles injured by eccentric contractions. *Journal of Physiology* **515 (Pt 2),** 609-619.

Warren, G. L., Lowe, D. A., Hayes, D. A., Karwoski, C. J., Prior, B. M. & Armstrong, R. B. (1993). Excitation failure in eccentric contraction-induced injury of mouse soleus muscle. *Journal of Physiology* **468,** 487-499.

Watanabe, I., Svensson, P. & Arendt-Nielsen, L. (1999). Influence of segmental and extra-segmental conditioning, stimuli on cortical potentials evoked by painful electrical stimulation. *Somatosensory & Motor Research* **16,** 243-250.

Waterman-Storer, C. M. (1991). The cytoskeleton of skeletal muscle: is it affected by exercise? A brief review. *Medicine and Science in Sports & Exercise* **23,** 1240-1249.

Weerakkody, N. S., Whitehead, N.P., Canny, B.J., Gregory, J.E., and Proske, U. (2001). Large-fiber mechanoreceptors contribute to muscle soreness after eccentric exercise. *Journal of Pain* **2,** 209-219.

Whitehead, N. P., Allen, T. J., Morgan, D. L. & Proske, U. (1998). Damage to human muscle from eccentric exercise after training with concentric exercise. *Journal of Physiology* **512,** 615-620.

Whitehead, N. P., Weerakkody, N. S., Gregory, J. E., Morgan, D. L. & Proske, U. (2001). Changes in passive tension of muscle in humans and animals after eccentric exercise. *Journal of Physiology* **533,** 593-604.

Williams, P. E. & Goldspink, G. (1973). The effect of immobilization on the longitudinal growth of striated muscle fibres. *Journal of Anatomy* **116,** 45-55.

Williams, P. E. & Goldspink, G. (1978). Changes in sarcomere length and physiological properties in immobilized muscle. *Journal of Anatomy* **127,** 459-468.

Willis, W. D., Sluka, K. A., Rees, H. & Westlund, K. N. (1996). Cooperative mechanisms of neurotransmitter action in central nervous sensitization. *Progress in Brain Research* **110,** 151-166.

Wise, A. K., Morgan, D. L., Gregory, J. E. & Proske, U. (2001). Fatigue in mammalian skeletal muscle stimulated under computer control. *Journal of Applied Physiology* **90,** 189-197.

Woledge, R. C., Curtin, N. A. & Homsher, E. (1985). Energetic aspects of muscle contraction. *Monographs of the Physiological Society* **41,** 1-357.

Wood, S. A. (1997) Factors affecting the size of the phasic stretch reflex. PhD thesis, Monash University.

Wood, S. A., Gregory, J. E. & Proske, U. (1996). The influence of muscle spindle discharge on the human H reflex and the monosynaptic reflex in the cat. *Journal of Physiology* **497,** 279-290.

Wood, S. A., Morgan, D. L. & Proske, U. (1993). Effects of repeated eccentric contractions on structure and mechanical properties of toad sartorius muscle. *American Journal of Physiology* **265,** C792-800.

Woods, J. J. & Bigland-Ritchie, B. (1983). Linear and non-linear surface EMG/force relationships in human muscles. An anatomical/functional argument for the existence of both. *American Journal of Physical Medicine* **62,** 287-299.

Woolf, C. J. & Salter, M. W. (2000). Neuronal plasticity: increasing the gain in pain. *Science* **288,** 1765-1769.

Woolf, C. J. & Thompson, S. W. (1991). The induction and maintenance of central sensitization is dependent on N-methyl-D-aspartic acid receptor activation; implications for the treatment of post-injury pain hypersensitivity states. *Pain* **44,** 293-299.

Wrigley, T. & Grant, M. (1995). Isokinetic dynamometry. In *Sports Physiotherapy: applied science and practice* (ed. M. Zuluaga), pp. 259-287. Churchhill Livingstone, New York.

Yeung, E. W., Bourreau, J. P., Allen, D. G. & Ballard, H. J. (2002). Effect of eccentric contraction-induced injury on force and intracellular pH in rat skeletal muscles. *Journal of Applied Physiology* **92,** 93-99.