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Pain sensitivity and referred pain in human tendon, fascia and muscle tissue

by

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Preface:

This PhD project is based on studies conducted between 2004 and 2006 at Center for Sensory Motor Interaction, Aalborg Denmark. Study III was written as a joint collaboration between human findings (SMI) and animal findings. The animal findings were solely carried out in Nagoya, Japan whilst human studies were solely performed in Aalborg, Denmark.

Study I

Referred Pain and hyperalgesia in human tendon and muscle belly tissue. *Pain*. 120:113-123. 2006. W. Gibson, L. Arendt-Nielsen, T. Graven-Nielsen

Study II

Delayed onset muscle soreness at tendon-bone junction and muscle tissue is associated with facilitated referred pain. *Exp. Brain. Res.* 174:351-360. 2006. W. Gibson, L. Arendt-Nielsen, T. Graven-Nielsen

Study III

Increased pain from muscle fascia following eccentric exercise: Animal and human findings. (Accepted with revisions; *Experimental Brain Research*). W. Gibson, L. Arendt-Nielsen, K. Mizumura, T. Taguchi, T. Graven-Nielsen

Study IV

Glutamate and capsaicin pain, hyperalgesia and modulatory interactions in human tendon tissue. (In submission). W. Gibson, L. Arendt-Nielsen, B. Sessle, T. Graven-Nielsen

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To my daughters: Ella-Margaret and Meg, my beautiful girls for providing endless distraction, fun, love and the perfect balance to my academic work. Thank you.

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Introduction

Musculoskeletal pain is widely prevalent (Urwin et al. 1998;Picavet and Hazes 2003;Salaffi et al. 2005) and is the most common cause of non-malignant pain in the general population (Katz 2002). The prevalence of musculoskeletal pain complaints within various populations ranges between 40% and 66% (Taylor 2005;Zapata et al. 2006) while selected samples can have a prevalence as high as 88% (Eriksen 2003). This represents a major burden to society in terms of pain/morbidity and associated healthcare costs (Yelin 2003).

The musculotendinous unit accounts for a significant portion of the musculoskeletal pain reported in the literature. Muscle pain as an entity is common with findings of 30% prevalence in a general practice population and 15% prevalence among factory sewing machine operators (Skootsky et al. 1989;Kaergaard and Andersen 2000). Tendon pain in the form of tendinopathy is a common, painful and frequently disabling (Khan and Cook 2003) subset within the body of musculoskeletal complaints. The prevalence of 'tendonitis' is reported between 3% and 19% (Salaffi et al. 2005;Forde et al. 2005;Kaplan et al. 2005) depending on the characteristics of the sample population. Thus, pain in the musculotendinous unit represents a significant clinical problem and as such deserves detailed investigation.

Clinical Background

Clinically, muscle pain is a frequent complaint of those suffering from myofascial pain syndromes (Carlson et al. 1993;Shah et al. 2005), fibromyalgia (Wolfe et al. 1990;Staud 2006a) and neck pain syndromes such as work related trapezius myalgia (Larsson et al. 2004;Rosendal et al. 2004;Sandberg et al. 2005). Common findings in muscle biopsies obtained from fibromyalgia and trapezius myalgia subjects include moth eaten fibres, changes in perfusion cross sectional area (Kadi et al. 1998a;Kadi et al. 1998b;Larsson et al. 2004) and increased DNA fragmentation (Sprott et al. 2004). Palpable taut bands in canine muscle (comparable to human myofascial trigger points) have been shown to display enlarged 'knot-like' fibres (Simons and Stolov 1976). In addition to these structural changes, local release of endogenous sensitising substances has been

demonstrated, with increased intramuscular, interstitial substance P and inflammatory infiltrates shown in fibromyalgia (Bengtsson et al. 1986;Sprott et al. 1998); increased interstitial levels of glutamate, lactate, pyruvate and serotonin in trapezius myalgia (Rosendal et al. 2004)and increased levels of bradykinin, serotonin and protons in myofascial pain subjects (Shah et al. 2005). This release is thought to result in peripheral sensitisation of nociceptors and ultimately to the perception of pain. Muscle nociceptors are known to exist in and around connective tissue and small arterioles (Reinert et al. 1998). The likely distribution of sites of nociceptive activity within different muscle structures following endogenous sensitisation is at present not known; indeed, relative sensitivity/afferent fibre population density in differing muscle structures has not been fully investigated. Whether, and to what intensity (as a reflection of afferent density) this nociceptive activity occurs in deep muscle afferents or in other structures such as the tissue of the fascia/epimysium or tendinous tissues may be important in terms of targeting therapy.

Clinical tendinopathy and resultant pain is not fully understood. Essentially, tendinopathy can be seen as a maladaptive response to external stresses (Leadbetter 1992). Eccentric or muscle lengthening activities are thought to be particularly damaging to tendon tissue and are strongly implicated in the development of tendinopathy (Lieber et al. 1997;Paavola et al. 2002;Sharma and Maffulli 2005). The classic structural changes seen in tendinopathy are increased ground substance, neovascularisation, collagen disruption and hypercellularity (fibroblastic proliferation: tendinopathy is not an inflammatory process) in the body of the affected tendon (Astrom and Rausing 1995;Kraushaar and Nirschl 1999;Rolf et al. 2001;Alfredson and Lorentzon 2002). Pain in tendons seems to be a result of these changes. It has been proposed that in particular, the neovascularisation is strongly associated with pain (Alfredson et al. 2003) such that sclerosing these vessels provides pain relief (Ohberg and Alfredson 2003;Alfredson and Ohberg 2005b). Again, as per muscle pain, it is likely that clinical tendon pain is a result of sensitisation of nociceptors via release of endogenous algescic substances. In line with this idea, microdialysis of tendinopathic tissue displays significant increases in glutamate and lactate (Alfredson et al. 2002;Alfredson and Lorentzon 2002) compared to healthy tendon

tissue. Recently, substance P (SP) and calcitonin gene-related peptide (CGRP) afferent immunoreactivity has been demonstrated at the tendon-bone/origin of tendons (Ljung et al. 2004). In addition, structural adaptation at the tendon-bone level has been suggested in lateral elbow tendinopathy (Edelson et al. 2001). This would imply that sensitivity of, and nociceptive activity in, the tendon bone-junction may be important in some tendinopathies. There is no current information regarding relative sensitivities between tendon and tendon-bone junction or as mentioned previously, muscle tissue.

Many studies have examined aspects of sensory manifestations in people experiencing muscle pain and to a very much lesser extent tendon pain. Clinical muscle pain is often associated with somatosensory changes such as increased mechanosensitivity (mechanical hyperalgesia) (Ohrbach and Gale 1989; Sandberg et al. 2005) and altered sensitivity to light touch (allodynia) (Leffler et al. 2003). Similarly, clinical tendinopathy is associated with increased mechanical sensitivity in the affected tendons (Astrom and Westlin 1992; Wright et al. 1992; Wright et al. 1994; Maffulli et al. 2003; Fredberg et al. 2004; Slater et al. 2005). Referral or spread of pain is frequently seen with clinical muscle pain (Vecchiet et al. 1991; Hong 1996; Hong C-Z 1998; Meyer 2002; Broadhurst et al. 2004; Al-Shenqiti and Oldham 2005; Fernandez-de-Las-Penas et al. 2006) but is largely unexplored for clinical tendon pain (Slater et al. 2005). Referred pain can be induced in anaesthetised (Laursen et al. 1999) limbs indicating that central pain processing mechanisms are involved in the perception of referred pain *and* central mechanisms are known to be involved in chronic pain maintenance/generation (Graven-Nielsen et al. 2000; Arendt-Nielsen and Graven-Nielsen 2003; Sarlani et al. 2004; Staud 2006b). Therefore investigating referred pain mechanisms may provide insight into relevant pain processing mechanisms in tendinopathy and muscle/fascia pain. It may also add to the body of knowledge regarding assessment and diagnosis of tissue injury/dysfunction based on likely referred pain patterns.

Investigative techniques

Social, motivational, cognitive and emotional factors are important aspects of a subjective clinical pain experience (Sullivan et al. 1998; Moseley 2004; Sullivan et al.

2005;Lame et al. 2005). These factors are not controllable in clinical settings and may be a considerable source of confounding error in the investigation of basic pain mechanisms involved in musculoskeletal pain complaints (Arendt-Nielsen and Svensson 2001).

Experimental pain research circumvents this problem by inducing a transient pain experience in healthy subjects and assessing the associated sensory manifestations. This technique has been frequently used to assess nociceptive characteristics and sensory-motor manifestations in muscle tissue (Kellgren J.H. 1938;Jensen and Norup 1992;Simone et al. 1994;Svensson et al. 1995;Arendt-Nielsen et al. 1997;Baker et al. 1997;Graven-Nielsen et al. 1997a;Graven-Nielsen et al. 1997b;Graven-Nielsen et al. 1997c;Graven-Nielsen et al. 1997d;Graven-Nielsen et al. 1998;Babenko et al. 2000;Bajaj et al. 2000;Barlas et al. 2000;Witting et al. 2000;Slater et al. 2003;Wang et al. 2004) providing valuable insights into muscle pain mechanisms and ultimately it is hoped toward a better understanding of clinical muscle pain. However, most of this investigative work has not discriminated between different structural components (muscle, tendon, tendon-bone junction and fascia/connective tissue) of the musculotendinous unit. As previously mentioned, muscle tissue is known to have a nociceptive innervation (Mense 1993) and it has been established that fascial and tendon tissue are also supplied with nociceptive afferent fibres (Stillwell D 1957;Yahia et al. 1992;Bjur et al. 2005;Danielson et al. 2006). Fascial tissue has not been comprehensively investigated and might well be a rich source of nociceptive afferent activity during clinical and experimental muscle pain states. Similarly, tendon tissue pain mechanisms remain relatively unexplored compared to muscle tissue despite an obvious clinical relevance.

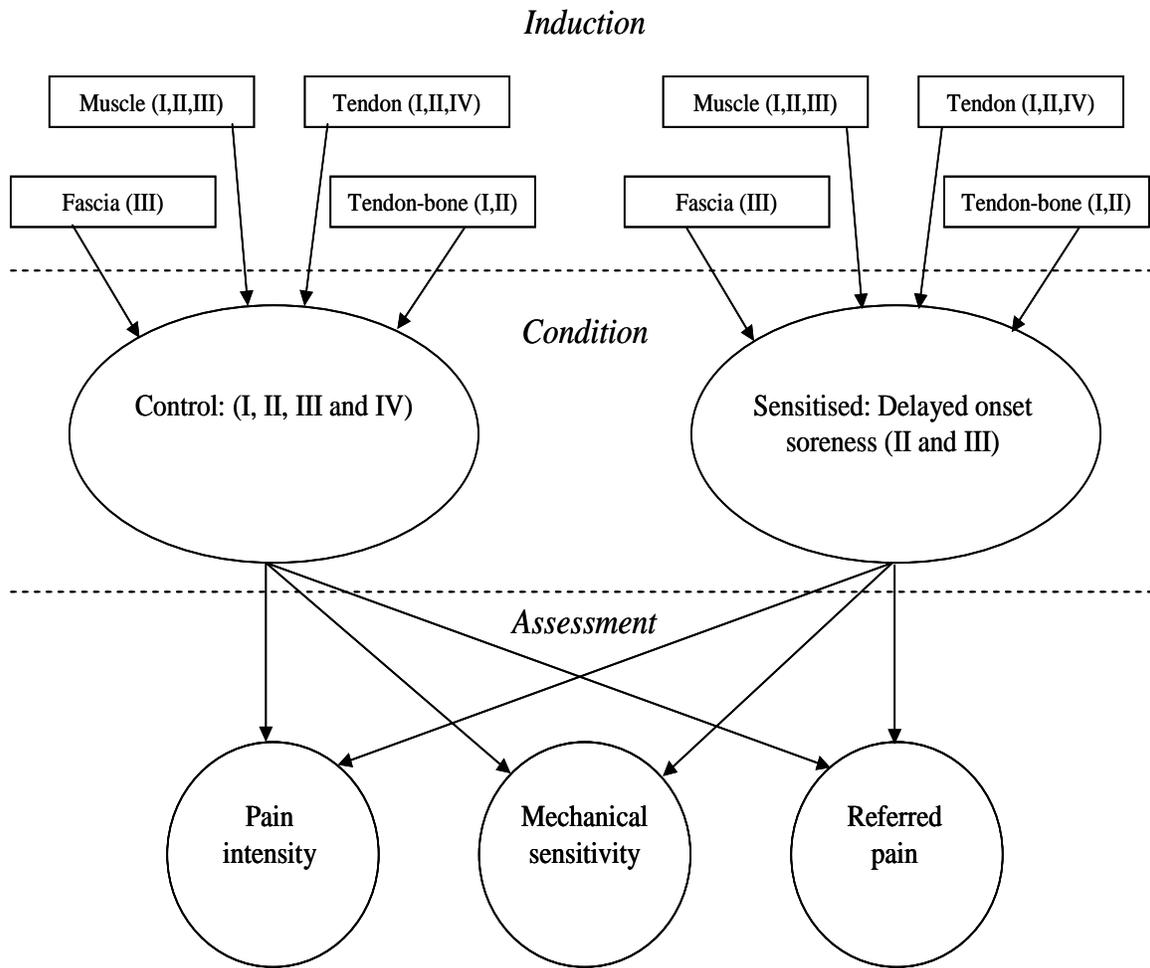
A small number of studies have utilised experimental pain induction techniques directed toward fascial and/or tendinous tissues (Kellgren J.H. 1938;Inman and Saunders 1944;Steinbrocker et al. 1953;Kawakita et al. 1991;Itoh and Kawakita 2002;Itoh et al. 2004) however these studies are of small sample size and do not yield comparative data regarding the muscle, tendinous structures and fascia. In order to fully understand clinical musculotendinous pain it is vital that in-depth knowledge of tissue sensitivity and therefore possible proportional contribution of each tissue type to the overall nociceptive

barrage be ascertained. It is important that the common sensory manifestations of clinical musculotendinous pain (mechanical hyperalgesia, referred pain) are assessed for each tissue type not only in control conditions, but also in sensitised conditions which may allow inferences to be made regarding tissue type and clinical pain states. Therefore, a detailed, comparative investigation in each tissue of sensory manifestations in response to standardised experimentally induced pain is required.

Aims of PhD project

This study aims to investigate the sensory manifestations to experimental pain induction in muscle, tendon, tendon-bone junction and fascial tissue in humans. This investigation shall quantify sensory manifestations often seen clinically and during experimental pain. Thus, pain sensitivity and mechanical sensitivity due to pain (assessment of hyperalgesic responses) and referred pain shall be the main focus areas of the study. This project will investigate these tissues under resting (control) and sensitised conditions (Fig. 1).

Fig. 1: Schematic representation of PhD project investigating sensory manifestations of experimentally induced pain in muscle, tendon and fascial tissues in a control (resting) condition to establish basic nociceptive characteristics as well as under sensitised conditions to investigate changes to nociceptive mechanisms during sensitisation. Assessment procedures consisted of pain intensity, mechanical sensitivity to pressure and pain distribution recording (referred pain).



1: Nociception from deep tissues

Nociceptive activity in animals and pain in humans from deep tissues is perceived following afferent activity in A δ (group III) and C-fibres (group IV) (Weddell and Harpman 1940;Mense and Stahnke 1983;Mense and Meyer 1985). Group III fibres are thinly myelinated and are characterised as having conduction velocities of between 2.5 and 30 m/s while group IV fibres are unmyelinated and have conduction velocities below 2.5 m/s (Mense and Meyer 1985). Nociceptive afferents are seen in the tissues as free nerve endings (Stacey M.J. 1969) and typically, are defined as being activated only by noxious thermal, chemical or high mechanical threshold stimulation (Kumazawa and Mizumura 1977;Hoheisel et al. 2005). Slowly conducting afferent fibres (group III and IV) and nerve endings have been observed in animal tendon, fascial (Stillwell D 1957;Andres et al. 1985;Mense and Meyer 1985) and muscle tissue (Stacey M.J. 1969). Histologically, sensory innervation and small fibre free nerve endings have been demonstrated in human fascial tissue (Weddell and Harpman 1940;Yahia et al. 1992) whilst intraneural microneurography observations in human muscle tissue (Marchettini et al. 1996) have identified putative nociceptive afferent activity. Substance P (SP) and calcitonin-gene related peptide (CGRP) immunoreactivity has been demonstrated in human tendon tissue (Bjur et al. 2005;Danielson et al. 2006) indicating a thin fibre sensory innervation (most likely serving a nociceptive function). In both tendon and muscle tissue, the nerve endings are mostly found in and around small arterioles and blood vessels in the tissue (Reinert et al. 1998;Bjur et al. 2005;Forsgren et al. 2005;Danielson et al. 2006). Thus, the peripheral apparatus required for transduction and transmission of nociceptive stimuli is present in muscle, fascial and tendinous structures.

Afferent activity from peripheral nociceptors is conveyed to the dorsal horn of the spinal cord, mainly to lamina I and III-V (Hoheisel et al. 1989;Todd et al. 2000). Thereafter, spinothalamic tract fibres (mostly but not exclusively) convey this activity to higher centres such as the thalamus (Craig et al. 1994) and cortex (Coghill et al. 1994). This is a reciprocal process however as nociceptive activity can be facilitated or inhibited by a number of supraspinal structures and mechanisms utilising descending pathways (Gebhart 2004).

Following injury or disease, peripheral nociceptors can become sensitised. This sensitisation occurs secondary to the release of endogenous substances (eg. inflammatory cytokines, neurotrophins, serotonin, bradykinin, glutamate, protons, prostaglandins and substance P) which are released locally in response to injury/inflammation and can directly or in combination lead to sensitisation of nociceptors (Mense 1981;Berberich et al. 1988;Nakamura-Craig and Gill 1991;Mense 1993;Issberner et al. 1996;Cairns et al. 2003a;Hoheisel et al. 2004;Hoheisel et al. 2005) with subsequent lowering of nociceptor response thresholds and often, increased background activity. This process likely explains the local tenderness, mechanical sensitivity and pain seen with injury or inflammatory conditions. In addition, this process can become self sustaining to an extent as local afferent activity can cause the antidromic release of substances such as SP and CGRP (Pedersen-Bjergaard et al. 1991;Loaiza et al. 2002) which may directly or indirectly lead to ongoing sensitisation.

Sustained nociceptive peripheral activity may lead to the development of central sensitisation (Woolf and Salter 2000). The most well known manifestations of this process are long-lasting neuronal activity secondary to stimulus, increased response to sub and suprathreshold stimuli and neuronal receptive field expansion (Hoheisel et al. 1993;Hoheisel et al. 1994). This central sensitisation mechanism probably accounts for the widespread pain and hyperalgesia/allodynia commonly seen in chronic pain patients in whom central sensitisation is thought to occur (Graven-Nielsen and Arendt-Nielsen 2002).

1.1 Clinical muscle, fascial and tendon pain

Peripheral sensitisation of nociceptors likely plays a role in clinical muscle pain. Microdialysis measurement from muscles of subjects with and without muscle pain demonstrate increased concentrations of potassium, serotonin, glutamate, bradykinin, SP, CGRP, cytokines and altered pH in muscle pain subjects (Rosendal et al. 2004;Shah et al. 2005;Rosendal et al. 2005). Central sensitisation also seems to be associated with muscle pain. Fibromyalgia (Staud 2006a) and myofascial pain syndrome patients frequently

complain of pain in the muscles and there is significant evidence for facilitated central pain processing mechanisms in people suffering with these conditions (Bendtsen et al. 1996;Graven-Nielsen et al. 2000;Staud et al. 2001;Staud et al. 2003). However it is not possible to say what relevant contribution fascial and muscle afferent activities make to clinical pain. No studies have examined the contributions of each tissue to the pain state.

Tendinopathy pain is associated with increased number and volume of blood vessels in the paratendinous tissue (Alfredson et al. 2003;Alfredson and Ohberg 2005b;Zeisig et al. 2006). The walls of these blood vessels are supplied with a SP and CGRP immunoreactive sensory innervation (Danielson et al. 2006). Altered local biochemical composition may suggest peripheral sensitisation is an important factor in tendinopathy pain perception (Alfredson et al. 2000;Alfredson et al. 2001b). Central pain processes may be involved in tendon pain states (Slater et al. 2005), however there are very few studies and little hard data is available.

1.2 Experimental pain techniques

Experimental pain induction affords the opportunity to investigate sensory manifestations of pain in a controlled environment. Essentially it involves standardised activation of nociceptive afferents in deep tissues and subsequent measurement of the various parameters of interest. This section shall review means of nociceptor activation employed in experimental pain studies.

1.2.1 Pain induction

There are two broad divisions of deep tissue pain induction techniques: those that utilise externally introduced stimuli (exogenous) and those that utilise internal or natural stimuli (endogenous). For a detailed description of the range of these techniques see Graven-Nielsen (2006).

Exogenous methods used to induce deep tissue pain are diverse ranging from mechanical stimulation (Kosek et al. 1996;Polianskis et al. 2002;Nie et al. 2005a) (studies *I to IV*) to

electrical stimulation (Kosek and Hansson 2002;Kosek and Hansson 2003;Koltzenburg et al. 2006) to injection of algogenic substances such as capsaicin (Sohn et al. 2000;Arima et al. 2000;Witting et al. 2000;Wang et al. 2002) (*study IV*), glutamate (Cairns et al. 2001;Svensson et al. 2003;Cairns et al. 2003a;Cairns et al. 2003b;Ge et al. 2005) (*study IV*), serotonin/bradykinin (Babenko et al. 1999a;Babenko et al. 1999b;Babenko et al. 2000) and hypertonic saline (Kellgren J.H. 1938;Steinbrocker et al. 1953;Graven-Nielsen et al. 1997b;Graven-Nielsen et al. 1997c;Graven-Nielsen et al. 1997d;Graven-Nielsen et al. 1997e;Graven-Nielsen et al. 1998;Hodges et al. 2003;Bennell et al. 2005) (*studies I to IV*). These techniques are useful as the tissue(s) of interest can be very specifically targeted.

Endogenous methods of pain induction are less plentiful and chiefly amount to pain induced through exercise (particularly lengthening or eccentric exercise) and through exercise with ischaemia. Eccentric exercise is the most widely employed method of endogenous pain induction in humans. Following this type of exercise, delayed onset soreness (DOS) over the musculotendinous unit is present after 24-48 hours (Newham 1988;Whitehead et al. 2001) allowing investigation of pain manifestations to be undertaken in this period. The site of nociceptive activity during DOS while thought to reside within the exercised muscle has not previously been investigated with regard to differing tissues within the muscle (*see study III*).

1.2.2 Selected pain induction techniques in fascia, muscle and tendon

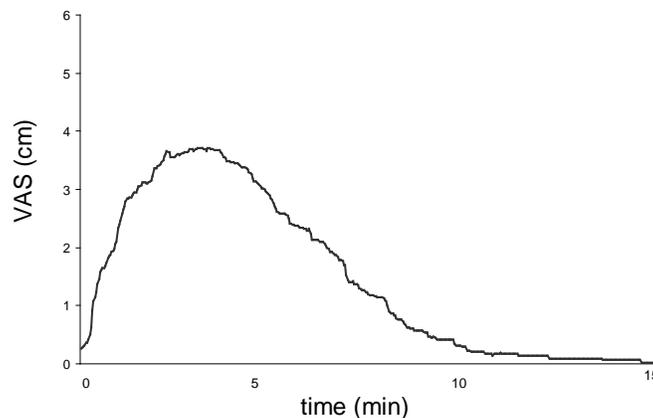
This section shall review the selected pain induction techniques used in the project.

1.2.3 Hypertonic saline

Hypertonic saline produces a transient local pain which is described as being similar to clinical muscle pain (Graven-Nielsen et al. 1997a;Capra and Ro 2000). Hypertonic saline injection is known to induce referred pain and has been used to do so in many structures including the following: muscle (Graven-Nielsen et al. 1997a;Graven-Nielsen et al. 1997b;Graven-Nielsen et al. 1997c), tendon (Kellgren J.H. 1938;Steinbrocker et al. 1953;Slater et al. 2003), ligaments (Lewis and Kellgren 1939;Inman and Saunders

1944;Feinstein et al. 1954), joint and joint related structures (Gerber et al. 1998;Hassett and Barnsley 2001;Bennell et al. 2004) (see also Table 1, Appendix 1). The pain intensity, quality and distribution of hypertonic saline has been shown to be reproducible for individuals over time and repeated injections (Graven-Nielsen et al. 1997a;Graven-Nielsen et al. 1997b). Thus it is a reproducible means of inducing experimental pain. The safety of hypertonic saline use is well established with a recent review finding over 6000 hypertonic saline injections performed in studies without side-effects (Graven-Nielsen 2006). Fig. 2 demonstrates a typical visual analogue scale (VAS) pain profile following hypertonic saline injection to tibialis anterior tendon.

Fig. 2: Average VAS profile following injection of 0.5ml, 5.8% hypertonic saline to the tendon of tibialis anterior muscle. (n=18). Data taken from study I.



Hypertonic saline induces activity in nociceptive group III and IV sensory endings (Kumazawa and Mizumura 1977;Hoheisel et al. 2004;Hoheisel et al. 2005) without effecting thick group I and II afferent fibres. However, it is not nociceptive specific and may cause activity in thermal, contraction and low threshold mechano-sensitive group III and IV fibres (Kumazawa and Mizumura 1977). The exact mode or site of action of hypertonic saline upon the nerve endings is unknown. It is speculated the hypertonic nature of the saline may act through the osmotic gradient to shrink the nerve endings and cause activity in the stretch inactivated channel, a variant of the transient receptor potential vanilloid 1 (TRPV1) receptor (Schumacher et al. 2000). Alternatively, the ion-channel TRPV4 (expressed peripherally and centrally) as a mediator of osmolarity may be implicated in hypertonic saline induced afferent activity (Alessandri-Haber et al. 2005).

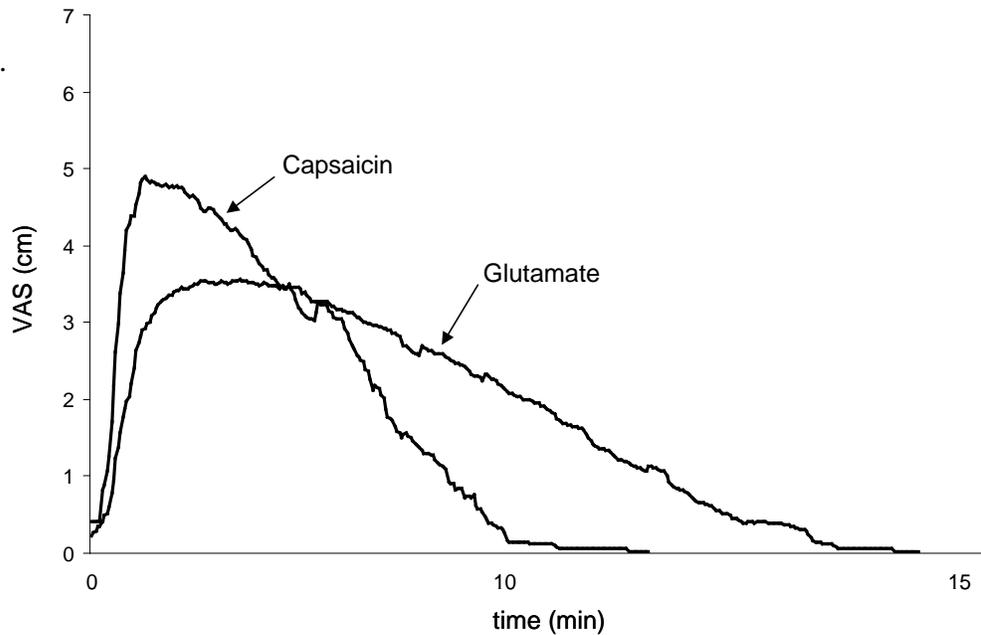
1.2.4 Glutamate and capsaicin

Glutamate is a natural amino acid present in the peripheral and central nervous system in humans and is a key mediator in nociceptive activity. Unlike hypertonic saline, the suggested mode of action of glutamate is well described. Sensory afferent activity induced by glutamate occurs at the ionotropic N-methyl-D-aspartate (NMDA), α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, kainate (AMPA and KA) (Coggeshall and Carlton 1998) and metabotropic (groups I, II and III) receptor types (Walker et al. 2001;Neugebauer 2002). It has been shown to cause pain in deep muscle tissue with a similar profile to that of hypertonic saline (Cairns et al. 2001;Svensson et al. 2003;Cairns et al. 2003a;Cairns et al. 2003b;Wang et al. 2004;Svensson et al. 2005;Cairns et al. 2006). Glutamate injection causes higher pain intensity in women and greater nociceptive afferent activity in female rats compared to males (Cairns et al. 2001). Due to this, in order to minimise any variability in the sample population, study IV was performed only on males. Application of glutamate to deep muscle results in increased activity in group III and IV fibres that is not restricted to purely nociceptive afferents (Cairns et al. 2003a). Tendon tissue has been shown to express sensory afferent NMDA receptors (Alfredson et al. 2001a) and increased tissue glutamate level is associated with tendinopathy (Alfredson et al. 2000;Alfredson and Lorentzon 2002) implying glutamate may be important in tendon pain. There are no prior studies profiling the characteristics of glutamate induced pain in human tendon tissue (*see study IV and Fig. 3*).

Capsaicin, familiar as the active constituent of chilli peppers is the agonist for the TRPV1 receptor found on peripheral and central afferent fibres (Caterina et al. 1997;Caterina and Julius 2001). Capsaicin has been employed on numerous occasions as a model to elicit deep muscle tissue pain (Arima et al. 2000;Witting et al. 2000;Graven-Nielsen et al. 2002;Wang et al. 2002;Qerama et al. 2004;Chang et al. 2004;Sohn et al. 2004;Qerama et al. 2005;Kumar et al. 2006) with induction of transient, moderate to intense pain. Nociceptive activity is elicited in group III and IV afferents, again in a non-specific fashion (Hoheisel et al. 2004). The TRPV1 receptor is known to respond to capsaicin, heat and protons (Caterina and Julius 2001). This is of interest as tissue injury and

inflammation is associated with low pH (McMahon and Koltzenburg 2006), meaning this may be a functionally important receptor in clinical pain states such as tendinopathy. No previous studies have examined capsaicin pain characteristics in tendon tissue (*see study IV and Fig. 3*).

Fig.3: Average VAS profiles following injection of 5 μ g (in 0.5ml solution) capsaicin and 0.5ml of 1M glutamate solution to tendon of tibialis anterior muscle. (n=12). Data taken from study IV.



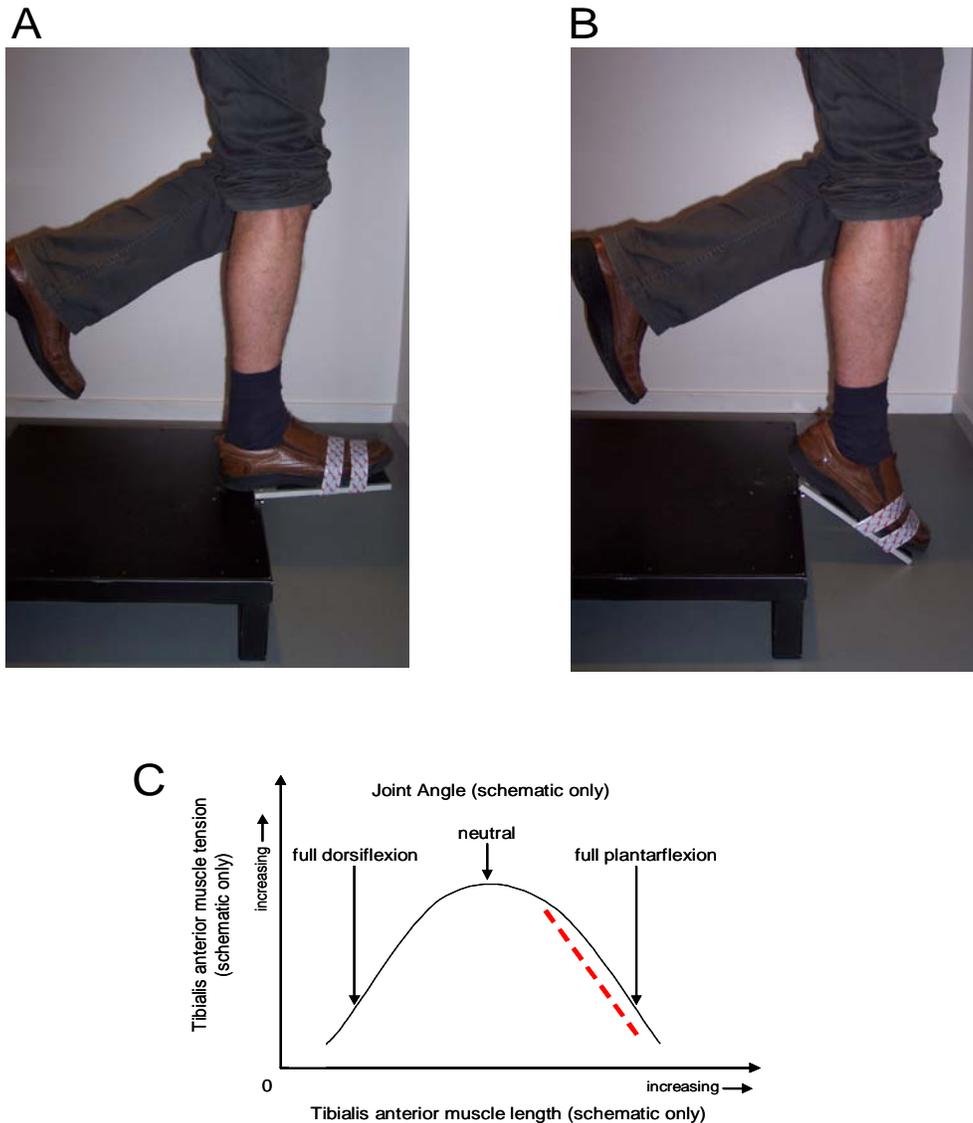
Additionally, utilising glutamate and capsaicin in a sequential fashion to induce pain has shown there may be modulatory activity between these two receptor types. Modulation of nociceptive activity following capsaicin injection has been achieved using NMDA and AMPA/KA antagonists (Sang et al. 1998; Lam et al. 2005). Facilitation of capsaicin response has been demonstrated in deep tissue immediately following glutamate injection (Teramoto et al. 2003; Wang et al. 2006). NMDA receptors are expressed in human tendon; however expression of TRPV1 receptors in this tissue is not yet established. Using capsaicin as a means of inferring TRPV1 expression and investigating modulatory interactions between these two receptor types in tendon tissue may reveal similar modulatory interactions to that seen in muscle.

1.2.5 Eccentric exercise induced delayed onset soreness

Delayed onset soreness becomes apparent approximately 24-48 hours after performing loaded eccentric exercise (Bajaj et al. 2000;Whitehead et al. 2001;Itoh et al. 2004;Prasartwuth et al. 2005). With DOS, subjects report tenderness on palpation over the affected muscle, soreness with movement but no pain at rest (Crenshaw et al. 1994;Bajaj et al. 2001;Nie et al. 2005a;Nie et al. 2005b;Nie et al. 2005c). In addition, maximum voluntary contraction force is decreased (Bajaj et al. 2000;Prasartwuth et al. 2005) and increases in stiffness and swelling of the muscle is seen (Crenshaw et al. 1994;Whitehead et al. 2001;Nosaka et al. 2002;Hirose et al. 2004). Loaded eccentric lengthening of muscles occurs when the muscle actively contracts to resist a force which is causing lengthening of the muscle. This form of lengthening muscular contraction is extremely efficient in terms of work-output to energy consumed (Komi et al. 1987;Caruso et al. 2001) compared to concentric (shortening) or isometric (static) work.

Eccentric exercise to induce DOS is a common endogenous method of experimental pain induction utilised in both upper and lower limb muscles eg. quadriceps (Crenshaw et al. 1994;Baker et al. 1997), triceps surae (Weerakkody et al. 2001;Weerakkody et al. 2003), hamstrings (Brockett et al. 2001), forearm extensors (Slater et al. 2003;Slater et al. 2005), interosseous muscle of the hand (Bajaj et al. 2001;Bajaj et al. 2002), biceps brachii (Nosaka and Clarkson 1996;Barlas et al. 2000) and trapezius (Nie et al. 2005b;Nie et al. 2005c). Eccentric lengthening protocols have also been utilised as a means of developing an animal correlate of human DOS models (Taguchi et al. 2005a;Taguchi et al. 2005b) in order to more fully investigate basic nociceptive mechanisms behind the eccentric-induced increased mechanical sensitivity. In humans, tibialis anterior muscle has not been utilised widely as a DOS model perhaps due to methodological difficulties. Only two previous studies have utilised eccentric exercise in tibialis anterior. In one (Warren et al. 2000), delayed soreness was not measured and in the other only a minority of healthy subjects actually went on to develop DOS (Birtles et al. 2003). Thus a new means of inducing DOS in tibialis anterior was developed (see Fig. 4 and studies II and III).

Fig 4: Illustration of new eccentric exercise protocol to induce DOS in tibialis anterior muscle. (A); starting position of eccentric exercise corresponding to neutral joint angle depicted in (C). (B); End point of eccentric exercise corresponding to full plantarflexion joint angle depicted in (C). On completion of the movement, the unexercised leg was extended until weightbearing and used to return the subject back to the starting position. (C) Schematic representation of the length-tension relationship of the tibialis anterior muscle and its associated joint angle during the eccentric exercise performed. The dotted red line indicates the descending limb of the length-tension curve (corresponding to increasing plantarflexion) where the main eccentric exercise effect is thought to occur. (Brockett et al. 2002; Bowers et al. 2004) and represents the joint angle range over which the new eccentric protocol worked.



In brief, subjects were instructed to stand with the heel of the experimental leg on the edge of a 13 cm high platform with flat hands resting lightly on a supporting surface at

shoulder level. The other leg was not in contact with the supporting surface. The foot of the experimental leg was strapped to a hinged metal footplate that supported the forefoot and allowed plantar and dorsi-flexion around the ankle joint. Subjects then slowly lowered the foot from the neutral ankle position to a fully plantar-flexed position; all the while tibialis anterior working to eccentrically control the lowering of bodyweight. The other leg was then brought into contact with the supporting surface and used to return to the starting position. In this way tibialis anterior muscle of the experimental leg was made to work solely eccentrically. This was a very efficient new method of inducing DOS in tibialis anterior. It may be that prior methods utilising a KinCOM dynamometer (Birtles et al. 2003) did not work the muscle toward sufficiently long lengths where the eccentric effect is primarily thought to occur (Jones et al. 1997).

It is commonly thought that eccentric exercise is caused by disruption at the level of the sarcomere (Morgan and Proske 2004) to the contractile apparatus. Alteration to the ultrastructure of the myofibril is reported, with Z-disc streaming (Fielding et al. 1993; Friden and Lieber 2001) and cytoskeletal protein loss observed (Lieber et al. 2002). This has historically been seen in terms of damage to the muscle (Stauber et al. 1990; Fielding et al. 1993) with increases in putative inflammatory markers such as serum creatine kinase (CK) and neutrophil levels taken as evidence of an inflammatory response (Nosaka and Clarkson 1996; MacIntyre et al. 1996; MacIntyre et al. 2000). The delayed onset soreness was attributed to peripheral sensitisation secondary to the assumed inflammation. However, more recent work brings this assumption into question. Serum CK profile does not match the soreness profiles; indeed serum CK can rise significantly post eccentric exercise in the absence of any evidence of an eccentric induced inflammatory response (Malm et al. 2004). Recent comparative studies between concentric and eccentric exercise found no evidence of an inflammatory response following eccentric exercise compared to control or concentric exercise groups (Malm et al. 2000; Malm et al. 2004). In reinforcement of this, anti-inflammatory medication has been shown to have little effect on inflammatory markers (Peterson et al. 2003) or perceived soreness post eccentric exercise (Semark et al. 1999; Loram et al. 2005). It is now proposed that the ultrastructural effects seen following eccentric exercise are more

properly viewed as an adaptive immunological response to exercise rather than tissue damage (Malm et al. 2000; Friden and Lieber 2001; Yu et al. 2002; Yu and Thornell 2002; Malm et al. 2004; Yu et al. 2004). Further it has been proposed that the tissue responsible for DOS may in fact be connective (fascia/epimysium) rather than muscle (Malm et al. 2004).

Regardless of whether eccentric exercise effects tissue damage or a muscle adaptation response, it is apparent that with time, sensitisation of thin fibre afferents by algescic substances is likely (Tegeder et al. 2002). The tenderness and soreness 24-48 hours post eccentric exercise would imply some degree of peripheral sensitisation of sensory thin afferents. Recent data from animal models confirms facilitation of peripheral thin afferent fibres 48 hours post eccentric exercise (Taguchi et al. 2005a; Taguchi et al. 2005b) (*see study III*). In addition, evidence for altered central pain processing mechanisms in DOS is gathering. Facilitation of temporal summation (Bajaj et al. 2000; Nie et al. 2005b), evidence of thick fibre involvement (Barlas et al. 2000; Weerakkody et al. 2001; Weerakkody et al. 2003) and facilitated referred pain (Study II) during DOS is established suggesting central sensitisation processes are at work.

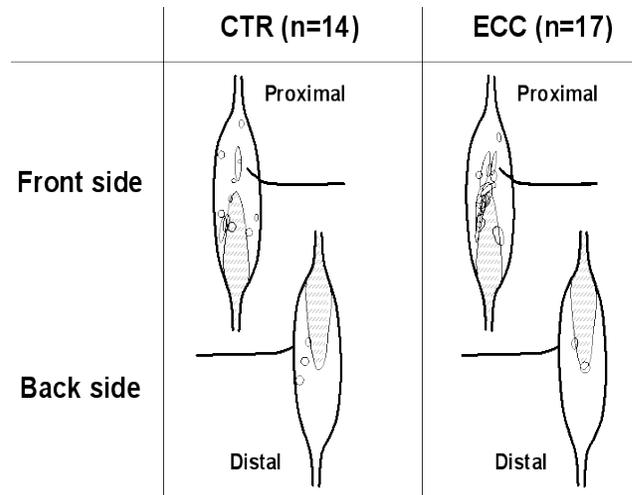
2. Mechanical sensitivity during experimental pain

2.1 Delayed onset soreness

Increased mechanical sensitivity (hyperalgesia) is a hallmark of eccentric exercise induced delayed onset soreness. It has become apparent that the hyperalgesia seen in DOS is not evenly distributed over the affected muscle. Whilst the most commonly reported site of tenderness in DOS is the mid muscle belly region (Bajaj et al. 2000; Weerakkody et al. 2001; Whitehead et al. 2001; Slater et al. 2003; Slater et al. 2005), other studies including animal studies report the musculotendinous junction being involved (Baker et al. 1997; Taguchi et al. 2005b; Nie et al. 2005c) which was confirmed in the current animal component of study III (see Fig. 5).

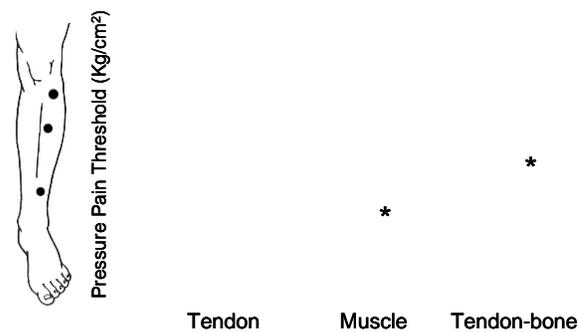
Fig. 5: Examples of receptive fields (outlined and indicated by probe) assessed in control (CTR) and eccentrically exercised (ECC) rat extensor digitorum longus muscles. Note that the identified mechanically

sensitised receptive fields in the ECC group are clustered mainly around the musculotendinous junction area. Data taken from animal component of study III.



In study II, 38 subjects with DOS had pressure pain thresholds measured at muscle belly, tendon-bone junction and distal tendon sites. Both muscle belly and tendon-bone junction sites were hyperalgesic but distal tendon was unaffected (see Fig. 6). This confirms previous findings but may not fully reflect DOS mechanical sensitivity patterns. Because only three sites were assessed and the sample size was such that sensitivity to change was high; it is possible that more extensive spread of hyperalgesia was missed or that the sites assessed were not the most sensitive areas.

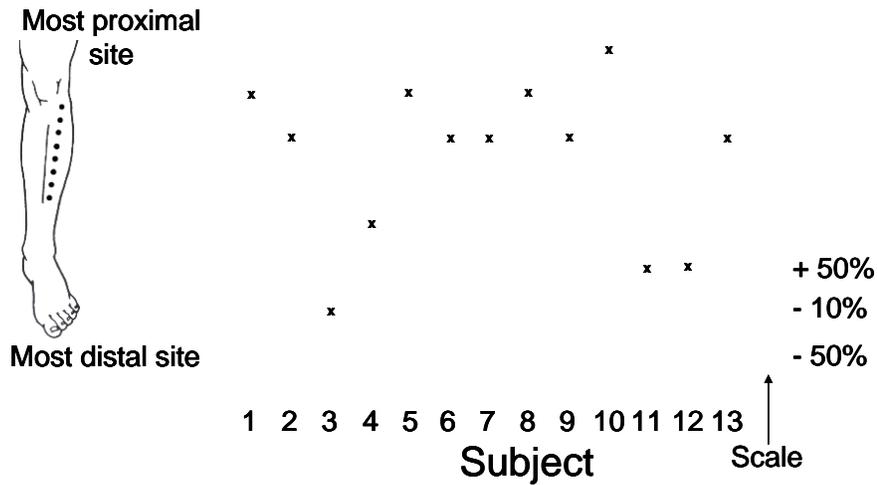
Fig. 6: Representation of mechanical sensitivity (represented by average PPT, n=38) pre and during DOS; assessed at distal tendon, muscle belly and tendon-bone junction sites. (*) significantly decreased compared to pre DOS sensitivity. Data taken from study II.



A progression of specificity in the search for mechanical sensitivity post eccentric exercise is seen in the receptive field assessment of presumed group IV afferent fibres post eccentric lengthening protocol in the animal component of study III. As shown in *Figure 5*, receptive fields found to be mechanically sensitised were located mainly around the musculotendinous junction area. However this may not reflect the full DOS spatial effect as the full muscle length was not assessed for mechanical sensitivity.

Widespread patchy distributions of sensitive loci during DOS has been demonstrated (Weerakkody et al. 2001; Weerakkody et al. 2003) and this is confirmed in the human component of study III. In this experiment it was important to specify the site of maximal mechanical sensitivity over the muscle during DOS. Eight sites distributed along the muscle from proximal tendon-bone junction area to distal musculotendinous junction were assessed. Post eccentric exercise, the change from baseline in mechanically sensitivity was assessed for each site and the site with the greatest relative change was deemed the area of maximal sensitivity and by extension the site of greatest DOS sensitisation. While muscle belly was a common site of maximal sensitivity there was considerable variation in the spatial distribution of these areas between subjects (see Fig. 7).

Fig.7: Visual demonstration of mechanical sensitivity (as represented by average PPTs, n=13) changes at eight sites along tibialis anterior muscle during DOS. Open circles represent increased sensitivity, filled represents decreased sensitivity (compared to pre eccentric exercise baseline). 'X' in open circles represents site of maximal sensitivity for each subject. Note the spatial distribution in site of maximal DOS induced mechanosensitivity. Data taken from study III.



This study established the site of maximal sensitivity during DOS; however, as the fascia directly overlies the muscle, it is not possible to make mechanosensitivity discriminations between fascia and deep muscle. Other more selective techniques are required (see Chapter 3).

There is no obvious explanation for this variation in mechanosensitivity during DOS. Numerous factors may be involved. Evidence points to type II fibres being preferentially utilised in a novel eccentric lengthening task (Warren et al. 2000) and it is reported that eccentric exercise preferentially induces damage/adaptation in type II muscle fibres in animals (Lieber and Friden 1988) and in humans (Warren et al. 2000). Additionally, it is known that muscle fibre type proportions vary between muscles and regions within muscles in humans (Henriksson-Larsen et al. 1983; Dahmane et al. 2005). It may be that the spatial variability in sensitivity post eccentric exercise found in study III was influenced by factors such as fibre type variability in the muscle and between subjects. It is possible this is a reflection of the specific properties of the muscle and the subject assessed.

2.1.1 Hypertonic saline

Local mechanical sensitivity changes during hypertonic saline induced pain have previously been investigated in muscle tissue, ligaments, tendon/fascia and joint and associated structures (see Appendix 1, Table 1). For muscle, the results are ambiguous as hyperalgesia is reported in response to both hypertonic and isotonic saline (Jensen and Norup 1992;Graven-Nielsen et al. 1998) or is found not to influence muscle mechanical sensitivity (Babenko et al. 1999b). There is no quantitative data for the studies performed on the other tissues mentioned in appendix 1 making their results difficult to interpret. There may be a number of mechanisms influencing peripheral sensitivity during saline induced pain. Injury induced by needle insertion may cause release of glutamate (Tegeger et al. 2002) or other endogenous substances which could lead to nociceptor sensitisation. While this is possible it does not explain the lack of change in mechanical sensitivity commonly seen post hypertonic saline injection (Babenko et al. 1999b;Slater et al. 2003;Slater et al. 2005). Afferent stimulation may lead to release of endogenous substances (glutamate, SP, CGRP) via a local axon reflex mechanism (Pedersen-Bjergaard et al. 1991;Tegeger et al. 2002;Loaiza et al. 2002) which may be a means of peripheral sensitisation. Lastly, it may be that afferent activity caused by the pressure algometer (used to assess mechanosensitivity) can summate with the afferent activity induced by the hypertonic saline in a centrally mediated process thereby leading to the perception of mechanical hyperalgesia (Graven-Nielsen et al. 1998).

Study I demonstrated significant reductions in pressure pain threshold (PPT) during pain in (and measured at) the tendon and tendon-bone junction sites. No change in PPT at the muscle belly was seen following pain induced at this site. This reduction in tendon/tendon-bone PPT was transient as values returned to baseline as soon as pain resolved. Hypertonic saline injected to the tendinous structures caused greater pain than muscle belly injection and it is likely the increased saline pain at these sites, in combination with the stimulation provided by the algometer, resulted in an afferent input summation effect causing pain to be perceived at lower pressures compared to baseline. From study I it may be inferred that hypertonic saline did not reliably induce peripheral sensitisation in tendon tissue. In this study, occasionally significant hypoalgesia was seen in distant sites following either hypertonic or isotonic saline injection at tendon or

tendon-bone junction. Physiological adaptation to repeated mechanical testing is reported and likely explains hypoalgesia following isotonic saline injection (Svensson et al. 2003). In contrast, it is thought that hypoalgesia at remote sites following painful stimulation is probably mediated through a descending noxious inhibitory control mechanism (Le Bars et al. 1979). This serves to highlight some of the difficulties and potential problems involved in repeated psychophysical testing of human subjects.

Study II examined mechanical sensitivity during DOS at muscle, tendon and tendon-bone sites. Subjects with DOS had a tendon, tendon-bone junction or muscle belly hypertonic saline injection. In this case no reduction in PPT at any site was seen during saline-induced pain. Due to presumed afferent sensitisation during DOS it would be expected that hypertonic saline injection may lead to increased mechanosensitivity via summation of afferent input or, following injection, through an antidromic sensitisation of already sensitised nociceptors. It has been suggested there may be a discrepancy between central afferent processing of saline pain and pressure stimulation during DOS (Weerakkody et al. 2003) with the main theory being that during DOS, it is actually thick-fibre afferents that mediate the soreness (Weerakkody et al. 2003). If this were the case it could explain why mechanosensitivity during DOS is seemingly unchanged by hypertonic saline injection: although non-specific in action, hypertonic saline does not excite thick fibre sensory afferents (Kumazawa and Mizumura 1977). However, basic animal studies (including study III) (Taguchi et al. 2005a; Taguchi et al. 2005b) provide good evidence that thin fibre sensory afferents *are* mechanically sensitised post eccentric lengthening exercise and as such it seems logical that hypertonic saline application could induce mechanical hyperalgesia during DOS. It has been postulated that post eccentric exercise the mechanical hyperalgesia may cause a sufficient nociceptive barrage such that further thin-fibre response to additional stimuli may not be possible; in effect there may be a sensory afferent 'saturation' (Marqueste et al. 2004). Lastly, due to the suggested 'all-or-nothing' response caused by hypertonic saline (Hoheisel et al. 2005) there remains the possibility that hypertonic saline cannot further sensitise afferents that are already sensitised.

2.1.2 Glutamate and capsaicin

Similar to hypertonic saline, the effects of deep muscle injection of glutamate are somewhat ambiguous, with increased (Svensson et al. 2003; Cairns et al. 2006) and unchanged (Cairns et al. 2003a; Ge et al. 2005) mechanosensitivity observed. Further, while glutamate levels are increased in both muscle and tendon clinical pain patients the relationship is ambiguous with increased glutamate levels related to increased mechanical sensitivity in trapezius myalgia patients but apparently unrelated to mechanical sensitivity in tendinopathy patients (Alfredson and Lorentzon 2003; Rosendal et al. 2004). In study IV glutamate had a similar pain profile to hypertonic saline (and no mechanical hyperalgesia was seen during saline pain). As suggested previously, if under some circumstances increased sensitivity can be mediated via a central summation of induced pain and pressure stimulation then the modest pain levels following glutamate injection may not have provided sufficient afferent input to allow this proposed summation to occur. Secondly, it remains unknown exactly which receptor types mediate mechano-transduction. There is speculation that the TRPV receptor family might be important in this regard (Lin and Corey 2005). Mice with TRPV4 receptor knock-out show decreased response to noxious mechano-stimulation (Suzuki et al. 2003) and mice lacking TRPV1 exhibit decreased response to the mechanical stimulus supplied by bladder filling (Birder et al. 2002). Given the established modulatory interactions between NMDA antagonists and TRPV1 receptor activity (capsaicin induced), it is apparent that the link between glutamate and mechanosensitivity is complex and as yet not fully understood.

Capsaicin intramuscular injection on the other hand reliably reduces PPT during induced pain (Arima et al. 2000; Witting et al. 2000; Qerama et al. 2004; Kumar et al. 2006). This increase in mechanosensitivity can have a prolonged duration of up to several hours (Arima et al. 2000; Witting et al. 2000).

In study IV injection of glutamate to the tendon did not change mechanosensitivity measured at the tendon. Hypertonic saline injected immediately after glutamate did not change mechanosensitivity during pain (in contrast to study I but the preceding injection of glutamate in this study makes direct comparison difficult). Capsaicin injection after

glutamate did not cause additional reduction in PPT during pain as compared to capsaicin injection alone. This was despite a facilitation of the pain (see chapter 3) perceived during capsaicin injection preceded by a glutamate injection compared to capsaicin injection alone. Therefore, it may be inferred that tendon tissue in a resting control condition does not display hyperalgesia subsequent to glutamate induced pain. Secondly, glutamate may cause sensitised conditions in the tendon tissue such that perceived pain to subsequent capsaicin injection is facilitated but this is not reflected by further increases in mechanosensitivity. Again, this highlights the complex relationship between glutamate and mechanosensitivity during control, and possibly also clinical conditions.

Capsaicin injection in study IV caused significant increases in mechanosensitivity at the tendon injection site and 2 cm proximal. Unlike previous studies however (Arima et al. 2000; Witting et al. 2000), the PPT reductions were transient and mechanosensitivity returned to baseline levels following resolution of pain. In addition, following capsaicin, both glutamate and hypertonic saline pain caused transiently increased mechanosensitivity which was manifest only during pain. Due to high perceived pain levels, the amount of capsaicin injected was 5µg which is ten times lower than that commonly injected in muscle pain studies (Graven-Nielsen et al. 2002). While it is not possible to exclude capsaicin induced peripheral sensitisation (there could have been short lived peripheral effects which, due to the small amount of capsaicin were resolved by the time pain resolved and post pain PPT recordings were taken) it seems most likely that hyperalgesia was appreciated again via a central summation effect. It has been demonstrated that phosphorylation of spinothalamic tract cell NMDA receptors occurs within 30 minutes post capsaicin injection (Zou et al. 2000) in rats. Phosphorylation of NMDA receptors is thought to be an important part of the central sensitisation process (Woolf and Salter 2000). It is possible that early stage central sensitisation could result in increased response to the subsequent combined stimuli of induced pain (glutamate and hypertonic saline) and pressure stimulation (hyperalgesia) as opposed to pressure stimulation alone (unchanged post-capsaicin-induced pain mechanical sensitivity).

3. Pain Intensity

One of the many factors influencing perceived pain may be innervation density of the painful tissue (Saxler et al. 2006). Increased pain from hypertonic saline injection at motor end-plates compared to control (non-end-plate) sites in the muscle are suggested to be reflective of increased innervation density at these sites (Qerama et al. 2004). The current studies (I to III) reveal significant differences in perceived pain intensity when comparing between tendon, tendon-bone junction, fascia and muscle which is suggestive of differences in sensory innervation density between these tissues.

3.1 Hypertonic saline

Studies I and II reveal an overall trend toward greater pain following hypertonic saline injection at tendon-bone and tendon compared to muscle belly. It was shown in study I that isotonic saline produced negligible pain in tendinous structures. Given the obvious differences in tissue composition and vascularity between muscle and tendon it was important to establish that simple mechanical deformation of the tissues by a saline bolus did not provoke pain. The greater pain perceived following hypertonic saline tendon injection compared to muscle is a stable consistent finding (see Fig. 8).

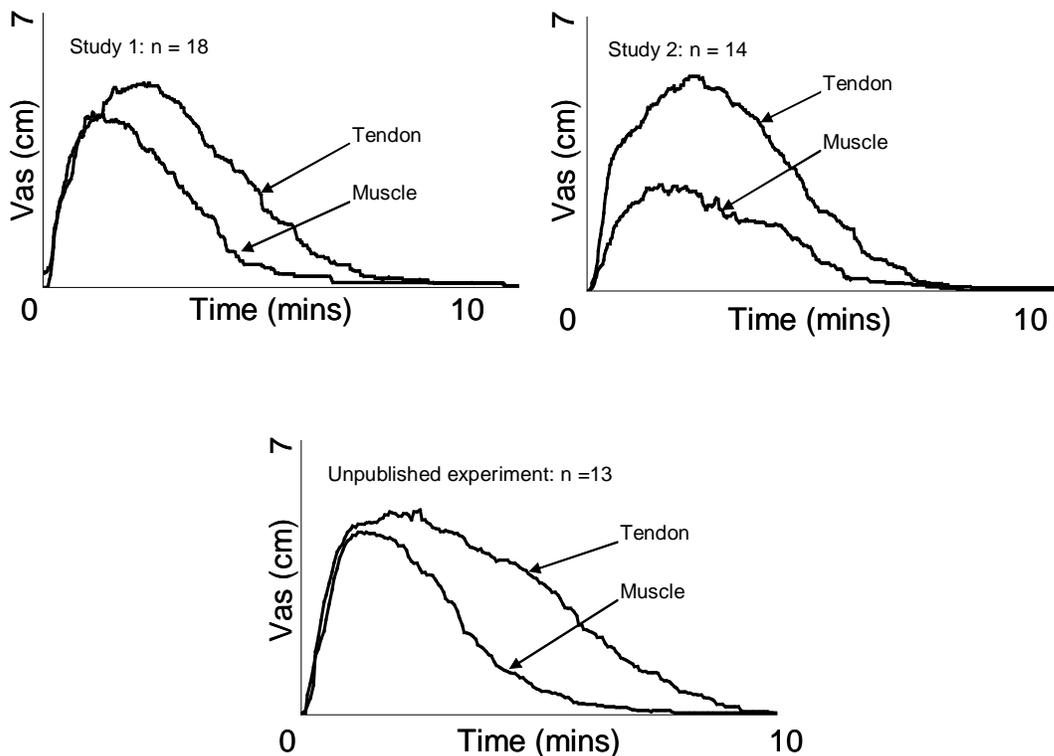


Fig.8: Demonstration of consistency of greater pain induced by hypertonic saline (5.8% 0.5ml) following tendon injection compared to muscle belly injection. All three average VAS graphs above show significantly greater area under the VAS-time curve while study 2 also displays increased pain intensity compared to muscle belly injection. Note: the lower graph is data taken from an unpublished manuscript.

Studies I and II established that the tendon-bone junction area was significantly more sensitive to hypertonic saline than the muscle belly. The possibility remains that following injection to this site; periosteal tissue which is known to be highly sensitive (Graven-Nielsen et al. 1997a) may have been affected by the saline. However, great care was taken in each study to avoid needle contact with (or if contact was made, retract the needle appreciably) the bone. If nociceptive innervation density is reflective of perceived pain, it is logical to suggest this area is more densely innervated than muscle belly. Nociceptors are not merely signallers of pain; they also serve a protective warning function (Mense 1993). The origin or tendon-bone junction of tibialis anterior is fleshy in nature (Moore 1992). Injection to this area will undoubtedly encompass musculotendinous junctions as well as tendon-bone transitional areas. The musculotendinous junction is the area most likely to be damaged in strain injuries (Nikolaou et al. 1987; Kirkendall and Garrett 2002; Cross et al. 2004). From a self-protection point of view, it is logical if this vulnerable area is more densely populated with nociceptors which could alert the system to potentially damaging situations.

Following studies I and II it can be inferred that tendon tissue is more sensitive than muscle belly to hypertonic saline injection. This fits well with observed higher innervation densities in rat peritendineum tissue compared with the associated muscle (Mense and Simons 2001). It may be implied that this current result reflects higher innervation density in tendon/paratendinous tissue than muscle. However, it should be noted that this series of studies aimed to inject at the surface of, and around the tendon. At no point were the injections deliberately targeted intra-tendinously to the body of the tendon. Therefore, it is most likely that this present finding reflects innervation in the

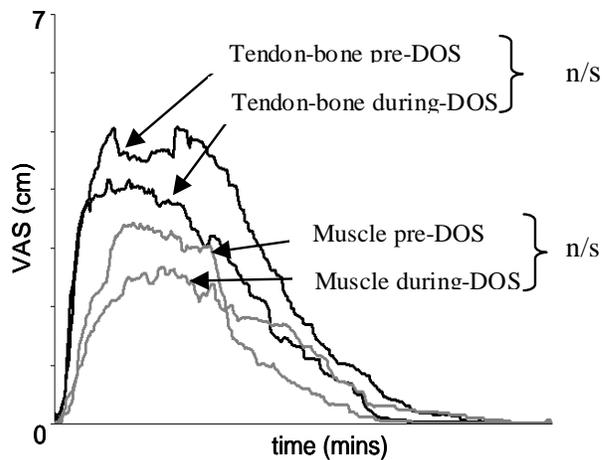
paratendon tissue and the most superficial layers of the tendon body. Clinically, paratendinous tissue is important as sclerosing the neovessels associated with tendinopathy in this outer layer of the tendon produces marked reductions in pain leading to the assumption that the pain of tendinopathy chiefly originates from nociceptive afferents in this tissue (Alfredson and Ohberg 2005a; Danielson et al. 2006). It must be acknowledged that the behaviour of the saline bolus may have had an effect on pain perceived. A hypertonic saline bolus injected to the muscle tends to distribute in a vertical plane (Graven-Nielsen et al. 1997d). The fluid mechanics of saline injected to the tendon is not known. If the saline were to sit in a pool, it may provide ongoing stimulation such that a temporal summation of pain may account for greater perceived pain. A similar process is suggested to account for high levels of pain post saline injection to the subcutis (Lindahl 1969).

A selective method of delivering targeted hypertonic saline injections to superficial fascia/muscle was developed in order to investigate comparisons between fascia and deep muscle sensitivity (*study III*). This study established the increased sensitivity to saline injection of muscle fascia/epimysium compared to deep muscle tissue. This finding confirms clinical anecdotal experience in which stimulation of fascia is consistently described as more painful than deep muscle tissue. Fascia/epimysium is continuous with the connective tissue of the muscle. Muscle connective tissue (endomysium, perimysium, epimysium, fascia) is a continuous structure that invests throughout the entire muscle and continues beyond the muscle to form tendons (O'Brien 1997). This pervasive tissue is a force transmitter unlike muscle fibres which are force generators. It is sensible that tissues involved in the transmission of force have high innervation densities of afferent fibres. This may be reflective of the 'early warning' function of nociceptors. Secondly, fascial/epimysium tissue may be particularly vulnerable during eccentric lengthening (see section 3.2) which occurs commonly in everyday activities. Again, highly developed afferent feedback may be important in minimising injury.

3.2 Hypertonic saline pain during DOS

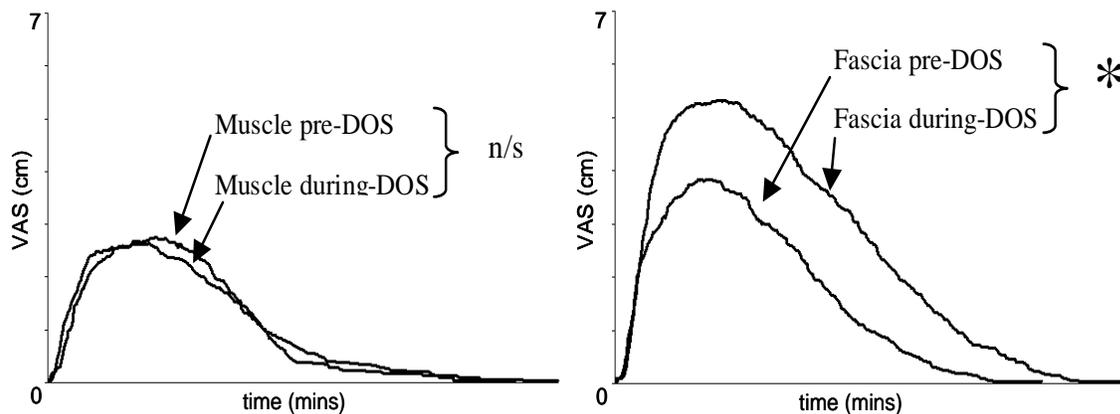
Study II demonstrated tendon and tendon-bone tissue cause significantly greater pain following saline injection than muscle belly under control conditions. Interestingly, despite tendon-bone and muscle being mechanically sensitised during DOS; repeat injections of saline in the presence of DOS showed no significant difference compared to baseline (pre eccentric exercise) conditions in response to saline (see Fig. 9).

Fig. 9: Average VAS profiles following muscle belly and tendon-bone junction hypertonic saline injection pre and during DOS. The lack of a facilitated pain response at these sensitised sites is noteworthy. (n=13). Data taken from study III.



As suggested previously, this may be reflective of a DOS induced afferent saturation (Marqueste et al. 2004). Due to this ‘saturation’ the thin fibre afferents putatively sensitised by DOS may be unable to respond to hypertonic saline with additional excitation. Therefore using this line of reasoning, hypertonic saline stimulation of the DOS affected afferents is unlikely to be reflective or comparable with results obtained during control conditions. However, by increasing the specificity of assessment site (see Fig. 8) and the tissue injected, study III demonstrated facilitated response to hypertonic saline in DOS affected tissues (see Fig. 10).

Fig. 10: Average VAS profiles (n=13) following hypertonic saline injection targeted toward muscle belly and fascial tissues. Profiles represent pain perceived in response to saline injection pre and during DOS. (*) Significantly increased pain intensity at fascia level during DOS. Data taken from study III.

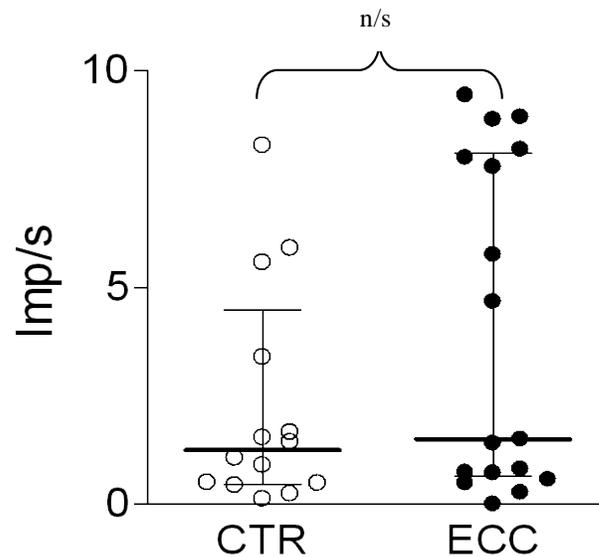


In this study, DOS was induced, the site of maximum mechanical sensitivity established and then selective injection of hypertonic saline targeting fascia/epimysium and muscle belly was undertaken. The animal component of this study examined the response of hypertonic saline superfused to the epimysium and muscle tissue of extensor digitorum longus (EDL) muscle in rats 48 hours post eccentric lengthening. One of the reasons previous studies may not have found changes in response to hypertonic saline injected to the muscle tissue pre and during DOS may have been due to not establishing and subsequently injecting the site most mechanically sensitised by the DOS. However, the human component of study III disputes this reasoning by establishing that despite injecting into the area of deep muscle tissue displaying the most mechanical sensitisation, there was no significant response difference between pre and during DOS (see Fig. 11). These injections were performed at the most sensitive point of the muscle during DOS and were therefore considered to be sites of maximal DOS manifestation. Notably though, injection of hypertonic saline directed toward fascia/epimysium at this maximal mechanically sensitised site of the DOS affected muscle *was* found to be significantly more painful than the same injection to the same tissue at the corresponding site of the contralateral unexercised leg. The implication of this is that fascial/epimysium connective tissue is preferentially sensitised by DOS subsequent to eccentric exercise.

However, this suspected sensitisation of peripheral fascial nociceptors may not fully explain the whole situation during DOS. The animal component of study III found no

significant difference between eccentrically exercised and control muscles when hypertonic saline was superfused to the identified receptive fields in a muscle-nerve preparation (see Fig. 11).

Fig. 11: Average afferent impulses per second recorded from an unexercised rat EDL muscle and an eccentrically lengthened, mechanically sensitised rat EDL muscle following exposure to hypertonic saline. Control (CTR) group n=14; eccentric (ECC) group n=17. Data taken from animal component of study III.



This may be explained by a number of factors. Firstly, rat EDL does not have a thickened epimysium forming a distinct fascia like the human tibialis anterior. Secondly, eccentric protocols in animals by definition are not voluntary, unlike human eccentric exercise. This means it is not possible to completely rule out some degree of muscle fibre tearing during the eccentric protocol potentially changing the nature of the induced sensitivity compared to the human component of the study. Thirdly, it is possible that some degree of afferent saturation (Marqueste et al. 2004) as previously discussed, may be involved thereby masking responses to hypertonic saline. All of these factors could potentially influence the response to superfusion of hypertonic saline to the muscle/epimysium. But of more interest, it may be that the exclusively peripheral nature of the muscle-nerve preparation does not allow for assessment of involvement of central processes during DOS. Even if hypertonic saline *does* induce an ‘all or nothing’ response in nociceptive

afferents and thus not be ideal for assessing peripheral sensitisation (Hoheisel et al. 2005), the acknowledged involvement of central pain processes during DOS (Bajaj et al. 2000) could result in increased pain being perceived to the potentially unchanged level of afferent input (compared to control injections) from the peripheral nociceptors following hypertonic saline injection. In effect, if fascial/epimysium tissue is sensitised by the eccentric exercise, the ongoing nociceptive activity from this tissue could sensitise the central (dorsal horn) neurones they communicate with. The result is that increased pain will be perceived to afferent stimuli. Thus, whether the perceived pain is reflective of central or peripheral 'increased pain gain' induced by fascial/epimysium sensitisation, it can be argued that the original source of the sensitising activity lies with fascial/connective tissue.

A selective DOS event in fascial/connective tissue may be explained by the manner in which eccentric damage/adaptation is thought to occur. Eccentric damage/adaptation occurs during the longer phase (longer muscle length) of the muscles length/tension relationship (Jones et al. 1997; Morgan and Proske 2004; Bowers et al. 2004). If, during the eccentric exercise, the muscle becomes inefficient at longer lengths due to sarcomere disruption, then connective tissue may be placed under increasing strain. It has been shown that absorbed energy of the musculotendinous unit during strain is significantly higher when the muscle is active as opposed to silent (Nikolaou et al. 1987), which, put simply, means that active, patent muscles are better able to deal with strain compared to muscles becoming inefficient and partially inactive (due to sarcomere disruption). Thus, during eccentric exercise, connective tissue may become vulnerable to excess strain forces if the muscle activity is impaired as myofibrillar disruption occurs. If this happens, it may induce the eccentric DOS effect in the fascia/epimysium connective tissue exposed to the increased strain/stress with the result that fascial/epimysium connective tissue nociceptive afferents become sensitised. This explanation fits nicely with the decrease in DOS perception seen following repeated bouts of eccentric exercise. Chronic adaptation to eccentric exercise has been shown in the form of sarcomerogenesis (Butterfield et al. 2005) and persistent shifts in the muscle length/tension relationship towards longer lengths (Prasartwuth et al. 2006). In turn, for the muscle, this would result in a greater

ability to perform work at longer lengths, resulting in stress shielding of fascia/connective tissue and subsequently less DOS induced/perceived.

3.3 Glutamate and Capsaicin induced pain

Functional metabotropic/ionotropic glutamate receptors in tendon tissue were implied by the results of study IV. Also, capsaicin caused moderate to high levels of pain in tendon tissue. This was despite the dose being 10 times (5 μ g) less than that used in previous muscle pain models (50 μ g) (Graven-Nielsen et al. 2002) to produce similar peak pain levels. This large difference in dose may be reflective of a higher TRPV1 afferent density population in tendon compared to muscle. Alternatively it may be as a result of the injected bolus forming a depot around the tissue, resulting in temporal summation. Either way, it points to TRPV1 receptors being functional in human tendon tissue and therefore the role of these receptors in clinical tendon pain should be further investigated.

The facilitated pain response of capsaicin injected immediately after glutamate compared to capsaicin injected alone confirms the findings of Wang et al. (2006) who demonstrated significant increases in pain from a capsaicin injection post glutamate injection compared to a capsaicin injection post isotonic saline injection. This may be due to sensitisation of peripheral receptors by glutamate. By using nociceptive reflex induced jaw muscle activity as a measure, it was found that pre-injection of a NMDA antagonist reduced nociceptive response to capsaicin injection in rat temporomandibular joint when compared to placebo pre-injection (Lam et al. 2005). Further, intravenous infusion of an AMPA/KA antagonist was found to reduce pain and hyperalgesia (compared to placebo infusion) caused by injection of intra-dermal capsaicin (Sang et al. 1998). Thus, there is interaction between TRPV1, NMDA and AMPA/KA receptors. Confirming this in human tendon tissue raises the possibility of developing appropriate receptor antagonist therapies which could reduce pain mediated not only via peripheral glutamate but also TRPV1 receptors.

4. Referred pain

Referred pain is a common clinical occurrence. Similarly in experimental pain studies it is frequently observed (Laursen et al. 1997;Graven-Nielsen et al. 1997a). A number of theories have been proposed attempting to explain this phenomenon. Evidence is mounting for a process by which central dorsal horn neurones, with time, become sensitised by peripheral noxious input and activate latent synaptic connections with surrounding neurones in the dorsal horn (Hoheisel et al. 1993) forming, in animals, new neuronal receptive fields and in humans the perception of referred pain (Graven-Nielsen 2006). Injection of bradykinin (causing activity in nociceptive afferent fibres) to rat muscle caused new receptive fields of dorsal horn neurons to appear, distinct from and outwith the boundaries of the original receptive fields mapped prior to nociception induced by bradykinin (Hoheisel et al. 1993). This has been suggested to be evidence of unmasking of latent synaptic connections in the dorsal horn between previously inactive (now active) synapses and neighbouring neurones. Further support for this ‘facilitation’ or ‘unmasking’ theory suggested to form the basis of referred pain is again provided by animal studies. Induction of an experimental myositis in rat gastrocnemius-soleus (GS) muscle had the effect of significantly increasing the population and spinal segmental distribution of dorsal horn-neurones excited by GS A-fibre stimulation (Hoheisel et al. 1994). The most logical explanation of this is activation of a previously silent network of widely distributed synaptic connections. In humans this may lead to referred pain perception as a result of the excitation of the neurones or misinterpretation of normal afferent activity in this sensitised, newly opened, expanded dorsal horn network (Graven-Nielsen 2006). It is apparent that time is likely to be an important factor in this process. A certain length of time of nociceptive activity/pain seems to be required to facilitate pain perception in humans (Graven-Nielsen et al. 1997c) and for the development of new receptive fields in animals (Hoheisel et al. 1993;Hoheisel et al. 1994). Study I confirmed the suspected importance of time in this proposed referred pain process. Tonic stimulation as compared to one-off stimulation (stimulation to referred pain appearance or subject tolerance) caused enlarged pain areas and more frequent perception of referred pain. This highlights the time taken for the peripheral input to make or open latent synaptic connections (see Fig. 12) if this is indeed the mechanism in operation behind referred pain.

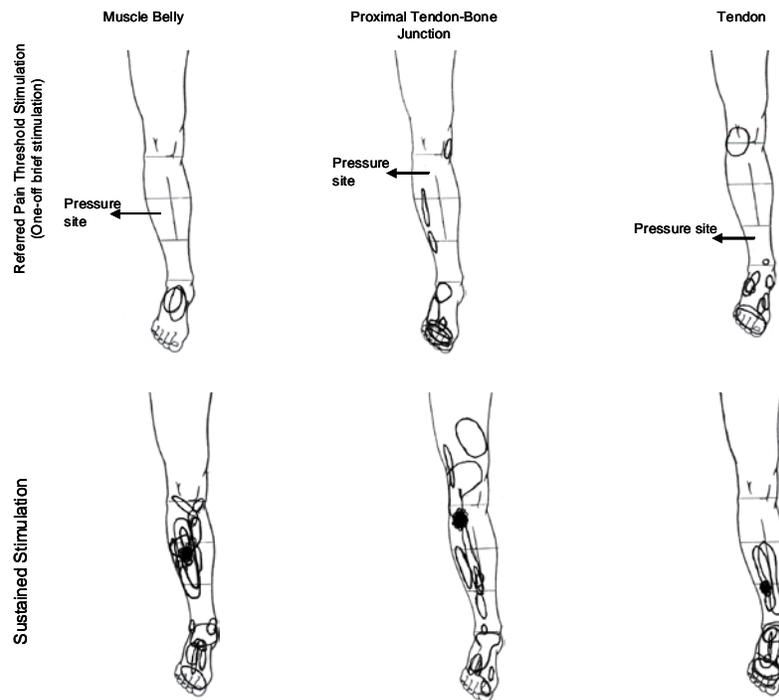


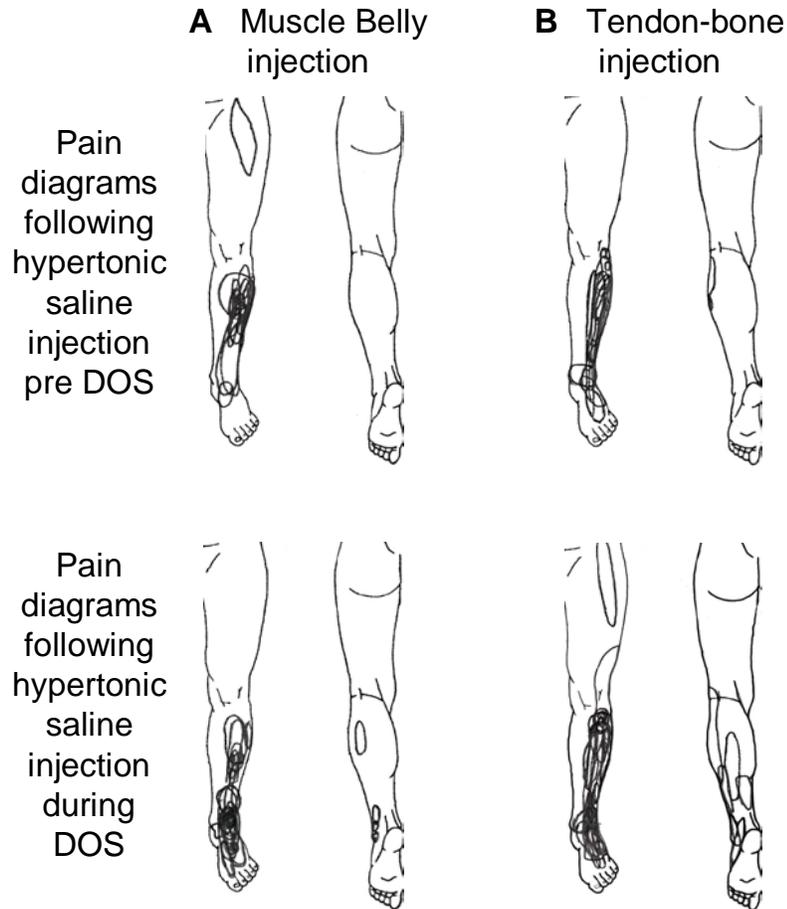
Fig12: Referred pain diagrams illustrating the temporal requirement of referred pain mechanism. Top row: Referred pain diagrams following a one-off brief mechanical stimulation. Bottom row: Referred and local pain diagrams following sustained mechanical stimulation. Data taken from study I.

Studies I, II and IV demonstrated that tendinous structures do exhibit referred pain with varying frequency (but roughly in line with muscle belly frequency). Referred pain is generally associated with higher pain intensities (Laursen et al. 1997;Graven-Nielsen et al. 1997b;Graven-Nielsen et al. 2000) which was observed at these sites in studies I and II. In study III, fascial tissue was shown to exhibit referred pain, however despite significantly higher pain intensities not to the same extent as muscle tissue. This may simply reflect fascial tissue nociceptive characteristics and is in line with previous findings (Kellgren J.H. 1938).

The finding of increased referred pain and enlarged pain areas during DOS to stimuli that was no more painful than pre DOS (study II) may be seen as providing further (indirect) support to the notion of referred pain being dependent on a central sensitisation process

opening latent synaptic networks/connections in the dorsal horn. As already stated there is an acknowledged role of central sensitisation during DOS (Bajaj et al. 2000;Barlas et al. 2000;Weerakkody et al. 2001;Weerakkody et al. 2003). Therefore, the increased referred pain response to hypertonic saline injection (with induced pain of no greater intensity than pre DOS) could have been a result of the previous ongoing (approximately 12 hours) nociceptive activity due to the DOS which may have caused central sensitisation and facilitated opening of latent neuronal connections to a far greater extent than the brief afferent barrage supplied by hypertonic saline injected in a non-DOS affected muscle. In study II, tendon-bone and muscle belly sites were shown to be affected by DOS (hyperalgesia). During DOS at these sites, hypertonic saline injection (despite being of similar intensity as control conditions) induced significantly higher frequency of referred pain (muscle belly) and significantly greater areas of enlarged pain (tendon-bone) compared to unexercised conditions (see Figure 13).

Fig 13: Top row shows pain areas following hypertonic saline injection to muscle (n=13) and tendon-bone (n=11) tissue in a control state. Bottom row shows the same injection to the same tissue during DOS. Note the areas of local pain (mid and proximal muscle areas) and the increased frequency of mapping of pain in the 'muscle belly' group at the anterior ankle area (typical referred pain area of tibialis anterior) compared to the pre DOS areas. During DOS, injection of tendon-bone tissue caused much greater area of pain including to areas not normally seen (posterior leg) with injection of this area. Data taken from study II.



This has not been shown before. Presently, increased referred or enlarged pain areas have been correlated with higher pain intensities (Graven-Nielsen et al. 2000). Potentially this mechanism could also apply to chronic, centrally sensitised patients (fibromyalgia, whiplash etc.) in which an acute stimulus of comparatively low intensity might lead to more widespread pain compared to a healthy control. Early results suggest this is in fact the case with chronic whiplash sufferers displaying greater areas of referred pain than healthy controls despite the induced pain being perceived at the same intensity (Kosek et al. 2006).

5. Summary

This PhD study investigated the sensory manifestations of tendinous, fascial and muscle tissues in relation to experimental pain. It has been established that sensitivity to

hypertonic saline in fascial and tendinous structures is higher than in muscle tissue (studies I, II and III). This is clinically relevant as it implies that the contribution of tendinous and fascial structures to the clinically prevalent entity 'muscle pain' needs to be carefully considered. Increased mechanical sensitivity at tendinous sites during experimental pain (saline, glutamate and capsaicin) is probably not the result of peripheral sensitisation (studies I and IV), instead a central summation between induced pain and mechanical stimuli may occur, leading to the perception of lowered mechanical pain thresholds. This may be important as chronic tendinopathic pain patients are likely to display central sensitisation of pain processing mechanisms and as such the commonly felt mechanical sensitivity in these conditions may be heavily influenced by central mechanisms. When the lack of an inflammatory process in these patients is considered (with possible subsequent lack of peripheral sensitising substances), this central mechanism of mechanosensitivity may be important and worthwhile targeting in future pain management strategies. Purely tendon structures are probably not sensitised by acute induction of DOS (study III). However, it was demonstrated that fascial/epimysium connective tissue does display increased sensitivity during the sensitised conditions of DOS and may be the main site of eccentric exercise induced DOS effect. This could give an insight into the damaging effects of repeated eccentric exercise which suggests that connective tissue may be exposed to excessive stress under these contraction conditions (thus becoming sensitised) when the myofibrillar contractile mechanism becomes inefficient. Further, in DOS sensitised conditions referred pain may be more frequently appreciated (compared to pre DOS) in response to stimuli of similar pain intensity as that induced pre DOS (study II). Lastly, functional glutamate and TRPV1 receptors are shown to be functional in tendon tissue (study IV) and there appears to be some modulation of response between these receptor types. This points to a need to investigate these receptors in clinical tendon pain. It is currently unknown (but certainly worth investigating) if modulation of activity in both these receptor types can be achieved by development of antagonists targeting NMDA receptors in clinical situations. This PhD project has illuminated some of the mechanisms and characteristics of pain in human tendon, fascial and muscle tissue. The results may have particular implications for treatment and

Fig 14: Eccentric exercise may be involved in the development of tendon/connective tissue injury secondary to a possible preferential effect of eccentric exercise in this tissue (subsequent to time and chronic repetition). Given time and continuation of the aggravating activity, clinical tendon pain may result. Potential treatments may include eccentric training to condition the muscle not to become disrupted at the level of the sarcomere in subsequent bouts of eccentric activity thereby protecting the connective tissue and tendon. Pain management strategies in future may look to the targeting of specific peripheral receptors such as NMDA and TRPV1 if future research confirms they do play a role in clinical tendon pain. Referred pain/widespread pain and central sensitisation may be closely related in patients who have chronic pain. Methods of allaying or avoiding this central sensitisation facilitation of referred pain may be necessary to treat the widespread pain commonly seen in chronic pain patients.

Future perspectives

This series of studies has elucidated some of the pain mechanisms behind fascial and tendon pain. The next step is to investigate the interaction between experimental tendon pain and motor control. Many common musculoskeletal complaints display alterations in motor control of surrounding musculature compared to healthy subjects: patellofemoral pain (Cowan et al. 2001), groin pain (Cowan et al. 2004) and low back pain (O'Sullivan et al. 1997) being just a few examples. There is however, no current information regarding tendinopathy and motor control. It is very important to investigate this as physiotherapists routinely prescribe strength training for tendinopathy patients and it is important to know what the motor effect of pain in these structures is. Additionally it would be very interesting to know if, and to what extent these possible motor control deficits during tendon pain can be reversed by removal of the pain. It may be that motor control alterations are immediately reversible following pain cessation or they may persist and require prolonged re-training. At present this is unknown and deserves to be investigated.

Eccentric exercise appears to be a key factor in tendinopathy. It has also become a mainstay of conservative treatment for tendinopathy and does seem to relieve pain although it is yet unknown as to how the pain reduction occurs. A mechanical effect on the neovessels (strongly associated with tendon pain) by the ongoing eccentric exercise has been proposed (Alfredson and Lorentzon 2003) but this seems unconvincing given how frequently eccentric activity occurs in everyday life without any apparent effect on

tendinopathic neovessels. Flowing from this current series of studies is the proposal that connective tissue is sensitised following eccentric exercise and that it is therefore exposed to excessive stress during eccentric exercise. It is worth investigating if the conservative eccentric training programmes simply allow the muscle to adapt to eccentric stresses by sarcomerogenesis with subsequent greater ability to maintain contractile efficiency during eccentric activity, thus affording connective tissue some degree of 'stress shielding' and healing in the tendon to occur.

Lastly, the effect of peripheral NMDA and TRPV1 receptor antagonists should be assessed in clinical tendon pain patients. If this can reduce perceived pain or mechanosensitivity in tendinopathic patients then further investigation of modulatory action between the two receptor types may be worth exploring as an avenue of future clinical pain management strategies for these patients.

Dansk sammenfatning

I dette Ph.D.-studie undersøges de sensoriske symptomer relateret til eksperimentel smerte for sene-, fascial og muskelvæv. Det er blevet fastslået, at følsomheden overfor hypertont saltvandsopløsning er større i senevævsstrukturer end i muskelvæv (studie I, II og III). Dette er klinisk relevant, da det tyder på, at bidraget fra senevævs- og fascialstrukturer til den klinisk udbredte term ”muskelsmerte” bør undersøges grundigt. Forøget mekanisk følsomhed i senevæv ved eksperimentel smerte (saltvandsopløsning, glutamin og capsaicin) er muligvis ikke resultatet af periferisk følsomhed (studie I og IV), men i stedet kan der forekomme en central summering mellem den påførte smerte og de mekaniske stimuli, der fører til en lavere mekanisk smertetærskel. Dette kan være vigtigt, da kroniske smertepatienter sandsynligvis er disponeret for at udvise en central smertefølsomhed overfor smertebehandlingsmekanismer, og derfor bliver den normale mekaniske følsomhed under disse forhold som regel stærkt påvirket af de centrale mekanismer. Samtidig kan der konstateres en manglende inflammatorisk proces i disse patienter (med mulig efterfølgende mangel på de periferiske følsomhedsstoffer), og derfor bør den centrale mekanisme for mekanisk følsomhed undersøges nærmere i fremtidige smertestyringsstrategier. Senestrukturer i sig selv er muligvis ikke følsomme overfor akut induktion af DOS (studie III). Derimod er det blevet demonstreret, at seneforbindende væv viser en forøget følsomhed under sensitive forhold af DOS og må derfor betragtes som stedet, hvor DOS-effekten viser sig ved gentagne excentriske øvelser. Dette kan give et indblik i den ødelæggende effekt ved gentagne excentriske øvelser, der kan betyde, at det forbindende væv må være udsat for et unormalt stort stressniveau under kontraktion, (hvilket bevirker, at det bliver sensitivt), når de myofibrillare kontraktionsmekanismer bliver ineffektive. Desuden er refereret smerte ofte mere anerkendt for DOS-konditioner (sammenlignet med før DOS) i relation til stimuli af tilsvarende smerteintensitet som den før-DOS-inducerede (studie II). Slutteligt er det påvist, at funktionelt glutamin og TRPV1-receptorer er funktionsdygtige i senevæv (studie IV), og der forekommer reaktionsmoduleringer mellem disse receptortyper. Derfor bør disse receptorer undersøges i klinisk senevævssmerte. På nuværende tidspunkt er det ikke påvist, (men det er bestemt værd at undersøge), om aktivitetsmodulation i begge disse receptortyper kan opnås ved udvikling af antagonist

rettet mod NMDA-receptorer i kliniske situationer. Denne Ph.D. afhandling belyser nogle af de mekanismer og karakteristika for smerte i menneskets sene-, fascial- og muskelvæv. Resultaterne har speciel betydning for behandling og mulig forebyggelse af senevævssmerter.

Appendix 1:

Review of research papers investigating mechanosensitivity and referred pain patterns in muscle, ligament, joint/joint associated structures and tendon/fascia following injection of hypertonic saline. All mechanosensitivity tests were assessed in the vicinity of the injection site and all were assessed during pain.

Table 1

Tissue investigated	Reference(s)	Local mechanical sensitivity during pain	Referred pain established
Muscle: Temporalis, tibialis anterior, trapezius, extensor carpi radialis brevis, triceps surae. Generally placebo controlled except (*)	Jensen and Norup (1992); Graven-Nielsen et al. (1998); Graven-Nielsen et al. (1998a); Ge et al. (2003); Graven-Nielsen et al. (2003); Slater et al. (2003); Weerakkody et al. (2003)*	Quantitative testing supplying ambiguous results: Hyperalgesic (Jensen and Norup, Graven-Nielsen et al. 1998), hypoalgesic (Slater et al.) and unchanged (Graven-Nielsen et al. 1998a, Ge et al., Weerakkody et al.) sensitivity reported.	Consistent and well defined referred pain patterns described during experimental muscle pain.
Joint and associated structures: Knee joint, Acromioclavicular joint, subacromial space, sternoclavicular joint, infrapatellar fat pad. No placebo control	Steinbrocker et al. (1953); Gerber et al. (1998); Hassett and Barnsley (2001); Bennell et al. (2004).	No deep tissue quantitative assessment. Bennell et al. utilized Von Frey filaments to assess superficial sensibility (no change). Gerber et al. and Steinbrocker et al. reported subjective 'tenderness' during pain.	Referred pain frequently seen following experimental painful stimulation of joint.
Tendon and fascia (references marked with '*' investigated both tendon and fascia): Tibialis anterior, biceps distal tendon, tendo-Achilles, common forearm extensor tendon origin (CEO). No placebo control	Kellgren (1938)*; Inman and Saunders (1944)*; Steinbrocker et al. (1953); Slater et al. (2003).	Slater et al. found no change in sensitivity at common extensor tendon without placebo trial. All others did not supply quantitative data.	Ambiguous results for tendon: local (Kellgren, Steinbrocker et al.) and referred (Slater et al.) pain patterns reported. Fascia described as producing predominantly local pain.
Ligament: Cervical, thoracic and lumbar interspinous ligaments. No placebo control.	Kellgren (1939); Lewis and Kellgren (1939); Inman and Saunders (1944); Sinclair et al. (1948); Hockaday and Whitty (1967).	No quantitative data. Varying reports of subjective 'hyperalgesia' locally and distant.	Well defined reproducible pain reproduction from interspinous ligaments.

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