AN INVESTIGATION INTO STRAIN WITHIN THE PATELLAR TENDON

by

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ABSTRACT

Tendon injuries have, for many years, frustrated clinicians and patients alike due to their longevity and resistance to therapy. In recent years there has been good response in the extensor tendons of the lower limb to an intense painful eccentric exercise protocol. As yet there is no established reason known why a tendon should develop degeneration within its structure or why it should respond to the eccentric exercises. We do however know that, like bone, tendons are biologically active and rapidly adapt to the mechanical environment to which they are exposed. Recent investigations have revealed that within a tendon such as the Achilles or the patellar tendon there may be regions that experience different strains to the rest of the tendon. Much of this work has been in vitro and an ultimate goal would be the development of a non-invasive method by which intra-tendinous strain might be measured.

The basis of this thesis is the validation of an existing grey-scale speckle pattern matching software programme developed for tracking motion through serial ultrasound images. Through in vitro and in vivo work we have developed its use for tracking the unique type of speckle found in tendons. By verifying, in vitro, that the displacements tracked in phantoms and tendons alike are representative of reality we provide confidence in the use of an exciting tool for measuring tendon motion in vivo. Furthermore, we have established the method by which the tracking can be adapted to accurately represent tendon strain in vitro which again provides assurance for its reliability when applied to examine tendon strain in vivo.

The methods of data collection and analysis developed in this study provide the foundations for an exciting avenue of research into tendon biomechanics.

In Memory

MUM AND DAD

You encouraged me to start this journey and your memory has inspired me through both difficult and good times

‘Haply I think on thee and then my state

Like to the lark at break of day, arising

From sullen earth, sings hymns at heaven’s gate;

For thy sweet love remember’d such wealth brings

That then I scorn to change my state with kings’

(William Shakespeare)

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BACKGROUND TO RESEARCH

INTRODUCTION

Over the last 2 to 3 decades there has been a marked increase in the number of people turning to sport and in particular running as a form of recreation. Concurrent with this has been an increasing number of people presenting to sports injury clinics with tendon injuries. Damage to these structures is frustrating for both the injured party and the clinician alike due to their longevity and unpredictable response to treatment. Despite much time and effort from a variety of research backgrounds there is, as yet, no definitive aetiology or management protocol.

It is the intention of this work to examine the available literature to find what is known about tendon disease and has been confirmed with effective research and what is still unknown including 'facts' that may be nothing more than medical folklore. In so doing it is possible to find 'gaps' in the knowledge that can lead to a better understanding of the condition as a whole.

One such 'gap' is the understanding of mechanics of movement within the tendon as a whole, in vivo. Recent research in vitro has led to an acceptance that there is differential movement within some tendons. Use of Real time Ultra Sound Scanning (U/S) to examine tendon movement under load has allowed workers to evaluate the mechanical properties of the tendon, as a complete structure, in vivo. By combining the latter technique and using new pattern matching software to analyse the resultant U/S cine footage it is hoped to show the former differential movement and how that may vary depending on the individual, the load and the angle of the joint when the work is applied. It is further hoped that it will be possible to show that this differential movement is of extreme importance due to the large shears placed upon the tissues by the differential intra-tendinous movement when under functional load.

ANATOMY

Tendons are a regular dense connective tissue which attaches muscle to bone in order to form the myotendinous unit. While extremely strong and stiff in the direction of muscle pull they retain sufficient flexibility to cope with movement in other planes. They are of different sizes and shapes dependant largely on function but generally are either flattened (named aponeurosis), or rounded and of a length dictated, in the main, by the function of the muscle to which they are attached: e.g. shorter fatter tendons for the more powerful muscles such as the quadriceps or longer and slimmer in those units where space is at a premium and precision is necessary such as the finger flexors(Kannus 2000). As a rule the flexor tendons will be round or oval and the extensors more flattened (Benjamin and others 2008). Grossly they are white and glistening due in part to the low vascularity(O'Brien 1992) and to the high concentrations of collagen within the matrix(Junqueira and Carneiro 1980).

The cells of tendons, tenoblasts and tenocytes have parallel orientation to the collagen fibres, their cytoplasm in turn nearly enveloping them. The cells produce energy, the proteins of the collagen and the substance of the matrix. The fibroblasts/tenoblasts of tendons contain actin and myosin which may be linked to some of the physiological properties of the tendon(Ippolito and others 1977). It is argued that an active contraction-relaxation mechanism may be able to work as a shock absorber in the case of sudden or forceful contractions of the associated muscle. A further possible explanation for the presence of alpha-smooth muscle actin may be involvement in the maintenance of the normal architecture or 'crimp'(Murray and Spector 1999), while yet another possibility is to allow contraction/movement of the cells where there is injury and/or repair(Torres and others 2000).

The parallel fibres within the tendons are made up primarily of collagen (80-90% of the total dry mass) and elastin (2%). The elastin, like actin, may contribute to recovery of the crimp in the tissue after stretch(Butler and others 1978). These fibres are part of a ground substance or extracellular matrix (ECM) which fills the spaces between the cells. The ECM consists of water and proteins such as glycosaminoglycans (GAGs), proteoglycans (PGs) and other components which bind the water to form a hydrophilic gel(Junqueira and Carneiro 1980; Kannus 2000;

Kjaer 2004). The most abundant PGs are decorin and cartilage oligometric matrix protein (COMP). The bound water molecules and proteins provide a structure which is both supportive and facilitates the flow of nutrients to the fibres and cells. The PGs assist in fibril fusion(Kjaer 2004) and it appears that adaptations of the matrix contribute to the viscoelastic properties of connective tissues although the experimental evidence is not yet complete(Mosler and others 1985; Purslow and others 1998).

COLLAGEN

The smallest unit in the collagen fibre is the tropocollagen molecule, composed of three polypeptide chains bound into a triple helix to form the collagen molecule(Butler and others 1978). Various combinations of amino acids comprise the tropocollagen subunit and the particular combination defines the collagen type. Type is often associated with which area of the body is under examination and would appear to be influenced by the mechanical environment therein (Benjamin and Ralphs 1998). For tendons under longitudinal load the most commonly occurring collagen type or combination of amino acids is Type I. This consists of two alpha-1 and one alpha-2 left-handed helical chains combined in a right-handed super-helical pattern bound with hydrogen bonds. (See Figure 1)

The whole tendon is a highly organised hierarchical structure: fibrils are comprised of tropocollagen subunits arranged in a quarter stagger, displaced successively about the origin and exhibiting a repeated periodicity of 67 nm. Numbers of fibrils are further united to form collagen fibre, which is visible under the light microscope and at this point a regular crimping may be observed via polarised light(Diamant and others 1972; Gathercole and Keller 1991). The degree of crimp is not always identical and may vary not just between different tendons but also within each tendon. It was found to vary with the age of the animal examined and this was linked with the extensibility of the structure.

The average diameter of the fibrils will increase with age in the early months of life(Friedman and others 1975) and also with function. There has been found to be an increase in the numbers of smaller diameter fibrils in a stress shielded tendon (Majima and others 2003) or in the case of one that has been injured. Areas immediately adjacent to the rupture of an Achilles tendon

showed fewer large diameter fibrils both in the core and the periphery(Magnusson and others 2002). It is useful to note here that the helical formation seen in the tropocollagen molecule is further seen in the fibrils to form miniature ropes(Bozec and others 2007).

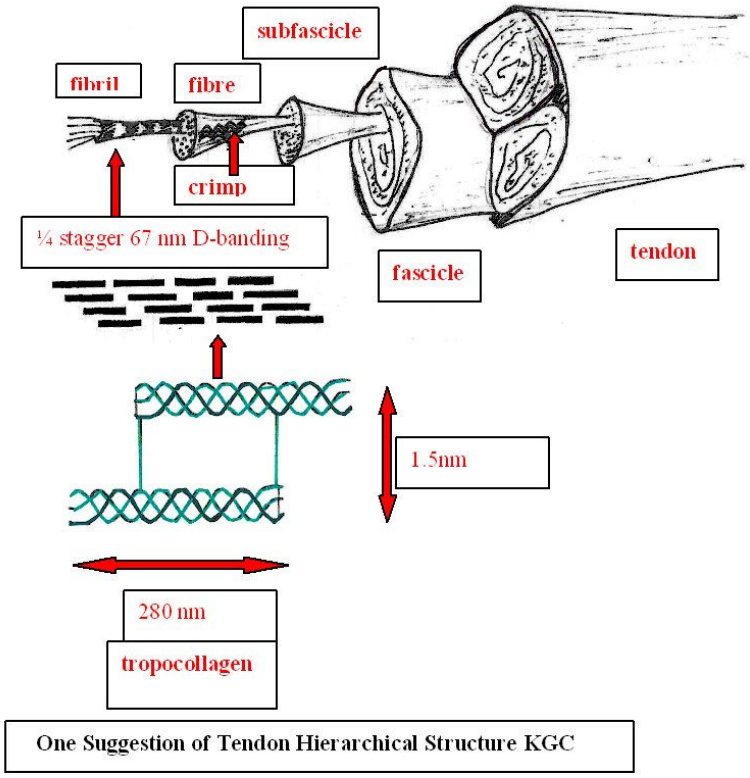


Figure 1 Diagram of tendon hierarchy

The fibres of round tendons are mostly orientated longitudinally which has been suggested to be a reaction to the angle of the stress under which they are placed(Josza and Kannus 1997). There is not yet conclusive evidence to confirm this in the literature, however, new techniques, using spectroscopy and loading of gel matrices are indicative (Kostyuk and Brown 2004). It has been noted that some fibres can be situated perpendicular to the line of action of the tendon and it is thought that this may protect the tissue from damage resulting from horizontal and rotational forces occurring in normal activity(Kannus 2000).

Where there is differential loading within the same tendon the collagen type and tendon structure will alter dependant on whether the load applied is compressive or tensile (Benjamin and Ralphs 1998). This is observed in tendons that work around a joint or pulley, such as the tendon of extensor hallucis longus which grooves in the sustentaculum tali. Here the tissue is held in one position at all times, and those parts in contact with the bone experience a compressive load rather than tension as in the rest of the tendon. Where there is compression there is fibro-cartilage and type II collagen and where there is tension there is type I collagen which is more commonly associated with tendons. Changing the loading, in vivo, can change the collagen from type I to II and back again when the loads are reversed. It is not known why such changes are seen but it has been suggested that it may be mechanical, in order to prevent the fibres splaying under the compression, or to control the swelling of large proteoglycans in order to make the structure tolerant to pressure (Benjamin and Ralphs 1998).

Further into the macroscopic scale within the tendon, numbers of fibres are further united to form sub-fascicles or the primary fibre bundle, surrounded by a layer of loose connective tissue, the endo-tendon. Numbers of these are further bound in endo-tendon to make up the secondary bundles or fascicles which in turn bind together to form the tertiary bundles. Finally the whole mass is bound within the epitendon to form the final structure(Kannus 2000; Sisson and Grossman 1975). It is of interest that some tendons show convergence of the fascicles towards their bony attachments(Fallon and others 2002) which would allow for the application of power by a number of muscle fibres on a relatively small area of tendon. Fascicles can slide independently of one another(Fallon and others 2002) and it has been suggested that this sliding may not wholly be limited to inter-fascicular level but may also be seen between the fibrils(Screen and others 2004). Interactions within the tendon under loading will be further explored in the mechanical properties section.

It is worth noting that this is one possible classification and different authors have used a variety of ways of classifying this structure. When reading the literature, it is important to be aware of which level within the hierarchy the author in question is referring.

Surrounding some tendons are external sheaths of varying type depending on the tendon. The paratenon and synovial sheaths are loose connective tissues surrounding the tendon structure which produce fluid to ease and lubricate the movement of the structure and vascularisation here facilitates the supply of nutrients to the tissues.

VASCULARISATION

The blood supply to tendons and, in particular, to the extensor tendons of the lower limb is important to explore ,as lack of vascularity in specific areas has been proposed as a potential cause of degeneration. This has commonly been purported as a potential aetiology for disease in the Achilles tendon. The methods used to demonstrate regions of hypo-vascularity have been injections of dyes into the vessels of cadavers(Carr and Norris 1989; Lagergren and Lindholm 1959) and more recently the demonstration of high levels of lactate after exercise(Alfredson and others 2002). It has since been suggested that the use of dyes in cadavers to illustrate vascularity is not an valid method to demonstrate fully all the vessels within the area (Astrom and Westlin 1994b) and techniques such as Doppler flowmetry are better at visualising the blood supply both at rest and while exercising in vivo(Astrom 2000; Astrom and Westlin 1994a; Astrom and Westlin 1994b). This technique was used to show that the Achilles tendon was well perfused throughout its length in both normals and those affected by degeneration. In fact the injured tendons appeared to have elevated blood flow when compared to the normals(Astrom and Westlin 1994a)

The length of the Achilles tendon is supplied by vessels derived from the paratenon and originally from the posterior tibial artery(Theobald and others 2005). Vessels may also enter tendons within the endotenon and those within the tendinous tissue may well be small and thin walled(Benjamin and others 2008)

INNERVATION

Although there is little written about the innervation of tendons it has been suggested that generally nerves originate from nerve trunks within the associated muscles, peritendinous and cutaneous tissues(Josza and Kannus 1997). Some branches from the paratenon follow the epitendon within the body of the tendon and then anastomose with branches of nerves from the associated muscle.

There have been four major types of nerve endings associated with tendons:

1. Type I: Ruffini corpuscles; which are pressure sensors and note both static and dynamic changes in tension. These receptors will adapt slowly to a change.

2. Type II: Vater-Pacini corpuscles; again are pressure sensors but in this case there is rapid adaptation to a change, they note acceleration and deceleration movements and work as dynamic mechanoreceptors at the beginning and end of a movement.

3. Type III: Golgi tendon organs; tension receptors which signal position and respond to both active contraction of the associated muscle and to passive stretching, adapting slowly to changes.

4. Type IV: free nerve endings; considered to be pain receptors and are most commonly found in the peritendinous tissues.

Most of the mechanoreceptors appear to be found at the myo-tendinous junction or the tendinous insertion along with some of the free endings(Josza and others 1993). It has been suggested by Stillwell in his report in 1957(Stillwell 1957) that there are also an array of longitudinally arranged nerve trunks within the body of a tendon which give off free endings in all areas and arise both from the associated muscle and from other sources e.g. the paratenon or in the case of more superficial tendons cutaneous nerve trunks. It is a possibility that these endings are

associated with the perception of pain(Josza and Kannus 1997; Stillwell 1957). Freeman and Wyke(Freeman and Wyke 1967)further subdivided these Type IV endings into:

1. Unmyelinated receptor endings which probably are involved in the sensitivity to pain.
2. Unmyelinated efferent terminals which may be involved in vasomotor control, explaining their close proximity to the vessels.

There is recent evidence to show that damaged tendons may exhibit new neural in-growth alongside neo-vessels and that these appear to coincide with the painful area of the tissue(Alfredson and others 2003). There is some evidence to suggest that the nerves may be sympathetic in nature rather than sensory(Danielson and others 2008), although the study in question examined the vessels found dorsal to the tendon rather than within it.

THE PATHOLOGY OF TENDON DISEASE

GROSS PATHOLOGY

Macroscopically the tissues of tendons which have ruptured spontaneously appear to be grey in colour with a watery appearance compared to the glistening white surface of the uninjured intact tendon.

HISTOPATHOLOGY

1991 marked a watershed in the thinking behind the potential aetiology of chronic tendon injury. An examination of 891 spontaneously ruptured tendons which included Achilles, patellar, biceps brachii, EPL and other tendons, found that 97% were degenerate prior to injury. This compared to an age matched cadaveric control group of 445 individuals who had died of accidental causes where only 35% showed signs of degeneration(Kannus and Jozsa 1991). The authors' conclusions were that it was common to find evidence of degeneration within the tendons of people over 35 and that pre-existing pathology was almost always associated with any tendon rupture. A similar picture was noted in a comparative study looking at the histology of 28 patellar tendons awaiting surgical treatment for treatment of 'Jumper's Knee' or patellar tendinopathy and comparing them with 20 age matched controls(Khan and others 1996). All of

the tendinopathic tendons here exhibited mucoid degeneration with no inflammatory cells compared with 3 out of the 39 control tendons.

The histopathology of tissue harvested from ruptured tendons reveals a variety of different types of degeneration and is apparently linked to the area of the body from which the tissue is derived(Kannus and Jozsa 1991). Hypoxic degenerative tendinopathy is seen in 44% of cases. This can be defined as tissues showing 'altered size and shapes of the cell nuclei and mitochondria in the early stages and later hypoxic or lipid vacuoles and occasionally necrosis'. The ruptured tendons show evidence of oedema with swelling of the collagen fibres and there may be some splitting or fraying of the collagen. It is conceivable, however, that this swelling is a response to the rupture rather than a degenerative change preceding it. It has been suggested that exposed proteoglycans from within the matrix may be responsible for drawing fluid into the injured area(Khan and Cook 2000).

Of injured tissues 21% show evidence of mucoid degeneration where there are areas of mucus and 'vacuoles' containing granules which contain glycosaminoglycans. Remaining relatively common degenerative pathologies consist of tendolipomatosis where deposits of fat are laid down within the tissue, more often seen in the older tendon, and calcifying tendinopathy. This latter condition is most commonly seen in the older rotator cuff area especially the tendon of supraspinatus(Kannus and Jozsa 1991).

A variable picture may present in tendinosis and there may be evidence of microtears sometimes involving only a few fibres or sometimes bundles often with red blood cells and fibrin deposited in the area. All seem to have irregular vascularity with hyper-vascularised tissue and narrowing or obliteration of the new vessels by proliferating tenoblasts and collagen fibres(Jarvinen and others 1997b) It is suggested that this picture is indicative of healing within the tendon but the result may be granulation and finally scarring(Josza and Kannus 1997). It has been shown that a comparison of histopathology of tendinosis, ruptured tendons and normal tendons reveals a

similar picture of degeneration in both the tendinosis and the ruptured tissue with the most severe degeneration being seen in the latter(Tallon and others 2001).

Against the majority of publications, showing chronic tendon injury to be degenerate rather than inflammatory, one study of 60 patients undergoing repair of ruptured Achilles tendons(Cetti and others 2003) found neutrophils infiltrating the site. This sign of inflammation was found in cases operated on a mere 7 hours post-rupture and the evidence seemed to suggest that the severity of the inflammation diminished with time post-rupture. The conclusion here was that the inflammation was not second to the rupture itself but was allied to the degenerative process, supported by the fact that inflammatory severity paralleled the severity of the degeneration and necrosis. These findings were seen throughout the length of the affected tendon not just at the site of the rupture The authors of this study claim that cells previously thought by most other investigators to be dying tenocytes are in fact neutrophils. Most authors still work on the premise that tendinopathy is largely a degenerative condition rather than an inflammatory one. More recently a group, based in Denmark, who have used the above study by Cetti et al to support their use of glucocorticoids to treat tendon injuries with some initial success and have gone on to promote the inflammatory aetiology (Fredberg and others 2004; Koenig and others 2004). There is little in the way of histological evidence other than that of Cetti et al, however, to support this thinking. Tenocytes when exposed to cyclic strain increased their production of PGE2 and other potential mediators of inflammation and this has been proposed as further evidence of an inflammatory pathology(Fredberg and Ostgaard 2008) but the studies that have shown this(Almekinders and others 1993; Yang and others 2005) have had doubts raised as to their physiological relevance for the strain levels are way beyond those generally experienced in vivo(Arnoczky and others 2007).

HYPERVASCULARITY

A number of groups have recently explored the finding of neovascularity within tendinopathies with a view to finding treatments(Ohberg and Alfredson 2002) and exploring possible aetiology(Pufe and others 2005). It has been shown that tendinopathic tendons that present with

pain are more likely to have neovascularisation shown using Doppler U/S scans(Ohberg and others 2001) and there is evidence that tendons that have become asymptomatic after a 12 week course of eccentric calf exercises no longer have any neovessels which is suggestive that this factor may be relevant to the progress of the disease(Ohberg and Alfredson 2004). The relevance of neo-vascularity within tendinopathic tendons has been explored further in possible aetiologies of tendon disease where it has been suggested that neovessels may not only be a consequence of the injury but may in fact contribute to the mechanical and pathological changes seen(Pufe and others 2005). The rationale behind this being that vascular endothelial growth factor (VEGF) has been shown to be raised in degenerate Achilles tendons compared to the normal down regulation found in tendon tissue. VEGF can increase matrix metalloproteinases (MMP) and inhibit tissue inhibitors of MMP (TIMP) and thus cause breakdown of the matrix. Injection of VEGF within a rat tendon caused alteration of the material properties of that tendon reducing overall stiffness and vulnerability to rupture(Pufe and others 2005).

TERMINOLOGY OF TENDON DISEASE

Throughout the literature there have been many terms used to describe tendon disease and injuries, often interchangeably. This has led to confusion regarding the mechanisms of injury involved.

In 1998 Maffulli, Khan and Puddu(Maffulli and others 1998) helped to clarify the issue with their article; 'Overuse Tendon Conditions: Time to change a confusing terminology'. In this work they suggested that tendinopathy be used as the universal term to describe chronic injuries to tendons, often ascribed to overuse, and thereafter other terms should be used to describe conditions as appropriate to the pathology described.

Tendinitis should only be used when the condition is of inflammatory origin. This has not been reported in overuse conditions where the problem is seen to be degenerative, demonstrated in vivo using microdialysis(Alfredson and others 2001; Alfredson and others 2000) and on examination of biopsies of tissue as described previously(Jarvinen and others 1997b; Kannus and

Jozsa 1991; Tallon and others 2001).It is still possible that there may be, in the acute state, an initial inflammatory phase which progresses on to a tendinosis, but this has not been, as yet, confirmed by research. Maffulli et al suggest that referring to chronic tendon injuries as tendinitis leads to a misunderstanding of the problem, its treatment and outcome by practitioners and athletes alike(Maffulli and others 1998).

Tendinosis is the description of choice for the common degenerative condition seen in tendon overuse injury and was first suggested by Puddu in 1976(Puddu and others 1976). This may not necessarily be symptomatic but can be confirmed by histopathology and more recently has been described in views derived by MRI and U/S scanning. Areas of tendinosis (degeneration) are a fairly consistent finding in tendon rupture, often there are no symptoms prior to rupture. Investigation with histopathology reveals areas of lysis, loss of parallel orientation of the fibres, reduced fibre diameter and collagen density. There is often evidence of microtears where the damaged areas are seen to be surrounded by erythrocytes, fibrin and fibronectin deposits. Visualising the collagen fibres reveals irregular crimping and increased waviness(Jarvinen and others 1997b). If it is difficult to examine the tendon using ultrasound, MRI or biopsy it has been suggested that the most appropriate term to use in the case of chronic tendon injury is tendinopathy(Alfredson 2003). If further investigation reveals, either with imaging or biopsy, degeneration within the tissue then tendinosis may be applied. In the case of painful tendons showing signs of enlargement both on examination and with U/S scan but no hypoechogenicity these will be refered to as tendinopathic in this thesis.

Paratenonitis is a universal term used to include inflammation of the structures surrounding the tendon, peritendinitis, tenosynovitis and tendovaginitis. It presents with acute oedema and a fibrinous exudate often leading to crepitus. This inflammation may go on to spread and infiltrate the tendon tissue as well leading to limitation of the tendon mobility(Jarvinen and others 1997b).

TENDINOPATHY AS A DYNAMIC PROCESS

One of the latest publications from Australian workers has attempted to classify the tendons that we see clinically within a model for tendon pathology(Cook and Purdam 2008) . Their proposal is that not all tendons present with the same symptoms and clinical signs because they are at a different stage within the disease process rather than being a different overall pathology. Of more interest is their suggestion that within this continuum of disease a tendon can move backwards as well as forwards depending on its exposure to loading. It is this that explains why some tendinopathies respond differently to treatment and why there are those that may not have pain but appear to have existing pathology under imaging(Cook and others 2000).

The proposal of this model is that there are three main stages of tendinopathy:

REACTIVE TENDINOPATHY

This is proposed to be a proliferation of the cells and the matrix in order to adapt to sudden increase in load. Here there is a resulting increase in cross-sectional area which allows the tendon better to deal with the increased stress. There is no degeneration but there is observable thickening visible clinically and on imaging from increased fluid within the matrix. There may or may not be pain associated with the swelling. This may resolve if the load is reduced or there is increased recovery time between sessions.

TENDON DYSREPAIR

The picture is similar to the above but there is seen to be greater matrix breakdown and some change within the cellular population to include chondrocytic cells and myofibroblasts. There may also be an increased vascularity and neural ingrowth. The authors suggest that this is best detected when areas of hypoechogenicity are seen on imaging. Their suggestion is that at this stage there is still some reversibility possible with conservative measures i.e. management of training and prescription of exercises.

DEGENERATIVE TENDINOPATHY

Along with large changes to the matrix and effects to the cells including apoptosis there are said to be often large vascular and neuronal ingrowths. This is commonly the middle aged athlete

who presents with repeated bouts of tendon pain and proliferation that may resolve and then recur with changes in load. It is these authors premise that these tendon will never fully resolve. It is these tendons that, they suggest, if the pathology or loading is great enough may rupture(Kannus and Jozsa 1991). It is interesting to remember at this point that this study noted that only 30% of the ruptured group had any preceding symptoms such as pain or swelling. Does this suggest that 70% of the ruptured tendons were not at the final degenerate stage of the tendinopathy or are pain and enlargement not always correlated with the end stage in the cycle?

Cook and Purdam (Cook and Purdam 2008)have used as a basis for this continuum model that proposed for osteoarthritis by Pollard, Gwilym and Carr(Pollard and others 2008). Can we use the changes seen in joint cartilage to model those for the extracellular matrix in tendons? Perhaps it is important to correlate normal adaptations to loading between chondrocytes and cartilage and tenocytes and tendon.

It has been observed that tenocytes exposed to compressive load rather than tensile will become more rounded and start to look more like chondrocytes. They also alter the protein production for the matrix from predominantly decorin to aggrecan and the predominant collagen from type I to type II(Ker 2007). Areas vulnerable to tendon disease, at least those of insertional tendinopathies have been suggested to be those that are exposed to more compressive loading(Maganaris and others 2004) and so perhaps it is correct to use a model for cartilaginous degeneration.

INVESTIGATIVE PROCEDURES

RADIOGRAPHY

X-rays can be used to pick out soft tissue shapes and swelling to some degree and with use of contrast mediums, they were previously used for tenography, arthrography or bursography(Kainberger and others 1997). Generally there is insufficient detail in the case of tendons to use plain films to diagnose any pathology other than calcification, insertional changes or in the case of the larger tendons possibly rupture. Coupled with this are the potential hazards

linked with cumulative exposure to x-rays which make this an inappropriate diagnostic tool in general with tendinopathy(Pope 1992).

MAGNETIC RESONANCE IMAGING

Felix Bloch and Edward Purcell were awarded the Nobel Prize in 1952 for their discovery of Nuclear Magnetic Resonance (NMR). At this stage it was used in spectroscopy to analyse the chemical composition of different fluids and solids. Paul Lauterbaur invented a method known as back projection imaging in order to distinguish different tissues within a body and Peter Mansfield increased the speed at which the radio signals from the nuclei were acquired in order to reduce the time taken to produce an image from hours to minutes. Both Lauterbaur and Mansfield were awarded the Nobel prize for medicine in 2003 for their part in the development of modern magnetic resonance imaging (MRI). It was in the mid seventies that Damadian, Minkoff and Goldsmith performed the first ever MRI scan on a human being. MRI exploits the fact that the body is largely made up of water and fat, thus there are a high proportion of hydrogen atoms within the tissues of the body. When placed inside the coils of MRI tube the majority of the protons of the hydrogen atoms will align with that field. By applying a radiofrequency (RF) pulse specific only to hydrogen to the area of interest of the body we can make the protons 'spin' or precess at a specific frequency in one specific direction, the frequency of Larmour. This frequency is calculated based on the tissue being imaged and the strength of the main magnetic field.

Larmour frequency = Gyromagnetic ratio (a constant specific to the nucleus) x Main magnetic field strength

A RF pulse of sufficient duration to cause a 90º shift is called a 90-degree pulse.

When the radiofrequency pulse is switched off the protons will return to their natural plane and release energy which is perceived as a signal by the coil and generates an electrical current. This

is digitised by an analogue to digital converter into mathematical data which can be converted by a Fourier transform into an image.

The images can be affected by what is known as T weighting, either T1 or T2.

T1 refers to spin relaxation time. This is the time it takes for 63% of the magnetisation of the field to return to the original longitudinal direction after excitation.

T2 refers to spin-spin relaxation time. This is where a 90º pulse is used initially as in T1 but as the protons relax a second 180º pulse is applied which causes the protons to spin again hence spin-spin relaxation. The important time is then how long it takes for the bulk (63%) of the magnetisation to dephase in the transverse plane.

Different tissues have different MR properties with respect to T1 or T2. By altering the weighting of the image it is possible to alter the contrast dependant on which structure we wish to predominantly view. This means that an image with T1 weighting will show fluid as dark, fat as bright and other tissues as grey, whereas T2 weighting an image will show fluid as bright and fat and other tissues as shades of grey.

Magnetic resonance imaging provides a good quality image of the soft tissues which are easy to identify and thus to visualise any abnormalities within them(Pope 1992). It is, however, not always easy to identify tendon pathology due to the low water levels within tendons. Although the tendon may be seen it is harder to define the 'fibrillar' structure and alterations to that which may arise in tendinopathy. Generally tendons and ligaments will appear as dark due to the rapid decay of the signal in a semisolid structure. In the case of pathology the structure may appear white or grey due to the increased water content. More recently, however, an artefact known as 'magic angle imaging' where the tendon is at 55º to the magnetic field has been shown to reveal far more of the intra-tendinous architecture by increasing the signal from the tendon (Lambe and others 2006). A significant limitation in the use of MRI is the financial burden and the limited numbers of trained personnel available to both run the machines and interpret the scans, although

accessibility is improving all the time. Perhaps this may be eased further by the advent of the small part coils for use on peripheral structures. It is also important to note that an average scan may take 30 minutes and involves the patient remaining still throughout that time to prevent artefacts. The large magnetic fields prohibit the use of MRI scanning in individuals with any sort of metal implant from artificial joints or pacemakers to clips used to repair aneurysms.

ULTRASOUND SCANNING

In 1880 Jacques and Pierre Curie first discovered the piezoelectric effect. That is that certain crystals will mechanically deform when subjected to an electrical voltage and similarly when mechanically deformed will produce a voltage in proportion to that deformation. In 1937 the first U/S imaging unit was designed by an Australian K T Dussik and in 1949 a B mode scanner was made by Howry and Bliss. Since then with many technical advances U/S has been used increasingly to visualise general organ pathology and as a useful diagnostic tool in obstetrics.

The basic principles of U/S are that using a transducer with an array of piezoelectric crystals a voltage is applied creating mechanical deformation which produces ultrasonic waves in the range 1 – 30 MHz depending on the crystals within. When the head of the probe is applied via a gel contact to the body the sound waves will travel through the tissues at a speed (c) which is affected by the density (ρ) and elastic modulus of the tissue through which it is travelling. There are minor variations in the density and modulus of different tissues. The acoustic impedance (z), which is a property that is a function of both density and modulus, of a given tissue and can be used to characterise that tissue.

$z=\rho c$ and $(c=f\lambda)$ (where λ is wavelength)

$\Rightarrow z=\rho(f\lambda)$

Throughout the tissues of the body acoustic impedance varies and it is this differential material property that enables us to visualise the structures below the probe. When the sound waves meet an interface between tissues that have different acoustic impedance the sound will be reflected

back at an angle dependant on the angle of incidence to the boundary. The reflected sound waves, by means of the piezoelectric effect on the crystals will create a voltage and this can then be converted into the image on the screen.

There are a number of issues that need to be considered when using U/S as diagnostic tool. As the sound waves move through soft tissues there will be a level of attenuation (weakening of the sound as it propagates) which encompasses absorption and the reflection and scattering of the sound as it meets tissues interfaces. This is measured in decibels (dB) and the level of attenuation will limit the imaging depth. As the frequency of the source increases this will increase the attenuation and thus decrease the depth to which it can penetrate.

If one increases the transmitting voltage that will increase the intensity of the beam, but one can compensate for lower output intensity by increasing the receiver 'gain'. The gain control can be used to help allow for the effects of attenuation on the signal from deeper structures. Thus later arriving signals from deeper levels can have increased gain to ensure that the image is presented with equal intensity throughout. This increase in gain with depth is known as the Time Gain Compensation slope (TGC). In order to view the image in real-time the image displayed on the screen is constantly being updated. These sequential images are averaged over time in order to smooth the image and reduce the appearance of noise and this is known as persistence. The level of persistence required will vary depending on what is being imaged. Faster moving structures should have a lower persistence in order to retain more accurate representation of the actual movement but slower moving structures will benefit from higher persistence as that will reduce noise and smooth the final image. It is worth remembering this when using U/S images for movement analysis as a more accurate account of the actual movement is represented by reducing the frame averaging.

Perceived inaccuracies in U/S imaging with inexperienced sonographers usually result from artefact within an image being mistaken for abnormalities or pathology.

Common U/S artefacts (Kremkau 2002) may arise as a result of:

- Reverberation is where multiple reflections are seen below the real reflecting surface. This results when a standing wave arises between the source and the reflecting surface and the later arriving signals are perceived by the image processor as resulting from deeper structures. Sometimes structures above the reflecting surface will be mirrored on the image below it as a result of echoes reflecting between the two structures.
- 'Comet-tail' artefacts arise as a result of reflections arising between two closely occurring structures which cause closely spaced reverberations below the lowest structure.
- Refraction is where the source beam is bent by a structure with a widely different propagation speed than that expected for soft tissues. This can result in incorrect positioning of a structure on the image or the doubling of a single object.
- Acoustic shadowing may occur where a strongly reflecting object such as an area of calcification weakens the signal to structures below such that there is a darkened shadow on the image.
- Enhancement similarly occurs below a structure that has weak attenuation levels causing the image below it to appear brighter.
- Anisotropy will make an object appear bright when imaged at one angle and dark when imaged from a different angle. This is seen at smooth boundaries where the angle of reflection and incidence are the same so the probe will only receive the echoes if the source beam strikes it at the right angle. In tendons imaging from the wrong angle can result in apparent dark areas within the structure which may be mistaken for pathology. This can be avoided by imaging the suspicious area from different angles to see if the dark area is persistent.

Operator education and experience will prevent incorrect diagnosis from artefacts seen within the images.

Recently, use of higher frequency U/S probes, from 10 MHz upwards, has allowed skilled practitioners to evaluate tendons and musculoskeletal structures with even more success. With

some of the better machines it is even possible using the Panoramic Program to visualise the entire structure at the same time (see Figure 2).

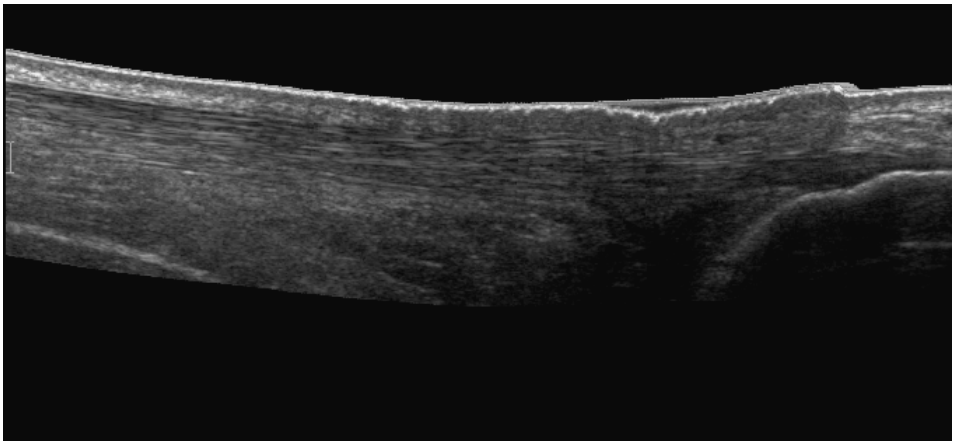


Figure 2 Panoramic view of a normal Achilles tendon (courtesy Dr R W Kerslake Consultant Radiologist QMC, Nottingham)

There are a number of benefits to using U/S:

- accessibility of machines
- comparatively low cost
- low levels of side effects to either patient or clinician
- the ability to visualise structures during movement – enabling analysis of movement within tissues and allowing patient involvement in assessment of injury
- real-time

Scans can show either longitudinal or transverse images of the tendon and can depict irregularities within the tissue from degeneration, swelling, ruptures or thickening(Fornage 1986). (See Figure 3 which shows a comparison of a normal with a degenerate Achilles tendon, the right tendinopathy is thickened to twice the size of the normal in this individual)

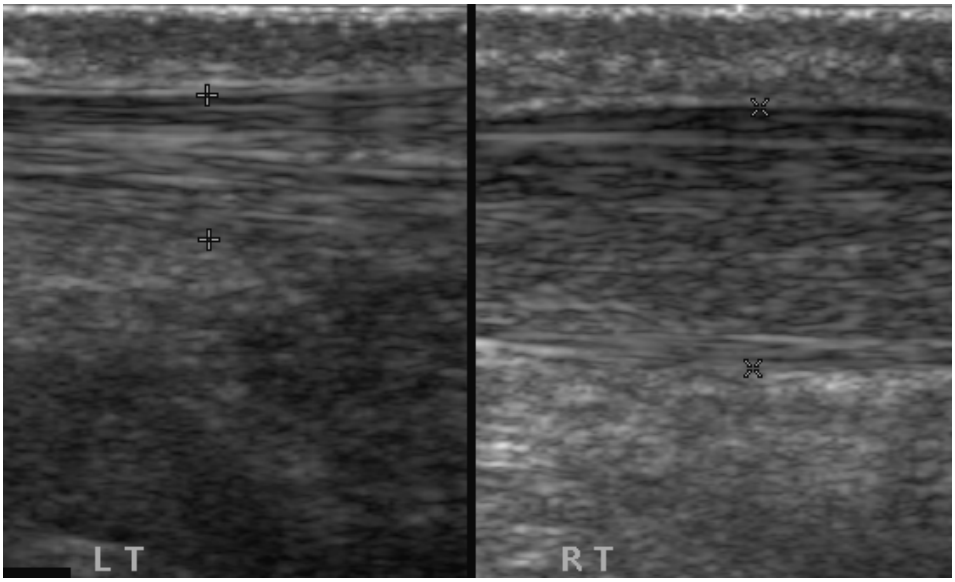


Figure 3 Longitudinal grey scale images of normal and degenerate Achilles tendons (courtesy Dr R W Kerslake Consultant Radiologist QMC, Nottingham)

It has been suggested that one possible advantage of radiographic images over U/S is the visualisation of calcification either within the tendon or at the tendon to bone insertion as in the case of enthesitis(Pope 1992). Calcification, however, is clearly visible on U/S provided there is no other bony interface between it and the probe.

Of huge importance to clinicians is the opportunity to read the image of the potentially injured structure while the patient is still there. This allows clinical signs and images to be appreciated in tandem during the diagnostic process. If injections are indicated as a treatment of choice the treatment may be administered accurately using U/S to visualise the injection into or around the structure as required. (see Figure 4 illustrating patellar tendinosis)

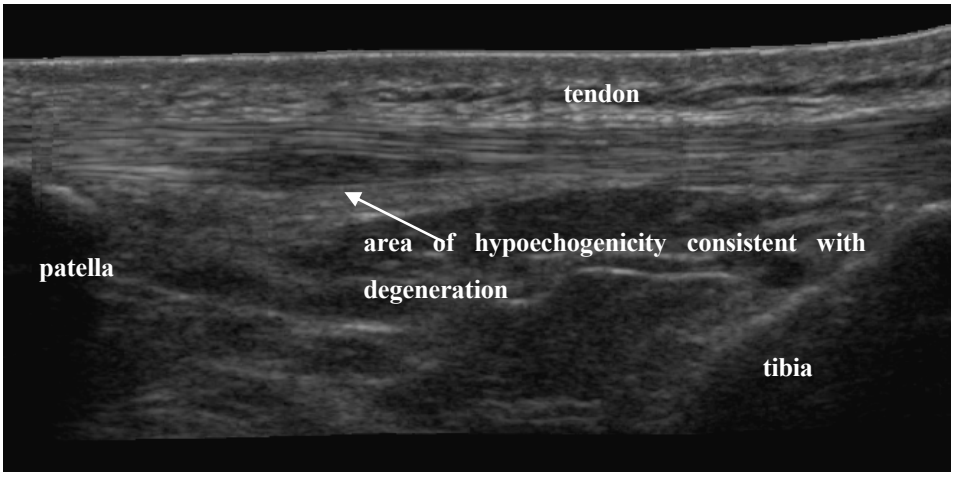


Figure 4 Panoramic greyscale ultrasound scan of patellar tendon showing area of tendinosis(courtesy Dr R W Kerslake Consultant Radiologist QMC, Nottingham)

One of the major benefits of U/S in tendon research has been its use to track movement in tissues using anatomical landmarks(Maganaris and Paul 1999) and tracking software(Revell and others 2005).This has allowed studies to evaluate the mechanical properties of tendons in vivo and then to see how differences may be effected due to exercise status(Kubo and others 2004b; Kubo and others 2001; Kubo and others 2002a) rest(Kubo and others 2004a; Reeves and others 2002) and age(Kubo and others 2003; Maganaris 2001; Reeves and others 2003a; Reeves and others 2003b; Reeves and others 2004). The effects that researchers have found to these factors will be explored fully in the main section looking at the mechanical properties of tendons.

As with all computer based technology the quality of the cheaper portable machines is improving all the time. This means that more clinicians are embracing U/S as an adjunct to their clinical expertise both in order to confirm a diagnosis and as a means of charting the recovery process and natural history. Any technology or method that could then be applied to U/S imaging in order to screen for potential problems is likely to be useful not just in the large centres on excellence but also in the field.

DOPPLER ULTRASOUND

The Doppler Effect arises from changes in frequency due to the movement of the source, the receiver or a reflector(Kremkau 2002). When it is the reflector that is moving one can describe the Doppler Shift as being the difference between the source frequency and that received. In medical physics this phenomenon has been used to detect blood flow by using the moving red blood cells as reflectors. The Doppler rate is proportional to the flow rate. On a Colour-Doppler image the regions of blood flow or tissue movement are represented in colour while the stationary areas are presented in grey scale. This has been used more recently in tendons to illustrate the differences in levels of blood flow in injured tendons compared with normals(Astrom and Westlin 1994a) and to illustrate the existence of neo-vessels in tendinopathy (see Figure 5)(Alfredson and others 2003; Ohberg and others 2001) and then both to treat them(Alfredson 2004; Ohberg and Alfredson 2002) and to explore the effects of treatment protocols on the neovessels seen(Ohberg and Alfredson 2004).

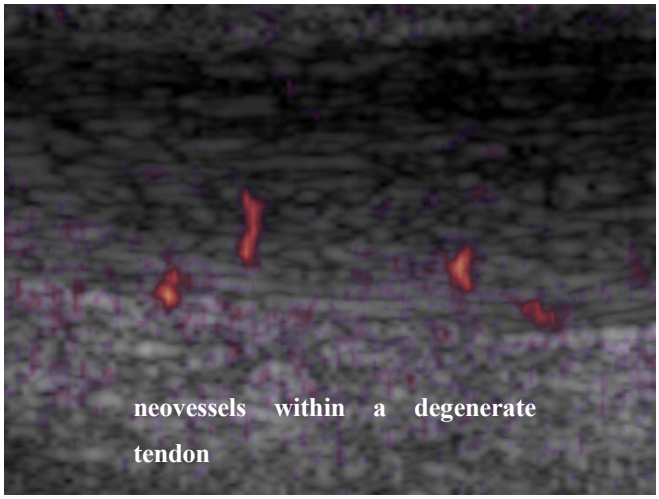


Figure 5 Doppler U/S image of tendinopathy (courtesy Dr R W Kerslake Consultant Radiologist QMC, Nottingham)

STUDIES TO COMPARE U/S, MRI AND OTHER INVESTIGATIONS

In order to compare the diagnostic benefits of different types of U/S and MRI it is important to establish a standard scoring system that may be universally applied both in the research and clinical setting(Robinson and others 2001). The Victorian Institute of Sport in Australia derived the VISA-A scoring system with this in mind. This is a questionnaire based measure that identifies impact of the condition in the patient's/participant's mind with respect to pain in general, activities of daily life and their participation in the sporting arena. It provides a more comprehensive marker than previously used visual analogue scales (VAS).

To try and find the gold standard for tendon investigation with respect to diagnostic techniques various studies have been performed.

The goal of such research is to find a technique that can be used:

- To screen asymptomatic individuals to identify those at risk of injury.
- To grade tendinopathies to predict outcome.

Comparisons have been made between MRI and U/S(Khan and others 2003; Khan and others 1999; Moller and others 2002), Power Doppler U/S and MRI(Richards and others 2001) and even palpation by clinicians(Cook and others 2001a).

Both MRI and U/S provide a useful tool in the diagnostic process, and the results of both imaging techniques appear to correlate well with one another(Cook and others 2000; Khan and others 2003; Khan and others 1999). Various studies have attempted to see how the extent of the lesion found on imaging correlates with clinical outcome(Archambault and others 1998; Khan and others 2003; Khan and others 1999; Moller and others 2002; Peers and others 2003). Despite an early indication that grading of the pathology seen in an U/S scan may be linked with the outcome (Archambault and others 1998), later studies have shown that U/S and Power Doppler U/S were unable to predict the clinical outcome or severity of a tendinopathy (Khan and others

1999; Moller and others 2002; Peers and others 2003). The grading of the pathology seen on an MRI scan did, however, appear to predict the outcome (Khan and others 2003).

Some asymptomatic tendons do show signs of 'degeneration' using both U/S and MRI scanning and some painful tendons may appear normal(Cook and others 2001b; Khan and others 2003). However, when examining asymptomatic tendons from a cadaveric control group Kannus and Josza found evidence of degeneration (Kannus and Josza 1991). It is reasonable to suggest that a positive diagnosis on imaging may still indicate pathology, possibly, as yet, sub-clinical. It has been shown that when screened by U/S there is four times the risk of developing jumper's knee in those with an area of hypoechogenicity within the tendon(Cook and others 2000). While imaging techniques are not absolute predictors of outcome they can provide a useful tool in screening(Fredberg and Bolvig 2002). It is hoped that by predicting a tendon injury prior to it becoming symptomatic it might be possible for the athlete and the clinician to rectify the problem before symptoms arise. This was attempted recently using eccentric exercises as a prehabilitative technique to stop a lesion visible on U/S becoming painful or progressing(Fredberg and others 2008). Disappointingly the results appeared to indicate that eccentric work had no effect on whether identified hypoechogenic Achilles tendons became symptomatic and it actually increased the risk of pain occurring in hypoechogenic patellar tendons. It is worth noting at this point, however, that the regime prescribed in this study was only 20 minutes 3 times a week whereas a treatment regime is usually prescribed as 45-90 repetitions twice a day. Also the patellar work was not using a decline board which has been identified as producing superior results in the case of patellar tendinopathy(Young and others 2005).

While there are little differences in the value of the image produced between U/S and MRI the main value to the clinician of U/S for the examination of tendons must be the portability of the machines and the ease with which the scans can be obtained. A patient presenting in clinic can be scanned as part of the assessment process rather than being sent away to await an MRI scan at some point in the future. The ease with which this technology can be accessed has also led to its

use for assessment of the properties of tendinous tissue. An U/S scan can be used to store and evaluate movement of the tissues in real-time. It is this use of U/S to find the material properties of tendons and the accessibility of U/S scanners that has led to U/S being the imaging of choice for this particular study.

EPIDEMIOLOGY

There is general acceptance in the literature that, in Western society, interest in recreational sport and running in particular has increased greatly over the last three decades (Jarvinen 1992; Kaufman and others 1999)and that this is linked to a similar increase in injuries, generally associated with overuse(Leach and others 1981; Nyyssonen and P 2000). Attraction into sport and recreation is not merely due to the variety of sports available and the innovation of the gym and leisure industry but is also promoted by the medical profession as a positive health benefit(Requa and others 1993). In this country we have seen, over the last decade, the introduction of the GP exercise referral scheme and, more recently, government led initiatives to encourage the marriage of sport and education in the community. Running is often the activity of choice due to its convenience and low financial outlay(Taunton and others 2002). It is worth noting that running while commonly accepted as beneficial for health, at least from a cardiovascular point of view, has been reported as having similar rates of injury as the competitive team sports(Requa and others 1993). While running has been shown to reduce the rate of heart disease and lower limb disability in older athletes it is linked with increased risk of 'Achilles tendinitis'(Kujala and others 1999). The most common overuse injury linked with running or jogging is patello-femoral pain syndrome but the tendon injuries of iliotibial band syndrome, patellar tendinopathy and Achilles tendinopathy are reported to occur next most often(Clement and others 1981; Requa and others 1993; Taunton and others 2002).

Reviews of the literature describing the epidemiology of tendon disease can be found readily but research into the true incidence of tendon pathology and any changes to this over the years are sadly lacking(Houshian and others 1998).

Age influences types of tendon injury seen: in children the tendon tissue is less vulnerable than the epiphysis and so most injuries here tend to involve damage to the insertion or the growth plate itself e.g. Sever's disease or Osgood-Schlatter's disease. In the older athlete, possibly in part, due to the increased likelihood of participation in an endurance sport, there would appear to be more evidence of overuse injuries damaging the body of the tendon itself.

One way of anticipating rates of Achilles tendinopathy might be to look at studies investigating the prevalence of Achilles ruptures and presume some degree of association between them. It has already been shown that there is degeneration present in nearly all tendons undergoing rupture(Jozsa and Kannus 1997).There is evidence of similar histopathology in both the ruptured and the tendinopathic groups(Astrom and Rausing 1995), although the degenerative changes may be more pronounced in the cases of rupture.

Studies looking at the incidence of Achilles tendon (TA) rupture in relation to participation in sporting activity in Scandinavia between 1979 and 1994 (Houshian and others 1998; Leppilahti and others 1996) indicate that there was a sixfold increase in the numbers of tendon ruptures seen. 80% of these injuries were classed as related to sport. Similar findings were seen in Denmark (Houshian and others 1998), where, in a Danish county, between 1984 and 1996 the numbers presenting per year with rupture of a TA in 1984 doubled by 1996. 73% of these injuries were again classified as being related to participation in sport.

AETIOLOGY OF TENDON DISEASE

HISTORY

Until recently it was believed that overuse injuries in any tendon resulted from persistent overstretching of the tendon fibres beyond 4 – 8 % of its normal length resulting in micro-tears from repetitive trauma which outstrips the body's ability to repair (Gibbon and others 1999). As a consequence of this injury healing commenced with inflammation (Kannus and Natri 1997 in Josza and Kannus)(Josza and Kannus 1997). This has resulted in the common terminology used in most tendon injuries: tendonitis, epicondylitis, etc(Khan and Cook 2000). It was further

suggested that as most injuries in the Achilles appeared to be in the middle third another possible cause may be insufficient oxygenation due to the limited vascularity at this site(Gibbon and others 1999). Further 'evidence' of lack of oxygen being responsible for tendon degeneration was taken from the type of degeneration commonly seen as being that seen where tissue has been deprived of oxygen(Kannus and Jozsa 1991).

CURRENT THINKING

DEGENERATION NOT INFLAMMATION

As previously mentioned, in the section on pathology, the most common type of overuse injury has now been found to be degenerative not inflammatory. This has been confirmed by histopathology(Jarvinen and others 1997b; Kannus and Josza 1991) in vivo by micro-dialysis(Alfredson and others 2001), where there is evidence of glutamate and glutamate NMDAR1 receptors, which may be linked with the experience of pain, but there is no evidence of raised levels of PGE (2) a common chemical mediator in inflammatory pathologies. This has been described earlier in the section on pathology. Periodically it is suggested that there is an early inflammatory stage in the progress of tendon disease which may not be obvious in many cases due to the late presentation by which time the condition has progressed to the chronic degenerative stage(Fredberg and Stengaard-Pedersen 2008; Koenig and others 2004). As mentioned, the evidence base behind this premise is at present scant but may be worth bearing in mind for future work(Kannus and others 2004).

BIOCHEMICAL MEDIATORS AND LINKS WITH TENDINOPATHY

It has been suggested that one possible route to tendon degeneration is the response of the cells to high levels of Reactive Oxygen Species (ROS), commonly referred to as free radicals. It is proposed that fibroblasts release increasing levels of ROS as a response to exercise. Over a period of time the tissues adapt to these levels with no adverse effects but if the athlete should suddenly increase their training levels before adaptation can occur the high ROS levels may act as inducers of apoptosis(Bestwick and Maffulli 2000). In terms of management, the significance would be that certain cytokines such as interleukin-1 alpha (IL-1) and tumour necrosis factor-

alpha (TNF) may influence the production of ROS by fibroblasts(Meier and others 1989). There has been some early promising results with use of TNF-alpha inhibiters for the treatment of back pain(Tobinick and Britschgi-Davoodifar 2003) and similar intervention with some success in tendinopathy(Fredberg and Ostgaard 2008). It is interesting to further note that some workers in America claim that Sever's Disease will respond quickly and well to treatment with a product containing a combination of Vitamin E and Selenium which it is claimed reacts with the ROS(Kidsheelpain.com 2002-7) there are no links on this site to published evidence to support this theory.

Micro-dialysis has, over the last 3 – 4 years, proved useful as a means of investigating biochemical markers and their levels within the intact tendon, both in normal tissues, after exercise, and in those exhibiting signs of tendinosis(Alfredson and others 2002; Alfredson and others 2001; Alfredson and others 2000; Langberg and others 1999). It appears that acute exercise instigates adaptation to exercise by the collagen in the soft tissues, and the increased formation of Type I collagen(Langberg and others 1999). This is shown by investigating the change in concentration of the biomarkers, PICP (a collagen pro-peptide), ICTP (a collagen degradation product) and PGE2 (an inflammatory mediator), in the peritendinous space before and after 3 hours of running.

It is, however, useful to note that tenocytes strained cyclically over a 24 hour period, in vitro, have been shown to express vascular endothelial growth factor (VEGF) a messenger which promotes angiogenesis(Petersen and others 2004). This factor is also found in higher concentrations in degenerate and ruptured tendons than in healthy tissues(Pufe and others 2005) which suggests that VEGF may be linked to the ingrowth of new vessels in the diseased tendons. It has been further shown by the same research group that VEGF can influence the expression of matrix metalloproteinase (MMP) and the inhibition of tissue inhibitor of metalloproteinase-3 (TIMP-3) in tendon cells and that alteration of the levels of these factors can affect the material properties of tendon and may, in fact be causative in the degenerating process(Pufe and others 2005). Certainly there are a number of investigations that suggest that neovascularistaion may be

linked to the pain in tendon disease(Alfredson 2005; Alfredson and others 2003; Clementson and others 2008; Gisslen and others 2005; Ohberg and others 2001) and some successful treatments linked to the reduction of the vasculature either by injection of a sclerosant or with exercise(Alfredson and Cook 2007; Clementson and others 2008; Khan 2002; Ohberg and Alfredson 2004; Ohberg and others 2004).

HYPOXIA AS A PRECURSOR TO TENDON DAMAGE

Histopathology has suggested that much tendon degeneration is hypoxic as mentioned in the section on pathology (Josza and Kannus 1997) and it has been thought that this may be due to low blood supply to the vulnerable areas linked with increased O_2 demand during exercise(Jozsa and others 1989; Paavola and others 2002a). Evaluation of normal and injured tendons, during exercise, using Doppler flowmetry has shown that blood flow is evenly distributed throughout the body of the tendon - although there was a significant reduction at the calcaneal insertion(Astrom 2000). Uses of Doppler flowmetry in conjunction with exercise are limited by possible artefacts that may arise associated with gross movements. Because of this other methods such as indocyanine green dye and near infrared spectroscopy have been developed with some success(Boushel and others 2000b). Using this technique a comparison of O_2 saturation with blood flow to the Achilles, during exercise, did not reveal any mismatch i.e. the O_2 supply to tendons during exercise is sufficient, in normal tendons, at least(Boushel and others 2000a).

As previously mentioned, recent work using Doppler ultrasound and power Doppler to investigate blood flow at the site of injury has, suggested that, in the case of chronic tendinopathy, at least, there is often increased blood flow(Astrom 2000; Ohberg and others 2001; Peers and others 2003). This is in the form of randomly orientated neovessels which may represent a failed response to healing(Peers and others 2003) or, indeed, may be the cause(Pufe and others 2005). It has been shown that the neovessels may be associated with neural in-growth and contribute to the source of the pain. As mentioned under Anatomy (Section 2) it has been shown that the nerve fibres associated with neovessels are predominantly sympathetic rather than sensory in origin. It is suggested that this new sympathetic innervation may, in the patellar

tendon, enhance the blood flow to the already present vascularisation of the area ventral to the tendon. It is also useful to note that the sympathetic nerves can enhance the appreciation of pain(Danielson and others 2008). In order to evaluate the contribution of the vessels seen in injured tendons to the pain, large studies have been carried out(Zanetti and others 2003) and suggest that while neovascularity can be used as a predictor of pain in tendinopathy it has not, however, proved as useful as normal grey scale U/S images as a predictor of outcome. Further work has argued against the correlation between pain and neovascularity(Peers and others 2003) and it is possible that it may just show evidence of the degree of degeneration present. While there appear to be conflicting views regarding the actual function of the in-growth of new vessels within degenerate tendon tissue most authors appear to agree that this is a significant area of research with respect to potential aetiology or progress of the disease.

CURRENT TREATMENTS

CONSERVATIVE

Traditionally treatment for 'tendinitis' consisted of addressing 'the inflammation' using various types of anti-inflammatory modalities, ranging from non-steroidal anti-inflammatory drugs and injections of corticosteroid to the various electrical modalities applied by physiotherapists(Galloway and others 1992). Having concluded at this time that there is no evidence of inflammatory cells in chronic tendinopathy, this approach needs to be reconsidered. Concurrently most practitioners would advocate a rehabilitation regime of stretching, strengthening and correction of any biomechanical faults including gait, if appropriate. This has in recent years been predominantly overridden by the introduction of a regime of painful eccentric exercises.

THE ECCENTRIC EXERCISE PROGRAMME

As most athletes experience pain with tendinopathy during deceleration or on change of direction it has been suggested that this is the point at which the most damage is likely to occur. This is often referred to as the eccentric phase of an activity, as the muscle is lengthening in a controlled fashion in order to decelerate the body as opposed to concentric activity where the muscle

shortens in order to move the joint through range. Similarly as the myotendinous unit experiences more applied force during this phase than during concentric or isometric work it seems logical to prepare for this with eccentric exercises(Stanish and others 1986).The original articles proposing this type of exercise as a treatment for tendinopathy have suggested that it may be linked with an increase in tensile strength of the tendon(Stanish and others 1986). The principle argument is that applying load when the tendon is at full stretch will alter its ability to withstand the stress by increasing the number of bonds between the helices(Fyfe and Stanish 1992).

The programme of eccentric exercises is based on three main aspects:

1. Increasing the length of the myotendinous unit at rest.
2. Increasing the tensile strength of the tendon by increasing the load applied on a progressive basis.
3. Increasing the force developed during the exercise by increasing the speed of the contraction.

Adaptation of tendons to a loading cycle will be further explored in the review under mechanical properties and although it seems unlikely that the strength is derived exactly as described by Fyfe and Stanish there is evidence of constant remodelling of the tissue as a reaction to the exercise levels under which it is placed. Perhaps by forcing the patient to load the tendon rather than protect it the recovery process is accelerated in some way.

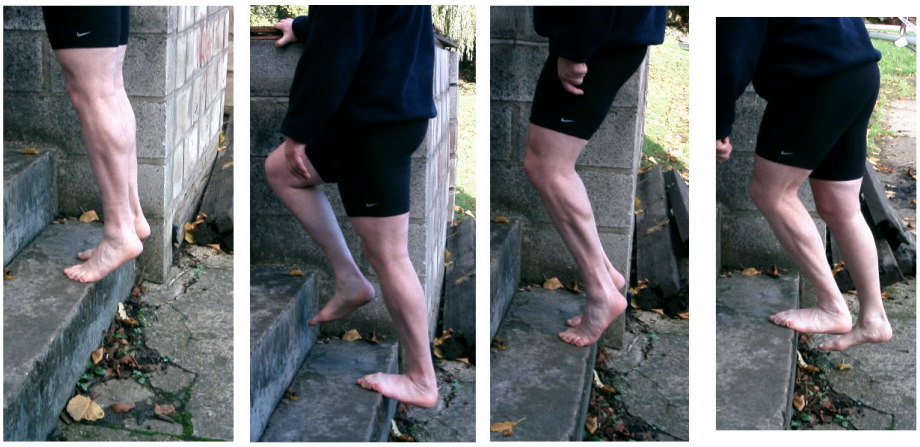


Figure 6 Eccentric exercises for gastrocnemius and soleus

The images above (Figure 6) illustrate a patient performing the eccentric exercise protocol. He rises up on both toes, or just on the unaffected one, (the concentric phase) and then lowers below the step weight bearing only through the affected leg (the eccentric phase). The first two pictures show this procedure with a straight knee focusing on the gastrocnemius muscle and the last two are with the knee bent focusing on soleus muscle.

Eccentric exercise has now been used clinically with marked success (see Figure 6)but it is still unclear as to why this should be so(Alfredson and others 1998; Fahlstrom and others 2003; Mafi and others 2001; Silbernagel and others 2001). Some work has been done to test the hypothesis that eccentric work is more effective for recovery than any other form of exercise but it has proved hard to operate with control groups due to the ethics of denying a treatment that appears to work and there is some apparent difficulty in prescribing rehabilitation that is completely concentric or isometric(Cannell and others 2001; Mafi and others 2001).

It is difficult to see how a tendon can ‘be aware’ of whether the muscle work is isometric, concentric or eccentric so is the type of work really important or is it the level and range at which subjects are encouraged to work that is more significant? As clinicians we rarely advise patients

with injuries to work through the pain but this seems to be a crucial part of this rehabilitation. Perhaps in so doing there is some modification of pain perception?

Unfortunately we do not yet know exactly where the pain in tendinopathy is derived from. It has been proposed that levels of glutamate which appear to be higher in painful tendons than in normals(Alfredson 2003; Alfredson and others 2000; Alfredson and Lorentzon 2003) may be implicated in the pain mechanism. By examining the levels of glutamate in degenerate tendons before and after a successful course of eccentric exercise there was no reduction in glutamate level associated with the reduction in pain(Alfredson and Lorentzon 2003). It is interesting, however, to note that a course of successful exercise has been associated with a reduction in neo-vascularity(Ohberg and Alfredson 2004).

SURGICAL

Surgery has been used as a treatment in some cases of chronic recalcitrant tendinopathy with a view to treating the areas of degeneration and in some cases the paratenon.

At some sites tendinopathy may be attributed to external forces impinging on the tissue i.e. around the shoulder. Surgery here would involve decompression of the impinging tissue. In cases where there is no evidence of tendon impingement the aims of surgery are varied and have been listed in Josza and Kannus (Josza and Kannus 1997)

1. To remove any granulation tissue or scarring.
2. To induce scarring to increase the strength of the tissue.
3. To encourage revascularisation of the tissue.
4. To relieve any extrinsic pressure (as above).
5. To relieve the tensile overload.
6. To repair any gross intrinsic damage.
7. To replace or add to any damaged areas by insertion of graft if appropriate.

Unfortunately much of the literature regarding surgical outcomes for tendinopathy is confounded by poor study design. Often the studies are retrospective cohorts which limit the control of the patient's treatment post-operatively and the surgery used in each individual, there is often no control group and results are often subjectively assessed by the patient and the surgeon.

In general, the response to surgery has been positive provided the outcome was measured some time after the surgery(Leppilahti and others 1998). Where the surgery is less destructive and the main aim is to excise any possible adhesions between the tendon and the paratenon the results appear to be favourable somewhat sooner, after 7 months(Paavola and others 2002b).

A systematic review of studies of surgical outcome in patellar tendinopathy by Coleman et al(Coleman and others 2000), has explored the limitations of current research in this field. Each study reviewed was assessed using the Coleman Methodology Score (CMS) system which allocates points between 0 and 100, where 100 is a design with very little influence of chance, confounding factors or bias on the results. Correlating the CMS with the reported success rate of the surgery revealed negative correlation or successful results where the study design was poor. There appears to have been little done to establish the merits if any of one type of surgery over another.

INJECTION

Corticosteroid

Historically clinicians would sometimes attempt to treat 'tendinitis' with a peri-tendinous injection of corticosteroid. The effects of injecting a solution of methyl-prednisolone and marcaine have been compared with the injection of marcaine alone(DaCruz and others 1988). There was found to be no significant difference in the outcomes of the two groups and the most telling factor in recovery rate appeared to be the correlation between a low level of presenting symptoms and full recovery. At best the literature is poor regarding the effects of corticosteroid injections in tendinopathy. There has been little evidence to support its use as a treatment, although, similarly there is no evidence to support the suggestion that steroid injections may be linked with spontaneous rupture(Shrier and others 1996). There are, however, two studies which

show a reduction in strength in collagen fascicles(Haraldsson and others 2006) and in rabbit Achilles tendons as a whole(Hugate and others 2004) when exposed to cortico-steroids both intra and peri-tendinous. More recently the use of steroid injections into the tendon has been proposed again based on the suggestion that there is, in fact, an inflammatory component to the disease(Fredberg and others 2004; Koenig and others 2004). The evidence base to these proposals is scant but there does seem to be some early improvement to the condition in the subjects. The injections were U/S guided intra-tendon(Koenig and others 2004) and peri-tendinous(Fredberg and Ostgaard 2008) and in both cases the blood flow within the tendon to the neovessels was reduced and the results were reported to be successful. Neither study was controlled, blinded or randomised, and both had low numbers of subjects therefore one should be wary of any conclusions that might be drawn here.

Scelorosants

Sclerosants have been used recently for injection in and around the tendon. As a continuation of the work by Ohberg, Lorentzon and Alfredson(Ohberg and others 2001) where they linked neovascularity with pain, Doppler U/S guided injections of a sclerosing agent (polidocanol) into the neovessels have been used to ablate the neovasculature. Some studies have demonstrated a reduction in pain levels and aided return to normal activity(Ohberg and Alfredson 2002). There were similar good results to pre-injury level within 12 weeks as that found when open surgery was used to ablate the vessels (Alfredson and others 2007). It has not been possible to find studies that perform a randomised, blinded and controlled study of this technique but the technique does appear promising and compared to surgical options relatively risk free(Clementson and others 2008).

High Volume U/S Guided Injection

Following a similar rationale workers in the United Kingdom have used High Volume Image Ultrasound Guided Injections of normal saline between the tendon and the paratenon with the intention of interfering with neovessels entering the body of the tendon(Chan and others 2008). Patients were selected from a group for whom a 3 month eccentric exercise protocol of

gastrocnemius and soleus had failed. Early indications for success of this technique are good and the above cohort study reported improvements both in the short term (4 weeks) and the longer term (30 weeks) follow up. Similar improvements were seen in a pilot to assess the same approach in patellar tendinopathy(Crisp and others 2008). Neither of the above investigations were randomised or blinded trials but both reported similar outcomes to the surgical or sclerosing treatments trialled by Alfredson et al(Alfredson and others 2007). It is argued that the treatment does not irritate the soft tissues in the area and visualisation of the injection under U/S appears to show the paratenon being peeled away from the underlying tendon tissue. Presumably this ablates the origin of the neovessels within the tendon itself. At a 2008 tendinopathy study day run by Alfredson, Malliaras and himself, Otto Chan one of the proponents of this approach has said that at follow up after 2 weeks there is no evidence of re-growth of the vessels as opposed to the injections of polidocanol by Hakan Alfredson and others where there is increased neovascularity at this stage.

Aprotonin

Aprotinin, a protease inhibitor, has been suggested as a useful, injectable treatment for tendinopathy(Capasso and others 1997). It is a drug that has been used within the management of cardiac surgery for some years and was applied in the nineties to the problem of 'tendinitis'. The principle behind the treatment is that as the connective tissues in and around the joints became inflamed proteases are released leading to breakdown of the collagen structure and promotion of the inflammatory phase. Aprotinin was used as a protease inhibitor in order to accelerate the repair process and interrupt the inflammatory cycle (Capasso and others 1993). It seems strange that this should be seen as relevant in the case of chronic tendinopathy where evidence of active inflammation is limited. This treatment has been trialled again recently with no evidence yet of an improved outcome over the control group(Brown and others 2006).

Autologous Blood

Autologous blood has also been suggested and trialled as an injectable treatment for tendinopathy(Edwards and Calandruccio 2003; James and others 2007). The theory behind this

treatment is that the blood contains cellular and humoral factors which will initiate the healing cascade in degenerate tissue. Previous work has suggested that injection of blood into the tendon will lead to an initial increase in the strength as assessed in vitro in rabbits(Taylor and others 2002).

This therapy has been trialled in the treatment of tendinosis of the common extensor origin of the elbow ('tennis elbow')and out of 28 subjects treated with 1 – 3 injections of autologous blood at the site of the degeneration as identified by U/S 22 (79%) reported that their symptoms had completely resolved during strenuous activity(Edwards and Calandruccio 2003). Problems with this study, as identified by the authors themselves include lack of blinding of both the operator and the subject and lack of a control group. It would obviously be difficult to blind the subject to whether they had had any blood harvested and the authors felt that most people would be reluctant to donate blood that was not to be used for their treatment.

A further study using dry-needling and autologous blood injection was done which showed that there was a significant change in the level of hypo-echogenicity seen on grey scale U/S and the level of neo-vascularity on Doppler U/S in those subjects with improvement in their symptoms(Suresh and others 2006). Disappointingly there are again no controls used which begs the question: is the autologous blood responsible for the improvement? Or is it the dry needling that leads to the improvement in the symptoms? One could argue that the dry needling alone may be sufficient to disrupt the neo-vascularity or cause local trauma that stimulates the healing cascade.

Stem Cell Therapy

Stem Cell Therapy as used to treat tendinopathy in Equine Medicine should also be considered under this heading as it seems likely that it may only be a matter of time before autologous mesenchymal stem cells are considered for the treatment of tendinopathy in humans(Andres and Murrell 2008). There have been some encouraging results using stem cells to promote healing in the early stages (3 weeks) of tendon repair in rabbit Achilles(Chong and others 2007) but when the tissue samples were examined at later stages 6 and 12 weeks there was little difference

between those repairs treated with mesenchymal stem cells and the control group. There are not yet any studies showing stem cell therapy to be superior to any other treatment to date(Taylor and others 2007) but in vivo studies in the horse show some promising results for return to racing when compared to horses monitored after similar injury levels(Smith and Webbon 2005).

C h a p t e r 2

MECHANICAL PROPERTIES OF TENDONS

KNOWN MECHANICAL PROPERTIES OF TENDONS

Tendons essentially attach muscle to bone in order to enable the muscles to initiate and control joint movement. They are described as viscoelastic, that is, the deformation varies dependant on both the load and the rate with which it is applied. Young's modulus (E) measures the resistance of a solid to changes in its length. E = tensile stress/tensile strain (where tensile stress is the external force divided by the cross-sectional area perpendicular to that force and the tensile strain is the resulting change in length divided by the original length.) In the case of tendon tissue the stress/strain graph will not be linear in the way that it would for a spring but in fact is curvilinear corresponding to the differential strain pattern throughout loading (Figure 7).

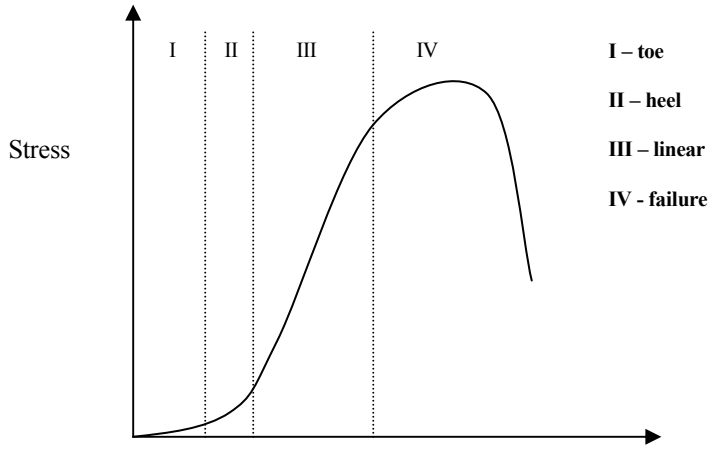


Figure 7 Typical example of a graph of stress v strain for a tendon

As a tendon is unloaded it does not retrace the same line on the force/displacement graph but rather will show a central shift to the right. This is known as the hysteresis loop (Figure 8) and the area within this represents energy stored within the tendon and lost during recoil, as heat(Wilson and Goodship 1994). It was suggested that this heating effect may be responsible in part for some of the degeneration often seen associated with overuse (in horses) but it has since

been shown that fibroblasts derived from tendon tissues can function at higher temperatures than their counterparts within the skin etc(Birch and others 1997). If thermal effects are responsible for degeneration it may not be due to cell death but rather compromised production of the components of the matrix is a possibility after repeated exposure to heat.

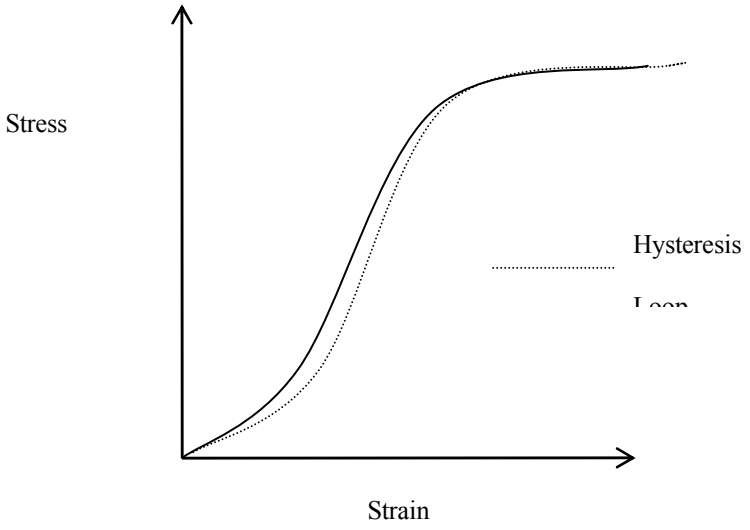


Figure 8 Hysteresis loop of a tendon being loaded and unloaded

It has also been illustrated that during exercise there is an increase in blood flow to the body of the tendon(Boushel and others 2000a). The authors here have been able to relate this to O2 uptake and oxidative metabolism but could there also be a convective heat transport from the tendon limiting the rise in temperature?

TENDONS AS LIVING SPRINGS

The compliance of tendons, and especially those of the lower limb allow for storage of large amounts of energy which reduces the work of walking and running by as much as a 50%(Alexander 1988; Alexander 1992; Alexander and Bennet-Clarke 1977). The camel exhibits an example of this in the tendon of plantaris, situated between the knee and the heel and redundant in man. Plantaris tendon measures approximately 1.3 metres in length where plantaris

muscle, which sits within the tendon, is only approximately 2 millimetres in length. It has become a passive spring using virtually no energy at all during locomotion(Alexander 1992). From this we can see that the compliance of the tendons involved in gait is crucial to the work of the attached muscles.

METHODS TO EXAMINE MECHANICAL PROPERTIES IN TENDONS

Research over the last 20 to 30 years investigating the mechanical properties of tendons has been aimed at establishing possible mechanisms of function and loading, response of the tissue to the mechanical environment and more recently variations in the mechanical properties as potential aetiologies of disease. Generally the original work was done in vitro on animal tissues or that derived from human cadavers, which enabled researchers to establish response of tendinous tissue to varying size and types of loading. It was also discovered that tendons, like bone, are a dynamic structure undergoing constant remodelling in response to the loads and activity placed upon them(Ker 2002; Michna and Hartman 1989).

More recently it has been possible, using imaging modalities such as real-time ultrasound, to examine deformation of the tissues under load in live subjects and from this derive the mechanical properties in vivo(Kubo and others 2002b; Maganaris 2001; Reeves and others 2003b). The main advantages of such techniques are to rule out the possible confounding factors that may arise from the effects of freezing and or desiccation on the tissue, and the potential slippage at the fixed ends of the specimen. It has also made it possible to perform longitudinal studies to establish the effects of exercise and specific types of functional loading on human tendons.

IN VITRO TESTING

As it is easier to define the parameters, by far the bulk of research has been done in vitro, both at macro and microscopic level. This may be as simple as taking a piece of tendon clamping it at both ends and applying load until rupture. By plotting the load against deformation over time one can derive such things as force/displacement, the modulus of elasticity and the tensile strength.

Problems with using cadaveric tissue:

- The original problem with stretching tendons was thought to be principally due to difficulty in clamping effectively the ends of the tissue. This may be in the form of slippage or of alteration of the tissue at the site of clamping due to 'squeezing out' the fluid or, if freezing is used to help overcome this and prevent slippage, there may be stress at the interface between the normal tissue and the frozen part(Ker 2007). This can be overcome by using a video extensometer or other non-contact method to measure the strain within the central portion of the sample.
- If we assess tissues ex vivo the strains may be different to that applied in situ about a joint. One way of overcoming this has been to retain the attachments of the tendon to the muscle and bony insertions and then to monitor the effects of applying load about the joint itself (Almekinders and others 2001; Lyman and others 2004). In these particular papers the strain was measured by means of inserting strain gauges at certain points within the tissue to derive differential strain within the same tendon. One must query whether the application of the strain gauge for measurement affect the values found.
- Similarly tissue from cadavers may not react in exactly the same way as it does in the live subject not only due to joint position but the interactions with the attached muscles and neural input etc. In most cases basic effects such as dehydration have been guarded against by ensuring continual bathing in a saline solution and, in some cases, temperature controlled environmental baths in order to simulate in vivo conditions. There is, however, thought to be some swelling of the tendon when immersed in a bath of saline and some have used liquid paraffin as an alternative(Ker 2007). It is impossible, however, to fully mimic the in vivo status.

When dealing with human subjects one limiting aspect of in vitro research is the inability to retest the same tissue after changing the exercise status of the subject. In animal studies one can overcome this by monitoring matched groups which are sacrificed at different points within the

study in order to see the effects of age(Diamant and others 1972), exercise and enforced rest(Almeida-Silveira and others 2000). Clearly this is not an option in humans.

IN VIVO TESTING

In order to overcome some of the problems listed above real-time U/S scanning has been used, with much success, to visualise the movement in the superficial structures of muscle and tendon during activity and under load. Initially studies have relied on the identification of a known point within the structure, such as a bony landmark or the musculo-tendinous insertion, in order to visualise the amount by which the structure moves as a whole. When examining the tendon during an isometric muscle contraction, where the joint position remains fixed, the only movement witnessed can be attributed to change in length of the tendon as a whole. By using this, predetermining the cross sectional area of the tendon, the moment arm and measuring the load using force plates or load cells, one can plot the load/deformation graph and by extrapolation of this calculate Young's modulus etc.

It is important in such studies to allow for the effects on load of co-contraction of the antagonist muscle groups and any changes in the moment arm that may arise as the load is applied(Maganaris and others 1998). Workers have tried to compensate for the influence of the antagonists by calculating what proportion of the maximum contraction and thus load has been applied to the tendon concerned using surface EMG on the agonistic and antagonistic muscles. This is not without pitfalls as it is generally accepted that the value of surface EMG is primarily one of qualitative assessment rather than quantitative (De Luca 1997), so to extrapolate values with any degree of precision is difficult to say the least.

Calculations of the cross-sectional area of the tendons under test if derived in the unloaded tendon will change as the load is applied. The degree to which this will affect the final results is unknown. Generally, however, if the antagonistic muscles are accounted for, the results have been pretty close to those found in vitro(Maganaris and others 2004).

TENDON STRUCTURE AND ITS RELATION TO THE MECHANICAL PROPERTIES

Two essential questions exist:

- Firstly, how is the stress/strain curve and the viscoelasticity of tendinous tissue explained at structural level?
- Secondly, are differences within these values linked to the vulnerability of certain areas within the tendon to tendinopathy?

Using the light microscope and polarised light it is possible to see regular crimping or zigzag patterns within the collagen fibres and this is related to the waviness apparent when a tendon is disrupted or 'teased out'. In the early part of loading these patterns will change and ultimately disappear until the fibres become straight. It has been suggested that this deformation may be responsible for the early or 'toe' region of the stress/strain curve, zone I in Figure 7 (Cribb and Scott 1995; Diamant and others 1972; Stolinski 1995).

Investigation further into the stress/strain curve requires examination deeper within the hierarchical structure using techniques such as X-ray diffraction. It appears that beyond the toe region into the 'heel', zone II, there may be a reversible straightening of kinks within the molecules and within the gap regions of the fibrillar structure(Fratzl and others 1998; Misof and others 1997). Beyond this and into the linear part under higher strains, until the line starts to plateau, the deformations are still reversible. The general consensus is that strain here may correspond to straightening of the helical portions of the collagen and/or of interfibrillar movement with resistance formed by cross-bonding and the other parts of the matrix(Fratzl and others 1998; Ker and others 2000; Mosler and others 1985). As bonds start to break the effects become irreversible and one by one the fibrils will slide apart until there is complete rupture of the tendon. Only 40% of the strain within the linear region of the tendon stress strain graph can be explained by increase in the 67 nm D-period of the fibril and it is suggested that the remainder must be attributed to other structural change, perhaps inter-fibrillar movement(Fratzl and others 1998) and as yet unknown properties of the extra-fibrillar matrix(Puxkandl and others 2002). One explanation of this anomaly however is that no-one as yet has been able to accurately assess

the length of each fibril and it is possible that each fibril may in fact be longer than the tendon itself which would mean a fibrillar strain lower than that of the tendon as a whole(Ker 2007).

As tendons deform there is assumed to be relative motion between the collagen fibres and the proteoglycan and water gel. This motion is resisted by viscous drag, the size of which depends on the relative speed of the motion. Energy is lost as heat as a consequence of this viscous drag which may result in the characteristic hysteresis loop previously illustrated. Tendons are therefore stiffer at high strain rates (Purslow and others 1998).

DIFFERENTIAL STRAIN WITHIN THE SAME TENDON

When one considers that a tendon will modify its properties depending on the mechanical environment it would seem reasonable to consider that, as the collagen type within a tendon will vary with the type of strain under which it is placed, so would the mechanical properties vary at different points within the same tendon.

By positioning small strain gauges at different points within the tendons of cadaveric samples it has been possible to explore the strain at different points of the tendon as a function of the joint range(Almekinders and others 2001; Lyman and others 2004). These studies have concluded that the areas within the patella and Achilles tendons that are more vulnerable to injury are not as has been suggested under greater strain but in fact less. These are not the conclusions of the authors Basso, Amis, Race and Johnson who explored the differential strain within the patellar tendon by use of suture material placed at specific points within the tendon and orientated along the line of pull of the fibres. They concluded that the change in length of the fibres anteriorly and posteriorly stayed the same throughout the joint range but because the original length of the posterior fibres was so much shorter than those anteriorly, as the knee went further into flexion, this translated to significantly higher strain levels posteriorly than anteriorly at higher ranges of flexion(Basso and others 2002).

Further work exploring the mechanical properties of individual fascicles harvested from different sites within the patella tendon has shown that the mechanical properties vary within the

tendon(Haraldsson and others 2005). The typically more injury prone posterior proximal part is less stiff than that harvested from the anterior portion. This can be explained by the conclusions that the posterior portion is under less strain as the joint moves further into flexion than the anterior part(Almekinders and others 2001).

The reduced strain experienced at the injury susceptible sites may be in part responsible for the vulnerability due to 'stress shielding'. Stress shielding is more commonly associated with areas in bone deprived of physiological loading by implants often after joint replacement. The implant reduces the mechanical stress in the surrounding bone causing bone loss (Cristofolini and others 1998). Extending this principle to the tendon suggests areas where lower strain levels are experienced will show reduced stiffness as a result. The analogy is not entirely accurate as the proposal is that the superficial fibres are 'protecting' the deeper fibres from the stress and thus stress-shielding them(Almekinders and others 2001).

If the strain, however, at these sites is low then, one must ask, despite the reduced stiffness, what is the trigger for injury? Is it possible that in certain situations the tissue is compromised by its inability to adapt to sudden increases in strain? In order to explore this further we need to be able to examine if there is any increase in the strains at these sites in the physiological setting. It is worth noting that the loads used by Almekinders et al (Almekinders and others 2001)were much lower than one might generally expect in every day life to be applied to the patella tendon, whereas Basso et al (Basso and others 2002)used loads of a more functional level.

Perhaps a further explanation may be the possibility of intra-tendinous shear due to differential movement between fascicles (Lyman and others 2004). It is possible that this may generate heat and subsequent damage (Maganaris and others 2004) or some other mechanical form of breakdown. Certainly differential movement between the soleus and gastrocnemius aponeuroses has been visualised using ultrasound (Bojsen-Moller and others 2004) and suggested as a possible source of vulnerability. It seems a real probability that tendons designed to be strong in tension are weak in shear.

It has been suggested by Arnoczky, Lavagnino and Egerbacher that when a collagen fibre is damaged by load this can affect the stimulus on the tenocytes such that they are understimulated, these authors have shown that looking at tenocytes in situ reveals isolated fibril damage leading to upregulation of collagenase protein and mRNA expression which is similar to that seen in tendinopathy. They argue that this may lead to a cascade which causes degeneration of the matrix in certain areas(Arnoczky and others 2007).

SUMMARY OF THE LITERATURE

Tendon injury can be a chronic and frustrating problem for both clinicians and those affected. The latter may range from elite athlete through recreational sportsman to the sedentary individual. Many different ideas have been proposed regarding treatments which have been aimed at addressing what is known to be a degenerative pathology. Recently there have been promising results with a conservative treatment based on a rigorous eccentric loading regime(Alfredson and others 1998; Shalabi and others 2004; Silbernagel and others 2001). When subjects respond favourably to this 12 week exercise programme and are able to return to sport and normal daily function there is found to be a normalisation of tendon structure and reduced thickness(Ohberg and others 2004). There is also a loss of neovascularisation from the affected areas of tendinosis(Ohberg and Alfredson 2004). It has been found that in degenerate tendons but not in normals there is an increase in the levels of the markers associated with production of type I collagen(Langberg and others 2007). It is possible that the beneficial effects behind this treatment may be increased production of type I collagen or ablation of the neovessels thought to be associated with pain in this condition (Alfredson and others 2003).

Further successful treatments are based on ablation of the neovasculature. Use of U/S guided injection of polidocanol a sclerosing agent (Ohberg and Alfredson 2002), Colour Doppler (CD) guided surgery (Alfredson and others 2007) and U/S guided high volume injections of saline in order to strip the paratenon and, in so doing, ablate to ventral ingrowth of neovessels from the tendon (Chan and others 2008), have also shown promising results. This would suggest that the neovasculature, or neurogenic growth linked with it, are certainly relevant to the presence of pain

in tendinosis (Alfredson and others 2003) if not the actual cause of breakdown of the matrix(Pufe and others 2005).

While treatment of existing cases is starting to look more promising we are not much closer to understanding the aetiology of tendon disease and from this deriving effective screening procedures and pre-habilitation. Use of U/S screening in asymptomatic tendons has been attempted and areas of hypoechogenicity have been noted and linked with increased likelihood of developing disease by some clinicians (Fredberg and Bolvig 2002) but unfortunately attempts to protect these subjects using a regime of eccentric overload did not prevent development of painful tendinosis but rather seemed to increase the risk of becoming symptomatic (Fredberg and others 2008). Interestingly, there did seem to be some reduction in the development of hypoechogenicity in those that had normal tendons at the start of the regime.

Generally overuse tendinopathy has been associated with overload and has often been linked with training errors (Almekinders and Temple 1998). This is not surprising when we note the capability of tendon to adapt to the mechanical load under which it is placed(Kubo and others 2004a; Kubo and others 2004b; Reeves and others 2003a). It would seem logical that the breakdown and remodelling of the structure must operate to a finite timescale which means that exposure to overload will result predominantly in breakdown of the tissue. It has been suggested that some fibril damage may in fact be interpreted by the tenocyte as underload and this in itself cause up-regulation of some of the chemo-factors leading to matrix breakdown (Arnoczky and others 2007).

Certain regions within the tendons commonly associated with tendinopathy in the lower limb are seen to be more vulnerable than others and it has been suggested that these areas are, in fact, under loaded, not overloaded. This is thought to be due to anatomical ‘stress-shielding’ at these points. It has been shown, in vitro, that there is certainly some differential strain occurring within the load bearing tendons (Almekinders and others 2001; Basso and others 2002; Lyman and others 2004) and this is thought to have resulted in different mechanical properties of the

fascicles derived from different areas (Haraldsson and others 2005). There is, however, some disagreement as to whether the strain in the vulnerable areas is lower or higher than elsewhere. The main limitation of these investigations must be that they have been carried out in vitro or have involved the insertion of devices within the tendon tissue (Dillon and others 2008). It would be much more useful to the debate if we were able to visualise differential movement within the tissue and therefore strain in vivo.

Assuming that there are variations in strain and properties within the tendons in vivo, one must question if there are any specific ranges or load that show alterations in the commonly occurring strain pattern and so might compromise the tissues leading to degeneration. That is, do the vulnerable areas experience increased load to that which they are used in daily life when under stress in athletic pursuits?

With the availability of pattern matching software, which uses a process of hierarchical block matching in order to optimise both accurate tracking and complex motion within the field (Revell and others 2004) it is possible to analyse movement throughout cine of U/S footage. It is proposed that the main goal of this study should be to analyse images of tendons, in vivo, under physiological load and throughout joint range to see if there is in fact any consistent variation in the strains between areas of vulnerability and the rest of the tissue.

C h a p t e r 3

AIMS AND OBJECTIVES OF THE THESIS

The original aims and objectives of this thesis were developed after experiencing the success of the eccentric exercises as a treatment for tendinopathy in a clinical setting, and from the recent findings regarding the mechanical properties within the literature. It was exciting to note the adaptability and differentiation not just of different tendons throughout the body but of different regions within the same tendon, perhaps dependant on the mechanical environment to which each part was exposed.

A new method of tracking grey-scale speckle within ultrasound scans had just been developed by another member of this research group(Revell and others 2004) and provided an exciting opportunity to analyse the strains not just of the tendon as a whole but of discrete areas within each tendon.

The aims and objectives of this study were to test the following hypotheses:

HYPOTHESIS 1

The Hierarchical Variable Sized Block Matching (HVBM) tracking software will be able to accurately track known movement at a depth equal to that found in the lower limb tendons in vivo.

HYPOTHESIS 2

The HVBM software will be able to accurately track known movement of the speckle produced by tendons with U/S.

Hypotheses 1 and 2 were tested in the Biomechanics Laboratory using an existing Instron material's testing machine and a Diasus Ultrasound machine. As this would not involve human subjects it would not require any Ethical approval. It is described under the **Calibration of Tracked Movement,** Chapter 5.

HYPOTHESIS 3

As the patellar tendon deforms under physiological load when there is an isometric quads contraction it will be possible to collect U/S data of sufficient quality in order to track the displacement with the HVBM software and calculate strains in the tendon.

In order to test Hypotheses 3 an in vivo test was designed using a modified leg extension machine to apply isometric loading to the patellar tendons of 8 volunteers. The Biomechanics Diasus Ultrasound Machine would again be used to collect the images for analysis. This would also be run in the Biomechanics Laboratory and would enlist normal volunteers. In order to run this part of the study it would be necessary to apply for Ethical Approval from the M3 School Ethics Board.

This will be described under Chapter 6 **Visualisation of Movement within the Patellar Tendon using an Isometric Quadriceps Contraction.**

HYPOTHESIS 4

The displacement values derived by the HVBM software when used on an area of strained tendon tissue will be representative of that occurring in reality.

This will be described in Chapter 7, **Validation of HVBM tracking as a measure of strain in tendons.**

HYPOTHESIS 5

At different levels of loading and at different points in the joint range the differential strain pattern observed within the tendon will alter, predisposing some areas to be more vulnerable to injury than others.

In order to test the last Hypothesis 5 it became important to use a bespoke muscle testing machine. This would ensure that the same joint range parameters were examined in each subject and that the same proportions of maximum voluntary contraction were assessed in each individual. The Cybex Dynamometer belonging to the Centre for Sports Medicine at The Queen's Medical Centre was made available for the purposes of this study. Because this part of the study was to be carried out on the hospital site, Queen's Medical Centre and because it was

hoped that at some point in the future it would be possible to use this study design to assess the strain map for patellar tendons in a patient group it was necessary to apply for full Ethical Approval from the local medical Ethics Committee. This investigation will be described in Chapter 8, **Pilot Study of Investigation of Differential Strain in the Patellar Tendon under Load**.

As the work progressed some of the investigations were adapted in order to address issues as they arose. The initial plan had been that this would be more of a clinical study with the emphasis of the investigation being on Hypothesis 5 and the Pilot Study. As the analysis were actually performed it became obvious that the main focus of the study should be developing the method and validating its results in order that the results of any clinical investigations would be well founded.

C h a p t e r 4

METHODS TO TRACK TENDON MOVEMENT

In order to understand the analysis of the tracking it is important to first describe how the two versions of the HVBM tracking software described within this work are used, how they may be modified, and why certain parameters may be beneficial to adjust for the grey scale pattern produced by tendons.

It is then necessary to describe how the output text files produced by this software were analysed in order to produce meaningful data regarding the displacement and strain of the tissues and phantoms.

THE ORIGINAL HIERARCHICAL VARIABLE SIZED BLOCK MATCHING SOFTWARE ('PROJECT')

This software was produced by James Revell as part of his Doctorate awarded in 2004(Revell 2004). The original programme 'project' was designed to track blocks of grey scale speckle pattern throughout a series of bitmap (bmp) images. It was possible to vary the overall size of the bmp analysed, the final block size and the number of iterations, or number of times the initial block was subdivided in order to find the smallest size of block for which to search. These are restricted such that each block in the progressive iterations must be a factor of the overall size of the analysed image and block sizes are defined in pixels. It is this ability of the software to search through variable block sizes for repeating patterns that sets it apart from previous types of tracking software. This combines the accuracy when tracking a large block of pixels with the ability to follow complex displacements within the field when tracking a small block(Revell and others 2004).

The useful data files produced by 'project' are in text format, each file representing the smallest blocks examined within the first analysed bmp. They are x displacement, y displacement, correlation coefficients and vectors, visual representations of the displacements of the final

blocks throughout the series (see Figure 9 one bitmap from a cine of sponge immersed in agar after the vector representations have been overlaid).

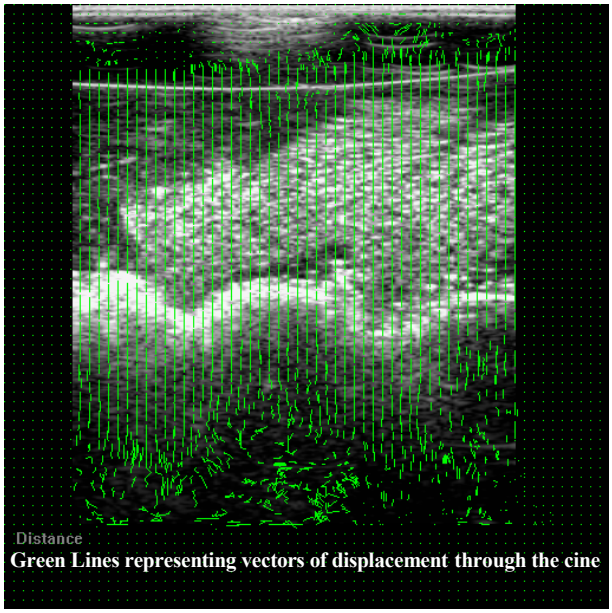


Figure 9 Vectors on a cropped bmp from the original cine of a sample of agarose and sponge as it is compressed by the U/S probe

It is a useful first check of the accuracy of the tracking to view the vectors in order to see that the general movements have been followed. Analysis of the x and y displacements and the correlation files involves inputting the text data into an array in order to be able to match the region of interest in the original bmp with the values in the output files.

By looking at the correlation files one can assess how well 'project' has been able to match the final blocks throughout the cine with the original grey scale speckle. This is produced in the form of correlation coefficients which can then be used to see where within the image the tracking has been most successful.

Analysis of the displacements can then be made by looking at the region of interest within the x and y data arrays.

THE SECOND VERSION OF HVBM SOFTWARE (‘PROJECT2’)

As this study progressed a more advanced version of the HVBM soft ware was produced (‘Project2’). The original version was entirely encrypted code with no opportunity to influence the analysis beyond choosing the final block size and number of iterations used. ‘Project2’ had the advantage over ‘Project’ that although it too was encrypted it referred to a secondary script file (‘Advance’) for refinements in its processing and this script file was able to be edited in order to affect those adjustments. See Appendices for settings within ‘Advance’. This progression made ‘Project2’ adaptable for operators to use it in the most appropriate way for their own particular data type(Revell and others 2005). This version also produced arrays of xx strain, yy strain, shear strain and rotational strain. The original version ‘Project’ was used for most of the work in Chapter 6 as this was started prior to ‘Project2’ becoming available. ‘Project2’ was used in Chapters 5, 7 and 8, the calibration work (Chapter 5) being repeated when the new software was produced in order to find how best to adjust the code for tendons.

The settings within ‘Advance’ were altered to see the effects on the tracking of displacements with SDFT within the **Calibration** study, Chapter 5. The best match to the actual displacement was using the default values as selected by the programme author, apart from Drift, which improved the accuracy of displacement tracking in Equine tendon when enabled. The Correlation threshold was increased to 0.93, for although the author of the code had felt a low correlation would be suitable for tendons due to the high definition of the high frequency probes used the nature of the speckle produced by tendons and the potential effects on the speckle by movement or strain within the tissue potentially reduced the accuracy of the tracking. This will be discussed further in Chapter 5. See Appendices for actual variable settings within ‘Advance’ and how they affect the running of ‘Project 2’.

THE CALCULATION OF STRAIN FROM DISPLACEMENT FILES

Although ‘Project2’ has the facility to calculate strain in the tissue it tracks as part of its programme, it was decided that an encrypted code did not offer enough clarity within its

calculations to rely on its results. It was felt more appropriate to use the displacement values produced to calculate the strain in a more transparent way.

Because we are looking at an original bmp of 512 x 512 pixels which is then subdivided into final blocks of 8 x 8 or 16 x 16, the final arrays will then be a minimum of 32 x 32 blocks of numbers. In order to process this a tool must be used which can manipulate large arrays of data. In the initial calibration of the TMM Xcel was used. This involved inputting the data in by hand into one work book and then performing a cross workbook calculation to find the average displacements. In order to deal with the large volumes of data output expected from the majority of the in vivo work MATLAB was employed and code was written in order to process the output arrays from 'Project' and 'Project 2' see Appendices.

CALCULATION OF STRAIN FROM THE HVBM OUTPUTS USING MATLAB

The initial calculation of strain from the 'project' output files used code which loaded all the data files, calculated the strains in the x and the y directions and then the shear strain.

(See Appendices)

Strain was calculated using a basic formula for Engineering Strain:

$\varepsilon = \Delta L/L$

where: ε = engineering strain

ΔL = change in length

L = original length

By calculating the change in the distance between three mid block points within the array and dividing this by 2 times the original number of pixels between each of the mid block points one can derive the strain for that particular portion of the structure. In Figures 10 and 11 below the final 8 x 8 pixel blocks are shown to illustrate the blocks as they have been used.

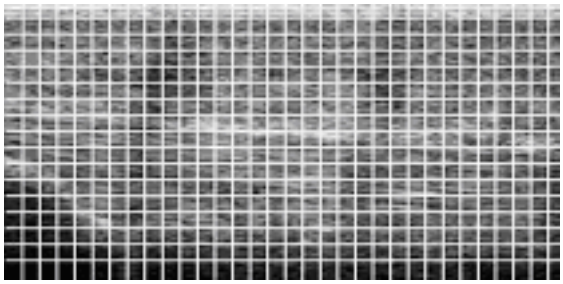


Figure 10 Part of bmp image of patellar tendon showing 8 x 8 pixel final blocks to be tracked

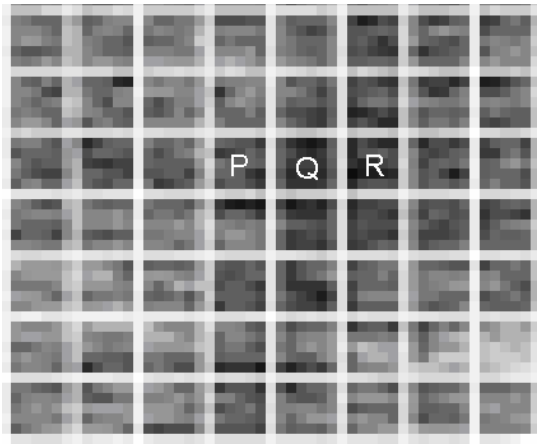
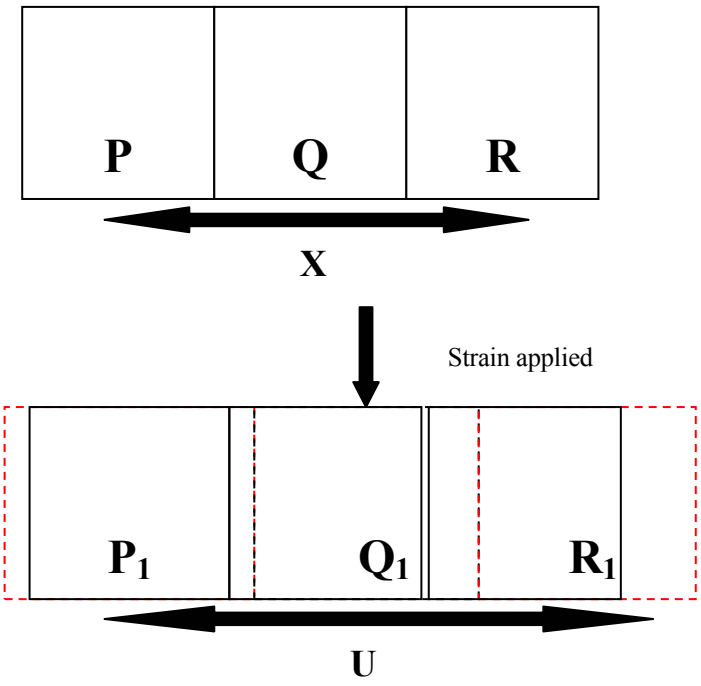


Figure 11 Sample of 3 consecutive blocks for strain calculation



To calculate change in length (ΔL) for tissue lying between the midpoint of blocks P, Q and R:

ΔL (U - X) = (displacement of R – displacement of P)

(Strain xx (ε) = ΔL/ L)

Strain xx (ε) = (U – X) / X

Original length for each block of tissue (L) is taken to be the smallest (final) block size searched for by the HVBM.

L = 2 (final block size *)

* in pixels

This calculation was repeated using displacements in the y direction to find the strain in the y direction, yy strain. In order to find shear strain it was calculated how the tendon was strained between the mid points of x blocks in the y direction and strained between the mid points of y blocks in the x direction. This calculated the xy strain and the yx strain.

The final calculation for shear was taken to be:

ε_{xy} = the mean of the xy and yx strains described above

where: ε xy = engineering shear strain (Solecki and Conant 2003).

In order that the region of interest (roi) within the original bmp could be isolated from the outputs of strain, colour-map grids of the strain field were produced (Figure 12). It was then possible to overlay the colour-map over the original bmp to locate areas of potential interest within the structure being assessed (Figure 13).

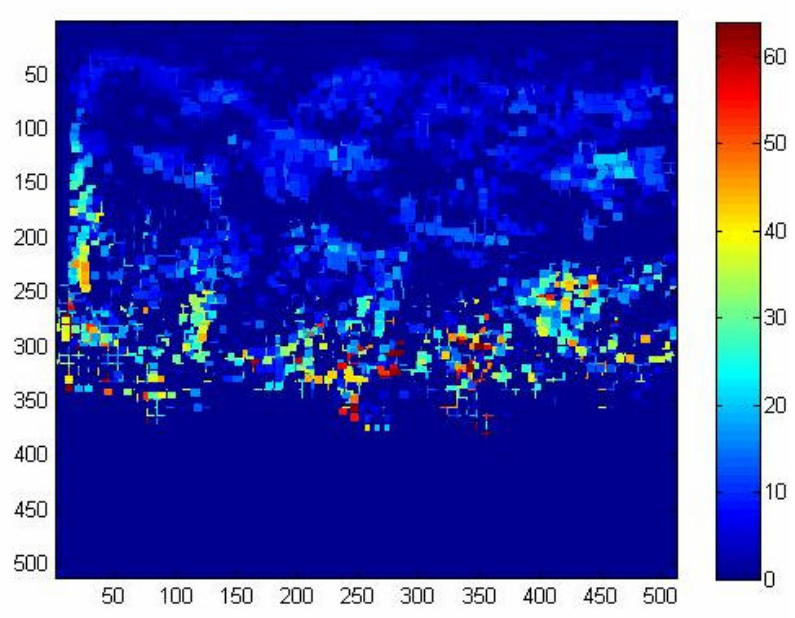


Figure 12 Colour map of % xx strain across the patellar tendon image (where the grid represents the x and y co-ordinates of the original image and the colour bar represents % xx strain)

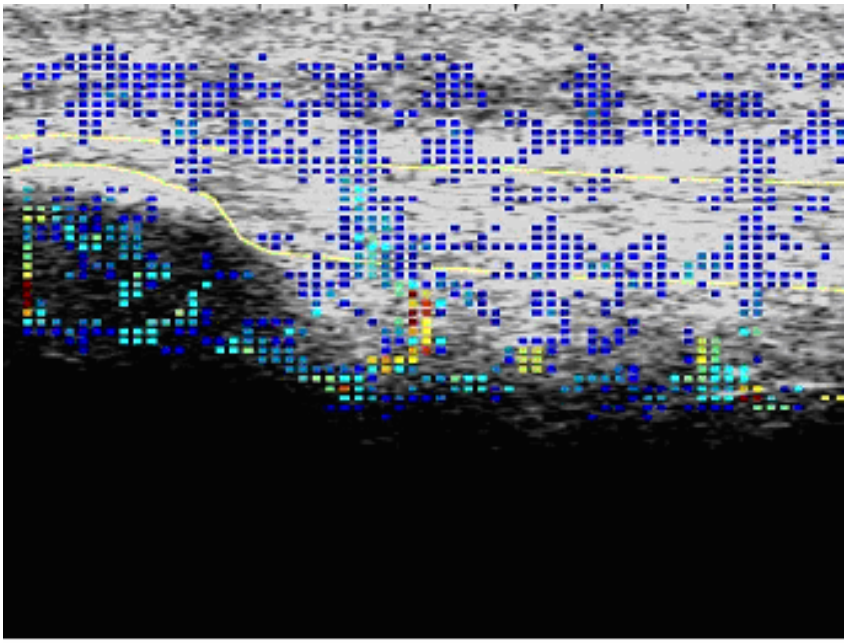


Figure 13 Colour map of % xx strain overlaid onto region of interest marked on patellar tendon

As seen from Figures 12 and 13, what at first may appear to be interesting areas of strain in the lower part of the colour map may not in fact be tendon at all but infra-patellar fat-pad or shear at the tissue interface.

This code ran as one large programme, which took a long time to run for each set of data. It was also difficult to easily relate the information within the calculated arrays to actual areas within the image in order to calculate the average strains for any one specific area of the image. Using this very basic method of strain calculation also meant that any block that had been poorly tracked could have a marked effect upon the results. In order to be able to smooth the results in such a way that outliers would be allowed for and to speed up the running time it was necessary to alter the code.

To improve the efficiency of the code it was appropriate to divide the process into a series of functions which would then run as part of an overall function. It was also necessary to be able to define the main region of interest from the original bmp and so one of these functions needed to produce the original cropped image in order that the operator could select the appropriate region of interest to be examined (Figure 14) to then select the appropriate portion of the output displacements and strain.

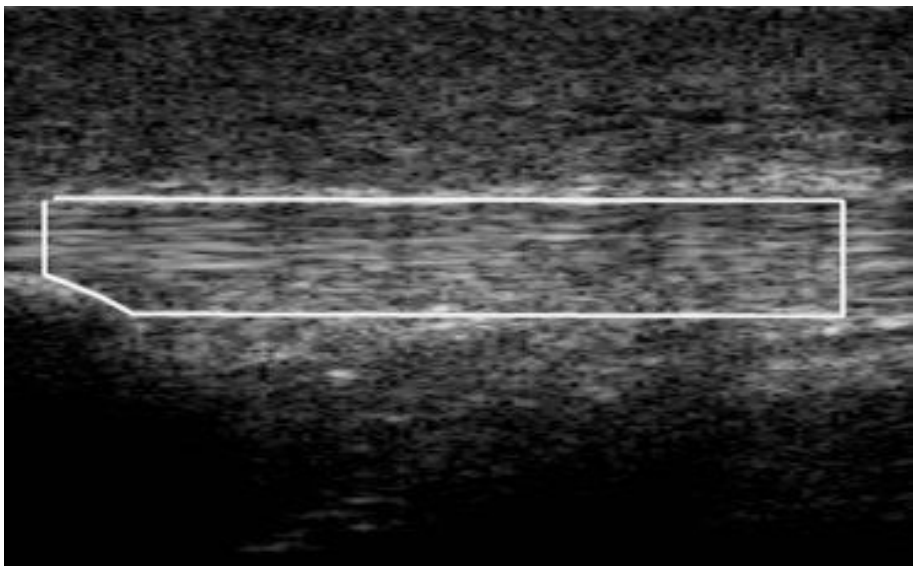


Figure 14 Image of patellar tendon with region of interest (tendinous tissue) marked for cropping extract important strain and displacement outputs

The code writing to produce the appropriate functions required a good working knowledge of Matlab or C-code in order to produce it in the time frame required and so the basis of a number of functions were produced by Dr DS McNally for general use in conjunction with the HVBM. These were then adapted and modified for this particular project in order to extract the most useful data for scrutiny (See Appendices).

In essence the data arrays were loaded into large 3 dimensional arrays on which the calculations could be performed in order that when the region of interested was located from the original bmp

all the data stored within the large arrays could be 'punched out' and then analysed through time (see Figure 15).

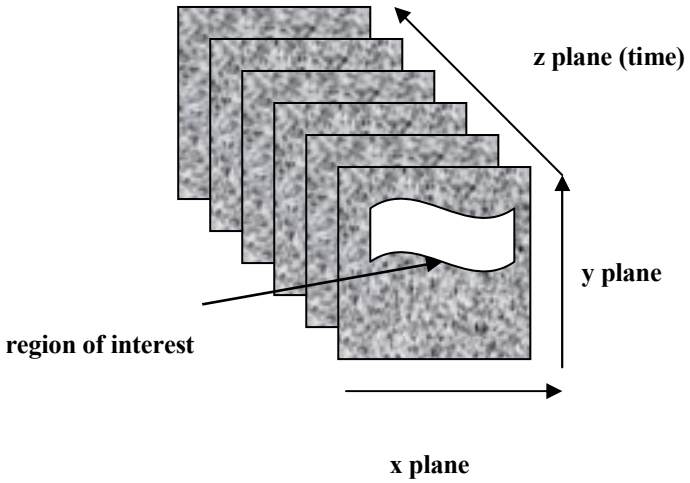
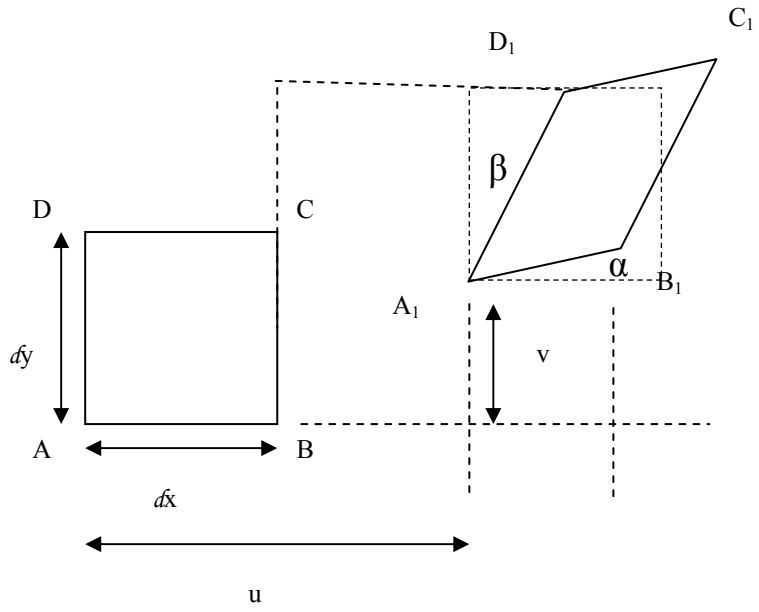


Figure 15 Three dimensional array representing displacement/ strain arrays through time within a cine loop

The strain calculations are basically the same as those listed above for the earlier code but rather than relying on 3 specific blocks (any one of which may be an outlier to the general trends for that region of interest) it is possible to determine for an infinitesimal point within the structure the calculations for the xx, yy, xy and yx strain values for that point.



u = displacement in the x direction

v = displacement in the y direction

$\varepsilon_{xx} = du/dx$

$\varepsilon_{yy} = dv/dy$

ε_{xy} = shear strain = $(\alpha + \beta) / 2$

$\varepsilon_{xy} = \varepsilon_{yx} = (du/dy + dv/dx) / 2$

(Feynman 1964; Solecki and Conant 2003)

This enables us to calculate the strains based on the gradients of the lines of best fit (using the polyfit function within Matlab) for each different description of the strain.

ε_{xx} is a description of how the x displacements vary through x

ε yy is a description of how the y displacements vary through y

ε xy is a description of how the x displacements vary through y

ε yx is a description of how the y displacements vary through x

As this is a function made up of smaller component functions to determine displacements, speeds of displacements and strains in the x and y directions, it is easy to break down and extract specific information with respect to the frames concerned and the different regions within the image. The function as a whole outputs graphical illustration of these values through time (Figure 16) and superimposes strain maps onto the image as a whole through time (Figure 18).

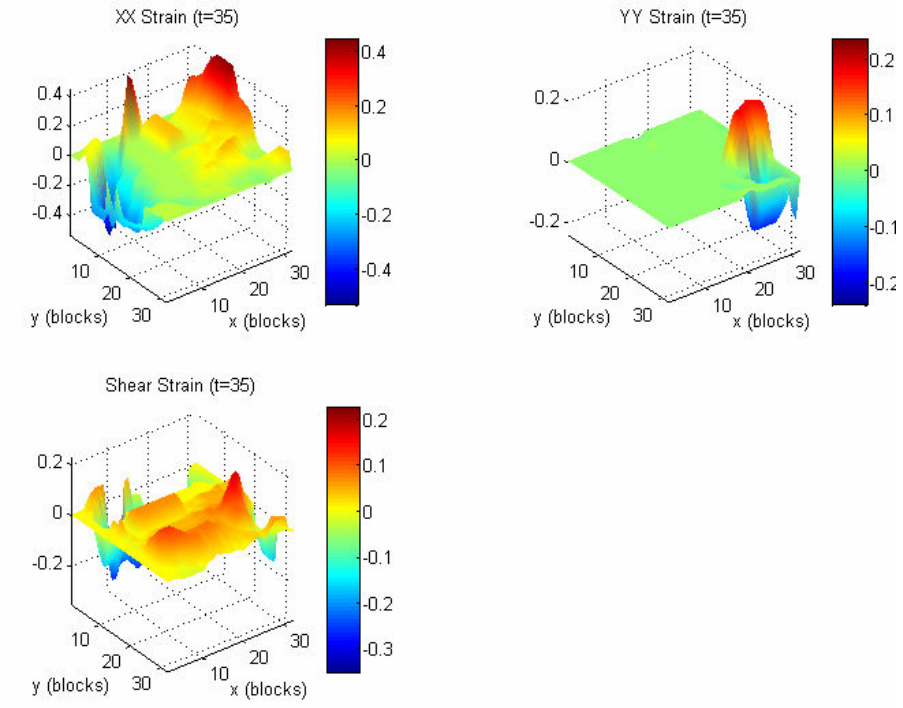


Figure 16 Strain graphs representing the amount of xx, yy and shear strain for a patellar tendon at the final time point after the subject has recruited the quadriceps muscle to work at maximum, isometrically

The above three graphs represent the distribution of xx, yy and shear strain across the tendon field where the x and y axes of the graphs are equivalent to the x and y co-ordinates, of the final blocks, within the original bitmap image of the sequence. The z axis is representative of the level of strain. The negative strain values obviously relate to compressive strain in all cases and it was important to ensure within the following chapters that this is an accurate representation of what happens to the tissues and not an artefact.

By looking at the graph of yy strain and relating it to the original image of the tendon as in Figure 17 we can see that the yy strain above, both tensile and compressive is in an area of the field that does not relate to tendon. That is, a large proportion of the original image is not the tendon itself.

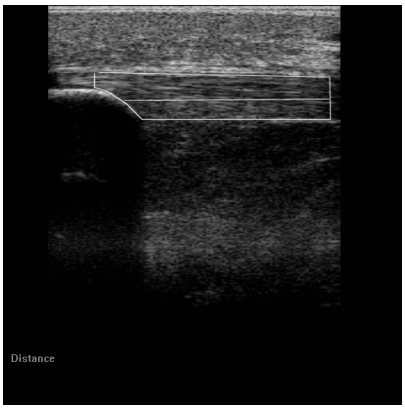


Figure 17 Original bitmap of sequence of patellar tendon being strained as a result of an isometric quads contraction (tendon outlined prior to cropping)

When examining the graphs of xx strain and shear strain from Figure 16 it is difficult to assess whether the strains represented, both tensile and compressive relate to tendon tissue or not and so further images were produced to give an impression of where the strains were occurring with respect to the actual cine. In this case the strains were represented by colours superimposed upon the images throughout the sequence as the strain varied, see Figure 18. As in the graphs in Figure 16, by using red to represent tensile strain through to blue to represent compressive strain, although not intended to be used to glean actual numbers, this gave a visual snapshot of where xx

strain, yy strain and shear strains were occurring as the tissue moved and as the strains were calculated by the Matlab code.

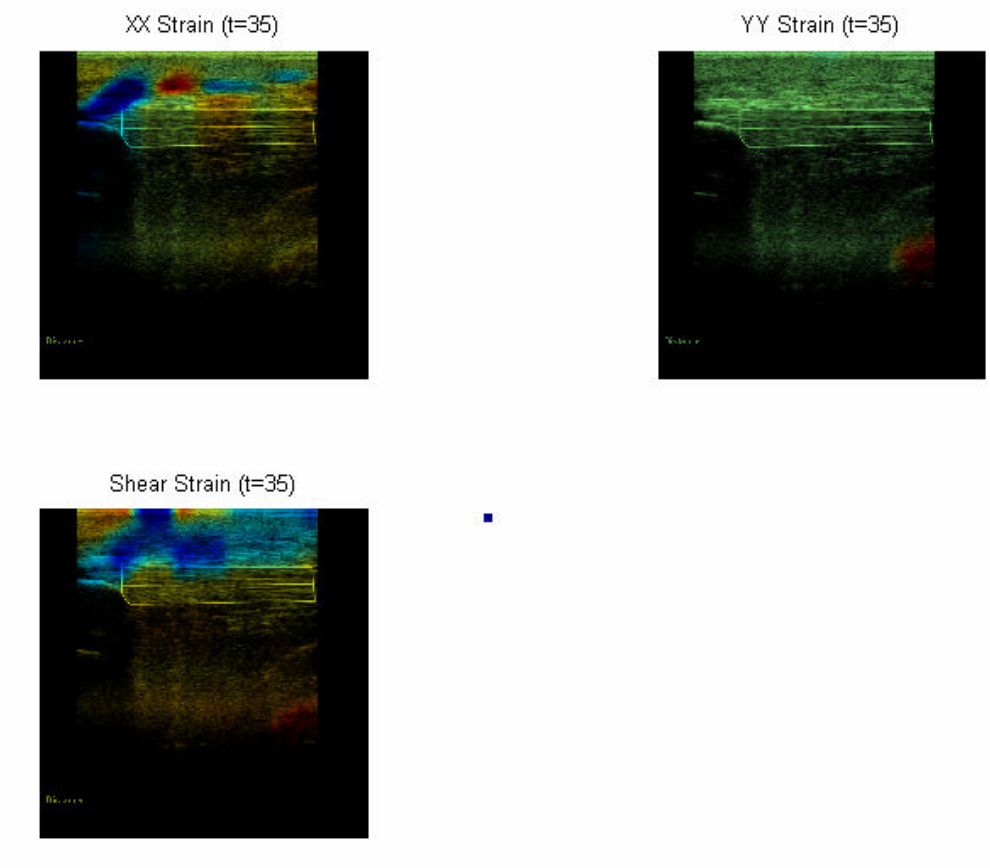


Figure 18 Illustrations of xx, yy and shear strain at the final time point superimposed onto original image

Finally by asking the operator to crop the region of interest (ROI) from the original bmp as illustrated in Figure 17, the function will then produce the mean values of xx strain, yy strain and shear strain for that ROI through time, velocity in both the x and the y direction through time and the displacements tracked in the x and the y direction through time (Figure 19).

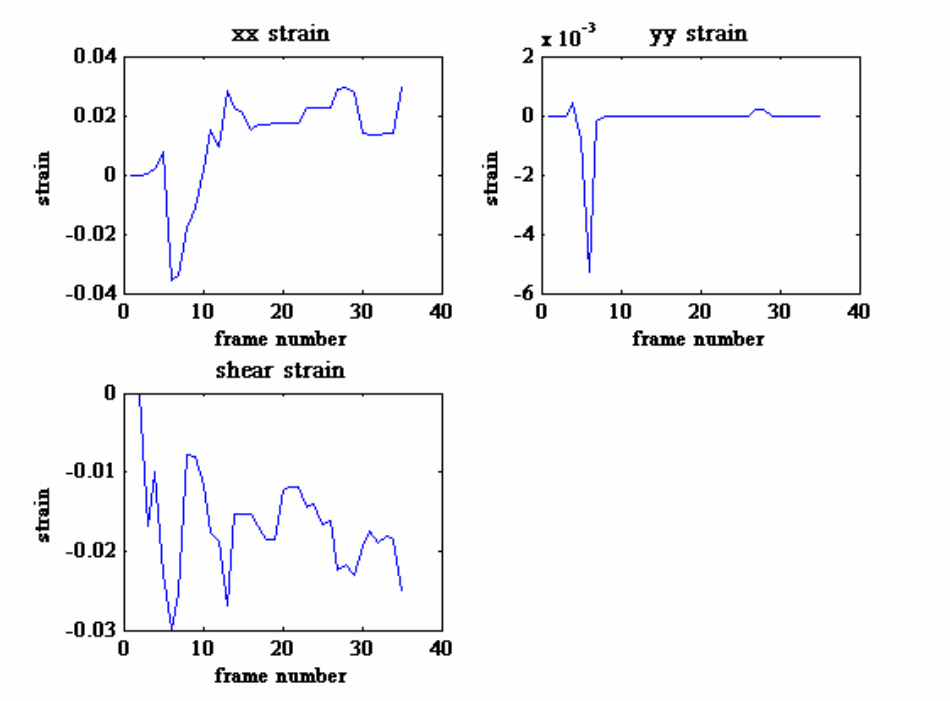


Figure 19 Graphs of xx, yy and shear strain v frame for the selected region of interest after cropping

The above graphs represent an example of the strain as it varies with each consecutive frame throughout the cine.

From these outputs one can select further regions of interest from within the tendon field and compare the strains to those found elsewhere. By cropping all of the tendon visualised within the first image the average value of the strain throughout that part of the patellar tendon may be found, using the code described above. This would be representative of the strain within the middle and proximal parts of the tendon as the U/S footprint was unable to capture the whole tendon in one image.

Chapter 5

CALIBRATION OF TRACKED MOVEMENT

In order to use the HVBM software with confidence in vivo it was important to test its ability to track known movements using tissue mimicking phantoms to validate its capability of producing reliable analysis of movements at similar tissue depths to that required for the in vivo work. It was then necessary to track known displacements in tendon tissue proper in order to modify the software settings to those optimum for the speckle produced by tendon fibrils in ultrasound scanning. Equine superficial digital flexor tendon (SDFT), a tendon known to be vulnerable to similar injuries to that seen in humans(Firth 2000), was dissected out to use for this. In Hypothesis 1 and 2 it was expected that the HVBM software 'Project2' would be able to both track tendon speckle accurately and at a depth of 10 to 20 mm which is roughly comparable to that of the patellar and Achilles tendons in humans.

METHOD

TISSUE MIMICKING MATERIAL (TMM)

Detection of normal and abnormal structures on ultrasound B-mode images must be done in the presence of material 'speckle'(White and Wambersie 1999) The material must be easy and safe to make whilst being able to rely on the uniformity of the mixture on each production. It was not important to have a medium that retained its form over a prolonged period as the collection of the data was planned to be run for a matter of seconds, but it did need to be stable enough to repeat the experiment up to three times to ensure repeated tracking. The original phantom produced by a previous member of our research group was made from a mixture of agarose, n-propanolol and evaporated milk made up to the recipe recommended by Madsen, Frank and Dong(Madsen and others 1998). Unfortunately this phantom, although providing good material speckle from the emulsified milk droplets also contained mercury and an organophosphate within its preservative, which was hazardous, both to store and to use. Trials were made using synthetic sponge within the agar, fine needles within a silicone base and inclusions of more concentrated areas of gelatine

within a gelatine base. All these materials have been used as the base of phantoms trialled for their acoustic impedance values(Zell and others 2007). Fruit inclusions within gelatine were also trialled. The images produced by these phantoms are illustrated below (Figures 20 - 22).

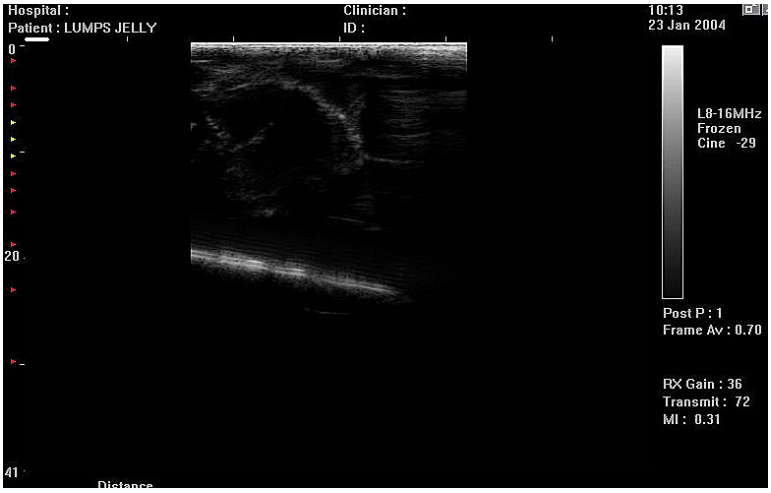


Figure 20 Concentrated areas of gelatine within jelly

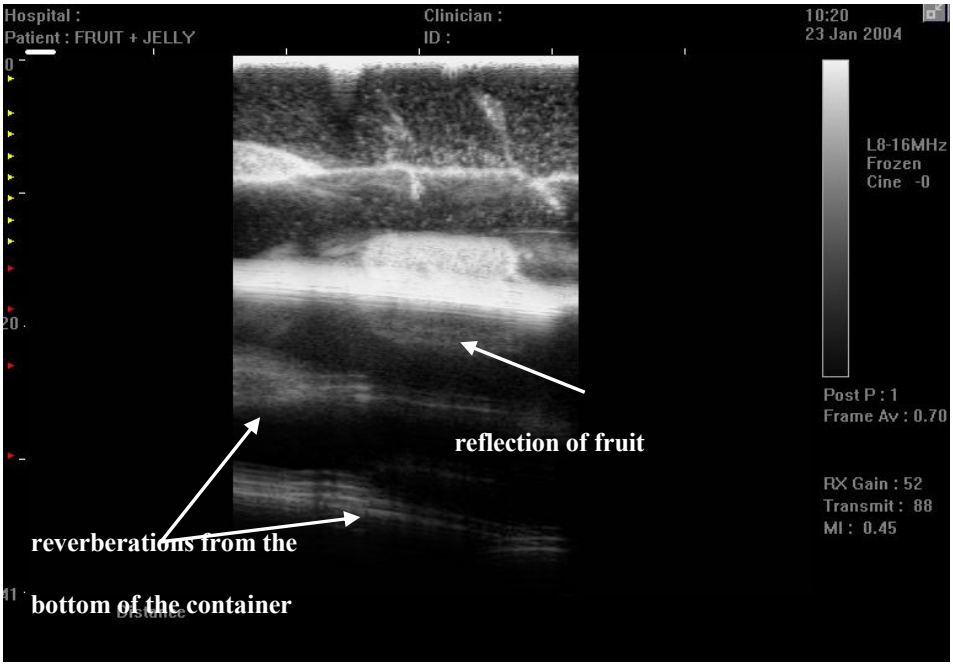


Figure 21 Fruit pieces in gelatine

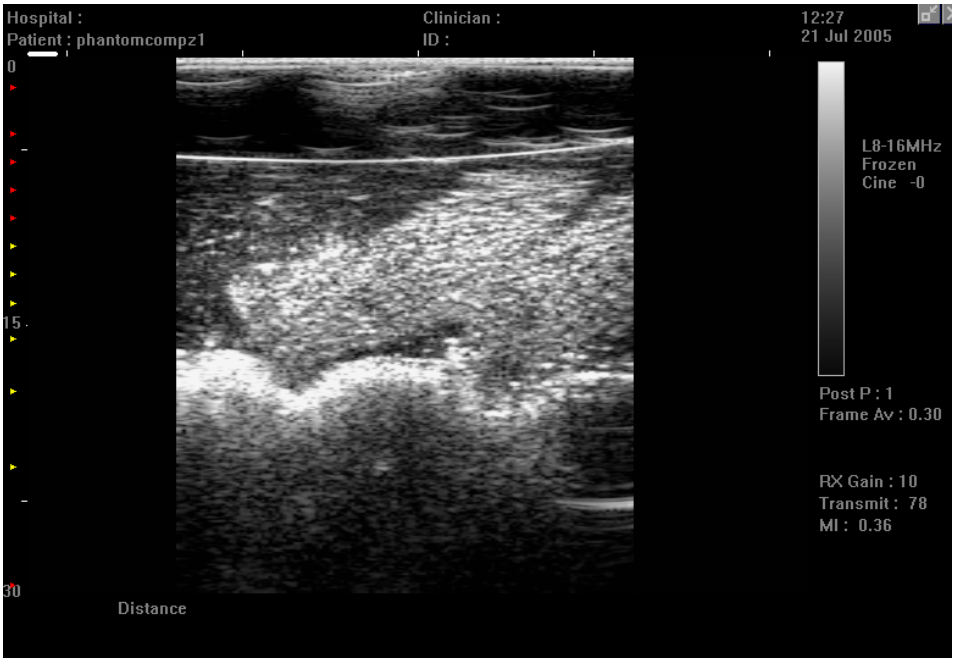


Figure 22 Sponge within agarose gel

There is no image of needles within the silicone here as the quality was too poor.

RESULTS OF THE TMM TRIAL

- The gelatine based phantoms were found to degrade easily when exposed to pressure from the transducer head and the aqueous gel applied as a connecting medium and did not provide adequate material speckle. It is also important to note that as it was necessary to maintain them within a container to retain their form it was difficult to prevent reflections from the base of the container illustrated in the fruit and gelatine image as shown in Figure 21.
- The silicon phantom with fine needle inserts did not produce an image with sufficient detectable speckle to either visualise or to track and so was discarded.

The 4% agar solution was made by dissolving agar powder in water and heating to 80º C continuously stirring until the solution became clear and all the powder was dissolved. At this

stage pieces of synthetic sponge were immersed within the solution to absorb agar and provide the speckle. The material was then allowed to cool until it became a solid and could be removed from its container for testing. There was not seen to be any obvious degradation as a result of usage or time. Agar gel and sponge provided a resilient and easily produced TMM which provided good speckle. Although there was some dehydration over a period of several weeks should it be required for use again it was easy to reconstitute by heating to 80º C and allowing it to set within a mould for further use.

EQUINE SUPERFICIAL DIGITAL FLEXOR TENDON (SDFT)

Portions of SDFT were harvested from cadaveric equine legs acquired from The University of Nottingham Veterinary School and stored at -20˚C until required. The tendon was allowed to thaw prior to testing, while wrapped in tissue soaked in saline to maintain hydration of the sample. While testing it was kept moist using regular spraying with saline. It was not important to mimic exactly in vivo conditions as the tissue would not be being strained or tested for its properties in any way. The purpose of the tendon was purely to provide speckle as produced by the tendons in vivo to test the software's ability to track a known displacement.

TESTING PROCEDURE USING TMM

At all times throughout this Thesis a Diasus, 'Dynamic Imaging', U/S machine was used to collect the images in the experimental work. The frames were cropped to 512 x 512 pixels and were captured at a rate of 8.6 Hz. The linear array transducer was 8 – 16 MHz which can visualise to a depth of 40 mm. To displace the probe with respect to the TMM and to displace the SDFT with respect to the probe an Instron 5960 Dual Column Table Top Universal Testing System was used with a position measurement accuracy of ±0.01mm or 0.05% of displacement whichever is greater.

The transducer was clamped vertically above the TMM and a large amount of ultrasonic gel was used to ensure air free contact between the two. The transducer clamp was secured to an Instron materials testing machine in such a way that the downward stroke of the Instron was applied to

the aqueous gel via the transducer head. The downward stroke was set to move by 2mm which produced a series of images of movement of the U/S transducer with respect to the TMM. There was no or only negligible compression in the y direction, i.e. the probe moved toward the top surface of the TMM without deforming it (Figure 23). The HVBM software was then used to track and analyse the resultant movement. Calculation of the displacement was made by taking an average of the displacement outputs in the y direction for the area correlating to the TMM within the original image. (N.B. in this context, x and y correlate to the pixel co-ordinates axes on the U/S images.)

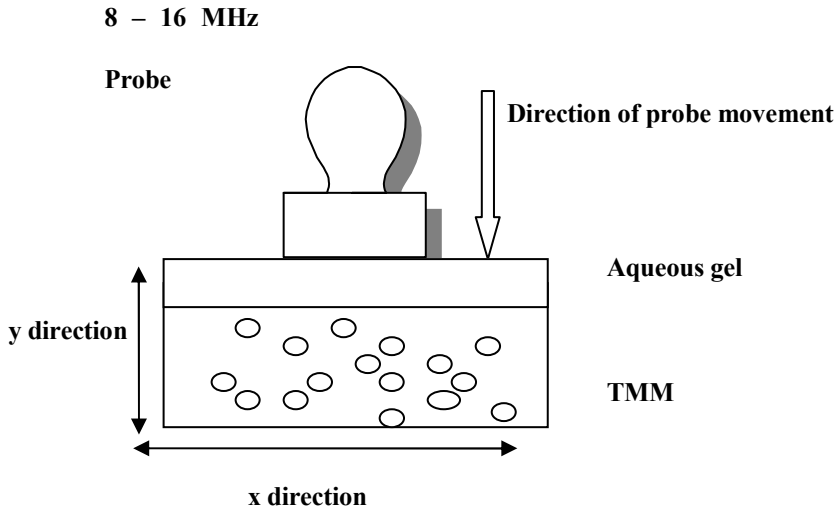


Figure 23 Probe displacement in the y direction

TESTING PROCEDURE USING SDFT

Examination in vivo of the amount of movement of the patellar tendon when the quadriceps applied a maximum isometric contraction at 90˚ of flexion revealed that although there was some variation in the amount we could expect the tendon to move between different subjects, generally displacement was in the region of 1 – 4 mm. To comply with this the displacement of the SDFT with respect to the U/S probe was set to 2 – 4 mm. Rates of 0.2 mm/sec and 1 mm/second were

set to 2mm displacement and 5 mm/sec was set to 4 mm. Essentially the variations were selected to see how well the HVBM coped with different rates of displacement.

A portion of Equine deep digital flexor tendon was harvested, as described, and clamped between the jaws of the upper Instron clamp. The lower end of the tendon was similarly clamped but not attached to the Instron in order to ensure the sample hung vertically. The U/S probe was then clamped in such a way that it remained stationary as the Instron moved the tendon (Figure 24) upwards at the 3 different rates, 0.2, 1 and 5mm per second. The bmp images produced by the Diasus after recording the movements were then analysed using the HVBM software and as in the case of the TMM the average displacement output was calculated from the regions of the output arrays areas correlating to the main body of the tendon within the original image.

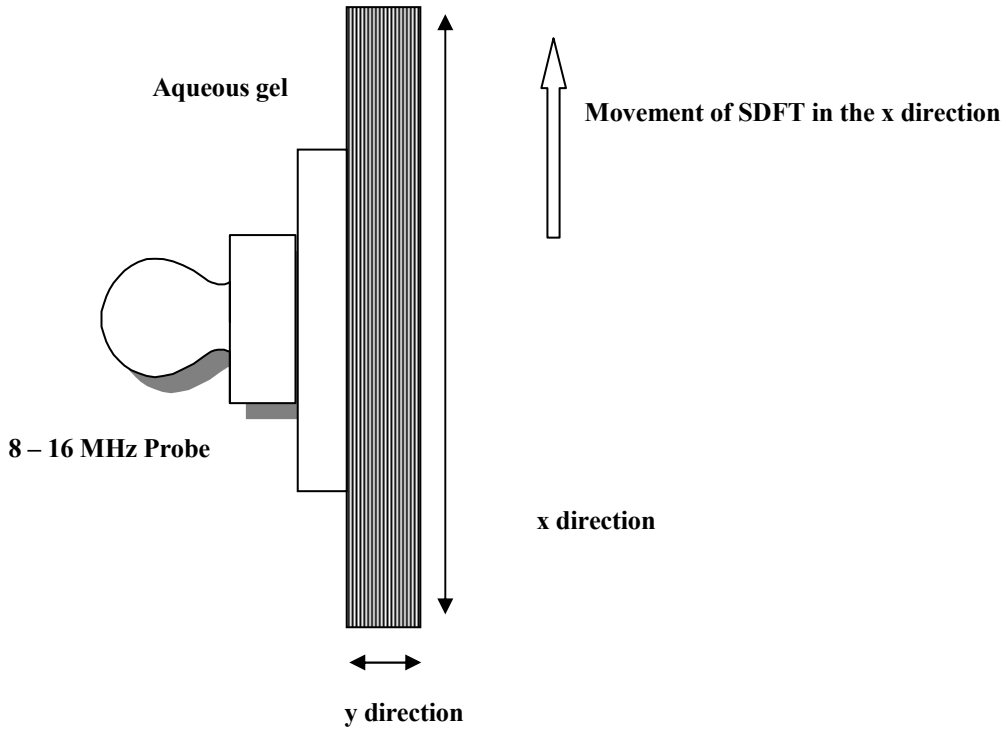


Figure 24 Probe capturing displacement in the x direction with respect to the image produced

ANALYSIS

The HVBM programme tracking parameters were set to analyse cropped images of 512 x 512 which included the relevant area of each frame. For the TMM analysis the default settings were used so that the final box resolution was set at 8 pixels with 4 iterations.

The outputs of x-movement and y-movement for each frame are given in pixels and this was converted to mm by assessing the number of pixels between 2 fixed points 20mm apart from one of the bit map images from the cine loop being analysed. These points are located at the top left corner of each Diasus image. This gives the operator a scale when assessing the extent of the footprint and the depth at which they are imaging. The scale in this image was found to be 13.5 pixels/mm (Figure 25) but it was re-calibrated for each new set of images.

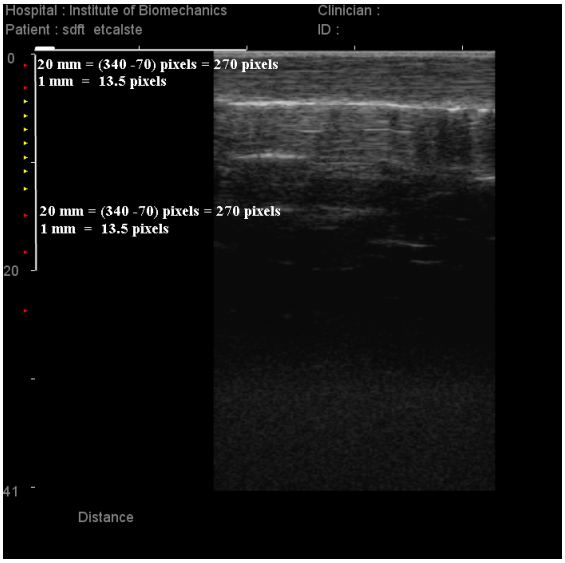


Figure 25 Diagram of calculation of pixels/mm in both the x and the y direction

When the movement of the tendon with respect to the probe was analysed it was important to ensure that the HVBM could track displacement consistently with even the smaller final block sizes so block sizes of 8,16 and 32 pixels were assessed. Obviously the smaller the final block the better able the software will be to track discrete variations in displacement within the field.

The compromise of this, however, is that the smaller blocks may lead to less accurate tracking. Further changes were made to compare enabling of the various smoothing parameters in the secondary script file working with 'Project2', 'Advance' described in Chapter 4, **Analysis**.

RESULTS

BULK MOTION WITH RESPECT TO TMM

The motion of the Instron was set to produce a triangular profile with a maximum displacement of 2 mm over at a rate of 0.5 mm per second.

Over a series of 3 compressions the cine was set to record 66 frames allowing for the recording of 8.68 seconds of footage. The average displacements of all the blocks throughout the TMM field on each series of images from tracking outputs were found to be: 2.02mm, 2.07mm and 2.09mm. (N.B. error of the Instron displacement ± 0.01mm)

In the case of tracking in the y direction movement with respect to TMM there is excellent correlation with reality, correlation factor 0.9972 (Figure 26).

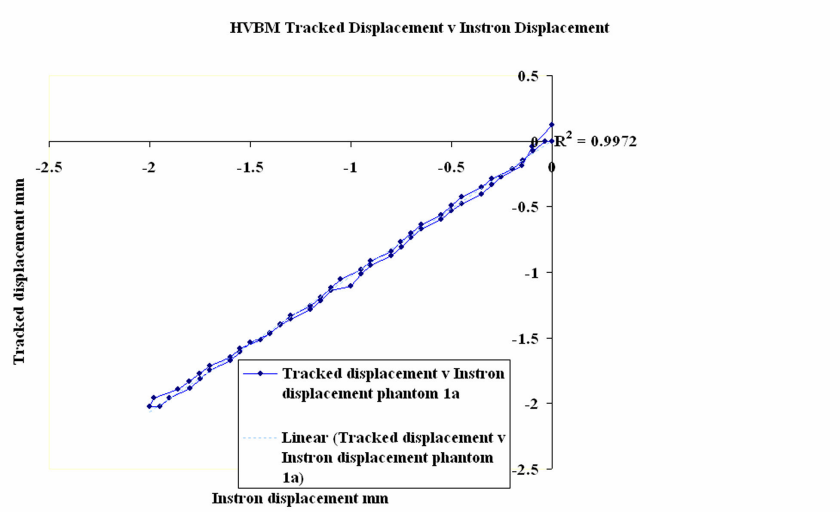


Figure 26 Sample graph of tracked displacement v Instron displacement

BULK MOTION WITH RESPECT TO SDFT

As described in the Method the Instron was set to displace the tendon at rates of 5mm/second, 1mm/second and at 0.2mm/second. The cine produced for each rate was analysed using the HVBM and the average displacement outputs for the region correlating to the SDFT within the original image was calculated and compared against the real Instron displacement.

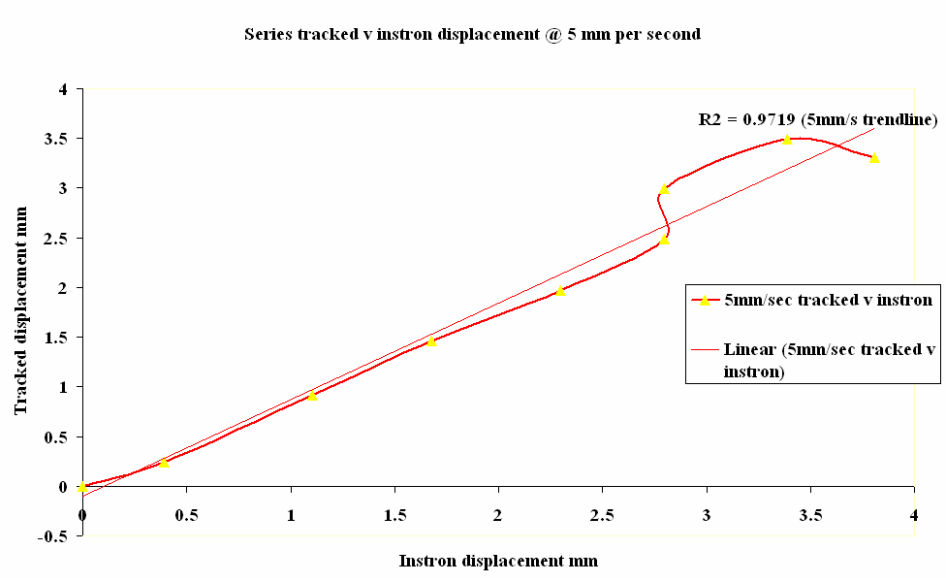


Figure 27 Instron v tracked displacement @ 5 mm/second

In Figure 27, where the tracked displacement for a rate of 5 mm per second is plotted against the Instron displacement, we see there is a good correlation coefficient of 0.9719 and the HVBM tracking of the displacement matches the actual Instron displacement through to the final stages of displacement. It is conceivable that at a displacement of 3 mm there may be areas within the overall region of the SDFT that have left the image and similarly new portions that have joined it which may confound the tracking somewhat. Alternatively the speed of displacement may lead to anisotropy. That is the speckle may alter due to small alterations in the angle of the tendon with respect to the probe. Especially when we consider that the dissected tendon is not a uniform diameter throughout its length.

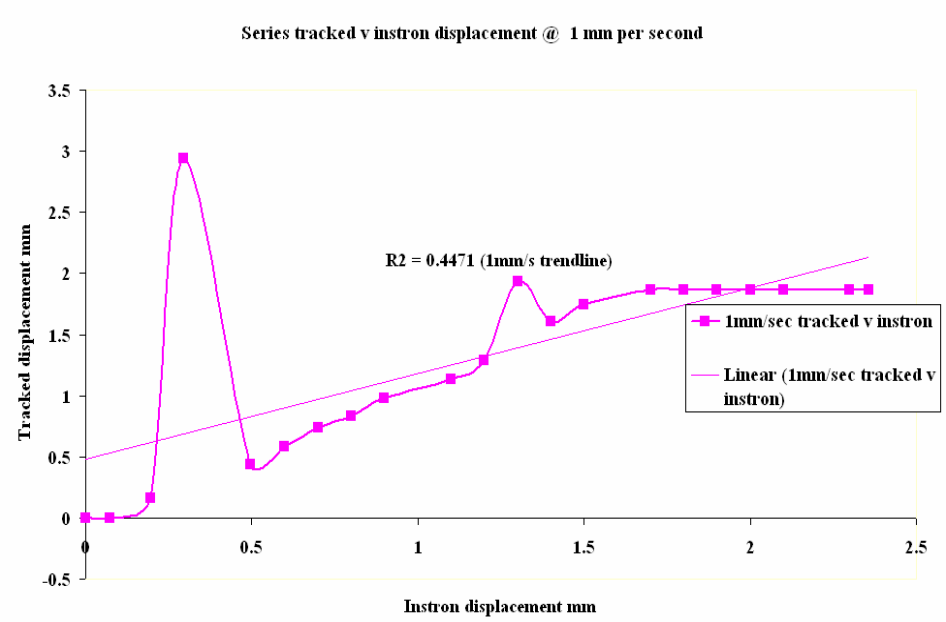


Figure 28 Instron v tracked displacement @ 1 mm/second

When the rate of displacement is altered to 1mm per second, Figure 28, the apparent linear regression is poor and the correlation coefficient is low ($R^2 = 0.4471$) but this is largely owing to two values which may be due to an error in the pattern matching at these time points. If they are removed the resultant graph has much improved correlation (Figure 29).

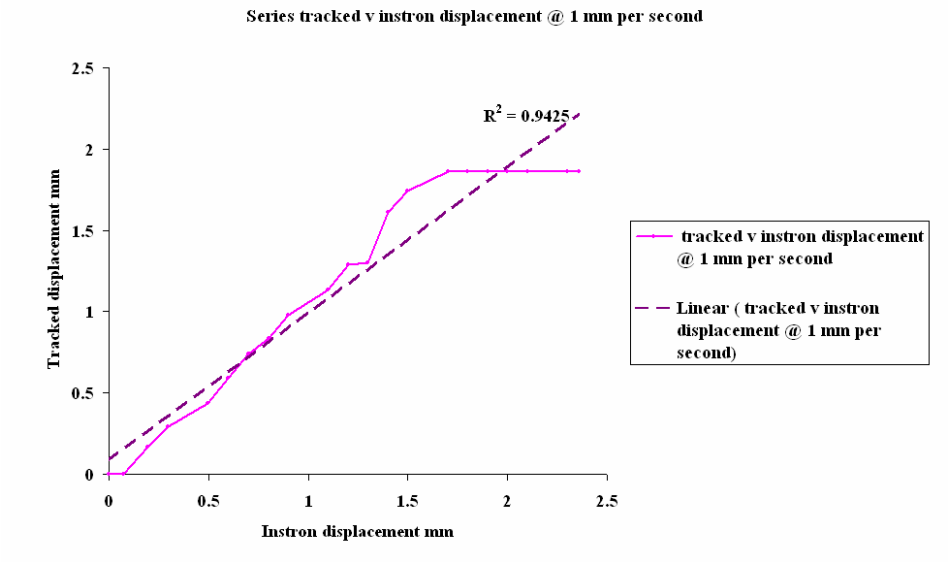


Figure 29 Instron v tracked displacement @ 1mm/second (4th and 12th values removed)

The correlation coefficient then becomes $R^2 = 0.9425$. It is important not to ignore these rogue values and be aware that when using the software to track in vivo the overall displacement through time will be more important than that seen at any one time point, and strain and displacement should perhaps be smoothed through time to account for random errors in tracking.

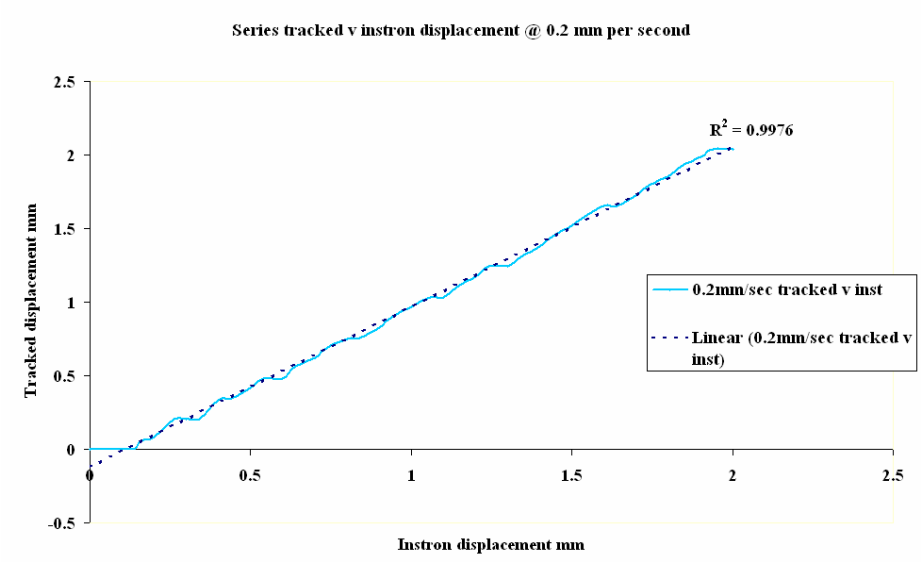


Figure 30 Instron v tracked displacement @ 0.2 mm/second

At a displacement rate of 0.2 mm per second as in Figure 30 there is good correlation between the displacement as tracked by the HVBM and that actually produced by the Instron, with a correlation coefficient of 0.9976.

DISCUSSION

BULK MOTION WITH RESPECT TMM

The results show good correlation between the actual displacement of the probe as recorded by the Instron and that tracked by the HVBM software. This indicates that on tissues such as inter-vertebral disc material where the movement is in the plane of the U/S beam and at a depth of 10 - 20 mm we can be confident that the tracked analysis at its default settings is a true and accurate representation of the actual movement.

BULK MOTION WITH RESPECT TO SDFT

The general profile of the tracked motion recorded on the tendon is good. This indicates that the tracking is able generally to match well at all the rates assessed. There is some suggestion of loss of tracking toward the end of the sequence at 5mm per second. One would be able to gauge this

by examining the original cine and visualising at which frame the movement reaches its conclusion in reality. Similarly examination of the vectors should illustrate where the tracking coincides with the actual displacement overall.

Looking at the tracking at 1mm per second there are time points within the sequence where the tracking is not a good match for the real displacement. Should this occur in vivo then one must examine the vector images closely in order to see whether the tracking is a believable match for the actual displacement throughout all frames. If outliers pertain to discrete areas within the overall image one must bear in mind that tissues tend to move in a similar fashion to the general trend of that particular tissue.

It is interesting that displacement at the slowest rate of 0.2 mm per second appears to produce the most reliable results. This may be because there is less movement of the original footprint of the tissue outwith the field, or it may be that as the rate of displacement increases there is more risk of an artefactual change to the grey speckle due to anisotropy. This may result in loss of the original blocks of speckle either for one or two frames within the cine (as seen at 1mm per second) or perhaps loss of the original speckle altogether towards the end of the cine (as seen at 5mm per second).

Examination of the vector images will give an idea of the reliability of the tracking overall in vivo, however, when examining regions within the field it will be important to check that the displacement continues in an orientation and at a rate that matches the overall trend for that region. One might argue that this will not allow us to see all discrete movements throughout the field but I would suggest that tissues are unlikely to suddenly alter the direction or rate at which they move unless there is a rupture or some similar event.

When examining the in vivo displacements it is important to look at the motion of the tissue through time and not just to rely on one snapshot in time. Perhaps it would be useful to slow the speed of muscle contraction when examining tendon strain in vivo although this must be balanced with what is nearest to functional loading in rate.

It may be that altering the capture rate of the U/S images might be able to improve the correlation of the tracking to the real displacement; however this was not an option available to us as Diasus has a preset rate of capture of 8.6 frames per second.

'STRIPES OF STRAIN'

When the original SDFT analysis was examined using the default settings of a smallest block size of 8 x 8 pixels and 4 iterations there existed in the strain maps what appeared to be 'stripes' of strain. This was in a structure that was undergoing no real strain but rather a pure displacement and so was concluded to be an artefact (Figure 31).

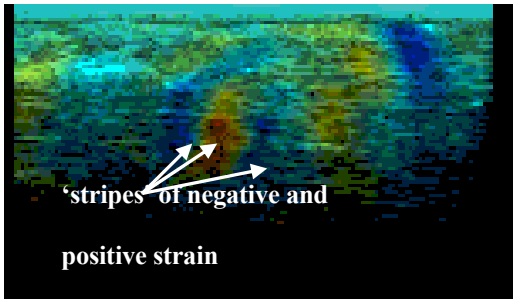


Figure 31 Strain map over original image with 'stripes' of strain

To address this the analysis of the HVBM outputs was adjusted in order to smooth the displacement at greater levels by increasing the number of blocks over which the displacement was averaged and the strain was smoothed. This had little effect on the outcomes and is not illustrated as there was no apparent difference to the outputs.

It was then surmised that perhaps the apparent variations in displacements were occurring within the tracking. There were no such stripes seen on the TMM analysis. As the speckle from tendons, unlike the TMM, are largely strips of variable speckle in the x plane of the U/S image rather than points of speckle. It was surmised that perhaps when examining displacements in that plane there is only sufficient speckle variation where the 'ends' of each 'fibril' occur within a block (Figure 32).

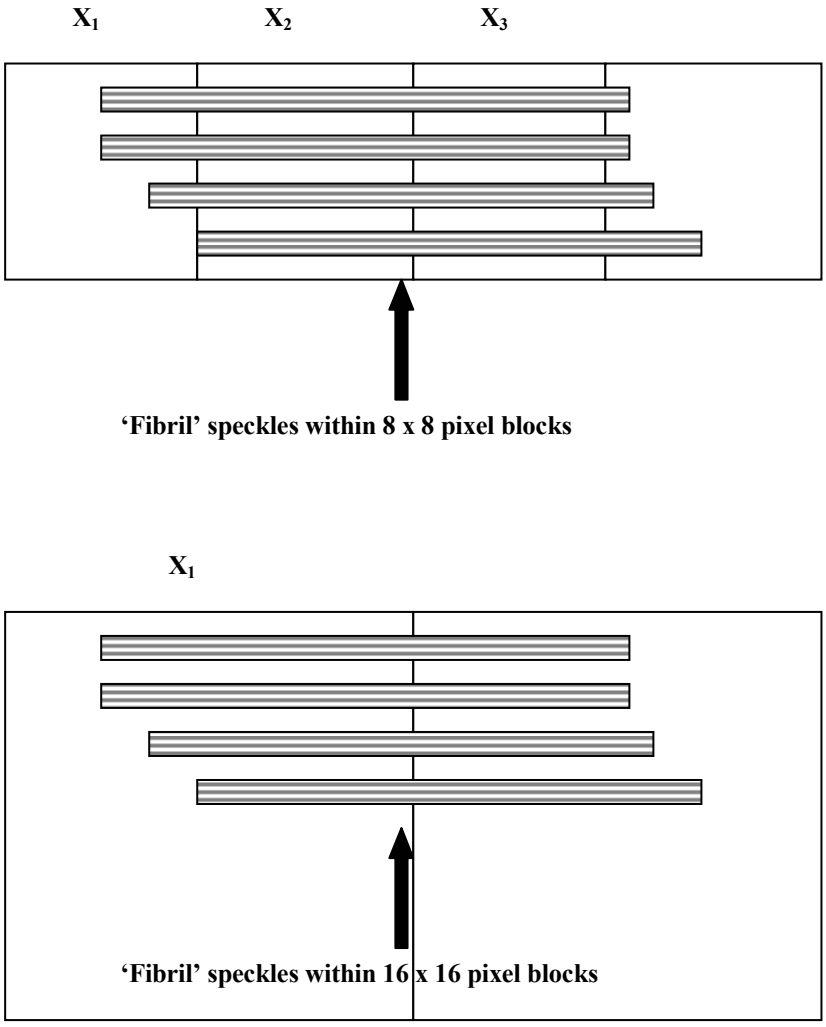


Figure 32 Diagram of tendon speckle within final blocks of 8 x 8 pixels and then 16 x 16 pixel blocks

We can see from Figure 32 that blocks X_2 and X_3 are indeed very similar in terms of speckle pattern. By increasing the size of the smallest block the speckle has a higher chance of varying between each block and therefore the displacements are seen as the same throughout the field

rather than occurring in ‘jumps’ as it had with the smaller block size. When this was applied to the HVBM analysis the strain became zero and was uniform throughout the field (Figure 33).

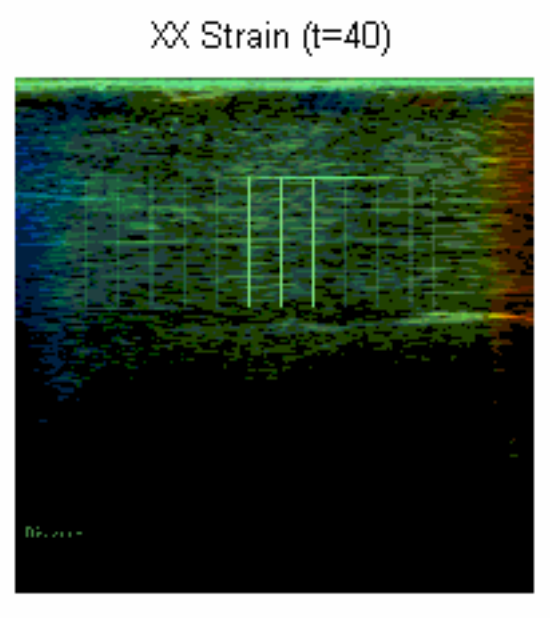


Figure 33 Strain map over original image with no apparent strain

When viewing the tracked results of the TMM in the y direction of the image and comparing them to the SDFT which illustrates displacement in the x direction, there is a much higher degree of accuracy for the HVBM matching the former than the latter. This may be for one of two reasons or, in part, for both:

The TMM provides a high quality image of discrete grey speckle pattern as opposed to tendons which, when viewed longitudinally, provide longer strips of speckle.

When imaging a displacement in the y direction, especially if there is no deformation of the material, the original grey speckle blocks will remain unaltered but will change position within the field. This is easier for the software to compute with respect to each block in the field than a pattern which may slightly alter, not due to any deformation, but anisotropy.

Secondly there will also be some loss of blocks or part of blocks out with the field as the probe moves in the x direction. The enabling of drift within the HVBM provided the closest match with the SDFT results and this allows for movements in and out of the original field of view without accumulative error due to smoothing but perhaps this still cannot provide 100% accuracy.

CONCLUSION

For the analysis of displacement of tendons, in vivo, the HVBM tracking software shows the ability to track tendon well at rates of 0.2mm to 5 mm per second. The tracked displacements at these rates show a correlation factors when plotted against the recorded displacement of the Instron testing machine of 0.9719 and 0.9976 respectively. It is important to note that when the test rate was 1mm per second 2 outliers on the plot were clearly errors and did not match reality. This means that one must not just accept the outputs at any one point in time but must assess the movement throughout the cine to ensure that the tracking follows an expected displacement path. This would potentially mean that it was limited to tracking of predictable displacements rather than sudden changes in direction or rate.

This should allow us to apply it to established methods(Kubo and others 2001; Reeves and others 2003b) of applying isometric muscle work to visualise movement in the lower limb tendons in vivo with confidence.

In summary:

When relying on 'Project2' to track movements of tendon tissue one must adhere to the following caveat in order to produce reliable data.

- Default settings in 'Advance' with enabling of drift and correlation threshold set to 0.93.
- Smallest block size should be 16 x 16 pixels in order to allow for the strip like nature of tendon speckle.

- Due to possible anisotropy of tendon speckle with movement one should examine the tracked movement of the ROI throughout the cine loop in order to rule out any tracking errors.

C h a p t e r 6

VISUALISATION OF MOVEMENT WITHIN A PATELLAR TENDON IN VIVO USING AN ISOMETRIC QUADRICEPS CONTRACTION

It is widely accepted that one can assess tendons in vivo using U/S in order to record the movement of a tendon as its associated muscle contracts (Kubo and others 2002b; Maganaris and Paul 1999; Reeves and others 2004). By measuring the increase in length of the tendon using bony landmarks or musculo-tendinous junctions and in some cases skin markers, it is possible to calculate values such as overall strain in the tendon as a whole and, knowing the load and cross-sectional area, Young's modulus.

Prior to the use of in vivo methods of establishing tendon properties we relied on in vitro research using animal tissues or those of human cadavers. This meant that the subject material was limited to what happened to be available rather than that from subjects of particular interest. Prospective studies of how human tissues might adapt to exercise or rest would not be an option due to ethical limitations. Using in vivo techniques workers have been able to discover that human tissues are likely to be dependant, in part, on genetic make up, and that athletes may excel at their particular sport due to the level of elasticity in their tissues. It was found that there was more elasticity in the structure of vastus lateralis (V.L.) in athletes who excelled at sprinting as opposed to long distance running (Fukunaga and others 2000). Further investigation within a group of sprinters revealed that although the compliance of the lower limb structures did not vary at high force production levels there was increased compliance in the V.L. at low force with respect to that of the controls (Kubo and others 2000).

Of special interest are the in vivo studies of human tendons with respect to alteration to exercise levels. It was found that after only 20 days enforced bed rest there was an increase in the compliance of the tendons of the knee extensors (Kubo and others 2004a) but not of the plantar flexors. After a period of 90 days, however, there was a reduction in Young's modulus for the tendons of both the knee extensors and the plantar flexors (Reeves and others 2002). Similarly 14

weeks of strength training will increase tendon stiffness even in elderly individuals (Reeves and others 2003a).

The limitations of the in vivo studies to date have been that it is difficult to describe accurately the tissue movement and strain unless there are obvious landmarks or skin markers. These would preclude examination of intra-tendinous movement unless markers are inserted within the tissues and that would potentially affect the results in the same way that inserting strain gauges would. In recent months there has been much interest in the literature in finding ways in which to describe intra-tendinous strain and investigations have included finite element analysis (Lavagnino and others 2008), estimation of strain using optic fibres inserted within the tendon tissue (Dillon and others 2008) and ultrasound elastography (Farron and others 2009).

Our hypothesis was that by applying the HVBM software to track U/S images charting displacement in the patellar tendon when strained by an isometric quadriceps contraction it would be possible to calculate intra-tendinous strain. This would provide an accurate, non-invasive, simple method which could revolutionise in vivo tendon investigations and may ultimately be applied within the clinical setting.

It would be useful in the future to use the HVBM as part of the assessment process for looking at all tendon properties rather than relying on landmarks. At this stage the most important thing to assess was that the displacements were accurately followed and the strain calculated. The final goal of this work would be to visualise any variation found in the strain within tissue as a whole. For these purposes the only relevant values would be the original length of the portion of tendon being examined and its change in length. A simple design to allow the subject to apply an isometric contraction in order to strain the tendon is all that is required.

The aim of this part of the investigation was a trial to see if it would be possible to acquire reliable U/S footage of sufficient quality for the HVBM software to track tendon movement accurately, as suggested in Hypothesis 3. The plan was to use only one researcher to operate the U/S, collect the data and direct the subject, helping to clarify the possibility of this as a one

person operation. It was hoped that later on in the project it would be possible to look at any changes to the resulting strain that alteration in joint angle and muscle loading might make, but this was not necessary for the purposes of this test.

METHOD

PRODUCTION OF A BASIC RIG TO ASSESS MOVEMENT OF TENDON UNDER LOAD

A rig was designed using a standard leg extension machine as found in any gym or leisure club. As all the major previous works looking at in vivo tendon properties used isometric muscle contractions, where the muscle works against a fixed point, there was no need for the rig to move freely through the joint range, although there was the facility for later investigations to vary the fixed point for the subject to work against in order to look at different points within a range of movement. It was however, necessary to record the load applied by the subject to ensure that the work output was maximum or a designated proportion of that individual's maximum and that it was repeatable.

A standard leg extension machine was acquired from a local gym that was upgrading its equipment. The weights stack was removed and the wire from the pulley was connected to the wall of the lab via a load cell. This was manufactured by Tedea Huntleigh Model 615 designed to record tension or compression up to loads of 200kg. Prior to this the load cell was calibrated in tension using calibrated weights suspended from the cell. By plotting the weights against voltage a formula was produced to calculate load from output signal (Figure 34).

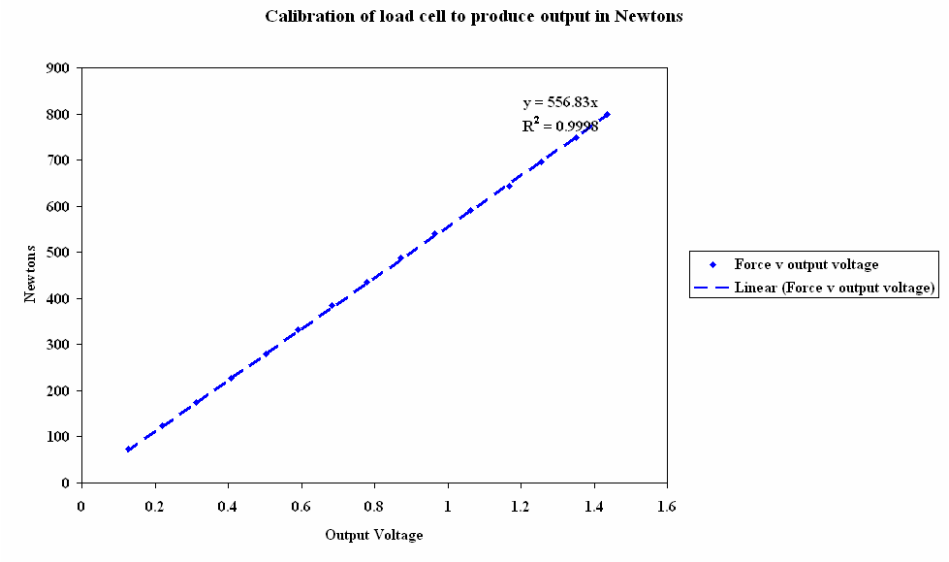


Figure 34 Graph of load cell calibration

ETHICS

Although normal subjects and no patients would be involved it was still necessary to apply to the Ethics Committee of the School of M3. After amending the information form for subjects to include a photograph of the experimental set up, this was granted (Appendices).

SUBJECTS

Eight volunteers were recruited from colleagues within the Engineering Department and were informed as to the nature of the study both verbally and in writing, as in Appendices. Informed consent was given in written form and the subjects were shown, on the rig, what would be expected of them during the experiment.

(Average age of subjects 33 years, 5 women and 3 men)

EQUIPMENT

A Diasus U/S Scanner (Dynamic Imaging) was used, as in the Calibration Study Chapter 4, equipped with an 8 – 16 MHz probe and connected via the University network to the Biomechanics Linux server in order to collect and store the images.

When first practising with the collection of U/S data of the patella tendon being strained, during a quadriceps contraction, I used my own knee. It was relatively straight forward to keep the probe still and in position throughout the work cycle. When I started using other subjects however, without the feedback of the sensation of the probe on the skin it was very difficult to maintain the probe in the start position. Generally as soon as the work was applied the probe would slip laterally. In order to try and prevent this I acquired several blocks of foam from Breasley Foam in Wirksworth and moulded them to sit comfortably over the patella. A small cut was made through which the probe was passed and the foam then provided support for its weight. Tape was applied in order to fix the foam block in position over the knee and thus the position of the probe to be maintained over the patellar tendon with the left hand leaving the right hand free to operate the U/S machine. The leg in question was strapped to the bench around the lower third of the thigh and at the distal third of the tibia. The bench was set such that the knee would be fixed at 90° flexion. Unfortunately individual variations in anatomy could still affect the exact angle at the knee (Figure 35).

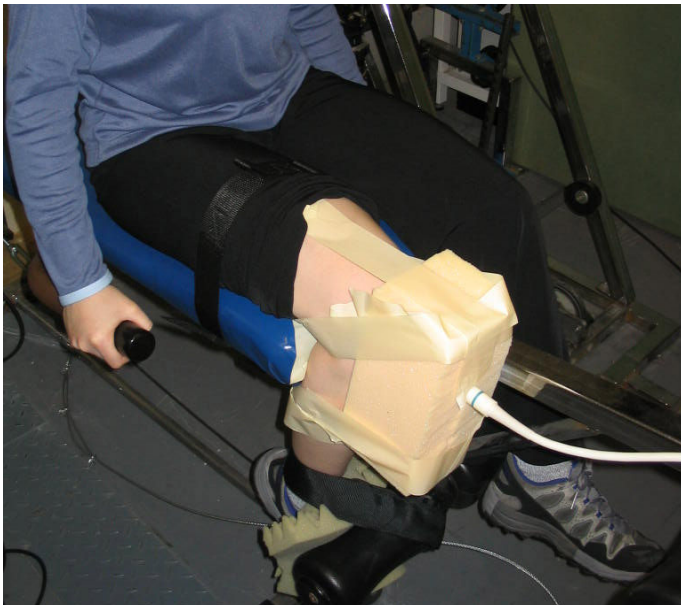


Figure 35 Subject seated on leg extension machine with foam block helping to 'hold' the probe in position (the operators hand is not in view for the purpose of this picture)

PROCEDURES

Using a data logger attached to the load cell to record the load applied, each subject was asked to produce 2 – 3 maximal isometric knee extensions and a 5s cine loop was recorded for each contraction and stored.

The cine loops were then analysed using the HVBM software as described under **Analysis**, Chapter 4.

EXAMINATION OF VECTORS

The vector images were viewed (Figure 36) to ensure that the tracking appeared to match the visual displacement of the tendon as it was loaded. Sequences of data where the vectors did not appear to correlate with the actual displacement of the tissue were rejected. Similarly those sequences where the probe had not maintained contact with the skin and visualised the tendon throughout the cine were rejected.

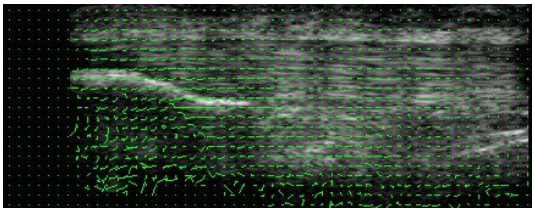


Figure 36 U/S image of patellar tendon with superimposed vectors tracking movement

VISUAL MAPPING

It was decided that although the vectors were able to approximate where the tracking matched the movement through the cine it was difficult to give an objective measure of how well it matched. In order to try and quantify how well the tracking of the HVBM matched the actual displacements the outputs were compared to that plotted in pixel co-ordinates using a landmark and followed its progression throughout the cine. Unfortunately it only became apparent how

useful this might prove after the some of the trials had been run and not all the cine collected had an obvious landmark like the patella throughout.

RESULTS

From each of 8 subjects 3 trials of isometric quads contractions were recorded with the U/S potentially leading to 24 data sets. Of that 24, 5 were rejected as the quality was too poor to merit further analysis. The main reason for rejection was that the probe had slipped during the trial such that it had lost contact and the tendon could not be visualised fully throughout the loading cycle.

EXAMINATION OF VECTORS

In total 19 trials of Patellar tendon strain using maximal isometric quadriceps contractions were collected and analysed using the HVBM software and the vectors examined for correlation. In 15 of these the general tracking of the movement seen appeared to match the overall displacement. In all of the sets of data where the vectors did not appear to be aligned to the general movements there was a large general excursion in the y direction with respect to the U/S co-ordinates. In 6 of the 15 well matched vectors there also appeared to be a large amount of y displacement and in those one must question the accuracy of the x displacement visually. Examination of the original cine and the vector cine appeared to show that the maximum application of an 'isometric' quadriceps contraction could affect the angle of knee flexion and so lead to overall displacement of the whole limb and displacement of the patellar tendon in the y direction. Generally this was a gradual displacement as the work was applied rather than a sudden jump. If we refer to the work of Hansen et al (Hansen and others 2006) it has been noted that there may be considerable longitudinal tibial movement during an isometric quadriceps contraction and this may contribute to approximately 45% of the overall patellar-tibial displacement. The above authors question the accuracy of other patellar tendon studies where this has not been taken into account.

VISUAL MAPPING

Only one trial per subject was selected for comparison between the tracked displacements and that visualised from the landmark excursion. Rejections were based on lack of a suitable landmark for visual recognition and obviously poor correlations from the vectors. In some cases there was still loss of visualisation of the patella at maximum displacement but in order to maximise the numbers available they were still used and the y displacements tracked using either the superficial or deep edge of the tendon.

In all 8 trials examined the trend of the displacements between that tracked and that observed by eye were similar but did not match exactly. Generally the x displacements mapped visually were far greater than that tracked by the HVBM. In all cases the y displacement appeared better tracked than the x displacements (Figure 37).

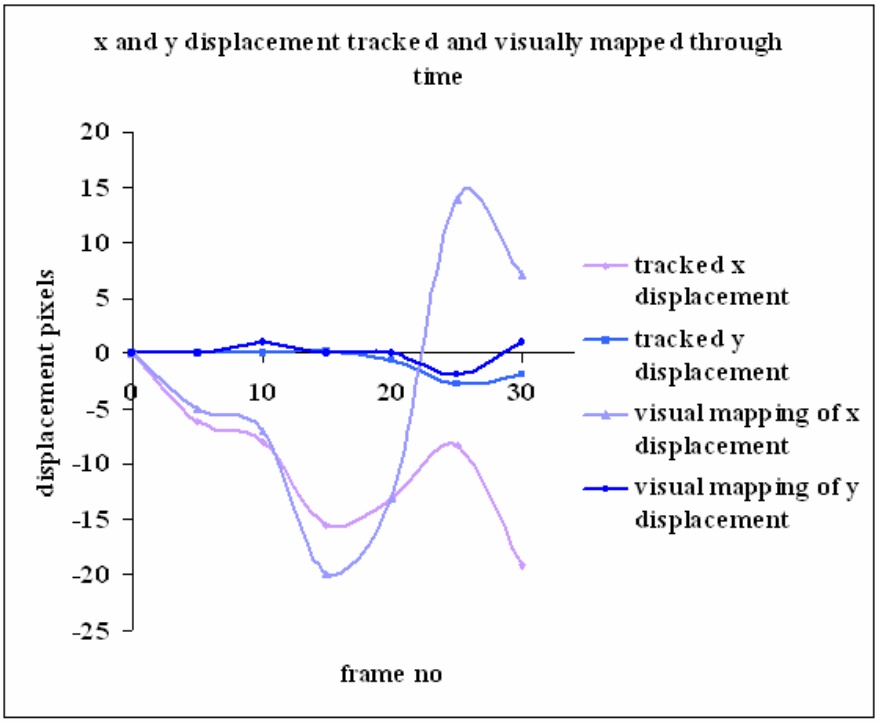


Figure 37 Example of tracked and visual displacements in both the x and the y direction

It is interesting to note that in this example the x displacements seem to lose all correlation after the 20th frame at the point were the both y displacements increase. The y displacements however remain similar.

It is easy to assume that any discrepancies between those displacements tracked by the HVBM and by visual mapping are due to faults with the speckle matching programme but as the image changes due to the strain it becomes harder to be certain that the points selected from the landmark in the first frame are indeed the same ones seen in the successive images. It was important to test the repeatability of the visual mapping before presuming that it is the reliable measure to test the tracking against.

TESTING THE RELIABILITY OF VISUAL MAPPING

In order to investigate inter and intra tester reliability 3 subjects were asked to read off the pixel co-ordinates for a point on the patella in successive images from the same cine and, from this, the pixel displacements were calculated in both the x and the y direction. Each subject was asked to repeat this on 2 trials, mapping within the same cine. They had a rest away from the images for a minimum of 1 hour. In the case of the third subject, the test was repeated on a third occasion using an area from the main part of the tendon rather than the patella in order to illustrate any differences a less discrete landmark may cause (Figure 38).

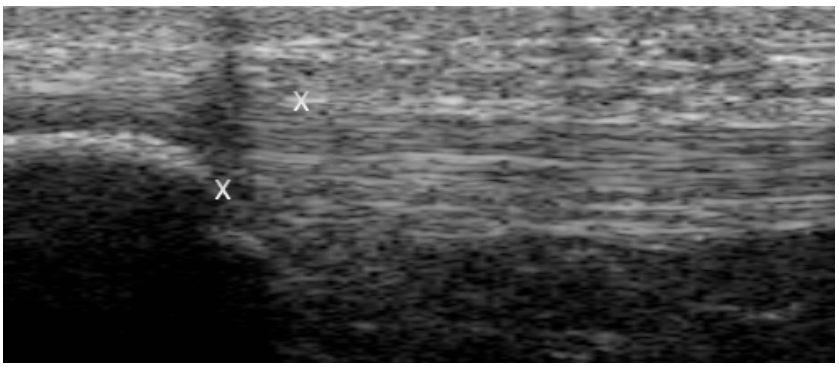


Figure 38 Illustration of different points used for tracking by Subject 3

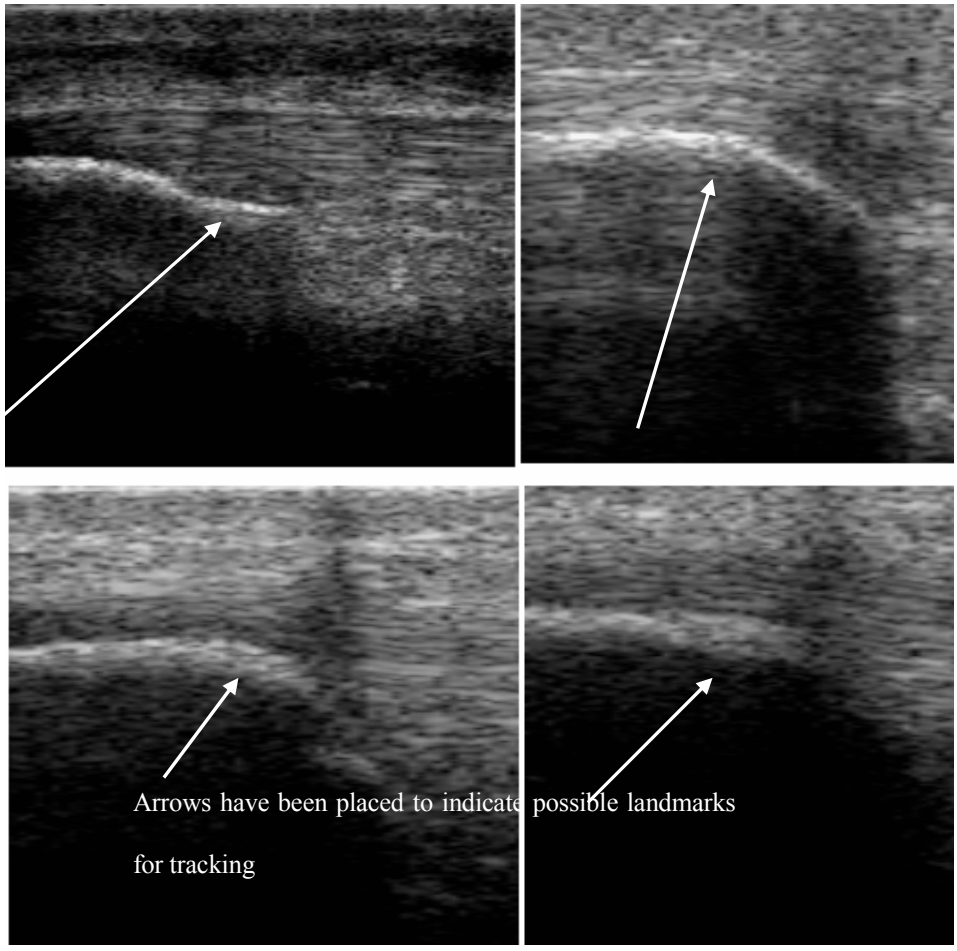


Figure 39 Illustration of differing U/S appearances of patella in 4 subjects

We can see from Figure 39 that in some individuals the patella provides a well defined landmark which one can follow easily throughout the sequence but in others the same area provides little in the way of definition to follow. In the first and second image above the pole of the patella provides and defined bright white bony point which retained its form throughout the cine. In the case of the last two images above however there was less bony definition to the edge of the patella and as the patellar tendon stretched so the apparent 'bony edge' of the patella became less obvious and harder to track.

RESULTS

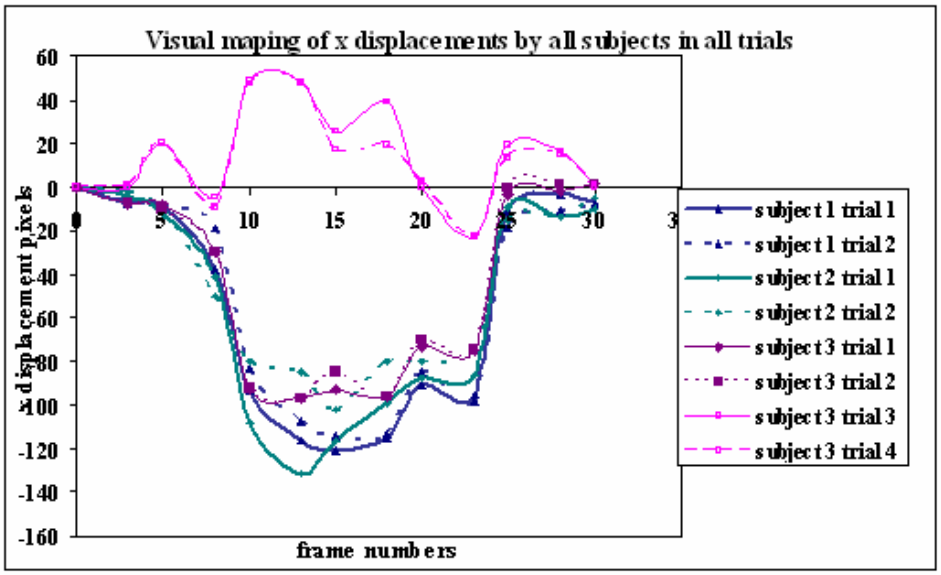


Figure 40 inter and intra tester trial of x displacement

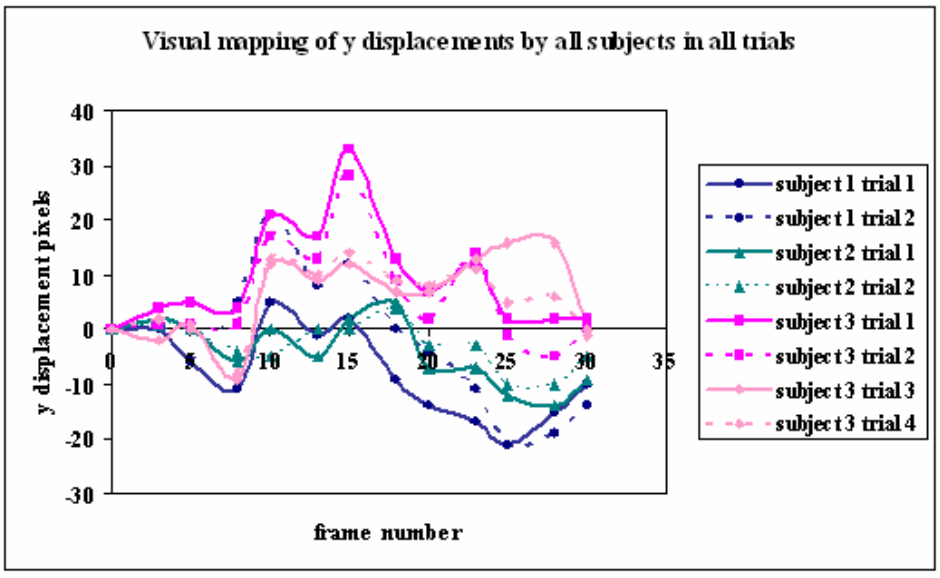


Figure 41 inter and intra tester trial of y displacement

The above two graphs illustrate the visual tracking of the same point through the same cine loop on 2 different occasions. In the case of one operator, subject 3, trials 3 and 4 are done using a mid tendon point within, again, the same cine on a further 2 occasions. It is easy to see from Figures 40 and 41 that there is a large variation between different operators when visually tracking the displacements throughout a series of U/S images.

In the direction of movement there is a consensus of the general movement between operators when tracking a well-defined landmark such as the pole of the patella, but there may be up to a maximum disagreement of 46 pixels between the visual maps of x displacement (see subject 2 in Figure 40). If we refer back to Figure 37 we see that the maximum disagreement between the visually mapped point and that tracked (at frame 25) was 23 pixels from the x displacement. When attempting to follow an area within the general body of the tendon there is no apparent correlation at all either with the other visual predictions or that tracked.

In the y direction perpendicular to the general movement there is again general consensus both between the human eye and the tracking with respect to the direction of the movement (Figure 37) but there is a maximum discrepancy of 37 pixels seen at the 15^{th} and the 25^{th} frame between different individuals mapping by eye (Figure 41).

It seems possible that the tracking is better in the y direction for similar reasons to those regarding the improved tracking in the y direction from the **Calibration Study,** Chapter 4. That is that the U/S image generally is more stable in that direction as there is less general deformation and movement of the tissue causing less likelihood of anisotropy. One might similarly expect increased accuracy in the y direction in the case of visual mapping as the structure of a tendon is made up of horizontal lines of grey pattern perpendicular to the y direction the edges of which are easier to locate by eye and then find in subsequent images.

The most important conclusion to draw from this test is that while visual mapping serves as an easy way to predict the usefulness of HVBM in tracking displacement in vivo it in no way provides an absolute calibration technique due to its own inbuilt limitations. As the visual

mapping test used only one cine loop on repeated occasions we must conclude that the inconsistencies arise from failure of the operator to follow the point accurately by eye. One could argue from the calibration of displacement that the software is more representative of reality than that followed by the human eye. This is borne out when comparing the mapping of a defined structure like the patella compared with a point within the tendon, as in the case of subject 3 but not so when one compares the mapping between different subjects (Figure 41).

STRAIN MAPS DERIVED FROM DATA

Using strain analysis with Matlab code the strain maps for each trial where the vectors and visual track appeared to match well were calculated. It was noted that there was little correlation between the strains produced in the tendons of different individuals despite the fact that all were produced using a maximum contraction for that individual. It would be possible for there to be some variation between subjects but one would expect a general pattern to evolve. Combined with this there appeared to be some rather unpredictable areas of strain within the tendon tissue in general. This seemed to show that there were areas of negative or compressive strain, shown in blue shading in the superimposed strain maps, within the tendon and large positive or tensile strain elsewhere, shown in red.

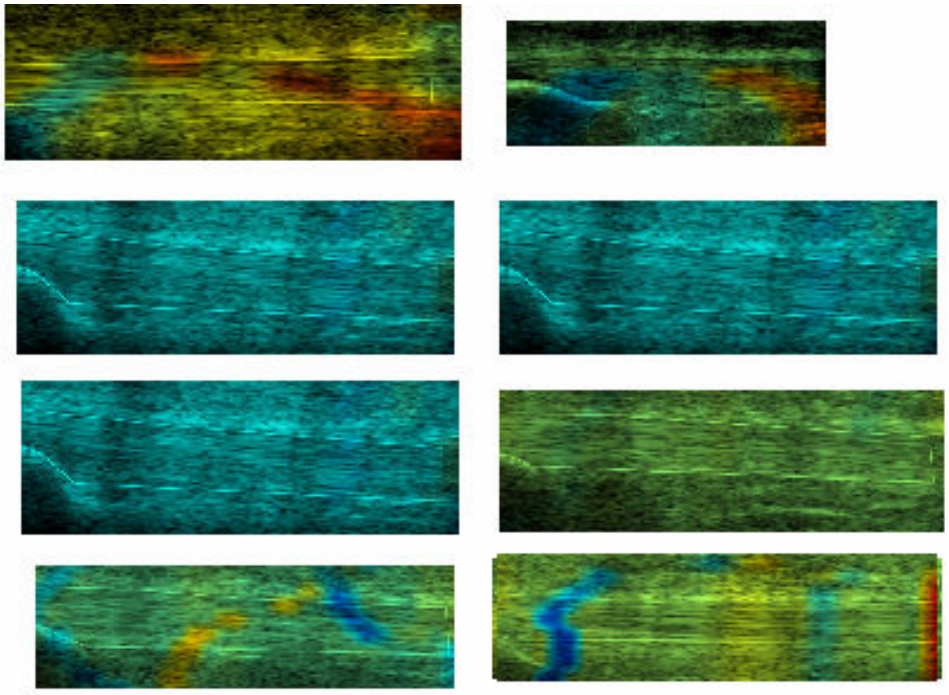


Figure 42 xx strain maps of 8 different subjects superimposed on the original image

Figure 42 represents the xx strain as it presented in the final time point for 8 individual subjects. The blue pigment generally represents the lowest strain moving through the spectrum to red for the highest level of tensile strain. The values represented are not mentioned for this is to show the variation in the strain values recorded throughout a number of different trials. The important features to note are that there appears to be no general consistency between all 8 maps either in overall colour or in the distribution of strain throughout the structure.

DISCUSSION

The intention of this part of the investigation was to find how suitable the set up using the leg extension machine would be for a reliable investigation of strains within the patellar tendon under loading. From the results above we can see that although it is generally possible to collect U/S data of a standard sufficient for some tracking. This is dependant on certain limitations:

Despite care being taken when collecting the data, to try and ensure minimal out of plane movement and loss of contact with the skin by the probe with visualisation of the tendon throughout, out of a total of 24 trials only 15 displayed tracking of the x displacement of sufficient quality. This was using the vectors to correlate the movement to the tracking in order to ascertain if further analysis of the data would be worthwhile in those where the tendon was visualised through the sequence. Of those rejected 5 lost contact with the probe and did not provide sufficient visualisation of the tendon for examination and the other 4 did not appear to have the movements followed by the vector pattern. In 6 of the 15 trials with good vector correlation there was a large amount of y displacement seen which, it seems likely, may affect quality of the images with respect to tracking the x displacement due to potential anisotropy.

Of the 8 sets of data where the tracking was compared with a visual map of the displacement, the general trend of the tracking matched that of the visual mapping but there was no exact match in the values. The correlation again appeared to be affected when the y displacements increased and it is likely that this may represent errors in both the HVBM tracking and the visual maps.

The main conclusion to draw from this investigation is that it is of paramount importance that when acquiring in vivo U/S data of sufficient quality to provide reliable tracking, y displacement must be minimised. For this reason the basic leg extension machine was too limiting as it had little or no facility to allow for differences in anatomy between the subjects being tested which meant that there was increased likelihood of general movement of the limb in question when applying an isometric quads contraction. For this reason it was decided that when exploring patellar tendon strain further, it would be preferable to use a machine that had the facility to be adapted for each individual, as can be found in isokinetic machines.

For the **Pilot Study of Investigation of Differential Strain in the Patellar Tendon under Load** in Chapter 8 the Cybex Dynamometer would provide a better standard of fixation and so, it was hoped, minimal y displacement.

Using the Cybex would also mean that it would be possible to examine the effects of joint range on patellar tendon strain for each subject as it has been suggested that this may be significant in the development of tendon pathology (Almekinders and others 2001) and therefore important to include for any investigation into strains within this tendon.

In principle this means of assessing patellar tendon strain in vivo by using an isometric quads contraction would be suitable for tracking displacements and strains using the HVBM software provided any out of plane movements were kept to a minimum.

From the early strain maps produced in this part of the study there were what appeared to be large areas of random and unexpected compressive (negative) strain within the tissue. It was difficult to believe that this was in fact representative of the real situation. It was apparent from this that it was important to further calibrate the HVBM tracking in tendinous tissue but this time to look at its ability to track a tendon under strain. This further validation would ensure confidence in the results produced in vivo and will be described in Chapter 7, **Validation of HVBM as a Measure of Strain in Tendons.**

In summary: the HVBM software should be used with care in vivo

- It should only be used where there is movement in the direction being tracked and any movements in other directions are minimal.

- The probe must maintain contact with the skin throughout the sequence.

- For assessment of displacement due to an isometric muscle contraction a machine which may be adjusted optimally for each subject to be tested should be used.

C h a p t e r 7

VALIDATION OF HVBM AS A MEASURE OF STRAIN IN TENDONS

It became apparent as the 'in vivo' work progressed that some rather unexpected results were emerging in the strain maps of the patellar tendons, with areas of negative strain or compression throughout the tendon. It was important to ensure that the tracking and analysis was as useful when studying a tissue under strain as it was when tracking a tissue as it moves unstrained. It was necessary at this point to further calibrate the HVBM software's ability to track tendon when placed under a known quantity of strain.

One of the most difficult parts of in vitro testing of tendons is ensuring that the results are not compromised by the slippage of the tendon within the clamp (Ng and others 2005). Although the material properties of the tendon itself were not being tested it was still important to ensure that the movement and the strains applied by the Instron Materials Testing Machine were exactly the same as those experienced within the tendinous tissue and then visualised by the U/S.

Methods of clamping used by other workers have included serrated clamps (Tak-Man Cheung and Zhang 2006), cryo-clamps (Ramachandran and others 2005; Wieloch and others 2004) and modifications of standard clamping devices using sandpaper, cardboard, cyanoacrylate and freezing with liquid nitrogen (Ker 2007; Ng and others 2005). For the purpose of this study it was important to minimise the slippage of the tendon in total. It was not planned, however, to strain the tissue to failure or to assess the properties of the sample and so the most important aspect was to try to apply the load evenly across the structure. Most devices work by gripping the outside of the structure and squeezing which may mean that the core of the material would be allowed to slip in relation to the periphery. One method used in the materials testing industry in order to clamp rope or string is to include bollards within the structure of the clamp (Figure 43).

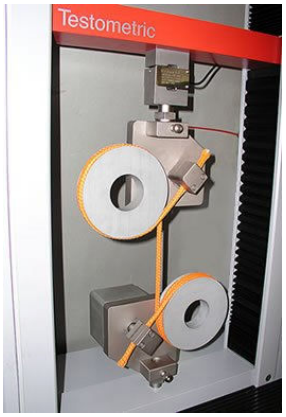


Figure 43 Example of a bollard clamping device for testing rope/string structures (image courtesy of http://www.testometric.co.uk/)

Although the tendon would not be long enough to wrap around the bollard as in the device illustrated above it was surmised that even a half turn around the bollard would help to distribute the loading through the structure. This idea was originally tested on a pre-existing rope testing clamp (Figure 44) within the laboratory and it was found that there appeared to be minimal slippage when the strain was applied.

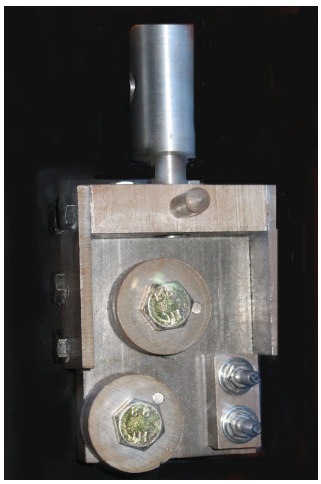


Figure 44 Double bollard clamp designed for clamping rope used initially to clamp SDFT

The main problem with using this existing device was that the length of the tendon would mean that it could only be wrapped around one bollard and so was not be aligned with the line of pull from the Instron testing machine and therefore we could not guarantee the actual strain applied.

It was also obvious from the trial that, as the loading increased, due to the width and the slippage of the tissue it may be difficult to maintain its wrap around the bollard and it would be useful to have a retaining device to maintain its position. Two custom made clamps were designed to function with the Instron Materials testing machine and built in the School workshop for the purpose of straining tendons (Figure 45). They were made of stainless steel in order that they should not be affected by either the saline used to keep the tissue moist during testing or the exudates inevitably produced by clamping a moist tissue. It also allowed for ease of cleaning after testing in keeping with the laboratory regulations when testing any animal tissue. The bollards were designed to be 4 cm diameter and 2 cm depth with a furrow within the section in order to help retain the structure of an equine tendon, specifically the superficial digital flexor tendon (SDFT). A retaining screw was placed at the outside edge of the bollard to prevent any slippage of the tendinous structure outwith the depression in the bollard. The clamping surface was scored in such a way to grip the tendon and was held in place by hand tightened nuts and bolts. Which allowed the tendon to be squeezed between the clamping edges progressively as fluid was squeezed out of the structure.

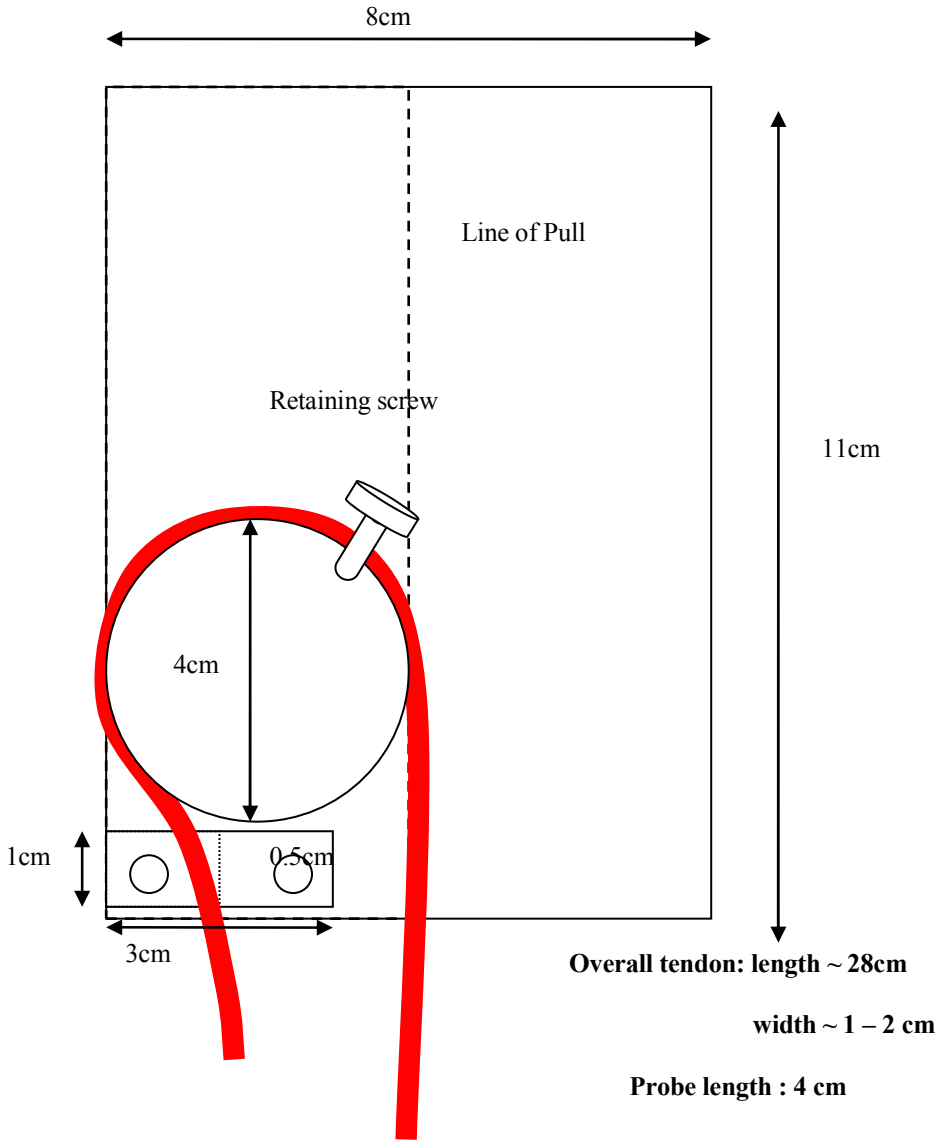


Figure 45 Design for bollard clamp to test tendon in tension

METHOD

Portions of SDFT were again harvested from equine cadavers acquired from the University of Nottingham Veterinary School and stored at -20˚C until required. The tendon was allowed to thaw, prior to testing, while wrapped in tissue soaked in saline to maintain hydration of the sample. While testing it was kept moist using regular spraying with saline. It was not important

to mimic exactly in vivo conditions, as the aim of the investigation was not to test the tissue but rather to test the tracking ability of the HVBM on a strained sample.

The tendon was then wrapped around the bollard and clamped in position using both the clamp and the retaining device as illustrated in the photograph. The Diasus U/S machine was again set up as in the previous chapter for calibration of displacement with the 8 - 16 MHz probe (Figure 46).

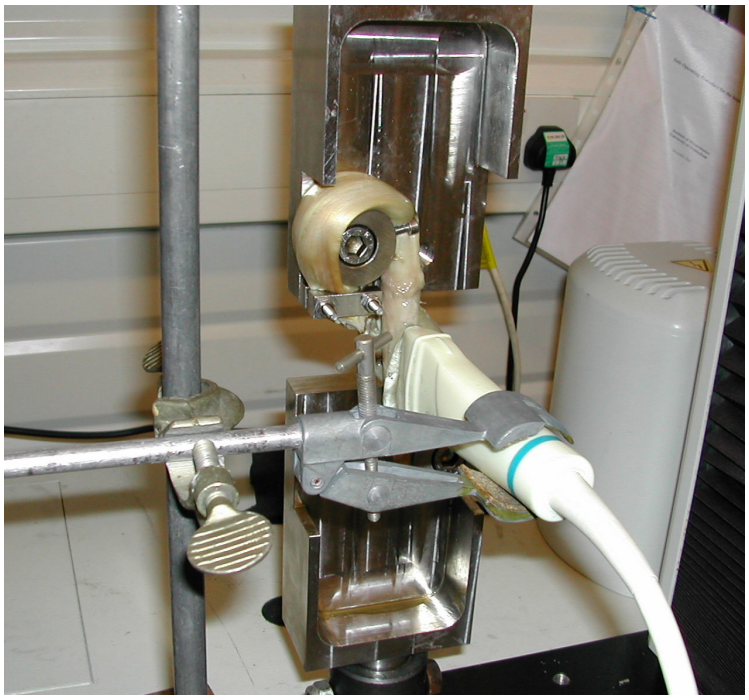


Figure 46 SDFT clamped using bespoke bollard clamps and imaged using 8 - 16 MHz probe fixed in position

The Instron 5960 Dual Column Table Top Universal Testing System was used, with a strain measurement accuracy of ±0.5% of reading, was set to extend at a uniform rate of 1mm/second for 4 seconds and the Diasus was set to save 50 frames within the loop which would record for a time of 6 seconds. This was then run over 6 separate trials in order to ensure that the results were repeatable.

The HVBM (Project2) analysis was then run using the parameters optimised by the results of the displacement calibration, with drift enabled and a smallest block size of 16 pixels with 4 iterations. The strain was then calculated using Matlab as described under **Analysis**, Chapter 4.

RESULTS

After analysis of the results the tracking of the strain applied by the Instron to the SDFT was not an accurate representation in any of the six test runs. It was noted that most of the trials had the appearance from the tracked data of a material under compressive loading not, as it actually was, tension (Figure 47).

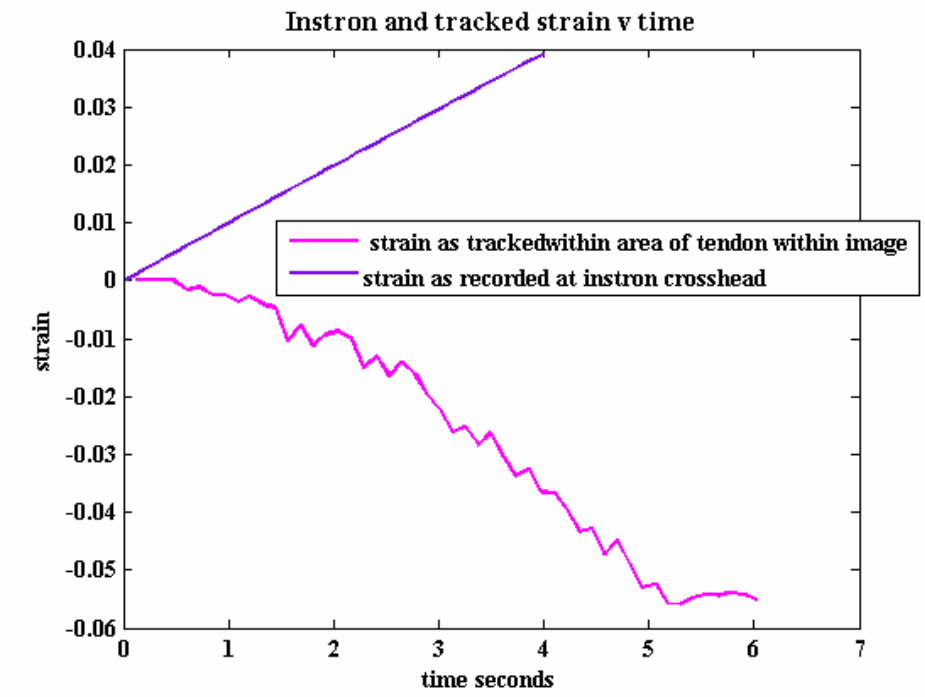


Figure 47 Third trial of strain as calculated from the tracked displacement within SDFT

In 4 of the 6 tests there did appear to be some degree of correlation with the magnitude and gradient of the strain but the strain as calculated from the tracked displacement was still negative or compressive. Further investigation of the strain maps of each field revealed a repeated pattern

where there was apparent negative strain to the left of the tendon field with positive strain on the right, as one views the original image, see Figure 48.

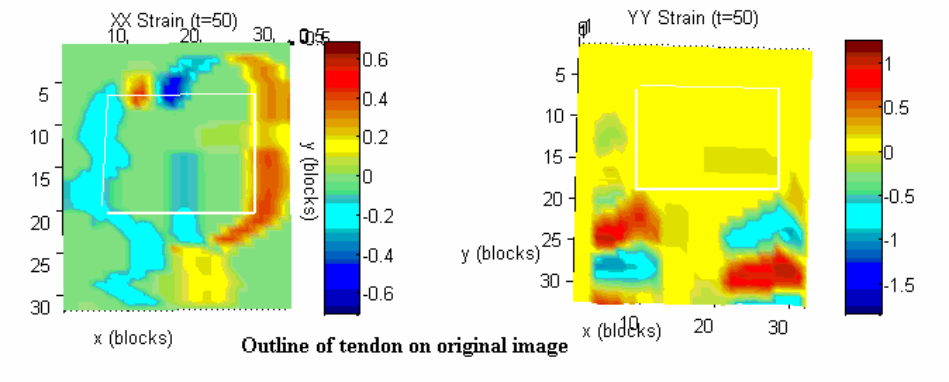


Figure 48 Graphs of strain across the tendon field showing most to be outside the edges, with negative strain, to the left, and positive strain, to the right

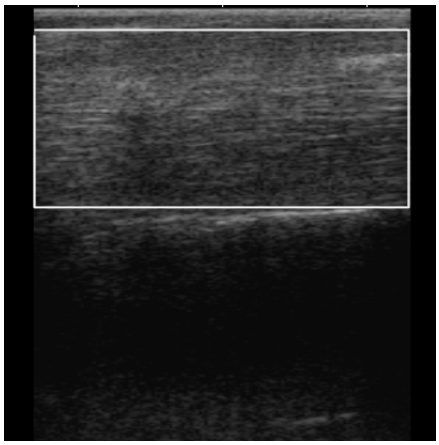


Figure 49 Cropped first image of SDFT in trial of tensile strain

From Figures 48 and 49 we can see that the apparent bands of strain are at the borders of the tendon field and within the area (below) the tendon and so are suggestive of an artefact.

Analysis of the displacements for each test through time showed a ramp which matched the ramp of displacement applied by the Instron although the magnitude was generally a proportion of that

applied overall. This, however, is what one would expect in a strained sample as opposed to one that has just been moved past the probe.

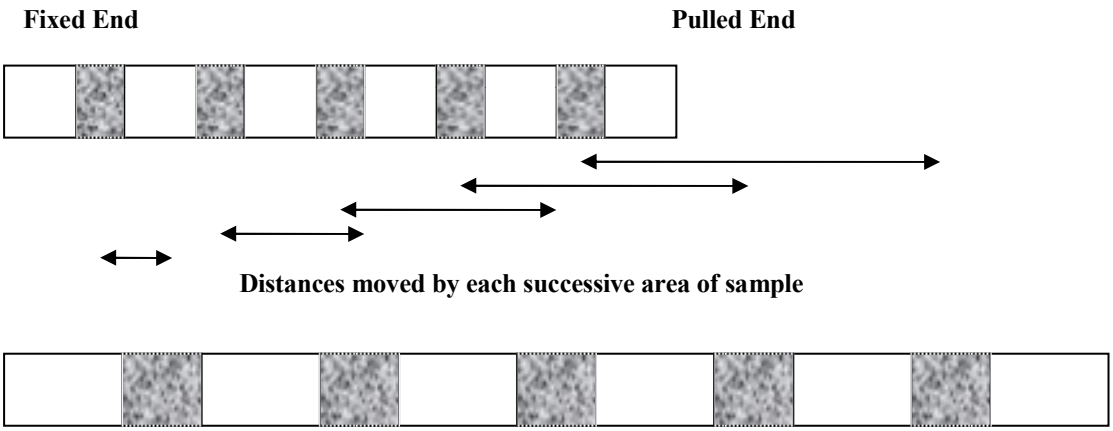


Figure 50 Illustration of a sample being strained from one end

Figure 50 shows that when a sample is strained from one end with the other end fixed, the points further away from the fixed end will move less than those at the top from which the strain is applied.

A projected displacement was calculated for the Instron at the midpoint of the probe, assuming strain is consistent throughout the tendon, for comparison with that found with the tracking.

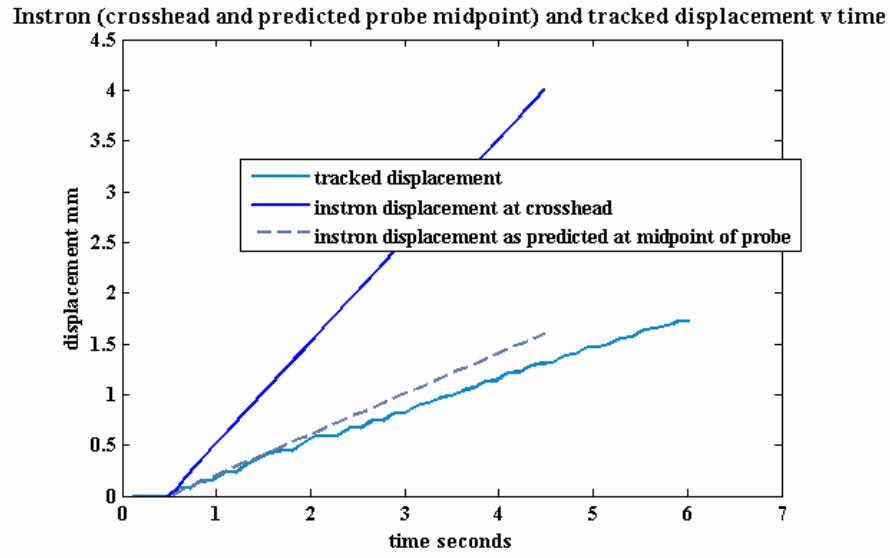


Figure 51 Example of displacement at Instron crosshead and that predicted at the midpoint of the probe compared with that tracked

As the midpoint of the probe was estimated to be 0.6 of the distance from the top clamp to the bottom it was possible to plot potential displacement at that point in the structure (Figure 51). This position was estimated as it was only intended as a way of checking that the displacements had tracked correctly in this case, the work in Chapter 5 had been used to calibrate this more accurately. All displacements were found to follow a similar line when plotted as above. This suggested that the HVBM software has essentially followed the speckle even as the tissue is stretched. It is important thus to probe further to find why the strain results are not as expected from the Instron strain results. Is the error in the tracking or in the analysis of the outputs?

It was important at this stage to look at the displacements as a whole through time to see why the strain graphs should present with bands of large amounts of positive and negative strain at either end with little strain in the main body of the tendon. This same pattern was seen in all 6 trials.

While the HVBM software was able to match the displacements of the tissue as a whole it could only follow the displacement of the central area of the field and was unable to track at the leading

edge where the pattern was leaving the image. There appeared to be less of a problem when new blocks of speckle entered the image and tracking gave a believable value for displacement here.

It was also, it appeared, unable to follow the variations in displacement within the tissue field and this led to a central uniform block of displacement. Where there was no perceived movement at either edge of the field that would then appear as compression to the left of the image and tensile strain to the right.

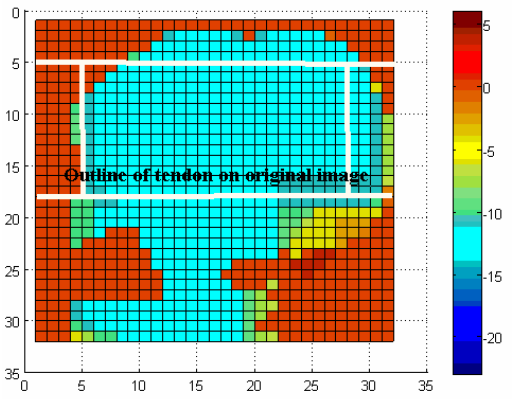


Figure 52 Displacement map showing uniform displacement throughout

Figure 52 illustrates the uniform displacement seen here of the main body of the tendon field. If we refer to the original cropped image in Figure 49, one can see that the main upper portion displaced by 30 pixels to the left is the main part of the tendon, without the two blocks immediately adjacent to the probe face and the left and right borders.

One possible explanation for this apparent mass displacement is that although changing the smallest block to be tracked from 8 to 16 blocks meant a more uniformly and accurately tracked displacement by allowing for the longer type of the speckle seen in tendons, it did however mean that after 4 iterations the largest block within which to search for the pattern is 128 pixels and this is a quarter of the size of the entire image. It is likely that that is insufficient for the HVBM software to track spatially discrete movements.

In order to see whether this was in fact the case the U/S cine loops were re-analysed using 8 pixel blocks for the smallest block size. This led to an improved gradation of the displacement seen through the field but it also, as in the case of the **Calibration of Tracked Movement**, where tracking was purely of movement, there was an increase in numbers of areas within the field which did not appear to track correctly. This obviously, had potentially profound effects on strain estimations found within the tendon. There still remains the fact that the overall strains depicted in the in vitro results here and in those from the in vivo results in Chapter 6 were often negative or compressive.

DEALING WITH NEGATIVE STRAIN

While it was apparent that the large negative strain areas on the left of the image were primarily due to incompletely tracked blocks as they left the image, there still appeared to be overall negative strain for the remaining field. It was important to confirm first if the outputs produced in the text files of data were presented in such way to match the blocks as they were analysed within the image. That is, are the blocks on the left of the image as we visualise them represented by the displacements numbers on the left of the array.

In order to do this it was necessary to produce an image where the speckle was only visible on one side of the image. This would ensure that displacements tracked would only be recorded on one side of the output array.

METHOD

A sponge was immersed within a water bath and the Diasus 8 -16 MHz probe was submerged to a depth of 3 - 4 mm in order that one half of the probe was in contact with the sponge. The probe was then used to compress the sponge and then release it and the resultant cine was stored and analysed as described in the **Analysis** chapter. This is illustrated diagrammatically in Figure 53 and with the first image in the cine in Figure 54.

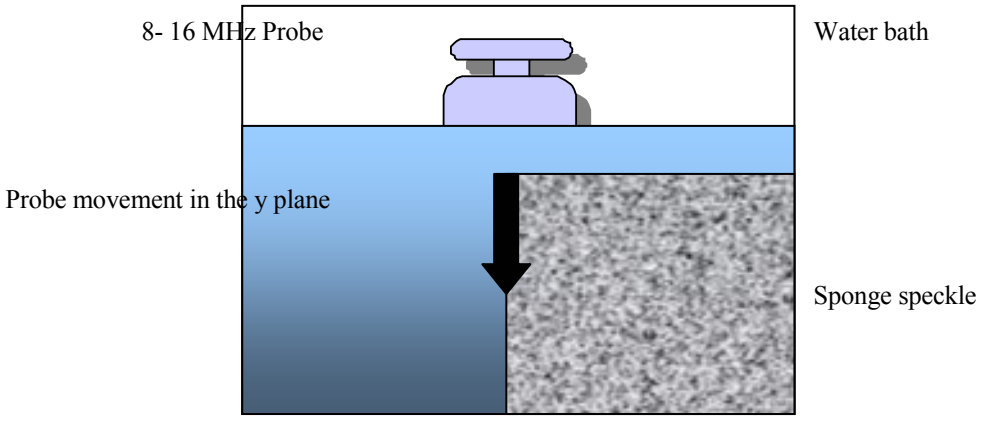


Figure 53 Probe displacement of sponge to confirm matching of output arrays to image

Figure 54 Image with concentrated speckle on the right of the image

RESULTS

Displacements as predicted were seen in the right hand side of the output arrays (Figure 55), with only minimal displacements on the left from movements within the water that led to random areas of speckle to be tracked.

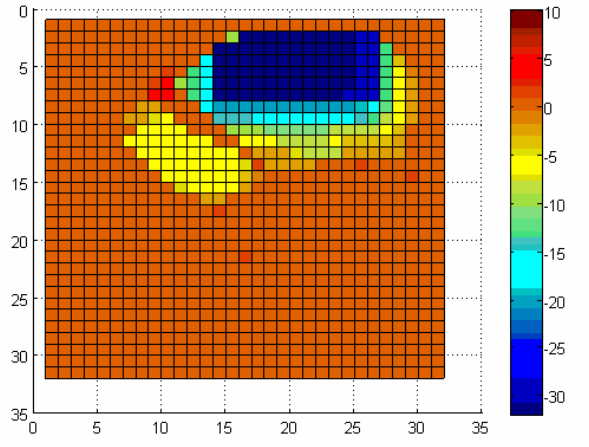


Figure 55 Displacement map of displaced speckle on the right of the image (small amounts of random speckle in water on the left also tracked in yellow)

Having confirmed that the basic displacements were being recorded as expected with respect to the original image, it was then important to address the calculation of strain from the displacement arrays. When the code was first produced it was tested on synthetic arrays to ensure correct calculation of the strain from known 'displacements'. However, now that 'real' data from U/S speckle was being used there still seemed to be a discrepancy between the apparent strain seen visually and recorded by the materials testing machine and that tracked by the HVBM.

TRACKING OF TENSILE STRAIN BETWEEN TWO DEFINED AREAS OF SPECKLE

In order to have a better idea of the direction of the speckle to be tracked and the type of strains being produced it was decided to create a basic elastic phantom with 2 areas of discrete speckle produced by plastic adhered to the elastic. This would allow us to see that the speckle moved apart and then to ensure the recording of tensile strain by the HVBM and Matlab analysis code (Figure 56).

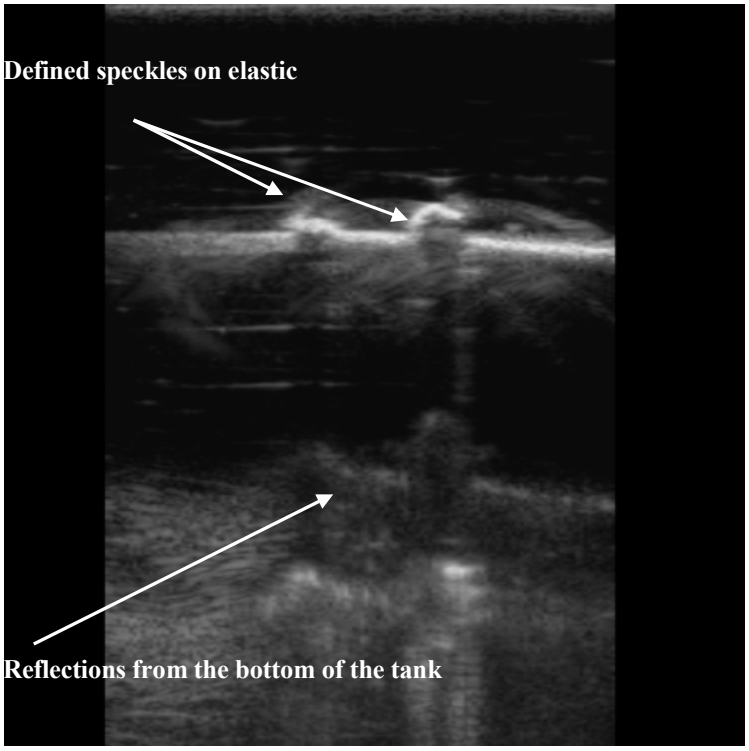


Figure 56 Image of elastic with 2 defined speckles in water bath

The elastic was then stretched, pulling the two areas of speckle apart, and the resultant U/S cine was collected and then analysed using Project2. This was repeated twice.

Despite angling the probe so that it was not at right angles to the tank there were still some reverberations produced of the image. It was important to be aware of this when looking at the displacement array and the strains in order that outputs from the speckle from the reverberations were disregarded.

As there were two very distinct areas of speckle it was easy to track visually and mark the displacements of the edges using pixel co-ordinates this enabled us to compare the displacements with a visual assessment but because of the easy numbers involved i.e. the strain between the two nodes (areas of speckle) it was also possible to calculate basic xx strain from both the visual displacements and those derived from the HVBM.

RESULTS

Initial comparison of the strains produced by hand from visual mapping of speckle displacements and from the tracking both by calculation within the Matlab programme and using a simple calculation of engineering strain from the tracked displacements showed that in the first trial the strains were both positive (Figure 57). In the second, however the tracked strain was negative and the calculation by hand from the visual mapping was positive as one would expect, given that the speckles were seen to move apart. It is important however to note that the discrepancy is not in the calculation of the strain but in the actual tracking of the speckles as simple calculation from the tracked displacements was negative as well (Figure 58).

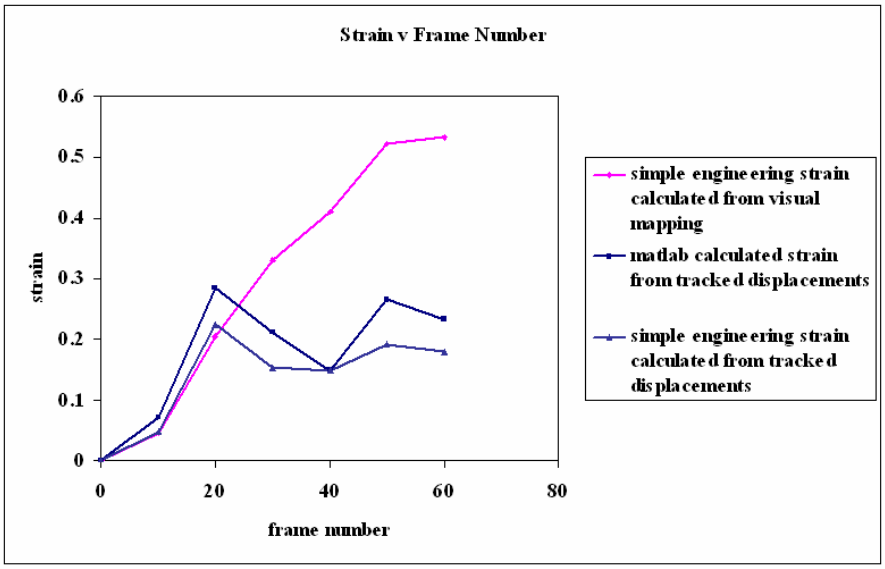


Figure 57 Trial 1 comparison of strains calculated from tracked displacements both in Matlab, by hand and from visual mapping

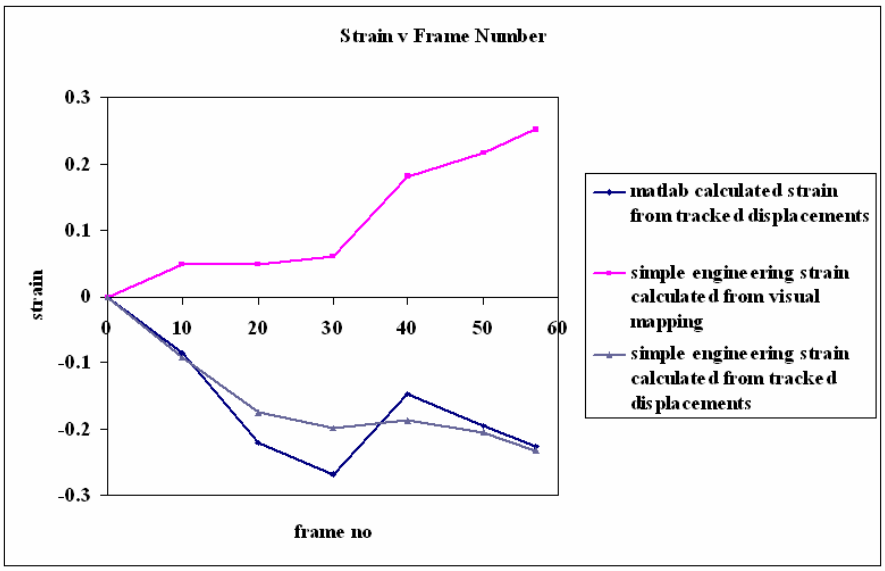


Figure 58 Trial 2 comparison of strain calculated from tracked displacements both in Matlab, by hand and from visual mapping

DISCUSSION

These results illustrated very easily that the calculation of strain using the Matlab code produced comparable results to those produced by hand from a simple formula for engineering strain.

$\varepsilon = \Delta L / L$ where:

L = original length

ΔL= change in length

ε = strain of elastic

Examination of the displacement data from both trials showed that in the second trial the strain calculated from the tracked data is negative and given that this is U/S footage of two areas being stretched apart this cannot be correct. Comparing the strain found by hand from the visually mapped displacements and that calculated by hand from the tracked displacements we can see that the problem is with the tracking. Indeed further examination revealed that the main problem here was inaccurate tracking of the first area of speckle.

It was important to note that this area of the 2nd trial had the largest amount of y displacement when taken with respect to the x displacement, such that, here the movement in the y direction was equal to or in fact greater than that in the x direction. When the other displacements were investigated for both speckles and speckle 2 in the 2nd trial the y displacements were generally less than that in the x direction (Figures 59 and 60).

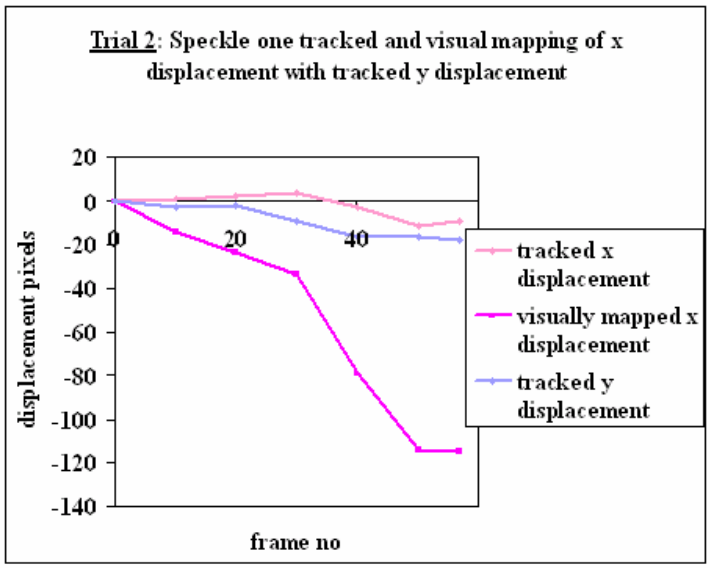


Figure 59 Trial 2: comparison of tracking of x and y displacements of speckle one

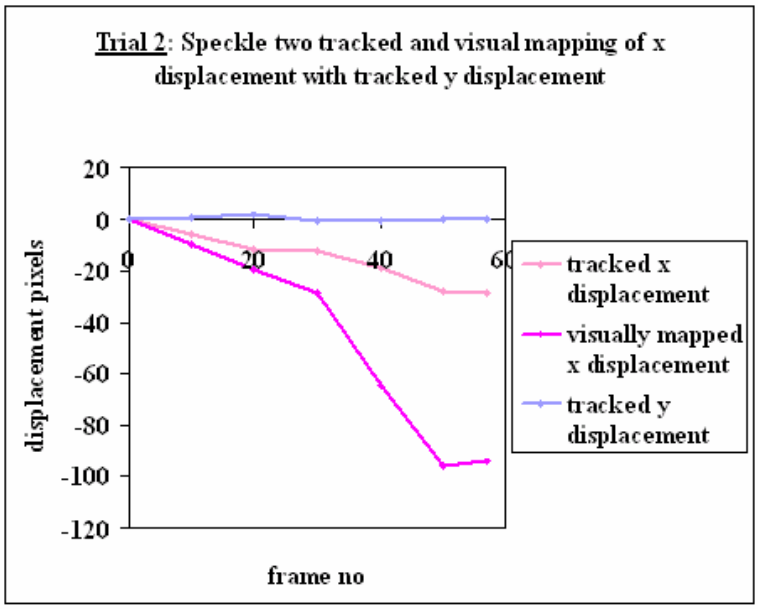


Figure 60 Trial 2: comparison of tracking of x and y displacements of speckle two

If we examine the tracked displacements in trial one there is one frame in which the y displacement appears to be greater than the x displacement for speckle 2, Figure 61. Otherwise for speckles one and two the visual mapping and the tracked displacements follow the same general trend.

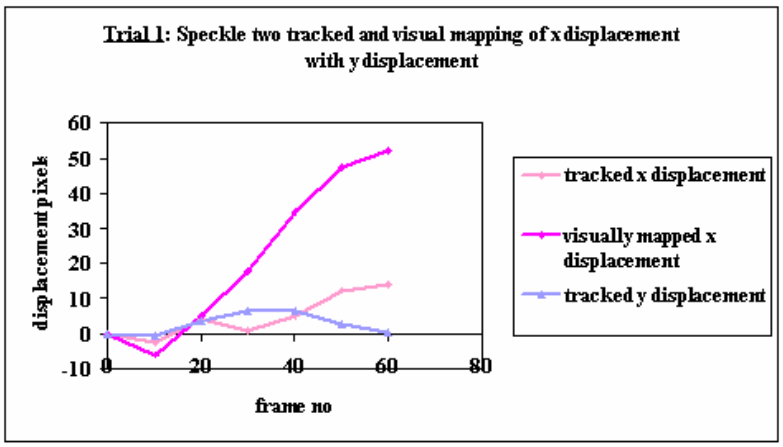


Figure 61 Trial 1: comparison of tracking of displacement of Speckle 2

One must assume from this that when we are using the HVBM software for tracking in the case of unknown strains we must pay special attention to the amount of movement that is occurring in the y direction with respect to that in the x. The above results suggest that if the y displacement is the same or greater than that in the main direction of movement it is likely that the tracking may fail to follow the speckles accurately in the main direction of interest.

It would be reasonable to express concern regarding the application of such a tool to tendons, in vivo, for one might expect that there should be some yy strain when a tendon is strained due to Poisson's ratio. For as a visco-elastic structure such as a tendon is stretched one would assume a degree of compressive strain in the y direction. If we look at the SDFT samples, however, in this chapter there appeared to be minimal yy strain if any. The equine SDFT is a large tendon and the strains applied were of the order of 4 % which presumably lead to minimal deformation of the structure as a whole.

One might argue that this would not necessarily be the case in the human patellar tendon, in vivo, as the tendon is not free as it is in vitro but is bounded by other tissues such as subcutaneous fascia and the infra-patellar fat pad. However, the reality is that both are loose connective tissues or fascia which as its name suggests is designed to allow mobility and free movement of structures such as the tendon. If we recall the graph of tendon strain from Chapter 2, the strains we are examining are from the first part or the toe part of the graph. In the next study in the Chapter 8 one of the subjects did have a substantial amount of scarring in the skin overlying the patellar tendon which appeared to affect the tendons ability to glide when the work was applied but when the strain was calculated it was found to be 4.8% xx strain and yy strain was negligible for maximum work at 90˚.

When the data from the SDFT was examined, however, although there is some y movement but this is generally minimal when compared to that in the x direction where the appropriate strain is occurring and consequently this is not enough to explain the levels of incorrect tracking being found. If, however, we reconsider the block sizes, there is often increased risk of inaccurate

tracking in tendons with a final block of 8 pixels but when this block size was increased to 16 pixels as described previously in the displacement calibration the tissue is tracked accurately but as one mass displacement and strain is not perceived.

One possibility is that the final block size is not the problem when the HVBM tries to track a strained tissue. A 16 pixel block is, after all, only just over 1 mm^2. If, however, we consider a search through 4 iterations the initial block size will be 128 pixels which is a quarter of the size of the overall image. If the program only searches through 3 iterations the initial block size will be 64 pixels which was the starting block size when the default settings of 8 pixels final block with 4 iterations are used.

After re-running the HVBM software on all the strained SDFT, with a 16 pixels final block and 3 iterations, the mean final strain calculated from the tracked outputs at the end of each trial was within 0.0002 of the mean strain recorded at the Instron crosshead.

Dark blue areas denote portions of the tendon that have move outside the field being imaged

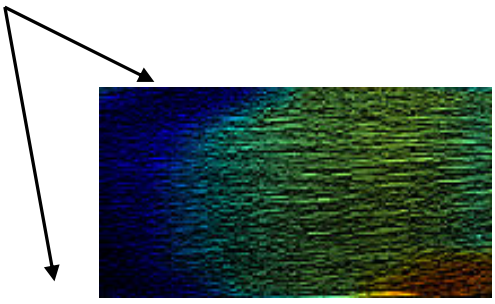


Figure 62 Colour map of xx strain superimposed on the tendon field showing blue areas where the original blocks have moved outwith the images

When examining the displacement field for the tissue analysed in this way we see the areas where there is no tracked displacement to the left and the edges of the tendon speckle changing in such a way that suggests the outer part of the tendon is being pulled first and then followed by the core of the tissue (Figure 62). This is in keeping with that expected when using most normal

clamps, for the clamp will obviously grip onto the outer part of the tissue causing deformation of the material and some slippage of the core.

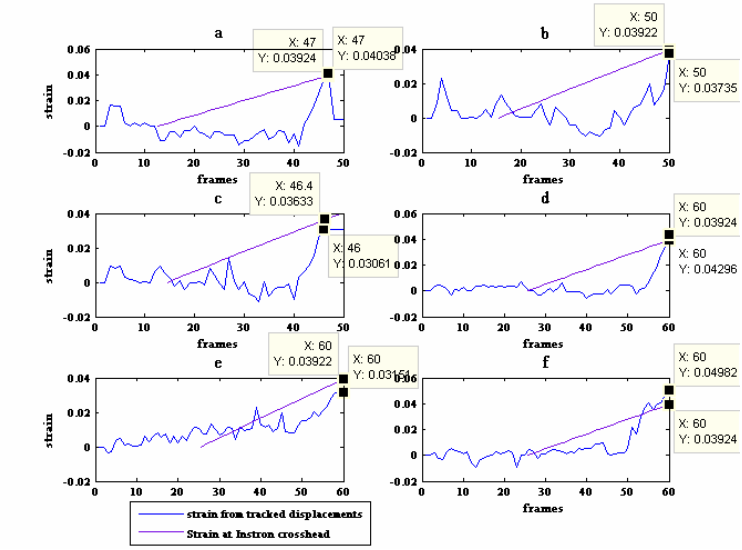


Figure 63 Graph of xx strain recorded at the Instron crosshead and tracked xx strain from the core of the SDFT v time for each trial

Trial	Instron X-head Strain	Strain from Tracked Displacements
a	0.03924	0.04038
b	0.03922	0.03735
c	0.03633	0.03061
d	0.03924	0.04296
e	0.03922	0.03151
f	0.03924	0.04982

Figure 64 Final Strains recorded at Instron Crosshead and from Tracked Displacements

The final strain tracked in the core of the SDFT here was comparable with that recorded at the Instron crosshead, indicating minimal overall slippage of the main body of the tendon certainly with respect to the early trials (Figure 64). The mean strain recorded at the Instron crosshead was 0.03875 while that calculated from the HVBM tracked displacements was 0.03877, a difference

of 0.00002. When one considers that the Instron accuracy is published to be ±0.05% of the strain recorded i.e. ±0.00019 this can be considered well within the limit of the Instron.

The strain within the SDFT as calculated from the tracked displacements did not appear to be linear as recorded at the crosshead but appeared to occur within the last few frames and this was similar in all the trials, as seen in Figure 63. A possible explanation is that the HVBM is tracking the movement and strain as it occurs within the main core of the tissue and that this is occurring later than at the periphery (Figure 62). A better explanation may be that there is slippage within the clamps despite the bollards. A more accurate way to have recorded the true mid-tendon strain would have been to use a video tensiometer but unfortunately this was not possible, due to financial constraints. Finally, it may be that there is some loss of the original grey scale patterns during the straining process which then resolves when the structure has reached its final position.

CONCLUSION

It is reasonable to conclude that 'Project2', the final version of the HVBM tracking software, provides a realistic representation of movement and the final strain within tendon tissue provided it is used as described and with attention to certain caveats.

In Summary:

The final settings that are most appropriate for tracking strain within a tendon are:

- An initial block size of 16 pixels but only 3 iterations to a final block size of 64 pixels.
- The 'Advance' settings may be left as the default settings, set by the author of the code, apart from Drift, which should be enabled and an increase in the correlation threshold to 0.93 as concluded from the results of the initial calibration of movement in tendons.
- After analysis the displacements in both the direction of movement and that perpendicular to it must be examined in order to ensure that the displacements outside the direction of interest are not greater than that being examined.

- It is important to look at the overall strains and displacements throughout the field to ensure that the tracking is a believable representation of what is being tested.

- The value of strain from the tracked displacements is reliable only when the tendon has stopped moving.

The overall conclusion is that this is potentially a useful and reliable method of analysing ultrasound cine of tendon strain in vivo, provided that the above settings and comments are adhered to.

Chapter 8

PILOT STUDY TO INVESTIGATE PATELLAR TENDON STRAIN UNDER PHYSIOLOGICAL LOAD

INTRODUCTION

The major part of this thesis has been the validation and investigation of the application of a novel U/S speckle tracking program for use in the examination of tendons. It has been shown that provided there is attention to the restrictions mentioned within the previous chapters it is a potentially useful method for the investigation of strains within tendons.

This Chapter describes the application of this analysis within an in vivo investigation, in the manner in which a full study might be run. To this end a pilot investigation of strain in tendons under physiological load is described.

PROTOCOL

SUMMARY OF TENDON STRAIN INVESTIGATIONS TO DATE

In recent years it has become more widely accepted that, in the majority of cases of tendinopathy associated with overuse, there is little if any evidence of inflammation but rather the tissue is degenerate (Jarvinen and others 1997a; Jozsa and Kannus 1997; Paavola and others 2002a; Tallon and others 2001). There have been changes arising from this in the conservative management of the disease toward an exercise based protocol and, partly because of this; some research has shifted to examine how the tendon may remodel. It has been illustrated, in vitro, that different tendons within the body have different properties. Furthermore, it has also been seen that, within the same tendon, different areas may also demonstrate different attributes and this is proposed to be a response to the different loading patterns applied to them (Almekinders and others 2001; Benjamin and Ralphs 1998; Haraldsson and others 2004; Haraldsson and others 2005; Lyman and others 2004).

The questions then arise:

- Is there consistently a different amount of axial strain occurring in the posterior fibres than in the anterior region?
- Does the distribution of strain vary depending on the level of loading and / or the joint angles at which that load is applied?
- More specifically if there is variation of the mechanical properties throughout an individual tendon and this is defined by the predominant mechanical environment under which that individual's tendons are placed does this influence the vulnerability to tendinosis?

(That is, if for the majority of the time the knee is generally working within the range of 0˚to 45˚ and the tendon modifies its structure and properties accordingly, then for short periods it is exposed to higher loads at 90˚ (i.e. in certain sporting situations) does this make some portions of the tissue more vulnerable than others?

- Is the vulnerability to tendon disease in specific areas due to a mismatch between regional properties in different situations or due to shear within the matrix of the tendon due to differences in the axial strains between different regions?

To date, most research into the differential properties found within the same tendon has used tissues from cadavers, as listed in the above references. There has been one recent investigation where, using the insertion of optical fibres within the tendon, it has been possible to examine how the loads and potentially strain in different areas are affected by joint position and muscle work (Dillon and others 2008). One potential limitation this work is the placement of a foreign body within the tissue being investigated, it should be considered that this in itself may have effects on the very properties being investigated. It has been possible, for some time to investigate, with U/S, the strain occurring in a whole tendon, in vivo (Kubo and others 2004a; Kubo and others 2003; Kubo and others 2002b; Maganaris 2001; Maganaris and Paul 1999; Reeves and others 2003a; Reeves and others 2003b). These studies, however, have relied on the use of anatomical landmarks and/or skin markers in order to visualise the movement of the tendon as the physiological load is applied. This means that it has not been possible to look at discrete areas within the tendon.

It is anticipated that the results of this study will show different amounts of strain in the posterior fibres of the proximal patellar tendon and those of the anterior region as predicted by modelling (Lavagnino and others 2008) and that there may be effects on this distribution of strain by changes in joint range and load (Hypothesis 5). It is possible that subjects training regularly in weight bearing sport may have different strain ratios between the regions when compared with those in sedentary subjects or to those taking part in a non-weight bearing sport but the numbers included here preclude investigation of this.

OBJECTIVES OF THIS STUDY

- To find if it is feasible to consistently produce images of sufficient quality to be analysed by the HVBM.
- To establish if there is a difference in the strain experienced between the anterior and posterior fibres in the patellar tendon and if this pattern is affected by changes in joint range and in load.

In a future study this technique could be used to establish if there is any difference in the strain patterns seen in the normal sedentary population, the normal athletic population and ultimately in those with established tendon disease.

ETHICS

Full ethical approval was applied for and granted by the Derbyshire Research Ethics Committee. A full NHS Ethics application was made for both patellar and Achilles tendons to be studied in both normal subjects and within a patient group of those suffering from an existing tendinopathy. This was done with a view to future investigation using this methodology.

METHOD

Normal subjects were recruited from colleagues and local sports clubs. They were fully advised of the experimental protocol both verbally and in writing (see Appendices). Reasons for exclusions were existing tendon pathology, knee pain of other derivation and pregnancy. Subjects were advised fully of the planned investigation and consented by the researcher (see

Appendix). The patellar tendon was examined using the U/S scanner to ensure that there was no evidence of any pre-existing tendon disease. Those exhibiting any signs of pathology, on an ultrasound scan, were advised to see their own GP to whom a letter would have been sent explaining the situation and advising referral to Professor Batt at the Centre for Sports Medicine.

Subjects were linked to data by allocation of encoded unique labels registered on the subject consent form. They were informed of this link prior to being consented.

All participants were then familiarised with the Cybex dynamometer, an isokinetic machine, which was set-up appropriately for the testing of quadriceps function. As a warm-up procedure they were directed to perform 10 isotonic, concentric knee flexions and extensions at a preset rate of 60º per second. They were then shown how it would feel to work against the fixed Cybex arm, and allowed to practice at maximum output and at 30% of this, at 90º, 45º and at 5º of knee flexion.

For the collection of experimental data the ultrasound probe was fixed to the leg by means of a sponge support, as in Chapter 5, and re-usable strap. Aqueous gel was applied to the probe as an interface.

Each participant was then instructed to perform a series of 3 isometric contractions at each of approximately 30% of maximum and at maximum output. This was performed with the knee in 90˚ of flexion, repeated at 45° and then again at 5° knee flexion. Prior to collection of the U/S data this was practiced again at maximum for each joint range in order that the subject was familiar with the appropriate level at which to work. If necessary participants were able to practice until they felt confident in the exercise. In most cases one or two practices were sufficient. Finally this regime was repeated with the U/S probe in position and the subject worked at each variation of joint range and load while the operator attempted to co-ordinate the collection of U/S cine data for each work trial. This should have resulted in 18 cine data sets for each candidate, allowing for 2 sets of back up data for each combination of work output and joint

range. In reality the numbers saved were 129 out of a potential 180 (18 per subject). Errors in timing and probe contact were responsible for the 61 data sets not saved (see the results section).

Ultrasound cine data was labelled at the time of collection and stored for later analysis. Subjects were then directed to stretch the quads and hamstring muscle groups.

The analysis was performed as described within Chapters 3, 4 and 6, in order to develop a strain map for the tendon under different loads.

SUBJECT GROUP

The 10 subjects taking part ranged in age from 27 to 48, average age 38.2, and were comprised of 7 women and 3 men. Of the 7 women 4 trained or competed in sporting activities more than 3 times a week and 3 of those were involved in weight bearing sport. Of the men only one trained regularly and in a non- weight bearing sport. None were found to have tendon degeneration when the patellar tendon was scanned.

Initial measurements of tendon data indicated that there was a large degree of variation in the load produced between different subjects (from 64 N to 273 N, maximum loading @ 90˚ knee flexion). Similarly the strains were seen to range when assessed at 90˚ between 1.5% and 7.5%. It was decided that, in order to further assess the reliability of the tendon strains derived with the HVBM in vivo, it would be appropriate to perform a repeatability test as part of the pilot.

This was done by analysing the footage of one subject working at a maximum level in 90º knee flexion over four separate trials and then comparing the results of the whole tendon strain and the strains found for the anterior and the posterior fibres.

RESULTS

It was essential to look at how much of the data produced in a clinical trial was of sufficient quality to be analysed by the HVBM software and produce meaningful results. It was then obviously important to look at reasons for failure in those sets of data that were discarded.

As there has been recent work, in vivo and in vitro, exploring potential differences between the anterior and posterior fibres of the patellar tendon (Dillon and others 2008; Lavagnino and others 2008) it was decided to analyse, where possible, the strains in the imaged portion of the tendon and then to divide it to see how feasible it would be to find discrete differences in the strains between regions. For the purpose of this Pilot Study it was decided that it would be appropriate to keep any regional cropping to two basic divisions. The imaged tendon represents only the proximal and middle portion of the tendon due to the size of the probe footprint and so to try to explore the areas investigated by others (Dillon and others 2008; Lavagnino and others 2008), a simple split into anterior and posterior using the patellar pole as the guide for the division was considered appropriate.

QUALITY OF THE DATA PRODUCED

129 of a potential 180 cine loops were stored for analysis. Data loss most often occurred due to poor co-ordination between the researcher collecting the data and the subject's application of the work. There was also, in some cases, loss of contact between the probe and skin. It was hardest to maintain contact with the knee in 5º flexion and in most cases it was easier to discard the foam support at this point and hold the probe in position against the patellar pole. Although the Cybex screen has commands directing work and rest periods most people found it easier to listen to the commands from the researcher. As the study progressed timing became more proficient and by the last 3 subjects the timing of work and data collection was fairly successful.

For each subject load and joint range variation there was at least one set of data stored and in most cases there were two and so it was expected that it should still be feasible to achieve the 60 useful cine loops required for all 10 subjects. After full analysis, however, only 44 of the required 60 were proved to produce reliable strain data.

The original cine was first viewed to check the general quality of the cine and the estimated point were maximum strain was reached. Vector analysis, as described in Chapter 6, was then

employed as a filter in order to find which cine loops were most likely to produce reliable results. The vectors were analysed using a simple scoring system of 0 - 3.

0	no apparent tracking
1	only minimal track with cine
2	faithful tracking but mostly y displacement
3	good obvious correlation of x displacement

This assessed the films for optimal correlation between the vectors of the tracking and the movement visualised on the cine. When the vectors were assessed to be vertical throughout most of the cine it was marked as a score of 2, or if there seemed to be minimal or no tracking of the tendon region by the vectors a score of 1 or 0 was given. In all these cases a 2nd or, if required, a 3rd set, of back up data, was then assessed in the same way. When it was not possible to give a vector score of 3 for any of the cine stored for a particular subject, load and joint position then the cine was analysed further anyway and if the y displacements or strain were greater than that in the x direction the results rejected.

Of sixty potential cine loops it was necessary to resort to back up data in seventeen cases. Forty three loops were then scored with a 3 after visual inspection of the vectors.

Of the 17 data sets that were not allocated with a vector score of 3, 11 were considered to have excessive amounts of y displacement; i.e. were scored with a 2. Unfortunately in the case of data from these subjects it was often found that there were similar problems in 'the back up data', which had been collected at the time. In some cases there was not a 3rd set of data saved, as those subjects with large amounts of y displacement were often the ones who had problems maintaining contact with the probe throughout the exercise. In five cases the vectors were scored with a 1 and there was one situation were there did not appear to have been any tracking, a score of 0. In the case of the five minimal tracks there was not found to be any backup material that could provide better images to replace them and the further analysis was done despite the minimal vector correlation. Of the forty four sets of strain data finally deemed to be reliable three

were originally scored with a 2 but on further analysis it was found that the y displacement had occurred after the final strain was reached. Similarly three of those given a 3 were finally rejected after full analysis as in two there was to be yy strain or y displacement that exceeded that of the xx strain or x displacement. In the third the software did not appear to have tracked within the tendon region, probably due to oversaturation of the image. Thus although the vectors give a reasonable idea of the reliability of the tracking it does not prove a full proof filter and can only be used as a rough guide prior to full analysis.

After deciding on the most appropriate data sets to analyse for each subject, the x and y displacements were loaded into the Matlab programme and plotted as a map of the original image in order to see where, within the images, the HVBM had appeared to track movement. This was to ensure that there was an appropriate pattern of tracked displacement and that the region of tracked displacement coincided with the tendon area. In nine of the cine loops it appeared that the tracking had not fully followed the tendon but rather was of the area below, i.e. the fat pad or loose connective tissue. In these cases the images were saturated or very white, over the tendon field whereas the fat pad was dark. Although movement within the dark area below the tendon was difficult to visualise, the displacements were often consistent with what one would have expected under this type of strain, and so were probably realistic i.e. a dark image is more appropriate for the HVBM tracking even if it appears difficult to view (Figure 65).

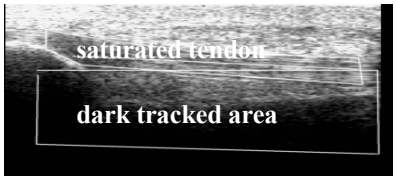


Figure 65 Image showing white 'saturated' tendon with dark area tracked below

A region of interest derived from the displacement maps, was drawn onto the original image so that it would provide a guide when selecting the area for strain analysis. It also enabled

visualisation of the outer edges of the strain field in relation to that tracked area, as the strain maps were presented. This acted as a 2nd confirmation of where the HVBM had tracked most accurately. This is illustrated in Figures 66 and 67.

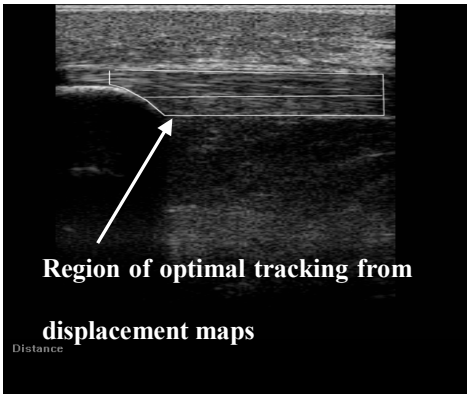


Figure 66 Region of interest marked on tendon as predicted by displacement maps

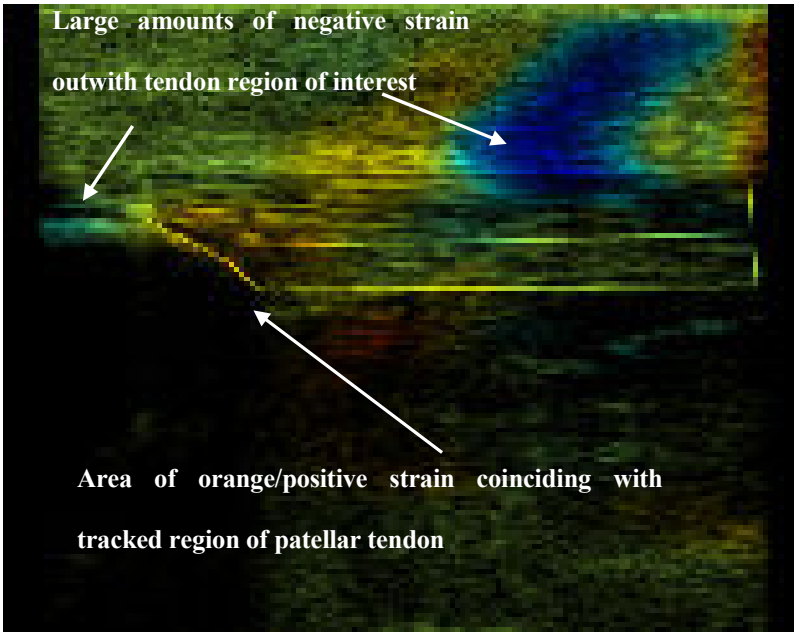


Figure 67 xx strain map superimposed over original image with defined region of interest as in Figure 66

Criteria for acceptable data were set such that the y displacement or yy strain should not exceed that of the x displacement or xx strain. It was predicted that where this was the case the tracking of movement in the x direction would not be reliable from the results in Chapter 7. In some cases where the y displacement did not occur until after the maximum xx strain appeared to have been reached it was still possible to record a reliable x displacement and xx strain. This was checked with the original cine. In the case of 13 sets of trials, the HVBM had failed to track the cine to a level where strain calculations could be performed, in these cases the backup data failed similarly.

From Figure 68 one can see that the large areas of extreme strain, often associated with the edges of tracked displacement, are outwith the actual region of interest for the tendon. On the marked area there is also a dividing line placed to help identify the anterior and posterior fibres of the tendon.

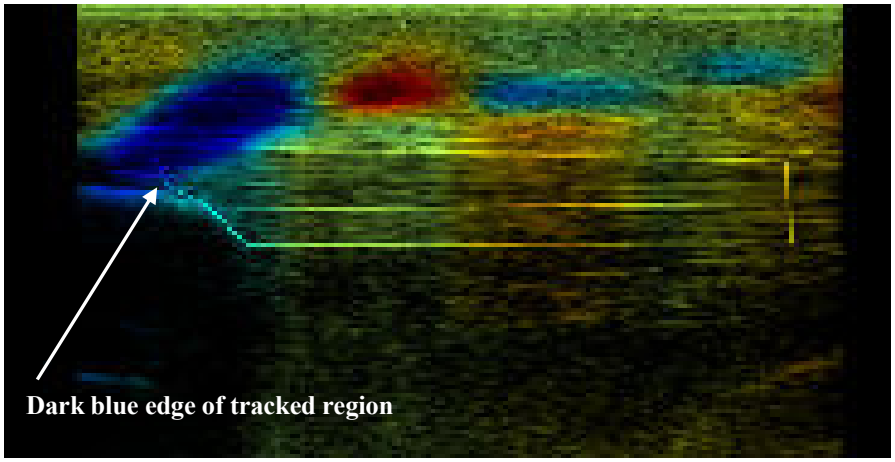


Figure 68 xx strain map superimposed on original image demonstrating dark blue edge of tracked displacement

In Figure 68 we can see the proximal edge of the tendon region has an area of apparent compressive strain represented by dark blue, which is because the most proximal part of the

image has not been tracked. When calculating the average tendon strains this area would need to be avoided.

After visualising the portion of the tendon that was most likely to provide reliable data from both the displacement and strain maps average strains through time were produced for the areas representing the whole of imaged tendon, the anterior and the posterior fibres.

WHOLE TENDON STRAIN WITH RESPECT TO SUBJECT AND TRIAL

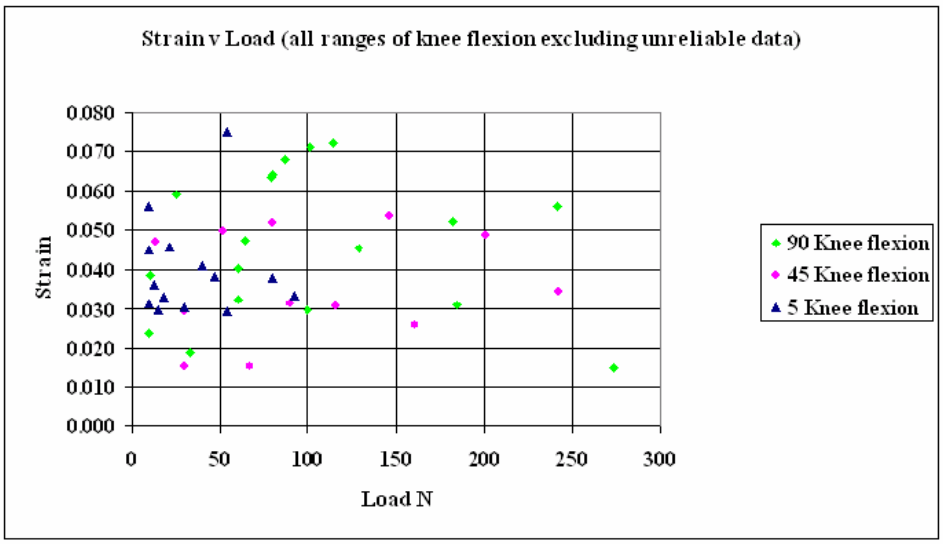


Figure 69 Graph of strains found in tendons of each subject at each trial

From Figure 69 we can see that the strains found within the proximal and middle parts of the tendons, as cropped after adhering to the guidelines in the previous chapter, vary from 0.015 to 0.075, or represent strain from 1.5% to 7.5%. Reviewing other studies we can see that this fits within the values that we might expect for this subject group. When a cohort of male athletes, aged 30 (SD 7) years, was used to define the mechanical properties of the patellar tendon, in vivo, and here a typical strain value of 7% was found when a maximum isometric quads contraction was applied with the knee in 90º of flexion (Hansen and others 2006). This study allows for any extraneous movement of the tibia with respect to the femur by visualising both

tendon insertion points on the U/S footprint, one could argue that this is not necessary in our strain calculation as it is based on length change within the tendon tissue alone. The main limitation is however that the area of the tendon that is visualised with the probe in our study only includes the proximal and mid portions and the full tendon strain may well be different when all parts of the tendon can be included, as in the work of Hansen et al. In an earlier study using an older subject group in the age range of 65 to 77 years but with a mix of male and female, as in the current work, the strains recorded with the knee in 90º flexion after applying a maximal isometric quads contraction were in the region of 9 ± 2.2 % but this dropped to 5 ± 2.4 % in the trained group after 14 weeks of training (Reeves and others 2003a) which is reported to be closer to those tendon strains often found in a younger cohort. When we filter the data from this Chapter to include only those strains produced with maximum loading at 90º flexion they have an average of 5.2 ± 1.8 %, see Figure 70. Therefore this data is in agreement with previous studies but provides information about local regions within the tendon rather than from insertion to insertion.

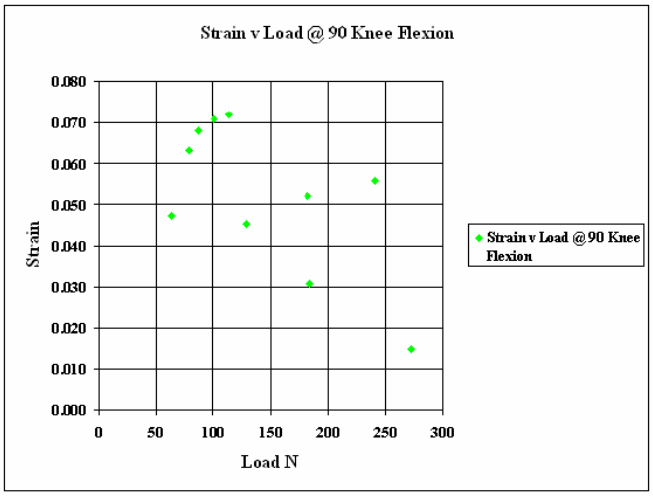


Figure 70 xx strain values @ 90º flexion with maximum load

ANALYSIS OF STRAINS IN ANTERIOR FIBRES, POSTERIOR FIBRES AND IMAGED TENDON AS A WHOLE

When the part of the tendon that had tracked effectively was identified and marked on the first image, it was subdivided such that the posterior fibres, level with and below the pole of the patella, were easily identified and the area above this was allocated to the anterior fibres, see Figure 71. This ensured that the values could be allocated accurately with respect to the original image and in the future if required.

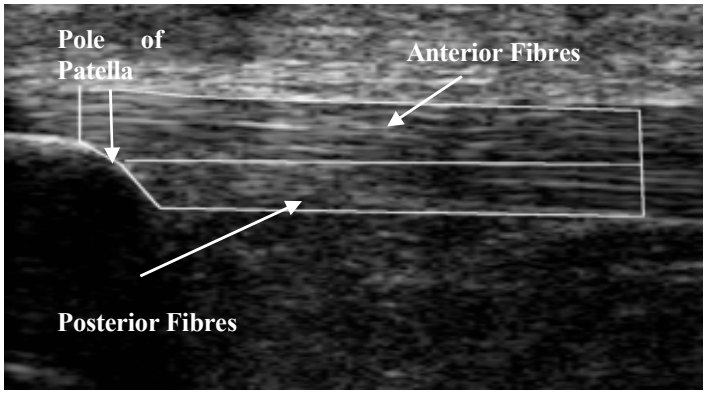
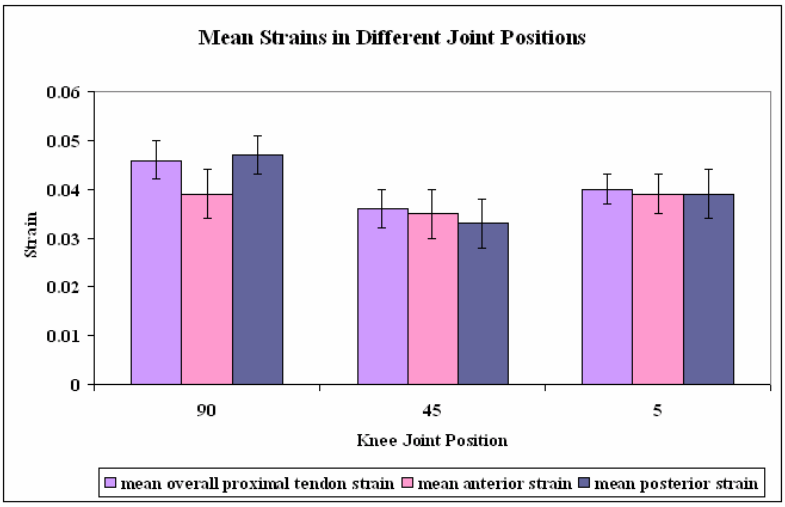


Figure 71 Regions of interest marked to identify anterior and posterior fibres for cropping

Of the forty four data sets considered reliable, higher strains were recorded in the posterior fibre area in 20 cases, in 1 case similar strains were found throughout the tendon and in the remaining 23 higher strains were found in the anterior fibre region. This is looking at the results for all three joint positions.



joint position	mean strain all fibres	standard error	mean anterior fibre strain	standard error	mean posterior fibre strain	standard error
90	0.046	0.004	0.039	0.005	0.047	0.004
45	0.036	0.004	0.035	0.005	0.033	0.005
5	0.04	0.003	0.039	0.004	0.039	0.005

Figure 72 Mean strains of Imaged Tendon at each joint position with standard error bars

It is however interesting to note the mean strains of the whole imaged tendon and that of the posterior and anterior portions at each joint position (Figure 72). It appears that there may be some change in the distribution as the range increases. If we further analyse the difference between the strains in the anterior and posterior portions at each joint position (Figure 73) it would seem possible that the differential increases as the knee flexion increases.

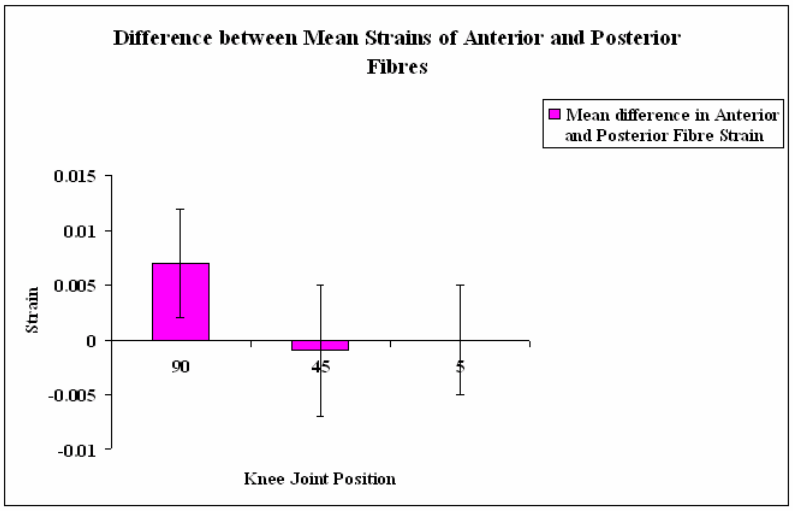


Figure 73 Difference Between the Mean Strains of the Anterior and the Posterior Fibres at Each Joint Position

This was examined using a Wilcoxon Signed Rank test (Figure 74) for related samples in order to see if the differences in fibre strains were significant.

Wilcoxon Signed Rank Test		
Joint Position	**Median Difference between Ant and Post Fibres**	**p value**
90	0.008	0.122
45	0.002	0.875
5	-0.003	0.650

Figure 74 Wilcoxon Signed Rank Test for the Median Strains of the Anterior and Posterior Fibre Strains at each Joint Position

We can see from this that the differences are not significant at any of the joint positions for this data. This will be discussed further in the discussions section.

RESULTS OF THE REPEATABILITY TRIAL

Although the strain values were not exactly matched in all the trials the regional patterns are similar. The average strain recorded at 90˚ knee flexion and maximum load was 0.029±0.007, and that within the anterior fibres was 0.025±0.008 and 0.037±0.01 in the posterior portion (Figure 75).

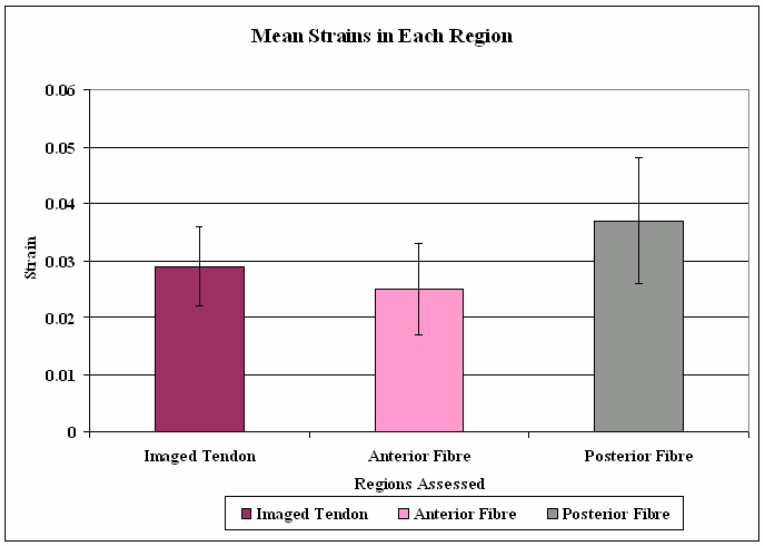


Figure 75 Representation of Mean strains for all four trials at maximum load and 90˚ flexion with standard error bars

We can see this further illustrated below in Figure 76 which represents graphically the regional strain and the mean strains for each cropped region of the tendon in all four trials.

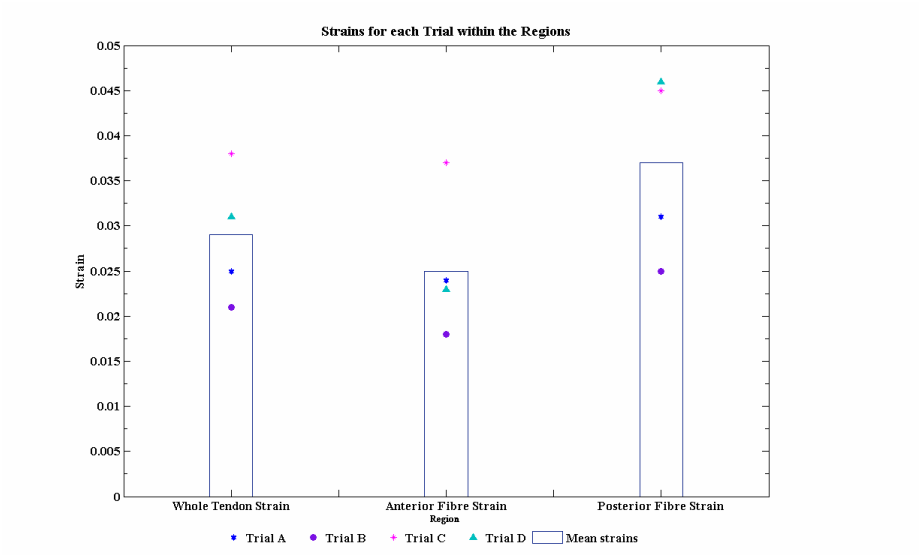


Figure 76 Regional strain for all repeatability trials and mean strain for each region

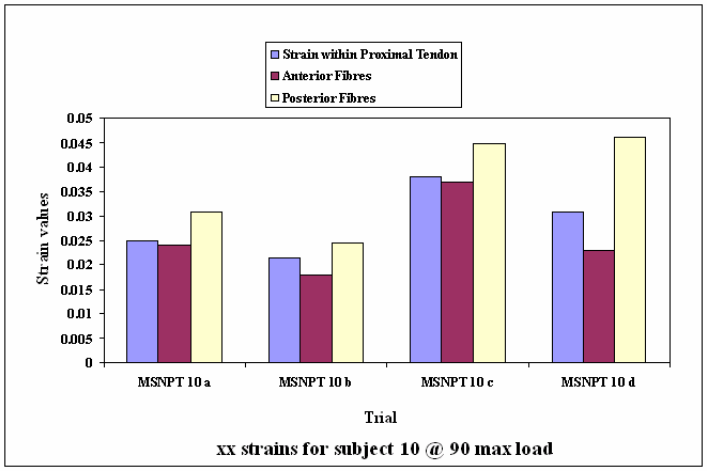


Figure 77 Graphical illustration of the regional xx strains in all four trials of maximum isometric quads work @ 90º flexion from Subject 10 in order to investigate repeatability

When examining the original cine and the vectors in the second trial, although there did not seem to be much y displacement, there was found to be greater yy strain than xx strain between frames 8 and 23. After examining the displacements and strains throughout the tendon field and the full

cine, it was found that there was an area of more reliable tracking within the proximal half of the tendon region visualised. The results shown within the above graph (Figure 77) represent this proximal portion of the tendon image.

DISCUSSION

It was clear as the analysis progressed that the strain values acquired from the U/S cine loops could only be as reliable as the quality of the images that were to be analysed. U/S cine was discarded due to two main reasons:

1. Some of the images, generally from the earlier tests, were not of a quality that the HVBM could track easily; often this was due to high saturation in the region of the tendon. An U/S image of a tendon may appear easier to read when the gain is highest leading to excessive saturation. The software, however, can best track images that may appear dark to the human eye as it relies on variation of the grey scale throughout the structure, which is less so in the case of a very white image.

2. There were in some cases levels of y displacement that exceeded the x displacement. This was not always apparent on the initial vector analysis and there may be levels of yy strain throughout the area of the image that correlated to soft tissue, both tendon and infra-patellar fat pad, which exceeded the xx strains recorded. It is interesting to note again here the work of Hansen et al. (Hansen and others 2006). They concluded that in the traditional in vivo calculation of tendon strain from visual analysis of series U/S images from the patellar tendon it is difficult to see how much extraneous tibial movement sometimes occurs. When they used a larger footprint in order to visualise both the patellar and tibial insertion they found that as much as 45% of the recorded tibia-patellar movement may result from movement of the tibia alone and this may well compromise the accuracy of the results. It is not unreasonable to suggest that in the case where there exists excessive y displacement or yy strain, in some of the results of this Chapter, this may be due to excursion of the tibia. Therefore I would argue that by

excluding the situations where the y displacement and the yy strain exceeded that within the x direction we have addressed some of the potential problem associated with a smaller probe footprint.

It was apparent that the best data was recorded towards the end of the study. By this stage the operator gained experience in the use of the U/S machine in general, optimising the image acquired for the HVBM analysis. There was also improved co-ordination of directing load application with saving of the cine. In the case of the last 3 subjects a different U/S machine had become available and although it was exactly the same make and model as the original version it may have had slightly less general usage and the probe may have been in a better state of repair.

In general, the trials which were discarded due to excessive y displacement or yy strain were often found within the same individuals. This may have been due to the fact that the dynamometer was not set in the optimum position for those individuals, allowing excessive movement when applying the work. In the case of future work in this area it is important to ensure that the bespoke set-up for each subject is optimum for minimising excessive y movement when applying the work. It would be useful to make final adjustments after a trial imaging with the U/S.

It was hypothesised that analysis of the patellar tendons from a regional point of view may support the recent work suggesting that there is a difference in the strains experienced between the posterior fibres of the tendon and those of the anterior region (Dillon and others 2008; Lavagnino and others 2008).

It was further hypothesised that any differential may alter when in different loading positions. Examination of the results here suggest that in the ranges between 0º to 45º there is little difference between the strains within the fibres but when the joint angle goes further into range the posterior fibre strains are increased, leading to a mismatch between the tendon properties and the strain in some individuals.

Full analysis of the results, however, did not show any significant difference between the means of the strains of the anterior and posterior fibres in any of the joint positions, although the results at 90˚ did at first appear promising. The mean difference between the anterior fibre strain and posterior fibre strain was found to be 0.007 (95% C.I. -0.003 to 0.017). This was not statistically significant ($p < 0.14$ paired t-test). A post-hoc power analysis (s.d. = 0.019, $\bar{x}$ =0.007, power 0.8, α 0.05) suggests that a sample size of 60 would be required to detect this difference. It is important also to note here that the potential differences in the strains vary from 0.3% to1.7%, and in the repeatability trials the overall strain varied between by 1.7% between trials 2 and 3. One could therefore argue that the differences seen in the strains in the different regions are due to random variations. However, the variations in the strains in the repeatability trial are between different repetitions of the same exercise and so are dependent on whether exactly the same conditions were fulfilled in each trial. That is, that exactly the same loads were applied in each and that the tendon strain had not been affected by the repetition of the exercise. If we refer back to figure 77 we can see that there does appear to be some increase in the strains as the exercise is repeated, with the exception of the 2nd trial which was that in which the yy strain exceeded the xx strain when the whole of the imaged tendon was considered and only the proximal half of the image could be used. What is more encouraging is the fact that each trial showed that the posterior fibre strain was greater than the anterior fibres.

After analysing the data fully it was apparent that the strains fluctuated throughout the loop. It is impossible to say if this variation is due to errors in the tracking, associated with the movement and potential anisotropy in the image, as we saw in Chapter 7 or if this is a reflection of the nature of the way in which work is applied by the muscle during an isometric contraction. In order to try and ensure that the strains identified were those where the maximum loading for that trial had been applied the original cine was viewed to establish at which point the loading was seen to be greatest. This was not an ideal way of identifying maximum strain. Due to this the quantitative measures of the strain outputs cannot be taken as absolutely reliable. Had funding and equipment permitted the output of the dynamometer should have been connected to the images on the U/S screen in order that the moment of maximum load application could be

identified with a particular frame within the cine. In other studies, in order to achieve true maximum quadriceps activation neuromuscular stimulation via the interpolated twitch technique has been used (Reeves and others 2003a). Future work from this pilot would benefit from the application of similar methods, especially with respect to the repeatability.

CONCLUSION

As a trial to see if the HVBM software provides us with a potentially useful tool to investigate strain in patellar tendons under load in vivo this work generally supports its application. It is important, however, as in the conclusions of Chapter 7 to be aware that the reliability of this software is completely dependent on the adherence to certain caveats:

- It is essential that the quality of the U/S images to be analysed is high and any operator must be skilled both in the use of U/S imaging and in the type of images required for optimal tracking. It is important to be aware of the requirements of the HVBM for tracking. Images that track well are somewhat darker than those one might acquire in order to visualise the tissue for clinical reasons.

- It is vital, when exploring displacements and strains in one direction to minimise any movements in the direction perpendicular to that or indeed any out of plane displacement. In order to comply with this the researcher must be fully familiar with setting up the dynamometer for each individual and be prepared to adapt the set-up as the procedure progresses depending on the images being seen.

- To ensure that the region of vulnerability to tendon disease is included, within that tracked fully, it would be helpful to use a probe with a larger footprint and to place the proximal end further over the patella itself. This would help to avoid losing this area within the untracked area while still being able to include the majority of the rest of the tendon body. In an ideal study design I would recommend that the U/S probe was large enough to include both the patellar and tibial insertion points in order that the strain through the whole tendon could be identified. This should then be subdivided into a

minimum of 4 - 6 regions in order to fully explore possible regional differences and any variation that the cropping points might make to the results.

- Future investigations should be run with more than one operator. By choice, a clinician skilled in the use of U/S would handle the data acquisition while a physiotherapist would be ideally suited to setting up the dynamometer and directing the work output.

- Any full study would require a larger sample size to find significant differences in the inter-regional strains ($n > 60$). Although the potential differences between the regional strains appear small it is important to note that when we consider the maximal difference of 0.017 this is 36% of the mean overall tendon strain, and therefore certainly worthy of further investigation.

Despite the guarded conclusion to this study and provided the above advice is adhered to, I believe that it has been shown that the HVBM potentially provides an exciting progression in the investigation of tendons in vivo. There is certainly no other non-invasive measure of inter-regional strain as yet available for use in vivo.

Chapter 9

SUMMARY AND FUTURE WORK

SUMMARY

Before starting this thesis it was expected that, in essence, this would be a clinical study looking at the strains in the patellar tendon and how they might be affected by changes in joint ranges and loading. It was surmised that the different populations of sedentary individuals, those involved in weight bearing sport and those with pathology would present with different distributions in the strains within their tendons. There is much debate regarding what is a 'normal' strain distribution with each tendon and research to date presents conflicting opinions (Almekinders and others 2001; Basso and others 2002; Dillon and others 2008; Haraldsson and others 2005; Lavagnino and others 2008).

When dealing with any biological tissue the results are likely to vary between individuals and we would expect to see a trend with respect to properties rather than an absolute value. Due to this and the untested nature of this area of investigation it was paramount that any method should be subjected to rigorous testing in order that any results could be presented with confidence. Thus the overall thesis has shifted to become a validation of method and the starting point for exciting investigations in the future.

In Chapters 5 and 7 we examined the ability of HVBM software to be able to track movements in phantoms, tendons, and tendons exposed to tensile strain. Provided the speckle is organised and is not removed from or added to, throughout the cine we can be confident that the tracking is an accurate representation of reality. 'Project2' the final version of the HVBM software has been shown to track grey speckle pattern within U/S images successfully both in phantoms and in tendon tissue whether it is merely moving or actually being strained. More care must be taken when the movement of interest is in the x direction as there is obviously difficulty in following blocks of speckle once they have left the image field and allowances must be made for this.

In the case of tendon, a highly anisotropic tissue, the texture and echogenicity changes considerably with the angle of the probe in relation to the structure. This means that y displacements and yy strain within the tissue has the potential to affect the pattern, and thus tracking and strain evaluation, in the x direction. This was illustrated in both Chapters 6 and 8 where the tracking was used on images captured in vivo. It is however, possible to prevent this provided care is taken with the acquisition of the images. The U/S probe should be held firmly in position, which should be confirmed visually on the screen throughout the cine. Essentially this means that the U/S operator must focus on handling the probe and must therefore have assistance with other equipment. When applying any physiological load, extraneous movement of the limb should be minimised and the exercise machine should be adjusted fully for each individual, therefore a fully customisable machine like the Cybex is ideal. In Chapter 8 it is apparent that good quality images can present interesting data regarding the xx strains throughout the patellar tendon. The assessment of regional strain in this final research chapter was more qualitative in nature due to the difficulties in minimising extraneous movements with one operator but it should be possible with practice and more than one researcher to produce results that add exciting new information to this area of research.

FUTURE WORK

An obvious progression from the work in this thesis would be a full clinical trial using normal subjects, in order to investigate further the proposals in currently of interest in the literature that there is comparatively a high/low strain level in the vulnerable proximal posterior region of the patellar tendon (Almekinders and others 2001; Basso and others 2002; Lavagnino and others 2008). It could be argued that one cause of the disagreement between different research groups might be answered by investigating Hypothesis 5 in Chapter 3 where it is proposed that the variations in strain between this region and elsewhere within the tendon are not the same in all situations and that it is influenced by the joint angle and/or load under which it is placed. This would go some way to explaining why a tissue that is clearly biologically dynamic in nature may

not in the case of tendinopathy respond to the strain under which it is placed by altering its properties and so, where there is high strain reduce the level by becoming stiffer.

It would then be useful to go further by dividing a normal cohort of subjects into two groups where one regularly takes part in weight bearing sport and the other is sedentary. We already expect the tendon as a whole to 'stiffen' as a result of regular exercise (Reeves and others 2003a), but is this so throughout the tendon or is there a lag in the ability to adapt within the posterior proximal portion.

Investigation of the patellar tendon is not the only application of this tool and potentially of interest are the investigation of other tendons in people such as the Achilles and other species such as the SDFT in horses. Preliminary data was collected to confirm the feasibility of studying these latter two further.

ACHILLES TENDON STRAIN

In order to visualise the Achilles under simple physiological load a subject was asked to perform simple eccentric plantar flexion with the knee straight as the images of tendon movement were captured. It was surprisingly easy, in this one case, to maintain contact with the skin and image the tendon fully throughout the series. At the time, it was not known that the final blocks to the left of the image would not be tracked well and so as the calcaneal insertion was used as the orientation point the vulnerable middle third of the tendon was not tracked adequately. There were periods at the start and the end of the sequence where the yy strain was such that the xx strains could not be taken as reliable but in the middle section of the cine where the yy strain was minimal the xx strain overall was found to be a believable ~ 4 % (Figure 78).

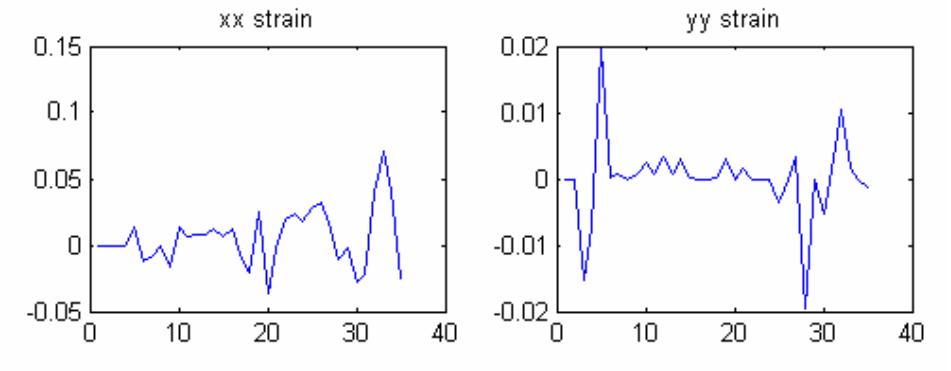


Figure 78 Graph of xx and yy strain of whole tracked region of interest through time

The image of the tendon was, as in the case of the patellar tendon samples, split into two regions with anterior and posterior fibres (see Figure 79). There was little variation between these regions and the tendon as a whole in this case but this is only one tendon and one type of load which only focuses on the distal third and not the area of prime vulnerability. It does, however illustrate that it would be feasible to proceed further with investigation of this tendon and also loading other than that of an isometric muscle contraction.

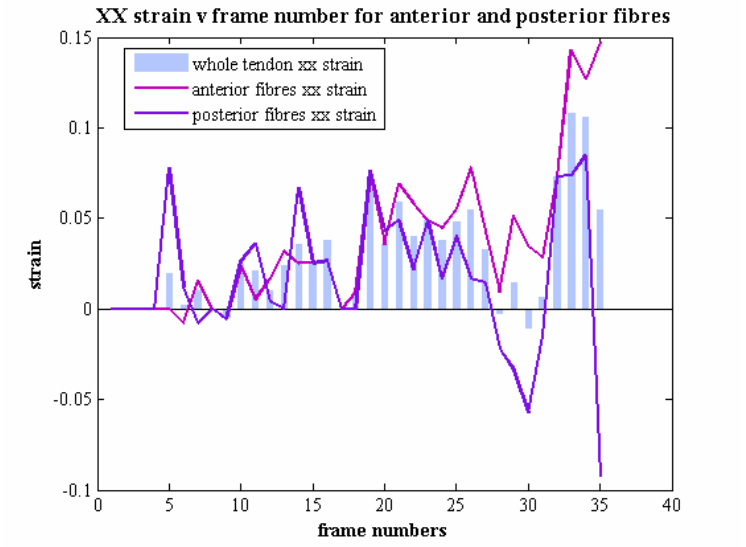


Figure 79 Regional xx strain in the distal third of an Achilles tendon

STRAIN IN AN EQUINE SDFT IN VIVO

It presented a more challenging proposition to try and find a way of loading an equine tendon, in vivo, in a controlled manner and while visualising the SDFT with U/S. It was decided that the best way would be to lift the contra-lateral forelimb while imaging the displacement within the SDFT as it absorbed the extra load. The images were once again analysed as they had been in Chapter 7 and the resultant strain for the whole tendon region selected from the image was, as in the human Achilles ~ 4 % (Figure 80).

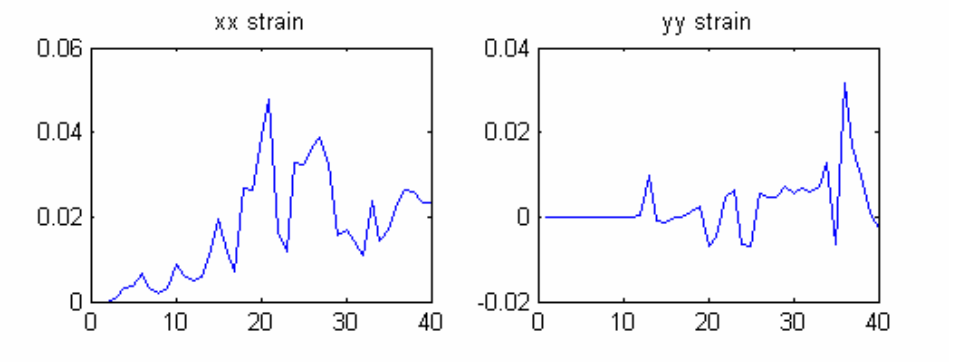


Figure 80 Graph of xx and yy strains as seen in a region representing the whole SDFT

Visual examination of the movements as they occurred in the original cine revealed what appeared to be different rates of displacement within the tendon, so regions were again explored for any differential in the xx strain (Figure 81).

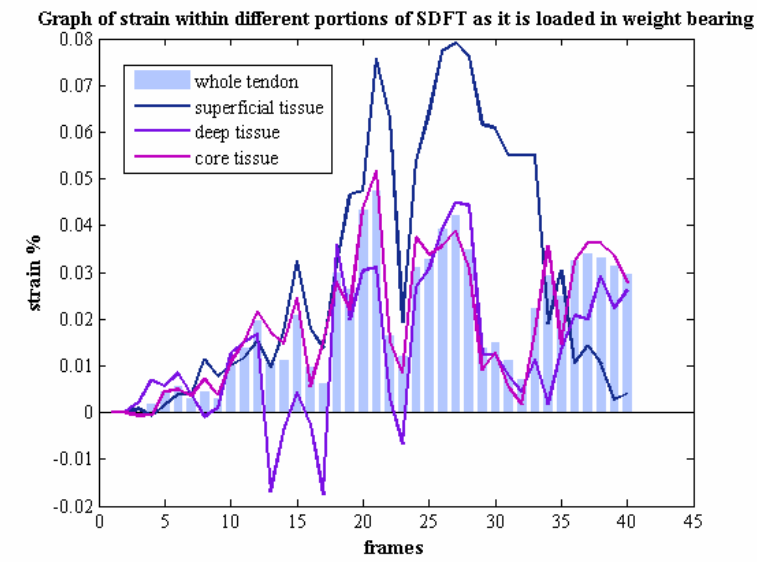


Figure 81 Graph of regional xx strain within and equine SDFT as its load is increased through weight bearing

It is interesting to note that the superficial portion of the tendon does appear to strain more than either the core or the deeper fibres. It is generally the core that is vulnerable to injury in horses. If however we examine the shear strain that is experienced at the interfaces between the different portions of tissue (Figure 82) we see that there is a large level of shear strain at the interface between the core and the superficial fibres. Could this indicate a possible potential for injury, it is almost certainly something that is worth exploring further.

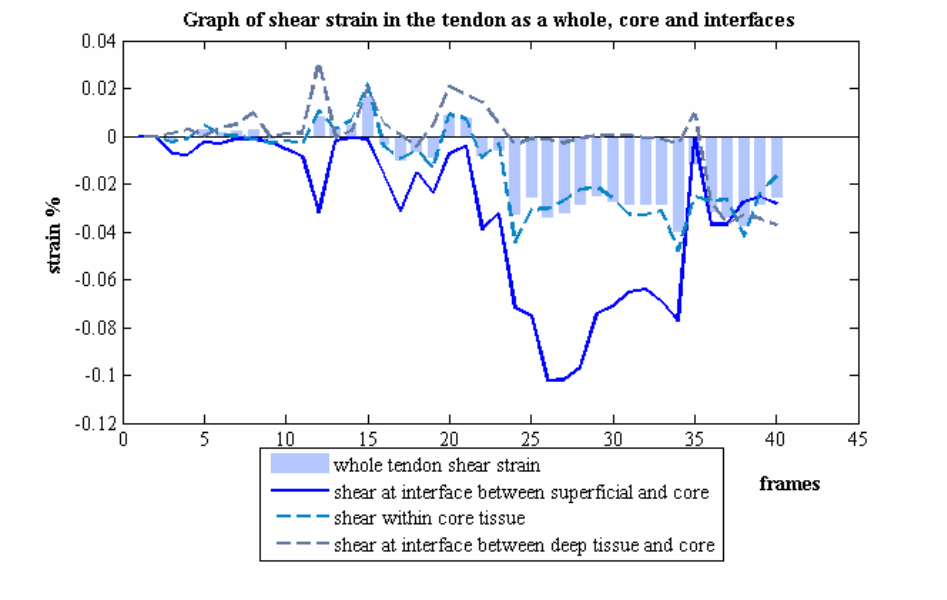


Figure 82 Graph of shear strains at the interfaces between different areas within the SDFT

It is certainly easier to explore the interfaces between regions within the SDFT than it is those of the human patellar and Achilles tendons because the structure as a whole is so much larger. The most exciting thing to take from the above case study is that it is, in principle, feasible to load an equine SDFT in vivo with little interference to the xx strain calculations from extraneous y displacement and yy strain.

Having validated this impressive tool the options for its further employment are immense.

BIBLIOGRAPHY

Alexander RM. 1988. Elastic Mechanisms in Animal Movement. Cambridge: Cambridge Press.

Alexander RM. 1992. Exploring Biomechanics. New York: Scientific American Library. 247 p.

Alexander RM, Bennet-Clarke HC. 1977. Storage of elastic strain energy in muscles and other tissues. Nature 265:114-117.

Alfredson H. 2003. Chronic midportion Achilles tendinopathy: an update on research and treatment. Clinics in Sports Medicine 22(4):727-741.

Alfredson H. 2004. Preliminary results of colour Doppler-guided intratendinous glucocorticoid injection for Achilles tendonitis in five patients. Scandinavian Journal of Medicine & Science in Sports 14(4):269-270.

Alfredson H. 2005. The chronic painful Achilles and patellar tendon: research on basic biology and treatment. Scandinavian Journal of Medicine and Science in Sports 15(4):252-259.

Alfredson H, Bjur D, Thorsen K, Lorentzon R. 2002. High intratendinous lactate levels in painful chronic Achilles tendinosis. An investigation using microdialysis technique. Journal of Orthopaedic Research 20:934-938.

Alfredson H, Cook J. 2007. A treatment algorithm for managing Achilles tendinopathy: new treatment options. British Journal of Sports Medicine 41(4):211-216.

Alfredson H, Forsgren S, Thorsen K, Lorentzon R. 2001. In vivo microdialysis and immunohistochemical analysis of tendon tissue demonstrated high amounts of free glutamate and NMDAR1 receptors, but no signs of inflammation , in Jumper's Knee. Journal of Orthopaedic Research 19:881-886.

Alfredson H, Ljung BO, Thorsen K, Lorentzon R. 2000. In vivo investigation of ECRB tendons with microdialysis technique- no signs of inflammation but high amounts of glutamate in tennis elbow. Acta Orthop Scand 71(5):475-479.

Alfredson H, Lorentzon R. 2003. Intratendinous glutamate levels and eccentric training in chronic Achilles tendinosis: a prospective study using microdialysis technique. Knee Surgery Sports Traumatology Arthroscopy 11(3):196-199.

Alfredson H, Ohberg L, Forsgren S. 2003. Is vasculo-neural ingrowth the cause of pain in chronic Achilles tendinosis? Knee Surg Sports Traumatol Arthrosc 11(5):334-338.

Alfredson H, Ohberg L, Zeisig E, Lorentzon R. 2007. Treatment of midportion Achilles tendinosis: similar clinical results with US and CD-guided surgery outside the tendon and sclerosing polidocanol injections. Knee Surgery, Sports Traumatology, Arthroscopy 15(12):1504-1509.

Alfredson H, Pietila T, Jonsson P, Lorentzon R. 1998. Heavy-Load Eccentric Calf Muscle Training For the Treatment of Chronic Achilles Tendinosis. Am J Sports Med 26(3):360-366.

Almeida-Silveira MI, Lambertz D, Perot C, Goubel F. 2000. Changes in stiffness induced by hindlimb suspension in rat Achilles tendon. European Journal of Applied Physiology 81(3):252-257.

Almekinders LC, Banes AJ, Ballenger CA. 1993. Effects of Repetitive Motion on Human Fibroblasts. Medicine and Science in Sports and Exercise 25(5):603-607.

Almekinders LC, Temple JD. 1998. Etiology, diagnosis, and treatment of tendonitis: an analysis of the literature. Medicine and Science in Sports and Exercise 30(8):1183-1190.

Almekinders LC, Vellema JH, Weinhold PS. 2001. Strain Patterns in the patella tendon and the implications for patella tendinopathy. Knee Surg Sports Traumatol Arthrosc 10:2-5.

Andres B, Murrell G. 2008. Treatment of Tendinopathy: What Works, What Does Not, and What is on the Horizon. Clinical Orthopaedics and Related Research 466(7):1539-1554.

Archambault JM, Wiley JP, Bray RC, Verhoof M, Wiseman DA, Elliott PD. 1998. Can sonography predict the outcome in patients with achillodynia? Journal of Clinical Ultrasound 26(7):335 - 339.

Arnoczky SP, Lavagnino M, Egerbacher M. 2007. The mechanobiological aetiopathogenesis of tendinopathy: is it the over-stimulation or the under-stimulation of tendon cells? International Journal of Experimental Pathology 88(4):217-226.

Astrom M. 2000. Laser Doppler flowmetry in the assessment of tendon blood flow. Scand J Med Sci Sports 10(6):365-367.
Astrom M, Rausing A. 1995. Chronic Achilles tendinopathy: A Survey of Surgical and Histopathological findings. Clinical Orthopaedics and Related Research 316:151-164.
Astrom M, Westlin N. 1994a. Blood flow in chronic Achilles tendinopathy. Clin Orthop(308):166-72.
Astrom M, Westlin N. 1994b. Blood flow in the human Achilles tendon assessed by laser Doppler flowmetry. J Orthop Res 12(2):246-52.
Basso O, Amis AA, Race A, Johnson DP. 2002. Patellar tendon fiber strains: Their differential responses to quadriceps tension. Clinical Orthopaedics and Related Research(400):246-253.
Benjamin M, Kaiser E, Milz S. 2008. Structure-function relationships in tendons: a review. J Anat 212(3):211-28.
Benjamin M, Ralphs JR. 1998. Fibrocartilage in tendons and ligaments an adaptation to compressive load. J Anatomy 193(4):481-494.
Bestwick CS, Maffulli N. 2000. Reactive Oxygen Species and tendon problems: Review and Hypothesis. Sports Med and Arthroscopy Review 8:6-16.
Birch HL, Wilson AM, Goodship AE. 1997. The effect of exercise-induced localised hyperthermia on tendon cell survival. Journal of Experimental Biology 200(11):1703-1708.
Bojsen-Moller J, Hansen P, Aagaard P, Svantesson U, Kjaer M, Magnusson SP. 2004. Differential displacement of the human soleus and medial gastrocnemius aponeuroses during isometric plantarflexor contractions, in vivo. J Appl Physiol:1908-1914.
Boushel R, Langberg H, Green S, Skovgaard D, Bulow J, Kjaer M. 2000a. Blood flow and oxygenation in the peritendinous tissue and calf muscle during dynamic exercise in humans. J Physiol (Lond) 524(1):305-313.
Boushel R, Langberg H, Olesen J, Nowak M, Simonsen L, Bulow J, Kjaer M. 2000b. Regional blood flow during exercise in humans measured by near-infrared spectroscopy and indocyanine green. J Appl Physiol 89:1868-1878.
Bozec L, van der Heijden G, Horton M. 2007. Collagen Fibrils: Nanoscale Ropes. Biophys. J. 92(1):70-75.
Brown R, Orchard J, Kinchington M, Hooper A, Nalder G. 2006. Aprotinin in the management of Achilles tendinopathy: a randomised controlled trial. British Journal of Sports Medicine 40(3):275-279.
Butler DL, Grood ES, Noyes FR, Zernicke RF. 1978. Biomechanics of ligaments and tendons. Exercise and Sports Science Reviews 6:125-182.
Cannell LJ, Taunton JE, Clement DB, Smith C, Khan KM. 2001. A randomised clinical trial of the efficacy of drop squats or leg extension/leg curl exercises to treat clinically diagnosed jumper's knee in athletes: pilot study. 35(1):60-64.
Capasso G, Maffulli N, Testa V, Sgambato A. 1993. Preliminary results with peritendinous protease inhibitor injections in the management of Achilles tendinitis. Journal of Sports Traumatology and Related Research 15(1):37-43.
Capasso G, Testa V, Maffulli N, Bifulco G. 1997. Aprotinin, corticosteroids and normosaline in the management of patellar tendinopathy in athletes: a prospective randomised study. Sports Exercise and Injury 3:111-115.
Carr A, Norris S. 1989. The blood supply of the calcaneal tendon. J Bone Joint Surg Br 71-B(1):100-101.
Cetti R, Junge J, Vyberg M. 2003. Spontaneous rupture of the Achilles tendon is preceded by widespread and bilateral tendon damage and ipsilateral inflammation - A clinical and histopathologic study of 60 patients. Acta Orthopaedica Scandinavica 74(1):78-84.
Chan O, O'Dowd D, Padhiar N, Morrissey D, King J, Jalan R, Maffulli N, Crisp T. 2008. High volume image guided injections in chronic Achilles tendinopathy. Disability & Rehabilitation(1):1-12.
Chong AKS, Ang AD, Goh JCH, Hui JHP, Lim AYT, Lee EH, Lim BH. 2007. Bone Marrow-Derived Mesenchymal Stem Cells Influence Early Tendon-Healing in a Rabbit Achilles Tendon Model. J Bone Joint Surg Am 89(1):74-81.

Clement DB, Taunton JE, Smart GW, McNicol KL. 1981. A survey of overuse running injuries. The Physician and Sportsmedicine 9(5):47-58.
Clementson M, Loren I, Dahlberg L, Astrom M. 2008. Sclerosing injections in midportion Achilles tendinopathy: a retrospective study of 25 patients. Knee Surgery, Sports Traumatology, Arthroscopy 16(9):887-890.
Coleman BD, Khan K, Maffulli N, Cook JL, Wark JD. 2000. Studies of surgical outcome after patellar tendinopathy: clinical significance of methodological deficiencies and guidelines for future studies. Scand J Med Sci Sports 10:2-11.
Cook J, Purdam CR. 2008. Is tendon pathology a continuum? A pathology model to explain the clinical presentation of load-induced tendinopathy. Br J Sports Med:online ahead of print.
Cook JL, Khan K, Kiss ZS, Purdam C, Griffiths L. 2001a. Reproducibility and clinical utility of tendon palpation to detect patellar tendinopathy in young basketball players. British Journal of Sports Medicine 35(1):65 - 69.
Cook JL, Khan KM, Kiss ZS, Coleman BD, Griffiths L. 2001b. Asymptomatic hypoechoic regions on patellar tendon ultrasound: A 4-year clinical and ultrasound followup of 46 tendons. Scandinavian Journal of Medicine & Science in Sports 11(6):321-327.
Cook JL, Khan KM, Kiss ZS, Purdam CR, Griffiths L. 2000. Prospective imaging study of asymptomatic patellar tendinopathy in elite junior basketball players. Journal of Ultrasound Medicine 19(7):473-479.
Cribb AM, Scott JE. 1995. Tendon response to tensile stress: an ultrastructural investigation of collagen:proteoglycan interactions in stressed tendon. J Anat 187 (Pt 2):423-8.
Crisp T, Khan F, Padhiar N, Morrissey D, King J, Jalan R, Maffulli N, Chan O. 2008. High Volume Ultrasound Guided Injections at the Interface between the Patellar Tendon and Hoffa's Body are Effective in Chronic Patellar Tendinopathy: A Pilot Study. Disability & Rehabilitation(iFirst article):1-10.
Cristofolini L, Bini S, Toni A. 1998. In vitro testing of a novel limb salvage prosthesis for the distal femur. Clinical Biomechanics 13(8):608-615.
DaCruz D, Geeson M, Allen M, Phair I. 1988. Achilles paratendonitis: an evaluation of steroid injection. Br J Sports Med 22(2):64-65.
Danielson P, Andersson G, Alfredson H, Forsgren S. 2008. Marked sympathetic component in the perivascular innervation of the dorsal paratendinous tissue of the patellar tendon in arthroscopically treated tendinosis patients. Knee Surgery, Sports Traumatology, Arthroscopy 16(6):621-626.
De Luca CJ. 1997. The Use of Surface Electromyography in Biomechanics. Journal of Applied Biomechanics 13:135-163.
Diamant J, Keller A, Baer E, Litt M, Arridge RGC. 1972. Collagen: ultrastructure and its relation to mechanical properties as a function of ageing. Proceedings of the Royal Society of London Series B-Biological Sciences 180:293-315.
Dillon EM, Erasmus PJ, Muller JH, Scheffer C, de Villiers RVP. 2008. Differential Forces Within the Proximal Patellar Tendon as an Explanation for the Characteristic Lesion of Patellar Tendinopathy: An In Vivo Descriptive Experimental Study. Am J Sports Med 36(11):2119-2127.
Edwards SG, Calandruccio JH. 2003. Autologous blood injections for refractory lateral epicondylitis. Journal of Hand Surgery-American Volume 28A(2):272-278.
Fahlstrom M, Jonsson P, Lorentzon R, Alfredson H. 2003. Chronic Achilles tendon pain treated with eccentric calf-muscle training. Knee Surgery Sports Traumatology Arthroscopy 11(5):327-333.
Fallon J, Blevins F, Vogel K, Trotter J. 2002. Functional Morphology of the supraspinatus tendon. J Orthop Res 20(5):920-926.
Farron J, Varghese T, Thelen DG. 2009. Measurement of tendon strain during muscle twitch contractions using ultrasound elastography. IEEE Trans Ultrason Ferroelectr Freq Control 56(1):27-35.
Feynman RP. 1964. The Feynman Lectures on Physics

Volume 2. Mainly electromagnetism and matter. The Feynman Lectures on Physics. p 39-1 - 39-3.
Firth EC. 2000. Tendinopathy in domestic animals. Sports Medicine and Arthroscopy Review 8(1):105-113.
Fornage BD. 1986. Achilles tendon:US examination. Radiology 159(3):759 - 764.
Fratzl P, Misof K, Zizak I, Rapp G, Amenitsch H, Bernstorff S. 1998. Fibrillar Structure and Mechanical Properties of Collagen. Journal of Structural Biology 122(1-2):119-122.
Fredberg U, Bolvig L. 2002. Significance of Ultrasonographically Detected Asymptomatic Tendinosis in the Patellar and Achilles Tendons of Elite Soccer Players: A Longitudinal Study. Am J Sports Med 30(4):488-491.
Fredberg U, Bolvig L, Andersen NT. 2008. Prophylactic Training in Asymptomatic Soccer Players With Ultrasonographic Abnormalities in Achilles and Patellar Tendons: The Danish Super League Study. Am J Sports Med 36(3):451-460.
Fredberg U, Bolvig L, Pfeiffer-Jensen M, Clemmensen D, Jakobsen BW, Stengaard-Pedersen K. 2004. Ultrasonography as a tool for diagnosis, guidance of local steroid injection and, together with pressure algometry, monitoring of the treatment of athletes with chronic jumper's knee and Achilles tendinitis: a randomized, double-blind, placebo-controlled study. Scandinavian Journal of Rheumatology 33(2):94-101.
Fredberg U, Ostgaard R. 2008. Effect of ultrasound-guided, peritendinous injections of adalimumab and anakinra in chronic Achilles tendinopathy: a pilot study. Scandinavian Journal of Medicine & Science in Sports Online publication May 2008(Online early view).
Fredberg U, Stengaard-Pedersen K. 2008. Chronic tendinopathy tissue pathology, pain mechanisms, and etiology with a special focus on inflammation. Scandinavian Journal of Medicine & Science in Sports 18(1):3-15.
Freeman MAR, Wyke B. 1967. The innervation of the knee joint: An anatomical and histological study in the cat. J Anat 101:505-532.
Friedman B, Torp S, Baer E. 1975. Effects of Age and Mechanical Deformation on Ultrastructure of Tendon. Journal of Bone and Joint Surgery-American Volume A 57(4):580-580.
Fukunaga T, Kubo K, Kawakami Y, Kanehisa H. 2000. Effect of Elastic Tendon Properties on the Performance of Stretch-shortening Cycles. In: Herzog W, editor. Skeletal Muscle Mechanics: from mechanisms to function. Chichester: J. Wiley and Sons. p 289-303.
Fyfe I, Stanish WD. 1992. The Use of Eccentric Training and Stretching in the Treatment and Prevention of Tendon Injuries. Clinics in Sports Medicine 11(3):601-624.
Galloway MT, Joki P, Dayton OW. 1992. Achilles tendon overuse injuries. Clinics in Sports Medicine 11(4):771 - 782.
Gathercole LJ, Keller A. 1991. Crimp Morphology in the Fiber-Forming Collagens. Matrix 11(3):214-234.
Gibbon W, Cooper G, Radcliffe G. 1999. Sonographic incidence of tendon microtears in athletes with chronic Achilles tendinosis. Br J Sports Med 33:129 - 130.
Gisslen K, Alfredson H, Peers K. 2005. Neovascularisation and pain in jumper's knee: a prospective clinical and sonographic study in elite junior volleyball players * Commentary. Br J Sports Med 39(7):423-428.
Hansen P, Bojsen-Moller J, Aagaard P, Kjaer M, Magnusson SP. 2006. Mechanical properties of the human patellar tendon, in vivo. Clinical Biomechanics 21:54-58.
Haraldsson BT, Aagaard P, Krogsgaard M, Alkjaer T, Kjaer M, Magnusson SP. 2004. Region Specific Mechanical Properties of the Human Patella Tendon. J Appl Physiol.
Haraldsson BT, Aagaard P, Krogsgaard M, Alkjaer T, Kjaer M, Magnusson SP. 2005. Region Specific Mechanical Properties of the Human Patella Tendon. J Appl Physiol 98(3):1006-1012.
Haraldsson BT, Langberg H, Aagaard P, Zuurmond AM, van El B, DeGroot J, Kjaer M, Magnusson SP. 2006. Corticosteroids reduce the tensile strength of isolated collagen fascicles. American Journal of Sports Medicine 34(12):1992-1997.

Houshian S, Tscherning T, Riegels-Nielsen P. 1998. The Epidemiology of achilles tendon rupture in a Danish county. Injury:International Journal of the care of the injured 29(9):651-654.
Hugate R, Pennypacker J, Saunders M, Juliano P. 2004. The Effects of Intratendinous and Retrocalcaneal Intrabursal Injections of Corticosteroid on the Biomechanical Properties of Rabbit Achilles Tendons. J Bone Joint Surg Am 86(4):794-801.
Ippolito E, Natali PG, Postacchini F, Accinni L, De Martino C. 1977. Ultrastructural and immunochemical evidence of actin in the tendon cells. Clin Orthop(126):282-4.
James SLJ, Ali K, Pocock C, Robertson C, Walter J, Bell J, Connell D, Bradshaw C. 2007. Ultrasound guided dry needling and autologous blood injection for patellar tendinosis * COMMENTARY. Br J Sports Med 41(8):518-521.
Jarvinen M. 1992. Epidemiology of Tendon Injuries in Sports. Clinics in Sports Medicine 11(3):493-504.
Jarvinen M, Josza L, Kannus P, Jarvinen TLN, Kvist M, Leadbetter W. 1997a. Histopathological findings in chronic tendon disorders. Scand J Med Sci Sports 7:85-95.
Jarvinen M, Jozsa L, Kannus P, Jarvinen TL, Kvist M, Leadbetter W. 1997b. Histopathological findings in chronic tendon disorders. Scand J Med Sci Sports 7(2):86-95.
Josza L, Balint BJ, Kannus P, Jarvinen M, Lehto M. 1993. Mechanoreceptors in human myotendinous junction. Muscle & Nerve 16:453-457.
Josza L, Kannus B. 1997. Human Tendons: Anatomy, Physiology and Pathology. Champaign: Human Kinetics.
Jozsa L, Kannus P. 1997. Histopathological findings in spontaneous tendon ruptures. Scand J Med Sci Sports 7(2):113-8.
Jozsa L, Kvist M, Balint BJ, Reffy A, Jarvinen M, Lehto M, Barzo M. 1989. The role of recreational sport activity in Achilles tendon rupture. A clinical, pathoanatomical, and sociological study of 292 cases. Am J Sports Med 17(3):338-43.
Junqueira LC, Carneiro J. 1980. Basic Histology. Rio de Janeiro: Lange Medical Publications.
Kainberger F, Mittermaier F, Seidl G, Parth E, Weinstabl R. 1997. Imaging of tendons--adaptation, degeneration, rupture. Eur J Radiol 25(3):209-22.
Kannus P. 2000. Structure of the tendon connective tissue. Scand J Med Sci Sports 10(6):312-20.
Kannus P, Jarvinen TL, Jarvinen TA, Jarvinen M, Parkkari J, Khan K. 2004. Editorial. Scand J Med Sci Sports 14(2):69-71.
Kannus P, Josza L. 1991. Histopathological changes preceding spontaneous rupture of a tendon. J Bone Joint Surg Am 73:1507-1525.
Kannus P, Jozsa L. 1991. Histopathological Changes Preceding Spontaneous Rupture of a Tendon - a Controlled-Study of 891 Patients. Journal of Bone and Joint Surgery-American Volume 73A(10):1507-1525.
Kaufman KR, Brodine SK, Schaffer RA, Johnson CW, Cullison TR. 1999. The effect of foot structure and range of motion on musculoskeletal overuse injuries. The American Journal of Sports Medicine 27(5):585 - 593.
Ker RF. 2002. The implications of the adaptable fatigue quality of tendons for their construction, repair and function. Comparative Biochemistry and Physiology a-Molecular and Integrative Physiology 133(4):987-1000.
Ker RF. 2007. Mechanics of tendon, from an engineering perspective. International Journal of Fatigue 29(6):1001-1009.
Ker RF, Wang XT, Pike AVL. 2000. Fatigue quality of mammalian tendons. Journal of Experimental Biology 203(8):1317-1327.
Khan K, Bonar F, Desmond P, Cook J, Young D, Visentini P, Fehrmann M, Kiss Z, O'Brien P, Harcourt P and others. 1996. Patellar tendinosis (jumper's knee): findings at histopathologic examination, US, and MR imaging. Victorian Institute of Sport Tendon Study Group. Radiology 200(3):821-827.
Khan K, Cook J. 2000. Overuse tendon injuries: Where does the pain come from? Sports Med and Arthroscopy Review 8:17-31.
Khan KM. 2002. Ultrasound guided sclerosis of neovessels in painful chronic Achilles tendinosis: pilot study of a new treatment - Commentary. British Journal of Sports Medicine 36(3):176-177.

Khan KM, Forster BB, Robinson J, Cheong Y, Louis L, Maclean L, Taunton JE. 2003. Are ultrasound and magnetic resonance imaging of value in assessment of Achilles tendon disorders? A two year prospective study. British Journal of Sports Medicine 37(2):149-153.

Khan KM, Visentini PJ, Kiss ZS, Desmond PM, Coleman BD, Cook J, Tress BM, Wark JD, Forster BB. 1999. Correlation of ultrasound and magnetic resonance imaging with clinical outcome after a patellar tenotomy: prospective and retrospective studies. Clinical Journal of Sport Medicine 9:129 - 137.

Kidsheelpain.com. 2002-7. Kid's Heel Pain. p Website marketing Oscon a product aimed at treating enthesitis.

Kjaer M. 2004. Role of extracellular matrix in adaptation of tendon and skeletal muscle to mechanical loading. Physiological Reviews 84(2):649-698.

Koenig MJ, Torp-Pedersen S, Qvistgaard E, Terslev L, Bliddal H. 2004. Preliminary results of colour Doppler-guided intratendinous glucocorticoid injection for Achilles tendonitis in five patients. Scandinavian Journal of Medicine & Science in Sports 14(2):100-106.

Kostyuk O, Brown RA. 2004. Novel Spectroscopic Technique for In Situ Monitoring of Collagen Fibril Alignment in Gels. Biophys. J. 87(1):648-655.

Kremkau FW. 2002. Diagnostic Ultrasound. Philadelphia: W.B. Saunders.

Kubo K, Akima H, Ushiyama J, Tabata I, Fukuoka H, Kanehisa H, Fukunaga T. 2004a. Effects of 20 days of bed rest on the viscoelastic properties of tendon structures in lower limb muscles. British Journal of Sports Medicine 38(3):324-330.

Kubo K, Akima H, Ushiyama J, Tabata I, Fukuoka H, Kanehisa H, Fukunaga T. 2004b. Effects of resistance training during bed rest on the viscoelastic properties of tendon structures in the lower limb. Scand J Med Sci Sports 14(5):296-302.

Kubo K, Kanchisa H, Ito M, Fukunaga T. 2001. Effects of isometric training on the elasticity of human tendon structures in vivo. Journal of Applied Physiology 91:26-32.

Kubo K, Kanehisa H, Fukunaga T. 2002a. Effects of resistance and stretching training programmes on the viscoelastic properties of human tendon structures in vivo. Journal of Physiology-London 538(1):219-226.

Kubo K, Kanehisa H, Kawakami Y, Fukunaga T. 2000. Elasticity of tendon structures of the lower limbs in sprinters. Acta Physiologica Scandinavica 168(2):327-335.

Kubo K, Kanehisa H, Miyatani M, Tachi M, Fukunaga T. 2003. Effect of low-load resistance training on the tendon properties in middle-aged and elderly women. Acta Physiologica Scandinavica 178(1):25-32.

Kubo K, Kawakami Y, Kanehisa H, Fukunaga T. 2002b. Measurement of viscoelastic properties of tendon structures in vivo. Scandinavian Journal of Medicine & Science in Sports 12(1):3-8.

Kujala UM, Sarna S, Kaprio J, Koskenvuo M, Karjalainen J. 1999. Heart attacks and lower-limb function in master endurance athletes. Medicine and Science in Sports and Exercise 31(7):1041-1046.

Lagergren C, Lindholm A. 1959. Vascular distribution in the Achilles tendon; an angiographic and microangiographic study. Acta Chir Scand 116(5-6):491-5.

Lambe G, Coutts G, McArthur P, Dangerfield PH. 2006. MRI `Magic Angle' Imaging of Finger Tendons. The Journal of Hand Surgery: Journal of the British Society for Surgery of the Hand 31(2):149-153.

Langberg H, Ellingsgaard H, Madsen T, Jansson J, Magnusson SP, Aagaard P, Kjaer M. 2007. Eccentric rehabilitation exercise increases peritendinous type I collagen synthesis in humans with Achilles tendinosis. Scandinavian Journal of Medicine & Science in Sports 17(1):61-66.

Langberg H, Skovgaard D, Petersen LJ, Bulow J, Kjar M. 1999. Type I collagen synthesis and degradation in peritendinous tissue after exercise determined by microdialysis in humans. J Physiol (Lond) 521(1):299-306.

Lavagnino M, Arnoczky SP, Elvin N, Dodds J. 2008. Patellar Tendon Strain Is Increased at the Site of the Jumper's Knee Lesion During Knee Flexion and Tendon Loading: Results and Cadaveric Testing of a Computational Model. Am J Sports Med 36(11):2110-2118.

Leach RE, James S, Wasilewski S. 1981. Achilles tendinitis. Am J Sports Med 9(2):93-98.

Leppilahti J, Korpelainen R, Karpakka J, Kvist M, Orava S. 1998. Ruptures of the Achilles tendon: relationship to inequality in length of legs and to patterns in the foot and ankle. Foot Ankle Int 19(10):683-7.
Leppilahti J, Puranen J, Orava S. 1996. Incidence of Achilles tendon rupture. Acta Orthop Scand 67(3):277-279.
Lyman J, Weinhold PS, Almekinders LC. 2004. Strain behavior of the distal Achilles tendon - Implications for insertional Achilles tendinopathy. American Journal of Sports Medicine 32(2):457-461.
Madsen EL, Frank GR, Dong F. 1998. Liquid or Solid Ultrasonically Tissue-Mimicking Materials with Very Low Scatter. Ultrasound in Med & Biol 24(4):535-542.
Maffulli N, Khan K, Puddu G. 1998. Overuse tendon conditions: Time to change a confusing terminology. Arthroscopy: The Journal of Arthroscopic and Related Surgery 14(8):840 - 843.
Mafi N, Lorentzon R, Alfredson H. 2001. Superior short-term results with eccentric calf muscle training compared to concentric training in a randomized prospective multicenter study on patients with chronic Achilles tendinosis. Knee Surgery Sports Traumatology Arthroscopy 9(1):42-47.
Maganaris CN. 2001. In vivo tendon mechanical properties in young adults and healthy elderly. Active Life Span Research Symposium. The Plasticity of the Motor System: Adaptations to Increased Use, Disuse and Ageing, Manchester Metropolitan University, United Kingdom, 2001, p. 13–14.
Maganaris CN, Baltzopoulos V, Sargeant AJ. 1998. Changes in Achilles tendon moment arm from rest to maximum isometric plantarflexion: in vivo observations in man. The Journal of Physiology 510(3):977-985.
Maganaris CN, Narici MV, Almekinders LC, Maffulli N. 2004. Biomechanics and pathophysiology of overuse tendon injuries : ideas on insertional tendinopathy. Sports Med 34(14):1005-17.
Maganaris CN, Paul JP. 1999. In vivo human tendon mechanical properties. Journal of Physiology 521(1):307-313.
Magnusson SP, Qvortrup K, Larsen JO, Rosager S, Hanson P, Aagaard P, Krogsgaard M, Kjaer M. 2002. Collagen fibril size and crimp morphology in ruptured and intact Achilles tendons. Matrix Biology 21(4):369-377.
Majima T, Yasuda K, Tsuchida T, Tanaka K, Miyakawa K, Minami A, Hayashi K. 2003. Stress shielding of patellar tendon: effect on small-diameter collagen fibrils in a rabbit model. J Orthop Science 8(6):836-841.
Meier B, Radeke HH, Selle S, Younes M, Sies H, Resch K, Habermehl GG. 1989. Human fibroblasts release reactive oxygen species in response to interleukin-1 or tumour necrosis factor-alpha. Biochem J 263(2):539-45.
Michna H, Hartman G. 1989. Adaptation of tendon collagen to exercise. International Orthopaedics 13:161-165.
Misof K, Rapp G, Fratzl P. 1997. A new molecular model for collagen elasticity based on synchrotron x-ray scattering evidence. Biophysical Journal 72(3):1376-1381.
Moller M, Kalebo P, Tidebrant G, Movin T, Karlsson J. 2002. The ultrasonographic appearance of the ruptured Achilles tendon during healing: a longitudinal evaluation of surgical and nonsurgical treatment, with comparisons to MRI appearance. Knee Surgery Sports Traumatology, Arthroscopy 10:49 -56.
Mosler E, Folkhard W, Knorzer E, Nemetschekgansler H, Nemetschek T, Koch MHJ. 1985. Stress-Induced Molecular Rearrangement in Tendon Collagen. Journal of Molecular Biology 182(4):589-596.
Murray MM, Spector M. 1999. Fibroblast distribution in the anteromedial bundle of the human anterior cruciate ligament: The presence of alpha-smooth muscle actin-positive cells. Journal of Orthopaedic Research 17(1):18-27.
Ng BH, Chou SM, Krishna V. 2005. The influence of gripping techniques on the tensile properties of tendons. Proc Inst Mech Eng [H] 219(5):349-54.

Nyyssonen T, P L. 2000. Achilles tendon ruptures in South-East Finland between 1986-1996, with special reference to epidemiology, complications of surgery and hospital costs. Annales Chirurgiae et Gynaecologiae 89(1):53-57.
O'Brien M. 1992. Functional anatomy and physiology of tendons. Clinics in Sports Medicine 11(3):505 - 520.
Ohberg L, Alfredson H. 2002. Ultrasound guided sclerosis of neovessels in painful chronic Achilles tendinosis: pilot study of a new treatment. British Journal of Sports Medicine 36:173 - 175.
Ohberg L, Alfredson H. 2004. Effects on neovascularisation behind the good results with eccentric training in chronic mid-portion Achilles tendinosis? Knee Surgery Sports Traumatology Arthroscopy 12(5):465-470.
Ohberg L, Lorentzon R, Alfredson H. 2001. Neovascularisation in Achilles tendons with painful tendinosis but not in normal tendons: an ultrasonographic investigation. Knee Surg Sports Traumatol Arthrosc 9:233-238.
Ohberg L, Lorentzon R, Alfredson H, Maffulli N. 2004. Eccentric training in patients with chronic Achilles tendinosis: normalised tendon structure and decreased thickness at follow up * Commentary. Br J Sports Med 38(1):8-11.
Paavola M, Kannus P, Jarvinen TA, Khan K, Jozsa L, Jarvinen M. 2002a. Achilles tendinopathy. J Bone Joint Surg Am 84-A(11):2062-76.
Paavola M, Kannus P, Orava S, Pasanen M, Jarvinen M. 2002b. Surgical treatment for chronic Achilles tendinopathy: a prospective seven month follow up study. British Journal of Sports Medicine 36:178 - 182.
Peers KHE, Brys PPM, Lysens RJJ. 2003. Correlation between power Doppler ultrasonography and clinical severity in Achilles tendinopathy. International Orthopaedics 27(3):180-183.
Petersen W, Varoga D, Zantop T, Hassenpflug J, Mentlein R, Pufe T. 2004. Cyclic strain influences the expression of the vascular endothelial growth factor (VEGF) and the hypoxia inducible factor 1 alpha (HIF-1 alpha) in tendon fibroblasts. Journal of Orthopaedic Research 22(4):847-853.
Pollard TCB, Gwilym SE, Carr AJ. 2008. The assessment of early osteoarthritis. J Bone Joint Surg Br 90-B(4):411-421.
Pope CF. 1992. Radiologic evaluation of tendon injuries. Clinics in Sports Medicine 11(3):579 - 599.
Puddu G, Ippolito E, Postacchini F. 1976. A classification of Achilles tendon disease. Am J Sports Med 4(4):145-50.
Pufe T, Petersen WJ, Mentlein R, Tillmann BN. 2005. The role of vasculature and angiogenesis for the pathogenesis of degenerative tendons disease. Scandinavian Journal of Medicine and Science in Sports 15(4):211-222.
Purslow PP, Wess TJ, Hukins DWL. 1998. Collagen orientation and molecular spacing during creep and stress-relaxation in soft connective tissues. Journal of Experimental Biology 201(1):135-142.
Puxkandl R, Zizak I, Paris O, Keckes J, Tesch W, Bernstorff S, Purslow P, Fratzl P. 2002. Viscoelastic properties of collagen: synchrotron radiation investigations and structural model. Philosophical Transactions: Biological Sciences 357(1418):191-197.
Ramachandran N, Koike Y, Poitras P, Backman D, Uhthoff HK, Trudel G. 2005. Dual cryogenic fixation for mechanical testing of soft musculoskeletal tissues. Ieee Transactions on Biomedical Engineering 52(10):1792-1795.
Reeves ND, Maganaris CN, Ferretti G, Narici M. 2002. Influence of long-term bed rest on muscle architecture and tendon mechanical properties. Journal of Physiology-London 543:103P-104P.
Reeves ND, Maganaris CN, Narici MV. 2003a. Effect of strength training on human patella tendon mechanical properties of older individuals. Journal of Physiology-London 548(3):971-981.
Reeves ND, Narici MV, Maganaris CN. 2003b. Strength training alters the viscoelastic properties of tendons in elderly humans. Muscle & Nerve 28(1):74-81.

Reeves ND, Narici MV, Maganaris CN. 2004. In vivo human muscle structure and function: adaptations to resistance training in old age. Experimental Physiology 89(6):675-689.
Requa R, DeAvilla L, Garrick J. 1993. Injuries in recreational adult fitness activities. Am J Sports Med 21(3):461-467.
Revell J, Mirmehdi M, McNally D. 2005. Computer vision elastography: Speckle adaptive motion estimation for elastography using ultrasound sequences. Ieee Transactions on Medical Imaging 24(6):755-766.
Revell JD. 2004. Computer Vision Elastography. Bristol: University of Bristol. 155 p.
Revell JD, Mirmehdi M, McNally DS. 2004. Musculoskeletal motion flow fields using hierarchical variable-sized block matching in ultrasonographic video sequences. J Biomech 37(4):511-22.
Richards PJ, Dheer AK, McCall IM. 2001. Achilles Tendon (TA) Size and Power Doppler Ultrasound (PD) Changes Compared to MRI: A Preliminary Observational Study. Clinical Radiology 56(10):843-850.
Robinson J, Cook J, Purdam C, Visentini PJ, Ross J, Maffulli N, Taunton JE, Khan K. 2001. The VISA-A questionnaire: avalid and reliable index of the severity of achilles tendinopathy. British Journal of Sports Medicine 35:335 -341.
Screen HRC, Lee DA, Bader DL, Shelton JC. 2004. An investigation into the effects of the hierarchical structure of tendon fascicles on micromechanical properties. Proceedings of the Institution of Mechanical Engineers Part H- Journal of Engineering in Medicine 218(H2):109-119.
Shalabi A, Kristoffersen-Wilberg M, Svensson L, Aspelin P, Movin T. 2004. Eccentric training of the gastrocnemius-soleus complex in chronic Achilles tendinopathy results in decreased tendon volume and intratendinous signal as evaluated by MRI. American Journal of Sports Medicine 32(5):1286-1296.
Shrier I, Matheson GO, Kohl HW. 1996. Achilles tendinitis: Are corticosteroid injections useful or harmful? Clinical Journal of Sport Medicine 6(4):245-250.
Silbernagel KG, Thomee R, Thomee P, Karlsson J. 2001. Eccentric overload training for patients with chronic Achilles tendon pain - a randomised controlled study with reliability testing of the evaluation methods. Scandinavian Journal of Medicine & Science in Sports 11(4):197-206.
Sisson S, Grossman JD, editors. 1975. The Anatomy of the Domestic Animals. 4th ed. Philadelphia: W.B. Saunders.
Smith RKW, Webbon PM. 2005. Harnessing the stem cell for the treatment of tendon injuries: heralding a new dawn? British Journal of Sports Medicine 39(9):582-584.
Solecki R, Conant RJ. 2003. Displacement and Strain. Advanced Mechanics of Materials. New York: Oxford University Press, Inc. p 55 - 94.
Stanish W, Rubinovich R, Curwin S. 1986. Eccentric exercise in chronic tendinitis. Clinical Orthopaedics and Related Research 208:65 - 68.
Stillwell DL. 1957. The innervation of tendons and aponeurosis. Am J Anat 100:289-311.
Stolinski C. 1995. Disposition of Collagen Fibrils in Human Tendons. Journal of Anatomy 186:577-583.
Suresh SP, Ali KE, Jones H, Connell DA, Beall DP, Klauser AS. 2006. Medial epicondylitis: is ultrasound guided autologous blood injection an effective treatment? * Commentary. Br J Sports Med 40(11):935-939.
Tak-Man Cheung J, Zhang M. 2006. A serrated jaw clamp for tendon gripping. Medical Engineering & Physics 28:379-382.
Tallon C, Maffulli N, Ewen SWB. 2001. Ruptured Achilles tendons are significantly more degenerated than tendinopathic tendons. Medicine and Science in Sports and Exercise 33(12):1983-1990.
Taunton JE, Ryan MB, Clement DB, McKenzie DC, Lloyd-Smith DR, Zumbo BD. 2002. A retrospective case-control analysis of 2002 running injuries. Br J Sports Med 36(2):95-101.
Taylor MA, Norman TL, Clovis NB, Blaha JD. 2002. The response of rabbit patellar tendons after autologous blood injection. Medicine and Science in Sports and Exercise 34(1):70-73.

Taylor SE, Smith RKW, Clegg PD. 2007. Mesenchymal stem cell therapy in equine musculoskeletal disease: scientific fact or clinical fiction? Equine Veterinary Journal 39(2):172-180.
Theobald P, Benjamin M, Nokes L, Pugh N. 2005. Review of the vascularisation of the human Achilles tendon. Injury 36(11):1267-1272.
Tobinick EL, Britschgi-Davoodifar S. 2003. Perispinal TNF-alpha inhibition for discogenic pain. Swiss Med Weekly 133:170-177.
Torres DS, Freyman TM, Yannas IV, Spector M. 2000. Tendon cell contraction of collagen-GAG matrices in vitro: effect of cross-linking. Biomaterials 21(15):1607-1619.
White DR, Wambersie A. 1999. Tissue substitutes, phantoms and computational modelling in medical ultrasound. ICRU Report 61 p.
Wieloch P, Buchmann G, Roth W, Rickert M. 2004. A cryo-jaw designed for in vitro tensile testing of the healing Achilles tendons in rats. Journal of Biomechanics 37(11):1719-1722.
Wilson AM, Goodship AE. 1994. Exercise induced hyperthermia as a possible mechanism for tendon degeneration. Journal of Biomechanics 27(7):899-905.
Yang GG, Im HJ, Wang JHC. 2005. Repetitive mechanical stretching modulates IL-1 beta induced COX-2, MMP-1 expression, and PGE(2) production in human patellar tendon fibroblasts. Gene 363:166-172.
Young MA, Cook JL, Purdam CR, Kiss ZS, Alfredson H. 2005. Eccentric decline squat protocol offers superior results at 12 months compared with traditional eccentric protocol for patellar tendinopathy in volleyball players. Br J Sports Med 39(2):102-105.
Zanetti M, Metzdorf A, Kundert HP, Zollinger H, Vienne P, Seifert B, Hodler J. 2003. Achilles tendons: Clinical relevance of neovascularization diagnosed with power Doppler US. Radiology 227(2):556-560.
Zell K, Sperl JI, Vogel MW, Niessner R, Haisch C. 2007. Acoustical properties of selected tissue phantom materials for ultrasound imaging. Physics in Medicine and Biology(20):N475-N484.

APPENDICES

APPENDIX 1

MATLAB CODE: 'SHEARSTRAIN6.M'

```
%practice code to try and load multiple arrays and then apply new strain calc
filename_root = 'etcalst2c_';
%eg filename_root = 'pattendisomext2zb_';
% these variables are to be defined as inputs...
%
% 1)                    init_index is the number of the 1st file to read i.e. 0,1,2,3...10,11,12...24,25 etc
%                                   in the example where the 1st file zb_000x.txt, the init_index is 0
init_index = 0;
% 2)                                grid_spacing is number of pixels per grid on input
grid_spacing = 8;
% 3)                    dot_size is the size of the output strain dots (must be odd number 1,3,5,7 etc)
%                                   bigger dot size means larger colour spots on output
dot_size = 9;
% 4)max_allowable_strain is the upper bound limit on strain -THIS IS SWITCHED OFF SO SEE ALL RESULTS
%                                   strains above this value are reset to this limit
max_allowable_strain = 3;
% 5)                                translate_dots is a flag to switch displacement tracking
%                                   on the output images:  1 = on, 0 = off
translate_dots = 0;
num = init_index;
maxnum = 0;
i = 0;
rows = 0;
cols = 0;
colormap(jet);
%-------------------------------------------------------------------------------
% loading data by filename - each array is stored in matrix zb_000x, zb_001x...etc
while 1
   % constructing filename string from inputs...
   %                                - note MATLAB relies on using a lot of string construction
   %                    - techniques. Use them to build MATLAB commands word by word.
   %                    - Then execute the commands by using the 'eval' command.
   %                    - You can recognise them from the square brackets []
   if (num < 10)
      %num
      datax = [filename_root,'00',num2str(num),'x'];
      datay = [filename_root,'00',num2str(num),'y'];
   elseif (num >= 10 && num < 100)
      datax = [filename_root,'0',num2str(num),'x'];
      datay = [filename_root,'0',num2str(num),'y'];
   elseif (num >= 100 && num < 1000)
      datax = [filename_root,num2str(num),'x'];
      datay = [filename_root,num2str(num),'y'];
   else
      error('O-U-C-H! input files exceeded 1000! - sorry too many files - bye!')
      %break;
   end
```

```
   filenamex = [datax,'.txt'];
   filenamey = [datay,'.txt'];
   % loading data from input files...
   %                                                    - note the while loop bombs out when the input file
   %                                                  - cannot be read, and the number of files found is output
   %                                                  - to the screen. 'whos' lists current vaiables and their size
   if ~exist(filenamex)
      break;
   elseif ~exist(filenamey)
      break;
   else
      %disp (num,'GIll')
      % this bit loads the data from the files into MATLAB array variables
      %                                                      - all indexed automatically - hmmm - nice and easy
      %                                                - note the string construction stuff again (square brackets)
      %                                              - which is used to prepare the command and eval is then used
      %                                                                                    - to execute it
      %                                                                                          - ok...
      eval(['load ',filenamex]);
      eval(['load ',filenamey]);
      num = num + 1;
      maxnum = maxnum + 1;
   end
end
% checking status of input files after break from while loop
if (maxnum == 0)
   error('oh dear, can''t find any files... wrong filename?!!!')
   %break,
   %Had to comment out end as otherwise this fails here
   %end;
else
   clc;
   disp([num2str(maxnum),' files found'])
   whos
end
%-------------------------------------------------------------------------------
% calculating strains... what is required for cell displacements
%                                                                  for a point lying between cell 5 and 6:
%                                                                        final x length cell 2 = (x6-x5)
%                                                                        final x length cell 1 = (x5-x4)
%                                                    ave final length in x of adjacent cells flx = (x6-x4)/2
%   ave final length in y of adjacent cells fly = (y6-y4)/2
%   xxstrain = ((x(r,c+1)-x(r,c-1))/2*grid_spacing)
%   yystrain = ((y(r+1,c)-y(r-1,c))/2*grid_spacing)
%   yxstrain = ((x(r+1,c)-x(r-1,c))/2*grid_spacing)
%   xystrain = ((x(r,c+1)-x(r,c-1))/2*grid_spacing)
disp('starting calcs...  want to take a tea break?');
% finding how many rows and columns in input files
num=init_index;
if (num < 10)
   rows = size(eval([filename_root,'00',num2str(num),'x']),1);
   cols = size(eval([filename_root,'00',num2str(num),'x']),2);
elseif (num >= 10 && num < 100)
   rows = size(eval([filename_root,'0',num2str(num),'x']),1);
   cols = size(eval([filename_root,'0',num2str(num),'x']),2);
elseif (num >= 100 && num < 1000)
```

```
    rows = size(eval([filename_root,'',num2str(num),'x']),1);
    cols = size(eval([filename_root,'',num2str(num),'x']),2);
end
% sizing new arrays accordingly...
datastrain = zeros(rows,cols);
reldispx = zeros(rows,cols);
reldispy = zeros(rows,cols);
reldispyx = zeros(rows,cols);
xxstrain = zeros(rows,cols);
%yystrain = zeros(rows,cols);
%commented out yx and xy to try and get to run with new calc
%yxstrain = zeros(rows,cols);
%xystrain = zeros(rows,cols);
% this array is grid_spacing * the number of rows and columns - to
% give an image the same size as the original bitmaps
visualise = zeros(rows*grid_spacing,cols*grid_spacing);
%for trialling
%num=26;
maxnum=49;
for num = 1:maxnum
    disp(['array number gill = ',num2str(num)]);
    % starting over with data variable names....
    if num < 10
        datax = [filename_root,'00',num2str(num),'x'];
        datay = [filename_root,'00',num2str(num),'y'];
        datax_dx = [datax,'_dx'];
        datay_dy = [datay,'_dy'];
        data_xxstrain = [filename_root,'00',num2str(num),'_xxstrain'];
        data_yystrain = [filename_root,'00',num2str(num),'_yystrain'];
        data_yxstrain = [filename_root,'00',num2str(num),'_yxstrain'];
        data_xystrain = [filename_root,'00',num2str(num),'_xystrain'];
        data_shstrain = [filename_root,'00',num2str(num),'_shstrain'];
    elseif num >= 10 && num < 100
        datax = [filename_root,'0',num2str(num),'x'];
        datay = [filename_root,'0',num2str(num),'y'];
        datax_dx = [datax,'_dx'];
        datay_dy = [datay,'_dy'];
        data_xxstrain = [filename_root,'0',num2str(num),'_xxstrain'];
        data_yystrain = [filename_root,'0',num2str(num),'_yystrain'];
        data_yxstrain = [filename_root,'0',num2str(num),'_yxstrain'];
        data_xystrain = [filename_root,'0',num2str(num),'_xystrain'];
        data_shstrain = [filename_root,'0',num2str(num),'_shstrain'];
    elseif num >= 100 && num < 1000
        datax = [filename_root,num2str(num),'x'];
        datay = [filename_root,num2str(num),'y'];
        datax_dx = [datax,'_dx'];
        datay_dy = [datay,'_dy'];
        data_xxstrain = [filename_root,num2str(num),'_xxstrain'];
        data_yystrain = [filename_root,num2str(num),'_yystrain'];
        data_yxstrain = [filename_root,num2str(num),'_yxstrain'];
        data_xystrain = [filename_root,num2str(num),'_xystrain'];
        data_shstrain = [filename_root,num2str(num),'_shstrain'];
    end
    % calculations....
    for c = 1:cols
        if (c/10) == floor(c/10), disp(['cols complete = ',num2str(c)]), end
```

```
for r = 1:rows
   %disp(['r=',num2str(r),' c=',num2str(c)])
   % x relative displacements
   if c == 1
      reldispx(c,r) = 0;
   elseif c == cols
      reldispx(c,r) = 0;
   elseif c > 1 && c < cols
      calc1 = [datax,'(',num2str(c-1),',',num2str(r),')'];
      calc2 = [datax,'(',num2str(c+1),',',num2str(r),')'];
      calc = [calc2,' - ',calc1];
      reldispx(c,r) = 0.5 * (eval(calc));
   end
   % y relative displacements
   if r == 1
      reldispy(c,r) = 0;
   elseif r == rows
      reldispx(c,r) = 0;
   elseif r > 1 && r < rows
      calc1 = [datay,'(',num2str(c),',',num2str(r-1),')'];
      calc2 = [datay,'(',num2str(c),',',num2str(r+1),')'];
      calc = [calc2,' - ',calc1];
      reldispy(c,r) = 0.5 * (eval(calc));
   end
   % xxstrain calc
   % xxstrain = ('redispx'/8)
   xxstrain(c,r) = (reldispx(c,r)/grid_spacing);
   yystrain(c,r) = (reldispy(c,r)/grid_spacing);
   %yx relative displacements
   if r == 1
      reldispyx(c,r) = 0;
   elseif r == rows
      reldispyx(c,r) = 0;
   elseif r > 1 && r < rows
      calc1 = [datax,'(',num2str(c),',',num2str(r-1),')'];
      calc2 = [datax,'(',num2str(c),',',num2str(r+1),')'];
      calc = [calc2,' - ',calc1];
      reldispyx(c,r) = 0.5 * (eval(calc));
   end
   % xy relative displacements
   if c == 1
      reldispxy(c,r) = 0;
   elseif c == cols
      reldispxy(c,r) = 0;
   elseif c > 1 && c < cols
      calc1 = [datay,'(',num2str(c-1),',',num2str(r),')'];
      calc2 = [datay,'(',num2str(c+1),',',num2str(r),')'];
      calc = [calc2,' - ',calc1];
      reldispxy(c,r) = 0.5 * (eval(calc));
   end
   yxstrain(c,r) = (reldispyx(c,r)/grid_spacing);
   xystrain(c,r) = (reldispxy(c,r)/grid_spacing);
   shstrain(c,r) = (yxstrain(c,r) + xystrain(c,r))/2
   %end
   % visualisation...
   % visualisation matrix is 'grid_spacing' times bigger than xxstrain matrix
```

```
            % thus (column,row) (x,y) in strain matrix becomes
            % (grid_spacing*x, grid_spacing*y) in visualisation matrix
            vr = grid_spacing * r;
            vc = grid_spacing * c;
            % to include translation (dx,dy) of pixel in visualisation matrix
            % add on pixel displacement in input files for row r, column c
            string1 = [datax,'(',num2str(c),',',num2str(r),')'];
            string2 = [datay,'(',num2str(c),',',num2str(r),')'];
            if translate_dots == 1
               vr = vr + (eval(string2));
               vc = vc + (eval(string1));
            end
            % to make coloured area larger than 1 pixel,
            %for k = 1 - (dot_size - 1)/2: 1 + (dot_size - 1)/2;
            %for l = 1 - (dot_size - 1)/2: 1 + (dot_size - 1)/2;
            for k = (1 - dot_size)/2: (dot_size - 1)/2;
               for l = (1 - dot_size)/2: (dot_size - 1)/2;
                  vrfinal = vr + k;
                  vcfinal = vc + l;
                  % only calc visualisation(vrfinal,vcfinal) if conditions apply
                  if vrfinal >= 1 && vrfinal <= (rows * grid_spacing)
                     if vcfinal >= 1 && vcfinal <= (cols * grid_spacing)
                        visualise(vcfinal, vrfinal) =  xxstrain(c,r) * 64 / max_allowable_strain;
                     end
                  end
               end
            end
         end
      end
      visualise_num = visualise;

      string1 = ['xxstrain_',num2str(num),' = xxstrain;'];
      eval(string1);
      string2 = ['yystrain_',num2str(num),' = yystrain;'];
      eval(string2);
      string3 = ['yxstrain_',num2str(num),' = yxstrain;'];
      eval(string3);
      string4 = ['xystrain_',num2str(num),' = xystrain;'];
      eval(string4);
      %string5 = [];
      colormap(jet);
      image(visualise_num);
      getframe;
      % incrementing data count
      %num = num + 1;
   end
    %repeating images...
    %num=init_index;
    %for i = 1:maxnum
    %string1 = ['visualise_',num2str(num)];
    %end
   %colormap(jet);
   %image(eval(string1));
   %mov(i)=getframe;
   %num = num + 1;
   %end
```

```
%%movie2avi(mov,sprintf('%smov.avi',filename_root),'comp','none');
%end
```

APPENDIX 2A

MATLAB CODE: 'USSTRAIN2.M'

```
%Program to calculate and display strain data from x and y displacement files
%
%
%Note arrays are set up so that they correspond to the images i.e. A (y,x)
%  e.g.
%     >> A=zeros(4,6);
%     >> x=2;
%     >> y=3;
%     >> A(y,x)=1
%
%        A =
%
%           0    0    0    0    0    0
%           0    0    0    0    0    0
%           0    1    0    0    0    0
%           0    0    0    0    0    0



%set up constants (to be derived from file sizes and number)

prompt={'Strain fit length (2n+1 blocks):',
      'Velocity fit time (2n+1 frames):',
      'Trajectory smoothing time (2n+1 frames):',
      'Block size (pixels):',
      'Frames per second:'};
name='Constants';
numlines=1;
defaultanswer={'3','3','3','16','8.3'};
answer=inputdlg(prompt,name,numlines,defaultanswer);
answer=str2double(answer);
diff_span=answer(1);
vel_span=answer(2);
traj_span=answer(3);
block_size=answer(4);
fps=answer(5);

FilterIndex=0;
file_root='npt390a_';
file_path='test/';
[file_root,file_path,FilterIndex] = uigetfile('*.bmp','Select a file root','');

if FilterIndex > 0


[file_root file_extension] = strread(file_root, '%q %q ', 'delimiter', '_');
file_root=char(file_root);
file_root=[file_root '_'];
file_name=[file_path file_root '000.bmp'];
```

```
displacement = load_displacement_files(file_path,file_root);
x_size=size(displacement.x,2);
y_size=size(displacement.x,1);
time_points=size(displacement.y,3);

%smooth trajectories
displacement=smooth_trajectories(displacement,traj_span);

%calculate velocities
velocity=calc_velocityb(displacement, vel_span, fps);


%calculate strains
strain=calc_lsstrainb(displacement,diff_span,block_size);


%saving files
file_out=[file_path file_root 'out'];
save(file_out, 'velocity', 'strain');

% file_out=[file_path file_root 'XX_strain'];
% for t=1:time_points
%     sheet_name=['Frame ' int2str(t)];
%     xlswrite(file_out, strain.xx(:,:,t), sheet_name);
% end
%
% file_out=[file_path file_root 'YY_strain'];
% for t=1:time_points
%     sheet_name=['Frame ' int2str(t)];
%     xlswrite(file_out, strain.yy(:,:,t), sheet_name);
% end
%
% file_out=[file_path file_root 'SH_strain'];
% for t=1:time_points
%     sheet_name=['Frame ' int2str(t)];
%     xlswrite(file_out, strain.sh(:,:,t), sheet_name);
% end
%
% file_out=[file_path file_root 'X_velocity'];
% for t=1:time_points
%     sheet_name=['Frame ' int2str(t)];
%     xlswrite(file_out, velocity.x(:,:,t), sheet_name);
% end
%
% file_out=[file_path file_root 'Y_velocity'];
% for t=1:time_points
%     sheet_name=['Frame ' int2str(t)];
%     xlswrite(file_out, velocity.y(:,:,t), sheet_name);
% end


%display strain graphs
done=disp_strain_graphs(strain);
```

```
%display strain maps
done=disp_strain_maps(strain,file_name);

%get ROI
roi_map=choose_roi(displacement,file_name);

%display vs time graphs
done=disp_vstime_graphs(roi_map, displacement, velocity, strain);

end;
```

APPENDIX 2B

MATLAB CODE: FUNCTIONS USED TO MAKE UP 'USSTRAIN2'

THREE_CHAR.M

```
function str = three_char( num )
% --- three_char ---
% This function converts an integer between 0 and 999 inclusively into
% a 3-character string.
% ------------------------------------------------------------------------

if (num < 10)
  str = ['00',num2str(num)];
elseif (num >= 10 & num < 100)
  str = ['0',num2str(num)];
elseif (num >= 100 & num < 1000)
  str = [num2str(num)];
else
  error('O-U-C-H! input files exceeded 1000! - sorry too many files - bye!')
end
```

LOAD_DISPLACEMENT_FILES.M

```
function displacement = load_displacement_files(file_path,file_root);


% --- Count how many files called [filename_root aaa  'x.txt']
%     where <aaa> is a three-character number.
% ---------------------------------------------------------------------

f_count=0;
igo = 1;

while igo > 0
   fname = [file_path file_root three_char(f_count)  'x.txt'];
   igo = exist(fname);
   if (f_count == 0)
      cmmd1 = ['load ' fname];          eval(cmmd1);
   end
   f_count=f_count+1;
end

f_count = f_count - 1;
np = f_count;

for f_count = 0:(np-1)

   fname_x = [file_path file_root three_char(f_count)  'x.txt'];
   tx = load (fname_x);
   displacement.x(:,:,(f_count+1)) = tx;

   fname_y = [file_path file_root three_char(f_count)  'y.txt'];
   ty = load (fname_y);
```

```
   displacement.y(:,:,(f_count+1)) = ty;

end
```

SMOOTH_TRAJECTORIES.M

```
function displacement=smooth_trajectories(displacement,traj_span);


%calcualte size of array
x_size=size(displacement.x,2);
y_size=size(displacement.x,1);
time_points=size(displacement.x,3);



%calculate displacement.x

%create edges
start_edge=zeros(y_size,x_size,traj_span);
end_edge=zeros(y_size,x_size,traj_span);
for t=1:traj_span;
   start_edge(:,:,traj_span+1-t)=displacement.x(:,:,1)*2-displacement.x(:,:,1+t);
   end_edge(:,:,t)=displacement.x(:,:,time_points)*2-displacement.x(:,:,time_points-t);
end;


%create big displacement array
big=zeros(y_size,x_size,time_points+traj_span*2);
big(:,:,1:traj_span)=start_edge;
big(:,:,traj_span+1:time_points+traj_span)=displacement.x;
big(:,:,time_points+traj_span+1:time_points+traj_span*2)=end_edge;


%smooth X displacement
sample=zeros(1,traj_span*2+1,1);
wbhandle = waitbar(0,'Smoothing X Trajectories');
for x=1:x_size;
   %disp(['X Velocity ' num2str(x) ' / ' num2str(x_size)])
   waitbar(x/x_size);
   for y=1:y_size;
      for t=1:time_points;
         for t2=0:2*traj_span
            sample(t2+1)=big(y,x,t2+t);
         end;
         p=mean(sample);
         velocity.x(y,x,t)=p(1);
      end;
   end;
end;
close(wbhandle)


%calculate displacement.y
```

```
%create edges
start_edge=zeros(y_size,x_size,traj_span);
end_edge=zeros(y_size,x_size,traj_span);
for t=1:traj_span;
   start_edge(:,:,traj_span+1-t)=displacement.y(:,:,1)*2-displacement.y(:,:,1+t);
   end_edge(:,:,t)=displacement.y(:,:,time_points)*2-displacement.y(:,:,time_points-t);
end;


%create big displacement array
big=zeros(y_size,x_size,time_points+traj_span*2);
big(:,:,1:traj_span)=start_edge;
big(:,:,traj_span+1:time_points+traj_span)=displacement.y;
big(:,:,time_points+traj_span+1:time_points+traj_span*2)=end_edge;


%calculate Y velocity
sample=zeros(1,traj_span*2+1);
wbhandle = waitbar(0,'Smoothing Y Trajectories');
for x=1:x_size;
   waitbar(x/x_size);
   for y=1:y_size;
      for t=1:time_points;
         for t2=0:2*traj_span
            sample(t2+1)=big(y,x,t2+t);
         end;
         p=mean(sample);
         velocity.y(y,x,t)=p(1);
      end;
   end;
end;

close(wbhandle)
```

CALC_VELOCITYB.M

```
function velocity=calc_velocity(displacement,vel_span,fps);


% %create dumy displacement fields for testing
% for y=1:6;
%    for x=1:6;
%       for t=1:6;
%          displacement.x(y,x,t)=t;
%          displacement.y(y,x,t)=t*2;
%       end;
%    end;
% end;
% vel_span=2;
% fps=1;


%calcualte size of array
x_size=size(displacement.x,2);
y_size=size(displacement.x,1);
```

```
time_points=size(displacement.x,3);


%initialise strain arrays
velocity.x=zeros(y_size,x_size,time_points);
velocity.y=zeros(y_size,x_size,time_points);
velocity.s=zeros(y_size,x_size,time_points);
velocity.a=zeros(y_size,x_size,time_points);


%calculate velocity.xx

%create edges
start_edge=zeros(y_size,x_size,vel_span);
end_edge=zeros(y_size,x_size,vel_span);
for t=1:vel_span;
   start_edge(:,:,vel_span+1-t)=displacement.x(:,:,1)*2-displacement.x(:,:,1+t);
   end_edge(:,:,t)=displacement.x(:,:,time_points)*2-displacement.x(:,:,time_points-t);
end;


%create big displacement array
big=zeros(y_size,x_size,time_points+vel_span*2);
big(:,:,1:vel_span)=start_edge;
big(:,:,vel_span+1:time_points+vel_span)=displacement.x;
big(:,:,time_points+vel_span+1:time_points+vel_span*2)=end_edge;


%calculate X velocity
sample=zeros(1,vel_span*2+1,1);
wbhandle = waitbar(0,'Calculating X Velocity');
for x=1:x_size;
   %disp(['X Velocity ' num2str(x) ' / ' num2str(x_size)])
   waitbar(x/x_size);
   for y=1:y_size;
      for t=1:time_points;
         for t2=0:2*vel_span
            sample(t2+1)=big(y,x,t2+t);
         end;
         p=polyfit(-vel_span:vel_span,sample,1);
         velocity.x(y,x,t)=p(1)*fps;
      end;
   end;
end;
close(wbhandle)

%calculate velocity.y

%create edges
start_edge=zeros(y_size,x_size,vel_span);
end_edge=zeros(y_size,x_size,vel_span);
for t=1:vel_span;
   start_edge(:,:,vel_span+1-t)=displacement.y(:,:,1)*2-displacement.y(:,:,1+t);
   end_edge(:,:,t)=displacement.y(:,:,time_points)*2-displacement.y(:,:,time_points-t);
end;
```

```
%create big displacement array
big=zeros(y_size,x_size,time_points+vel_span*2);
big(:,:,1:vel_span)=start_edge;
big(:,:,vel_span+1:time_points+vel_span)=displacement.y;
big(:,:,time_points+vel_span+1:time_points+vel_span*2)=end_edge;


%calculate Y velocity
sample=zeros(1,vel_span*2+1);
wbhandle = waitbar(0,'Calculating Y Velocity');
for x=1:x_size;
   %disp(['Y Velocity ' num2str(x) ' / ' num2str(x_size)])
   waitbar(x/x_size);
   for y=1:y_size;
      for t=1:time_points;
         for t2=0:2*vel_span
            sample(t2+1)=big(y,x,t2+t);
         end;
         p=polyfit(-vel_span:vel_span,sample,1);
         velocity.y(y,x,t)=p(1)*fps;
      end;
   end;
end;

close(wbhandle)

for t=1:time_points;
   for y=1:y_size;
      for x=1:x_size;
         velocity.s(y,x,t)=sqrt(velocity.x(y,x,t)^2 + velocity.y(y,x,t)^2);
         if velocity.x(y,x,t) ==0 && velocity.y(y,x,t) == 0
            velocity.a(y,x,t)=0;
         else
            if abs(velocity.x(y,x,t)) > abs(velocity.y(y,x,t))
               velocity.a(y,x,t)=atan(velocity.y(y,x,t)/velocity.x(y,x,t));
            else
               velocity.a(y,x,t)=atan(velocity.x(y,x,t)/velocity.y(y,x,t));
               if velocity.a(y,x,t) > 0
                  velocity.a(y,x,t)= pi/2-velocity.a(y,x,t);
               else
                velocity.a(y,x,t)= -1*velocity.a(y,x,t)-pi/2;
               end;
            end;
         end;
      end;
   end;
end;
```

CALC_LSSTRAINB.M

```
function strain=calc_lsstrain(displacement,diff_span,block_size);


%create dumy displacement fields for testing
%[displacement.x displacement.y T]=meshgrid(1:6,1:3,1:5);  %meshgrid works for x and y
%diff_span=2;
```

```
%block_size=1;


%calcualte size of array
x_size=size(displacement.x,2);
y_size=size(displacement.x,1);
time_points=size(displacement.x,3);


%set up incremental arrays X, Y, T - these arrays increment in x, y or t
[X,Y,T]=meshgrid(1:x_size,1:y_size,1:time_points);


%initialise strain arrays
strain.xx=zeros(y_size,x_size,time_points);
strain.xy=zeros(y_size,x_size,time_points);
strain.yy=zeros(y_size,x_size,time_points);
strain.yx=zeros(y_size,x_size,time_points);
strain.sh=zeros(y_size,x_size,time_points);


%calculate strain.xx

%create edges
left_edge=zeros(y_size,diff_span,time_points);
right_edge=zeros(y_size,diff_span,time_points);
for x=1:diff_span;
   left_edge(:,diff_span+1-x,:)=displacement.x(:,1,:)*2-displacement.x(:,1+x,:);
   right_edge(:,x,:)=displacement.x(:,x_size,:)*2-displacement.x(:,x_size-x,:);
end;


%create big displacement array
big=zeros(y_size,x_size+diff_span*2,time_points);
big(:,1:diff_span,:)=left_edge;
big(:,diff_span+1:x_size+diff_span,:)=displacement.x;
big(:,x_size+diff_span+1:x_size+diff_span*2,:)=right_edge;


%calculate strain
sample=zeros(diff_span*2+1);
wbhandle=waitbar(0,'Calculating XX Strain');
for t=1:time_points;
   %disp(['XX Strain ' num2str(t) ' / ' num2str(time_points)])
   waitbar(t/time_points);
   for y=1:y_size;
      for x=1:x_size
         sample=big(y,x:x+2*diff_span,t);
         p=polyfit(-diff_span:diff_span,sample,1);
         strain.xx(y,x,t)=p(1)/block_size;
      end;
   end;
end;

close(wbhandle);
```

```
%calculate strain.yy

%create edges
top_edge=zeros(diff_span,x_size,time_points);
bottom_edge=zeros(diff_span,x_size,time_points);
for y=1:diff_span;
   top_edge(diff_span+1-y,:,:)=displacement.y(1,:,:)*2-displacement.y(1+y,:,:);
   bottom_edge(y,:,:)=displacement.y(y_size,:,:)*2-displacement.y(y_size-y,:,:);
end;


%create big displacement array
big=zeros(y_size+diff_span*2,x_size,time_points);
big(1:diff_span,:,:)=top_edge;
big(diff_span+1:y_size+diff_span,:,:)=displacement.y;
big(y_size+diff_span+1:y_size+diff_span*2,:,:)=bottom_edge;


%calculate strain
sample=zeros(diff_span*2+1);
wbhandle=waitbar(0,'Calculating YY Strain');
for t=1:time_points;
   %disp(['YY Strain ' num2str(t) ' / ' num2str(time_points)])
   waitbar(t/time_points);
   for x=1:x_size;
      for y=1:y_size
         sample=big(y:y+2*diff_span,x,t);
         p=polyfit(-diff_span:diff_span,sample',1);
         strain.yy(y,x,t)=p(1)/block_size;
      end;
   end;
end;

close(wbhandle);

%calculate strain.xy

%create edges
top_edge=zeros(diff_span,x_size,time_points);
bottom_edge=zeros(diff_span,x_size,time_points);
for y=1:diff_span;
   top_edge(diff_span+1-y,:,:)=displacement.x(1,:,:)*2-displacement.x(1+y,:,:);
   bottom_edge(y,:,:)=displacement.x(y_size,:,:)*2-displacement.x(y_size-y,:,:);
end;


%create big displacement array
big=zeros(y_size+diff_span*2,x_size,time_points);
big(1:diff_span,:,:)=top_edge;
big(diff_span+1:y_size+diff_span,:,:)=displacement.x;
big(y_size+diff_span+1:y_size+diff_span*2,:,:)=bottom_edge;


%calculate strain
sample=zeros(diff_span*2+1);
```

```
wbhandle=waitbar(0,'Calculating XY Strain');
for t=1:time_points;
   %disp(['XY Strain ' num2str(t) ' / ' num2str(time_points)])
   waitbar(t/time_points);
   for x=1:x_size;
      for y=1:y_size
         sample=big(y:y+2*diff_span,x,t);
         p=polyfit(-diff_span:diff_span,sample',1);
         strain.xy(y,x,t)=p(1)/block_size;
      end;
   end;
end;

close(wbhandle);

%calculate strain.yx

%create edges
left_edge=zeros(y_size,diff_span,time_points);
right_edge=zeros(y_size,diff_span,time_points);
for x=1:diff_span;
   left_edge(:,diff_span+1-x,:)=displacement.y(:,1,:)*2-displacement.y(:,1+x,:);
   right_edge(:,x,:)=displacement.y(:,x_size,:)*2-displacement.y(:,x_size-x,:);
end;


%create big displacement array
big=zeros(y_size,x_size+diff_span*2,time_points);
big(:,1:diff_span,:)=left_edge;
big(:,diff_span+1:x_size+diff_span,:)=displacement.y;
big(:,x_size+diff_span+1:x_size+diff_span*2,:)=right_edge;


%calculate strain
sample=zeros(diff_span*2+1);
wbhandle=waitbar(0,'Calculating YX Strain');
for t=1:time_points;
   %disp(['YX Strain ' num2str(t) ' / ' num2str(time_points)])
   waitbar(t/time_points);
   for y=1:y_size;
      for x=1:x_size
         sample=big(y,x:x+2*diff_span,t);
         p=polyfit(-diff_span:diff_span,sample,1);
         strain.yx(y,x,t)=p(1)/block_size;
      end;
   end;
end;

close(wbhandle);


%calculate total shear strian
%adjusted to represent mathematical shear strain and account for pure
%rotation (KGC)
strain.sh=(strain.xy+strain.yx)/2;
```

DISP_STRAIN_GRAPHS.M

```
function done=disp_strain_graphs(strain);

warning off %Hides OpenGL warning messages

%set up global variables

%g_az = 0;      g_el = 90;      %for 'overhead' view
g_az = -37.5;  g_el = 30;      %for 3-D view

x_size=size(strain.xx,2);
y_size=size(strain.xx,1);
time_points=size(strain.xx,3);

X=zeros(x_size,y_size,time_points);
Y=zeros(x_size,y_size,time_points);
T=zeros(x_size,y_size,time_points);


[X,Y,T]=meshgrid(1:x_size,1:y_size,1:time_points);



scrsz = get(0,'ScreenSize');
fig1_h=figure(1);
set(fig1_h,'Position',[100 100 scrsz(3)-200 scrsz(4)-200]);
set(fig1_h,'Name','Graphs of X, Y and Shear Strain');



for t=1:time_points;

   title_str=['XX Strain (t=',num2str(t),')'];
   subplot(2,2,1);
   surf(X(:,:,t),Y(:,:,t),strain.xx(:,:,t));
   shading interp;
   title(title_str);
   xlabel('x (blocks)');
   ylabel('y (blocks)');
   caxis([min(min(min(strain.xx))) max(max(max(strain.xx)))]);
   colorbar;
   axis([1 x_size 1 y_size min(min(min(strain.xx))) max(max(max(strain.xx)))]);
   axis ij;
   view([g_az g_el]);

   title_str=['YY Strain (t=',num2str(t),')'];
   subplot(2,2,2);
   surf(X(:,:,t),Y(:,:,t),strain.yy(:,:,t));
   shading interp;
   title(title_str);
   xlabel('x (blocks)');
   ylabel('y (blocks)');
   caxis([min(min(min(strain.yy))) max(max(max(strain.yy)))]);
   colormap jet;
   colorbar;
```

```
   axis([1 x_size 1 y_size min(min(min(strain.yy))) max(max(max(strain.yy)))]);
   axis ij;
   view([g_az g_el]);

   %title_str=['XY Strain (t=',num2str(t),')'];
   %subplot(2,2,3);
   %surf(X(:,:,t),Y(:,:,t),strain.xy(:,:,t));
   %shading interp;
   %title(title_str);
   %caxis([min(min(min(strain.xy))) max(max(max(strain.xy)))]);
   %colormap jet;
   %colorbar;
   %axis([1 x_size 1 y_size min(min(min(strain.xy))) max(max(max(strain.xy)))]);
   %axis ij;
   %view([g_az g_el]);

   %title_str=['YX Strain (t=',num2str(t),')'];
   %subplot(2,2,4);
   %surf(X(:,:,t),Y(:,:,t),strain.yx(:,:,t));
   %shading interp;
   %title(title_str);
   %caxis([min(min(min(strain.yx))) max(max(max(strain.yx)))]);
   %colormap jet;
   %colorbar;
   %axis([1 x_size 1 y_size min(min(min(strain.yx))) max(max(max(strain.yx)))]);
   %axis ij;
   %view([g_az g_el]);

   title_str=['Shear Strain (t=',num2str(t),')'];
   subplot(2,2,3);
   surf(X(:,:,t),Y(:,:,t),strain.sh(:,:,t));
   shading interp;
   title(title_str);
   xlabel('x (blocks)');
   ylabel('y (blocks)');
   caxis([min(min(min(strain.sh))) max(max(max(strain.sh)))]);
   colormap jet;
   colorbar;
   axis([1 x_size 1 y_size min(min(min(strain.sh))) max(max(max(strain.sh)))]);
   axis ij;
   view([g_az g_el]);

   pause(0.1)

end;

warning on;
done=1;
```

DISP_STRAIN_MAPS.M

```
function done=disp_strain_maps(strain,file_name);

warning off %hides OpenGL warining messages
```

```
x_size=size(strain.xx,2);
y_size=size(strain.xx,1);
time_points=size(strain.xx,3);



scrsz = get(0,'ScreenSize');
fig2_h=figure(2);
set(fig2_h,'Position',[100 100 scrsz(3)-200 scrsz(4)-200]);
set(fig2_h,'Name','Strain maps superimposed on "Frame 0" image');


%input image
image_1info=imfinfo(file_name);
[image_1,map1]=imread(file_name);
image_1= ind2gray(image_1,map1);
image_scale=image_1info.Width/x_size;
interp_type='bicubic';
image_1=image_1(1:y_size*image_scale,:);

%scale strainmap

min_strain = min(min(min(strain.xx)));
max_strain = max(max(max(strain.xx)));
a=1/(max_strain-min_strain);
b=-1*a*min_strain;
strainmap.xx=a*strain.xx+b;

min_strain = min(min(min(strain.yy)));
max_strain = max(max(max(strain.yy)));
a=1/(max_strain-min_strain);
b=-1*a*min_strain;
strainmap.yy=a*strain.yy+b;

min_strain = min(min(min(strain.xy)));
max_strain = max(max(max(strain.xy)));
a=1/(max_strain-min_strain);
b=-1*a*min_strain;
strainmap.xy=a*strain.xy+b;

min_strain = min(min(min(strain.yx)));
max_strain = max(max(max(strain.yx)));
a=1/(max_strain-min_strain);
b=-1*a*min_strain;
strainmap.yx=a*strain.yx+b;

min_strain = min(min(min(strain.sh)));
max_strain = max(max(max(strain.sh)));
a=1/(max_strain-min_strain);
b=-1*a*min_strain;
strainmap.sh=a*strain.sh+b;

caxis([0 1]);

imshow(0)
```

```
set(fig2_h,'Position',[50 50 scrsz(3)-200 scrsz(4)-200]);


%produce strain overlays
image_1 = double(image_1)/256;
for t=1:time_points;
 %change images to same class and size
 %image_1 = double(image_1)/256;

   image_2.xx=imresize(strainmap.xx(:,:,t),image_scale,interp_type);
   image_2.xx=gray2ind(image_2.xx,256);
   image_2.xx=ind2rgb(image_2.xx,colormap(jet));
   for clayer=1:3
      image_2.xx(:,:,clayer)=image_2.xx(:,:,clayer).*image_1;
   end;
   subplot(2,2,1);
   imshow(image_2.xx);
   title_str=['XX Strain (t=',num2str(t),')'];
   title(title_str);

   image_2.yy=imresize(strainmap.yy(:,:,t),image_scale,interp_type);
   image_2.yy=gray2ind(image_2.yy,256);
   image_2.yy=ind2rgb(image_2.yy,colormap(jet));
   for clayer=1:3
      image_2.yy(:,:,clayer)=image_2.yy(:,:,clayer).*image_1;
   end;
   subplot(2,2,2);
   imshow(image_2.yy);
   title_str=['YY Strain (t=',num2str(t),')'];
   title(title_str);

   %image_2.xy=imresize(strainmap.xy(:,:,t),image_scale,interp_type);
   %image_2.xy=gray2ind(image_2.xy,256);
   %image_2.xy=ind2rgb(image_2.xy,colormap(jet));
   %for clayer=1:3
   %    image_2.xy(:,:,clayer)=image_2.xy(:,:,clayer).*image_1;
   %end;
   %subplot(2,2,3);
   %imshow(image_2.xy);
   %title_str=['XY Strain (t=',num2str(t),')'];
   %title(title_str);

   %image_2.yx=imresize(strainmap.yx(:,:,t),image_scale,interp_type);
   %image_2.yx=gray2ind(image_2.yx,256);
   %image_2.yx=ind2rgb(image_2.yx,colormap(jet));
   %for clayer=1:3
   %    image_2.yx(:,:,clayer)=image_2.yx(:,:,clayer).*image_1;
   %end;
   %subplot(2,2,4);
   %imshow(image_2.yx);
   %title_str=['YX Strain (t=',num2str(t),')'];
   %title(title_str);

   image_2.sh=imresize(strainmap.sh(:,:,t),image_scale,interp_type);
   image_2.sh=gray2ind(image_2.sh,256);
   image_2.sh=ind2rgb(image_2.sh,colormap(jet));
```

```
   for clayer=1:3
      image_2.sh(:,:,clayer)=image_2.sh(:,:,clayer).*image_1;
   end;
   subplot(2,2,3);
   imshow(image_2.sh);
   title_str=['Shear Strain (t=',num2str(t),')'];
   title(title_str);


   pause(0.1);
end;

warning on;
done=1;
```

CHOOSE_ROI.M

```
function roi_map = choose_roi(displacement,file_name);

% %temporary settings
% displacement.x=zeros(64,64,11);
% file_name='test/npt390a_000.bmp';

x_size = size(displacement.x,2);
image_1info=imfinfo(file_name);
[image_1,map1]=imread(file_name);
image_1= ind2gray(image_1,map1);
image_scale=x_size/image_1info.Width;

figure(3);
imshow(image_1);
roi_map = roipoly;
imshow(roi_map);
roi_map=imresize(roi_map,image_scale);
```

DISP_VSTIME_GRAPHS.M

```
function done=disp_vstime_graphs(roi_map, displacement, velocity, strain);

% %set up temorpary values
%
% roi_map=zeros(64,64);
% roi_map(3:6,5:7)=1;
%
% displacement.x=ones(64,64,11);
% displacement.y=ones(64,64,11);
%
% velocity.x=ones(64,64,11);
% velocity.y=ones(64,64,11);
% velocity.s=ones(64,64,11);
%
% strain.xx=ones(64,64,11);
% strain.yy=ones(64,64,11);
% strain.sh=ones(64,64,11);
```

```
x_size=size(strain.xx,2);
y_size=size(strain.xx,1);
time_points=size(strain.xx,3);

mean_displacement.x=zeros(1,time_points);
mean_displacement.y=zeros(1,time_points);

mean_velocity.x=zeros(1,time_points);
mean_velocity.y=zeros(1,time_points);
mean_velocity.s=zeros(1,time_points);

mean_strain.xx=zeros(1,time_points);
mean_strain.yy=zeros(1,time_points);
mean_strain.sh=zeros(1,time_points);

for t=1:time_points;
   roi_count=0;
   for y=1:y_size;
      for x=1:x_size;
         if roi_map(y,x) == 1

            roi_count=roi_count+1;

            mean_displacement.x(t)=mean_displacement.x(t)+displacement.x(y,x,t);
            mean_displacement.y(t)=mean_displacement.y(t)+displacement.y(y,x,t);

            mean_velocity.x(t)=mean_velocity.x(t)+velocity.x(y,x,t);
            mean_velocity.y(t)=mean_velocity.y(t)+velocity.y(y,x,t);
            mean_velocity.s(t)=mean_velocity.s(t)+velocity.s(y,x,t);

            mean_strain.xx(t)=mean_strain.xx(t)+strain.xx(y,x,t);
            mean_strain.yy(t)=mean_strain.yy(t)+strain.yy(y,x,t);
            mean_strain.sh(t)=mean_strain.sh(t)+strain.sh(y,x,t);

         end;
      end;
   end;

   mean_displacement.x(t)=mean_displacement.x(t)/roi_count;
   mean_displacement.y(t)=mean_displacement.y(t)/roi_count;

   mean_velocity.x(t)=mean_velocity.x(t)/roi_count;
   mean_velocity.y(t)=mean_velocity.y(t)/roi_count;
   mean_velocity.s(t)=mean_velocity.s(t)/roi_count;

   mean_strain.xx(t)=mean_strain.xx(t)/roi_count;
   mean_strain.yy(t)=mean_strain.yy(t)/roi_count;
   mean_strain.sh(t)=mean_strain.sh(t)/roi_count;

end

%plot displacements;

fig4_h=figure(4);
```

```
set(fig4_h,'Name','Graphs of displacement vs time');

subplot(1,2,1);
plot(1:time_points,mean_displacement.x);
title('x displacement');

subplot(1,2,2);
plot(1:time_points,mean_displacement.y);
title('y displacement');

fig5_h=figure(5);
set(fig5_h,'Name','Graphs of velocity vs time');

subplot(2,2,1);
plot(1:time_points,mean_velocity.x);
title('x velocity');

subplot(2,2,2);
plot(1:time_points,mean_velocity.y);
title('y velocity');

subplot(2,2,3);
plot(1:time_points,mean_velocity.s);
title('speed');

fig6_h=figure(6);
set(fig6_h,'Name','Graphs of strain vs time');

subplot(2,2,1);
plot(1:time_points,mean_strain.xx);
title('xx strain');

subplot(2,2,2);
plot(1:time_points,mean_strain.yy);
title('yy strain');

subplot(2,2,3);
plot(1:time_points,mean_strain.sh);
title('shear strain');

% disp(mean_strain.sh);

done=1;
```

APPENDIX 3

FACTORS AVAILABLE FOR MODIFICATION IN 'PROJECT2' BY 'ADVANCE'

Generally the settings used for tendon assume low speckle to noise ratio (SNR) as images are of a high quality.

DIFFUSION A pre-processing tool which updates each pixel depending on the movement of its neighbours in order to selectively smooth regions of pixels based on iterative evaluation i.e. gets rid of any unpredictable jumps in the movement of a particular block with respect to the blocks around it is disregarded and replaced with a similar value to its neighbours The value is the number of iterations of processing required tendon default (2) disc default (5)	This is a filtering process which is speckle specific and smoothes the images in order to exclude inaccurate displacements due to 'noise' but not speckle or structure
ENHANCEMENT A pre-processing tool which utilises constant variance enhancement, a digital optical processing tool for use in sequences of low contrast intensity It is on (>0) or off (0) Default value (0) as the high frequency probe generally produces such high quality data	By employing enhancement the programme can correct for transducer gain artefacts
UPDATE Temporal updating is one way of allowing for movement outwith the frame in question and updating it with respect to the previous frame) one possible problem is that accumulative error that may arise from sequential	Trajectories will be added and removed dynamically to track all new data within a sequence.

corrections It is on (>0) or off (0) Default value (0) as this is only useful if looking solely at displacements for the vectors produced too irregular for meaningful strain calculations	
STACK_N_TRACK It is on (>0) or off (0) Default value (0) off because tendon/high frequency probe images are such high quality	This can produce a 'super-resolution' ultrasound image
TX_COMPENSATION It is on (>0) or off (0) Default value (0) Probe correction. off because it is assumed that we are not looking at overall displacement of structure for strain but the displacement of one area of the structure with respect to another	This was introduced in order to update the displacement of deeper tissues such as the tendon with respect to the skin layer and thereby account for any probe slippage
DRIFT It is on (1) or off (0) Default value (0)	This modifies the images as described under 'Update'. Drift uses temporal updating but also corrects for accumulated error by referring to the original frame
WMVF (Post-processing algorithm which smoothes the displacements found by tracking. This means that	Weighted vector median filter. Default value for disc material

spurious displacements identified by means of large differences to their neighbours are disregarded and replaced with more believable values)	(2) Default value tendon (0)
SMOOTH It is on (>0) or off (0) Default value (5)	Applies a 1D filter to each track
BACKGROUNDWHITE Default value for tendon (200) this refers to the background colour of the vector bmp	This sets the background of the vector field to either white (0) or (>0) for the ultrasound image
HISTORY Default value for tendon (5) i.e. looks over 5 successive images	Sets the amount of tracking temporal history
STRAIN If set to 0 then the first order differential (normal strain) of each vector is calculated e.g. $Ex = du/dx$ if set to 1 then a higher order is used >1% e.g. $Ex = du/dx + ½ ((du/dx)^2+(dv/dx)^2)$	This refers to the precision of the strain estimates Default value (0)
CUMSUM If set to 1 it determines the accumulative summation or total distance travelled from the first frame, if set to 0 it determines each consecutive distance	Default set (1)

The following 3 values fuse different tracker metrics in order to improve tracking in images with wide ranges in signal from different densities of speckle, signal and noise	
MSE (1) fuses the Fourier NCC and MSE (0) fuses the Fourier NCC, MSE and CD^2 metrics	Mean Square Error This determines which metrics to fuse
WAGNER To determine how the metrics are fused the speckle SNR is computed and this is based on the work of Wagner, Smith and Sandrik(Wagner et al., 1983)	
CORR_THRESHOLD	This is a threshold for the correlation values to determine use of a Fourier NCC based on the spatial content
For the above 3 values the original tracking would be used by the author of the programme for tendon images using a Fourier NCC (normalised cross correlation) due to the high SNR, for more complicated structures like discs he used a fused tracker	(MSE to 1, WAGNER to 0 and CORR_THRESH to 0.001)(MSE to 1, WAGNER 1 and CORR_THRESH to 0.93)

The settings within ‘Advance’ were altered to see the effects on the tracking of displacements with SDFT within the **Calibration** study, Chapter 4. The best match to the actual displacement was using the default values as selected by the programme author, apart from Drift, which improved the accuracy of displacement tracking in Equine tendon when turned on. The Correlation threshold was increased to 0.93 for although James Revell had felt a low correlation would be suitable for tendons due to the high definition of the high frequency probes used the nature of the speckle produced by tendons and the potential effects on the speckle by movement or strain within the tissue potentially reduced the accuracy of the tracking. This will be discussed further in Chapter 4.

APPENDIX 4

'VISUALISATION OF MOVEMENT WITHIN A PATELLAR TENDON IN VIVO USING AN ISOMETRIC QUADRICEPS CONTRACTION'

PATIENT INFORMATION SHEET

(font size reduced for Appendices)

INFORMATION SHEET FOR SUBJECTS

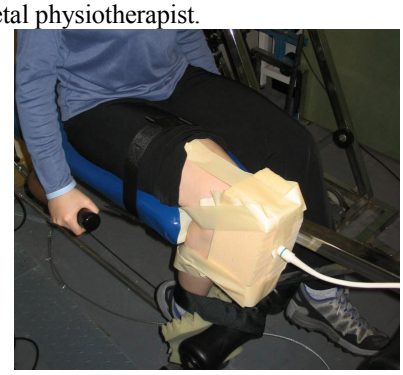
Examination of movement patterns within the patella tendon under load

- The aim of this investigation is to establish the mechanical properties of tendons under load. We will be using a leg extension machine as you might find in any gym in order to apply the load and visualising how the tendon moves using real time ultra sound. Pads will be applied to your leg in order to pick up any simultaneous activity in the hamstrings as you work the quadriceps.
- Do not participate in the study if you are aware of any existing or previous knee injury. Discuss any possible doubts regarding this with the researcher (Gillian Campbell MCSP SRP) prior to starting the activity.
- Before starting the test you will be shown what is expected of you throughout the warm-up, test and post activity stretch procedure. If you need any further information at any stage please discuss this with the researcher who will try to answer any queries you may have about the protocol. At this point you if you are happy to participate you will be asked to read and sign the consent form attached, after signing you still have the right to withdraw from the study at any stage. If at any point you have any further concerns please discuss this with the researcher.
- You will be expected to wear shorts and areas on the backs of your thighs will be cleaned with alcohol and may require a small area to be shaved in preparation for application of skin electrodes which will record the activity of both your hamstrings.
- Warm-up: You will perform a session of controlled sit to stand exercises for a period of one minute in order to prepare you and your muscles for activity.
- You will lie prone on the bench and your leg will be fixed to the apparatus by means of Velcro webbing at the thigh and ankle. While it is important that these fixings are tight please let the researcher know of any discomfort.
- You will be asked to apply maximum power to bend your knee. There will be 5 practice contractions followed by a rest period of approximately 1 minute and then 3 test contractions. You will be informed when the contractions are to be the test.
- After a further short rest period, when you will be able to get up and move around for about 5 minutes, you will be asked to SIT on the bench and once more will be strapped at the thigh and the ankle.
- The Ultrasound probe will be applied to your knee with aqueous gel at the probe/skin interface. The probe will be supported by means of a sponge fixed to your knee with tape. (Please inform the researcher of any known allergies prior to this).
- You will be asked to perform maximum power knee extension against a stationary bar. As a practice you will perform this 5 times. After a short, approximately 1 minute, rest period you will be asked to perform the 3 test knee extensions. You will again be made fully aware when it is the test contraction.
- After removing all the binding, electrodes and the ultrasound probe you will be able to move around again before being instructed on a set of gentle stretches for the hamstrings and quads muscle groups.

- While it is possible that there may be a small amount of ache in the front and back of the thigh used on the day of the experiment and up to 2 days after, this will be minimal and should not affect any normal daily activity.

ALTHOUGH IT IS UNLIKELY THAT YOU WILL EXPERIENCE ANY REAL DISCOMFORT, IT IS IMPORTANT THAT YOU INFORM THE RESEARCHER IF AT ANY STAGE DURING THE PROTOCOL YOU FEEL UNWELL, EXPERIENCE DISCOMFORT OR ARE IN PAIN. THE TEST WILL BE IMMEDIATELY STOPPED.

The test will be monitored throughout by Gillian Campbell BSc MCSP SRP who is an experienced musculoskeletal physiotherapist.



CONSENT FORM:

ANALYSIS OF MOVEMENT PATTERNS WITHIN THE PATELLA TENDON BY

MEANS OF U/S SCAN WHILE UNDER LOAD.

Name: …….

Address: ……..
……...
……
……

I …….. consent to take part in the above study. I confirm that the full experimental protocol has been fully explained to me and any questions have been answered to my satisfaction by the researcher.
I do not suffer from any of the conditions listed below:

- Heart conditions that preclude me from participating in exercise
- Previous history of knee injury
- Uncontrolled epilepsy
- Rheumatoid arthritis
- Pregnancy

I understand that I can withdraw from the experiment at any time
Signed: ……..
Date: …….

APPENDIX 5

PARTICIPANT INFORMATION SHEET

INVESTIGATION INTO THE BIOMECHANICS OF TENDONS

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish.
PART 1 tells you the purpose of this study and what will happen to you if you take part.
PART 2 gives you more detailed information about the conduct of the study.
Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

PART 1

(PROTOCOL: INVESTIGATION INTO THE STRAIN PATTERNS IN TENDONS UNDER PHYSIOLOGICAL LOADS: VERSION 2 16/03/06)

What is the purpose of the study?

- The aim of this study is to investigate the movement of different areas within tendons as the muscles work. This will allow us to see if some individuals are at more risk of injuring those tendons than others.
- This study is being conducted as part of the research being done by Gillian Campbell towards her PhD in Tendon Biomechanics.

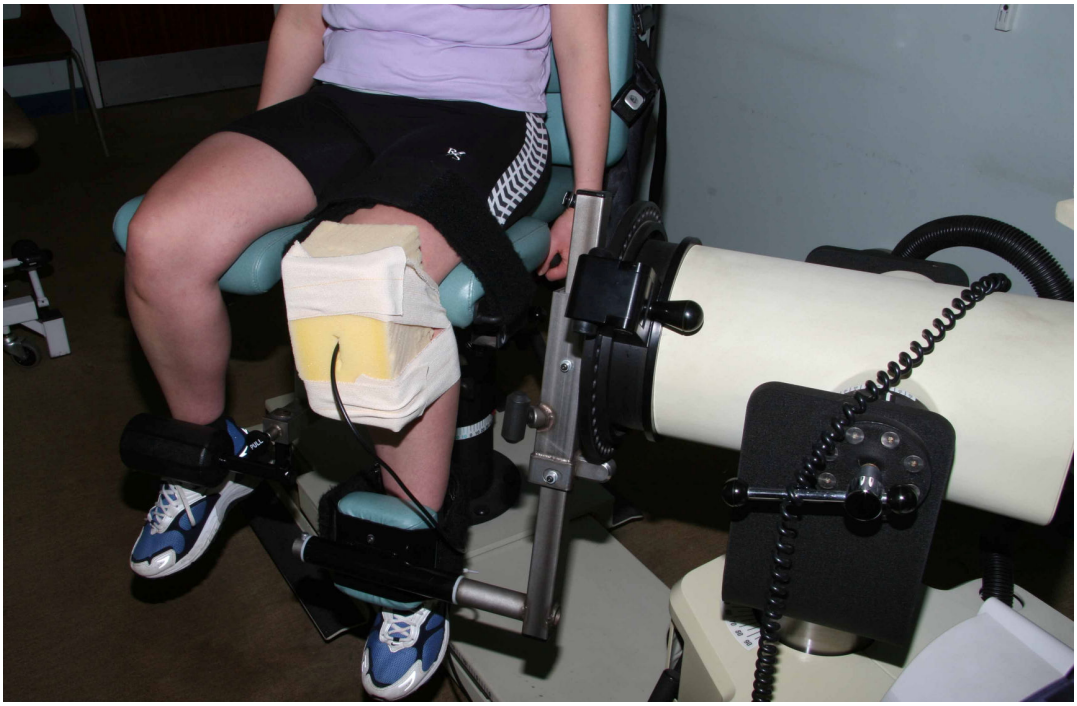
Why have I been chosen?

- We are particularly interested in looking at the tendons in normal individuals, both sedentary and sporting and at the tendons of patients previously diagnosed with tendinopathy. This is in order to compare their results to those with existing tendon damage and visualise any differences between the two groups. We hope to recruit around 40 to 80 volunteers in total from these different groups. You will be involved in the decision to allocate you to a particular group.

Do I have to take part?

- No. It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason. If you are a patient of the Centre for Sports Medicine any decision to withdraw will have no bearing on your standard of care.

What will happen to me if I take part?



- Normal candidates will first have their tendon scanned to ensure there is no existing degeneration.
- We will be using a machine called a dynamometer (as pictured above) which will allow you to straighten and bend your knee or ankle and then will record the amount of work you have applied. Applying the work will feel similar to using a weights machine in the gym. We will then visualise how the tendon moves using an ultra sound machine. Before starting the test you will be shown what is expected of you throughout the warm-up, test and post-activity stretch procedure. If you need any further information at any stage please ask the researcher who will try to answer any queries you may have about the protocol.
- The test should take about 1 hour from start to finish
- You will be expected to expose your knees and it will probably be most comfortable to wear loose clothing such as a t-shirt and shorts.
- Warm-up: The dynamometer will be adjusted to ensure that it is comfortable for you and Velcro strapping will be placed above the knee and around the ankle. You will then warm up by extending and flexing your knee or ankle against light resistance 10 times.
- After a short rest period, the Ultrasound probe will be applied to your knee or ankle with a water-based gel between the probe and the skin. The probe will be supported by means of a sponge fixed to your knee with tape. (Please inform the researcher of any known allergies prior to this).
- The test proper will then start: This will involve recording ultrasound footage of movement within your tendon as you work against the resistance of the machine. The machine will not move during this part of the test.

- You will be asked to apply work at 3 levels, up to your maximum. At all points you will dictate the amount of work applied. You will be asked to repeat this work at 3 different points through joint range. (In the case of the knee this will be at 90°, 45° and 5° of knee flexion. In between 5 second periods of work you will be able to rest in these positions for 1 minute.
- At the end of the above test period the Ultrasound probe will be removed and any excess gel wiped away. You will be released from the Cybex and shown a gentle stretch for the muscle that has been worked. There should be no discomfort at any stage of the procedure if you feel this to be the case, or you feel unwell in any way please inform the researcher and the test will be stopped immediately.
- The total time of testing should take approximately 1 hour and will not require any follow-up tests.
- Reasonable travel expenses may be claimed by filling out an expenses form and submitting it to the University of Nottingham. The researcher will guide you through this process. You will be expected to produce any bus or train tickets.

What are the possible disadvantages and risks of taking part?

- While it is possible that there may be some mild post testing muscle ache for up to 2 days this will be minimal and should not affect any normal daily activity.

What are the possible benefits of taking part?

- We cannot promise that the study will help you but the information we get might help improve the treatment of people with tendinopathy in the future.

What if there is a problem?

- Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in Part 2.

Will my taking part in the study be kept confidential?

- Yes. All the information about your participation in this study will be kept confidential. The details are included in part 2.

Contact details:

Gillian Campbell
Institute of Biomechanics
Wolfson Building
University Campus
University of Nottingham
Nottingham NG7 2RD

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This completes Part 1 of the information sheet. If the information in Part 1 has interested you and you are considering participation,

please continue to read the additional information in Part 2 before making any decision.

Part 2

Please do not take part if you are pregnant or suffer from any of the following conditions:

- Cardiovascular disease.
- Rheumatoid arthritis.
- Uncontrolled epilepsy.
- Existing tendon injury (other than patients of the Centre for Sports Medicine)

If you are aware of any existing knee or ankle injury please discuss this with the researcher (Gillian Campbell MCSP) prior to starting the activity.
Your GP will be informed that you are participating in this study.
At this point you if you are happy to participate you will be asked to read and sign the consent form attached, after signing you still have the right to withdraw from the study at any stage. If at any point you have any further concerns please discuss this with the researcher.
The test will be monitored throughout by Gillian Campbell BSc MCSP SRP who is an experienced musculoskeletal physiotherapist.

What will happen if I don't want to carry on with the study?

- If you withdraw from the study we will destroy all identifiable samples but will need to use the data collected up to your withdrawal.

What if there is a problem?

If you have a concern about any aspect of this study, you should speak to the researcher who will do her best to answer your questions.

(Contact details as above.)

If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained through the hospital.

Any complaint you might have about the way you have been dealt with during the study should be directed to the Centre for Sports Medicine. In the unlikely event that you suffer any harm and this is due to someone's negligence then you may have grounds for a legal action for compensation against Queen's Medical Centre, Nottingham but you may still have to pay your legal costs. The normal NHS complaints mechanisms will still be available to you if appropriate.

Will my taking part in this study be kept confidential?

The consent form you sign and the letter to your GP will be the only identifying record of your details. For patients of the Centre for Sports Medicine it will be noted in your medical records that you have taken part in the study.

Data collected, in the form of Ultrasound footage, will be encoded and numbered for storage and analysis. Each subjects coding will be noted on their consent form. Data will not be labelled with your name or address so that you cannot be recognised from it. All information collected during the course of the study will be kept strictly confidential.

Your GP will be sent a short letter informing him or her that you have agreed to take part. This is so that they will have knowledge of the study in the event that you feel the need to discuss any concerns about the test with him or her.

What will happen to the ultrasound footage?

- Once the ultrasound sequences have been analysed they will be stored electronically labelled only by means of the encoding as mentioned above.
- After the study is complete they will be stored on discs stored securely within the Centre for sports medicine.

Patients under the care of the Centre for Sports Medicine will continue their treatment as normal and their participation or decision to withdraw from this test will not affect their routine treatment in any way.

What will happen to the results of the research study?

The results of the study will be published in an academic thesis and in peer reviewed journals. No individual taking part will be identifiable in the results.

Who is organising and funding the research?

This study is part of an educational research project and as such is funded by the EPSRC via the University of Nottingham.

Who has reviewed the study?

This study was given a favourable ethical opinion for conduct in the NHS by the Derbyshire Research Ethics Committee.

Thank you for taking the time to read this information sheet. Please feel free to direct any queries you may have to Gillian Campbell as listed above.

APPENDIX 5

Study Number: 06/Q2401/1

Patient Identification Number for this trial:

CONSENT FORM

An Investigation into the Strain Patterns of Normal and Diseased Tendons Under Functional Load

Chief Investigator: Professor M.E. Batt

Principal Investigator: Gillian Campbell

Please initial box

1. I confirm that I have read and understood the information sheet dated 30/03/06 (version 3) for the above study. □
I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving reason and without my medical care or rights being affected. □

3. I understand that relevant sections of any of my medical notes and data collected during the study may be looked at by responsible individuals from the University of Nottingham, from regulatory authorities or from the NHS trust where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. □

4. I agree to my GP being informed of my participation in the study □

GP's name: ……

Address: ……
…..
…..
…..

5. I am not affected by any of the conditions listed:
- **a.** Heart conditions that preclude me from exercise
- **b.** Uncontrolled epilepsy
- **c.** Rheumatoid arthritis
- **d.** Pregnancy □

6. I agree to take part in the above study. □

……

Name of the patient
Date Signature

……
Name of the person taking consent
Date Signature
(if different from the researcher)

……
Researcher

When completed, 1 for patient; 1 for researcher site file; 1 original to be kept in medical notes(where subject is a patient of the Centre for SportsMedicine)

APPENDIX 6

SUBJECT	90º MAX						
	STRAIN	Ant fibres	Post fibres	LOAD		Vector score	Problems with data
MSNPT1	0.045	0.056	0.034	129		3	generally saturated image
MSNPT2	0.063	0.048	0.080	79		3	
MSNPT3	0.071	0.090	0.073	101		3	
MSNPT 4	0.015	0.013	0.023	273		3	
MSNPT5	0.068	0.066	0.061	87		3	very white images
MSNPT6	0.047	0.018	0.043	64		3	
MSNPT7	0.072	0.039	0.014	114		3	
MSNPT8	0.052	0.034	0.061	182		3	
MSNPT9	0.056	0.047	0.065	241		3	
MSNPT10	0.031	0.023	0.046	184		3	
SUBJECT	XX STRAIN	Prop diff ant fibres	Prop diff post fibres	LOAD	Ant Fibre/ Overall	Post Fibre/ Overall	
MSNPT1	0.045	-0.246	0.248	129	1.246	0.752	
MSNPT2	0.063	0.239	-0.266	79	0.761	1.266	
MSNPT3	0.071	-0.273	-0.033	101	1.273	1.033	
MSNPT 4	0.015	0.132	-0.564	273	0.868	1.564	
MSNPT5	0.068	0.026	0.099	87	0.974	0.901	
MSNPT6	0.047	0.610	0.098	64	0.390	0.902	
MSNPT7	0.072	0.459	0.812	114	0.541	0.188	
MSNPT8	0.052	0.343	-0.171	182	0.657	1.171	
MSNPT9	0.056	0.156	-0.158	241	0.844	1.158	
MSNPT10	0.031	0.255	-0.491	184	0.745	1.491	

90º MIN							
SUBJECT	STRAIN	Ant fibres	Post Fibres	LOAD			Problems with data
MSNPT1				40	poor tracking	1	oversaturated image
MSNPT2	0.059	0.063	0.057	25		3	
MSNPT3	0.019	0.025	0.031	33		3	
MSNPT 4	0.029	0.028	0.026	100		3	
MSNPT5				20		0	oversaturated, no tendon tracking
MSNPT6	0.038	0.022	0.056	11		3	
MSNPT7	0.024	0.022	0.026	10		3	
MSNPT8	0.040	0.019	0.049	60		3	
MSNPT9	0.064	0.069	0.056	80		3	
MSNPT10	0.032	0.027	0.038	60		3	
SUBJECT	XX STRAIN	Prop diff ant fibres	Prop diff post fibres	LOAD	Ant Fibre/ Overall	Post Fibre/ Overall	
MSNPT2	0.059	-0.063	0.044	25	1.063	0.956	
MSNPT3	0.019	-0.336	-0.645	33	1.336	1.645	
MSNPT 4	0.029	0.046	0.108	100	0.954	0.892	
MSNPT6	0.038	0.438	-0.454	11	0.562	1.454	
MSNPT7	0.024	0.089	-0.089	10	0.911	1.089	
MSNPT8	0.040	0.529	-0.210	60	0.471	1.210	
MSNPT9	0.064	-0.082	0.120	80	1.082	0.880	
MSNPT10	0.032	0.150	-0.167	60	0.850	1.167	

SUBJECT	45º MAX							
	STRAIN	Ant fibres	Post fibres	LOAD			Vector score	Problems with data
MSNPT1	0.030	0.023	0.012	116		y disp in all data	2	
MSNPT2				71			2	large amounts y displacement
MSNPT3	0.031	0.011	0.014	90			2	early y disp but improved latter part
MSNPT 4	0.049	0.047	0.053	201			2	y disp after early frames but full strain
MSNPT5				73			1	oversat tendon not tracked
MSNPT6	0.060	0.063	0.064	67			3	oversat tendon tracked but reliable?
MSNPT7	0.054	0.043	0.057	146			3	
MSNPT8				102			2	y > x disp throughout
MSNPT9	0.026	0.014	0.049	161			3	
MSNPT10	0.034	0.052	0.012	242			3	some yy strain through profile but not here
	XX STRAIN	Prop diff ant fibres	Prop diff post fibres	LOAD	Ant Fibre/ Overall	Post Fibre/ Overall		
MSNPT1	0.030	0.236	0.593	116	0.764	0.407		
MSNPT3	0.031	0.653	0.545	90	0.347	0.455		
MSNPT 4	0.049	0.031	-0.100	201	0.969	1.100		
MSNPT7	0.054	0.200	-0.070	146	0.800	1.070		
MSNPT9	0.026	0.453	-0.907	161	0.547	1.907		
MSNPT10	0.034	-0.521	0.663	242	1.521	0.337		

	45º MIN							
	STRAIN	Ant fibres	Post fibres	LOAD			Vector Score	Problems with data
MSNPT1	0.029	0.027	0.018	30			3	
MSNPT2				22			1	y disp excessive poor tracking
MSNPT3	0.015	0.016	0.029	30			2	y disp and strain altho not > x
MSNPT 4	0.015	0.017	0.017	67			3	
MSNPT5	0.035	0.037	0.038	13			1	white oversat image
MSNPT6	0.047	0.069	0.033	14			3	
MSNPT7				13			2	yy strain > xx strain
MSNPT8	0.056	0.056	0.053	30			3	yy strain > xx strain after fr 8
MSNPT9	0.050	0.050	0.050	52			3	
MSNPT10	0.052	0.050	0.057	80			3	max strain @ fr 16 after which yy strain inc
	XX STRAIN	Prop diff ant fibres	Prop diff post fibres	LOAD	Ant Fibre/ Overall	Post Fibre/ Overall		
MSNPT1	0.029	0.070	0.388	30	0.930	0.612		
MSNPT3	0.015	-0.078	-0.912	30	1.078	1.912		
MSNPT 4	0.015	-0.127	-0.156	67	1.127	1.156		
MSNPT6	0.047	0.308	0.308	14	1.463	0.692		
MSNPT9	0.050	0.005	0.005	52	0.996	0.995		
MSNPT10	0.052	-0.095	-0.095	80	0.957	1.095		

SUBJECT	5º MAX						
	STRAIN	Ant fibres	Post fibres	LOAD		Vector Score	Problems with data
MSNPT1	0.038	0.042	0.038	80	frame 5	3	
MSNPT2				30		2	y > x in both disp and strain
MSNPT3				28		1	poor tracking of tendon too white?
MSNPT 4	0.038	0.039	0.039	47		3	
MSNPT5				33		2	oversaturated and y > x
MSNPT6	0.046	0.029	0.082	21		3	
MSNPT7	0.075	0.081	0.066	54		3	
MSNPT8	0.041	0.043	0.035	40		3	
MSNPT9	0.029	0.035	0.027	54		3	
MSNPT10	0.033	0.039	0.018	92		3	y disp sim to x but min y strain
	XX STRAIN	Prop diff ant fibres	Prop diff post fibres	LOAD	Ant Fibre/ Overall	Post Fibre/ Overall	SUBJECT
MSNPT1	0.038	-0.113	0.003	80	1.113	0.997	MSNPT1
MSNPT 4	0.038	-0.018	-0.018	47	1.018	1.018	MSNPT3
MSNPT6	0.046	0.354	-0.795	21	0.646	1.795	MSNPT6
MSNPT7	0.075	-0.079	0.129	54	1.079	0.871	MSNPT7
MSNPT8	0.041	-0.059	0.131	40	1.059	0.869	MSNPT8
MSNPT9	0.029	-0.188	0.058	54	1.188	0.942	MSNPT9
MSNPT10	0.033	-0.176	0.472	92	1.176	0.528	MSNPT10

	5º MIN						
	STRAIN	Ant fibres	Post fibres	LOAD		Vector Score	Problems with data
MSNPT1	0.030	0.029	0.031	15	frame 8	3	
MSNPT2				10		2	
MSNPT3	0.045	0.054	0.027	10		3	
MSNPT 4				15		3	y > x throughout all cine
MSNPT5				13		2	oversaturated and y > x
MSNPT6	0.031	0.020	0.019	10		3	
MSNPT7	0.056	0.045	0.062	10		3	
MSNPT8	0.036	0.046	0.034	13		3	
MSNPT9	0.033	0.026	0.029	18		3	
MSNPT10	0.030	0.017	0.044	30		3	large amounts of y disp throughout
							procedure but first set has useable
							data after frame 2
	XX STRAIN	Prop diff ant fibres	Prop diff post fibres	LOAD	Ant Fibre/ Overall	Post Fibre/ Overall	
MSNPT1	0.030	0.032	-0.035	15	0.968	1.035	
MSNPT3	0.045	-0.190	0.394	10	1.190	0.606	
MSNPT6	0.031	0.349	0.394	10	0.651	0.606	
MSNPT7	0.056	0.200	-0.111	10	0.800	1.111	
MSNPT8	0.036	-0.273	0.053	13	1.273	0.947	
MSNPT9	0.033	0.222	0.125	18	0.778	0.875	
MSNPT10	0.030	0.426	-0.461	30	0.574	1.461	

Results of Repeatability Study			
	Strain	Anterior Fibres	Posterior Fibres
MSNPT 10 a	0.025	0.024	0.031
MSNPT 10 b	0.021	0.018	0.025
MSNPT 10 c	0.038	0.037	0.045
MSNPT 10 d	0.031	0.023	0.046
Mean strain	0.029	0.025	0.037
Standard Dev	0.007	0.008	0.011