Deep tissue biomechanics during pressure-induced pain

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Preface

This PhD project is based on studies conducted between October 2007 and September 2010 at Centre for Sensory Motor Interaction, Aalborg University, Denmark. Parts of the unpublished findings have been carried out at the Department of Neural Regulation, Research Institute of Environmental Medicine, Nagoya University, Japan.

Study I

Pressure-induced muscle pain and tissue biomechanics: A computational and experimental study; Sara Finocchietti, Mogens Nielsen, Carsten Dahl Mørch, Lars Arendt-Nielsen, Thomas Graven-Nielsen. *Eur J Pain 2011; 15(1):36-44.*
DOI: 10.1016/j.ejpain.2010.05.010

Study II

Effects of adipose thickness and muscle hardness on pressure pain sensitivity; Sara Finocchietti, Carsten Dahl Mørch, Lars Arendt-Nielsen, Thomas Graven-Nielsen. *Clin J Pain 2011;27(8):735-745.*
DOI: 10.1097/AJP.0b013e31820c5353

Study III

Pressure evoked pain from periosteum - a computational and experimental human study; Sara Finocchietti, Lars Arendt-Nielsen, Trine Andreasen, Thomas Graven-Nielsen. *Eur J Pain 2011, in press.*
DOI: 10.1016/j.ejpain.2011.08.001

Study IV

Tissue characteristics during temporal summation of pressure evoked pain; Sara Finocchietti, Lars Arendt-Nielsen, Thomas Graven-Nielsen. *Submitted to Experimental Brain Research.*

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1. Introduction

Musculoskeletal pain impairs quality of life and can also have a substantial socio-economic impact; it affects 33 % of adults and accounts for 29 % of the lost workdays, in addition to the distress caused to the patients and relatives (Becker et al., 1997). Musculoskeletal pain is associated with a variety of disorders that cause pain in bones, muscles, joints or surrounding structures. It is more prevalent than skin pain, 23% of pain patients visiting the general practitioner had muscle and deep tissue pain, in contrast to 0.1 % with skin pain (Hasselström et al., 2002). Acute deep tissue pain is initiated by stimulation of free nerve endings in the peripheral nervous system that transduce chemical, mechanical or thermal stimuli into neural signals (Kumazawa and Mizumura, 1977, Mense, 1993). Pain is a complex phenomenon; social, motivational, cognitive and emotional factors are also important features of a subjective pain experience (Sullivan et al., 1998, Moseley, 2004). Moreover, clinical musculoskeletal pain is usually associated with somatosensory changes such as mechanical hyperalgesia (increased mechanosensitivity) (Ohrbach and Gale, 1989, Treede et al., 1992, Sandberg et al., 2005).

A common practice to assess the pressure pain sensitivity is by manual palpation (Main and Watson, 1999), although this method is difficult to standardize (Greenspan and McGillis, 1991). Therefore, standardized measurements are needed in order to diagnose pressure-induced pain. One of the approaches to assess mechanical pain sensitivity is by pressure pain threshold (PPT) and pressure pain tolerance (PPTO); a pressure is applied and transmitted to the deep tissue through the skin and the subcutaneous tissue, activating the deep tissue nociceptors that are responsible for the pain sensation (Graven-Nielsen and Mense, 2001). A valid, reproducible tool to assess musculoskeletal pain sensitivity is the pressure algometer (Fischer, 1987). It has been used in several pain conditions such as the myofascial pain syndrome (Vanderweeen et al., 1996, Farella et al., 2000), tension type headache (Langemark et al., 1989), fibromyalgia (Carli et al., 2002, Maquet et al., 2004, Petzke et al., 2003), arthritis (Gerecz-Simon et al., 1989) and on distal limbs for neuropathic pain (Rolke et al., 2005). The pressure algometer is normally a force gauge with a well-defined probe area by which the pressure needed to evoke muscle pain can be recorded. In clinical and experimental settings 1.0 cm^2 probes with a flat, cylindrical tip have been recommended for measurement of PPT in muscle (Fischer, 1987) and are actually the most commercialized. Notwithstanding this, the scientific background behind the use of this kind of probes is lacking, and several factors may influence those measurements. They can be related to external factors like probe dimension and examiner skills (Greenspan and McGillis, 1991, Graven-Nielsen and Mense, 2001, Milne et al., 1988) or to intrinsic factors like tissue type (Rolke et al., 2005) and geometrical characteristics of the limb.

So far there is limited knowledge about how pressure is distributed in the tissue, but an interesting tool to explore this aspect is the finite element (FE) analysis (Takahashi et al., 2005, Takahashi and Mizumura, 2004). FE analysis is a method to express and solve differential equations for inhomogeneous structures by splitting into a finite number of elements with finite size. Nowadays, this method is implemented in computer software and among other applications it is used to describe the physical stress and strain in different structures. To date no studies have described the relation between structural mechanical properties, i.e. stress and strain, in tissues where the pressure stimulation is applied and related them to the pressure pain sensitivity.

1.a Aim of the Ph.D. project

The aim of this PhD project is to evaluate the mechanical changes of the deep tissues below the stimulation probe during pressure pain assessments. Changes of pressure-induced pain and stress/strain distribution has been evaluated in relation to 1) extrinsic and 2) intrinsic factors. (figure 1). A computer simulation model was developed and verified by human experimental pressure algometry data.

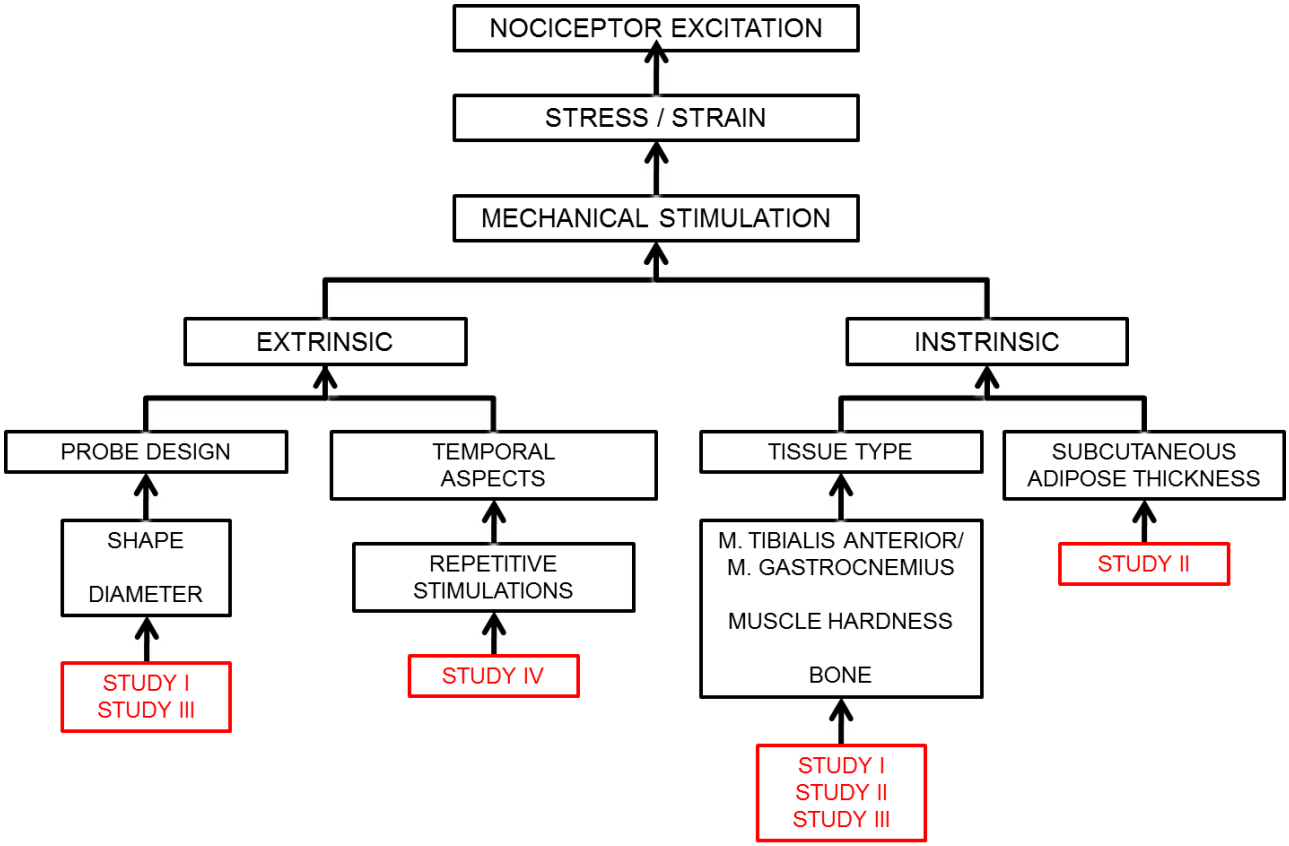

Figure 1. Schematic representation of the PhD project investigating mechanical changes of the deep tissues below the stimulation probe during pressure pain assessment.

2. Musculoskeletal pain physiology

The sensory receptors that respond to potentially damaging stimuli are the nociceptors. They mediate information to the spinal cord and brain that may cause the perception of pain. The existence of nociceptors was at first suggested by Charles Sherrington in 1906 (Sherrington, 1906); they are found in any area of the body from which pain can be perceived either externally or internally, such as skin, mucosa, muscle, join and viscera. In the deep tissue, nociceptive afferents are found as free nerve endings (Stacey, 1969) and are polymodal, meaning that they respond to thermal, chemical and/or mechanical stimulation (Kumazawa and Mizumura, 1977). Free nerve endings are unmyelinated axon terminals that are surrounded by a single layer of Schwann cell covering, except for the receptive areas, which are free of Schwann cell processes (Torebjörk, 1974, Mense, 1981) and have direct contact with the interstitial fluid. The cell bodies of these neurons are located in either the dorsal root ganglia or the trigeminal ganglia. The trigeminal ganglia are specialized nerves for the orofacial area, whereas the dorsal root ganglia associate with the rest of the body. The axons extend into the peripheral nervous system and terminate in branches to form receptive fields. In the dorsal horn of the spinal cord mainly lamina I and III-IV convey the peripheral afferent activity (Hoheisel et al., 1989, Mense and Craig, 1988). Then, through the spinotalamic tract, the activity is conveyed to higher centres such as the thalamus (Craig et al., 1994) and the cortex (Coghill et al., 1994). This is the principal process, however the nociceptive activity can be facilitated or inhibited by a number of supraspinal structures and mechanism utilizing descending pathways (Gebhart, 2004). Peripherally, nociceptors are usually divided in two different groups, thin myelinated (group III or Aδ) and unmyelinated (group IV or C) fibers. Studies in cats have detected unmyelinited afferent units that have receptive fields in two different tissues. Some units have one receptive field in deep somatic tissue (muscle, joint and periosteum) and another in the skin distal from the deep receptive field (Mense, 1981). Physiologically, group III and group IV fibers are differentiated on the basis of conduction velocity, 2.5-35 m/s for group III fibers and less than 2.5 m/s for group IV fibers (Graven-Nielsen and Mense, 2001, Djouhri and Lawson, 2004). Microneurographic recordings in volunteers have shown that those responses of human muscle nociceptors are similar to those of nociceptors in rats or cats (Marchettini et al., 1996). The pain associated with group III fibers can be associated to an initial sharp pain. The second phase is a more prolonged and slightly less intense feeling of pain as a result of group IV activity (Shibata et al., 1989). If there is massive or prolonged input to a group IV fiber by repetitive stimulations with constant stimulus intensity, there is progressive build up in the spinal cord dorsal horn, showing a phenomenon of neuronal wind-up (Mendell, 1966). A human correlate of wind up is temporal summation, which presents as increased pain perception in response to repetitive stimulation

of the same intensity. Both central and peripheral mechanisms seem to be involved. In fact, it is often difficult to distinguish between fast sensitization of peripheral muscle nociceptors (peripheral sensitization) and that of central neurons (central sensitization) (Nie et al., 2005). The latter is an important factor for the transition from acute to chronic pain and is therefore of importance for both basic research and clinical treatment (Graven-Nielsen and Arendt-Nielsen, 2010).

3. Deep somatic pain assessment

Manual palpation was traditionally the only method used to assess musculoskeletal pain hypersensivity (Jensen et al., 1992, Bendtsen et al., 1994). It is used to assess tenderness through tissue deformation (e.g. provoking pain with pressure or stretching), to determine painful areas and to qualify pain felt by patients. However, this method has many limitations. Studies on inter-examiner reliability related to manual palpation report poor reliability (Levoska, 1993, Maher and Adams, 1994) and many variations of manual techniques are possible (Hulet, 1972, Bendtsen et al., 1994, Simons et al., 1999). Manual palpation is not an objective measurement, but some standards are needed in order to compare different examiners, studies, and to evaluate treatment strategies (Isselée et al., 1997). In parallel, more advances devices, as the palpometer, which allows the measurement of pressure exerted during palpation, have been created (Bendtsen et al., 1994). Nevertheless, the best method to estimate tissue sensitivity thresholds in normal and sensitized muscles is the mechanical pressure stimulation (Arendt-Nielsen, 1997) and the instrument used to do so is the algometer. A pressure algometer is usually a force gauge with a well-defined probe area by which the pressure needed to evoke muscle pain can be recorded and can be manual or computer-controlled. Manual algometry has good inter-rater reliability, but measurement results can be biased by different examiners (Fischer, 1987, Antonaci et al., 1998). Hand-held algometers have been used extensively in clinics to study changes in the pressure-pain threshold and/or pressure-pain tolerance in e.g. fibromyalgia (Granges and Littlejohn, 1993), osteoarthritis (Wessel, 1995), headache (Bovim, 1992), reflex sympathetic dystrophy (Bryan et al., 1991), whiplash (Kasch et al., 2001), and in the early postpartum period (Shapira et al., 1995). Control of compression rate, area and localization during hand-held algometry are important for reliable assessment of pressure-pain thresholds (Brennum et al., 1989), but as the compression rate is adjusted by visual feedback, it may be difficult to maintain. An alternative to the handheld algometer, with the inherent variability related to manual pressure application, is the computer-controlled pressure algometer, where the pressure probe is attached to a mechanical construction and inclination angle and rate of pressure is computer controlled. Computerized pressure algometry excludes manual involvement of a researcher and guarantees stable stimulus configuration thus removing the inter-examiner part of the overall data variability. In addition, the body part assessed is stabilized using a kapok-filled vacuum cushion, in order to limit the body's movement during pain assessment. Moreover, the computer controlled pressure algometer allows to record pain intensity continuously on a visual analogue scale (VAS) and to estimate the stimulus-response function between pressure and pain intensity (figure 2). The actual relation between excitation of deep tissue

nociceptors and mechanical stimulation are not known. Likewise, the pressure distribution below a stimulation probe has never been estimated.

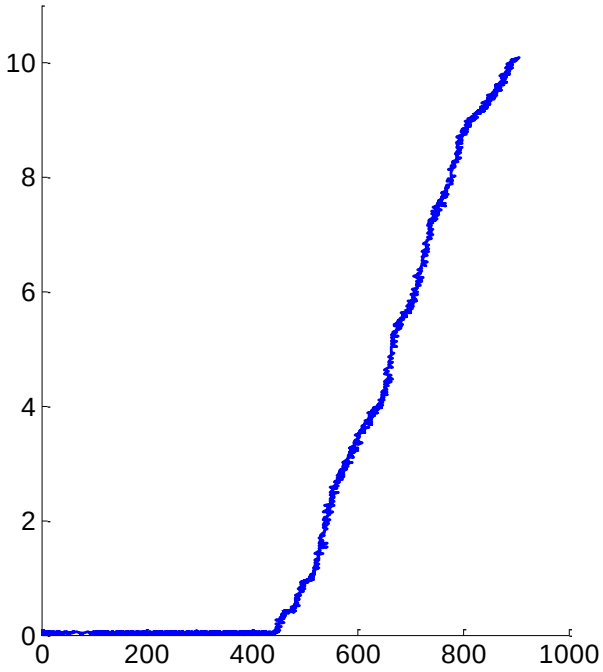

Figure 2: Stimulus-response function between pressure and pain intensity in one subject. The pain intensity is continuously recorded with an electronic visual analogue scale (VAS) and increases after pressure pain threshold. The maximum value of VAS is 10 and it is in correspondence to the pressure pain tolerance. Data taken from study I.

Other devices that can be used to quantify deep-tissue pain sensitivity are the pneumatic cuffs, which allow the pain assessment on a large volume of tissue. Pneumatic cuffs are widely used in medicine for indirect arterial pressure measurement and during surgery. Cuff pressure, intra-arterial pressure under the cuff, and therefore tissue pressure under the cuff, are directly related (Ernst and Matrai, 1987, Van Egmond et al., 1985). A cuff pressure algometry has also been developed (Polianskis et al., 2001). This technique is a reliable method to assess muscle sensitivity by the pressure-pain function in a large volume of tissue.

4. Human tissue mechanics

The mechanical properties of human tissue are complex, depending on e.g. age and gender. Human tissue is inhomogeneous, anisotropic and multi-layered and its mechanical behavior is described by a nonlinear load-deformation relationship (Fung, 1993, Daly and Odland, 1979). This means that human tissue mechanical properties differ according to the direction of measurement and energy is dissipated, usually by heating, when a load is applied.

4.a Stress and strain

In continuum mechanics, stress is a measure of the average force per unit area of a surface within a deformable body on which internal forces act (Spencer, 1980). These internal forces are produced between the particles in the body as a reaction to external forces applied on the body. External forces are either surface forces or inside forces. Because the loaded deformable body is assumed as a continuum, these internal forces are distributed continuously within the volume of the material body, i.e. the stress distribution in the body is expressed as a piecewise continuous function of space coordinates and time. The SI unit for stress is pascal (Pa), which is equivalent to one newton (force) per square meter (unit area). The unit for stress is the same as that of pressure, which is also a measure of force per unit area. In fact, pressure is the mathematically trivial case that describes a state where the stresses along the three Cartesian axes are equivalent in magnitude and the shear terms are zero.

Strain is the geometrical measure of deformation representing the relative displacement between particles in the material body (figure 3). It measures how much a given displacement differs locally from a rigid-body displacement. A strain is a normalized measure of deformation representing the displacement between particles in the body relative to a reference length and so is a dimensionless quantity, usually expressed as a decimal fraction or a percentage of the reference length (Spencer, 1980).

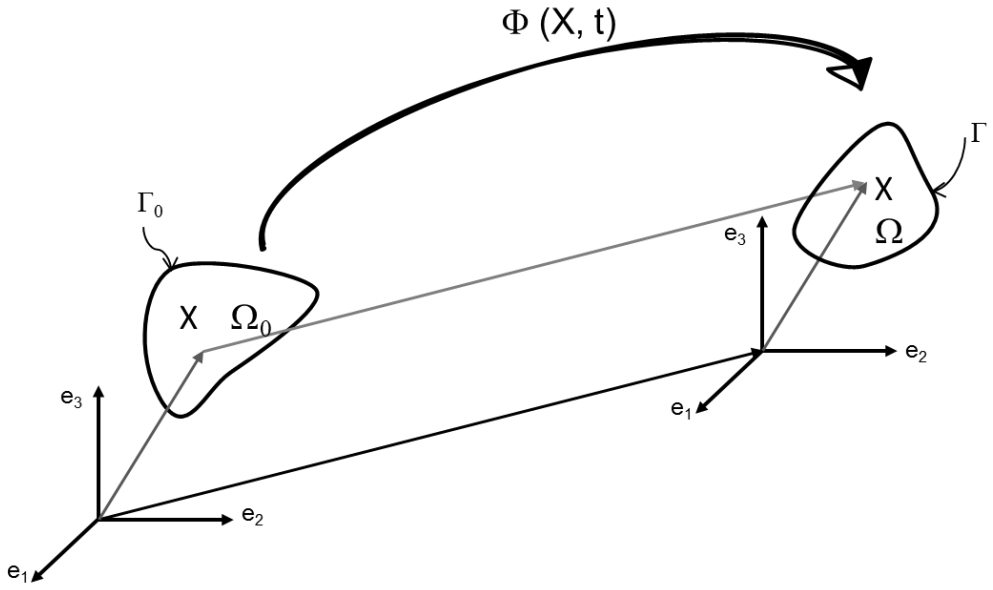

Figure 3: Motion of a continuum body. A continuum is a body that can be continually sub-divided into infinitesimal elements with properties being those of the bulk material. Undeformed (or reference) configuration of the continuum body is Ω_0 with boundary Γ_0, while the deformed (or current) configuration is Ω with boundary Γ. X is a generic infinitesimal element inside the continuum body. Φ (X, t) is the motion that takes the body from the reference to the current configuration. This motion is constituted by two components: A rigid-body displacement and a deformation. A rigid-body displacement consists of a simultaneous translation and rotation of the body without changing its shape or size. Deformation implies the change in shape and/or size of the body from an initial or undeformed configuration Ω_0 to a current or deformed configuration Ω.

This deformation could be resulting by elongation, shortening, volume changes, or angular distortion. In studies I to IV the stress and strain considered are the principal ones. Principal stresses or principal strains are the components of the tensor when the basis is changed in such a way that the shear components become zero (Shanley, 1967) (figure 4).

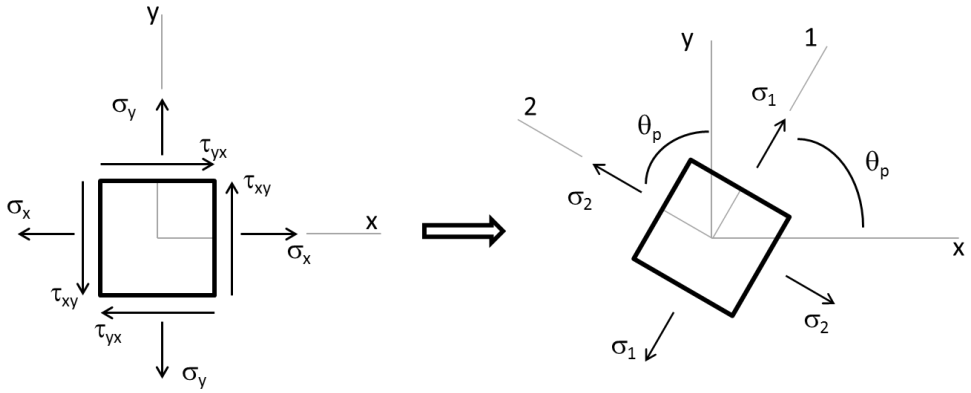

Figure 4: Principal stresses in 2D. Normal stresses σ are perpendicular to the surface of the body. Shear stresses τ are parallel to the surface of the body and exist when the shape of a material tends to change (usually by transversely-acting forces). The angle θ_p, at which the shear stresses τ_{xy} or τ_{yx} are equal to 0, allow to find the principal stresses (also called maximal normal stresses) σ_1 and σ_2.

4.b Biomaterials

Different materials models have been proposed to model the behaviour of human tissues. It is known from in-vitro and in-vivo studies that the skin, the subcutaneous adipose tissue and the muscle tissue are non-linear elastic material, so the stress and strain relationship is not a constant. Soft tissues are subject to large deformations and this elastic behaviour is necessarily nonlinear. Different models have been proposed. Weiss et al. (1996) proposed a transverse isotropic material to describe a three dimensional model of biological soft tissues and its finite element implementation for fully incompressible materials. Miller (1999) proposed a constitutive model of brain tissue using a viscoelastic material. Viscoelastic materials lose energy when a load is applied and consequently exhibit a time dependent strain. Notwithstanding this, rubber-like, NeoHookean hyperelastic materials have been shown to be the more suitable approximation (Tran et al., 2007), as their stress-strain curves are typical for materials with fibers; large strains for small stresses in conditions where tangled fibers are aligned, but larger stresses are required to achieve much higher strains where the already aligned fibers are stretched (Ceelen et al., 2008; Cherubini et al., 2008). Specifically, these materials are a special case of a Cauchy elastic material and their stress-strain relationship can be defined as non-linearly elastic. These materials are characterized by a relative low elastic modulus and high bulk modulus, are commonly subjected to large strains and deformations and are almost incompressible (Ogden, 1997). Mathematically, a material is said to be hyperelastic if there exists elastic potential, called strain energy density function that is a scalar function of one of the strain or deformation tensors, whose derivative with respect to a strain component determines the corresponding stress component.

$$\boldsymbol{S_{ij}} = \partial W / \partial \varepsilon_{ij} \tag{1}$$

Where S_{ij} is the stress tensor, W is the strain energy density function and ε_{ij} is the strain tensor. Different hyperelastic models have been proposed. In this project the Neo-Hookean model has been used with a strain energy density relationship defined as

$$\boldsymbol{W} = \frac{1}{2}\mu(I_1 - 1) + \frac{1}{2}K(J-1)^2 \tag{2}$$

where I_1 is the first invariant of the strain tensor and J is the volumetric ratio, μ is the shear modulus and K is the bulk modulus. The constants used for the skin, the subcutaneous adipose and the muscle tissue are shown in table 1.

	Bulk modulus (K) [kPa]	Shear modulus (μ) [kPa]
Skin tissue	30000	2000
Subcutaneous adipose tissue	36	1
Muscle tissue	92.8	5.952

Table 1. Neo-hookean materials moduli of three tissues proposed to model the behavior of human tissues, adapted on data from Tran et al. (2007).

4.c Finite Element method

The finite element method (FE) is a computer modelling technique widely used for understanding and experimenting with the mechanics of physical systems. It is used to find approximate solutions of partial differential equations as well as of integral equations. The solution approach is based either on solving the differential equation completely, or rendering the partial differential equations into an approximating system of ordinary ones, which are then numerically integrated. In solving partial differential equations, the primary challenge is to create an equation that approximates the equation to be studied, but is numerically stable, meaning that errors in the input and intermediate calculations do not accumulate and cause the resulting output to be meaningless. By developing a finite element model of a certain tissue it is possible to assess the propagation of pressure stimuli through this tissue, both to estimate the actual pressure inside the deep tissue such as the potential for excitation of the nociceptors and to relate the perception of muscle pain to structural mechanics variables, i.e. the stress and strain propagation within the tissues below a stimulation probe. This is done to understand which structures are predominantly stimulated by pressure stimulation.

4.d Model proposed

A FE model has been developed in order to assess the stress and strain distribution during pressure algometry measurements. Five main phases are needed to develop such a model

- Discretization: the problem domain is discretized into a collection of simple geometric objects. In this project, a transverse magnetic resonance image (MRI) (figure 5A) has been segmented using a custom-made graphical user interface using COMSOL scripting and MATLAB (Mathworks, USA). The

segmented tissue layers were imported in COMSOL (figure 5B) and extruded in the direction of the tibia axis on a length varying from 8 cm (study I) to 5 cm (study II, III and IV).

- Geometry meshing: a mesh is a collection of vertices, edges and faces that defines the shape of an object. In this project, a 2-dimensional mesh with triangular elements was at first created. The mesh was refined in the contact area with the probe, with an element size of 1 mm in the skin and subcutaneous adipose tissues, and 5 mm in the muscle tissue. The 2-dimentional mesh was then swept in the longitudinal direction creating a 3-dimentional tetrahedral mesh (figure 5C); the total number of elements was between 20000 and 70000 and the number of degrees of freedom was between 54000 and 280000 (study I, II, III and IV). For every model, convergence tests show that increased mesh densities do not improve significantly the geometry, loading and constrain parameters.

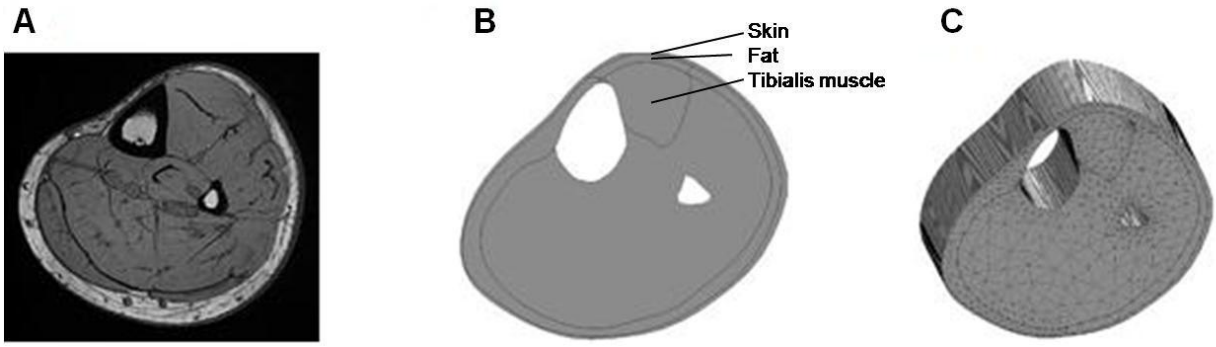

Fig. 5. The finite element (FE) model. (A) MR image of the calf, transverse plane, medial point. Three different layers are segmented, skin tissue, subcutaneous adipose tissue and tibialis muscle tissue. (B) FE model in two dimensions, transversal section. (C) Three dimensional FE meshed model. Data taken from study I.

- Develop element equations and assembly: the element equations are developed using the physics of the problem and assembled into a set of global equations that model the properties of the entire system. The element equations used are the ones for structural mechanics problems, dedicated to the analysis of components where it is necessary to evaluate deformations under loads.

  The material behaviour for the different tissues in the model was assumed to be homogeneous hyperelastic, slightly compressible through the NeoHookean material (see section 4b), whereas the bones were assumed to be rigid elements which could not deform. Periosteum and fascia are not geometrically modelled, but they are considered as the interface between the bone and the subcutaneous adipose tissue or between the muscle and subcutaneous adipose tissue.

- Application of boundary conditions. A solution cannot be obtained unless boundary conditions are applied. They specify the behaviour of the solution not only at boundary, but impose modifications at the global equations. In this project, all nodes of the bones were fixed, while the others were left free resulting in non-constricted boundary conditions. In this way the bone was considered as a rigid and uncompressible element. The probe was fixed in 2 directions and allowed to move in the direction orthogonal to the skin surface. A contact surface between the tip of the probe (*master boundary*) and the skin (*slave boundary*) was defined and a contact penalty factor was set constant and defined as the ratio between a given stiffness (1 GPa), and the maximum element size in the contact surface (1.6 mm), a bit bigger than the element size, in order to prevent geometrical errors in the computation. This procedure minimizes the error, prevents the slave to penetrate the master and allows an accurate but relative fast solution.
- Solving: the modified global equations are solved. Different simulations were performed combining simulation sites (muscle, near-bone, and bone), probe diameters (5, 10, 15 mm), probe design (flat, rounded), subcutaneous adipose thickness and muscle hardness (percentage of maximal voluntary contraction). The solver was linear parametric, with probe displacement step of 0.6 mm on the axis perpendicular to the skin. A UNIX multicore server (8x2.6 GHz Dual Core AMD Opteron) and 4 to 6 processors in parallel were used.

In order to validate the model, human experiments have been performed. The pressure indentation curve obtained from the FE model and the data recorded by computer-controlled pressure-induced muscle pain in healthy subjects was compared (figure 6). The experimental pressure-indentation curve fitted the outcome of the FE model with good accuracy; e.g. with 10 mm diameter flat probe, the linear regression coefficient (R^2) is 0.96 or with a 0.03 cm^2 (3 mm diameter) flat probe the two way mixed intra-class correlation coefficient (ICC) was 0.85. A similar agreement was present for all the other simulations, showing good reliability between the experimental and model data (study I, II, III, IV).

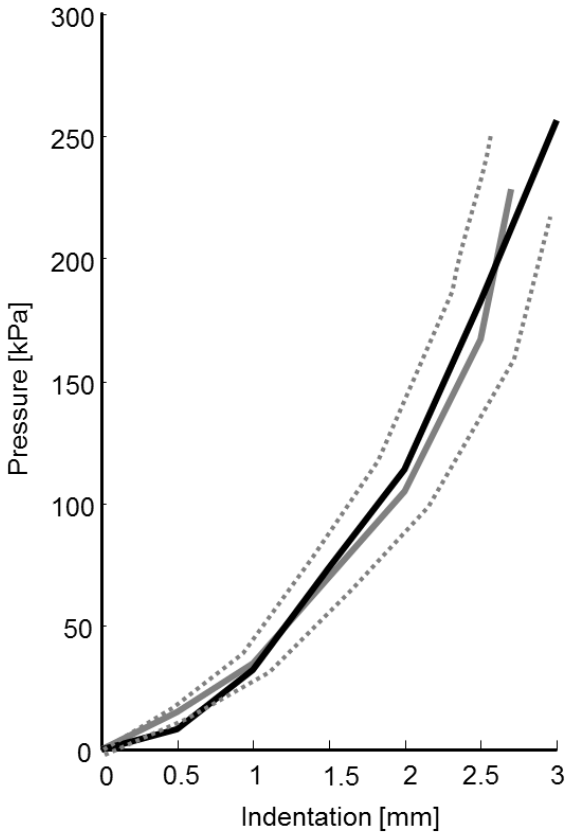

Figure 6: Pressure-indentation response curve for the simulation model and experimental data. The tissue indentation (displacement) is shown on the x-axis and the pressure intensity (kPa) on the y-axis. The solid grey line indicates the mean curve for experimental data, while the solid black line is from the simulation model. The grey dotted lines indicate the curve interval of the experimental data. Data taken from study III.

5. Tissue mechanics during pressure algometry

A critical question, not addressed in previous investigations, is what is mechanically quantitatively relevant in order to excite muscle nociceptors during pressure pain stimulation? This question has gone unanswered largely because of the difficulty in quantifying the mechanical state that develops in the deep tissue at the receptor ending during pressure stimulation. As addressed before, the skeletal muscle is an inhomogeneous and anisotropic tissue (Fung, 1993) and the receptive endings of mechanoreceptors are principally located in the collagenous extracellular matrix (especially in the perimysium) near vascular tissue (Reinert et al., 1998). The physiological principles that lead to nociceptor excitation are also still not fully clear (Mense, 1993), but a mechanical stimulation evoking pain from deep structures has to be related to the stress and strain distribution within the deep somatic structures. Excitation of a peripheral nociceptor by an adequate stimulus depends upon the presence of specific ion channels and receptors in the membrane of the receptive ending. Some nociceptors have cationic channels that are gated by mechanical loads, but the sensitivity to gating can be influenced by various ligands, e.g. bradykinin or prostaglandin and ATP (Mense, 1981, Berberich et al., 1988, Cairns et al., 2003, Hoheisel et al., 2004). The detailed mechanical state (characterized by stress and strain) that is relevant to activate mechanoreceptors during noxious muscle pressure is still unknown (Marchettini et al., 1996, Simone and Kajander, 1997), because of the difficulty in quantifying the "in vivo" mechanical state that develops at the receptor ending during loading. The present project has shown that muscle mechanonociceptors stimulated by external pressure indentation do not experience the externally applied load (i.e. force or displacement by themselves). Rather those receptors experience internally developed stress, related to the distribution of force, or local strain, which is related to the relative displacement. The role of strain was already outlined in animal studies (Paintal, 1960, Iggo, 1961), where group III and IV feline muscle afferents were stimulated and the dominant response of group III afferent found to be to compression. Particularly, skin evoked pain seems to be mainly related to the stress and this is in line with previous findings on a variety of other mechanically sensitive afferents: Cutaneous slowly adapting type I (Ge and Khalsa, 2002) and type II mechanoreceptors (Grigg, 1996) and cutaneous Aδ and C mechano-nociceptors (Khalsa et al., 1997). On the contrary, during painful pressure stimulation, more strain is distributed in deep tissue with the 10 mm probe instead of the 5 mm probe, indicating that deep tissue mechanonociceptors encode strain rather than displacement or stress (figure 7), this means that a specific tissue deformations is responsible of the pain sensation. The peak strain is distributed in the interface zone between the subcutaneous adipose tissue and the muscle tissue, more likely the fascia, a layer of connective tissue surrounding every muscle. In pressure-induced pain, the role of the fascia and more generally of "loose" connective tissue has already been suggested (Graven-Nielsen et al., 2004, Gibson et al., 2009).

Graven-Nielsen et al. (2004) addressed that receptors in the superficial parts of the fascia might be block by subcutaneous lidocaine injection, while Gibson et al. (2009) suggested that the fascia, rather than muscle tissue, is the main tissue adequate for pain evoked by mechanical stimulation. As a consequence the painful sensation due to pressure stimulation is most likely originated from fascia or superficial muscle and conducted via group III and IV afferent fibres.

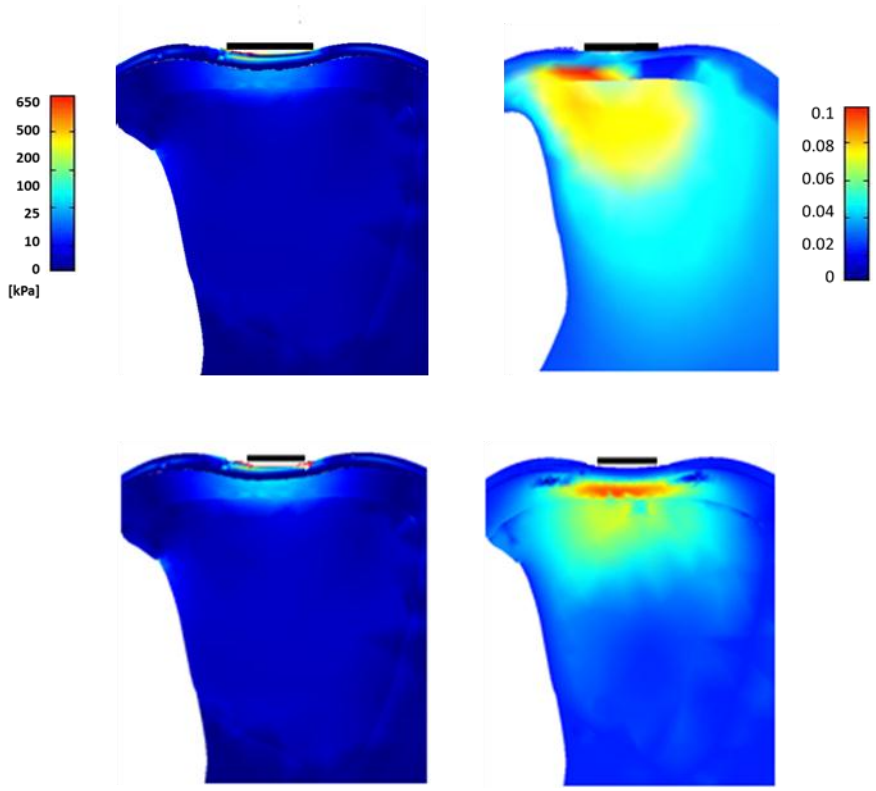

Figure 7: A cross sectional image of principal stress (left) and principal strain (right) distribution in the medial-lateral plane for two flat probes (1.0 cm, first row, and 0.5 cm diameter, second row) during stimulation equivalent to the pressure pain threshold. The colour coding shows that the stress profile seemed more flat and uniform while the strain profile usually had a clear maximum. The strain area increases in the superficial muscle tissue in relation to the probe's diameter. Data taken from study I.

6. Aspects influencing pressure pain measurements

Nociceptors may be activated by mechanical stimulation, but different factors can influence the distribution of stress and strain and thus the perception of pain during pressure pain assessments. The extrinsic factors, independent of the anatomy or tissue condition of the subjects are e.g. probe design and examiner skills (Reeves et al., 1986, Persson et al., 2004, Brennum et al., 1989). The intrinsic are related to the material and geometrical characteristics of the tissue assessed.

6.a Probe design

Probe shape and diameter are crucial factors in pressure pain assessment. Traditionally, the classical probes used in clinical studies have a contact area of 1 cm^2 and are flat, usually covered by a rubber disk in order to minimize the sharp contact edges, in line with the Fischer algesiometer (Fischer, 1987, Offenbächer and Stucki, 2000) which has been recommended for measurement of the muscle pain threshold. This kind of probe has been used in a large variety of studies e.g. to define the correlation between clinical pain intensity and duration in tempomandibular disorder patients (Svensson et al., 2001), to study the abdominal pressure pain threshold in children (Duarte et al., 2000), to evaluate tender points in shoulder muscles (Vanderweeen et al., 1996).

It has been suggested that the use of small probes (0.01-0.49 mm) is activating mainly intraepidermal nerve endings and has little effect on nociceptors in the deep tissue (Treede et al., 2002). Accordingly, Takahashi (2005) suggested that larger probes can give a better estimation of muscular pain threshold. Moreover, the sensation of sharpness that is usually felt on the interface between the probe and the skin was shown to be mediated by superficial tissue's nociceptors (Greenspan and McGillis, 1991). Another study addresses the quality of pain evoked by pressure stimulation; with the large area probe (> 1 cm^2) it was perceived as a pressure whereas with small area probes it was as a prick (Defrin et al., 2003). Other probe designs, such as rounded probes (Nordahl and Kopp, 2003), have been suggested to be more efficient. Nevertheless the mechanical stimulation efficacy in muscle tissue has not still been evaluated to show that it is superior to the conventional probe design.

Study I shows that in muscle tissue, the strain and consequently the nociceptors' excitation at a specified indentation (7 mm) seem most efficiently induced by large rounded probes compared with smaller and flatter ones (figure 8). At 7 mm indentation, VAS scores were between 5.1 and 6.6 in all conditions for all subjects.

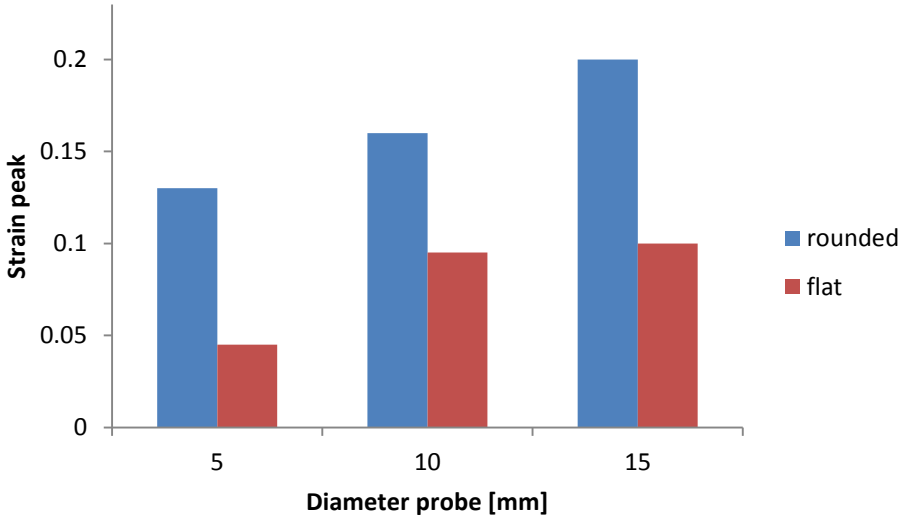

Figure 8: Peak principal strain in the superficial muscle tissue at 7 mm indentation on the tibialis anterior muscle with 6 different probes: 5 mm, 10 mm and 15 mm diameter, rounded or flat. Data taken from study I.

Moreover, the FE model suggests a strong relation of the strain to the depth of stimulation, larger probes deform a bigger portion of the deep muscle, while smaller ones mainly deform the superficial part of the tibialis anterior muscle (study I). On the contrary, in study III it is shown that a small probe (diam: 0.2 cm, area: 0.03 cm^2) compared to a large probe (diam: 1.1 cm, area: 1.0 cm^2) is better to elicit bone periost pain, at least for a superficial bone as the tibia, that is not covered with muscle, but only skin tissue and subcutaneous adipose tissue. Accordingly, in a previous study (Brennum et al., 1989) a small diameter probe of 0.28 cm^2 was used to evaluate difference in pressure pain threshold between males and females on fingers and toes, but arguments for the use of the probe were not provided; probably such a small contact area was suitable for the measurement on those small areas. The same may be argued for a later study (Slater et al., 2003), where a probe of 0.5 cm^2 was used on the radio-ulnar joint.

In addition, rounded or semi-rounded probes, without sharp edges, help to reduce the shear strains in the skin (figure 9). Bishop (1948) identified already the importance of edges on a stimulating probe, addressing that flat probes stimulate mainly the sharp corners. The same was stated by Nordhal and Kopp (2003). Rounded probes may also minimize the deformation in the epidermis and enable a preferential activation of deep afferents (Treede et al., 2002). In study I, the PPT measured with the rounded probe and the conventional probe showed similar degrees of inter individual variation and reproducibility. The complete adhesion between probe and skin tissue with the rounded probe seem to favour deep tissue strain; it is almost double for the rounded probe, showing a generally higher strain distribution in the muscle.

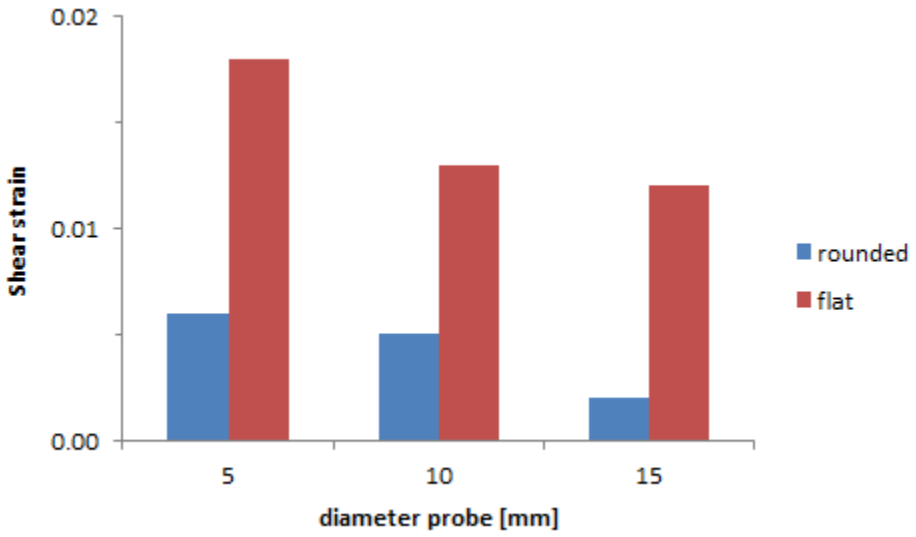

Figure 9: shear strain on the contact area between probe and skin. Rounded probes show smaller shear strain in comparison to flat ones. Data taken from study I.

6.b Muscle hardness

Hardness is the characteristic of a solid material expressing its resistance to deformation due to an applied force and may affect the strain and stress during pressure stimulation. Muscle hardness has been defined as the straightening part of the pressure-indentation curve (Horikawa et al., 1993). The initial part of the indentation–pressure curve with a small slope has previously been defined as the hardness of skin and subcutaneous tissue and the portion where the curve straightened has been defined as the hardness of muscle tissue (figure 10). Clinically, it is known that the hardness of soft tissues changes in pathologic situations, such as muscle damage (Gerwin, 2005), edema, degeneration, fibrosis and cancer (Mridha and Ödman, 1986, Garra et al., 1997). Experimentally, muscle hardness has been shown to increase with specific exercises as eccentric contraction of the tibialis anterior muscle (Andersen et al., 2006) or eccentric exercise performed with triceps surae (Murayama et al., 2000). Thus, it seems that various exercises can influence muscle hardness, but the effect on pressure distribution is not fully clarified.

Muscle hardness effect the pressure-induced pain sensation in muscle, influencing the transmission of externally applied pressure. Clinically, muscle hardness has been associated with muscle hyperalgesia, where pericranial muscles were significantly harder in patients with chronic tension-type headache compared with healthy controls (Ashina et al., 1999, Sakai et al., 1995). Moreover, in the trapezius muscle, increased muscle hardness was identified after 15 to 30 minutes of computer work, when the angle of the computer screen was in an unusual position for the head and neck during the working task (Horikawa, 2001).

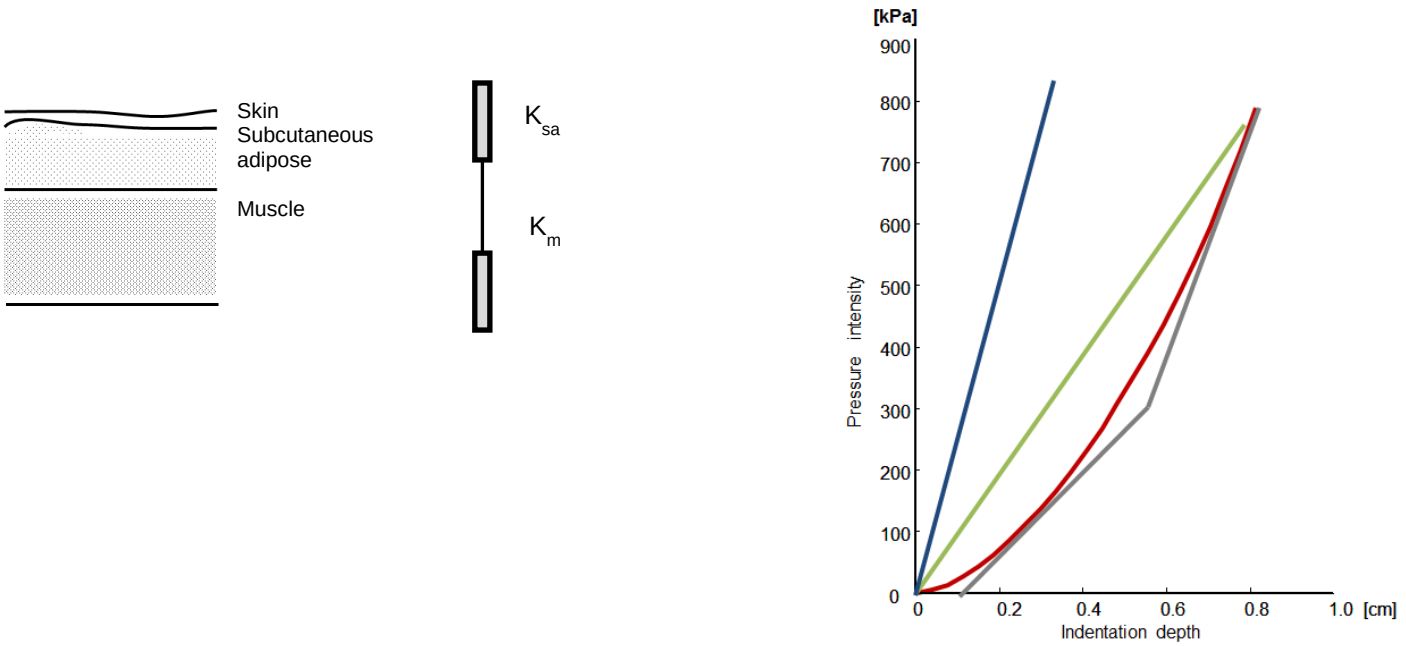

Figure 10: Pressure indentation curve of one subject in relation to pressure pain intensity on the tibialis anterior muscle (in red). The tissues can be simulated in a 2 layer spring model where the 2 elastic constant are the hardnesses (on the left, K_{sa} = skin + adipose hardness, K_m = muscle hardness). The blue line express the hardness of the muscle and his function is $P_m(ind) = K_m * ind + d$, while the green line is the hardness of skin/subcutaneous tissue and his function is $P_{sa}(ind) = K_{sa} * K_m / (K_{sa} + K_m) * ind$. Data taken from study II.

Study II shows that the hardness is increased by isometric contraction of tibialis anterior muscle, which was used to mimic increased muscle hardness. Furthermore, it was shown that harder muscles present lower strain peak in muscle (study II) and, as a consequence, higher pressure stimulation intensity are required to reach the pain threshold. This is in accordance with previous studies where pressure pain thresholds were found to increase during isometric contraction (Kosek and Ekholm, 1995).

The FE model provides an additional explanation to the relation between hardness and pain sensitivity, a harder muscle leads to a lower strain, making difficult to stimulate intramuscular mechano-nociceptors, suggesting that more pressure is needed to evoke pain. The model shows also that in a harder muscle, only superficial part of the muscle is actually strained, suggesting that probably the mechano-nociceptors are located in that area, or alternatively, in the fascia layer, the connective tissue surrounding muscles. So, nociceptors are excited differently in relation to the tissue characteristics as muscle hardness. This may be clinically relevant, so measurements and comparison between groups of subjects have to been cautiously done.

6.c Subcutaneous adipose thickness

The role of subcutaneous adipose tissue thickness in pressure-induced pain assessment is not completely clear. The literature related to this topic is also inconsistent. In obese people who were exposed to pain stimulation to determine pain threshold, an increased pressure pain threshold was observed (Zahorska-Markiewicz et al., 1983, Khimich, 1997). Contrarily, the studies using electrical stimulation reported lower pain threshold in obese compared to normal subjects, which indicates a negative correlation between degree of overweight and the nociceptive threshold (Roane and Porter, 1986, McKendall and Haier, 1983). A relationship between the endogenous opioids, nociception and obesity or eating behavior has been suggested, even if the mechanisms are unclear (McKendall and Haier, 1983). Moreover, it is known that other factors, such as muscle hardness, depend on the thickness of the soft tissues (Lu et al., 2005). Increased thickness in uniform tissue-mimicking phantoms will lead to greater deformations and potentially greater stresses and strains acting on the tissue.

In study II, the PPTs were shown not to be significantly different between subjects with normal and thick adipose tissue showing only a tendency for reduced pain sensitivity in the group with the thickest adipose tissue, in accordance with the FE model where the strain in muscle tissue was comparable in the two conditions. However, a simulation of a very thick adipose tissue indicated a reduced strain in muscle tissue, but no experimental data were available to validate this finding. Probably the magnitude of subcutaneous adipose tissue is an important factor for differentiating the strain distribution in muscle. The clinical implication for assessment of the tibialis anterior muscle may be limited at the fact that it rarely shows a thickness superior to 7 mm. A previous study (Takahashi et al., 2004) showed that the same pressure caused different stress/strain propagation on brachioradialis muscle depending on the subcutaneous adipose thickness, but the thickness boundary between the 2 groups (normal and fat) was 9 mm.

In conclusion, the magnitude of transcutaneous pressure transmitted to the muscle is smaller in the cases with very thick subcutaneous tissue.

6.d Tissue type

Pressure algometry has been used to assess the pain sensitivity of different tissues as bone (Ashina et al., 1999, Brennum et al., 1989, Slater et al., 2003, Duarte et al., 2000), muscle (Defrin et al., 2003, Kosek et al.,

1999, Kosek et al., 1993, Svensson et al., 2001, Takahashi et al., 2005), fascia (Gibson et al., 2009) and tendon (Gibson et al., 2006, Slater et al., 2010, Kaya et al., 2007).

Previous studies on the comparison between pain responses to external pressure stimuli on bone and muscle present inconsistent results. Two studies (Slater et al., 2003, Torgén and Swerup, 2002) reported that PPTs over bone was lower than on the muscle, but Kosek et al. (1993) showed that pressure pain threshold was not significantly higher at the muscle site than at the bony sites. In another study (Rolke et al., 2005) the difference between tissues was only significant when testing the lower limb and differences between upper and lower limb were only significant when testing the nail bed and bony prominences, not different muscles.

The type of tissue over which PPT was measured had a significant effect on the PPT values obtained in the current project. In study I and III, stress and strain have been estimated in relation to pressure pain stimulation on tibialis anterior muscle (belly and next to the bone site) and tibia bone. PPTs were found to be lower at the bone (study III) and next-to-bone (study I) compared to the muscle belly site. The strain was found to be higher on the tibia bone (periost), in comparison to the muscle sites (figure 11), suggesting that the nociceptors in the periost are more sensitive to mechanically elicited pain.

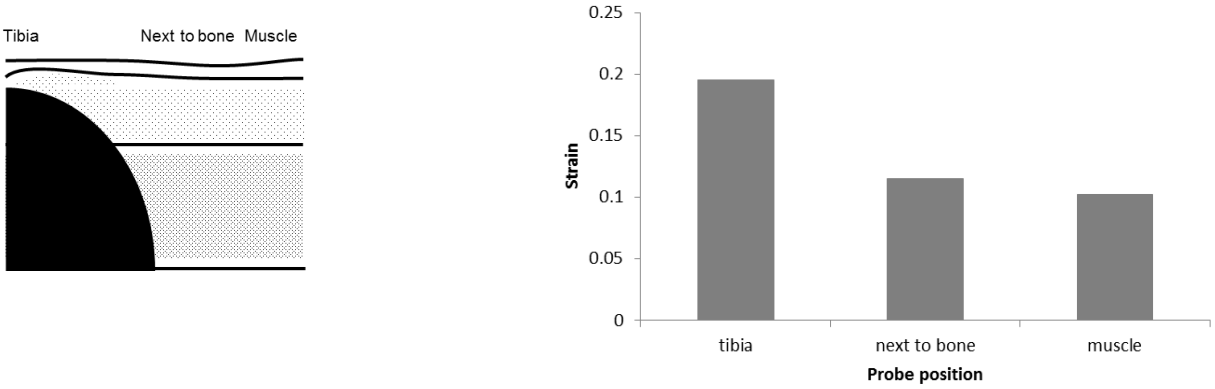

Figure 11: Graphical explanation of the three different probe positions: "muscle" on anterior tibialis muscle belly, "next to bone", 2.5 cm from the belly, "tibia", directly above the bone. On the right: Peak strain at PPT value for a 1 cm^2 flat diameter probe on the tibia bone, tibialis anterior muscle and a point in between, but still on the muscle. Data taken from study I and study III.

Bone presumably receives innervation from both group III and IV fibers, all of which could mediate sensory input from the periphery to the spinal cord (Mach et al., 2002). Both Lewis (1938) and Hockaday and Whitty (1967) showed that mechanical stimulation and injection of hypertonic saline into the region of the periosteum at the tibia bone cause pain. It is in fact believed that the sensation of pain due to pressure on bone depends primarily on the action of nociceptors in the periosteum, the membrane that covers the bones, and around joint surfaces, while areas such as the cortex and bone marrow are believed to be insensitive (Gennari et al., 1991). Moreover, periosteal tissue is known to be highly sensitive (Graven-Nielsen et al.,

1997) and has the greatest density of sensory innervation when compared to the marrow or mineralized bone (Mach et al., 2002).

In study I and study III, the strain was shown to be higher in the interface between subcutaneous adipose and muscle tissue in the tibialis anterior model, in correspondence of the fascia, which is a continuous structure that covers the muscle and continues further to form tendons (O'Brien, 1997). A previous study (Gibson et al., 2009) showed increased sensitivity to saline injection of muscle fascia compared to deep muscle tissue. Muscle fibers are force generators while the fascia is force transmitter. So this tissue may reflect the "warning" function of nociceptors; the fascia, with its elastic properties, responds with higher strains and contains receptors that responds optimally to pressures of high intensity (Sagada and Taguchi, 1971) in order to develop afferent feedback and minimize injuries.

In addition, Gibson et al., (2006) showed that hypertonic saline injections at the tendon and proximal tendon bone junction are more painful compared to the ones at muscle belly site, indicating that generally those sites are more sensitive and potentially have a higher nociceptive density. These findings address the role that connective tissues, such as fascia, periosteum and tendon, have on pain assessments. In fact, from a self-protection point of view, it is logical to suggest that these areas are more densely populated with nociceptors which could alert the system to potentially damaging situations.

The pressure pain sensitivity has been found different between muscles examined (Fisher, 1987). In that study, the most sensitive muscle was the upper trapezius, followed by the low back muscles. On the contrary, the lumbar paraspinalis and the gluteus medius muscle had the highest pressure threshold. Generally, the muscles situated in lower parts of the body were found characterized by a higher pressure pain threshold and were less sensitive (Fisher, 1987). These differences in pressure pain threshold, the expression of the pressure-pain sensitivity of individual muscles, were significant, but no mechanistic explanation was provided. Moreover, the structure of the muscle is also different in relation of the anatomical location. For example it is known that back muscles have a different structure in comparison to leg muscles, they are usually flat with an extensive fascia. In order to further investigate the difference in pain sensitivity among muscles, another study has been carried out (still unpublished). In brief, the pressure stimulation was applied perpendicular to the skin surface by hydraulic algometer which allows applying a force during magnetic resonance imaging. MRIs were carried out at 50% PPT and PPT (of a pre-measured pressure threshold). Preliminary results show that the compression in the muscle seems related to the muscle stimulated and not very deep structures (figure 12). At pressure pain threshold, on the tibialis anterior muscle, only the superficial part is deformed by the stimulation, while a wider and deeper part is compressed on the gastrocnemius. These differences can be related to variations in the properties and solicitation of the muscle.

For instance, it has been shown that the sarcomeres of the medial gastrocnemius are more stretchable than those of the tibialis anterior (Kurihashi et al., 2006). In human lower legs, ankle plantar flexors are composed of shorter muscle fibre bundles than ankle dorsiflexors and in tibialis anterior lengthening; ankle plantar flexors are more likely to sustain mechanical injury of muscle fibre bundles than ankle dorsiflexors (Friederich and Brand, 1990). Although no direct relation between hardness and sarcomeres' stretch has been reported, but considering that muscle hardness has been found increased with contraction (study II), it is possible to argue that a more stretchable muscle is also softer, while a less stretchable, eg. muscle tibialis anterior, is harder. Finally, another potential explanation of the difference in strain distribution between tibialis and gastrocnemius muscle might be a different fascia's thickness, although not validated.

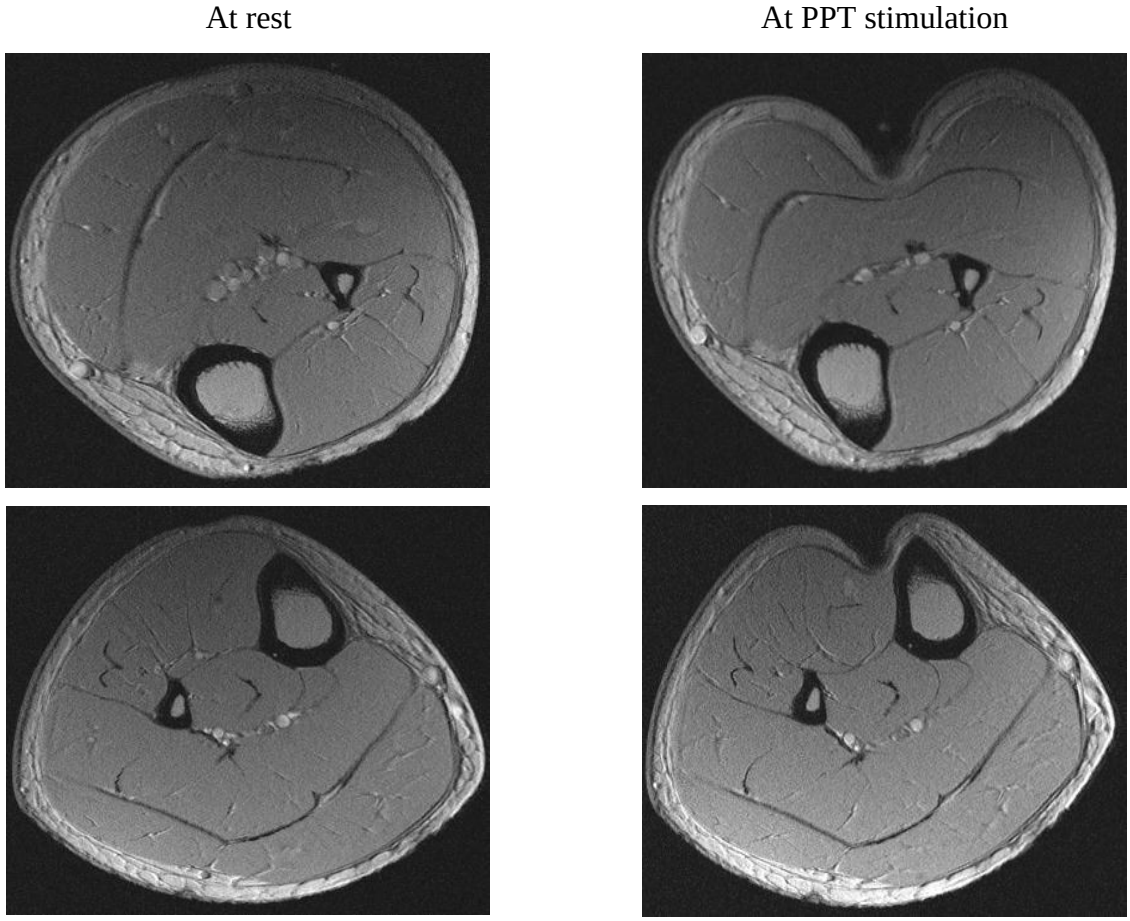

Figure 12: Magnetic resonance image (1.5 T, 28 mm) during pressure pain assessment using a specifically developed hydraulic pressure algometer. The images are recorded from a male subject at rest (left) and pressure pain threshold (right). On the first line, the pressure pain is assessed on the gastrocnemius muscle, while on the second line on tibialis anterior muscle. The PPT on tibialis anterior muscle was 338 kPa, while on gastrocnemius muscle was 401 kPa. Unpublished data.

6.e Temporal aspects

The phenomenon of windup of central neuronal activity, known as temporal summation in humans, is characterized by an increased pain perception to repetitive stimulation of the same intensity and is considered as one of the mechanisms facilitated in chronic pain (Graven-Nielsen and Arendt-Nielsens, 2010). The wind-up phenomenon is the increased neuronal firing of dorsal horn neurones to a train of stimuli and it is inhibited by an NMDA-antagonist (Dickenson and Sullivan, 1987). Temporal summation is considered the initial part of wind-up because repeated stimulation for at least 20 sec cause central hyper-excitability (Wall and Woolf, 1984), which is not seen after a short duration of temporal summation (Arendt-Nielsen et al., 2000). Moreover, both phenomena are inhibited by blocking the NMDA receptor (Dickenson and Sullivan, 1987).

In humans, repetitive mechanical pressure stimulations on muscle tissue with inter-stimulus intervals (ISI) of 1, 5, 10, or 30 s induce temporal summation of pain (Nie et al., 2005). Temporal summation of pain evoked by mechanical stimulation had frequency-dependent properties; in fact both central and peripheral mechanisms seem to be involved. Since the central temporal summation has frequency-dependent features, ISI longer than 3 s do not normally result in increased pain ratings (Vierck, 2006) while and inter-stimulus intervals of 3 to 5 s was found to facilitated temporal summation of pressure-induced pain in fibromyalgia patients compared with controls (Staud et al., 2001). Recently, facilitated temporal summation (2 s ISI) with pressure stimulation was reported in osteoarthritis pain patients compared with matched controls (Arendt-Nielsen et al., 2010). Nonetheless, peripheral sensitization of nociceptors in muscle is potential after sequential pressure stimulation and has been shown to be an important factor especially for repetitive stimulations at long ISI (30 s), although no frequency-dependent feature was found (Nie et al., 2005).

In study IV a model of repetitive pressure stimulations was investigated. This model mimics the temporal aspect exploiting the non-linear material properties of tissue; skin, subcutaneous adipose and muscle tissue have in fact a residual strain during stimulations at short ISI. As a result, the muscle strain slightly increases during repetitive stimulations, while the stress is invariant. This finding may then relate the temporal summation phenomenon to a simple peripheral sensitization or maintained stimulation. However, this small increase (18%) in peak principal strain during sequential stimulation doesn't have a practical influence on the pressure pain sensation, as the indentation depth didn't increase during repetitive stimulation, but the normalized VAS score increased from 0 to 3.7 (figure 13).

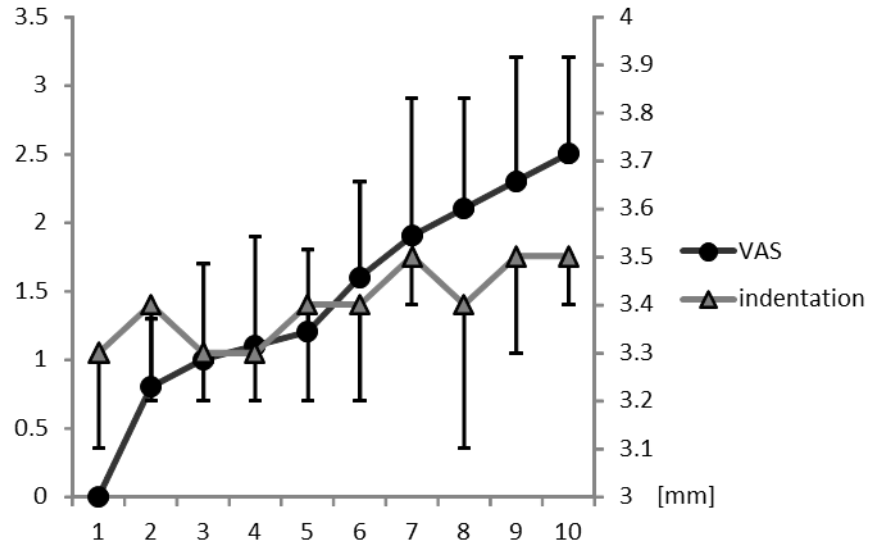

Figure 13: Mean (+/- SEM) indentation depth and mean VAS ratings (normalized to the first stimulus by subtraction) for repetitive stimulation at 3 s interstimulus interval (ISI). Data taken from study IV.

In addition, in order to have an increase of 2.5 cm in the pressure pain VAS scores during a single stimulation, the strain should increase by 47%. In addition, at the strongest stimulation intensity the corresponding average VAS score was 6.8 cm and the muscle strain needed was an increase of 85 % in comparison to the muscle strain at pressure pain threshold. Thus, the small increase (18%) in peak principal strain during sequential stimulation had not influence on the pressure pain sensation.

This may indicate that central mechanisms are, at least for small inter-stimulus intervals, involved in the temporal summation of deep tissues pain as found during the sequential pressure stimulation. The timing issue and thereby the temporal summation of the nociceptive primary afferent discharge is the crucial factor for indicating the magnitude of a pain sensation.

6.f Model Limitations

The limitations of the modelling approach are mainly related to the mechanical properties assigned to the model. The materials are considered homogeneous, but in reality they are slightly inhomogeneous (Fung, 1993, Kvistedal and Nielsen, 2004). However, these models take into account the hyper-elasticity and almost

incompressibility of the tissues. The simulation, especially for the flat probe, shows rough contact between probe and skin; this is due to the contact penalty factor but this indentation error in the simulation is less than 0.2 mm which is acceptable.

The model was validated for a homogenous group of subjects. The elastic properties of the tissues may however be changed in relation to age: previously, it was shown that young or elderly people have a less elasticity of the skin, related to the percentage of collagen (Cua et al., 1990).

Moreover, several studies suggest that females are more sensitive to experimental painful stimuli than males (Fillingim and Maixner, 1995, Berkley, 1997, Dao and LeResche, 2000). Psychological and physiological factors may be the cause. Temporal summation of mechanically evoked pain has been suggested to be higher in females compared to males and related to the stimulation frequency (Sarlani and Greenspan, 2002), but this result is contradictory to another study (Nie et al., 2005).

It is also known, that factors as previous experiences, cultural influences, internal hormonal environment, nociceptive input integration in the central nervous system, as well as modulation of the afferent input by descending supraspinal pathway influence the pain measurements, but this project is the first step to assess what mechanically is important to activate nociceptors during pressure pain measurements. More models on this issue are needed.

7. Conclusions and implications

Deep tissue mechanonociceptors stimulated by pressure indentation do not simply experience the externally applied load, but encode compressive strain rather than displacement or stress. The model below (figure 14) outlines the fundamental findings of this project and show how they may fit into the practical use of pressure algometry.

Extrinsic and intrinsic factors play an important role in pressure pain threshold measurements. The probe has to be chosen in relation to the tissue to be assessed with respect to pain sensitivity: large probes on muscles and small on bones. Rounded probes prevent shear strains and are more suitable for deep tissue pain assessments. Moreover also muscle hardness and subcutaneous adipose thickness influence pressure pain measurements. A harder muscle present lower strain peak and, as a consequence, higher pressure stimulation intensity are required to reach the pain threshold. In addition, the magnitude of transcutaneous pressure transmitted to the deep tissue is smaller in the cases with thicker subcutaneous tissue. Finally, also the timing issue is a crucial factor for the modulation of the pain sensation. The strain increases in relation to repetitive pressure pain stimulation but this increase is shown not to be sufficient to explain the pain increase (temporal summation) and central mechanism seem to be involved.

These findings are highly clinically relevant. Pressure algometry is a simple and valuable technique to assess pain, but measurements have to be cautionary done, considering the parameters previously described. The use of the correct probe in relation to the tissue assessed is important in diagnostic procedures and pharmacological profiling studies. This project can be considered the first step in basic research in order to study the mechanical factors that influence the deep tissue nociceptor excitation and consequently the pain sensitivity.

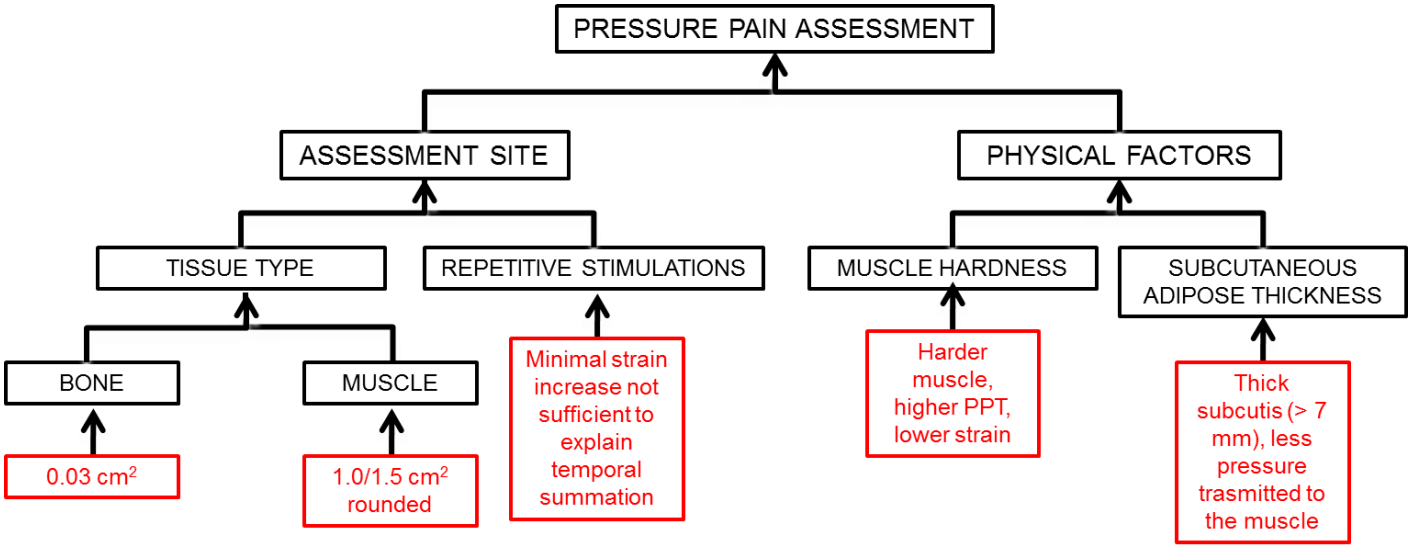

Figure 14: Main recommendations from the series of pressure algometry studies. In relation to physical factors, the comparison between groups has to been done with caution. Physical activity (i.e. muscle hardness) and subcutaneous adipose thickness should be evaluated prior the pressure assessment. On the other side, probes have to be chosen in relation to the site of measurement: small on the bones and large on the muscles. The timing issue is also a crucial factor, temporal summation can be assessed but peripheral aspects will also influence the measurements.

Future perspectives

This series of studies has highlighted some of the physical/mechanical mechanisms behind pressure pain assessments. The main step after this project is to ensure translation from research to applied situations such as innovation on pain assessment technologies, clinical evaluation and pharmacologic studies. Many musculoskeletal disorders would strongly benefit from clearly defined pressure pain evaluation protocols. Such a protocol should be developed by involving both researchers and practitioners to obtain the most suitable methods for the different kind of patients and situations.

Further research may clarify the impact of materials modelling method and parameters on the current findings. This may lead to more appropriate evaluation techniques for specific groups of patients, such as elderly people related for instance with specific physical skin properties.

8. Summary

Muscle pain is difficult to localize and is generally referred to deep structures. Pressure algometry has become a widely used technique to quantitatively assess deep tissue pain sensitivity in experimental and clinical studies. The algometer is a simple device, where pressure is applied and transmitted to the deep tissue through the skin and the subcutaneous tissue, eventually exciting deep-tissue nociceptors that initiate the pain sensation. So far there has been limited knowledge on the pressure distribution in the tissue and which tissue actually is mainly affected.

This Ph.D. project aimed to describe the relation between structural mechanical properties, i.e. stress and strain, in tissues where the pressure stimulation is applied and relate them to the pressure pain sensitivity. Three dimensional finite-element computer models have been used to describe the mechanical stress/strain distribution in the deep tissues of the lower leg during pressure stimulation. The relation between tissue indentation and pressure stimulation intensity was extracted from the simulation models and were validated with human experimental data. Study I showed that muscle mechano-nociceptors stimulated by pressure indentation do not experience the externally applied load by itself, but encode compressive strain rather than displacement or stress. Moreover, extrinsic and intrinsic factors seem to play an important role in pressure pain threshold measurements. The probe shape and diameter are extrinsic factors. The probe has to be chosen in relation to the tissue investigated: Small probe area on bones (III) and large probe area on muscles (I). The simulations showed that a larger area of the periosteal tissue was strained using a small probe (0.03 cm^2) compared to a larger one when stimulating on bone, while on the muscle, as the probe diameter increases, a wider portion of the muscle was strained. Moreover, rounded probes prevent shear strains and are more suitable for deep tissue pain assessments (I). Hardness and subcutaneous adipose thickness are intrinsic factors and influence pressure pain measurements. A harder muscle present lower strain peak and, as a consequence, higher pressure stimulation intensities are required to reach the pain threshold (II). In addition, the magnitude of transcutaneous pressure transmitted to the deep tissue is smaller in the cases with very thick subcutaneous tissue (II). Additionally, the strain distribution is also evaluated in relation to repetitive pressure stimulations at short ISI, the muscle strain increase during repeated stimulations although not sufficient to increase the pain facilitation due to temporal summation (IV).

Those findings are highly clinically relevant and comparison of pressure algometry assessments between groups of subjects has to be cautionary done.

8.a Dansk Sammenfatning

Muskelsmerter er svære at lokalisere, men trykalgometri er ofte anvendt i eksperimentelle og kliniske studier til kvantitativt at vurdere smerteføl somheden af dybtliggende væv (f.eks. muskler, led, og knogler). Trykalgometrimetoden er enkel: Et tryk påføres huden og overføres til dybtliggende væv gennem subkutanet væv, hvilket aktiverer smertereceptorerne (nociceptorerne), der er ansvarlige for smerteoplevelsen. Der er dog kun begrænset viden om, hvordan trykket fordeles i vævet, og dermed hvilket væv der egentligt stimuleres og hvor kraftigt dette stimuleres.

Formålet med dette Ph.D. projekt er at beskrive forholdet mellem mekaniske egenskaber, dvs. stress og strain, i væv, hvor trykstimulation appliceres, og relatere dem til tryk-smerteføl somhed. Tredimensionelle computermodeller (finite-element) anvendes til at beskrive den mekaniske tilstand (stress/strain) i dybtliggende væv under trykstimulation på underbenet.

Nociceptorerne der påvirkes af trykket i musklen stimuleres ikke med samme intensitet som eksternt påførte på huden og afkoder strain (kraftpåvirkningen) fremfor shear strain (forskydningskrafter) eller stress (indre spænding) i vævet (Studie I). Eksterne og interne faktorer påvirker tryksmertesensitiviteten og stress/strain fordelingen i vævene. Form og diameter af trykstimulationsproben er eksterne faktorer. Proben skal vælges i forhold til de væv der undersøges: En probe med stor diameter anvendes på muskler (Studie I) og lille diameter probe på knogler (Studie III). Halvrunde prober reducere shear strain og er derfor mere velegnede til vurdering af smertesensitiviteten i dybtliggende væv end de flade prober (Studie I). Derudover skal den temporale indflydelse ved gentagne stimuleringer også tages i betragtning. Strainfordelingen i musklen blev vurderet under gentagne tryk med korte tidsintervaller, der giver en progressiv forøgelse af smerten (temporal summation). Det er klarlagt at centrale mekanismer er involveret i dette fænomen da strain forøgelsen ved gentagende stimulationer ikke er tilstrækkelig til at forklare den progressive smerteforøgelse (studie IV). Muskelhårdhed og subkutan fedtvævstykkelse er interne faktorer der også påvirker tryksmertemålinger. En hårdere muskel giver et lavere maksimal strain i forhold til stimulation på en blød muskel, og som en konsekvens er tryksmertetærsklen højere end ved stimulation på en blød muskel (Studie II). Desuden er intensiteten af det transkutane tryk der fordeles til det dybtliggende væv mindre hvis det subkutane fedtlag er meget tykt (studie II).

De beskrevne observationer er klinisk yderst relevante. Sammenligning af tryksmertesensitivitet mellem patientgrupper med forskellige interne faktorer (f.eks. tykkelsen på subkutant fedtlag) der påvirker trykstimulationen bør fortolkes med forsigtighed. Desuden viser de indeværende studier at trykalgometrien med fordel kan optimeres ved valg af relevante probeudformning.

9. References

Andersen H. Pressure pain sensitivity and hardness along human normal and sensitized muscle. Somatosensory and Motor Research 2006;23(3):97-109.

Antonaci F, Sand T, Lucas GA. Pressure algometry in healthy subjects: inter-examiner variability. Scand J Rehabil Med 1998;30(1):3-8.

Arendt-Nielsen L, Nie H, Laursen MB, Laursen BS, Madeleine P, Simonsen OH, Graven-Nielsen T. Sensitization in patients with painful knee osteoarthritis. Pain 2010.

Arendt-Nielsen L, Laursen RJ, Drewes AM. Referred pain as an indicator for neural plasticity. Prog Brain Res 2000;129:343-356.

Arendt-Nielsen L. Induction and assessment of experimental pain from human skin, muscle, and viscera. 1997:393–425.

Ashina M, Bendtsen L, Jensen R, Sakai F, Olesen J. Muscle hardness in patients with chronic tension-type headache: relation to actual headache state. Pain 1999;79(2-3):201-205.

Becker N, Bondegaard Thomsen A, Olsen AK, Sjøgren P, Bech P, Eriksen J. Pain epidemiology and health related quality of life in chronic non-malignant pain patients referred to a Danish multidisciplinary pain center. Pain 1997;73(3):393-400.

Bendtsen L, Jensen R, Jensen NK, Olesen J. Muscle palpation with controlled finger pressure: new equipment for the study of tender myofascial tissues. Pain 1994;59(2):235-239.

Berberich P, Hoheisel U, Mense S. Effects of a carrageenan-induced myositis on the discharge properties of group III and IV muscle receptors in the cat. J Neurophysiol 1988;59(5):1395.

Berkley KJ. Sex differences in pain. Behav Brain Sci 1997;20(03):371-380.

Bishop GH. The skin as an organ of senses with special reference to the itching sensation. J Invest Dermatol 1948; 11(2):143-154.

Bovim G. Cervicogenic headache, migraine, and tension-type headache. Pressure-pain threshold measurements. Pain 1992;51(2):169-173.

Brennum J, Kjeldsen M, Jensen K, Staehelin Jensen T. Measurements of human pressure-pain thresholds on fingers and toes. Pain 1989;38(2):211-217.

Bryan AS, Klenerman L, Bowsher D. The diagnosis of reflex sympathetic dystrophy using an algometer. Journal of Bone and Joint Surgery-British Volume 1991;73(4):644.

Cairns BE, Svensson P, Wang K, Hupfeld S, Graven-Nielsen T, Sessle BJ, Berde CB, Arendt-Nielsen L. Activation of peripheral NMDA receptors contributes to human pain and rat afferent discharges evoked by injection of glutamate into the masseter muscle. J Neurophysiol 2003;90(4):2098.

Carli G, Suman AL, Biasi G, Marcolongo R. Reactivity to superficial and deep stimuli in patients with chronic musculoskeletal pain. Pain 2002;100(3):259-269.

Ceelen KK, Stekelenburg A, Mulders JLJ, Strijkers GJ, Baajiens FPT, Nicolay K, Oomens CWJ. Validation of a numerical model of skeletal muscle compression with MR tagging: a contribution to pressure ulcer reserarch. J. Biomech. Eng 2008; 130(6):6-15.

Cherubini C, Filippi S, Nardinocchi P, Teresi L. An electromechanical model of cardiac tissue: constitutive issues and electrophysiological effects. Progress in Biophysics and molecular biology 2008; 97(2-3):562-573.

Coghill RC, Talbot JD, Evans AC, Meyer E, Gjedde A, Bushnell MC, Duncan GH. Distributed processing of pain and vibration by the human brain. Journal of Neuroscience 1994;14(7):4095.

Craig AD, Bushnell MC, Zhang ET, Blomqvist A. A thalamic nucleus specific for pain and temperature sensation. 1994.

Cua AB, Wilhelm KP, Maibach HI. Elastic properties of human skin: relation to age, sex, and anatomical region. Arch Dermatol Res 1990;282(5):283-288.

Daly CH, Odland GF. Age-related changes in the mechanical properties of human skin. J Invest Dermatol 1979;73(1):84-87.

Dao T, LeResche L. Gender differences in pain. J Orofac Pain 2000;14(3):169.

Defrin R, Ronat A, Ravid A, Peretz C. Spatial summation of pressure pain: effect of body region. Pain 2003;106(3):471-480.

Dickenson A, Sullivan AF. Evidence for a role of the NMDA receptor in the frequency dependent potentiation of deep rat dorsal horn nociceptive neurones following C fibre stimulation. Neuropharmacology 1987;26(8):1235-1238.

Djouhri L, Lawson SN. A [beta]-fiber nociceptive primary afferent neurons: a review of incidence and properties in relation to other afferent A-fiber neurons in mammals. Brain Res Rev 2004;46(2):131-145.

Duarte MA, Goulart EMA, Penna FJ. Pressure pain threshold in children with recurrent abdominal pain. J Pediatr Gastroenterol Nutr 2000;31(3):280.

Ernst EE, Matrai A. Intermittent claudication, exercise, and blood rheology. Circulation 1987;76(5):1110.

Farella M, Michelotti A, Steenks MH, Romeo R, Cimino R, Bosman F. The diagnostic value of pressure algometry in myofascial pain of the jaw muscles. J Oral Rehabil 2000;27(1):9-14.

Fillingim R, Maixner W. Gender differences in the responses to noxious stimuli. 1995;4:209-221.

Fischer AA. Pressure algometry over normal muscles. Standard values, validity and reproducibility of pressure threshold. Pain 1987;30(1):115-126.

Friederich JA, Brand RA. Muscle fiber architecture in the human lower limb. J Biomech 1990;23(1):91-95.

Fung YC. Biomechanics: Mechanical Properties of Living Tissues: Springer; 1993.

Garra BS, Cespedes EI, Ophir J, Spratt SR, Zuurbier RA, Magnant CM, Pennanen MF. Elastography of breast lesions: initial clinical results. Radiology 1997;202(1):79.

Ge W, Khalsa PS. Encoding of compressive stress during indentation by slowly adapting type I mechanoreceptors in rat hairy skin. J Neurophysiol 2002;87(4):1686.

Gebhart GF. Descending modulation of pain. Neuroscience & Biobehavioral Reviews 2004;27(8):729-737.

Gennari C, Agnusdei D, Camporeale A. Use of calcitonin in the treatment of bone pain associated with osteoporosis. Calcif Tissue Int 1991;49:9-13.

Gerecz-Simon EM, Tunks ER, Heale JA, Kean WF, Buchanan WW. Measurement of pain threshold in patients with rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, and healthy controls. Clinical Rheumatology 1989;8(4):467-474.

Gerwin RD. A review of myofascial pain and fibromyalgia - factors that promote their persistence. Acupunct Med 2005;23:121-134.

Gibson W, Arendt-Nielsen L, Graven-Nielsen T. Referred pain and hyperalgesia in human tendon and muscle belly tissue. Pain 2006;120(1-2):113-123.

Gibson W, Arendt-Nielsen L, Taguchi T, Mizumura Z, Graven-Nielsen T. Increased pain from muscle fascia following eccentric exercise:animal and human findings. Exp Brain Res 2009;194(2):299-308.

Granges G, Littlejohn G. Pressure pain threshold in pain-free subjects, in patients with chronic regional pain syndromes, and in patients with fibromyalgia syndrome. Arthritis & Rheumatism 1993;36(5):642-646.

Graven-Nielsen T, Mense S, Arendt-Nielsen L. Painful and not painful sensation from human skeletal muscle. Exp Brain Res 2004;159(3):273-283.

Graven-Nielsen T, Mense S. The peripheral apparatus of muscle pain: evidence from animal and human studies. Clin J Pain 2001;17(1):2.

Graven-Nielsen T, Arendt-Nielsen L, Svensson P, Jensen TS. Experimental muscle pain: a quantitative study of local and referred pain in humans following injection of hypertonic saline. Journal of Musculoskelatal Pain 1997;5(1):49-69.

Graven-Nielsen T, Arendt-Nielsen L. Assessment of mechanisms in localized and widespread musculoskeletal pain. Nature Reviews Rheumatology 2010;6:599-606.

Greenspan JD, McGillis SLB. Stimulus Features Relevant to the Perception of Sharpness and Mechanically Evoked Cutaneous Pain. Somatosensory and Motor Research 1991;8(2):137-147.

Grigg P. Stretch sensitivity of mechanoreceptor neurons in rat hairy skin. J Neurophysiol 1996;76(5):2886.

Hasselström J, Liu-Palmgren J, Rasjö-Wrċċk G. Prevalence of pain in general practice. European journal of pain 2002;6(5):375-385.

Hockaday JM, Whitty CWM. Patterns of referred pain in the normal subject. Brain 1967;90(3):481.

Hoheisel U, Reinöhl J, Unger T, Mense S. Acidic pH and capsaicin activate mechanosensitive group IV muscle receptors in the rat. Pain 2004;110(1-2):149-157.

Hoheisel U, Lehmann-Willenbrock E, Mense S. Termination patterns of identified group II and III afferent fibres from deep tissues in the spinal cord of the cat. Neuroscience 1989;28(2):495-507.

Horikawa M. Effect of visual display terminal height on the trapezius muscle hardness: quantitative evaluation by a newly developed muscle hardness meter. Appl Ergon 2001;32(5):473-478.

Horikawa M, Ebihara S, Sakai F, Akiyama M. Non-invasive measurement method for hardness in muscular tissues. Med Biol Eng Comput 1993;31(6):623-627.

Hulet CV. A rectal-abdominal palpation technique for diagnosing pregnancy in the ewe. J Anim Sci 1972;35(4):814.

Iggo A. Non-myelinated afferent fibres from mammalian skeletal muscle. J Physiol (Lond) 1961;155:52-53.

Isselée H, Laat A, Lesaffre E, Lysens R. Short-term reproducibility of pressure pain thresholds in masseter and temporalis muscles of symptom-free subjects. Eur J Oral Sci 1997;105(6):583-587.

Jensen R, Rasmussen BK, Pedersen B, Lous I, Olesen J. Cephalic muscle tenderness and pressure pain threshold in a general population. Pain, 1992;48(2):197-203.

Kasch H, Stengaard-Pedersen K, Arendt-Nielsen L, Staehelin Jensen T. Pain thresholds and tenderness in neck and head following acute whiplash injury: a prospective study. Cephalalgia 2001;21(3):189-197.

Kaya T, Bal S, Gunaydin R. Relationship between the severity of enthesitis and clinical and laboratory parameters in patients with ankylosing spondylitis. Rheumatol Int 2007;27(4):323-327.

Khalsa PS, Lamotte RH, Grigg P. Tensile and compressive responses of nociceptors in rat hairy skin. J Neurophysiol 1997;78(1):492.

Khimich S. Level of sensitivity of pain in patients with obesity. Acta Chir Hung 1997;36(1-4):166-167.

Kosek E, Ekholm J, Hansson P. Pressure pain thresholds in different tissues in one body region. The influence of skin sensitivity in pressure algometry. J Rehabil Med 1999;31(2):89-93.

Kosek E, Ekholm J. Modulation of pressure pain thresholds during and following isometric contraction. Pain 1995;61(3):481-486.

Kosek E, Ekholm J, Nordemar R. A comparison of pressure pain thresholds in different tissues and body regions. Long-term reliability of pressure algometry in healthy volunteers. Scand J Rehabil Med 1993;25(3):117-124.

Kumazawa T, Mizumura K. Thin-fibre receptors responding to mechanical, chemical, and thermal stimulation in the skeletal muscle of the dog. J Physiol (Lond) 1977;273(1):179.

Kurihashi A, Tamai K, Saotome K, Takemura M, Fujiwara A, Fujita S. Difference in stretching of sarcomeres between medial gastrocnemius and tibialis anterior by tibial lengthening: an experiment in rabbits. Journal of Orthopaedic Surgery 2006;14(2):147-150.

Kvistedal Y, Nielsen P. Investigating stress-strain properties of in-vivo human skin using multiaxial loading experiments and finite element modeling. 2004;2.

Langemark M, Jensen K, Jensen TS, Olesen J. Pressure pain thresholds and thermal nociceptive thresholds in chronic tension-type headache. Pain 1989;38(2):203-210.

Levoska S. Manual palpation and pain threshold in female office employees with and without neck-shoulder symptoms. Clin J Pain 1993;9(4):236-241.

Lewis T. Study of Somatic Pain. Br Med J 1938;1(4023):321.

Lu MH, Zheng YP, Huang QH. A novel noncontact ultrasound indentation system for measurement of tissue material properties using water jet compression. Ultrasound Med Biol 2005;31(6):817-826.

Mach DB, Rogers SD, Sabino MC, Luger NM, Schwei MJ, Pomonis JD, Keyser CP, Clohisy DR, Adams DJ, O'Leary P, Mantyh PW. Origins of skeletal pain: sensory and sympathetic innervation of the mouse femur. Neuroscience 2002; 113(1):155-166.

Maher C, Adams R. Reliability of Pain and Stiffness Assessments in Clinical Manual Lumbar Spine Examination. Phys Ther 1994;74(9):801.

Main CJ, Watson PJ. Psychological aspects of pain. Man Ther 1999;4(4):203-215.

Maquet D, Croisier JL, Demoulin C, Crielaard JM. Pressure pain thresholds of tender point sites in patients with fibromyalgia and in healthy controls. European Journal of Pain 2004;8(2):111-117.

Marchettini P, Simone DA, Caputi G, Ochoa JÈL. Pain from excitation of identified muscle nociceptors in humans. Brain Res 1996;740(1-2):109-116.

McKendall MJ, Haier RJ. Pain sensitivity and obesity. Psychiatry Res 1983;8(2):119-125.

Mendell LM. Physiological properties of unmyelinated fiber projection to the spinal cord. Exp Neurol 1966;16(3):316-332.

Mense S. Nociception from skeletal muscle in relation to clinical muscle pain. Pain 1993;54(3):241-289.

Mense S, Craig AD. Spinal and supraspinal terminations of primary afferent fibers from the gastrocnemius-soleus muscle in the cat. Neuroscience 1988;26(3):1023-1035.

Mense S. Sensitization of group IV muscle receptors to bradykinin by 5-hydroxytryptamine and prostaglandin E2. Brain Res 1981;225(1):95-105.

Miller K. Constitutive model of brain tissue suitable for finite element analysis of surgical procedures. J Biomech 1999;32(5):531-537.

Milne RJ, Aniss AM, Kay NE, Gandevia SC. Reduction in perceived intensity of cutaneous stimuli during movement: a quantitative study. Experimental Brain Research 1988;70(3):569-576.

Moseley GL. Evidence for a direct relationship between cognitive and physical change during an education intervention in people with chronic low back pain. European Journal of Pain 2004;8(1):39-45.

Mridha M, Ödman S. Noninvasive method for the assessment of subcutaneous oedema. Medical and Biological Engineering and Computing 1986;24(4):393-398.

Murayama M, Nosaka K, Yoneda T, Minamitani K. Changes in hardness of the human elbow flexor muscles after eccentric exercise. Eur J Appl Physiol 2000;82(5):361-367.

Nie H, Arendt-Nielsen L, Andersen H, Graven-Nielsen T. Temporal summation of pain evoked by mechanical stimulation in deep and superficial tissue. The Journal of Pain 2005;6(6):348-355.

Nordahl S, Kopp S. Pressure pain threshold of the posterior aspect of the temporomandibular joint measured with a semi-spherical probe. J Orofac Pain 2003;17(2):145-150.

O'Brien M. Structure and metabolism of tendons. Scand J Med Sci Sports 1997;7(2):55-61.

Offenbächer M, Stucki G. Physical therapy in the treatment of fibromyalgia. Scand J Rheumatol 2000;29:78-85.

Ogden R. Non-Linear Elastic Deformations: Dover Pubns; 1997.

Ohrbach R, Gale EN. Pressure pain thresholds, clinical assessment, and differential diagnosis: reliability and validity in patients with myogenic pain. Pain 1989;39(2):157-169.

Paintal AS. Functional analysis of group III afferent fibres of mammalian muscles. J Physiol (Lond) 1960;152(2):250.

Persson AL, Brogardh C, Sjolund BH. Tender or not tender: test-retest repeatability of pressure pain thresholds in the trapezius and deltoid muscles of healthy women. J Rehabil Med 2004;36(1):17-27.

Petzke F, Clauw DJ, Ambrose K, Khine A, Gracely RH. Increased pain sensitivity in fibromyalgia: effects of stimulus type and mode of presentation. Pain 2003;105(3):403-413.

Polianskis R, Graven-Nielsen T, Arendt-Nielsen L. Computer-controlled pneumatic pressure algometry—a new technique for quantitative sensory testing. European Journal of Pain 2001;5(3):267-277.

Reeves JL, Jaeger B, Graff-Radford SB. Reliability of the pressure algometer as a measure of myofascial trigger point sensitivity. Pain 1986;24(3):313-321.

Reinert A, Kaske A, Mense S. Inflammation-induced increase in the density of neuropeptide-immunoreactive nerve endings in rat skeletal muscle. Experimental Brain Research 1998;121(2):174-180.

Roane DS, Porter JR. Nociception and opioid-induced analgesia in lean (Fa/−) and obese (fa/fa) Zucker rats. Physiol Behav 1986;38(2):215-218.

Rolke R, Andrews Campbell K, Magerl W, Treede RD. Deep pain thresholds in the distal limbs of healthy human subjects. European Journal of Pain 2005;9(1):39-48.

Sagada, S. and Taguchi, S. Electrophyiological studies of the free-fiber ending units of the cat mandibular periosteum. Bull Tokyo Dent Coll 1971;12(3):175–197.

Sakai F, Ebihara S, Akiyama M, Horikawa M. Pericranial muscle hardness in tension-type headache: A non-invasive measurement method and its clinical application. Brain 1995;118(2):523.

Sandberg M, Larsson B, Lindberg LG, Gerdle B. Different patterns of blood flow response in the trapezius muscle following needle stimulation (acupunture) between healthy subjects and patients with fibromyalgia and work-related trapezius myalgia. Eur J Pain 2005;9:497-510.

Sarlani E, Greenspan JD. Gender differences in temporal summation of mechanically evoked pain. Pain 2002;97(1-2):163-169.

Shanley RF. Mechanics of Materials. New York: McGraw Hill; 1967.

Shapira SC, Magora F, Chrubasik S, Feigin E, Vatine JJ, Weinstein D. Assessment of pain threshold and pain tolerance in women in labour and in the early post-partum period by pressure algometry. Eur J Anaesthesiol 1995;12(5):495-499.

Sherrington C. The Integrative Action of the Nervous System. Oxford University Press 1906.

Shibata M, Ohkubo T, Takahashi H, Inoki R. Modified formalin test: characteristic biphasic pain response. Pain 1989;38(3):347-352.

Simone DA, Kajander KC. Responses of cutaneous A-fiber nociceptors to noxious cold. J Neurophysiol 1997;77(4):2049.

Simons DG, Travell JG, Simons LS. Travell & Simons' Myofascial Pain and Dysfunction: The Trigger Point Manual: Williams & Wilkins; 1999.

Slater H, Gibson W, Graven-Nielsen T. Sensory responses to mechanically and chemically induced tendon pain in healthy subjects. European Journal of Pain 2010.

Slater H, Arendt-Nielsen L, Wright A, Graven-Nielsen T. Experimental deep tissue pain in wrist extensors--a model of lateral epicondylalgia. European Journal of Pain 2003;7(3):277-288.

Spencer AJM, Continuum Mechanics, Longman, London, New York, 1980.

Stacey MJ. Free nerve endings in skeletal muscle of the cat. J Anat 1969;105(Pt 2):231.

Staud R, Vierck CJ, Cannon RL, Mauderli AP, Price DD. Abnormal sensitization and temporal summation of second pain (wind-up) in patients with fibromyalgia syndrome. Pain 2001;91(1-2):165-175.

Sullivan MJL, Stanish W, Waite H, Sullivan M, Tripp DA. Catastrophizing, pain, and disability in patients with soft-tissue injuries. Pain 1998;77(3):253-260.

Svensson P, List T, Hector G. Analysis of stimulus-evoked pain in patients with myofascial temporomandibular pain disorders. Pain 2001;92(3):399-409.

Takahashi T, Mizumura K. 3-D finite element analysis of stresses in the epidermis and the muscle given by a transcutaneous pressure. Jpn J Physiol 2004;54:S175.

Takahashi K, Taguchi T, Itoh K, Okada K, Kawakita K, Mizumura K. Influence of surface anesthesia on the pressure pain threshold measured with different-sized probes. Somatosensory and Motor Research 2005;22(4):299-305.

Torebjörk HE. Afferent G Units Responding to Mechanical, Thermal and Chemical Stimuli in Human Non-Glabrous Skin. Acta Physiol Scand 1974;92(3):374-390.

Torgén M, Swerup C. Individual factors and physical work load in relation to sensory thresholds in a middle-aged general population sample. Eur J Appl Physiol 2002;86(5):418-427.

Tran HV, Charleux F, Rachik M, Ehrlacher A, Tho MC. In vivo characterization of the mechanical properties of human skin derived from MRI and indentation techniques. Comput Methods Biomech Biomed Engin 2007;10(6):401-407.

Treede RD, Rolke R, Andrews K, Magerl W. Pain elicited by blunt pressure: neurobiological basis and clinical relevance. Pain(Amsterdam) 2002;98(3):235-240.

Treede RD, Meyer RA, Raja SN, Campbell JN. Peripheral and central mechanisms of cutaneous hyperalgesia. Prog Neurobiol 1992;38(4):397-421.

Van Egmond J, Hasenbos M, Crul JF. Invasive v. non-invasive measurement of arterial pressure: Comparison of Two Automatic Methods and Simultaneously Measured Direct Intra-Arterial Pressure. Br J Anaesth 1985;57(4):434.

Vanderweeen L, Oostendorp RAB, Vaes P, Duquet W. Pressure algometry in manual therapy. Man Ther 1996;1(5):258-265.

Vierck Jr CJ. Mechanisms underlying development of spatially distributed chronic pain (fibromyalgia). Pain 2006;124(3):242-363.

Wall PD, Woolf CJ. Muscle but not cutaneous C-afferent input produces prolonged increases in the excitability of the flexion reflex in the rat. J Physiol (Lond) 1984;356(1):443.

Weiss JA, Maker BN, Govindjee S. Finite element implementation of incompressible, transversely isotropic hyperelasticity. Comput Methods Appl Mech Eng 1996;135(1-2):107-128

Wessel J. The Reliability and Vatidity of Pain Threshold Measurements in Osteoarthritis of the Knee. Scand J Rheumatol 1995;24(4):238-242.

Zahorska-Markiewicz B, Kucio C, Pyszkowska J. Obesity and pain. Hum Nutr Clin Nutr 1983;37(4):307-310.