After several decades of severe neglect, fascia is developing its own identity within medical research and is being found to play an important role in health and pathology. The number of research papers on fascia in peer-reviewed journals has shown a steady rise particularly since 2005 (see figure 1). The purpose of the program book for the Third International Fascia Research Congress is to provide you background material to understand recent developments in fascia research. To this end, the editors reviewed titles of thousands and content of hundreds of published papers. As our understanding of the breadth of scientific information relating to fascia expands, we find more and more highly related papers. We have selected almost twice as many papers as in the first two fascia congress program books. These are organized by topic area to parallel the scientific abstracts submitted to the Congress. We bring you papers which illustrate techniques and concepts as well as those which summarize important principles. While most are recent, some (6.1.1) are older ones recently discovered. In the interest of green publication, the full text is included only for those papers which are not freely available at www.pubmed.gov . We give you the Pubmed ID and encourage you to read the others online, and think you will find, as we do, that in the process of locating and downloading articles you will find another article you were not looking for but which turns out to be very interesting as well. At pubmed you will find over 15,000 papers by entering the term "fascia" in the search box; you can then easily select the 1300 of these which are available online as free fulltext.

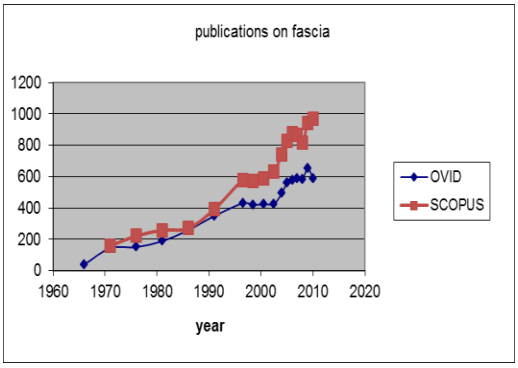


**Fig. 1: Number of peer reviewed scientific papers on fascia.** Papers on fascia indexed in Ovid Medline or Scopus have grown from 200 per year in the 1970's and 1980's to almost 1000 in 2010

As articles on fascia has grown, so have the number of people interested in those articles: Researchers, Clinicians who want to do research, and those who want to be aware of the latest research. I have provided information to guide you in evaluating papers in a series of twelve articles on research for the clinician, which is freely available at http://www.rolfresearchfoundation.org/other_resources . You may want to start with the second article, "The Conceptual Review of the Literature or How to Read More Articles Than You Ever Want to See in Your Entire Life." Article 5.1.4 in this program book illustrates some important things to be aware of when you read papers. In this paper, appropriately, the square root was taken of the ultrasound variable echogenicity which improved the p value from 0.02 to 0.006. Mathematical transformation of data is an important concept which is explained in my eighth article "Preliminary Data Analysis;" the whole range of these transformations from negative reciprocal to cube is termed the "ladder of powers" which are illustrated there. I provide guidance to choosing standard statistical tests in article nine "Primary Data Analysis" which will cover the vast majority of published medical studies. The multivariate mixed effects statistics in the paper by Ahn are well beyond methods used for one or two variables. The benefits from visually inspecting data in a research paper are illustrated in article five "Data Entry and Exploratory Data Analysis", which plots four hypothetical data sets which have exactly the same correlation coefficient, 0.70, but visual inspection shows that the data relationship is quite different in each case (Figure 2). Figures from this acupuncture paper show some similarity to the first two examples (Figure 3):

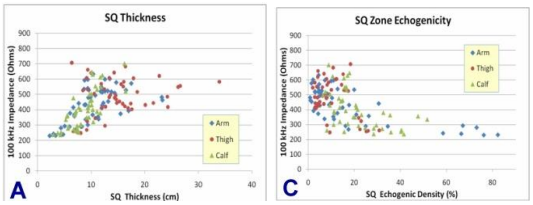

**Figure 3** Data sets illustrating a and b above from 5.1.4 Li W, Ahn AC. Subcutaneous fascial bands--a qualitative and morphometric analysis. PLoS One. 2011;6(9):e23987.

## 2 INTERSTITIAL FLUID FLOW

2.1.1 Dr Rolf Reed summarizes his studies on remodeling of loose connective tissues in the skin and in carcinomas, looking in particular at flow of fluid through that tissue. In the normal steady state, flow of interstitial fluid is "auto-regulated;" any increase in capillary hydrostatic pressure and movement of fluid from the capillary to the interstitial space is countered by a compensatory rise in the pressure in the interstitial space. A similar but opposite mechanism occurs when capillary hydrostatic pressure drops. During acute inflammation, the fluid flow out of the capillary can increase several hundred times within a few minutes. This flow is influenced by the loose connective tissue, which recent studies show is neither a passive nor a static framework. During acute edema formation, as after a second degree burn, frostbite or anaphylaxis, the interstitial fluid pressure actually falls within minutes of injury, which actively pulls fluid out of the capillary.

2.1.2 All organs in the body must be viewed in the context of the surrounding blood and lymphatic vessels as well as the loose connective tissue which has four main components: 1) a network of collagen fibers which is the primary 3 dimensional scaffold for the blood vessels 2) elastin microfibrils 3) ground substance including hyaluronan and 4) interstitial fluid. Properties of this tissue can be rapidly modified by several means. Connective tissue cells directly exert tension on both the collagen and the elastin fibers through β 1 integrin mediated contraction. This restrains the fluid retaining capacity of the ground substance which is normally underhydrated. While hyaluronan levels tend to remain stable, it is not a static tissue but rather has both rapid synthesis and turnover. Finally, the interstitial fluid depends on the fluid flux across the capillary and the removal through the lymph system. In humans the extracellular fluid volume is 15 L, with normal blood plasma volume of 3 L, and 6-10 L passes through the lymph system each day with resulting turnover of extracellular fluid every 48 hours.

2.1.3 Decreased lymphatic drainage of fluid from the extracellular space can be acquired (as after surgical disruption of the lymphatic system, or changes in venous circulatory system), or congenital. Humans with congenital primary lymphedema have swelling of the limbs, thickening of the skin and increase in both fluid and fat in subcutaneous tissue. This structural remodeling further impedes lymphatic flow. Studies on two mouse models, both of which lack dermal lymphatic capillaries, illustrate that the model which has increased collagen and fat in the cutaneous tissues has more swelling. This suggests that human therapies will need to address tissue remodeling as well as growth of new lymphatic vessels.

2.1.4 Dynamic mechanical stresses have long been recognized as important in the maintenance of supporting structures such as bone and muscle. Less well recognized is the need for stresses and pressure gradients to maintain function in all living tissues. In soft tissues interstitial flow is primarily driven by plasma leaving the blood capillaries, and that pressure gradient in turn is affected by large movements of the skeletal system and smaller motion from arterial pulsation, respiration and organ motion. This slow interstitial flow can have a direct mechanical effect on cells, as well as transporting proteins and other components of the biochemical environment. Increased interstitial flow stimulates fibroblasts to differentiate into myofibroblasts, increase production of collagen and other factors associated with fibrosis.

**Important concepts:** The collagen fibers in the extracellular matrix act as strings tying up a sponge, preventing it from expanding. Therapies designed to locally increase edema such as Chinese cupping may increase the adaptability of the fluid flow adjustment systems by temporarily increasing fluid flow. Therapies designed to reduce lymphedema must take into account the tissue changes which take place with prolonged decrease in interstitial flow, including the increased tissue compliance or "overstretching" of the interstitial matrix. Organs must be viewed in the context of the surrounding connective tissues and distant blood and lymphatic fluid flow, and specific organ pathology cannot be fully understood or treated without taking those tissues into account. We think fluid flow is a very important concept for future research but is only briefly touched upon in article 6.1.1 and abstracts 10.2.5 and 10.2.22.

## 3 CYTOLOGY/HISTOLOGY OF FIBROBLASTS

3.1.1 The myofibroblast is now recognized as the key player in tissue fibrosis which causes organ

destruction in recognized diseases of the heart, lung, kidney, and skin. It can develop from a number of cell types, most commonly the fibroblast. α smooth muscle actin in the stress fibers of the myofibroblasts generate mechanical forces which regulates tissue remodeling, both in normal development and in pathological processes. Experimental therapies for fibrosis have been directed toward decreasing myofibroblasts formation, reducing contractile forces, and stimulating apoptosis or de-differentiation to reduce numbers of myofibroblasts. Blocking specific integrins or controlling stiffness of the extracellular matrix may reduce the local stress on the myofibroblasts and have long term effects on fibrosis.

3.1.2 Fibroblasts synthesize, organize and remodel collagen, depending on the tension between the cell and the extracellular matrix. At low tension, the fibroblast in a resting state with low synthesis of collagen matrix assumes a morphology of a small cell body with dendritic extensions connected to other cells by gap junctions. When placed in a high tension matrix the fibroblasts assume a larger cell body of lamellar shape, and increase their collagen synthesis and cell proliferation. They can also further differentiate into myofibroblasts from this lamellar state. Each fibroblast can remodel nearby collagen matrix, and this local remodelling can spread throughout the matrix to result in large scale matrix contraction. By exerting traction on the matrix, the fibroblast can either cause motion of the collagen or movement of the fibroblast through the matrix. The central importance of these concepts led us to repeat this paper from the 2009 program book.

3.1.3 Studies in cell culture have shown that mechanical forces outside the cell can affect the shape of the nucleus, particularly nuclear invaginations which contain α-actin. Previous studies have shown that stretching affects the shape of the cell, and this study explored whether those changes would be carried to the nucleus. Stretching of areolar connective mouse tissue for 30 minutes caused the fibroblast nuclei to become wider, flatter and smoother with loss of the invaginations, as shown by confocal microscopy and histochemistry. This can be blocked by a Rho kinase inhibitor, suggesting cytoskeletin actin bundle contractility is important to this active process.

3.1.4 Fibroblasts produce and degrade matrix proteins, with an indirect effect on matrix stiffness. They can also differentiate into myofibroblasts which can contract and increase matrix tension. By changing shape, the fibroblast can affect stiffness and viscosity of connective tissue within minutes, consistent with the mechanotransduction models of microtubule network expansion and actomyosin generated tension proposed by Ingber (4.1.1). The fibroblast may also remodel cell matrix contacts in the direction of tissue stretch to reduce tension.

**Important concepts:** Connective tissue actively regulates matrix tension in response to stretch as a normal, dynamic physiological process. Understanding how cells respond to forces can lead to potential treatments to decrease fibrosis in cases where the forces remain high. Will studies on severe fibrotic processes within organs such as liver kidney and heart lead to possible treatments for less severe fibrosis which may be seen in other locations of the body? How important is the extracellular matrix, and do tissue fluid pressure and the collagen fibers discussed in chapter 2 affect the forces on and generated by the myofibroblast? Can local manual treatments affect the fibrotic process early on in its development? Yet to be explored are techniques to increase fibrosis in tissues which are too lax.

4 MECHANOTRANSDUCTION AND BIOMECHANICS

4.1.1 Thirty years of research from the laboratory of Dr Ingber are summarized here, starting with the use of tensegrity architecture to sense mechanical signals which control cell function and development. The living cell is a mechanical structure with a force balance between compression bearing microtubules and tension bearing bundles of actomyosin filaments. The cells are anchored to the extracellular matrix by clusters of integrin receptors which connect extracellular proteins to intracellular actin associated molecules. These receptors also serve to sense physical forces outside the cell and transmit that information through mechanical connections throughout the cell to the nucleus as well as multiple locations in the cell. This cytoskeleton provides both mechanical structure and direction to biochemical reactions within the cell. The cell can thus convert external mechanical signals into internal biochemical reactions

4.1.2 One hundred years ago, mechanical forces were thought to control development of the embryo. Molecular biology has led to discovery of genes and chemicals which drive development. This paper draws the connection between these views, showing that mechanical stresses directly affect molecular biochemistry and gene expression, with a key role afforded to cytoskeletal tension, traction and the balance of forces or

prestress among the microfilaments (tension elements) and microtubules (compression bearing elements) within the cell and the surrounding extracellular matrix which exerts traction at the cell surface. Osmotic pressure, surface tension and shear stress from fluid flow are identified factors in specific aspects of development, particularly the circulatory system. The role of mechanical forces can now be seen in studies with such methods as micromanipulation, surface tensiometry, magnetic forces, atomic force microscopy, traction force microscopy, microengineered adhesive substrates, and microfluidic systems.

4.1.3 The physiological effects of physical forces have long been recognized by clinicians – for example tension and skin aging, compression and bone formation, shear stress and vascular remodeling. Studies in cell mechanics have developed quantitative models of red and white blood cell motion and deformation which have provided a biomechanical basis for inflammation, cardiovascular disease, and organ perfusion. Particularly fruitful have been studies of fluid shear stress and the thin layer of macromolecules coating blood vessels. Extracellular matrix stiffness and cell deformation affect cell growth, differentiation, apoptosis as well as cell motility. Bone, muscle and cartilage remodel in response to mechanical forces, mediated through extracellular fluid flow. Future research is challenged to develop more integrative theories. With the deciphering of the human genome, we may get closer to a comprehensive theory which incorporates three dimensional geometry and physical forces to explain the folding, assembly and function of biosynthesized molecules.

4.1.4 Moving from the cellular level we find that traditional views of force transmission by muscles are also changed greatly by recent investigations. The classic view has been that individual muscles are independent force generators which connect to their tendon and from there to the bony origin and insertion. However, there is an areolar connective tissue layer which provides intermuscular force transmission between neighboring muscle bellies. In depth studies of the anterior compartment of the rat foreleg show the extent of these connections, with almost 40% of the generated force traveling laterally to other muscles rather than to the particular proximal or distal tendon of that muscle. Forces are also transmitted to non-muscular structures by the connective tissue of the neurovascular tract, continuity between muscle fascial layers and more superficial subcutaneous fascia, and connective tissues surrounding tendons. It is not clear to what extent muscle forces actually travel down these other pathways, with contradictory studies on cats and rats. Human studies (see 5.1.1 in the next section) point to force transmission between only selected muscles. However, the transfer of forces within a muscle may allow rest and repair for injured muscle fibers, with the surrounding fibers taking the load as described in 4.1.5 below.

4.1.5 Purslow reviews the specific anatomy of the connective tissue structures within muscles. The endomysium, perimysium and epimysium are collectively termed the intramuscular extracellular matrix. The mechanical environment and functional demands on the muscle affect degradation, growth, reformation and remodeling of these tissues, forming a dynamic balance in the adult. The outer layer, the epimysium, consists of collagen fibers oriented at 55 degrees to the long axis of the muscle and shows a consistency of collagen composition across different muscles, in contrast to the other two layers which vary between muscles. The collagen fibers of the inner layer, the endomysium, have a wide range of direction in relation to the muscle fiber; as the muscle elongates the predominate fiber direction changes from circumferential to longitudinal. As a result, the endomysium is very compliant to tensile forces. In contrast, because the endomysium is so thin (about 5 microns), it provides a much stiffer shear linkage between adjacent muscle fibers and provides lateral load sharing. Endomysium thickness and collagen structure varies little between muscles. The middle layer, the perimysium, is a shared structure between muscle fascicles which varies in thickness from 50 to 150 microns in different muscles; its collagen fibers also lie at 55 degrees to the muscle fiber at rest, but change to 80 degrees at short and 20 degrees at long sarcomere lengths. One intriguing explanation offered is that the perimysium allows muscles to change shape during contraction; depending on the type of muscle, more or less change is needed in daily function and thus the perimysium needs to vary similarly to accommodate these changes. Since intramuscular ECM is continually remodeled so as to be mechanically adapted for its roles in developing and growing muscles, it is additionally suggested, that influencing the processes governing intramuscular ECM turnover and repair could provide a useful avenue for exploring the reduction of fibrosis following muscle injury

**Important concepts:** A better understanding of mechanochemical control mechanisms may let us correct mechanical loading or mechanochemical signaling in adult conditions. Use of the experimental

methods developed may allow us to explore at the cellular level the effects of externally applied forces such as repetitive stress disorders or manually applied therapies. Changes in mechanical loading with activity are particularly relevant to the collagen fiber structure of muscles and the intramuscular extracellular matrix. Many clinical therapies use externally applied forces based on empirical observations (some ancient), but rarely if ever are these treatments anchored in the biomechanical environment of the cell or the structural requirements of the muscle. Recent developments in medical diagnostic imaging may allow direct observation of this biomechanical context (see chapters 9 and 10).

5 GROSS ANATOMY

5.1.1 The traditional biomechanical model of muscles as independent actuators has been challenged by studies in animals and in humans during surgery which show the possibility of transmission of forces from the entire surface of the muscle, not just at the ends through tendons. This study investigated whether intermuscular force transmission can be detected in the lower leg of the living human, where the anatomy of the muscles crossing the knee and the ankle allow for displacement of some muscles (gastrocnemii and flexor hallucis longus) while keeping others (the soleus) at a constant length by fixing the ankle position and moving the knee or the toe. In all seven subjects there was consistent force transmission detectable between the medial gastrocnemius and the soleus muscles. During submaximal plantarflexion, however, half the subjects had co-contraction of the flexor hallucis longus at 50% and half at only 10%; this varied movement pattern and the small tissue displacement at the interface with the FHL did not allow this study to confirm force transmission between the soleus and the flexor hallucis.

5.1.2 This is the first comprehensive review paper on fascial anatomy in a major anatomical journal. It extends from the gross to the molecular level. For expediency, it deals only with fascia in the limbs and back. Particular attention is given to deep fascia (as opposed to the loose connective tissue layer of the 'superficial fascia' under the skin) and thus consideration is given to structures such as the fascia lata, thoracolumbar fascia, plantar and palmar fascia, along with regional specializations of deep fascia such as retinacula and fibrous pulleys. Among the many functions of fascia considered in detail are its ectoskeletal role (as a soft tissue skeleton for muscle attachments) and its importance for creating osteofascial compartments for muscles. It discusses the presence and potential function of fascial myofibroblasts and gives particular importance to fascial expansions of entheses, such as the continuation of the Achilles tendon over the heel pad into the plantar fascia. This excellent review emphasizes the continuity of fascia between regions. In addition it appreciates the key role of fascia in coordinating muscular activity and acting as a body-wide proprioceptive organ.

5.1.3 In this richly illustrated report the authors describe the results of dissection of 15 unembalmed cadavers, that examined anatomical continuity between the muscles involved in flexion of the upper limb and associated fascia. The study demonstrated the widespread presence of specific myofascial expansions that originate from the flexor muscles and extend to the overlying fascia. "*The expansions allow reciprocal feedback between fascia and muscles: the fascia can perceive tension produced by a muscle due to its expansions, and can transmit it to a distance, informing the distal muscle about the state of contraction of the proximal muscle, possibly via muscle spindles activation*." As these muscles contract during flexion they exert tension into the anterior portions of the brachial and antebrachial fascia. The authors hypothesize that this tension activates proprioceptive nerve endings in the fascia, which contributes to perception of motor direction during movement. They further hypothesize that when the muscular fascia is in a dysfunctional state, these mechanisms may be altered, and the fascial proprioceptors may not be correctly activated and therefore give rise to some of the widespread extra-articular pain symptoms.

5.1.4 In other studies Chinese acupuncture points have been linked anatomically to neurovascular bundles, trigger points, and connective tissue fascial planes and separately to areas of reduced electrical impedance. This study combined these measures in subcutaneous tissue at segments of acupuncture meridians located at Large Intestine (LI) 13 in the arm, Liver (LV) 9 in the thigh, and Bladder (BL) 56 in the calf, compared to nearby control non-meridian segments. While there was an overall decrease in impedence at the meridians, this was due to changes at LI and it was not found at LV or BL. Subcutaneous collagenous bands identified by echogenicity on ultrasound were associated with decreased impedence. The graphic presentation

of data illustrates the substantial variability and nonlinearity of response when comparing ultrasound findings and impedance.

**Important concepts**: In real bodies, muscles may not transmit their full force directly via tendons into the skeleton, as is usually suggested by our textbook drawings. They rather distribute a large portion of their contractile or tensional forces onto fascial sheets. These sheets transmit these forces to synergistic as well as antagonistic muscles. Thereby they stiffen not only the respective joint, but may even affect regions several joints further away. The simple questions from musculoskeletal textbooks regarding “which muscles” are participating in a particular movement thus become almost obsolete. Muscles are not separate functional units, no matter how common this misconception may be. Rather, muscular movements may be generated by many individual motor units, which are distributed over some portions of one muscle, plus other portions of other muscles. The tensional forces of these motor units are then transmitted to a complex network of fascial sheets, bags, and strings that convert them into the final body movement. The papers in this section begin to map out the details of this network in specific parts of the body, both in cadavers and in living humans.

## 6 SURGERY AND SCARS

6.1.1 Deep fascia has parallel longitudinal collagen bundles and rudimentary elastic laminae, giving it both high tensile strength and elasticity. At the junction between the deep fascia and the muscle, without any special secretory cells, the fascia is able to maintain a lubricating layer of hyaluronic acid. However, when the epimysium is disrupted the overlying fascia does not remain distinct and does not create a gliding layer over the scar. This is consistent with more recent findings that while hyaluronic acid is a lubricant, breakdown products of this large molecule are themselves tissue irritants.

6.1.2 The deep fascia is a highly vascular structure with a superficial and a deep layer, each with an independent rich vascular network of capillaries, venules, arterioles, and lymphatic channels. The presence of mast cells in deep fascia suggests a protective role similar to other connective tissues. The deep layer has few elastin fibers but does have myofibroblasts, suggesting contractile ability. Any active contraction would need to be controlled by a nerve supply, and indeed one finds myelinated and unmyelinated nerve axons, and Schwann cells. The deep fascia is not just a tough barrier structure of collagen and elastin, but is a metabolically active vascular layer which provides gliding and protective functions.

6.1.3 In vitro and in vivo studies of tendons in the forearm show that blood vessels and connective tissue fibers forming vacuoles can clearly be seen connecting the tendon with the tendon sheath, a structure termed the multimicrovacuolar collagenous dynamic absorbing system. Some of these structures deform quite readily during small motion, while others require much greater tendon motion, and the overall architecture depends on how far the tendon needs to move in normal circumstances. The fibers of this system line up in the direction of motion with increasing traction. Under direct observation, these fibers appear to reversibly extend and contract, divide into other fibrils, and glide across other fibers at crossing points. Unexpectedly, the layer of fibers closest to the tendon is not observed to move more than the layers furthest away. A theory of tissue continuum is proposed to replace models of tissue hierarchy.

**Important concepts:** The architecture of the fascia allows continuity of nerves, blood and lymph vessels between the sliding tissues. Fascial layers are able to produce a lubricant, hyaluronic acid, which allows sliding between fascia and neighboring muscle. With trauma to the muscle, the overlying fascia no longer produces the sliding layer of hyaluronan. Blood vessels and connective tissue fibers are observed in vivo to directly connect tendon and tendon sheath despite up to 2 cm of motion between these tissues. The architecture of the connective tissue allows this continuity with extensibility. Anatomic and clinical studies will be necessary to identify and improve methods to maintain sliding after tissue trauma.

## 7 PATHOLOGY

Tendons and ligaments are not generally included in the list of tissues comprising fascia. However, there is much to be learned from study of pathology in related tissues. Furthermore, anatomical studies are beginning to show the widespread connection of tendons and ligaments to surrounding fascial tissues. Just as cartilage was included in a keynote presentation at the first fascia congress, so will tendon and ligaments be included in this congress.

7.1.1 Fiber bundles in ligaments are not straight, but have small or larger amounts of undulation or

twisting at resting length. When the ligament is stretched, those with less undulation reach the straight position first and begin to offer increased stiffness to stretch. As stretch increases, when the last fibers become straight the stiffness of the ligament rises sharply, resulting in a non-linear length-tension curve. At a constant load, ligaments continue to elongate or "creep" and at a constant length, they show a decrease in tension over time. The rate of elongation also affects tension in the ligament, with higher rate of stretch leading to higher tension. Repeated cycles of stretch-relax show gradually reducing tension, or hysteresis, with more creep the higher the frequency of the loading cycles. Complete recovery from each cycle may take 20-40 times longer than the cycle itself, such that persons with jobs requiring repetitive motion may not have completely recovered 24 hours later at the beginning of the next shift. Moderate activity over time results in increased ligament strength, and immobilization decreases strength. Ligaments not only stabilize joints, but have important sensory function for proprioception and ligamento-muscular reflexes. Acute overload from cyclic or static loads results in decreased muscular reflexes due to changed sensory threshold from creep, spasms elicited by damage to collagen fibers, hyper-excitability of reflexes which progressively increase in the first 6 hours of rest, and then slowly exponentially decrease to normal. Mechanical injury is affected by load magnitude, number of repetitions, cycle of work and rest, and work duration.

7.1.2 Tendon consists primarily of type I collagen fibrils with small numbers of fibroblast cells. While there are small increases in tendon cross section after short term loading of 2-3 months, longer term loading may increase tendon size by 30%, with corresponding increase in stiffness. The loading must be greater than 3% strain, and is close to levels which induce tissue damage. There also is increased collagen synthesis which changes the stiffness beyond that expected by size increase alone; this is probably related to transforming growth factor β-1 (TGF-β-1) and to insulin like growth factor-I (IGF-I) but the mechanism for this is not fully established as yet.

7.1.3 Tendon function has been studied using enzymatic degradation, microdialysis, non radioactive isotopes, and tendon biopsies. Acute exercise increases tendon collagen synthesis from a base rate of 1%/day to 2-3%/day, with both base and increased rates higher in men than in women. Injured tendon shows disturbance of the organization of collagen fibrils, increased cellularity and proteoglycan concentration, but surprisingly there is a lack of inflammatory cell infiltration. The individual fibrils themselves seem unchanged, suggesting the problem may be in the organization and extra-fibrillar material. Tendinopathy symptoms of activity related pain, focal tenderness and decreased strength and movement are treated successfully with heavy load eccentric training but the mechanism for this is not understood.

7.1.4 Proline is an amino acid which is incorporated into collagen. It can be labeled with a non radioactive isotope of Flourine and has been used as a tracer to follow fibrosis as a pathologic process. This study explores its use to follow changes in musculoskeletal connective tissue after exercise in rats. Tendon, muscle and bone all showed visual uptake of the tracer, but only trabecular and cortical bone showed significant increase in uptake from 1 to 4 hours after tracer injection. RNA measures of gene expression showed 2-5 fold increase in muscle and 2 fold increase in tendon after 3 days of treadmill running 20-60 minutes per day. Neither muscle, tendon or bone showed measurable effects of exercise on uptake of the collagen tracer. This project illustrates the range of molecular probes and imaging methods available for fascial studies.

7.1.5 Patients with chronic patellar tendinopathy improved in symptoms, quadriceps strength and size after 12 weeks of heavy resistance training. Biopsy showed that fiber density increased 70%, to normal levels, with increased numbers of small diameter fibers. Tendon stiffness and modulus determined from ultrasound measurement of tendon elongation and patellar tendon forces, however, decreased 10% from pre exercise.

**Important Concepts** Tendons normally have a slow turnover of their collagen, and many months of heavy loading close to damaging levels are needed to enlarge them. Injured tendons show changes in fiber density and in the organization of the collagen fibrils, and this too is improved by heavy load eccentric resistance training. Ligaments have a collagen structure, with fibers at varied angles and degrees of undulation. This allows the ligament to stretch over a range of loads, but they then require a rest period which can be 20-40 times longer than the loading time. Ligaments have sensory receptors for joint proprioception and to trigger ligamento-muscular reflexes which result in muscle activity protecting the joint. This too requires rest to recover. Molecular probes are being developed to follow collagen incorporation during exercise and repair.

## 8 PAIN AND INNERVATION

8.1.1 This article is designed to increase the clinicians understanding of the anatomical and physiological processes underlying common types of muscle pain. Musculoskeletal pain is a common reason for physician visits. It differs from cutaneous pain by being poorly localizable, tending to refer to other areas of the body, and by being difficult to tolerate. It tends to have a tearing, cramping or pressing quality, in contrast to stabbing, burning and cutting for cutaneous pain. Free nerve endings in muscles can be activated by mechanical stimuli and by adenosine triphosphate (ATP) which can leak from any type of damaged cell, low pH (6 to 7), or nerve growth factor (NGF) which is made in larger quantities by inflamed muscle cells. When activated, muscle nociceptors release neuropeptides which induce local edema and increase nociceptors sensitivity to further stimulation. If the nerve axon coming from the nociceptors is directly compressed, it generates an axon potential which travels in both directions – centrally to carry the signal of pain, and peripherally to the nerve ending to release stored neuropeptides. Sensitization of the muscle nociceptors results in greater response to stimulation. Stimuli that normally do not cause pain become painful, and those which normally cause pain become more painful. The processes whereby Central Sensitization (CS) emerges are outlined. One model involves excitation of muscle nociceptors leading to hyperexcitability of spinal sensory neurons, suggesting that low frequency activity in muscle nociceptors is sufficient to induce CS. Insufficient descending pain inhibition is another feature of CS. CS itself leads to increased excitation in the spinal cord and to referral of muscle pain – with fibromyalgia as an example. The authors note that: "*Clinical examination reveals sites of excessive sensitivity to palpation (tender points, TeP), at which mild externally applied pressure causes pain. Many of these TeP's are located at the myotendinous junction, rather than near the belly of the muscle, where myofascial trigger points are more likely to be found*." In contrast to myofascial trigger points, which have identifiable local thickening of muscle fibers, there is no pathology which can be found at these tender points. Fascial structures (painful myotendinous junction) may therefore contribute to peripheral sensitization and therefore to CS.

8.1.2 Carageenen injection into the rat lumbar fascia induced inflammation; thickness measured by ultrasound doubled. Injected animals showed increased mechanical sensitivity to touch and 15% decrease in gait stride length and intrastep distance. These changes were completely ameliorated by 10 minutes of lumbar stretching twice a day for 12 days. Saline injection and sham stretch did not show these effects. While previous animal models of low back injury have focused on spine injury, this study points to the importance of the lumbar connective tissues regarding both impairment of function and treatment.

8.1.3 The role of thoracolumbar fascia (TLF) as a source of low back pain has been difficult to assess. In this study both animal (rat) and human tissue were studied. In the rat a quantitative evaluation was made of calcitonin gene-related peptide (CGRP) and substance P (SP)-containing free nerve endings. The resulting data show that the TLF is a densely innervated tissue with marked differences in the distribution of the nerve endings over the fascial layers –outer layer (transversely oriented collagen fibers adjacent to the subcutaneous tissue); middle layer (massive collagen fiber bundles oriented obliquely to the animal's long axis); inner layer (loose connective tissue covering the paraspinal muscles). The subcutaneous tissue and the outer layer showed a particularly dense innervation with sensory fibers. SP-positive free nerve endings—assumed to be nociceptive—were exclusively found in these layers. The middle layer does not have these nerve endings which would presumably be excited by the shearing forces during normal activities in this layer. The suggestion is that because of its dense sensory innervation, including presumably nociceptive fibers, the TLF may play an important role in low back pain. *"The finding that most CGRP- and SP-ir (sensory) fibers are located in the outer layer of the fascia, and the subcutaneous tissue, may explain why some manual therapies that are directed at the fascia and the subcutaneous tissue (e.g. fascial release) are often painful."*

8.1.4 Eccentric contractions result in soreness 1-2 days after exercise, with 25% reduction in voluntary force and 30% decrease in pressure pain threshold. This mechanical hyperalgesia is due to both central and peripheral mechanisms. Injection of hypertonic saline was used to explore peripheral sensitivity in human volunteers. Injection into the fascia/epimysium was associated with increased pain after eccentric exercise, while injection into the deep muscle was not, suggesting that fascia rather than muscle tissue has an important role in perception of delayed onset muscle soreness.

**Important concepts:** Understanding the physiology of pain and pain perception can help the clinician

initiate and evaluate treatment although there are strong psychological components which are not addressed here. The clinician needs to know about muscle tender points (which have no identifiable underlying pathology) versus myofascial trigger points which do have clear pathological changes. Understanding fascial changes may lead to new therapeutic approaches such as the stretching used in study 8.1.2.

CHAPTER 9 THERAPY

9.1.1 The clinical application of mechanotransduction is described and explained by means of details of how cells sense and respond to mechanical loads The paper outlines ways in which load can be used therapeutically by means of exercise and manual methods that stimulate tissue repair and remodeling in tendon, muscle, cartilage and bone. Key features of this process are: 1) Mechanocoupling - that describes physical load (often shear or compression) that perturbs cells, transforming into various chemical signals - within and among cells 2) Cell–cell communication – a feature in which stimulus in one location leads to a distant cell registering a new signal, despite distant cell receiving no mechanical stimulus and 3) Effector cell response **–** the way mechanical loading stimulates protein synthesis at the cellular level, promoting tissue repair and remodeling . "*Tendon can respond favorably to controlled loading after injury. Research into the ideal loading conditions for different types of tendon injury is ongoing.*"

9.1.2 A theoretical framework is discussed for the role that fascia may play in apparently diverse passive manual therapies, along with a brief review of the relevant anatomy of fascia. Myofascial ('*soft tissue*') and manipulative ('*joint-based*') therapeutic interventions are compared and using measures of pain, function and '*autonomic activation*'. Evaluation of outcomes between these forms of therapy suggests that they are usually comparable in the quality, if not the quantity of the results, with little to distinguish such outcomes from a patients' perspective. The authors propose that biologically plausible mechanisms for these results – neuro-physiological as well as non-neurological - relate to the therapeutic stimulation of fascia, in its various forms.

9.1.3 In this study repetitive motion strain (RMS) and myofascial release (MFR) were modeled in vitro to investigate possible cellular and molecular mechanisms to potentially explain the immediate clinical outcomes associated with RMS and MFR. Cultured human fibroblasts were strained with 8 hours RMS, followed by 60 seconds of MFR. Fibroblasts were immediately sampled upon cessation of strain and evaluated for cell morphology, cytokine secretions, proliferation, apoptosis, and potential changes to intracellular signaling molecules. Among the findings it was noted that modeled injury (RMS) displayed enhanced apoptosis activity and loss of intercellular integrity. Simulated treatment with MFR, following RMS, resulted in normalization in apoptotic rate and cell morphology. These *in vitro* studies build the cellular evidence base needed to fully explain clinical efficacy of manual manipulative therapies.

9.1.4 Thirty patients were measured with high-frequency ultrasound (22 MHz) immediately before and after their first treatment (connective tissue massage – skin rolling) in the area in which they experienced pain or other discomfort and/or movement restriction. Highly significant differences were visualized in the structure of the collagen matrix in the dermis, before and after treatment. These changes reflect the palpated differences in tension, softness and regularity, possibly caused by changes in the mechanical forces of fibroblasts and increased microcirculation. Measurement of the distribution of collagen showed a reduction of the highest densifications in the dermis - mainly in the transition zone with the subcutis and an increase in thickness of the dermis.

9.1.5 Exercise to exhaustion caused muscle damage with tears to muscle fibers, and changes in muscle metabolites. Ten minutes of quadriceps massage to one leg following the exercise showed no effect on muscle glycogen and lactate. However, muscle biopsy showed activation of mechanotransduction signaling pathways within the cell immediately after massage, and nuclear changes several hours later showed increase mitochondrial formation in the massaged leg. These accompanied reduced inflammation and cell stress. The authors conclude "our findings suggest that the perceived positive effects of massage are a result of an attenuated production of inflammatory cytokines, which may reduce pain by the same mechanism as conventional anti-inflammatory drugs."

**Important concepts** Khan (9.1.1) and Simmonds (9.1.2) propose a conceptual framework to prescribe therapy based on its effects on fascia. This chapter describes effects of massage at the cellular level, from basic science studies (9.1.2) to in vivo human research (9.1.5) as well as structural effects on collagen matrix of the dermis. Papers in other chapters address rest for ligamentous injury (7.1.1), heavy resistive exercise for

tendons (7.1.2, 7.1.5) and tissue stretch for inflammation (8.1.2).

CHAPTER 10 RESEARCH METHODS, IMAGING AND HYPOTHESES

Musculoskeletal ultrasound is an important technique to image fascial tissues. Its use is discussed in papers 3.1.3, 3.1.4, 5.1.1, 5.1.4, and 9.1.4, and throughout the submitted abstracts: chapter 4 (6, 7, 9, 10) 5.2.8, 7.2.5, 7.2.6, chapter 8 (1, 2, 3, 11, 12), 9.2.22, 10.2.4, and 10.2.22. Lesser known but promising methods are presented in the following papers.

10.1.1 Imaging of tissue sections and living animals with particular focus on collagen can be performed to an accuracy of 1 micrometer and a depth of 1 mm, using multiphoton excitation flourescence microscopy with laser scanning (MPEF). Recent developments have reduced the associated phototoxicity and photobleaching by eliminating the need for flourescence using techniques of second harmonic generation (SHG), and third harmonic generation (THG) from a Ti: sapphire laser, and Coherent anti-Stokes Raman Scattering (CARS) from a Nd:YVO4 laser. Images of ex vivo muscle fascia with SHG detect parallel collagen type I fibrils and fiber bundles of 1 micron or less in diameter. SHG and CARS show these are arranged in collagen sheets 10-20 microns thick, and THG shows the border between collagen sheets. In this paper the three methods are combined in the same sample to give more detailed information about the collagen tissue.

10.1.2 Blood flow and collagen density in achilles tendons from rats exercised daily for 12 weeks were compared to controls. The collagen surrounding the tenocytes showed reduced collagen density and abnormal fibrillar collagen in localized regions by SHG imaging. Tenocytes were rounded with prominent cytoplasm. The normal rat tendons showed closely and regularly spaced fibrillar collagen with elongated slender tenocytes. Quantitative analysis of collagen density showed 70% of normal values. The authors interpret these findings as indicating that the tenocytes actively mediate degradation or remodeling of the collagen matrix, rather than the collagen changes reflecting accumulation of fatigue damage.

10.1.3 Collagen displays piezoelectric properties and this was invoked 50 years ago from studies of dried bone as a mechanism to explain the remodeling of bone in response to stress. However, when investigators began studying wet bone, they found the remodeling was directed by streaming potentials from the movement of fluid through the rigid bone channels (canaliculi connecting with Haversian canals) This paper models how collagen piezoelectricity impacts the streaming potential and suggests experiments to test this proposed effect.

10.1.4 Collagen type I is made up of tropocollagen molecules which have dimensions of 280 nm in length and 1.5 nm in diameter. 10 micron thick samples of fascial tissue were imaged with second harmonic generation (SHG) and Piezoresponse Force Microscopy (PFM). The mechanism of forward and back scattered SHG images is discussed. Fascia has an upper layer of quasi-randomly oriented fibers and a lower one with separate parallel collagen bundles 2-10 microns wide consisting of individual fibrils 30 nm in diameter organized in groups of 2-3 fibrils.

**Important Findings**

Piezoelectric properties of collagen in fascia are used to generate detailed images of collagen fibrils using second harmonic generation (SHG) with laser microscopy, with resolution showing individual fibrils of 30 nanometers. The same microscope set up can be used for Piezoresponse force microscopy at the same time. SHG images can also be combined with third harmonic generation and coherent anti-stokes Raman scattering. These techniques show changes in rat tendons after 12 weeks of exercise which suggest active degradation and/or remodeling of the collagen matrix by the tenocytes. The same piezo electric nature of collagen was once thought, incorrectly, to be a major factor in bone remodelling, and is now felt due to fluid streaming potentials. Hence this chapter returns the reader to Chapter 2, fluid flow, as another frontier of fascia research.