**REPUBLIC OF TURKEY**
**HACETTEPE UNIVERSITY**
**INSTITUTE OF HEALTH SCIENCE**

# A COMPARISON OF IMMEDIATE EFFECTS OF PASSIVE STRETCHING EXERCISES AND MANUAL THERAPY TECHNIQUES FOR REDUCING STIFFNESS ON MUSCLE AND TENDON UNIT OF QUADRICEPS FEMORIS MUSCLE

**Haris BEGOVIC**

**Program of Physical Therapy and Rehabilitation**
**Philosophy of Doctoral (PhD) Thesis**

**ANKARA**
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**ANKARA**
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|---|---|
| Department | : **Physiotherapy and Rehabilitation** |
| Program | : **Physiotherapy and Rehabilitation** |
| Title of the thesis | : **A COMPARISON OF IMMEDIATE EFFECTS OF PASSIVE STRETCHING EXERCISES AND MANUAL THERAPY TECHNIQUES FOR REDUCING STIFFNESS ON MUSCLE AND TENDON UNIT OF QUADRICEPS FEMORIS MUSCLE** |
| Full name of the student | : **Haris BEGOVIC** |
| Examination date | : **15. 01. 2014** |

This thesis was approved as a doctoral (PhD) thesis.

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APPROVAL:

This doctoral thesis was unanimously approved by the Examination Panel and Board of the Directors, Institute of Health Sciences in accordiance with the relevant postgraduate examination rules and regulations at the Hacettepe University.

Prof. Dr. Ersin FADILLIOĞLU
Director of the
Institute of Health Sciences

## ACKNOWLEDGEMENT

I thank to my advisor, Prof. Dr. Filiz CAN, who has given great effort for designing the hypothesis, methodology and all other parts of the thesis. Her scientific and moral supports have encouraged me to work hardly throughout of my PhD studentship period. She has always encouraged me to go deeply into basic medical sciences to strengthen physiotherapy both scientifically and clinically.

My special thanks to Prof. Dr. Necla OZTÜRK for her scientific, technical and moral support. She was always next to me during my laboratory research and been the saver in my most difficult moments. She taught me how much is important to understand muscle dynamics through biophysical rules and she enabled me to establish a strong connection between biophysics and physiotherapy.

My second special thanks should go to the Associate Professor Suha Yağcıoğlu. I managed to work on the laboratory due to his enabling to all equipments working actively.

Lastly, I would like to thank to my friend, mechanical engineer Riza Emre Ergun, who has manufactured the testing device for my research and, also to my family for their moral support.

# ABSTRACT

**HARIS BEGOVIC, A COMPARISON OF IMMEDIATE EFFECTS OF PASSIVE STRETCHING EXERCISES AND MANUAL THERAPY TECHNIQUES FOR REDUCING STIFFNESS ON MUSCLE AND TENDON UNIT OF QUADRICEPS FEMORIS MUSCLE, DOCTORAL THESIS IN THE PROGRAM OF PHYSIOTHERAPY AND REHABILITATION, ANKARA, 2014.** Stretching exercises and manual therapy techniques are commonly used in order to reduce stiffness of muscle and tendon. It is suggested that muscle dynamics consisting of excitation-contraction coupling, electromechanical delay and muscle fiber vibration and force output may be altered using these thechniques. Time delays between onset of electromyographic (EMG), mechanomyography (MMG) and Force (FORCE) have been using to assess the changes in the dynamics of muscle after various techniques. The aim of this study was to investigate acute changes in the electro-mechanical properties of quadriceps femoris muscle and tendon after passive streching and manual therapy techniques. A total of 56 healthy subjects aged between 20-35 years old ( have been divided into 4 groups as passive stretching, manual therapy, passive stretching + manual therapy and control group. Subjects in the interventional groups had only one treatment session and they have been assessed immediately after the session. There was no application for the control group. The delays between EMG and MMG signals and, Force-Output of quadriceps femoris during voluntary isometric contraction at three different knee flexion angles, 15°,30° and 45° were simultaneously measured on a device originally derived for positioning lower extremities and a custom made amplifier for signal detection. Signal records have been taken from Rectus Femoris (RF) and Vastus Medialis (VM) muscle fibers. For RF muscle, time delay between EMG-MMG and MMG-Force increased after stretching at 15° knee flexion, while time delay between EMG-Force (electromechanical delay) increased after manual therapy at 15° and 30° knee flexion. Manual therapy + stretching has lengthened the time delay between all onsets of EMG, MMG and Force output in isometric contraction at 15° knee flexion, while time delay between EMG-MMG and EMG-Force has incresed at 30° and 45° knee flexion. For VM muscle, time delay between EMG-Force increased at 15° and 45° knee flexion after stretching. Manual therapy has increased time delay between EMG-Force only at 15° knee flexion. Manual therapy + stretching has lengthened all time delays between EMG, MMG and Force at 15° knee flexion while time delay between EMG-MMG increased only at 30° knee flexion. Comparison of the all application groups revealed that the application of manual therapy has better effects than stretching or manual therapy + stretching when they were tested at 15° of knee flexion for both RF and VM muscles. Stretching was more efficient to reduce stiffness at muscle fiber level for RF muscle during force generation in 15° of knee flexion, whereas manual therapy had beneficial effects to increase time of overall process starting by EMG signal till the generation of force output at both 15° and 30° of knee flexion. For VM muscle, both stretching and manual therapy were efficient to increase time delay of overall process between EMG and Force at 15° of knee flexion. Combination of manual therapy + stretching has increased time delay of overall process in EMG signals in accordance with force output at 15° of knee flexion. Such increase in time delay between EMG signals and force output began to gradually decrease at 30° and 45° of knee flexion for both RF and VM muscles.

Key Words: Electromyography, mechanomyography, Quadriceps tendon, tendon stifness, rectus femoris, vastus medialis, stretching, manual therapy, friction massage

# ÖZET

**HARİS BEGOVİC, QUADRİCEPS KASININ SERTLİĞİNİ AZALTMADA KULLANILAN PASIF GERME VE MANUEL TEDAVI YÖNTEMLERİNİN KAS VE TENDON ÜZERİNE OLAN ETKİLERİNİN KARŞILAŞTIRILMASI, DOKTORA TEZİ, FİZİK TEDAVİ VE REHABİLİTASYON PROGRAMI, ANKARA, 2014.** Germe egzersizleri ve manuel tedavi teknikleri, kas ve tendon sertliğini azaltmak için yaygın olarak kullanılmaktadır. Bu tekniklerin kullanılmasıyla, kas dinamiklerini oluşturan uyarılma-kasılma çifti, elektromekanik gecikme, kas liflerinin vibrasyonu ve güç çıkışının değiştirilebileceği öne sürülmüştür. EMG ve EMG-Güç (EMG-Force) arasındaki zamansal gecikmeler, çeşitli tekniklerden sonraki kas dinamiklerinde ortaya çıkan değişiklikleri değerlendirmek için kullanılır. Bu çalışmanın amacı, manuel tedavi ve pasif germe sonrası Quadriceps kası ve Quadriceps tendonunun elektro-mekaniksel özelliğinde ortaya çıkacak akut değişiklikleri incelemektir. Yaşları 20-35 arasındaki toplam 56 sağlıklı birey, pasif germe, manuel tedavi, pasif germe+ manuel tedavi ve kontrol grubu olmak üzere 4 gruba ayrılmıştır. Uygulama gruplarındaki bireyler tek bir tedavi seansı almışlar ve seanstan hemen sonra değerlendirilmişlerdir. Kontrol grubundaki bireylere bir uygulama yapılmamıştır. 15°, 30° ve 45° olmak üzere 3 farklı diz fleksiyon açısında yapılan istemli izometrik kontraksiyonlar sırasında Quadriceps femoris kasının güç çıkışı(Force output) ve Elektromyografik(EMG) ve mekanomyografik (MMG) sinyalleri arasındaki gecikmeler, alt ekstremiteyi pozisyonlamak için özel olarak tasarlanmış bir cihaz ve bir amplikatörler vasıtasıyla eşzamanlı olarak ölçülmüştür. Sinyal kayıtları, Rectus Femoris (RF) ve Vastus Medialis (VM) kaslarından alınmıştır. Rectus femoris kası için EMG- MMG ve MMG-Force arasındaki zamansal gecikme, germe egzersizlerinden sonra ve 15° de artarken, EMG-Force (elektromekanik gecikme) arasındaki gecikme manuel tedaviden sonra 15° ile 30° diz fleksiyonunda artmıştır. Manuel tedavi + germe, 15° diz fleksiyonunda tüm sinyaller arasındaki (EMG-MMG-Force) zamansal gecikmeleri uzatırken, 30° ve 45°'lik diz fleksiyonlarında sadece EMG-MMG ve EMG-Force sinyallerindeki zamansal gecikmelerde artış olmuştur. VM kası için EMG-Force arasındaki zamansal gecikme, germe egzersizlerinden sonra 15° ve 45° diz fleksiyonunda artmıştır. Manuel tedavi uygulaması, EMG-Force arasındaki gecikmeyi, sadece 15° diz fleksiyonunda artırmıştır. Manual tedavi + germenin, 15° diz fleksiyonunda EMG, MMG and Force sinyalleri arasındaki zamansal gecikmeyi uzatırken, 30° diz fleksiyonunda sadece EMG-MMG arasındaki zamansal gecikmeyi uzatmıştır. Tüm uygulama grupları birbirleri ile karşılaştırıldığında, 15° diz fleksiyonunda, hem RF hem de VM kasları için manuel tedavi uygulamasının, manuel tedavi+ pasif germe uygulamasına göre daha etkili olduğu ortaya konulmuştur. 15° diz fleksiyonundaki güç yayılımı sırasında, RF kasının kas fibrili düzeyindeki sertliğini azaltmak için germenin daha etkili olduğu bulunurken, hem 15° hem de 30°'lik diz fleksiyonunda güç çıkışının yayılımına kadar EMG sinyalleri tarafından başlatılan tüm sürecin zamanını artırmak için manuel tedavinin daha yararlı etkilere sahip olduğu bulunmuştur. VM kası için hem germe hem de manuel tedavi, 15° lik diz fleksiyonunda EMG ve güç arasındaki tüm süreçlerdeki zamansal gecikmeyi artırmada etkili olmuştur. Manuel tedavi ve germe kombinasyonu, 15° diz fleksiyonunda güç çıkışı ile uyumlu olarak EMG sinyallerindeki tüm zamansal süreçlerin artış zamanını artırmıştır. Hem RF, hem de VM kası için EMG sinyalleri ve güç çıkışı arasında gecikme zamanındaki bu artış, 30° and 45°'lik diz fleksiyonunda dereceli olarak azalmaya başlamıştır.

Anahtar kelimeler: Elektromiyografi, mekanomiyografi, Quadriceps tendonu, tendon sertliği, rectus femoris, vastus medialis, germe, manuel tedavi, friksiyon masajı

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# ABBREVIATIONS

| | |
|---|---|
| CC | contractile components |
| EMD | electromechanical delay |
| EMG | electromyography |
| FA | force – activation |
| FV | force – velocity |
| FL | force – length |
| FΔL | force vs length curve |
| GTO | golgi tendon organ |
| LC | load cell |
| MTU | muscle tendon unit |
| MTJ | myotendinous junction |
| MMG | mechanomyography |
| MUAPs | muscle unit action potentials |
| MAS | musculoarticular stiffness |
| PEC | paralel elastic components |
| PCSA | physiological cross sectional area |
| QF | quadriceps femoris muscle |
| ROM | range of motion |
| RTD | rate of torque development |
| RF | rectus femoris |
| SEC | series elastic components |
| SA | stimulation – activation |
| VL | vastus lateralis |
| VM | vastus medialis |
| VI | vastus intermedius |
| VMO | vastus medialis obliqus |
| VLO | vastus lateralis obliqus |

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## 1. INTRODUCTION

Both stretching and massage techniques have been using frequently by orthopaedic and sport physical therapists in an attempt to acutely increase range of motion and to decrease muscle stiffness (1). Many orthopaedic problems such as patellofemoral pain syndrome resulted by patellar malalignment or degenerative knee joint are affected by quadriceps inflexibility caused by increased stiffness (2). In order to increase quadriceps flexibility, static stretching exercises, musculotendinous junction massage (transvers friction massage) and patellar mobilization techniques are commonly used in clinical practice. Each one of these techniques have different effects along the way on decreasing stiffness in muscles. Static stretching exercises have been investigated under different conditions using different methodologies (3, 4, 5, 6, 7, 8, 9, 10, 11). It has been shown that cyclic and static stretching did not produce any change on muscle-tendon unit (MTU) viscoelastic properties (5). Three weeks of static stretching program has not reveal any changes in tendon stiffness (8). Passive muscle stiffness is defined as the length-tension relationship of passive muscle or slope of the linear portion in the passive torque-angle curve when it is passively stretched (12). Passive muscle stiffness properties has been investigated in some studies (7,8,9) showing that MTU stiffness following repeated stretches was not due to changes in the tendon but rather to an increased compliance of the proximal muscular portion of the MTU (7). One of these studies showed that effect of static stretching was lasting at least for 10 minutes after its application and prolonged effect of stretching was more influential on reducing muscle stiffness rather than tendon stiffness (8). So, there are controversial results about effects of stretching and part of MTU (either muscle or tendon) being affected by stretching.

Various massage techniques like petrissage, tapotement, effleurage and friction massage have already been investigated (13, 14, 15, 16). In one of the study, there has been observed signals changes of the electromyographic (EMG) and mechanomyographic (MMG) amplitude and reduced force production when knee joint has been tested at 60°/s velocity in cybex machine after massage treatment (14). However they could not find any alteration in muscle power (16). Some authors have found reduction of α-motoneuron excitability (17) and increased muscle activity when they initiated massage therapy with deep pressure massage (18). Transverse friction massage is a commonly used technique by physical therapists to relieve pain and to prevent adhesions in soft tissues. When effects of transverse friction massage on distal portion of myotendinous junction (MTJ) of hamstring muscle have been investigated, it was found increased hip range of motion (ROM) in flexion. This represents as an indication of increased hamstring flexibility, increased muscle compliance and decreased muscle stiffness (15). Continuous application of massage of the MTJ could theoretically activate the inhibitory action of the Golgi Tendon Organs (GTO) or lead to a presynaptic inhibition of the I a sensory fibers from the muscle spindles of the activated muscle (19). Massaging for MTJ of distal portion of hamstring muscle was delivered without additional stretching and its effect on stiffness and flexibility was verified by measuring ROM of hip flexion in one of the study in the literature. However, they did not have any control group in their study and they had no chance to compare the efficacy of the massage. They recomended a future study which the one must include control group to determine combined effects of massage and static stretching in addition to duration of the technique. In the literature, there is a few study measuring musculo-articular stiffness (MAS) using simultaneous recording of EMG, MMG, FORCE and Ultrasonography (20, 21, 22, 23). Simultaneous measurements of EMG, MMG and FORCE may provide insight into the relationship between the mechanical and electrical events associated with a contracting skeletal muscle (24, 25). Stiffness of the tendon is a primer modulator of the musculoarticular stiffness (MAS) at low loads. Muscle stiffness is primarly a function of the numbers of attached cross-bridges between muscle fibers and it increases as isometric muscle torque increases (26). In this situation, simultaneous measurement of EMG and MMG with FORCE can be used to monitor the dissociation between the electrical and mechanical aspects of muscle function (27). MMG records lateral oscillations or grosslateral movements of the muscle fibers at the beginning and at the end of the muscle actions which will be generated by nonsimultaneous activation of the muscle fibers (28). Increase in muscle tension results with increase in intramuscular fluid pressure (IMFP), thus preventing oscillations of the muscle fibers leading to decrease in MMG amplitude. (24) The MMG amplitude is influenced by many factors like muscle stiffness, muscle tension, length, mass of muscle, intramuscular pressure, the viscosity of surrounding tissue, and the motor unit firing frequency (29). In one of the study, a significant correlation was observed between increased muscle-tendon tension and electromechanical delay (EMD) (30). Reduced electromechanical delay shows increase in muscle and tendon stiffness (30), while increased electromechanical delay shows decrease in muscle-tendon stiffness (31, 22). Thus, time delay between EMG-MMG ( time index of local motion prior to the elongation of the passive series elastic components), EMG-FORCE ( electromechanical delay as a time elapse between onset of action potential and force production) and MMG-FORCE (duration of overall events after cross-bridge formation) would be of high importance to observe muscle dynamics changes and behaviors after some physical therapy interventions like exercise, stretching, manual therapy or massage. However, there is no studies in the literature which shows clearly the effects of physical therapy interventions such as stretching or manual therapy techniques on the MTU assessed by time delay recording EMG, MMG and Force. If we could measure all these biomechanical responce of MTU after physical therapy interventions, we would know more details in all muscle dynamics changes and behaviours. This would give us beneficial results for clinical practice and to compare their acute or late effects. Despite there are many individuals suffering from Quadriceps muscle and tendon stiffness in the clinics, there is stil lack of knowlodge on efficacy of stretching and manual therapy and, their comperable effects in terms of muscle dynamics.

On these basis, the aims of the present study were to assess time delays between EMG, MMG and FORCE in three different interventional groups, which are: passive stretching, manual therapy ( transverse friction massage and patellar mobilization), and combined therapy consisted of passive stretching and manual therapy ( transverse friction massage and patellar mobilization). The results obtained from all these groups were compared with the results obtained from the control group which was non-interventional group but with the same testing procedure as in the interventional groups. The final aim of this study was to find out the most effective intervention to reduce muscle and tendon stiffness.

The hypothesises of this study were that when manual therapy, stretching and manual therapy + stretching applied there will be increase in time delays between EMG, MMG and Force onsets indicating changes in the muscle dynamics which happen during stiffness changes of muscle and tendon of Quadriceps Femoris (QF) muscle.

Hypothesis 1: Passive stretching increases time delay between EMG, MMG and Force.

Hypothesis 2: Manual therapy ( transverse friction massage + patellar mobilizatin) increases time delay between EMG, MMG and Force.

Hypothesis 3: Combined therapy consisted of passive stretching + manual therapy ( transverce friction and patellar mobilization) increases time delay between EMG, MMG and Force.

Hypothesis 4. Combined therapy consisted of passive stretching + manual therapy ( transverce friction and patellar mobilization) has better effects on the increasing time delay comparing with the each intervention alone.

**2. GENERAL KNOWLEDGE**

Muscle are attached to bone via tendons, which compromise densely packed connective tissue. Tendons, similar to muscles, are devided into fibriles, fibers, fiber bundles, and fascicles. However, the tendon function as a whole of withstand high tensile forces. A tendon consists of 55% to 70% water. In all, 60 to 85% of the dry weight of the tendon is composed of collagen. (32)

Muscle is that tissue type which most completely expresses cell contractility. It is a composite structure consisting of muscle cells or fibers, and the connective tissue network that transmits the pull of the muscle cells. The sarcomere, the basic contractile unit that makes up most of the muscle cells, is composed of actin and myosin. These contractile proteins are arranged in specific pattern, which gives muscle tissue characteristic striated apparance. Sarcomeres are further arranged in paralel to form myofibrils (muscle fiber sor cells), which in turn are arranged in bundles to form fascicles and finally a whole muscle. Other qualities of the muscle structure, particularly the contents of the sarcoplasm, cause muscle fibers to be classified as red ( fast-twitch), white (slow twitch), or intermediate fibers. These characteristics affect not only the contractile properties of the muscle fiber but also its response to exercise and immobilization (33, 34) (Fig. 2.1)

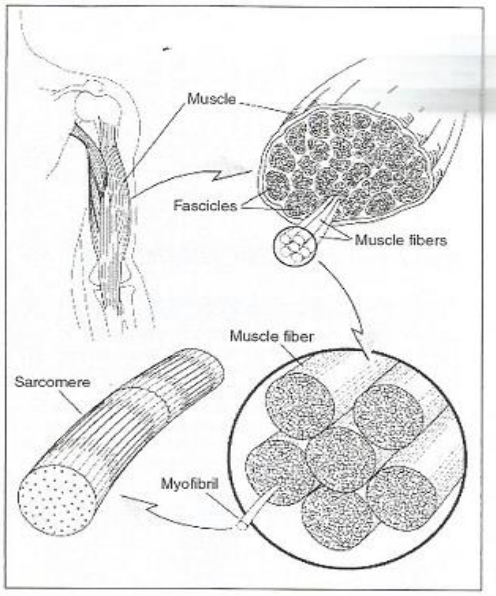


Figure 2.1. Structural hierarchy of skeletal muscle. (from Thomas A. Einhorn. Orthopaedic Basic Science. 2006-200. AAOS) (34)

Two layers of connective tissue cover the muscles: a thick outer layer called fascia, and a thinner layer called the epimysium. Lying underneath the epimysium are boundles of tissue called the fasciculi. Each fasciculus is covered by a connective tissue layer call the perimysium. The fasciculi, then are broken down into cells called muscle fibers.  Again, a layer of connective tissue called the endomysium surrounds each of these muscle fibers. The three layers of connective tissue: epimysium, perimysium and endomisyum play an important part in contraction of the muscle and provide access for blood vessels and nerves to reach the interior of the muscle. (32)

Each muscle is composed of a great number of subunits, muscle fibers, that are arranged in paralell and normally extend from one tendon to another. Muscle fibers are cable-like structures composed of tightly packed sub-units myofibrils, that fill-lup most of the volume of the fibers. The myofibrils are composed of sarcomeres arranged in series. The sarcomeres, defined as the basic units of the myofibrils, are responsible for force generation and shorthening of myofibrils. The basic unit is formed by two contractile proteins, or myofilaments, the myosin (thick) and actin (thin) filaments. Actin filaments are anchored at each end to the Z-disks. When a muscle is in a relaxed state there is some overlap between the myosin and actin filaments (35).

With the advent of electron microscopy in the early 1950s, the exact arrangement of the actin and myosin filaments within the sarcomere was discovered. By comparing micrographs of muscle in the relaxed and the contracted state, H.E.Huxley and co-workers in 1954 developed the ‘’sliding filament’’ theory, which immediately gained widespread acceptence. The manner in which this sliding occurs has been the subject of much study. When the muscle is relaxed, the lateral projections of the myosin filaments lie close to theirparent filament, whereas in contraction they project to contact the adjacent actin strands. In contracted muscle the actin filaments slide, in relation to the myosin, toward the center of the sarcomere, bringing the attached Z bands closer together and thus shortening the whole contractile unit. This and many other observations indicate that muscle contraction may be caused by the successive making and braking of cross-connections between thick myosin and thin actin filaments in a cyclical fashion, pulling the actin between the myosin toward the sarcomere center. If contraction is continued, actin filaments may overlap each other in the middle of the A band, and the Z bands may meet the ends of the myosin filaments. As the length of he sarcomere changes, so does the amount of overlap between the actin and the myosin. Since the numbers of possible cross links between the two depend on the amount of overlap, it might be expected that a muscle would generate different lengths without being allowed to shorten (1).

The three types of muscle contraction – isotonic, isometric and accentric can be correlated with the behavior of the fine structure of the contractile mechanism of actin and myosin. In isotonic contraction, in which a muscle shortens under a constant load to perform positive external work, actin and myosin cross-bridges are active in causing mutual sliding of the filaments. In isometric contraction, cross-bridges are made and broken repeatedly to maintain a constant muscle length under an external load. In eccentric contraction, a muscle generates tension while it is being actively lengthened by an external load. The precise behavior of the filaments has not been estabilished in this type of contraction, but it is probable that cross-bridges are active in the usual manner while the actin and myosin are sliding apart (36).

The last factor to be considered in the basic understanding of muscle structure and function is its innervation. Efferent, or motor, nerves supply each muscle via numerous axons. One axon may supply one or many muscle fibers by means of branching. The ratio of muscle fibers to axons in a muscle determines the fineness of the motion capabilities of that muscle. A single motor neuron and its axons together with all the muscle fibers it innervates is called a motor unit. When stimulated, the fibers belonging to a single motor unit contract either completely or not at all – the ‘’all or none’’ law. The force of contraction can vary because of circumstances as well, such as the physiologic state of the fibers and the length-tension relationship. Within any one motor unit all the fibers can be either red or white but not both.

An excitatory impulse generated naturally in the central nervous system or artificially by an electrical signal generator creates a so-called action potential (specific electrical field) at the relevant muscle fibers. The tensile force developed by a single fiber in response to a single action potential invading the motor endplates is called a twitch. As the frequency of the stimulating impulses increases, twitches begin to overlap. At frequencies above a certain limit, single twitches can no longer be discriminated and tetanic contraction develops. The frequency limit, where tetanic contraction or tetanic force generation occurs, varies among different fibers and individual motor units. This limit is normally observed in the range between 10 and 100 Hz. The higher the frequency of stimulation of the muscle fibers, the greater is the force produced in the muscle as a whole (35).

## 2.1. Muscles of the lower limb

Each muscle is unique in terms of its architecture, taken as functional groups (for example, hamstrings, quadriceps, dorsiflexors, plantar flexors). In terms of architecture, the typical properties of the various groups can be articulated.

## 2.2. Anatomic structure and architecture of Quadriceps Femoris muscle

Quadriceps femoris muscles are characterized by their relative high pennation angles, large Physiological Cross Sectional Areas (PCSA), and short fibers. In terms of design, these muscles appears suited for the generation of large forces (because force is proportional to PCSA). The hamstrings, on the outer hand, by virtue of their relative long fibers and intermediate PCSAs, appear to be designed for large excursions (because excursions are proportional to fiber length) (34).

The quadriceps femoris (QF) has two separate muscles, the two-joint rectus femoris (RF) and one-joint vastus muscles that attach via tendon to the patella, and are innervated by branches from the femoral nerve. The vastus muscles, or vasti, comprise three compartments: Vastus lateralis (VL), Vastus medialis (VM), and Vastus intermedius (VI). The RF muscle is typically described as a simple bipennate muscle, but detailed analyses have revealed a more complex structure. The proximal one-third of RF is unipennate while the distal muscle is radially bipennate. The fibers originate from an internal tendon and attach to the apponeurosis of insertion that surrounds the muscle belly. (37). The vastus medialis obliquus (VMO) and vastus lateralis obliquus (VLO) originate from septa along side the femur and approach the patella from direction that deviate from anatomic axis of the femur. The vastus medalis obliquus has a mean orientation that deviates 47° ± 5° medially from the femoral axis, and the vastus lateralis obliquus has a mean orientation that deviates 35° ± 4° laterally from the axis (38).

There is a major aponeurosis structure, the proximal extension of the quadriceps tendon, attached to RF that also extends inside the vastus muscles.

Because all of the four knee extensor muscles transmit force via this connective tissue, it is likely to have a role in normal and abnormal knee mechanics (37).

Rectus femoris initiates extension of the knee, but contributions from the vasts medialis and lateralis become important only during the latter part of knee extension. Each component of the quadriceps have independent functioning and selective strengthening of each component is emphasized during rehabilitation. Selective strengthening of vastus medialis increases dynamic medial support of the patella. Vastus lateralis provides direct lateral pull on the extensor mechanism (39).

**2.3. Form and architecture of tendons**

Tendons exhibit nonlinear viscoelastic behaviour in response to deformations or stresses, but most contribute somehow to the rather amazing resistance to fatique damage that tendons display.

The load carrying ability of tendons, resulting from their unique structural organization, is a hallmark requirement of those normal structures. It is of great importance that analogous to bones, both tendons and ligaments adjust their mechanical properties in response to their load history. Several studies have shown that with joint immobility (load deprivation), ligament properties deteriorate quickly and exponentially (they become less stiff and strong within a few weeks of immobility).

Some different morphological aspects and functional responses between suprapatellar and infrapatellar connective tissues have been described: the collagen density is statistically higher in quadriceps tendon compared to patellar ligament, and the maximum force in human quadriceps tendon exceeds that in patellar ligament by ratio of about 8:5 (40).

Frequently, tendons are classified as sheathed or synovial-covered tendons (such as the long flexors of the fingers) and unsheathed or paratenon-covered tendons (such as the Achilles tendon). These two tendon types have differences in their soft-tissue envelope and vascularity.

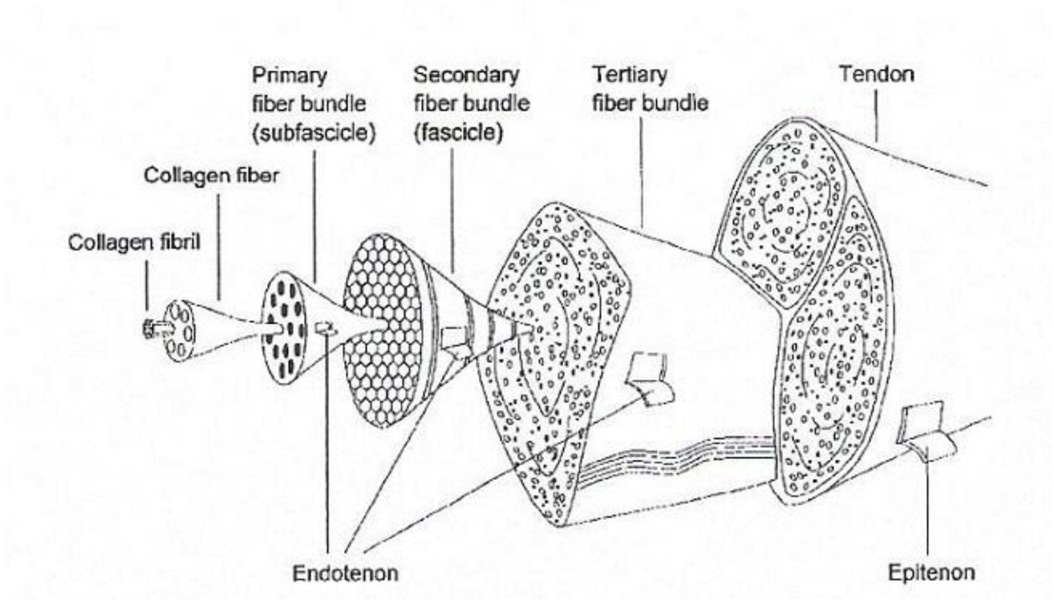


Figure 2.2. Shematic presentation of the tendon. (from Thomas A. Einhorn. Orthopaedic Basic Science. 2006-2007.AAOS) (34)

Tendon are formed primarily of collagen fibrils, which are evident ultrastructurally. These molecules form fibers that are visible on light microscopy. A collection of fibers forms a fiber bundle, a group of fiber bundles forms a fascicle, and fascicles are surrounded by a loose connective tissue network called the endotenon and the entire tendon is enveloped by a similar and contiguous (with the endotenon) structure called the epitenon. In addition to binding the tendon subunits together, both the epitenon and endotenon subunits together, both the epitenon and endotenon support blood vessels, lymphatics and nerves. Most tendons such as the Achilles tendon, patellar tendon, and flexor tendons of the finger contain multiple fascicles that spiral along the length of the tendon. This permits adjecent fascicles and fiber bundles to slide relative to one another along the longitudinal length of the tendon. (Fig. 2.2)

Although collagen is the primary component of tendon, respresenting 70% to 80% of the dry weight of tendon, it should be remembered that this respresents only the dry weight of tendon, and water is still the primary constitutent of tendons. Water accounts for 50% to 60% of the wet weight of a tendon and this component may be critical during normal and pathologic tendon structure.

Tendons consists mainly of Type I collagen (95%) with a small amount of Type III collagen (< 5%) normally present. There are also minor quantities of other collagen types (type V and VI collagen) (41).

The junction between tendon and bone may take two forms – fibrous or fibrocartilaginous. Fibrous insertions or indirect insertions (such as pes anserinus) are found in the methaphysis and diaphysis of long bones whereas fibrocartiloginous insertions or direct insertions (such as rotator cuff) are typical of tendon insertions into the epiphyses and apophyses of bone. In a fibrous insertion, the collagen fibers of the tendon insert into the periosteum during growth and development and directly into bone at maturation. Conversely, in a fibrocartiloginous insertion, there is a gradual transition from tendon to bone that is characteristically composed of four zones:

1. Tendon,
2. Uncalcified fibrocartilage,
3. Calcified fibrocartilage,
4. Bone.

This gradual transition dissipates load at the insertion site and ensures that the collagen fibers in the tendon bend gradually with joint motions.

Tendons are considered to have a rich nevre supply and are typically innervated by the nevre(s) in its associated muscle(s) in addition to local cutaneous and other nerves. Nerve end-organs including Golgi organs, Pacinian corpuscles, and Ruffini ending generally lie adjacent to the myotendinous junstion whereas free nevre ending are typically adjecent to the bone-tendon junction (32,34).

## 2.4. Tendon functions

Tendons have mostly mechanical functions. These functions of the tendon can be described as seen below:

1. The primary and most obvious function of tendons is to transmit force generated from muscle to bone
2. Tendons can center the action of several muscles into a single line of pull.
3. They can distribute the contractile force of one muscle to several bones.

4. Tendons allow muscles to be at a distance to their insertion.
5. They allow muscle pull to travel through narrow areas of the body and allow the direction of pull to be changed in conjunction with a pulley
6. They provide static and dynamic stabilization of the joints and they act as passive viscoelastic stabilizers to the joint when they are not active (passive tone)
7. Most of the tendons store elastic energy during locomotion by cyclically lengthening and shortening

Of particular importance of tendon is their nonlinear anisotropic biomechanical behaviour. Tendons are much less extensible than muscle, so tendon is stiffer than the muscle in high- speed traction (42). Under low loading conditions, tendons are relatively compliant. With increasing tensile loads, tendons become increasingly stiff until they reach a range where they exhibit nearly linear stiffness. At this point, elastic elongation is occuring as a result of slippage of the fibers; tearing then occurs through molecular slippage (increased gap between adjacent molecules). Beyond that range, tendons then continue to absorb energy up to the point of their tensile failure. This initial low-load, nonlinear behavior as called the ″toe region” is caused, in part, by the recruitment of ‘’ crimped’’ collagen fibers, as well as the viscoelastic behaviors and interactions of collagen and other matrix materials. (Fig 2.3)

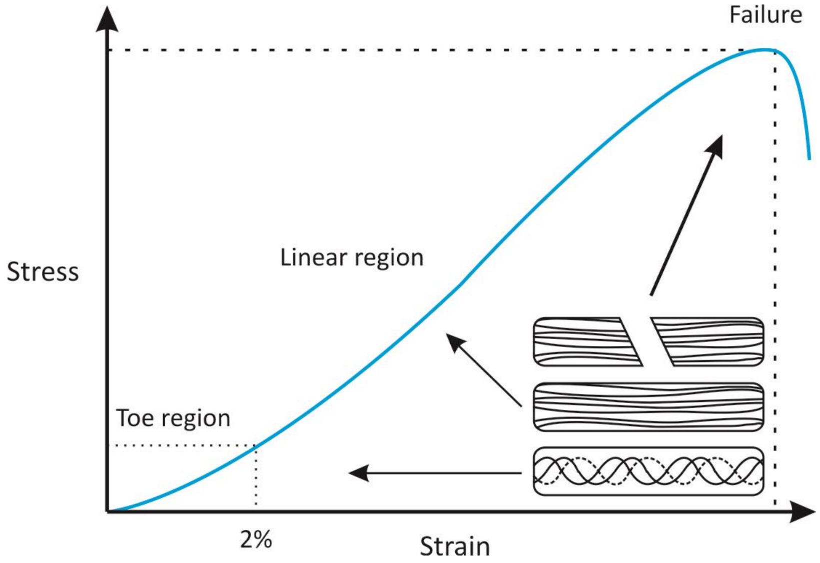


Figure 2.3. A shematic load-elongation curve (stres-strain) for tendon. Indicating three distinct regions of response to tensile loading.(from Thomas A. Einhorn. Orthopaedic Basic Science. 2006-2007.AAOS)

Material properties of tendon are represented by a stress-strain curve and are biomechanical characteristics of the tendon substance itself. These properties are therefore normalized to the cross-sectional area of the tendon and its original length. Stress is defined as force per unit area (usually Newton/mm² in soft-tissue testing) whereas strain is defined as the change in length divided by its original length. A stress-strain curve will look similar to a load elongation curve except that its values have been normalized to the dimensions of the tendon being tested. From the stres-strain curve the elastic modulus (slope of the curve), the tensile strength (stress at failure), the ultimate strain (strain at failure), and the strain energy density (area under the curve) can be calculated. In human tendons, the elastic modulus has been measured from 1,200 to 1,800 MPa, the ultimate tensile strength from 50 to 105 MPa, and the ultimate strain from 9% to 35%.

Tendons are viscoelastic, meaning they posses time-dependent and history-dependent properties. Therefore their mechanical behaviors depend on the manner, in which they have been loaded (loading rate, loading limits, loading history) and on their environments (such as temparature and water content). This behavior can be represented by several properties including load relaxation, creep, and hysteresis (34).

**2.5. The Hill musle model of muscle and tendon**

Many models coexist with Hill’s Model like Huxley Model, Huxley and Simmons Crossbridge Model, the Morgan Intersarcomere Dynamics Model, and Zahalak’s Distribution Moment Model. The Hill model is powerful and appropriate for describing muscle mechanics for the purposes of modeling and understanding most voluntary human movements.

The basic Hill model consists of the contractile component (CC), the series elastic component (SEC), and the paralel elastic component (PEC). The model represents muscle behavior rather than structure and each component has its mechanical characteristics (43,44) (Fig 2.4).

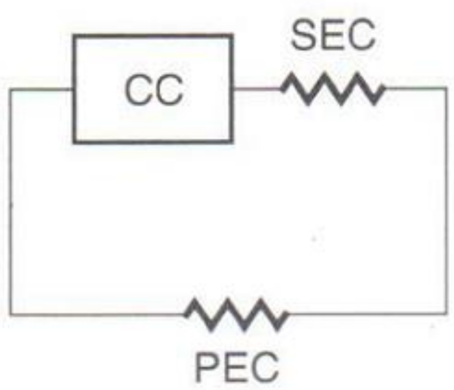


Figure 2.4. The three-component Hill muscle model consists of a contractile component (CC), series elastic component (SEC), and parallel elastic component (PEC). (from D.Gordon E.Robertson. Research methods in muscle biomechanics)

**2.5.1. Contractile components (CC)**

In the Hill model, the CC is the ‘’active’’ element that turns nervous signals into force. The magnitude of the CC force produced depends on its mechanical characteristics, which can be expressed in four separate relationships:

1. Stimulation – activation (SA)
2. Force – activation (FA)
3. Force – velocity (FV)
4. Force – length (FL) (34)

#### 2.5.1.1. Stimulation – activation, SA (excitation – contraction coupling process)

The first of the CC's mechanical properties concerns how the nervous system signal (stimulation) is related to the muscle's intrinsic force capability or potential (activation). Physiologically, this property reflects the excitation-contraction coupling process, in which α-motor neuron action potentials (APs) trigger motor unit action potentials (MUAPs) that travel along muscle fibers. These MUAPs are carried inward through the transverse tubule system to the sarcoplasmic reticulum, where they cause the release of calcium ions into individual sarcomeres. This portion of the excitation contraction coupling sequence can be considered the stimulation, because it is independent of the actual force production mechanism in the sarcomere at the level of the crossbridges, which link the thick and thin filaments containing the contractile proteins myosin and actin, respectively. The actin-myosin complex responds to the calcium ion influx by changing from its resting state (no cross-bridge attachment and no force potential) to an activated state in which force production can occur. This is the activation part of the stimulation-activation process. Stimulation represents the input to the process and the activation represents the response, or output (45).

When a motor unit is initially activated, there is a time delay between the onset of the neural AP and the activation at the crossbridge level. This time delay has two components, the first of which is the transit time for the MUAP to travel from the myoneural junction to the sarcoplasmic reticulum. The second component is the length of time for the calcium ions to be released from the sarcoplasmic reticulum and become attached to the thin filaments, a process that, when completed, removes the inhibition from crossbridge attachment imposed by the troponin-tropomyosin complex. When the force response from the motor unit is no longer necessary, the α motor neuron stops sending impulses. However, for a brief period, there is still a supply of calcium ions within the sarcomeres, allowing the crossbridges to remain activated even in the absence of stimulation. The duration of this deactivation process is longer than the activation process, and it is dictated by the time it takes fort he sarcoplasmic reticulum to reabsorb the free calci um ions within the sarcomeres (34,45, 46).

#### 2.5.1.2. Force – activation

Force – activation is a state in which force can be produced, rather than an actual force level. The force – activation relation is direct and linear (e.g. 10, 20, or 50% activation represents 10, 20 and 50% force, respectively.

#### 2.5.1.3. Force – velocity (FV)

Influence of CC velocity on force production.

#### 2.5.1.4. Force – length (FL)

The basic shape of the FL relation illustrates that isometric force production is greatest at intermediate CC lengths and declines as the CC is either lengthened or shortened.

### 2.5.2. Series elastic component (SEC) : tendon

Any force the contractile component (CC) produces, is expressed across the series elastic components (SEC). One obvious contributor to the SEC elasticity is the tendon that joins the muscle fibers to the skeleton. In this perspective tendon is considered passive connective tissue.

Other structures contributing to the elasticity;

a. Aponeurosis or inner tendon which connects tendon to muscle fibers
b. Connective elements within the muscle fibers (e.g. Z – lines)

SEC elasticity results from all elastic elements in tendon with the active force –generating elements in the muscle. Recents studies indicate that the aponeurosis contribute to a large extent to the series elasticity.

#### 2.5.2.1. SEC elastic behavior

In physics, the elasticity of a material is usually quantified by its stiffness, (k), calculated as the change in applied force divided by the resulting change in the length of the material.

$$\mathbf{K = \Delta F / \Delta L}$$

Increased SEC extension results with increase in the slope of (force-length curve) FΔL. Increased slope of the FΔL means increase in the stiffness (46). (Fig 2.5)

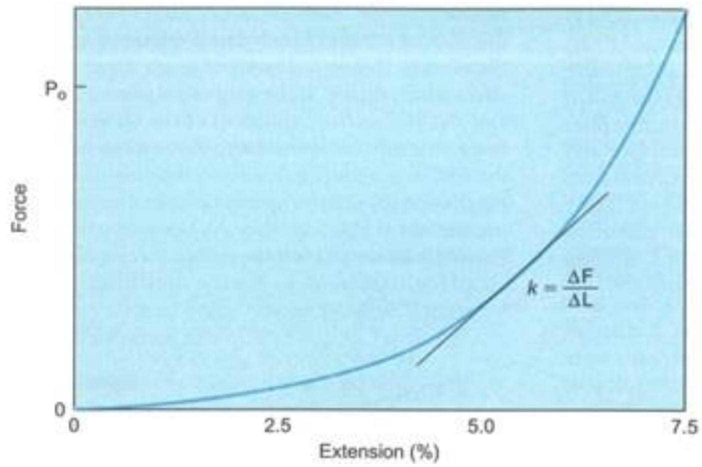


Figure 2.5. The SEC FΔL relationship. (from D.Gordon E.Robertson. Research methods in muscle biomechanics) (46)

### 2.5.3. Parallel elastic component (PEC)

Muscle display elastic behavior even if the CC is inactive and producing no force. If an external force is applied across an inactive, passive muscle, it resists, but stretches to a longer length. This is not a response of the SEC, because no force is being produced by the inactive CC. Instead, this inactive elastic response is produced by structures that are ‘in parallel’ to the CC. The paralel elastic component (PEC) is usually correlated with the fascia that surrounds the outside of the muscle and separates muscle fibers into distinct compartments. Like the SEC, the FΔL relationship of the PEC is highly nonlinear in nature, with increasing stiffness as the muscle lengthens.

The PEC elasticity is considered a passive response, yet it can play a role during active force production too. In an active isometric force situation, the measured force response is a combination of the active CC force and the passive PEC force associated with the isometric length of the muscle, depicts the FL relations of the active CC, the passive PEC, and the summed CC and PEC responses. At shorter lengths, the PEC is not streched and, thus the muscle force response will be entirely caused by the active CC. As the muscle is placed at longer lengths, the PEC is stretched and its force response is added to the active CC response. The exact shape of the summed response across the range of muscle lengths depends on the relative overlap of the CC and PEC response (34, 45).

### 2.6. CC – SEC Interactions during an isometric twitch

Given stimulative pulse of few milliseconds cause a peak twitch force in time of 20 to 50 ms. This discrepency in time course results from CC and SEC dynamics interaction and the interplay of their mechanical properties.

| **Given stimulus---CC becomes active via (SA, FA, FV, and FL)--FΔL changes at SEC** |
| --- |

If total muscle length is kept constant (shortened CC + lengthened SEC), it is isometric contraction. With decreasing stimulation, the force continually falls, meaning that the SEC is recoiling (shortening) and the CC is therefore lengthening.

During isometric contraction, the actual force produced by the CC is less than its capability.

During isometric contraction, the CC shortens at the same high velocity, as the CC and SEC velocities must offset each other to keep the total muscle in an isometric state. (46,47) (Fig 2.6).

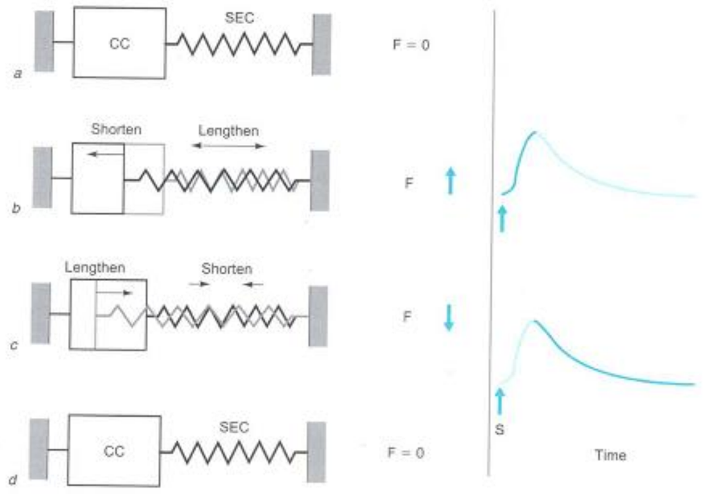


Figure 2.6. CC-SEC dynamic interaction during isometric twitch. (from D.Gordon E.Robertson. Research methods in muscle biomechanics)

Biomechanical effects of various massage techniques are believed to be achieved by increasing muscle-tendon compliance by mobilising and elongating muscle tendon unit. Various massage techniques which produce mechancal effects on tendon compliance mean that compliance of SEC is being changed. Its change is going to effect also muscle mechanics affecting contractile components. Passive and active stiffness properties are going to be changed and it is important to investigate how are affected muscle's electromechanical characteristics after massage itself and combined with stretching exercises (48).

## 2.7. Skeletal muscle mechanics

There are two main ways in which a muscle may work naturally. It may contract and produce no movement, called isometric contraction, or it may produce movement during contraction, called isotonic contraction. There are two types of isotonic contractions, concentric contraction and ecentric contraction.

### 2.7.1. Isometric contraction

When a muscle works isometrically, it keeps constant its muscular length and slightly lengthens its non-contractile components and in doing so, no movement occurs at any of the joints over which that muscle passes. It is easiest and in fact usual for an induced isometric contraction to be performed when a muscle is resting at the innermost part of its range, i.e. with the muscle attachments approximated, but with practice the skill can be developed so that it is possible isometrically to contract a muscle or muscle group at any part of the range. Isometric contraction can be taught to a muscle by the application of a manual resistance which is exactly equal to the contraction which the muscle produces (49).

## 2.8. Concentric Contractions (Isotonic Shortening)

When a muscle is activated and required to lift a load that is less than the maximum tetanic tension it can generate, the muscle begins to shorten. Contractions that permit the muscle to shorten are known as concentric contractions. In concentric contractions, the force generated by the muscle is always less than the muscle's maximum force ($P_0$). As the load the muscle is required to lift decreases, contraction velocity increases. This occurs until the muscle finally reaches its maximum contraction velocity, V max.

### 2.9. Eccentric ontraction (isotonic lengthening)

As the load on the muscle increases, it finally reaches a point where the external force on the muscle is greater than the force that the muscle can generate. Even though the muscle may be fully activated, it is forced to lengthen because of the high external load. There are two main characteristics regarding eccentric contractions. First, the absolute tensions achieved are very high relative to the muscle’s maximum tetanic tension-generating capacity; second, the absolute tension is relatively independent of lengthening velocity. These characteristics suggest that skeletal muscles are very resistant to lengthening, a property necessary for many normal movement paterns.

### 2.10. The active length - tension mechanics

It has been known that the force developed by a muscle during isometric contraction (when the muscle is not allowed to shorten) varies with its starting length. The isometric length-tension curve is generated by maximally stimulating a skeletal muscle at a variety of discrete lengths and measuring the tension generated at each length. The length tension relationship reflects the fact that tension generation in skeletal muscle is a direct function of the magnitude of overlap between the actin and mysoin. (46)

### 2.11. The passive length-tension relationship

The tension is generated if a muscle is stretched to various lengths without stimulation. Near the optimal length, passive tension is almost zero. However, as the muscle is stretched to longer lengths, passive tension increases dramatically. These relatively long lengths can be attined physiologically, and therefore, passive tension can play a role in providing, resistive force even in the absence of muscle activation. (46)

### 2.12. Force-velocity relationship describes isotonic muscle contraction

Unlike the length-tension relationship, the force-velocity relationship does not have a precise, anatomically identifiable basis. The force-velocity relationship describes the force generated by a muscle as a function of velocity under conditions of constant load (isotonic condition). It can also be stated in the reverse, such that the velocity of muscle contraction is dependent on the force resisting the muscle. (46)

### 2.13 Tendon Stiffness

Stiffness is a property of the tendon which is principally associated with the unit's ability to transfer forces. A stiffer tendon being able to transfer muscle forces to the bone more rapidly than a less stiff (or more compliant) tendon. (50)

Tendons are spring-like structures and their tensile stiffness is adaptable to the mechanical environment they operate in, increasing in response to chronic loading and decreasing with chronic unloading. Findings from the in vivo studies are mixed as to whether these adaptations are due to changes in the tendon's cross-sectional area, material properties or both. The increase in stiffness may be explained by increases in tendon size and/or Young's modulus (stiffness = Young's modulus tendon cross-sectional area/tendon length), and the later may be due to tendon microstructural changes, including an icreased collagen fibril diamater, an increased fibril packing, an increased collagen cross-linking, and a reduced collagen crimping.

A compliant tendon will allow, relatively slow transmission of forces from muscle to bone, thereby exhibiting the tendon as a ''viscoelastic damper'' (51).

### 2.14. Relationship between tendon stiffness and sarcomere length

Most human muscles (including the gastrocnemius muscle) act on the ascending limb of the sarcomere length-tension relation, thus more fibre shortening would shift the muscles' operating range to the left, away from the optimal region, causing a reduction in force. (Fig 2.7). However, the number of sarcomeres in-series will also influence this theoretical effect and may vary between young and older adults. The more compliant tendon of older adults may result in a slower transmission of contractile forces to the skeleton, which will be seen as a slower rate of torque development at the level of the whole joint system.

The increased tendon stiffness found after the resistance training intervention might be expected to increase the velocity of force transmission. The increased tendon stiffness post-intervention may also have implications for the muscle's operating range. It might be speculated that the muscle fibers woud shorten less, causing a shift towards the optimal sarcomere operating range (assuming the muscle acts on the ascending limb) (Fig 2.7) (52).

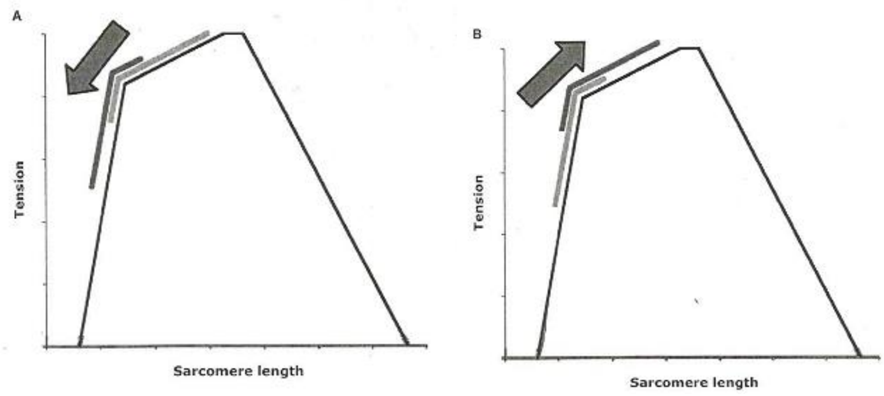


Figure 2.7. Shematic sarcomere length-tension relations to illustrate theoretical changes in a muscles operating range wth changes in the stiffness of the in-series tendon. Assuming all other conditions remained constant, a reduction in tendon stiffness would result in greater sarcomere shortening and a left shift of the sarcomere length-tension relation (A), whereas an increase in tendon stiffness would result in less sarcomere shortenng and a right shift of the sarcomere length-tension relation (B). (from N.D. Reeves. Adaptation of the tendon to mechanical usage.)

Reduction in tendon stiffness - greater sarcomere shortening - left-shift of the sarcomere length-tension relation

Increase in tendon stiffness - less sarcomere shortening - right shift of the sarcomere length-tension relation.

One of the studies showed that there is a strain variation along the tendon aponeurosis. This finding of nonhomogenous behavior has important implications for muscle function. Muscle fibers attached to more extensible aponeurotic regions would shorten more upon contraction than fibers attached to less extensible aponeurotic regions. The extra shortening will result in shifting the force-length relation of muscle-tendon unit to the right, which will result in a force enhancement in units operating in the descending limb of the force-length relation and in a force reduction in units operating in the ascending limb of the force-length relation (53).

It was found that the patellar tendon is significantly stiffer in adults, compared to children (54). The increase in stiffness can be attributed to a relative increase in both cross-sectional area of the tendon and an increased Young's modulus in females, while increased Young's modulus only in males (51). Tendon mechanical properties have a key role in the time course of torque development. In fact, tendon stiffness affects the time required to stretch the series elastic components (SEC) and will therefore affect both the electromechanical delay (EMD) and the rate of torque development (RTD) (55).

Electromechanical delay is interpreted in terms of changes in musculo-tendinous stiffness and it is a measure of the time delay or time elapse between muscle activation and muscle force production . The EMD is considered to be influenced by several structures and mechanisms, including the time course of:

1-The propagation of the action potential over the muscle membrane

2-The excitation-contraction coupling process

3-The stretching of the series elastic component (SEC) by the contractile components (56).

## 2.15. The mechanism underlying the surfce electromyography

### 2.15.1. Assessment of neuromuscular responses using surface EMG

Surface EMG has been used extensively in biomechanical applications to describe and quantify a muscle or muscle group's activity or performance about the knee.

Surface EMG can assist the clinician or researcher in determining when a muscle is activated, the timing of that activation in relation to a stimulus or event, and its sequential firing with other muscles.

Unlike the classical neurological EMG, where an artificial muscle response due to external electrical stimulation is analyzed in static conditions, the focus of kinesiological EMG can be described as the study of the neurmuscular activation of muscles within postural tasks, functional movements, work conditions and treatment / training regimes**.**

### 2.15.2. The motor unit

The smallest functional unit to describe the neural control of the muscular contraction process is called a Motor Unit. It is defined as the cell body and dendrites of a motor neuron, the multiple branches of its axon, and the muscle fibers that innervates it.

### 2.15.3. Excitability of muscle membranes

The excitability of muscle fibers through neural control represents a major factor in muscle physiology. This phenomenon can be explained by a model of a semi-permeable membrane describing the electrical properties of the sarcolemma. An ionic equilibrium between the inner and outer spaces cell forms a resting potential at the muscle fiber membrane (approximately -80 to -90 mV when it is not contracted). This difference in potential which is maintained by physiological processes (ion pump) results in a negative intracellular charge compared to the external surface. The activation of an alpha-motor anterior horn cell (induced by the central nervous system or reflex) results in the conduction of the excitation along the motor nevre. After the release of transmitter substances at the motor endplates, an endplate potential is formed at the muscle fiber innervated by this motor unit. The diffusion characteristics of the muscle fiber membrane are briefly modified and $Na^+$ ions flow in. This causes a membrane depolarization which is immediately restored by backward of ions within the active ion pump mechanism, the repolarization (57).

### 2.15.4. The action potential

If a certain threshold level is exceeded within the Na+ influx, the deloparization of the membrane causes an Action Potential to quickly change from -80 Mv up to +30 Mv. It is monopolar electrical burst that is immediately restored by the repolarization phase and followed by an After Hyperpolarization period of the membrane. Starting from the motor end plates, the action potential spreads along the muscle fiber in both directions and inside the muscle fiber through a tubular system.

This excitation leads to the release of calcium ions in the intra-cellular space. Linked chemical processes (Electro-mechanical Coupling) finally produce a shortening of the contractile elements of the muscle cell.

The EMG signal is based upon action potentials at the muscle fiber membrane resulting from the depolarization and repolarization processes as described above (58).

### 2.15.5. The “raw” EMG signal

An unfiltered (exception: amplifier bandpass) and unprocessed signal detecting the superposed Muscle Unit Action Potentials (MUAPs) is called a raw EMG Signal. (Fig 2.8)

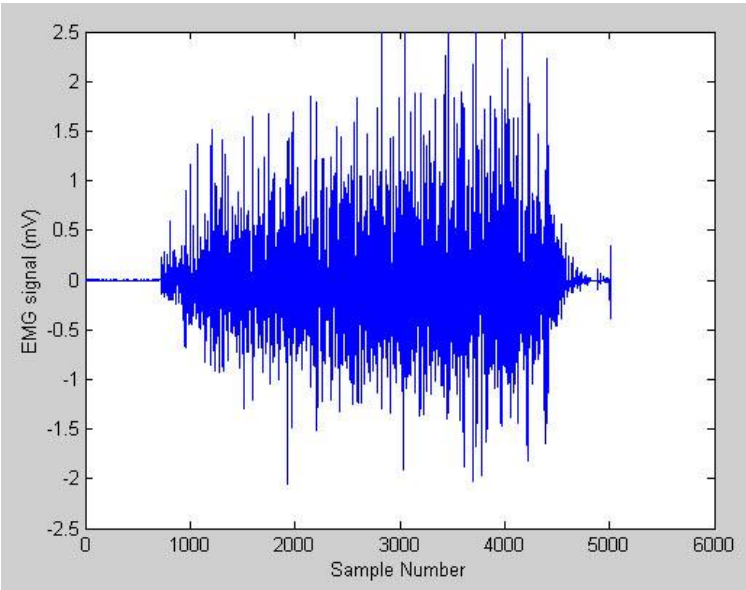


Figure 2.8. Example of Raw EMG signal

When the muscle is relaxed, a more or less noise-free Baseline can be seen. The raw EMG baseline noise depends on many factors, especially the quality of the EMG amplifier, the environment noise and the quality of the given detection condition. (59)

### 2.15.6. Factors influencing the EMG signal

There are some factors which influence the EMG signals. All these are below:

1. Tissue characteristics
2. Physiological cross talk
3. Changes in the geometry between muscle belly and electrode site
4. External noise
5. Electrode and amplifier

### 2.15.7. Temporal measurement of "reflexive activation"

The most basic information that can be derived from the EMG signal is whether or not a muscle is active or at rest. The timing and phasing of this muscular activity has been used to determine muscular response characteristics such as reaction time, electromechanical delay (EMD), and firing patterns in response to stimulus (59).

### 2.15.8. Linear envelope detection

Linear envelope detection may be used to quantify the amount of muscle activity as measured by the area of the EMG burst. The rate of muscle activation is reflected in the rising phase of the EMG burst.

The slowly changing EMG waveform associated with linear envelope detection is often preferred to facilitate the extraction of area, slope, onset, and shape characteristics of the muscle activity profile. Each measure is thought to reveal specific information about how to muscle is controlled during both static and dynamic contractions (60).

### 2.15.9. Determining the EMG onset

Determining the exact time a muscle becomes activated after a stimulus can be influenced by a number of factors.

To accurately determine the onset of muscle activity, the clinician or researcher must be able to confidently and consistently identify when EMG activity begins or significantly deviates from static or baseline activity. To do so, the EMG signal must exceed a threshold that can be defined in some sway, either visually (subjective) or by a statistically predetermined level (objective). Manual inspection of the EMG signal on the computer screen is the only way an investigator can have 100% confidence that every onset was correctly determined. However, this can be a time-consuming and tedious process. There is also the mistaken perception that manual onset determination is subjective and unreliable. Manual determination of EMG onset does require experience, but the agreement between experienced investigators can be qute high (59,60).

### 2.15.10. Electromechanical delay (EMD)

Determination of electromechanical delay (EMD) is completely objective. Electromyography has also been used extensively to quantifiably measure the time elapse between the change in electrical activity and the actual force generation in the muscle. It is important to realize that the EMG signal reflects only the electrical activity of the muscle, which is not synonymous with the production of tension. In fact, a natural EMD exists between neural activation of the muscle as recorded electrically by EMG and the actual generation of force. EMD can be measured using a force transducer (or similar device like Load Cell) interfaced with the EMG to detect and quantify when muscle tension is developed after neural activation. This delay can be quite variable due to factors such as fiber-type composition and firing rate dynamics of the muscle, velocity of movement, viscoelastic properties and length of the muscle and tendon tissues, activity state, and coactivity of other muscles (59). Several structures and mechanisms that contribute to electromechanical delay are

a) neuromuscular synapsis latency and the propagation of the action potential over the muscle memebrane

b) the excitation-contraction coupling process, and

c) the time for elongation of the SEC by the contractile elements (25).

**2.16. The Mechanisms Underlying The Surface Mechanomyography (MMG)**

The surface mechanomyogram (MMG) records and quantifies the low-frequency lateral oscillations of active skeletal muscle fibers (61). Accelerometers are frequently used tools for measurement of low-frequency lateral oscillations of muscle fibers. (Fig 2.9)

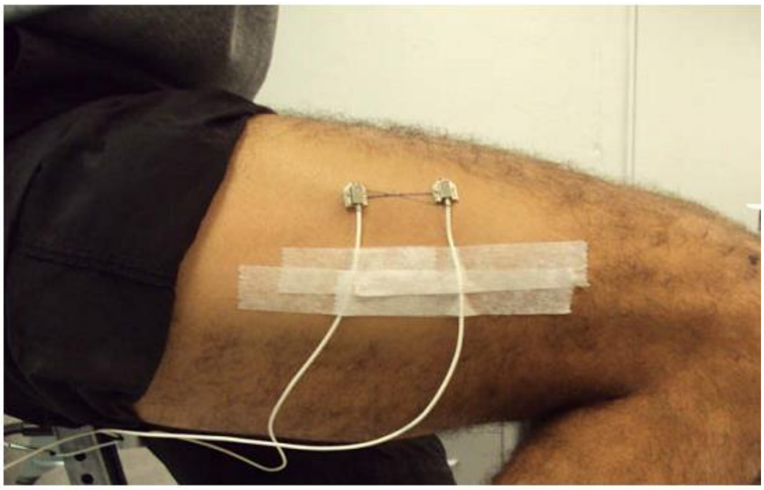

Figure 2.9. Example of the proximal and distal accelerometer placement on the vastus lateralis muscle.

It is important to acknowledge the fact that various terms have been used to describe MMG, including soundmyography, phonomyography, acoustic-myography, accelerometermyography, and vibromyography. Although the use of these terms was heavily influenced by the type of sensor used to detect the signal, it created confusion in the literature with regard to what was actually being measured. Thus, in 1995, the term'' surface mechanomyogram'' was determined by CIBA Foundation Symposium to distinguish the MMG signal from other mechanical signals that are unrelated to muscle activity (46).

Low frequency lateral oscillations are generated by;

1) a gross lateral movement of the muscle at the initiation of a contraction that is generated by non-simultaneous activation of muscle fibers,

2) smaller subsequent lateral oscillations occurring at the resonant frequency of the muscle, and

3) dimensional changes of the active muscle fibers.

The MMG amplitude, however, is influenced by many factors, including muscle stiffness, tension, length, mass, intramuscular pressure, the viscosity of the surrounding medium, and the motor unit firing frquency (62).

However, the frequency content of the MMG signal was closely related to the muscle's resonant frequency, which suggested that MMG could potentially be used as a noninvasive measure of muscle stiffness (46).

Muscle stiffness is primarily a function of the number of attached crossbridges. Greater muscle stiffness at slower velocities may limit the oscillations of the muscle fibers and MMG amplitude whereas, at faster velocities, the unloading of slow-twitch fibers results in less muscle stiffness and greater muscle fiber oscillations and MMG amplitude (29).

It is important to point out that the mechanism underlying the MMG signal during an electrically-stimulated contraction are very different from those during a voluntary muscle action (dynamic or isometric).

During an isometric twitch, the MMG signal was generated by lateral movement of the whole muscle, and its maximum amplitude occurred when the muscle length was slightly less that the required for maximum force production.

During voluntary muscle action, the motor unit activities are usually not synchronized, and the twitches from each motor unit are summed to create a complex MMG signal.

During an electrically stimulated contraction, all fibers are stimulated to contract simultanously, and the response of the muscle is dependent on the stimulation frequency and the muscle's ability to contract and relax at a rate that matches the stimulation rate.

The simultaneous recording of surface EMG and MMG can provide unique information about the electro-mechanical properties and motor control strategies during several types of muscle actions, including both electrically and voluntary evoked isometric contractions (25).

## 2.17. Measurement of Force

There are many tools available to the clinicians for the measurement of force and moment of force. Although all of these tools can be called transducers, it can be devided into force platforms, pressure distribution sensors, internally aplied force sensors, and isokinetic devices (46). In many biomechanical researches, CYBEX 600 Isokinetic Dynamometer is used for measurement of isokinetic muscle actions of leg extensors. Using CYBEX, it is possible to measure torque output at different velocitis and different knee angles (63). Subjects are generally placed in seated position with a restraining strep over the pelvis and trunk in accordance with the device's user guide (64). On the other hand, in accordance to study design, many biomechanical researchers prefer to use special custom-made devices combined with Load Cells (LC) for force detection. (Fig 2.**10)**

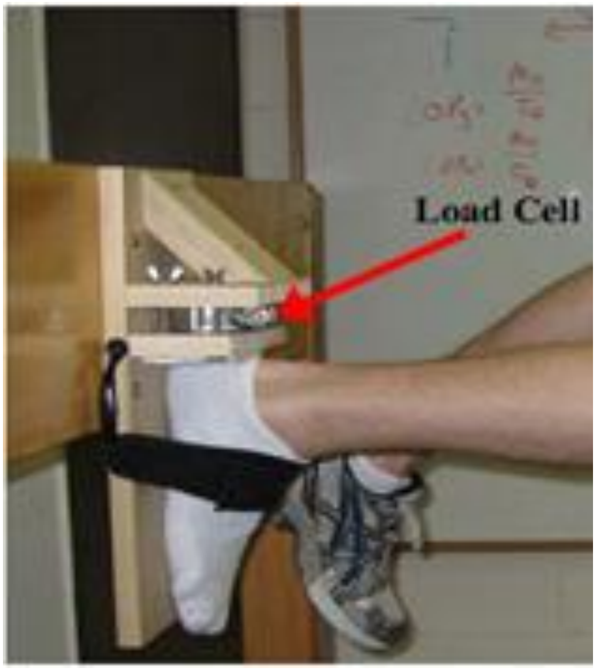


Figure 2.10. Example for the measurement of hamstring force with load cell placed on the back side of calcaneus.

Tension-compression load cells can be with wide range of sensitivity between 0 N and more than a 1000 N accordingly to the force generation since it can be preffered to record and force generation capability that person can exert. Combination of force measurement with electromyography and mechanomyography is of high importance in biomechanics science for better understanding muscle dynamics with inner changes (electromechanical characteristics) and force generation capabilities.

### 2.18. Manual therapy (transverse friction massage) and stretching exercises

Massage is the manipulation of the soft tissue of the body with the hands using varying degrees of force (65). Transverse friction massage is a specific type of connective tissue massage applied precisely to the soft-tissue structure of tendons, muscles and ligaments, for a specific purpose. Increased muscle – tendon compliance is believed to be achieved by mobilising and elongating shortened or adhered connective tissue with friction massage. Improved muscle compliance results in a less stiff muscle – tendon unit Doing mechanical pressure on tissues produces:

- decrease in tissue adhesions,
- increase of muscle compliance,
- increase in range of motion,
- decrease in passive stiffness, and
- decrease in active stiffness (66).

Doing deep – friction massage in which the forces are applied perpendicular to the fibers for separating each fiber, mechanically assisting in the alignment of newly formed collagen during healing. It has been used to promote local hyperemia, analgesia, and the reduction of adherent scar tissue to ligaments, tendons and muscles (67). Massage can increase ROM, it may be an alternative or compliment to static stretching during the warm-up, cool-down, and specialized flexibility training sessions. It is applicable to do massage on both muscle belly and musculotendinous junction. Massaging musculotendinous junction could theoretically activate the inhibitory action of the Golgi tendon organ (GTO) or lead to a presynaptic inhibition of the Ia sensory fibers from the spindles of the activated muscles. In a study where is investigated muscle-tendon unit massage of hamstring muscle is not verified with a control group. Further studies using control groups should determine both the cumulative effects of massage and static stretching and, the persistance of the technique (15).

### 2.19. Stretchıng Exercıses

Stretching is traditionally used as a part of warm-up program to increase flexibility or pain-free range of motion (ROM) about a joint in an attempt to promote better performances, and / or reduce the risk of injury (68,69). It is reported that static stretching reduce the force and power producing capabilities of the leg extensors during voluntary maximal concentric isokinetic musle actions, also increase of active and passive range of motion and increase in MMG amplitude. Because MMG showed increase after strecthing, MMG would be usefull in investigation of the mechanical changes that may occur, such as changes in muscle stiffness. It is also reported that strength is decreased after stretching exercises by mechanisms:

1. Pre and post sinaptic neural inhibition,
2. Changes in the structural and functional characteristics of the MTU.

After passive stretching, some modifications at the sarcomer level in contractile (cross-bridges efficiency and structural elastic filaments of titin) elements have been reported (70). It is generally accepted that passive stretching decreases muscle and / or tendon stiffness, thus changing the viscoelastic properties of the muscle-tendon unit. Decrease in muscle-tendon unit stiffness is the result of several mechanisms, involving both the contractile (changes in cross-bridge characteristics) and the connective (perimisium, endomysium, aponeurosis and tendons) elements (25).

## 3. METHODS AND MATERIALS

This study was carried out to investigate the changes in the electromechanical properties of quadriceps femoris after three different physical therapy interventions and to find out the best intervention to reduce muscle and tendon stiffness. A total number of 56 healthy voluntary subjects with a mean age of 27.5 (ranged between 20 – 35) have been recruited to the study. Each subject was clinically assessed and questioned to record anthropometric changes and to eliminate if any pathological condition is present. Subjects were devided into 3 interventional groups and 1 control group;

1. Stretching group (N=15): clinical and laboratory assessments were done before and immediatelly after 20 minutes passive stretching exercise of Quadriceps Femoris muscle(stretching exercise 20 minutes in duration) as pre- and post- measurements.
2. Manual Therapy Group (Transverse friction massage + Patellar mobilization group) (N=14): clinical and laboratory assessments were done before and immediatelly after 20 minutes application of friction massage on Qudriceps Femoris muscle-tendon unit + patellar mobilization as pre- and post-measurements. .
3. Combined Therapy Group ( Stretching + Manual Therapy Group) (N=15): clinical and laboratory assessments were done before and immediatelly after the 20 minutes application of the friction massage +patellar mobilization + stretching exercises of Quadriceps Femoris muscle as pre- and post-measurements. .
4. Control group (N=12): Clinical and laboratory assessments were done before and immediatelly after 20 minutes of resting period. No any intervention was applied between pre- and post- measurements.

### 3.1. Subjects

The subject population consisted of young (aged between 20-35 years), healthy female and male vounteers without consideration of race, ethnicity or religion. None of them was involved in any sports activity regular basis. All of them were subjects who have job and working 5 days a week, 8 hours daily. The subjects were healthy subjects willing to participate in this study. None of them among 56 subjects rejected the interventions and / or physical therapy.

### 3.2. Inclusion/Exclusion Criteria

A subject was excluded if she/he: 1) did not meet the age criteria, 2) was involved in sports activity regular basis, 3) had previous surgical intervention for their lower extremity, 4) had any history of metabolic, neurologic, and orthopaedic dissease, 5) had previous physical therapy 2 years before, 5) was scared of testing procedures or had anxiety before testing because of sophisticated looking devices used for laboratory assessment.

### 3.3. Statement of informed consent

Once subjects were recruited, a brief interview was conducted in order to assess the inclusion criteria. Before beginning the initial testing session, the subject was asked to read an informed consent agreement outlined the procedures, protocols, and potential risks of the study. The informed consent agreement form was in accordance with the standards set by the Ethical Review Board at the Hacettepe University. Approval referance number :B.30.2.HAC.0.05.07.00/776; Project number: HEK12/81. (Annex I) If the subject met the inclusion criteria and signed the informed consent agreement, she / he was assigned to a cohort and included in the study.

### 3.4. Study Design

Study design was firstly started at the Hacettepe University, Faculty of Health Sciences, School of Physiotherapy and Rehabilitation and Faculty of Medicine, Department of Biophysics. Designing the software in MatLab program and its integration with the measurement device was primarily conducted at the Department of Biophysics.

Once subjects were recruited, screened, and consented for the study, the subjects participated in a baseline data collection session at the Maltepe University, Department of Biophysics. Physical characteristics such as gender, age, body height and weight were assessed. After collection of informations about subject's medical history, clinical assessment was initiated.

### 3.5. Clinical assessment

Clinical assessment has consisted of tests for determining muscle shortness and goniometric measurements for range of motion.

The following measurements has been performed for each subject:

1. The Thomas Test: Thomas test is a test to evaluate the shortness or thightness of rectus femoris which is the part of Quadriceps Femoris muscle. It has been proven that it has good interrater reliability and it is a valid test for assessing rectus femoris thightness. It must be tested for the both rectus femoris on right and left side to compare the level of tightness.

Figure 3.1. Assessment of the shortness of rectus femoris muscle.

Lying in supine position with hips nearly off the edge of the table, the patient holds the controlateral knee into the chest to prevent lumbar hyperextension, anterior pelvic tilt compensations. A negative test result is when the thigh of the free leg remains flat on the table with the knee flexed. (Fig 3.1)

2. Goniometric measurement of knee angle in the Thomas Test position: subject was lying in the supine poston with less than half of the thigh off the examination table. In this position knee angle of the tested side was measured using the standart goniometer. (Fig 3.2)

Figure 3.2. Measurement of the knee angle wıth the standart goniometer on the tested side

3. Goniometric measurement of the knee flexion range of motion in prone position: subject was lying in the prone position on the examination table. In this position, active and passive knee flexion range of motion was measured using the standart goniometer. (Fig 3.3)


Figure 3.3. Goniometric measurement of the active and passive knee flexion range of motion

4. Goniometric meassurement of the active and passive range of motion of the ankle dorsiflexion and plantar flexion: Subject was lying in the prone position and knee was flexed at 90° . Using standart goniometer, active and passive measurement of the dorsi- and plantar- flexion was meassured. (Fig 3.4)

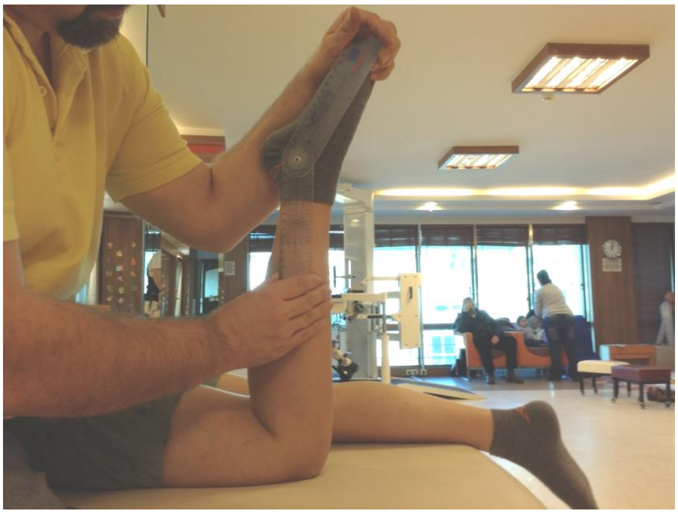

Figure 3.4. Measurement of the active and passive range of motion of the knee dorsiflexion and plantarflexion

5. Test for shortness or tightness of the gastrocnemius muscle: Subject was lying in the supine position on the examination table. Pushing the subject's forefoot into the dorsiflexion, first feel of the tightness was determining for tightness or shortness of the gastrocnemius muscle. (Fig 3.5)

Figure 3.5. Assessment of the shortness or tightness of the gastrcnemius muscle is supine positon.

**3.6. Laboratory Assessment**

Quadriceps setting exercise is frequently used by phyisical therapist in clinical practice. However, there was no industrial device to use it in clinical practice or laboratory settings. Thus, we designed and constructed a device for positioning and stabilizing of the subjects in a neutral position and for giving preferred knee flexion angle during isometric quadriceps angle. The subjects were able to perform quadriceps setting exercises in a correct way since we have provided neutral positioning of the subjects, stabilizing of their lower limbs and maintaining preferred knee flexion angle with this device. We could manage to record all electromyographic measurements without having any enterference or loosening of the position during the test. This device was practical to use in clinical settings in addition to the laboratory conditions, because it was portable and adjustable.

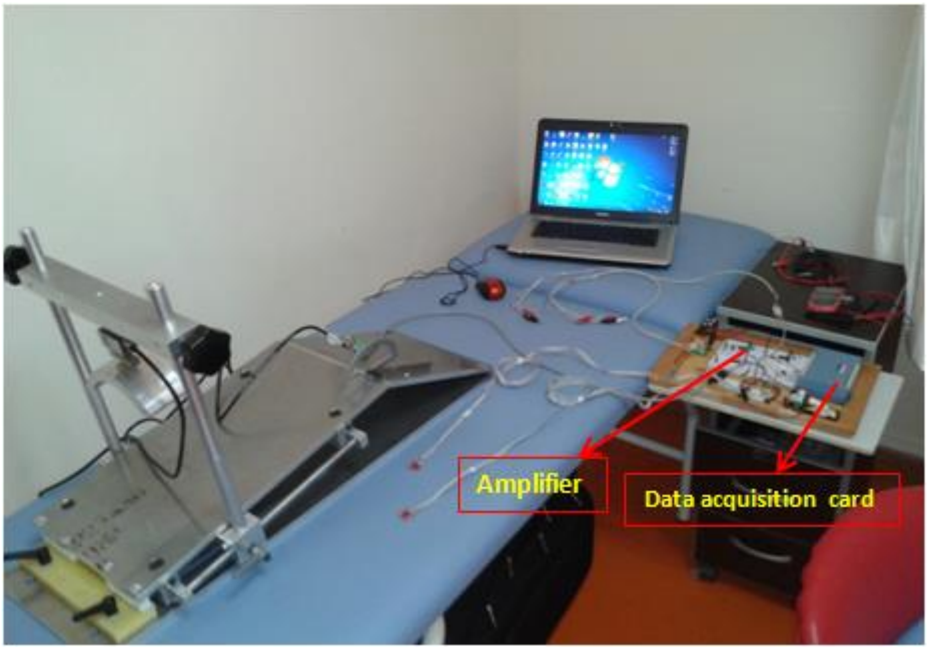


Figure 3.6. Overlook of the laboratory testing system

The device was made by heavy aluminium with two sliding boards, some keys to restrict sliding boards for accurately adjusting to preferred knee angle and load cell mounted on a horizontal bar which could slide up and down. Load cell was also moveable along the horizontally mounted bars so that we could meassure moment arm along the length of tibia (Fig 3.6).

### 3.6.1. Force detection

Load Cell: The force output generated by the qudriceps femoris muscle during isometric contractions was recorded by means of a calibrated load cell (model CAS-500N) operating linearly between 0 and 500 Newton (Fig 3.7).


Figure 3.7. CAS-S model Load Cell.

The force signal was amplified by 500 Newton (N) and filtered by a low-pass filter with a low-pass cut-off frequency of 100 Hertz (Hz) and then stored in a personal computer after A/D conversion (model MC: Measurements Computing, USB-1608G) with a sampling frequency of 5 KHz  (Figure 19).

Figure 3.8. 16-Bit USB-1608G Data acquisition card (A Measurement Computing, DAQ).

USB-1608G Data acquisition card (A Measurement Computing, DAQ) was installed on a personal computer and used for recording analog signals such as EMG, MMG and force (Fig 3.8).

### 3.6.2. EMG and MMG recording

EMG electrodes were applied to the subject's dominant leg. Two surface EMG electrodes (standart Ag/AgCL electrodes) were placed over the belly of the rectus femoris muscle at the 1/2 part of the distance between anterior spina iliaca superior and superior pole of the patella, spaced 25 mm centre-to-centre. Also two electrodes were placed over the belly of the vastus medialis muscle at 80% of the line between spina iliaca anterior superior and joint space in front of the anterior border of the medial ligament (Fig 3.9). Electrodes were parallel to the muscle fibers of rectus femoris and vastus medialis. To reduce skin impedance and to allow proper electrode fixation, electrode sites were shaved and cleaned with isopropyl alcohol. Electrode gel was also used to reduce the skin empendance. The skin impedance was lower than 5Ω.

A ground electrode was put on the lateral line of the knee joint. The electrodes were then connected via electrical cables to a custom made amplifier. In order to keep full contact of the electrodes with the skin and to have clear electromyography signals, electrodes were held with hypafix tape.

The MMG signal was detected using an ADXL335-Small, Low Power, 3-Axis ± 3g Accelerometer. Accelerometers were placed between the two EMG electrodes and fastened by hypafix tape. During placement of accelerometers, precaution was taken so that X-axis of acceleometers was paralel to the fibers of the RF and VM.

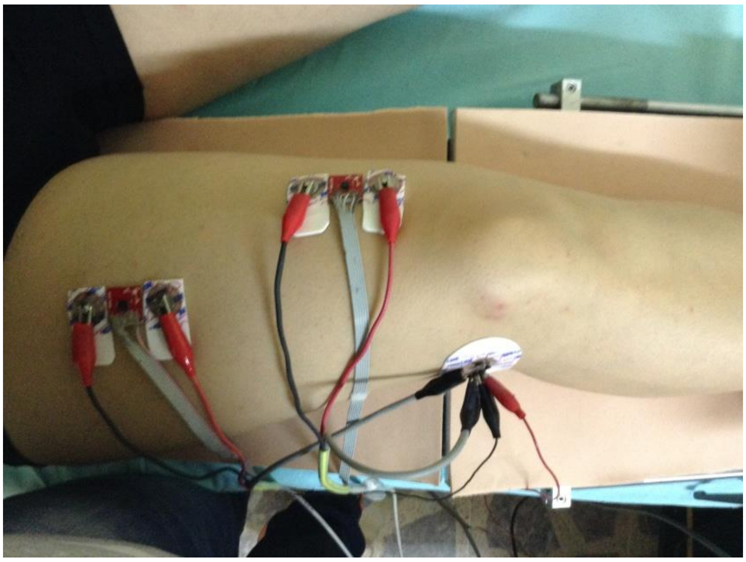

Figure 3.9. Placement of the EMG electrodes and accelerometers on Rectus Femoris and Vastus Medialis muscles.

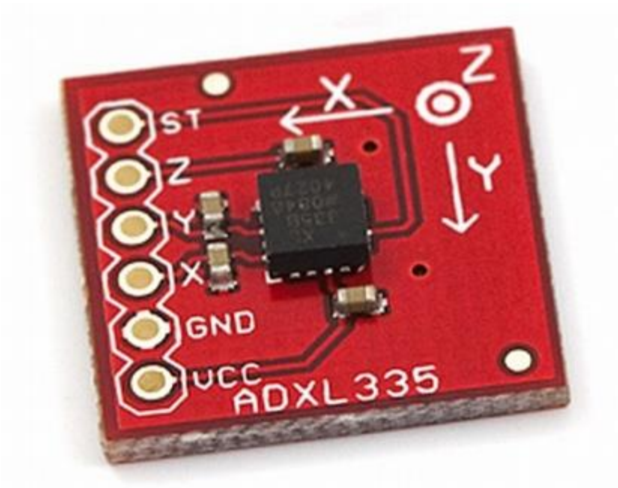


Figure 3.10. Accelerometer used in the experiments. (Sparkfun electronics, Triple Axis Accelerometer Breakout - ADXL335)

The ADXL335 is a triple axis MEMS accelerometer with extremely low noise and power consumption - only 320uA. The sensor had a full sensing range of +/-3g. The surface EMG and MMG signals were amplified by 1000 and 200 times, respectively by a custom designed amplifier. The freqeuncies above 500 Hz and below 10 Hz in EMG signals were filtered. MMG signals were filtered above 100 Hz and below 5 Hz.Then EMG and MMG signals were digitized by a 16-bit data acquisition card (MC: Measurements Computing, USB-1608G) with a sampling rate of 5 KHz. (Fig 3.10).

Force, EMG and MMG signals were acquired and recorded simultaneously during isometric contractions of QF muscle.

### 3.6.3. Data collection procedure

Before coming to the recording, subjects were instructed to wear high-cut socks and spandex shorts. Subjects were also instructed to not use any body lotion 24 hours prior to assessment. Subject was seated with the dominant leg positioned on the device at randomly decided starting knee angle. To prevent external rotation of leg tight strap was secured and stretched over the middle of the tibia towards to medial side. The testing position of the subject was in accordance with the frquently used exercise position for isometric strengthening of the quadriceps femoris muscle All subjects were tested at three different knee flexion angles, 15°, 30° and 45°. (Fig 3.12)

Prior to test, the subjects were instructed how to generate quadriceps isometric contraction. A familiarization session was given to allow the subjects to practice in isometric experimental protocols. After the familiarization session, subjects were started with real trials accordingly to testing procedure.

Verbal encouragement was given to the subjects to maintain 5 seconds submaximal isometric contraction at desired level. Subjects were instructed to apply isometric contraction as quickly as possible.

All subjects performed 4 sets of isometric contractions at each testing knee angle before and after interventional procedures. Each set consisted of 5 isometric contractions with duration of 5 seconds. Between each isometric contraction, there was 10 seconds for resting period. Between each set, there was 2 minutes of resting period in order to prevent muscular fatigueness which might affect the subsequent trial. This procedure was repeated for each targeted knee angle. During the test contractions EMG, MMG and FORCE were recorded simultaneously via designed program in MATLAB (Fig 3.11).

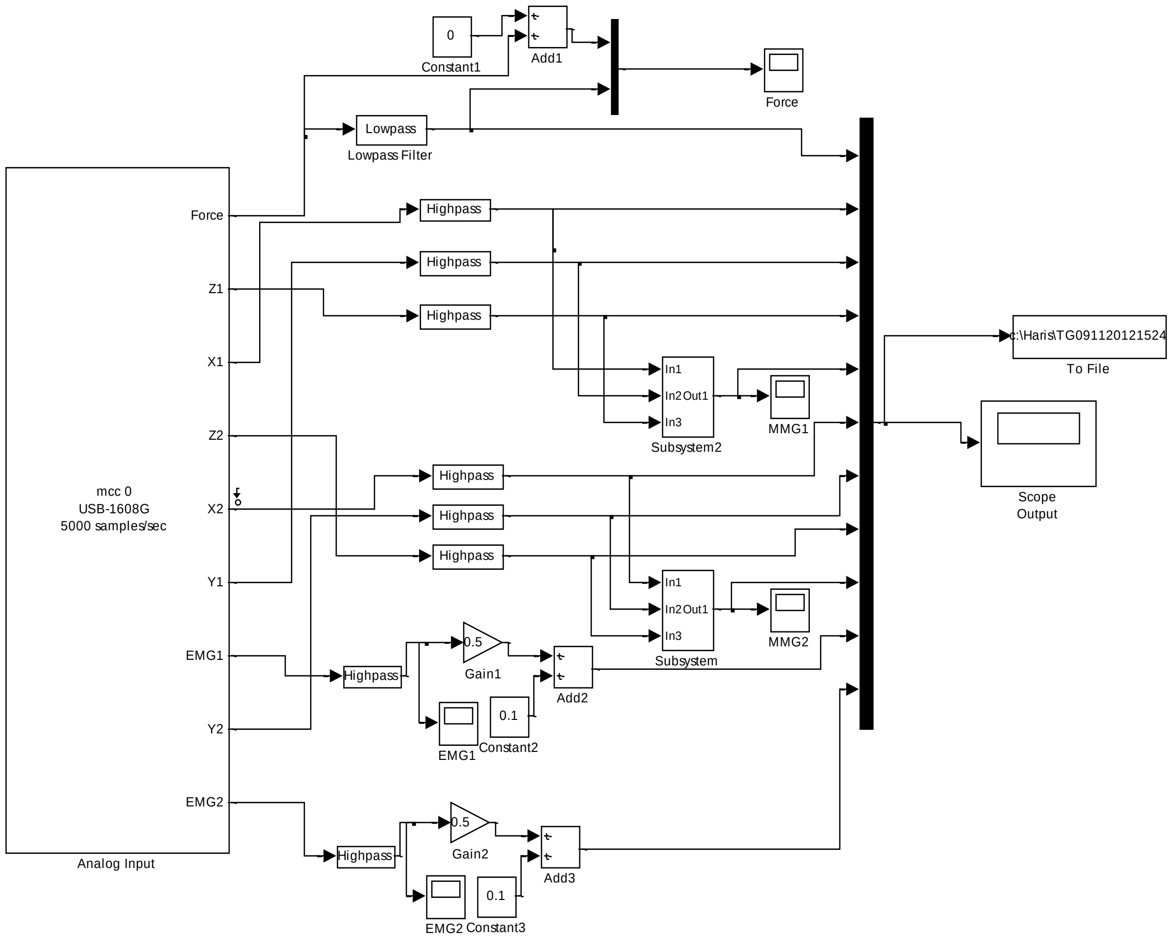


Figure 3.11. Designed Simulink operating program in MATLAB

Positioning of the three knee flexion angle was settled randomly. The subjects did not have any information about at what flexion angle they will start to be tested and they will continue so on. After finishing the assessment at any knee flexion angle (15°, 30° and 45°), the mechanical knee flexion angle of the device was settled again for the other two flexion angles. Load-cell was re-adjusted on the tibia because of altered position of the knee for the other knee flexion angles. Then they had the same test procedures for electromyographic and mechanomyographic signals and force output. (Fig 3.12)

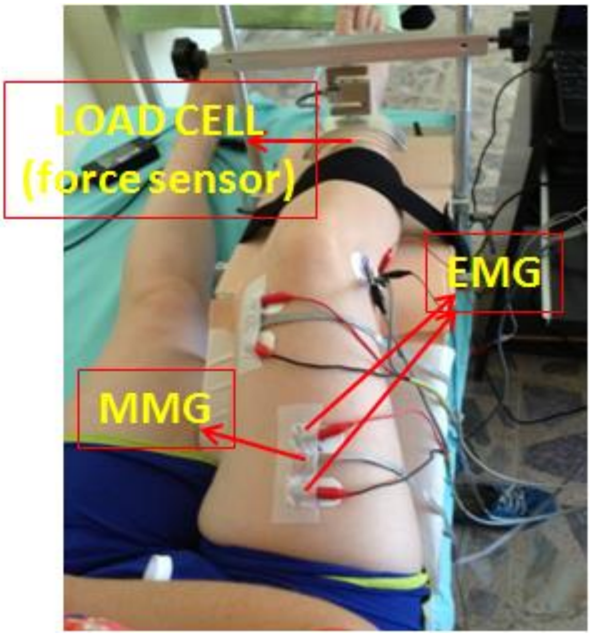


Figure 3.12. Experimental setup.

### 3.6.4. Physical therapy intervention

Physical therapy interventions as stretching and manual therapy (transverse friction massage + patellar mobilization) were applied to the intervention groups.

The passive stretching group had only static stretching application for the Rectus Femoris muscle. The passive static stretching was applied by the same physical therapist for a period of 30 seconds with 1 minute intervals as 10 sets. (Fig 3.13)

Figure 3.13. Application of passive static stretching exercise for the RM muscle

In the Manual Therapy Group, transverse friction massage has been applied directly on the Quadriceps tendon for about 10 minutes. (Fig 3.14)

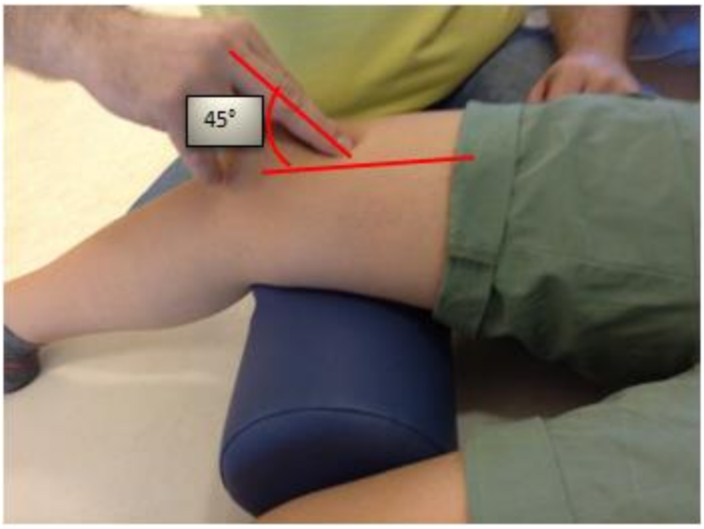


Figure 3.14. Application of the transverse friction massage on the quadriceps tendon

In addition to transverse friction massage to quadriceps tendon, patellar mobilization in both directions (supero-inferior and medio-lateral) was applied. The duration of patellar mobilization was not more than 5 minutes. (Fig 3.15)

Figure 3.15. Application of the patellar mobilization

The Combined Therapy Group ( The stretching + Manual therapy Group) has got both applications.

After the application of the intervention, the subjects in the groups have been re-tested. Preparing the subjects for the re-test experiments took about 10 minutes after the intervention (1,32,33).

### 3.7. Data Analysis

All signals were processed off-line using a program written in MatLab (Version 19). The program was writen so as to detect the onset times of the simultaneously recorded EMG, MMG and Force signals and calculate the delays between these signals. This program consisted of three subcomponents:

1. Detecting the onset times of electromyography (EMG), mechanomyography (MMG), and Force for each contraction: Initially EMG signals were rectified. Then, standart deviation for each EMG, MMG and FORCE signal were calculated for 10 milisecond's duration just prior to contraction. A threshold level of 3 standart deviations from the base line was set for each force, EMG and MMG signal. The time that the signal crossed the threshold level was detected. In order to define a crossing time as the onset time, a condition for signal to stay 10 miliseconds above the threshold level was required (Fig 3.16).
2. Calculation of delays: The delay between the EMG and MMG was calculated by subtracting the onset time of MMG from the onset time of EMG. The delay between the EMG and force was calculated by subtracting the onset time of FORCE from the onset time of EMG. The delay between the MMG and force was calculated by subtracting the onset time of FORCE from the onset time of MMG.
3. The program also allowed to check and to correct manually the calculated onset times of Force, EMG and MMG.

Delays between EMG-MMG, EMG-FORCE, and MMG-FORCE were expressed in milliseconds and denoted by

- $\Delta$t [EMG – MMG]
- $\Delta$t [EMG – FORCE]
- $\Delta$t [MMG – FORCE]

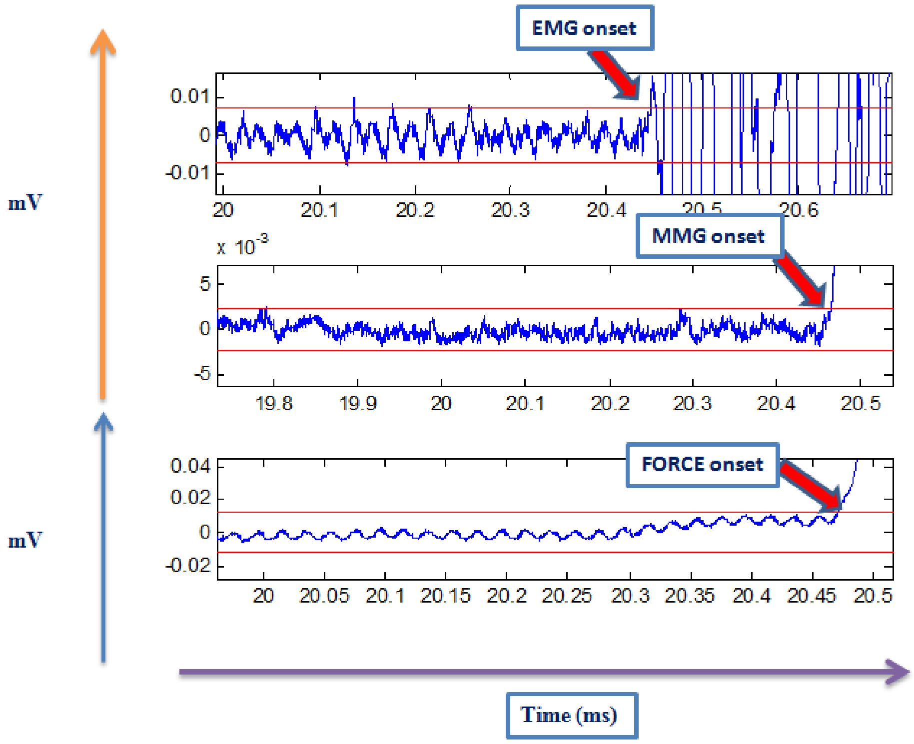


Figure 3.16. Detection of the onset times of the EMG, MMG and FORCE signals

### 3.8 Statistical Analysis

Statistical analysis was performed on raw values using a Statistical Software Package (SPSS version 19). According to the power analysis performed on previous similar studies in the literature, the number of subjects recruited to this study should be at least 5 person. In order to increase level of significance and to have better sensitivity for statistical analysis, it has been preferred to include more than 5 subjects for each group. A sample size of 5 participants in each one interventional group was selected to ensure a statistical power higher than 080. A One-way analysis of variance (ANOVA) for repeated measures was used to determine possible differences among the PRE and POST assessment. Two-way Analysis of Variance (ANOVA) Test was used to determine intersubjects homogenity of variance between PRE and POST in control group. The level of significance was set at $p < 0.05$. The results are expressed as mean ± standart deviation.

## 4. RESULTS

Demographic data of the subjects recruited into control and 3 interventional groups are expressed as mean ± standard deviation. Number of subjects, their mean age, weight and height in addition to their dominant extremity are shown in the table 1. Goniometric measurements of the knee and the ankle joints (expressed as average value ± standard deviation) are shown in the table 2 and 3. Muscle tightness tests results (expressed as negative or positive) are shown in table 4. 1

Table 4.1. The physical characteristics of the groups taken from demographic measurements of the subjects.

| DEMOGRAPHIC CHARACTERISTICS | STRETCHING GROUP | MASSAGE GROUP | COMBINED THERAPY GROUP | CONTROL GROUP |
|---|---|---|---|---|
| SUBJECTS (N) | 15 (female:7; male:8) | 14 (female: 8; male:6) | 15 (female:7; male:8) | 12 (female:50; male:7) |
| AGE (years) | 27.1 ± 3.7 (min:22; max:33) | 27.3 ± 4.3 (min:22; max:35) | 24.3 ± 4.0 (min:20; max:34) | 26.9 ± 4.0 (min:23; max:35) |
| WEIGHT (Kg) | 72.3 ± 12.5 (min:46; max:95) | 64.9 ± 7.8 (min:55; max: 85) | 68.6 ± 11.1 (min:57; max:82) | 69.4 ± 10.3 (min:57, max:80) |
| HEIGHT (cm) | 173.3 ± 8.3 (min:170: max:184) | 167.9 ± 6.7 (min: 158; max: 178) | 171.8 ± 8.9 (min:160; max:190) | 174 ± 5.6 (min:170; max:185) |
| DOMINANT SIDE Right (R) / left (L) | R | R | R | R |

*R: Right side.

In this study, the total number of subjects recruited into 4 groups was 56. All subjects were tested on their right (R) leg presented as dominant side. Mean age, weight and height for each group are shown in table 1 (Table 4.1).

Table 4.2. Goniometric measurements of the active and passive range of motion of right and left knee joints.

| | STRETCHING GROUP | MASSAGE GROUP | COMBINED THERAPY GROUP | CONTROL GROUP |
|---|---|---|---|---|
| SUBJECT (N) | 15 | 14 | 15 | 12 |
| RIGHT KNEE FLEXION ACTIVE | 129.3° ± 6.2° (min:120°; max:140°) | 134.6° ± 7.5° (min:125°; max:150°) | 134.7° ± 5.8° (min:125°; max:145°) | 138.8° ± 5.3° (min:130°; mac:145°) |
| RIGHT KNEE FLEXION PASSIVE | 141.3° ± 6.7° (min:130°; max:155°) | 146.8° ± 6.7° (min:140°; max:160°) | 147.0° ± 6.2° (min:135°; max:155°) | 152.9° ± 5.8° (min:140°; max:160°) |
| RIGHT KNEE EXTENSION ACTIVE | 5.00° ± 00° (min:0°; max:5°) | 6.00° ± 2.2° (min:0°; max:10°) | 7.56° ± 2.9° (min:0°; max:10°) | 6.43° ± 2.4° (min:0°; max:10°) |
| RIGHT KNEE EXTENSION PASSIVE | 6.00° ± 3.2° (min:0°; max:15°) | 6.67° ± 2.5° (min:0°, max:10°) | 7.30° ± 2.9° (min:0°; max:10°) | 6.43° ± 2.4° (min:0°; max:10°) |
| LEFT KNEE FLEXION ACTIVE | 129.3° ± 6.2° (min:120°; max:14°0) | 133.6° ± 6.9° (min:125°; max:150°) | 134.7° ± 5.8° (min:125°; max:145°) | 138.8° ± 5.3° (min:130°; mac:145°) |
| LEFT KNEE FLEXION PASSIVE | 141.3° ± 6.7° (min:130°; max:155°) | 146.0° ± 6.3° (min:140°; mac:160°) | 146.3° ± 6.4° (min:135°; max:155°) | 152.9° ± 5.8° (min:140°; max:160°) |
| LEFT KNEE EXTENSION ACTIVE | 5.00° ± 00° (min:0°; max:5°) | 6.00° ± 2.2° (min:0°; max:10°) | 7.30° ± 2.9° (min:0°; max:10°) | 6.43° ± 2.4° (min:0°; max:10°) |
| LEFT KNEE EXTENSION PASSIVE | 6.00° ± 3.2° (min:0°; max:15°) | 6.67° ± 2.5° (min:0°, max:10°) | 7.30° ± 2.9° (min:0°; max:10°) | 6.43° ± 2.4° (min:0°; max:10°) |

*SUBJECT ( N ): number of subjects, RIGHT KNEE FLEXION ACTIVE.: right knee flexion active, RIGHT KNEE FLEXION PASSIVE.: right knee flexion passive, RIGHT KNEE EXTENSION ACTIVE.: right knee extension active, RIGHT KNEE EXTENSION PASSIVE.: right knee extension passive, LEFT KNEE FLEXION ACTIVE.: left knee flexion active, LEFT KNEE FLEXION PASSIVE.: left knee flexion pasive, LEFT KNEE EXTENSION ACTIVE.: left knee extension active, LEFT KNEE EXTENSION PASSIVE.: left knee extension passive.*

All measurements in all the groups were within the normal active and passive range of motion for flexion and extension of the knee joints. There were no extreme recordings taken from the subjects and they had similar knee range of motion resuults. (Table 4.2)

Table 4.3. Goniometric measurements of the active and passive range of motion of right and left ankle joints

| | STRETCHING GROUP | MASSAGE GROUP | COMBINED THERAPY GROUP | CONTROL GROUP |
|---|---|---|---|---|
| SUBJECT (N) | 15 | 14 | 15 | 12 |
| RIGHTANKLE FLEXION ACTIVE | 17.6° ± 4.6° (min:10°; max:25°) | 15.7° ± 3.3° (min:10°; max:20°) | 20.3° ± 6.9° (min:5°; max:30°) | 15.4° ± 4.5° (min:5°; max:20°) |
| RIGHTANKLE FLEXION PASSIVE | 26.0° ± 5.3° (min:15°; max:30°) | 25.4°± 3.7° (min:20°; max:30°) | 27.0° ± 7.0° (min:15°; max:40°) | 23.8° ± 3.8° (min:15°; max:30°) |
| RIGHTANKLE EXTENSION ACTIVE | 49.0° ± 10.2° (min:30°; max:70°) | 45.4° ± 3.7° (min:40°; max:50°) | 55.0° ± 9.8° (min:40°; max:70°) | 48.8° ± 6.8° (min:40°; max:60°) |
| RIGHTANKLE EXTENSION PASSIVE | 59.0° ± 10.2° (min:40°; max:80°) | 57.1° ± 3.2° (min:50°; max:60°) | 63.3° ± 8.6° (min:50°; max:75°) | 58.3° ± 6.2° (min:50°; max:70°) |
| LEFT ANKLE FLEXION ACTIVE | 17.0° ± 4.9° (min:10°; max:25°) | 15.7° ± 3.3° (min:10°; max:20°) | 20.3° ± 6.9° (min:5°; max:30°) | 15.4° ± 4.5° (min:5°; max:20°) |
| LEFT ANKLE FLEXION PASSIVE | 25.0°± 6.3° (min:15°; max:30°) | 25.4° ± 3.7° (min:20°; max:30°) | 27.0° ± 7.0° (min:15°; max:40°) | 25.4° ± 5.5° (min:15°; max:30°) |
| LEFT ANKLE EXTENSION ACTIVE | 47.7° ± 12.5° (min:15°;max:70°) | 45.4° ± 3.7° (min:40°; max:50°) | 55.0° ± 9.8° (min:40°; max:70°) | 48.8° ± 6.8° (min:40°; max:60°) |
| LEFT ANKLE EXTENSION PASSIVE | 57.7° ± 11.9° (min:30°; max:80°) | 47.1° ± 3.2° (min:50°; max:60°) | 63.7° ± 8.1° (min:50°; max:75°) | 58.3° ± 6.2° (min:50°; max:70°) |

*SUBJECT (N): number of subjects, RIGHTANKLE FLEXION ACTIVE.: right ankle flexion active, RIGHTANKLE FLEXION PASSIVE.: right ankle flexion passive, RIGHTANKLE EXTENSION ACTIVE.: right ankle extension active, RIGHTANKLE EXTENSION PASSIVE.: right ankle extension passive, LEFT ANKLE FLEXION ACTIVE: left ankle flexion active, LEFT ANKLE FLEXION PASSIVE: left ankle flexion pasive, LEFT ANKLE EXTENSION ACTIVE: left ankle extension active, LEFT ANKLE EXTENSION PASSIVE: left ankle extension passive.*

All the results were within the normal active and passive range of motion of flexion and extension of ankle joints in all the groups. The measurements recorded from the subjects in all the groups were similar. (Table 4.3)

Table 4.4. Measurements of right and left knee angle in Thomas test position for assessing the tightness of rectus femoris (by Standard goniometer) and muscle tigthness test for iliopsoas and gastrocnemius muscles (expressed as negative or positive)

| | STRETCHING GROUP | MASSAGE GROUP | COMBINED THERAPY GROUP | CONTROL GROUP |
|---|---|---|---|---|
| SUBJECT (N) | 15 | 14 | 15 | 12 |
| RIGHT KNEE ANGLE | 60.0° ± 5.7° (min:50°; max:70°) | 60.0° ± 6.5° (min:50°; max:75°) | 61.3° ± 5.2° (min:50°; max:70°) | 62.5° ± 3.4° (min:60°; max:70°) |
| LEFT KNEE ANGLE | 60.0° ± 5.0° (min:50°; max:70°) | 59.3° ± 5.1° (min:50°; max:65°) | 61.3° ± 5.2° (min:50°; max:70°) | 62.5° ± 3.4° (min:60°; max:70°) |
| RIGHT THOMAS TEST | ( - ) | ( - ) | ( - ) | ( - ) |
| LEFT THOMAS TEST | ( - ) | ( - ) | ( - ) | ( - ) |
| RIGHTGASTROCNEMIUS | ( - ) | ( - ) | ( - ) | ( - ) |
| LEFT GASTROCNEMIUS | ( - ) | ( - ) | ( - ) | ( - ) |

SUBJECT (N): number of subjects, RIGHT KNEE ANGLE: right knee angle in Thomas Test position, LEFT KNEE ANGLE: left knee angle in Thomas Test position, RIGHT THOMAS TEST: shortness test of right Iliopsoas muscle, LEFT THOMAS TEST: shortness test for left iliopsoas muscle, RIGHTGASTROCNEMIUS: shortness test for right gastrocnemius muscle, LEFT GASTROCNEMIUS: shortness test for left gastrocnemius muscle.

Right and left knee angles in Thomas Test position were measured with a standard goniometer. Mean values of right and left knee angle measurements for each group are shown in the table 4.4. Thightness tests for the right and the left Iliopsoas and Gastrocnemius muscles were found negative for all the subjects in all groups. (Table 4.4)

Table 4.5. Comparative analysis between PRE and POST intervention measurements for the knee flexion ROM measured actively and passively on the right (dominant) side within the groups.

| BETWEEN GROUPS<br><br>(1 way ANOVA) | PRE TEST<br><br>KNEE FLEXION ACTIVE | POST TEST<br><br>KNEE FLEXION ACTIVE | *One-way ANOVA*<br><br>*P value* | PRE TEST<br><br>KNEE FLEXION PASSIVE | POST TEST<br><br>KNEE FLEXION PASSIVE | *One-way ANOVA*<br><br>*P value* |
|---|---|---|---|---|---|---|
| STRETCHING GROUP<br><br>N=15 | 129.3°±6.2 | 129.3°±6.5 | p>0.05 | 141.3°±6.7 | 141.3°±6.7 | p>0.05 |
| MANUAL THERAPY GROUP<br>N=14 | 134.6°±7.5 | 142.5°±7.5 | p>0.05 | 146.8°±6.7 | 151.1°±7.6 | p>0.05 |
| COMBINED THERAPY GROUP N=15 | 134.7°±5.8 | 135.7°±5.6 | p>0.05 | 147.0°±6.2 | 148.7°±6.7 | p>0.05 |
| CONTROL GROUP<br><br>N=12 | 134.3±4.7 | 134.7±5.5 | P>0.05 | 143.5±5.9 | 143.9±6.1 | p>0.05 |

*One-way ANOVA, Significant p<0.05

Goniometric measurement of the active and passive knee flexion range of motion revealed that there were no any significant differences between PRE and POST measurements. (Table 4.5)

**RESULTS RELATED TO RECTUS FEMORIS MUSCLE**

Table 4.6. Comparison of PRE and POST measurement values of Δt [EMG-MMG, Δt [EMG-FORCE] and Δt [MMG-FORCE] onsets for Rectus Femoris muscle within the control group.

| | 1 way ANOVA CONTROL GROUP N=12 | 15° | | | | | 30° | | | | | 45° | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PRE | POST | R | F | *p* | PRE | POST | R | F | *p* | PRE | POST | R | F | *p* |
| RF | Δt [EMG-MMG] | 15.7 ±8.03 | 16.56±8.93 | 72.234 | 0.933 | 0.34 | 15.60±7.41 | 18.12±9.03 | 334.618 | 1.995 | 0.16 | 19.15±8.82 | 18.65±8.53 | 313.164 | 1.841 | 0.18 |
| | Δt [EMG-FORCE] | 73.38±17.28 | 70.72±19.21 | 68.301 | 8.744 | 0.00 | 62.14±18.31 | 62.53±17.24 | 316.072 | 0.046 | 0.83 | 62.87±19.94 | 59.68±1448 | 224.265 | 0.947 | 0.33 |
| | Δt [MMG-FORCE] | 57.33±16.62 | 54.87±18.64 | 75.196 | 0.345 | 0.56 | 46.30±16.08 | 44.79±13.81 | 303.740 | 3.391 | 0.07 | 43.72±18.55 | 41.08±12.4 | 248.042 | 2.850 | 0.09 |

*One-way ANOVA, Significant $p<0.05$

In the Control Group, there was a statistical significant increase in the time delay between EMG and MMG onsets measured after resting period of 15 – 20 min ( $p < 0.05$) only at 30° of knee flexion. All other measured onsets did not reveal any statistical significance between the first and the second measurement values for the time delay ($p>0.05$)

In addition to One-way ANOVA test, it has been used Two-way ANOVA Test (Test of between subject effect), to analyze the homogenity of variables between the first and second measurements for RF muscle in the control group. (Table 4.6)

Table 4.7. Comparison of first and second measurement values of the time delays

Δt [EMG – MMG], Δt [EMG – FORCE] and Δt [MMG – FORCE] in the control group for RF muscle.

| 2 way ANOVA with Δt PRE and POST variables | | | | | | | |
|---|---|---|---|---|---|---|---|
| Tests of Between-Subjects Effects | | | | | | | |
| GROUP | MUSCLE | DEGREE | SOURCE | df | R | F | *P* |
| CONTROL | RF | 15° | INTERCEPT | 1 | 2623205.455 | 10896.900 | 0.000 |
| | | | PRE- POST | 2 | 363.860 | 1.511 | 0.211 |
| | | 30° | INTERCEPT | 1 | 1943036,586 | 9588,677 | 0.000 |
| | | | PRE- POST | 2 | 380.588 | 1.878 | 0.153 |
| | | 45° | INTERCEPT | 1 | 2032976.582 | 9736.287 | 0.000 |
| | | | PRE- POST | 2 | 204.342 | 0.979 | 0.376 |

* Two-way ANOVA, Significant $p<0.05$

2-way ANOVA Test results in the Control Group revealed that there were no differences between the first and second measurements for all the onsets (Δt EMG-MMG, Δt EMG-FORCE and Δt MMG-FORCE) at three testing knee flexion angles (15°, 30° and 45°) indicating homogenity of the variables between the subjects when they tested for RF muscle. Under this condition, found significance between PRE and POST in the time delay between EMG – MMG dissapeared within the group. (Table 4.7)

The significant differences have found between the first and second measurement values in the time delay between EMG-MMG onsets for the control group analyzed with One-way ANOVA test, disappeared when analyzed with Two-way ANOVA test. Under this situation, it was concluded that there was no statistically significant difference in the time delay in Δt [EMG-MMG], Δt [EMG-FORCE] and Δt [MMG-FORCE] for the control group.

Table 4.8. Comparison of PRE and POST values of Δt [EMG-MMG], Δt [EMG-FORCE] and Δt [MMG-FORCE] time delays for Rectus Femoris muscle within the stretching group.

| | 1 way ANOVA STRETCHING GROUP N=12 | 15° | | | | | 30° | | | | | 45° | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PRE | POST | R | F | *p* | PRE | POST | R | F | *p* | PRE | POST | R | F | *p* |
| RF | Δt [EMG-MMG] | 19.7±6.44 | 21.8±8.78 | 59.549 | 9.223 | 0.003 | 20.25±7.46 | 21.28±8.17 | 61.156 | 2.239 | 0.14 | 20.98±7.23 | 20.50±8.12 | 59.073 | 0.509 | 0.48 |
| | Δt [EMG-FORCE] | 71.17±14.93 | 72.50±20.42 | 320.780 | 0.684 | 0.41 | 63.85±20.69 | 63.55±16.6 | 351.627 | 0.035 | 0.85 | 58.11±13.8 | 60.53±20.41 | 303.992 | 2.495 | 0.11 |
| | Δt [MMG-FORCE] | 53.88±14.26 | 49.61±20.11 | 305.856 | 7.409 | 0.01 | 44.09±19.69 | 42.73±15.01 | 306.809 | 0.789 | 0.37 | 36.86±13.53 | 40.41±18.21 | 257.545 | 6.331 | 0.01 |

*One-way ANOVA, Significant if $p<0.05$

In the Stretching Group, there was statistically significant increase in the time delay between EMG and MMG onsets measured after stretching exercises compared with the delay measured before stretching at 15° of knee flexion ($p < 0.05$). This result was found to be accompained by statistically significant decrease in time delay between MMG and Force onsets after the streching exercise ($p < 0.05$). At 45° knee flexion, there was only statistically significant increase in the time delay between MMG and Force onsets ($p < 0.05$). At 30° knee of flexion, there was no statistical significant difference between EMG, MMG and Force onsets before and after the stretching. (Table 4.8)

Table 4.9. Comparsion of PRE and POST intervention values of Δt [EMG-MMG], Δt [EMG-FORCE] and Δt [MMG-FORCE] onsets for Rectus Femoris muscle within the manual therapy group.

| RF | 1 way ANOVA MANUAL THERAPY GROUP N=12 | 15° | | | | | 30° | | | | | 45° | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PRE | POST | R | F | *p* | PRE | POST | R | F | *p* | PRE | POST | R | F | *p* |
| RF | Δt [EMG-MMG] | 18.19±8.9 | 20.14±15.42 | 154.861 | 7.738 | 0.18 | 20.01±11.39 | 20.54±10.31 | 159.052 | 5.052 | 0.89 | 21.00±11.59 | 22.44±12.14 | 191.556 | 0.723 | 0.48 |
| | Δt [EMG-FORCE] | 68.42±26.78 | 80.39±37.13 | 1152.391 | 9.158 | 0.001 | 67.04±31.4 | 71.02±32.11 | 1223.42 | 2.716 | 0.05 | 69.68±33.2 | 70.24±27.99 | 988.181 | 0.029 | 0.97 |
| | Δt [MMG-FORCE] | 51.638±24.55 | 59.920±29.04 | 818.171 | 7.317 | 0.94 | 49.006±29.53 | 50.515±29.58 | 952.012 | 0.306 | 0.54 | 48.662±31.63 | 48.194±24.53 | 788.772 | 0.062 | 0.10 |

*One-way ANOVA, Significant if $p<0.05$

In the Manual Therapy Group, there was statistically significant increase in the time delay between EMG and Force (electromechanical delay) onsets measured after manual therapy compared to the delay measured before manual therapy at 15° knee flexion ($p<0.05$). At 30° knee flexion, there was an increase in the time delay between EMG and Force (electromechanical delay) measured after manual therapy compared to the delay measured before manual therapy ($p<0.05$). Time delays between EMG – MMG and MMG – Force did not show any statistical significance at 15° and 30°, respectively. At 45° knee flexion, there was no any statistical difference in the time delay between any onsets. (Table 4.9)

Table 4.10. Comparison of PRE and POST intervention values of Δt [EMG-MMG], Δt [EMG-FORCE] and Δt [MMG-FORCE] onsets for Rectus Femoris muscle within combined therapy group.

| | 1 way ANOVA COMBINED THERAPY GROUP N=12 | 15° | | | | | 30° | | | | | 45° | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PRE | POST | R | F | *p* | PRE | POST | R | F | *p* | PRE | POST | R | F | *p* |
| RF | Δt [EMG-MMG] | 16.62±8.18 | 20.09±11.52 | 100.733 | 14.719 | 0.001 | 17.48±8.29 | 19.87±10.11 | 86.212 | 8.693 | 0.001 | 16.69±7.59 | 20.90±10.46 | 84.105 | 26.240 | 0.001 |
| | Δt [EMG-FORCE] | 69.17±14.35 | 78.99±27.39 | 490.492 | 23.778 | 0.001 | 61.61±13.31 | 64.66±19.46 | 282.737 | 4.327 | 0.04 | 59.38±12.75 | 64.69±40.36 | 910.605 | 3.857 | 0.05 |
| | Δt [MMG-FORCE] | 52.57±12.67 | 58.91±21.85 | 326.254 | 14.862 | 0.001 | 44.12±13.47 | 45.03±15.71 | 215.757 | 0.510 | 0.48 | 42.65±12.23 | 41.62±15.01 | 188.262 | 0.696 | 0.40 |

*One-way ANOVA, Significant if p<0.05

In the Combined Therapy Group (manual therapy + stretching group), there were statistically significant increases in the all time delays after application of combined therapy at 15° knee flexion. ( $p < 0.05$). At 30° of knee flexion, there were statistically significant increases in time delay between EMG-MMG and EMG –Force onsets measured PRE and POST intervention ( $p < 0.05$), whereas the time delay between MMG and Force onsets was not significant. At 45° knee flexion, there were statistically significant increases in the time delay between EMG – MMG, and EMG – Force onsets after intervention ($p < 0.05$), while time delay between MMG and Force did not reveal any significance (Table 4.10).

Table 4.11. Comparison of averages between Δt [EMG-MMG], Δt [EMG – FORCE] and Δt [MMG – FORCE] of POST measurements between control and intervention groups at 15° of knee flexion for RF muscle.

| MUSCLE and DEGREE | Δt (ms) | GROUP | MEAN (ms) | GROUP | MEAN (ms) | *p* | R | F |
|---|---|---|---|---|---|---|---|---|
| RF 15° of knee flexion | Δt EMG -MMG | CONTROL | 15.56±8.93 | STRETCHING | 21.81±8.78 | 0.001 | 1022.614 | 7.622 |
| | | | | MANUAL THERAPY | 20.14±15.42 | 0.01 | | |
| | | | | COMBINED THERAPY | 20.09±11.52 | 0.01 | | |
| | Δt EMG-FORCE | CONTROL | 70.72±19.21 | STRETCHING | 72.50±20.42 | 0.9 | 5099.603 | 6.862 |
| | | | | MANUAL THERAPY | 80.39±37.13 | 0.001 | | |
| | | | | COMBINED THERAPY | 78.99±27.39 | 0.01 | | |
| | Δt MMG-FORCE | CONTROL | 54.87±18.64 | STRETCHING | 49.61±20.11 | 0.07 | 5464.658 | 10.45 |
| | | | | MANUAL THERAPY | 59.92±29.05 | 0.1 | | |
| | | | | COMBINED THERAPY | 58.91±21.85 | 0.25 | | |

*One-way ANOVA, Significant if $p<0.05$

At 15° of knee flexion for the RF muscle, One-way ANOVA between POST measurements of control and intervention groups revealed that the time delay between EMG – MMG significantly increased in all intervention groups compared to the control group ($p<0.05$). The time delay between EMG – Force increased both in the manual therapy and the stretching groups compared to the control group ($p<0.05$), whereas the stretching group showed no significant increase in time delay between EMG-Force. The time delay between MMG – Force did not reveal any significance between the control and the intervention groups. (Table 4.11)

Table 4.12. Comparsion of averages between Δt [EMG-MMG], Δt [EMG – FORCE] and Δt [MMG – FORCE] of POST measurements between the control and the intervention groups at 30° of knee flexion for RF muscle.

| MUSCLE and DEGREE | Δt (ms) | GROUP | MEAN (ms) | GROUP | MEAN (ms) | *p* | R | F |
|---|---|---|---|---|---|---|---|---|
| RF 30° of knee flexion | Δt EMG -MMG | CONTROL | 18.12±9.03 | STRETCHING | 21.28±8.17 | 0.001 | 387.879 | 4.326 |
| | | | | MANUAL THERAPY | 20.54±10.31 | 0.04 | | |
| | | | | COMBİNED THERAPY | 19.87±10.11 | 0.20 | | |
| | Δt EMG-FORCE | CONTROL | 62.53±17.24 | STRETCHING | 63.55±16.6 | 0.96 | 497.944 | 6.870 |
| | | | | MANUAL THERAPY | 71.02±32.11 | 0.001 | | |
| | | | | COMBİNED THERAPY | 64.66±19.46 | 0.74 | | |
| | Δt MMG-FORCE | CONTROL | 44.79±13.81 | STRETCHING | 42.73±15.01 | 0.69 | 387.731 | 7.091 |
| | | | | MANUAL THERAPY | 50.51±29.59 | 0.02 | | |
| | | | | COMBİNED THERAPY | 45.03±15.17 | 0.10 | | |

*One-way ANOVA, Significant if p<0.0

At 30° knee flexion for RF muscle, One-way ANOVA Test results showed that the time delay between EMG – MMG onsets significantly increased in the stretching group and the manual therapy group in comparison with the control group ( $p<0.05$ ), while the combined therapy group did not reveal any significance. The Time delay between EMG – Force and MMG – Force significantly increased only in the manual therapy group compared to the control group ($p<0.05$) (Table 4.12).

Table 4.13. Comparison of averages between Δt [EMG-MMG], Δt [EMG – FORCE] and Δt [MMG – FORCE] of POST measurements between control and intervention groups at 45° of knee flexion for RF muscle.

| MUSCLE and DEGREE | Δt (ms) | GROUP | MEAN (ms) | GROUP | MEAN (ms) | *p* | R | F |
|---|---|---|---|---|---|---|---|---|
| RF 45° of knee flexion | Δt EMG - MMG | CONTROL | 18.65±8.53 | STRETCHING | 20.50±8.12 | 0.20 | 539.218 | 5.417 |
| | | | | MANUAL THERAPY | 22.44±12.14 | 0.001 | | |
| | | | | COMBINED THERAPY | 20.90±10.46 | 0.08 | | |
| | Δt EMG-FORCE | CONTROL | 59.68±14.48 | STRETCHING | 60.53±20.41 | 0.99 | 5489.649 | 6.984 |
| | | | | MANUAL THERAPY | 70.24±27.99 | 0.001 | | |
| | | | | COMBINED THERAPY | 64.69±40.36 | 0.23 | | |
| | Δt MMG-FORCE | CONTROL | 41.08±12.4 | STRETCHING | 40.41±18.12 | 0.98 | 3159.979 | 9.471 |
| | | | | MANUAL THERAPY | 48.19±24.53 | 0.001 | | |
| | | | | COMBINED THERAPY | 41.62±15.10 | 0.99 | | |

*One-way ANOVA, Significant if $p<0.05$

The time delays between EMG-MMG, EMG-Force and MMG-Force at 45° knee flexion for RF muscle were significantly increased only in the manual therapy group compared with the control group ($p<0.05$). (Table 4.13)

**RESULTS RELATED TO VASTUS MEDIALIS MUSCLE**

Table 4.14. Comparison of first and second measurement values Δt [EMG-MMG], Δt [EMG – FORCE] and Δt [MMG – FORCE] onsets for Vastus Medialis for the control group.

| | 1 way ANOVA CONTROL GROUP N=12 | 15° | | | | | 30° | | | | | 45° | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PRE | POST | R | F | *p* | PRE | POST | R | F | *p* | PRE | POST | R | F | *p* |
| VM | Δt [EMG-MMG] | 18.10±8.39 | 18.15±8.25 | 69.201 | 0.003 | 0.96 | 16.16±6.68 | 18.47±9.14 | 62.786 | 7.765 | 0.01 | 19.42±9.28 | 18.11±7.65 | 72.352 | 2.261 | 0.13 |
| | Δt [EMG-FORCE] | 76.12±21.03 | 70.68±20.59 | 432.817 | 6.073 | 0.01 | 62.98±19.26 | 65.71±18.93 | 365.094 | 1.819 | 0.18 | 65.82±23.65 | 61.62±15.57 | 401.208 | 4.176 | 0.04 |
| | Δt [MMG-FORCE] | 57.42±21.2 | 55.23±21.7 | 460.595 | 0.914 | 0.34 | 47.96±18.55 | 48.41±19.37 | 359.354 | 0.050 | 0.82 | 46.80±20.46 | 43.95±16.22 | 340.334 | 2.290 | 0.13 |

*One-way ANOVA, Significant $p<0.05$

There were statistical significant decreases in time delay between EMG and Force for VM muscle at 15° and 45° of knee flexion in the Control Group ( $p<0.05$ ).

In regards to time delay measurement values between EMG and MMG onsets in 30° of knee flexion for the VM muscle, there was a significant increase in the second measurement. (Table 4.14)

To ensure the homogenity of variables between first and second resting period in the Control Group for RF muscle it was used TWO-way ANOVA (Tests of between-subjects effects).

Table 4.15. Two-way ANOVA between PRE and POST measurements of the time delay Δt [EMG-MMG], Δt [EMG – FORCE] and Δt [MMG – FORCE] in the Control Group for VM muscle.

| 2 way ANOVA with Δt PRE and POST variables | | | | | | | |
|---|---|---|---|---|---|---|---|
| Tests of Between-Subjects Effects | | | | | | | |
| GROUP | MUSCLE | DEGREE | SOURCE | df | R | F | *P* |
| CONTROL | VM | 15° | INTERCEPT | 1 | 2579709.907 | 8064.961 | 0.000 |
| | | | PRE- POST | 2 | 677.877 | 2.119 | *0.121* |
| | | 30° | INTERCEPT | 1 | 2025064.515 | 7773.703 | 0.000 |
| | | | PRE- POST | 2 | 132.720 | 0.509 | *0.601* |
| | | 45° | INTERCEPT | 1 | 2079774.108 | 7667.530 | 0.000 |
| | | | PRE- POST | 2 | 199.817 | 0.737 | *0.479* |

*Two-way ANOVA, Significant if p<0.05

2-way ANOVA test results in the control group revealed that there was no differences in all variables for VM muscle between the first and the second measurement values (Δt EMG-MMG, Δt EMG-FORCE and Δt MMG-FORCE) at three testing angles (15°, 30° and 45°) which indicated homogenity between subjects. (Table 4.15)

Table 4.16. Comparsions pf PRE and POST intervention values of Δt [EMG-MMG], Δt [EMG – FORCE] and Δt [MMG – FORCE] onsets for Vastus Medialis muscle of the stretching group.

| | 1 way ANOVA STRETCHING GROUP N=12 | 15° | | | | | 30° | | | | | 45° | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PRE | POST | R | F | *p* | PRE | POST | R | F | *p* | PRE | POST | R | F | *p* |
| VM | Δt [EMG-MMG] | 20.70±7.64 | 22.03±10.2 | 82.054 | 2.606 | 0.11 | 19.90±8.12 | 21.36±8.53 | 69.556 | 3.952 | 0.05 | 20.41±8.29 | 21.54±8.98 | 74.596 | 2.164 | 0.14 |
| | Δt [EMG-FORCE] | 71.84±15.16 | 75.68±21.59 | 348.887 | 5.159 | 0.02 | 63.75±21.34 | 65.17±20.18 | 430.229 | 0.609 | 0.44 | 58.39±15.78 | 63.49±23.64 | 402.051 | 8.157 | 0.001 |
| | Δt [MMG-FORCE] | 50.72±16.11 | 53.69±20.67 | 344.377 | 3.104 | 0.08 | 44.32±20.65 | 43.85±16.69 | 349.277 | 0.081 | 0.78 | 38.51±14.67 | 41.65±20.02 | 306.255 | 4.059 | 0.04 |

*One-way ANOVA, Significant if p<0.05

In the stretching group, for VM muscle at 15° and 30° of knee flexion, the time delay between EMG – MMG significantly increased after stretching. The time delay between EMG – Force increased significantly only at 15° and 45° knee flexion while time delay between MMG – Force increased significantly after stretching only at 45° knee flexion. (Table 4.16)

Table 4.17. Comparison of PRE and POST measurement values of Δt [EMG-MMG], Δt [EMG – FORCE] and Δt [MMG – FORCE] for Vastus Medialis muscle in the manual therapy group.

| | 1 way ANOVA MANUAL THERAPY GROUP N=12 | 15° | | | | | 30° | | | | | 45° | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PRE | POST | R | F | *p* | PRE | POST | R | F | *p* | PRE | POST | R | F | *p* |
| VM | Δt [EMG-MMG] | 20.69±12.46 | 22.99±14.54 | 170.500 | 5.115 | 0.13 | 24.93±23.81 | 23.77±18.71 | 369.914 | 4.050 | 0.78 | 23.91±14.19 | 22.97±13.14 | 204.548 | 2.657 | 0.76 |
| | Δt [EMG-FORCE] | 70.46±27.91 | 80.36±32.99 | 1056.980 | 6.131 | 0.001 | 73.57±35.83 | 70.66±33.27 | 627.311 | 0.590 | 0.60 | 69.56±35.39 | 70.79±31.37 | 1165.134 | 0.084 | 0.92 |
| | Δt [MMG-FORCE] | 52.21±23.27 | 57.60±25.7 | 739.179 | 4.035 | 0.07 | 51.60±27.21 | 47.64±25.01 | 668.157 | 1.732 | 0.21 | 46.30±29.38 | 48.10±26.1 | 866.504 | 1.069 | 0.78 |

*One-way ANOVA, Significant if p<0.05

In the manual therapy group, for VM muscle, at 15° knee flexion, the only time delay between EMG – Force significantly increased after manual therapy ( p<0.05). (Table 4.17)

Table 4.18 Averages and standard deviations analyzed with 1 way ANOVA for Δt [EMG-MMG], Δt [EMG – FORCE] and Δt [MMG – FORCE] onsets measured as PRE and POST for Vastus Medialis muscle within the combined therapy group.

| | 1 way ANOVA COMBINED THERAPY GROUP N=12 | 15° | | | | | 30° | | | | | 45° | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PRE | POST | R | F | *p* | PRE | POST | R | F | *p* | PRE | POST | R | F | *p* |
| VM | Δt [EMG-MMG] | 18.73±8.24 | 21.07±12.41 | 113.464 | 5.715 | 0.02 | 17.31±8.01 | 19.66±10.05 | 84.048 | 8.372 | 0.001 | 20.20±9.33 | 20.90±11.59 | 111.347 | 0.554 | 0.46 |
| | Δt [EMGFORCE] | 70.63±13.87 | 81.11±28.94 | 534.009 | 24.383 | 0.001 | 64.02±18.45 | 67.66±23.53 | 456.776 | 3.691 | 0.06 | 63.69±14.23 | 63.63±20.34 | 310.896 | 0.001 | 0.97 |
| | Δt [MMG-FORCE] | 51.89±13.07 | 60.01±24.13 | 388.345 | 20.194 | 0.001 | 46.96±16.6 | 48.79±19.91 | 341.250 | 1.259 | 0.26 | 43.61±12.77 | 42.83±15.27 | 198.991 | 0.390 | 0.53 |

*One-way ANOVA, Significant if p<0.05

In the combined therapy group for VM muscle, at 15° knee flexion, all time delays between EMG – MMG, EMG – Force and MMG – Force significantly increased after combined therapy (massage + stretching) ( $p<0.05$). At 30° of knee flexion, the only time delay between EMG – MMG significantly increased after manual therapy ($p<0.05$). At 45° of knee flexion, time delay between any onsets did not reveal any significance ($p>0.05$). (Table 4.18)

Table 4.19. Comparison of averages between Δt [EMG-MMG], Δt [EMG – FORCE] and Δt [MMG – FORCE] of POST measurements between control and intervention groups at 15° of knee flexion for VM muscle.

| MUSCLE and DEGREE | Δt (ms) | GROUP | MEAN (ms) | GROUP | MEAN (ms) | *p* | R | F |
|---|---|---|---|---|---|---|---|---|
| VM 15° of knee flexion | Δt EMG -MMG | CONTROL | 18.15±8.25 | STRETCHING | 22.03±10.27 | 0.01 | 886.715 | 6.343 |
| | | | | MANUAL THERAPY | 22.99±14.54 | 0.001 | | |
| | | | | COMBINED THERAPY | 21.07±12.41 | 0.06 | | |
| | ΔtEMGFORCE | CONTROL | 70.68±20.59 | STRETCHING | 75.68±21.59 | 0.23 | 4877.404 | 6.709 |
| | | | | MANUAL THERAPY | 80.36±32.99 | 0.001 | | |
| | | | | COMBINED THERAPY | 81.11±28.94 | 0.001 | | |
| | ΔtMMG-FORCE | CONTROL | 55.23±21.7 | STRETCHING | 53.69±20.60 | 0.91 | 1863.275 | 3.445 |
| | | | | MANUAL THERAPY | 57.60±25.7 | 0.72 | | |
| | | | | COMBINED THERAPY | 60.01±24.13 | 0.15 | | |

*One-way ANOVA, Significant if p<0.05

At 15° knee flexion for VM muscle, the time delay between EMG – MMG significantly increased in the stretching and manual therapy group in comparison to control group when it was analyzed with One-way ANOVA, ($p<0.05$). The time delay between EMG – Force significantly increased in both manual therapy group and combined therapy group compared to the control group, ( $p<0.05$), while the time delay between MMG – Force did not show any significance in any of the intervention groups for VM muscle at 15° knee flexion. (Table 4.19)

Table 4.20. Comparison of averages between Δt [EMG-MMG], Δt [EMG – FORCE] and Δt [MMG – FORCE] of POST measurements between control and intervention groups at 30° of knee flexion for VM muscle.

| MUSCLE and DEGREE | Δt (ms) | GROUP | MEAN (ms) | GROUP | MEAN (ms) | *p* | R | F |
|---|---|---|---|---|---|---|---|---|
| VM 30° of knee flexion | Δt EMG -MMG | CONTROL | 18.47±9.14 | STRETCHING | 21.36±8.53 | 0.08 | 1197.484 | 7.733 |
| | | | | MANUAL THERAPY | 23.77±18.71 | 0.001 | | |
| | | | | COMBINED THERAPY | 19.66±10.05 | 0.75 | | |
| | Δt EMG-FORCE | CONTROL | 65.71±18.93 | STRETCHING | 65.17±20.18 | 1.00 | 1518.011 | 2.427 |
| | | | | MANUAL THERAPY | 70.66±33.27 | 0.20 | | |
| | | | | COMBINED THERAPY | 67.66±23.53 | 0.86 | | |
| | Δt MMG-FORCE | CONTROL | 48.41±16.22 | STRETCHING | 43.85±16.69 | 0.11 | 1337.834 | 3.184 |
| | | | | MANUAL THERAPY | 47.64±25.01 | 0.98 | | |
| | | | | COMBINED THERAPY | 48.79±19.91 | 1.00 | | |

*One-way ANOVA, Significant if $p<0.05$

At 30° of knee flexion for VM muscle, the only time delay between EMG – MMG significantly increased in the manual therapy group compared to the control group (,$p<0.05$), whereas time delays between EMG – Force and MMG – Force did not reveal any statistical significance in any of the intervention groups at 30° of knee flexion. (Table 4.20)

Table 4.21. Comparison of averages between Δt [EMG-MMG], Δt [EMG – FORCE] and Δt [MMG – FORCE] of POST measurements between control and intervention groups at 45° of knee flexion for VM muscle.

| MUSCLE and DEGREE | Δt (ms) | GROUP | MEAN (ms) | GROUP | MEAN (ms) | *p* | R | F |
|---|---|---|---|---|---|---|---|---|
| VM 45° of knee flexion | Δt EMG -MMG | CONTROL | 18.11±7.65 | STRETCHING | 21.54±8.98 | 0.01 | 865.225 | 7.619 |
| | | | | MANUAL THERAPY | 22.97±13.3 | 0.001 | | |
| | | | | COMBINED THERAPY | 20.90±11.59 | 0.03 | | |
| | Δt EMG-FORCE | CONTROL | 61.62±15.57 | STRETCHING | 63.49±23.64 | 0.85 | 3766.980 | 6.676 |
| | | | | MANUAL THERAPY | 70.79±31.37 | 0.001 | | |
| | | | | COMBINED THERAPY | 63.63±20.34 | 0.81 | | |
| | Δt MMG-FORCE | CONTROL | 43.95±16.22 | STRETCHING | 41.65±20.02 | 0.63 | 1923.574 | 4.829 |
| | | | | MANUAL THERAPY | 48.10±26.1 | 0.14 | | |
| | | | | COMBINED THERAPY | 42.83±15.27 | 0.94 | | |

*One-way ANOVA, Significant if p<0.05

At 45° knee flexion, for VM muscle, the time delay between EMG – MMG significantly increased in all the intervention groups, (stretching, manual therapy and combined therapy groups) compared to the control group (p<0.05). Time delay between EMG – Force significantly increased only in the manual therapy group compared with the control group (p<0.05), whereas the time delay between MMG – Force did not show any statistical significance in any of the intervention groups compared to the Control Group. (Table 4.21)

Table 4.22. Averages of the Δt [EMG-MMG], Δt [EMG – FORCE] and Δt [MMG – FORCE] described as a percentage change and compared between groups with respect to each other for RF muscle at 15° of knee flexion.

| | | | STRETCHING | MANUAL THERAPY | COMBINED THERAPY |
|---|---|---|---|---|---|
| RF 15 ° | **CONTROL** | Δt [EMG – MMG] | **32%** | 22% | 21% |
| | | Δt [EMG –FORCE] | **3%** | **14%** | **12%** |
| | | Δt [MMG – FORCE] | -10% | 9% | 7% |
| | **STRETCHING** | Δt [EMG – MMG] | 0% | -8% | -8% |
| | | Δt [EMG –FORCE] | 0% | 11% | 9% |
| | | Δt [MMG – FORCE] | 0% | 21% | 19% |
| | **MANUAL THERAPY** | Δt [EMG – MMG] | 8% | 0% | 0% |
| | | Δt [EMG –FORCE] | -10% | 0% | -2% |
| | | Δt [MMG – FORCE] | -17% | 0% | -2% |
| | **COMBINED THERAPY** | Δt [EMG – MMG] | 9% | 0% | 0% |
| | | Δt [EMG –FORCE] | -8% | 2% | 0% |
| | | Δt [MMG – FORCE] | -16% | 2% | 0% |

For RF muscle, at 15° of knee flexion, stretching exercises showed the biggest effect on increase the time delay between EMG-MMG (32%), while manual therapy has the biggest effect on increasing the time delay between EMG-Force (14%), in respect to the control group. When massage + stretching applied as a combined therapy, there were stronger effect (12%) than applied stretching alone (3%) on increase the time delay between EMG-Force but lesser effect than applied manual therapy alone (14%). (Table 4.22.)

Table 4.23 Averages of the Δt [EMG-MMG], Δt [EMG – FORCE] and Δt [MMG – FORCE] described as a percentage change and compared between groups in respect to each other for RF muscle at 30° of knee flexion.

| | | | **STRETCHING** | **MANUAL THERAPY** | **COMBINED THERAPY** |
|---|---|---|---|---|---|
| RF 30 ° | **CONTROL** | Δt [EMG – MMG] | **17%** | 13% | 10% |
| | | Δt [EMG –FORCE] | 2% | **14%** | 3% |
| | | Δt [MMG – FORCE] | -5% | 13% | 1% |
| | **STRETCHING** | Δt [EMG – MMG] | 0% | -3% | -7% |
| | | Δt [EMG –FORCE] | 0% | 12% | 2% |
| | | Δt [MMG – FORCE] | 0% | 18% | 5% |
| | **MANUAL THERAPY** | Δt [EMG – MMG] | 4% | 0% | -3% |
| | | Δt [EMG –FORCE] | -11% | 0% | -9% |
| | | Δt [MMG – FORCE] | -15% | 0% | -11% |
| | **COMBINED THERAPY** | Δt [EMG – MMG] | 7% | 3% | 0% |
| | | Δt [EMG –FORCE] | -2% | 10% | 0% |
| | | Δt [MMG – FORCE] | -5% | 12% | 0% |

For RF muscle, at 30° of knee flexion, stretching has biggest effect on increasing the time delay between EMG-MMG (17%), while manual therapy has a stronger effect on increase the time delay between EMG-Force (14%) when compared to the control group. (Table 4.23)

Table 4.24. Averages of the Δt [EMG-MMG], Δt [EMG – FORCE] and Δt [MMG – FORCE] described as a percentage change and compared between groups in respect to each other for RF muscle at 45° of knee flexion.

| | | | STRETCHING | MANUAL THERAPY | COMBINED THERAPY |
|---|---|---|---|---|---|
| RF 45 ° | **CONTROL** | Δt [EMG – MMG] | 10% | **20%** | **12%** |
| | | Δt [EMG –FORCE] | 1% | **18%** | 8% |
| | | Δt [MMG – FORCE] | -10% | **17%** | 1% |
| | **STRETCHING** | Δt [EMG – MMG] | 0% | 9% | 2% |
| | | Δt [EMG –FORCE] | 0% | 16% | 7% |
| | | Δt [MMG – FORCE] | 0% | 31% | 13% |
| | **MANUAL THERAPY** | Δt [EMG – MMG] | -9% | 0% | -7% |
| | | Δt [EMG –FORCE] | -14% | 0% | -8% |
| | | Δt [MMG – FORCE] | -24% | 0% | -14% |
| | **COMBINED THERAPY** | Δt [EMG – MMG] | -2% | 7% | 0% |
| | | Δt [EMG –FORCE] | -6% | 9% | 0% |
| | | Δt [MMG – FORCE] | -11% | 16% | 0% |

For RF muscle, at 45° of knee flexion, manual therapy has the strongest effect on increasing all time delays between EMG-MMG, EMG-Force and MMG-Force (20%, 18% and 17%,respectively) when compared with applied stretching alone (10%, 1% and -10%, respectively) and combined therapy (12%, 8% and 1%, respectively). When massage + stretching applied as a combined therapy, the biggest effect was observed on increasing EMG-MMG for 12%, while stretching applied alone showed lesser effect (10%). (Table 4.24)

Table 4.25. Averages of the Δt [EMG-MMG], Δt [EMG – FORCE] and Δt [MMG – FORCE] described as a percentage change and compared between groups with respect to each other for VM muscle at 15° of knee flexion.

| | | | **STRETCHING** | **MANUAL THERAPY** | **COMBINED THERAPY** |
|---|---|---|---|---|---|
| VM 15 ° | **CONTROL** | Δt [EMG – MMG] | **21%** | **27%** | **16%** |
| | | Δt [EMG –FORCE] | **7%** | **14%** | **15%** |
| | | Δt [MMG – FORCE] | -3% | 4% | 9% |
| | **STRETCHING** | Δt [EMG – MMG] | 0% | 4% | -4% |
| | | Δt [EMG –FORCE] | 0% | 6% | 7% |
| | | Δt [MMG – FORCE] | 0% | 7% | 12% |
| | **MANUAL THERAPY** | Δt [EMG – MMG] | -4% | 0% | -8% |
| | | Δt [EMG –FORCE] | -6% | 0% | 1% |
| | | Δt [MMG – FORCE] | -7% | 0% | 4% |
| | **COMBINED THERAPY** | Δt [EMG – MMG] | 5% | 9% | 0% |
| | | Δt [EMG –FORCE] | -7% | -1% | 0% |
| | | Δt [MMG – FORCE] | -11% | -4% | 0% |

For VM muscle, at 15° of knee flexion, manual therapy was shown the strongest effect (27%) on increase the time delay between EMG-MMG. When the time delay between EMG-FORCE was considered, the combined therapy was more influential (15%) then the stretching (7%) and manual therapy (14%) applied as a single treatment. (Table 4.25)

Table 4.26. Averages of the Δt [EMG-MMG], Δt [EMG – FORCE] and Δt [MMG – FORCE] described as a percentage change and compared between groups in respect to each other for VM muscle at 30° of knee flexion.

| | | | STRETCHING | MANUAL THERAPY | COMBINED THERAPY |
|---|---|---|---|---|---|
| VM 30 ° | **CONTROL** | Δt [EMG – MMG] | **16%** | **29%** | **6%** |
| | | Δt [EMG –FORCE] | -1% | **8%** | 3% |
| | | Δt [MMG – FORCE] | -9% | -2% | 1% |
| | **STRETCHING** | Δt [EMG – MMG] | 0% | 11% | -8% |
| | | Δt [EMG –FORCE] | 0% | 8% | 4% |
| | | Δt [MMG – FORCE] | 0% | 9% | 11% |
| | **MANUAL THERAPY** | Δt [EMG – MMG] | -10% | 0% | -17% |
| | | Δt [EMG –FORCE] | -8% | 0% | -4% |
| | | Δt [MMG – FORCE] | -8% | 0% | 2% |
| | **COMBINED THERAPY** | Δt [EMG – MMG] | 9% | 21% | 0% |
| | | Δt [EMG –FORCE] | -4% | 4% | 0% |
| | | Δt [MMG – FORCE] | -10% | -2% | 0% |

For VM muscle, at 30° of knee flexion, manual therapy had the strongest effect on increasing the time delays between EMG-MMG and EMG-Force, 29% and 8%, respectively. Stretching applied as a single therapy was able to increase the time delay between EMG-MMG in a higher percentage (16%) than the combined therapy (6%). (Table 4.26)

Table 4.27. Averages of the Δt [EMG-MMG], Δt [EMG – FORCE] and Δt [MMG – FORCE] described as a percentage change and compared between groups with respect to each other for VM muscle at 30° of knee flexion.

| | | | STRETCHING | MANUAL THERAPY | COMBINED THERAPY |
|---|---|---|---|---|---|
| VM 45 ° | **CONTROL** | Δt [EMG – MMG] | **19%** | **27%** | **15%** |
| | | Δt [EMG –FORCE] | 3% | **15%** | 3% |
| | | Δt [MMG – FORCE] | -5% | **9%** | -3% |
| | **STRETCHING** | Δt [EMG – MMG] | 0% | 7% | -3% |
| | | Δt [EMG –FORCE] | 0% | 11% | 0% |
| | | Δt [MMG – FORCE] | 0% | 15% | 3% |
| | **MANUAL THERAPY** | Δt [EMG – MMG] | -6% | 0% | -9% |
| | | Δt [EMG –FORCE] | -10% | 0% | -10% |
| | | Δt [MMG – FORCE] | -13% | 0% | -11% |
| | **COMBINED THERAPY** | Δt [EMG – MMG] | 3% | 10% | 0% |
| | | Δt [EMG –FORCE] | 0% | 11% | 0% |
| | | Δt [MMG – FORCE] | -3% | 12% | 0% |

For VM muscle, at 45° of knee flexion, manual therapy has shown the strongest effect on increase the time delays between EMG-MMG, EMG-Force and MMG-Force, 27%, 15% and 9%, respectively. Stretching applied as a single treatment was able to increase time delay between EMG-MMG in a higher percentage (19%) than applied combined therapy (15%). (Table 4.27)

## 5. DISCUSSION

This study was carried out to determine the alterations of the electromechanical properties of the Quadriceps femoris muscle after stretching exercises, manual therapy (transverse friction massage and patellar mobilization) and combined therapy (combination of all these techniques) applied in order to reduce the stiffness. For these purposes, the electromechanical time delays described as time delays between onsets of the surface EMG, MMG and Force signals of the Rectus Femoris and Vastus Medialis muscles have been assessed during isometric contractions of the Quadriceps femoris muscle at three knee flexion angles of 15°, 30° and 45°. Tests have been performed before (PRE) and after (POST) the interventions divided into the three different groups and have been found out their immediate effects on the electro-mechanical properties of quadriceps muscle and tendon stiffness. The results have been recorded as time delay (Δt) between signal onsets of the EMG and MMG (Δt EMG-MMG), EMG and FORCE (Δt EMG-FORCE) and MMG and FORCE (Δt EMG-FORCE) indicating electromechanical properties. Results from the three intervention groups were compared within the groups (PRE and POST) and also with the control group (POST). The percentage of alteration in Δt EMG-MMG, Δt EMG-FORCE and Δt MMG-FORCE after the interventions were calculated for each intervention group and was compared with the control group. Thus, it was intended to find out which intervention will have better effect on the electromechanical time delay (Δt EMG-FORCE) indicated as a time elapse between onset of action potential and actual force production (Force). Because the increased time delay in Δt EMG-FORCE shows reduced muscle-tendon stiffness, it has clinical importance for the physical therapy interventions applied in order to reduce muscle stiffness.

### Stretching Group

Our results showed that the stretching exercises had a significant effect on increasing the time delay between EMG and MMG (Δt EMG-MMG) of Rectus femoris muscle and only when tested at 15° of knee flexion. It means that stretching exercises showed effects on the sarcomer level, producing increases in the time elapsed between the start of the action potential propagation and cross-bridge formation related to the actin and myosin filaments. This could be explained as a decrease in active muscle stiffness produced by stretching exercises. When observed the effects of the stretching exercises on the overall time elapsed between the start of the action potential propagation and actual force generation in Rectus Femoris muscle, there was no effect what means that electromechanical delay was not affected. These findings were found as the immediate effects of the stretching exercises within the groups when they compared between PRE and POST assessments of stretching intervention. When POST assessments were compared with the POST assessments of the control group, it was found that stretching exercises had beneficial effects on increasing time elapsed between the start of the action potential propagation and cross-bridge formation between actin and myosin filaments at 15° and 30° of knee flexion. Results compared within the group and compared with the control group are consistent with each other indicating that stretching exercises are efficient only at the sarcomer level indicating decrease of the active muscle stiffness of the Rectus femoris muscle.

For Vastus medialis muscle, our results showed that the stretching exercises had a significant effect on increasing the overall time elapsed between the start of the action potential propagation and actual force output (moment), called as electromechanical delay (Δt EMG-FORCE), when tested at 15° and 45° of knee flexion. While active stiffness (Δt EMG-MMG) showed a tendency to be decreased in all three tested angles, significant result was found only when tested at 30° of knee flexion. Passive stiffness (Δt MMG-FORCE) was found to be significantly decreased only when tested at 45° of knee flexion. Comparison with the control group revealed that time delay, Δt EMG-MMG, increased at all three angles tested at 15°, 30°and 45° of knee flexion while significant increase was found to be only at 15° and 45° of knee flexion. Increased time delay between EMG and MMG could be explained that stretching exercises were efficient to decrease active muscle stiffness by effecting active contractile components and showing the effect on the sarcomer level. In addition to the decrease in active muscle stiffness, significant increase in the electromechanical delay at 15° and 45° of knee flexion shows that stretching exercises were also efficient to decrease both active and passive muscle stiffness even if it was found that the time delay between the MMG and FORCE (Δt MMG-

FORCE), indicating passive muscle stiffness, was found to be significant only at 45° of knee flexion.

Goniometric measurements revealed that the knee flexion range of motion measured both actively and passively was not significantly changed stretching intervention.

These results suggest that stretching exercises (10 x 30 seconds) had significant effect on excitation – contraction coupling at both sarcomer and muscle-tendon unit level for Rectus femoris muscle only at 15° of knee flexion. A comparison with the control group revealed that the electromechanical delay (Δt EMG-FORCE) which means the time delay between onset of the propagation of action potential and force output remained increased at 15° of knee flexion, but was not statistically significant. This means that stretching exercises for Quadriceps muscle tends towards increase electromechanical time delay which would indicate decrease in both active and passive stiffness of the Quadriceps muscle. The methodology of our study is particularly in accordance with the study of Esposito et al, (25) because the author has used a methodology to state series of the events from the onset of action potentials by EMG, first vibration of the fibers by MMG and force output by FORCE sensors. One of the difference was that we recorded series of these events during repeated voluntary isometric contractions, but Esposito F. (25) did it during elicited tetanic contractions with electrical stimulator. Another difference was that our measurements were obtained at the different knee flexion angles, 15°, 30° and 45°. It is known that when the knee is closer to full extension, less isometric torque is produced, selective regional activation of motor units occurs within a particular muscle, differential activation of the four parts of quadriceps femoris muscle occurs, biomechanical considerations and/or differences in the overlap of actin and myosin filaments occurs (26). In our study, we used a moderate level of isometric force production, because intramuscular fluid pressure (IMFP) is not sufficient to affect the MMG amplitude (71). On the other hand, load cell that we used to measure force output was sensitive only between 0 and 500 N.

Several studies had investigated the effects of stretching exercises on muscle stiffness and muscle-tendon unit viscoelatic properties under different conditions, using different methodology (5,7,8,9,72,73,74,75,76). The most common used stretching technique by athletes and physiotherapists during rehabilitation is static stretching in order to reduce stiffness and increase flexibility. When biomechanically evaluation was done on cyclic and static stretching and stretching training on viscoelastic properties, it was found that cyclic and static stretching did not produce any change on the muscle-tendon unit (MTU) stiffness (5) There was also observed no changes in the stiffness of tendon structures after 3 weeks stretching program. (74) Some studies investigated the effects of stretching exercises on the passive stiffness of the MTU (7,8,9). It has been suggested that passive muscle stiffness can be defined as the length-tension relationship of muscle when it is passively stretched and the slope of the linear portion of the passive torque-angle curve is defined as passive stiffness (12). When acute effects of stretching on the passive stiffness of the human gastrocnemius muscle-tendon unit was investigated, it was found that MTU stiffness decreased following repeated stretches. However, it was not due to changes in the tendon, but rather to an increased compliance of the proximal muscular portion of the MTU. The anatomical muscle becomes less stiff and overall stiffness of the MTU decreases (7). When acute and prolonged effects of static stretching were investigated, it was found that 5 min static stretching reduced MTU and muscle stiffness and this positive effect endured 10 minutes at least. Prolonged effects of static stretching also reduced MTU stiffness as a consequence of the decrease in muscle stiffness, not to that in tendon stiffness. (8) As similarly in this study, we used 5 min (10 rep x 30 s) repeated stretching protocol and we investigated the immediate effect of stretching. However, we did not find out the prolonged effect of stretching, as they have done. Because it has been proven that acute effects of 5 minutes stretching is good enough to see reducing stiffness in the muscle-tendon unit and prolonged effect showed the same results (8). We did not want to re-test the subjects for the records and make them exhausting with the test. 4 weeks static stretching program on human gastrocnemius muscle showed significant decrease in MTU stiffness, in particular muscle stiffness as a consequence of change in properties of the intramuscular connective tissue such as endomysium, perimysium and epimysium instead of lengthening muscle fibers.(9,75), When the MMG and FORCE signals were recorded before and after (every 15 min, up to 2 h) a stretching routine during tetanic stimulations from the medial gastrocnemius muscle, the early return to MMG pre-stretching values was found. It was suggested that changes in viscoelastic paralel components recovered after few minutes.(75). In one of the study, muscle-tendon series compliance was presented with rate of force development, electromechanical delay and musculotendinous junction displacement using ultrasound during MVC and elicited contractions to investigate the effect of 4x45 s static stretching protocol. It was found that the effect of acute static stretching on both voluntary and elicited forces did not produce any changes in tendon properties. (76) In our study, contrary to Esposito G.2011(25), there was no any significant change in electromechanical delay for the Rectus femoris muscle (after stretches, from 71,17 to 72,50 ms, p>0.05) as a possible reason to be that we detected electromechanical delay (EMD) during repeated voluntary isometric contractions while Esposito et al (25) used electrostimulation. Elelectromechanical delay measured in-vivo mainly indicated the time course or time elapsed between the start of the action potential propagation and actual force development (56). If reduced EMD shows increase in tendon stiffness and energy utilization (30), then, almost remained unchanged EMD (from PRE to POST) in our study shows that 10x30 seconds repeated stretching has no any effect on the decrease of the overall time elapsed. The findings of the unchanged EMD were evident when tested at all three angles, 15°, 30° and 45° of knee flexion for Rectus femoris muscle. As a contrary effects time delay between EMG-MMG termed as excitation-contraction coupling was found to be significantly lengthened (after 10x30 scstretches) at 15° of knee flexion (p<0.05) for the Rectus femoris muscle. After stretching, some modifications at the sarcomer level in contractile (cross-bridges efficiency) and structural (elastic-filaments of titin) elements have been reported. In our study, lengthened time delay between EMG and MMG at 15° of knee flexion, shows decrease in active muscle stiffness produced by stretching. It was suggested that elastic filament of titin described as a structural elements was likely to be affected by stretching. (77). However, in our study, there was no changes in time delay between EMG and MMG when tested at 30° and 45° of knee flexion. The time delay between MMG and FORCE (period between muscle surface displacement and last mechanical event as force production) for Rectus femoris was found to be significantly decreased from PRE to POST at 15° of knee flexion (p<0.05) while time delay between MMG and FORCE significantly increased at 45° suggesting that there was not stretching induced changes in perimysium, endomysium, aponeurosis or tendon mechanical characteristics and lower knee angles while there might be produced some changes when measured during repeated voluntary isometric contraction with higher knee flexion angles.

In conclusion, during voluntary isometric contractions of RF muscle, stretching induced significant change detected as increase in time delay in EMG – MMG. It shows decrease in active muscle stiffness and modifications at sarcomer level including contractile and structural elements.

To detect the effect of 10x30 s stretching applied protocol, EMD may not be a measurable parameter during voluntary isometric contractions. Doing the measurements during the voluntary isometric contraction could be one of the possible restrictions in our study. EMD remained unchanged showing that static stretching is not effective to change the overall time elapsed between action potential propagation and actual force generation. Significant decrease in the time delay between the MMG and FORCE at 15° of knee flexion, unchanged results at 30° and significantly increase at 45° of knee flexion for Rectus femoris muscle, show that stretching may produce some changes in passive stiffness including series elastic components when measured at higher knee flexion angles.

**Manual Therapy Group**

For Rectus femoris muscle, results showed that manual therapy (transverse friction massage and patellar mobilization) had a significant effect on increasing the time delay between EMG and FORCE (Δt EMG-FORCE), called as electromechanical delay when tested only at 15° and 30° of knee flexion. It means that the transverse friction massage + patellar mobilization are effective techniques to increase the time elapsed between the start of action potential propagation and actual force production. It could be explained that the stiffness of the overall anatomical structure, including both muscle and muscle tendon unit is decreased. At what extend is effected separately active stiffness and passive stiffness cannot be explained since there was no any statistically significant result about time delay between EMG – MMG and MMG – FORCE within the group. Even without any statistical significant result regarding the active and passive muscle stiffness, it could be observed that the biggest time delay was between MMG and FORCE which indicates that the transverse friction massage applied over the quadriceps tendon and patellar mobilization tended to decrease the passive muscle stiffness. A comparison with the non-intervention control group revealed that the transverse friction massage + patellar mobilization were effective to statistically increase the time delay between the EMG and FORCE (electromechanical delay) when tested at all three angles, 15°, 30° and 45° for Rectus femoris muscle. When the time delay between the MMG and FORCE was measured and compared with the control group, it was found to be significantly lengthened only when tested at 30° and 45° of knee flexion while the tested 15° of knee flexion did not show any significance but with tendencies towards the increased time delay. It could be explained that passive muscle stiffness which is related to the connective tissue, such as endomysium, perimisyum and epimysium is effectively decreased when tested at 30° and 45° of knee flexion. Active muscle stiffness which is presented by the delay between the EMG and MMG ($\Delta t$ EMG – MMG), was significantly decreased by the friction massage + patellar mobilization when tested at all three angles, 15°, 30° and 45° of knee flexion but in a lesser extend than it was found to be for the time delay between MMG and FORCE.

For Vastus medialis muscle, friction massage + patellar mobilization significantly increased the overall time delay between the start of action potential propagation and actual force production (electromechanical delay) only at 15° of knee flexion. There was no any statistically significant result about the time delay between EMG – MMG and MMG – FORCE which refer to the active and passive muscle stiffness, respectively. Time delay between the EMG and FORCE (electromechanical delay) compared with the non-intervention control group was significantly lengthened when tested at 15° and 45° of knee flexion. Passive stiffness which was presented by the time delay between MMG and FORCE was not affected by friction massage + patellar mobilization at any tested angle while the active stiffness presented by the time delay between EMG and MMG was significantly decreased at all three tested angles. It means that the friction massage applied over the Quadriceps femoris tendon with patellar mobilization produced significant changes at the sarcomer level.

Results from the goniometric measurements for active and passive range of motion of the knee joint, did not produce any effect in order to increase the knee flexion range of motion, what means that the decrease in the stiffness was not accompained by an increase of the range of motion.

Various studies have investigated effects of massage (13,14,18,17,15,78,79,80) with focusing on electromyographic changes. It is generally accepted that massage produces reduction (inhibition) of motoneuron activity. (81) Massage produces inhibitory effects (decrease in H-reflex amplitude) and H-reflex was found to be reduced in triceps surae muscle showing a reduction in the α-motoneuron excitability. (17). In another study, it was found that application of 3 s petrissage massage on peroneous longus and brevis muscles produced large reflex induced response in tensor fascia latae. Changes in the amplitude of the EMG and MMG of the tensor fascia latae were observed as changes located at the distance from the massaged muscle (13). It was also investigated whether massage produces a force reduction in quadriceps femoris (14) and plantar flexors (78) when tested during the concentric contraction. When the effects of massage for Quadriceps femoris muscle was studied, massage was applied in random cross over fashion using strokes with grade 1 or 2 and effleurage grade 3 for totally 30 minutes. The main finding was that force was reduced when it is tested at 60°/s velocity while 120°/s showed decline in isokinetic force. 180° and 240°/s of velocity did not reveal any difference (14). It was concluded that massage effects are velocity dependent and effects of the massage are best observed during the slower velocities. We did not use isokinetic test in different velocities for assessing the effects of massage, but we did take attention to use sub-maximal voluntary isometric contraction because of avoiding tetanic effects of the maximal isometric contraction. It might not be correlated with the study used isokinetic test, but we may attribute that this unwanted effect with the higher speed in their study might be related with the tetanization of the quadriceps muscle. It is one of the reason why we used submaximal isometric contraction instead of using maximal contraction in our testing procedure.

Tapotement and petrissage are commonly used massage techniques by physical therapists. In duration of 3 minutes with the frequency of 4 Hz and 3 minutes petrissage as different massage techniques were investigated. Both of these techniques, have shown no differences in either the drop-jump or the concentric calf rise while increases in ankle ROM indicated an increase in muscle compliance as a consequence of an increase in muscle temperature altering tissue viscosity and tissue compliance (78). In order to investigate the effects of decreasing pressure and increasing pressure massage, it was hypothesized that locally applied pressure can reduce muscle tension. In this study, subjects were asked to allow only pressure levels that were below the pain threshold when tested. Massage was performed using slow strokes with adjacent thumbs in a distal-to-proximal direction on quadriceps femoris using 3 strokes lasting for 15 seconds. Main findings were that gradually increasing pressure massage prevents increased muscle activity while decreasing pressure massage which start with deep pressure enhanced muscle activity (18). Because of this finding from this study, we preferred to use moderate pressure during application of transverse friction massage on quadriceps muscle-tendon unit.

Transverse friction massage is frequently used modality by physiotherapists with aims to reduce the pain and prevent the adhered tissues. It can be applied on musculotendinous junction with top of the fingers making transverse movements over the tendon under angle of 45°. This technique was investigated on the musculotendinous junction of the distal portion of hamstring muscle with duration of 10 and 30 seconds (15). Continuous massaging the musculotendinous junction could theoretically activate the inhibitory action of the Golgi tendon organs (GTO) or lead to a presynaptic inhibition of the Ia sensory fibers from the spindles of the activated muscle (19). The most important finding from the study (15) was that friction massage for the medial part of hamstring with duration of 10 and 30 seconds was increased hip flexion ROM for 5,9% and 7,2%, respectively. This was indication that brief duration massage study provides an increase in hamstring flexibility, suggesting an increased muscle compliance or decreased muscle stiffness. (15). This study was the only study in the literature which has used friction massage like we used in our study since the majority of the studies related with massage have investigated the effects of classical massage like stroking or kneading instead of friction massage. One of the hypothesis was that the increased stiffness of the Achilles Tendon, increased contraction velocity and increased calcium sensitivity in muscle fibers was found after plyometric training, suggesting that training may reduce the time delay between EMG and FORCE. It was found that there was significant correlations between tendon stiffness and voluntary EMD and jump height. This study revealed that plyometric training reduced voluntary EMD with which tendon stiffness and unwanted early muscle activation. (30).

It is anticipated that the stiffness of the tendon is primarily modulator of the musculoarticular stiffness (MAS) at low loads (82). So stiffer MTU will generate internal forces more rapidly by contractile elements. (20) On the other hand , one of the study showed that if stiffer MTU increases internal forces in muscle more rapidly, than patients who suffer from anterior knee pain will show increase in patello-femoral joint reaction forces by unwanted faster contraction of quadriceps femoris. It could have one of the detrimental effects on cartilage tissue and pain production observed in patello-femoral joint. (83). Stretching also used as modality to reduce the stiffness has shown that reduction of stiffness in MTU after stretching was primarily produced by reduction in the stiffness of the muscle, but not the stiffness of tendon (84) All these inconsistant results triggered us to investigate how can be changed the contractile components of muscle by applying localized transverse friction massage combined with the patellar mobilization on muscle-tendon unit of Quadriceps Femoris muscle.

MMG signals which are primarily produced by vibration of the muscle fibers, EMG signals which are reflection of motor unit recruitment and firing rate and force signals showing moment force produced by muscle are series of the events providing unique insight into the active skeletal muscle (85,25). The main aim of our research in massage group was to investigate the interaction between contractile components and series elastic components after massaging musculotendinous junction of the quadriceps femoris muscle using simultaneously recording of EMG, MMG and FORCE. Massaging musculotendinous junction of the hamstring muscles showed immediate effects on increase of ROM while results from our study did not show any significant results in change of ROM. In our study, we observed internal muscle dynamics after the transverse friction massage + patellar mobilization application within the group compared as PRE and POST and also compared with the non-interventional control group. When compared within the group, we observed that the time delay between EMG-FORCE (electromechanical delay) increased when tested at 15° and 30° of knee flexion for RF muscle. It indicates that an increase in the EMD shows decrease in both active and passive muscle stiffness. At what extend is effected separately active stiffness and passive stiffness can not be explained since there was no any statistically significant result about time delay between EMG – MMG and MMG – FORCE within the group. Increase in time delay between MMG and FORCE also indicates elongation of the series elastic component, the transfer of the tension at the insertion point and the subsequent force generation (25). When compared with the control group we found that time delays between EMG-MMG and EMG-FORCE were lengthened at all three testing angles showing that transverse friction massage on MTJ has significant effect starting at the point of T-tubules depolarization and followed by events until the first mechanical vibration at the muscle surface (active muscle stiffness) and subsequent force generation (FORCE output). Similar effects have been found to be evident in one of the study when stretching applied, but tested during tetanic electrical stimulation. (25) In our study time delays were much longer because we measured during voluntary isometric contraction. When the time delay between the MMG and FORCE was measured and compared with the control group, it was found to be significantly lengthened only when tested at 30° and 45° of knee flexion while the tested 15° of knee flexion did not show any significance. It could be explained that passive muscle stiffness which is related to the connective tissue, such as endomysium, perimisyum and epimysium is effectively decreased when tested at 30° and 45° of knee flexion. Even if non-simultaneous motor unit activations are present during voluntary isometric contraction, in our study, the effects of transverse friction massage on the MTU were obviously evident when it was compared within the group and with the control group.

Results from the Vastus medialis muscle revealed that electromechanical delay (Δt EMG-FORCE) was lengthened only at 15° of knee flexion when analyzed within the group while electromechanical delay was found to be lengthened at 15° and 45° of knee flexion when compared with the control group. Significant result regarding the lengthening electromechanical delay from within group analysis at 15° of knee flexion shows that transverse friction massage + patellar mobilization were effective to decrease the overall stiffness (including both contractile and series elastic components) when assessed on subject base and when the knee angle is closer to extension. When compared with the control group, the time delay between the EMG and MMG, which presents active muscle stiffness was found to be significantly lengthened at all tested three angles. Passive stiffness which were presented by the time delay between MMG and FORCE was not affected by friction massage + patellar mobilization at any tested angle while the active stiffness presented by the time delay between EMG and MMG was significantly decreased at all three tested angles. It means that the friction massage applied over the Quadriceps femoris tendon with patellar mobilization produced significant changes at the sarcomer level. One of the study examined electromechanical delay on vastus medialis obliques in patients with PFPS in comparison with the control group and it was found that electromechanical delay was significantly longer in patients with PFPS. They also showed significant differences between VMO and VL with lengthened EMD for VMO. (86,87)

Leg flexion angle closer to full extension results in less isometric force production due to selective regional activation of motor units within a particular muscle, differential activation of the four parts of quadriceps femoris muscle, biomechanical considerations and/or differences in the overlap of actin and myosin filaments (24)

Because the muscle length affects isometric torque production and possibly motor unit activation strategies, leg flexion angle may also influence the pattern of EMG and MMG amplitude. (24) This influence can be seen in our study results since we have got different results with the different knee flexion angles.

A motor unit firing rate, which is closely correlated with EMD will be reduced in flexion angle closer to full extension suggesting an increase in EMD.

Regarding the results from the comparison with the control group it can be concluded that the MTU transverse friction massage combined with patellar mobilization were effective techniques to reduce both active (Δt EMG-MMG) and passive stiffness (Δt MMG-FORCE) of Rectus femoris muscle, showing a bigger influence on reducing its passive stiffness. Results from the Vastus medialis muscle showed that the MTU transverse friction massage and patellar mobilization increased electromechanical delay (Δt EMG-FORCE) when the knee angle is closer to full extension, while excitation-contraction coupling (stiffness at the sarcomer level or active muscle stiffness) is affected at all three angles, 15°, 30° and 45°. The reason for not having any changes in passive muscle stiffness (Δt MMG-FORCE) might be associated to the anatomical position and size of the Vastus medialis muscle, which is much smaller than Rectus femoris and with smaller attachment considering its tendon which emerges into the common tendon of all parts of the quadriceps muscles, so called Quadriceps femoris tendon.

**Combined Therapy ( Stretching+ Manual Therapy ) Group**

Combined treatment of transverse friction massage, patellar mobilization and stretching exercises revealed that the active muscle stiffness (Δt EMG-MMG) was reduced at all tested angles while passive muscle stiffness (Δt MMG-FORCE) found to be reduced only at 15° of knee flexion. A comparison with the control group revealed that the electromechanical delay (Δt EMG-FORCE) was increased showing reduced stiffness of the overall muscle with the biggest influence on the reducing the active muscle stiffness at 15° of knee flexion for the Rectus femoris muscle. For Vastus medialis muscle, it was found that both active and passive muscle stiffness were reduced together with increased electromechanical delay at 15° of knee flexion while 30° of knee flexion revealed only a decrease in active muscle stiffness. Comparison with the control group revealed only an increase in the electromechanical delay when tested at 15° of knee flexion without any significant result regarding the active or passive muscle stiffness.

Goniometric measurements of active and passive range of motion of the knee joint did not reveal any significance when it was measured after the combined treatment.

Reduced active muscle stiffness (Δt EMG-MMG) indicated that the chemical events such as action potential propagation along the sarcolemma and cross-bridge formation are significantly affected. Decreased passive muscle stiffness (Δt MMG-FORCE) indicates that the time between the first sarcomer motion and elongation of the passive series of elastic components of the muscle-tendon unit as well as the time required to stretch a series of elastic components represented by electromechanical delay (Δt EMG-FORCE) was lengthened (25). Time delay between MMG – Force is a useful monitor of the duration of the overall events after cross-bridge formation, i.e. the elongation of the series elastic component, the transfer of the tension to the tendon insertion point and the subsequent force generation. (25). As the angle increases length-tension relationship (88) could be one of the reasons for not having affected electro-chemical and electro-mechanical events in muscle, especially when results are compared with the control group for both Rectus femoris and Vastus medialis muscles. It is well known that if an activated muscle is stretched prior to shortening , its performance during the concentric phase of action is enhanced. (23). Torque production at the knee is dependent on the knee angle with greater torque in the mid range positions, which has been attributed to joint angle dependent changes in muscle length of the different quadriceps component (89). As torque production increases, intramuscular pressure increases and electromechanical delay reduces because of the stiffness since there is negative correlation between time delay and MTU stiffness. ( 24,89 ) This could be one of the explanations why electromechanical delay was not affected at 30° and 45° of knee flexion when compared with the control group for both RF and VM muscles.

One of the recent studies (78) has investigated myotendinous junction massage, tapotement massage with and without stretching on ankle plantar flexors in comparison with the control group. The authors has studied variables which were H/M ratio, plantar flexors twitch contractile properties (peak torque, time to peak torque, half relaxation time (HRT), soleus M-wave amplitude and electromechanical delay for intervention and control group. It was found that EMD (representative of compliance in the musculotendinous unit) was prolonged when tapotement massage combined with stretching and MTJ massage combined with stretching (78). Even if their methodology was different from our study, there is similarity between results indicating that stiffness was effectively decreased when massage was combined with static stretching exercises.

**Percentage changes**

For the Recrus Femoris muscle, comparison with the control group revealed that the stretching applied as a single treatment showed the biggest effect on decreases the stiffness at the sarcomere level (active stiffness) for 32% at 15°, 17% at 30° and 10% at 45° of knee flexion. There is observed tendency towards decreasing effect on the active stiffness by going into the deeper knee flexion angles. When manual therapy (transverse friction massage + patellar mobilization) applied and compared with the control group, there was found increase in the overall time delay by 14% at 15° and 30° and 18% at 45° of knee flexion. Opposite to the active stiffness, overall time delay (electromechanical delay) seems to be increasingly affected by manual therapy showing decreased stiffness even in the deeper knee flexion angles when manual therapy applied. Throughout this overall time delay, transverse friction massage + patellar mobilization showed the biggest influence on decreasing active muscle stiffness producing changes at the sarcomer level by 22%, 13% and 20% at 15°, 30° and 45° of knee flexion, respectively. Influence on decreasing the passive muscle stiffness producing changes on serie elastic components was 9%, 13% and 17% at 15°, 30° and 45° of knee flexion, respectively. Combined treatment showed the biggest effect on decreasing active muscle stiffness producing changes at the sarcomer level by 21%, 10% and 12% at 15°, 30° and 45° of knee flexion, respectively.

For Vastus Medalis muscle, stretching exercises applied alone and compared with the control group showed the biggest effect on decreasing active muscle stiffness producing changes at the sarcomer level by 21% , 16% and 19% when tested at 15°, 30° and 45° of knee flexion, respectively. Manual therapy compared with the control group decreased overall muscle stiffness in all three tested knee angles, including biggest influence on decreasing active muscle stiffness producing changes at the sarcomer level for 27%, 29% and 27% at 15°, 30° and 45° of knee flexion, respectively. During this time delay, influence on decreasing passive muscle stiffness producing changes at the level of series elastic components was found to be 4% and 9% at 15° and 45° of knee flexion, respectively. Combined treatment compared with the control group decreased active muscle stiffness producing changes at the sarcomer level for 16%, 6% and 15% when tested at 15°, 30° and 45° of knee flexion, respectively.

These results suggest that stretching ( 10 x 30 sc) protocol had biggest effect on sarcomer level presented as the the time elapse between the propagation of action potential and first fiber vibration for both tested Rectus femoris and Vastus medialis muscles. Better said, there is a longer time delay produced between the action potential propagation along the sarcolemma underneath the EMG electrodes and the cross-bridge activation, i.e. the first mechanical event occurring immediately at the muscle belly, before the tensioning of the series elastic components (25). Some authors suggested that the EMG – MMG latency could describe the excitation – contraction coupling changes after particular events, such as fatigue or mechanical strain (90). In light of this knowledge, for Vastus Medialis muscle, such a big increase in the time delay between EMG – MMG showing a decrease in active muscle stiffness after transverse friction massage + patellar mobilization application could be affected by fatigue induced changes after repeated isometric contractions during testing we used in our study. It is well known that in patients with

Patellofemoral Pain Syndrome, vastus medialis obliqus (VMO) weakened in relation to vastus lateralis. (83,91,92) After repeated isometric contractions of Quadriceps Femoris, VMO could be fatigued, so, we observed quite big increase in the time delay between EMG – MMG caused by manual therapy for Vastus medialis muscle. It could be concluded that application of transverse friction massage over the Quadriceps femoris muscle combined with patellar mobilization produced a significant effect on decreasing active muscle stiffness suggesting changes at the sarcomer level for Rectus femoris muscle, while, the biggest effect was on Vastus medialis muscle because of its anatomical location considering its smaller size, distal placement and smaller tendon length that immerges into the Quadriceps femoris tendon.

Massaging the Quadriceps muscle-tendon unit we also observed lengthened the time of the overall process indicating that massage was quite efficient to reduce stiffness by increasing time between action potential propagation and force produced by quadriceps muscle at 15° of knee flexion. This trend was also observed at 30° and 45° of knee flexion but with lesser changes at the level of serie elastic components indicating that viscoelastic characteristics of endomysium , perimysium and aponeurosis could not be affected as the knee goes into the deeper flexion angles.

Combined treatment had a lesser effect than the stretching applied and manual therapy applied alone on decreasing the active muscle stiffness by increasing the time delay between the EMG and Force. As the angle increases, the length – tension relationship could be one of the reasons for not having affected electromechanical delay (12) in deeper knee flexion angles. Deeper knee flexion angles (30° and 45°), revealed that in combined therapy with added stretching exercises to transverse friction massage and patellar mobilization reduced the effect of massage possibly caused by increased length-tension relationship and consequently masking the produced effect of transverse friction massage on Quadriceps femoris muscle.

Reduced effect of massage by stretching application could be also as a consequence of repeated stretching that we used in our study. In rehabilitation static stretching is more preferred technique for decreasing the stiffness and increasing the flexibility. In the stretching group where stretching is applied as a single treatment, it was found that stretching was effective only at the sarcomer and series elastic component level. When stretching applied together with massage, decrease in stiffness (12%) was higher than stretching applied alone (3%) but lesser than massage applied alone (14%) indicating that the stretching masks the effect of the manual therapy while manual therapy applied alone is the best way to decrease the stiffness. (4)

**CONCLUSIONS**

1. There were no changes in range of motion (ROM) of knee and ankle joint after stretching exercises, manual therapy and combined therapy.
2. Stretching exercises are efficient techniques to increase overall time delay showing a decrease in the muscle stiffness, but their biggest effect was on decreasing active stiffness at the sarcomer level with lesser impact on series elastic components. These findings were detected within the groups and when compared with the control group.
3. Manual therapy (transverse friction massage + patellar mobilization) showed different results for Rectus femoris and Vastus medialis muscles. In Rectus femoris muscle, manual therapy was found to be effective in decreasing overall muscle stiffness, but with the biggest impact on decreasing the passive muscle stiffness effecting series elastic components.
4. For Vastus medialis muscle, manual therapy was effective in decreasing overall muscle stiffness, but with the biggest impact on active muscle stiffness affecting the sarcomer level.
5. The combined treatment for both Rectus femoris and Vastus medialis muscles, revealed to be efficient to decrease overall muscle stiffness, but with the biggest impact on reducing the active muscle stiffness. It is supposed that the stretching exercises, mask the effectiveness of the transverse friction massage and patellar mobilization on the series elastic components when applied as a combined treatment.
6. Stretching exercises produced the biggest percentage of change in reducing the active stiffness by 23% for Rectus femoris and 21% for Vastus medialis muscles.
7. Manual therapy produced a significant change in percentage of reducing overall muscle stiffness by 18%, active stiffness by 22% and passive stiffness by 17% of Rectus femoris muscle.
8. For Vastus medialis muscle, transverse friction massage + patellar mobilization produced the biggest change of percentage in reducing the active muscle stiffness by 29%.
9. Combined treatments produced the biggest change of percentage in reducing active muscle stiffness by 21% for Rectus femoris muscle and 16% for Vastus medialis muscle.

### Study limitations

It should be noted that we could not measure maximum voluntary isometric contraction because our load-cell was sensitive only between 0 – 500 Newton (50 Kg). Measurement of the rate of force development would be of high importance to find out the interaction between the stiffness, peak force and rate of force development.

Investigation of late response was not possible because the subjects involved in our study were full-time working people and they were not able to participate regularly in massage and stretching sessions applied by physical therapists.

### Future directions

**Further researches are needed;**

1. To identify the effects of transverse friction massage and deep friction massage when tested during both isometric and isokinetic contractions,
2. To compare changes in muscle dynamics with measured displacement of the muscle tendon unit junction observed by the ultrasound imaging,
3. To find out late ressponses (at least after 3 weeks) of the friction massage techniques,
4. To find out gender dependent changes in muscle dynamics.

### Clinical relevance

Muscle stiffness is commonly seen in the clinical practice as one of the most challenging problems for the physical therapists and other clinicians. Manual therapy techniques such as transverse friction massage and patellar mobilization, combined with stretching or not, have frequently used techniques to solve this problem. Some clinicians prefer to use transverse friction massage which can be applied to muscle and tendon separately while some of them prefer to use stretching exercises. All these techniques can be used in order to decrease the stiffness, but the effects of these techniques on the muscle or tendon stiffness and stiffness induced changes in electrophysiological properties are unknown. On regarding to our study results, it can be recommended that transverse friction massage applied over the Quadriceps femoris tendon and patellar mobilization are very efficient techniques to reduce the stiffness in both muscle and tendon parts even without any application on the muscle belly. The effects of these techniques are best seen and preserved at 15° and 30° of knee flexion what means that these techniques can be useful for the sports players which use a smaller knee range of motion such as basketball players. Stretching applied alone are only efficient to reduce active muscle stiffness at the sarcomere level without any significant effect on the tendon. When all these techniques, stretching, massage and mobilization, applied together, it is expected to get decreased both active and passive stiffness at the maximum level but our results showed that stretching exercises masked the effects of massage and mobilization so it is recommended to apply massage after the stretching protocols.

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## ATTACHMENTS

### Annex 1. Research Ethics Review Board Approval

**HACETTEPE ÜNİVERSİTESİ**
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01 Ekim 2012

Sayı: B.30.2.HAC.0.05.07.00/776

***ARAŞTIRMA PROJESİ DEĞERLENDİRME RAPORU***

**Toplantı Tarihi** : 26.EYLÜL 2012
**Toplantı No** : 2012/09
**Proje No** : HEK 12/81 (Değerlendirme Tarihi 05.06.2012)
**Karar No** : HEK 12/81 - 07

Üniversitemiz Sağlık Bilimleri Fakültesi Fizyoterapi ve Rehabilitasyon Bölümü öğretim üyelerinden Prof. Dr. Filiz CAN'ın sorumlu araştırmacısı olduğu Uzm. Fzt. Haris BEGOVİÇ ve Prof.Dr. Necla ÖZTÜRK ile birlikte çalışacakları HEK 12/81 kayıt numaralı ve ***"Quadriceps Kasının Sertliğini Azaltmada Kullanılan Pasif Germe ve Manual Tedavi Yöntemlerinin Kas ve Tendon Üzerine Olan Etkilerinin Karşılaştırılması"*** başlıklı proje önerisi Kurulumuzda değerlendirilmiş olup, etik açıdan uygun bulunmuştur.

1.Prof. Dr. Nurten Akarsu (Başkan)

2. Prof. Dr. Nüket Örnek Buken (Üye)

3. Prof. Dr. Hakan S. Orer (Üye)
KATILMADI

4. Prof. Dr. Sevda F. Müftüoğlu (Üye)

5. Prof. Dr. Cenk Sökmensüer (Üye)

6. Prof. Dr. Volga Bayrakçı Tunay (Üye)

7. Prof. Dr. Songül Vaizoğlu (Üye)

Y.S. Erdal

8. Prof. Dr. Yılmaz Selim Erdal (Üye)

9 Prof. Dr. Melahat Görduysus (Üye)

10. Doç. Dr. R. Köksal Özgül (Üye)

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12. Doç. Dr. Ayşe Lale Doğan (Üye)

13 Doç. Dr. S. Kutay Demirkan (Üye)

14. Yrd. Doç. Dr. H. Hüsrev Turnagöl (Üye)
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15. Av. Meltem Onurlu (Üye)