

**INTRAOPERATIVE MEASUREMENT OF HUMAN
SPASTIC GRACILIS MUSCLE ISOMETRIC FORCES AS A
FUNCTION OF KNEE ANGLE**

by

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ABSTRACT

INTRAOPERATIVE MEASUREMENT OF HUMAN SPASTIC GRACILIS MUSCLE ISOMETRIC FORCES AS A FUNCTION OF KNEE ANGLE

Spasticity is a neuromuscular disease which is associated with increased muscle tone, stiffness and impaired motor control and consequently functional limitations. Improved understanding of spasticity requires the collection of substantial directly measured length-force characteristics of spastic muscles. Studies including direct measurement of human muscle force are very rare due to limited access to the muscle. With the method developed in this study, isometric length (knee angle)-force characteristics of human spastic Gracilis muscle are measured intraoperatively for the first time in literature. Experimental data is collected during the surgical operations performed by Prof. Dr. Yener Temelli and his group in Istanbul University School of Medicine. In 7 subjects (average age: 8 ± 4.6), isometric muscle forces are measured by buckle force transducers at five different knee angles (of 120° , 90° , 60° , 30° and 0°). Mean peak Gracilis muscle force and mean optimal knee angles are measured to be 41.19 ± 41.07 N and $30\pm 31.6^\circ$ respectively. Knee-angle force characteristics of 7 subjects showed inter-subject variability and peak Gracilis muscle forces were not correlated with the anthropometric data of subjects. Gracilis muscle exerted non-zero force in each condition indicating that functional joint range of motion is at least as wide as from full knee extension to 120° of knee flexion. A finding of major importance is that knee angle-force characteristics of spastic Gracilis muscle are found to be not representative of the pathological condition occurring at the joint and are comparable with the ones obtained from healthy subjects in a previous study of our research group. Moreover, length history of muscle was shown presently to affect muscle force in most of the subjects.

Keywords: Cerebral palsy, Gracilis muscle, Intraoperative muscle force measurement.

ÖZET

İNSAN SPASTİK GRASILIS KASI İZOMETRİK KUVVETİNİN DİZ AÇISI FONKSİYONU OLARAK ÖLÇÜLMESİ

Spastisite artan kas gücü ve fonksiyonel hareket kısıtlamaları ile sonuçlanan nöromusküler bir hastalıktır. Spastisite hastalığının daha iyi anlaşılabilmesi, yeni tedavi yöntemlerinin bulunması ve kullanılan tedavi yöntemlerinin geliştirilmesi için kapsamlı spastik kas uzunluk-kuvvet verisinin toplanması gerekmektedir. İnsan kası uzunluk-kuvvet karakteristiğinin direkt ölçümü kasa ulaşılabilirliğin kısıtlı olmasından dolayı bir kaç araştırma ile sınırlı kalmıştır. Bu çalışmada geliştirdiğimiz yöntem sayesinde, insan spastik gracilis kasının izometrik uzunluk (diz açısı)-kuvvet karakteristiği intraoperatif olarak literatürde ilk kez ölçülmüştür. Bütün kuvvet ölçümleri Prof. Dr. Yener Temelli ve ekibinin İstanbul Üniversitesi Tıp Fakültesi'nde uyguladıkları aponörotomi ameliyatları sırasında yapılmıştır. 7 denegın (ortalama yaş: 8 ± 4.6) spastik gracilis kası izometrik kuvveti, hedef kas maksimal şekilde uyarıldıktan sonra tendon üstü kuvvet çevirici yardımıyla, 5 farklı diz açısında (120° , 90° , 60° , 30° ve 0°) ölçülmüştür. Sonuç olarak 7 denekte ölçülen ortalama maksimal Gracilis kası kuvveti 41.19 ± 41.07 N, ortalama optimal diz açısı ise $30\pm 31.6^\circ$ olarak belirlenmiştir. Ölçülen kuvvetler ve diz açısı-kuvvet karakteristikleri denekler arasında yüksek varyasyon göstermiştir. Deneklerin antropometri dataları ve maksimal Gracilis kası kuvvetleri arasında bir korrelasyon bulunmamıştır. Spastik Gracilis kası diz açısının 0° ve 120° olduğu durumlarda sıfır olmayan kuvvetler üretmiş ve fonksiyonel eklem aralığının en az bu açılar kapsayacak kadar geniş olduğunu göstermiştir. Spastik Gracilis kası diz açısı-kuvvet karakteristiğinin eklem aralığında görülen patolojik durumu yansıtmadığı gözlenmiş ve bu karakteristik bir önceki çalışmada ölçülen sağlıklı hastaların Gracilis kası karakteristiklerine benzer olarak belirlenmiştir. Kas geçmişinin kas kuvveti üzerinde etkisi çoğu denekte önemli ölçüde gösterilmiştir.

Anahtar Sözcükler: Serebral Palsi, Gracilis kası, İntraoperatif kas kuvveti ölçümü

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LIST OF ABBREVIATIONS

BFT	Buckle Force Transducer
EDL	Extensor Digitorum Longus
FCU	Flexor Carpi Ulnaris Muscle
MFT	Myofascial Force Transmission
MTS	Material Testing Machine

1. INTRODUCTION

Muscle is an activatable soft tissue whose function is to generate and exert force and produce movement. Muscles can cause either locomotion of the organism itself or movement of internal organs. They are classified as skeletal, cardiac or smooth muscles.

1.1 Skeletal Muscle

Skeletal muscle can be considered as activatable functional units whose single cells are muscle fibers. The complete muscle is surrounded by a fascia and a connective tissue named epimysium which consists of irregularly distributed collagen fibers, connective tissue cells and fat.

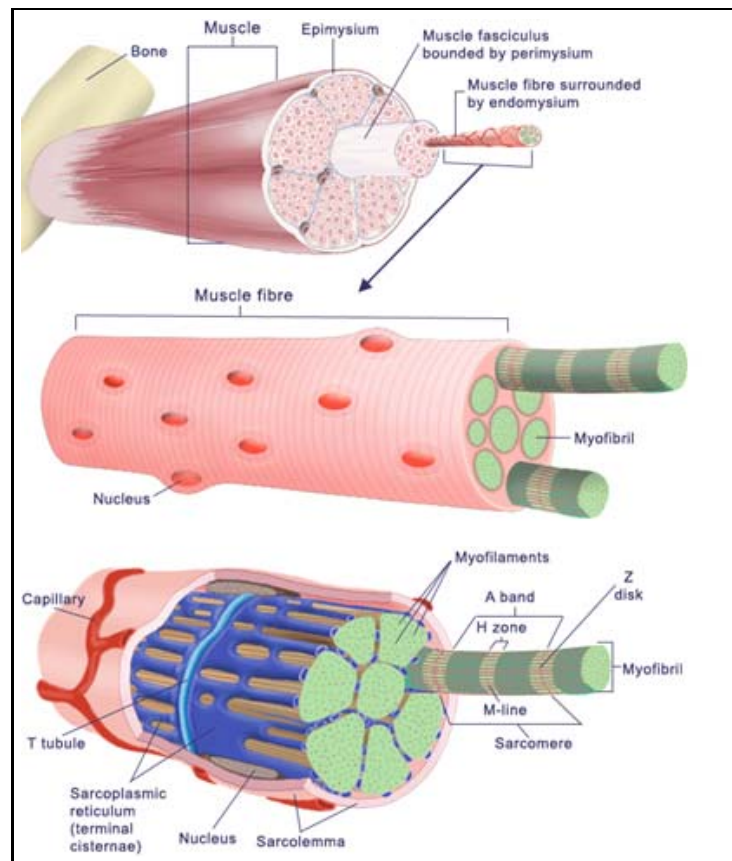


Figure 1.1 A schematic view of a muscle, a muscle fiber and a myofibril. Modified from [1].

The bundle composed of a number of muscle fibers is called fascicle. Each fascicle is surrounded by a connective tissue structure called perimysium. One fascicle is composed of many muscle cells (fibers) which are within a cell membrane called sarcolemma. Muscle cells are surrounded by the endomysium, a thin sheet of connective tissue. Within each muscle fiber, several thousand myofibrils are arranged in parallel. Myofibrils are serially repeated lines of the fundamental contractile unit, the sarcomere. The sarcomere consists of parallel protein filaments; thin (actin) and thick (myosin) filaments. Filaments attach to a disk-like protein structure, termed the Z disk, which marks the ends of the sarcomere. Actin filaments are divided into two parts by Z-disks whereas the myosin filaments are located in the center of the sarcomere. Myosin filaments give a dark image to the striated pattern which is called A-band. Actin filaments make up the light patterns of the striation called I-bands. The area within the A-band with a lower refractive index is called H-band. The myosin filaments are connected to each other with a system of fixed transverse filaments called M-bridges (Figure 1.2).

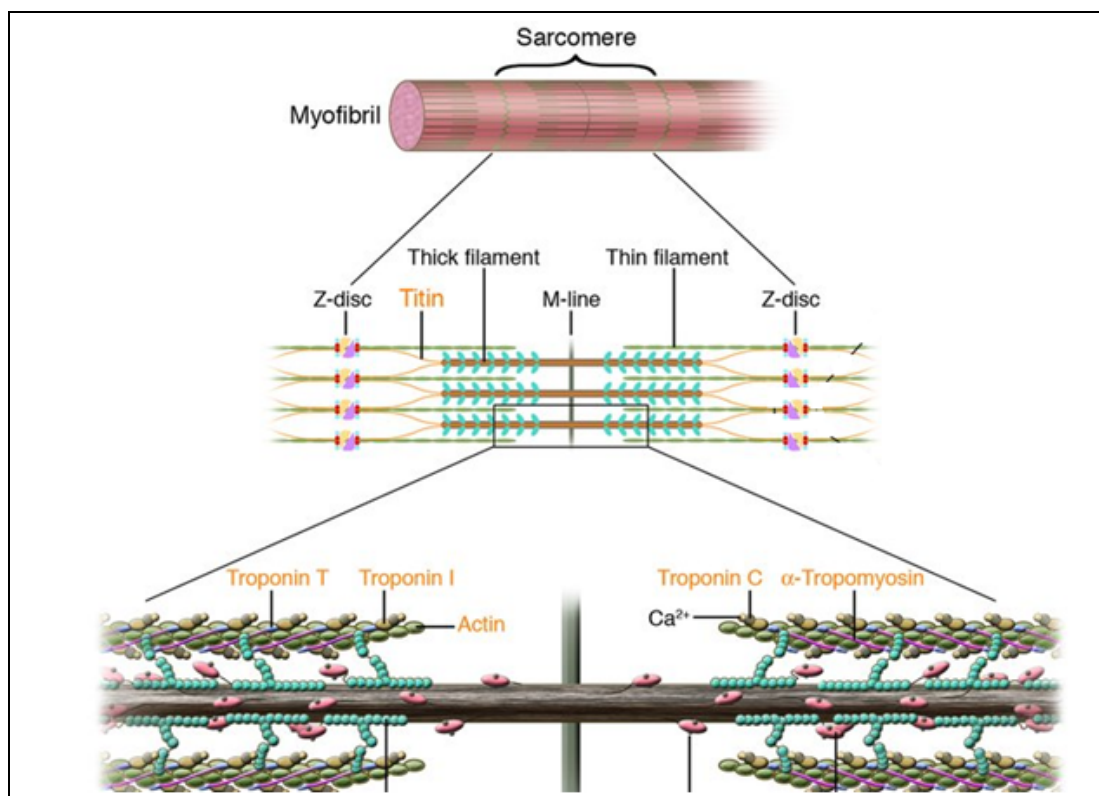


Figure 1.2 A schematic view of a myofibril and a sarcomere. Modified from[2].

1.1.1 Skeletal Muscle Force Production

The relationship between the structure of the sarcomere and the contractile mechanism, including the role of ATP as the energy source for contraction, is explained by "sliding filament theory" originally proposed by Huxley and Hanson[3]. According to this theory, the contractile mechanism depends on the presence and interaction of myoproteins (actin and myosin) and two other proteins; tropomyosin and troponin which regulate the actin-myosin interaction. Tropomyosin is a long fibrous protein that lies in the groove formed by the actin chains whereas troponin molecules are located along the actin. Troponin is composed of three subunits: Troponin C (contains sites for calcium ion (Ca^{+2} binding), Troponin T (contacts tropomyosin) and Troponin I (blocks the cross-bridge attachment site in the resting state).

When the muscle shortens or lengthens (either actively or passively), active filaments slide along myosin. The sliding of the filaments is dependent on the cross-bridging of myosin heads to actin filaments. The mechanism of the sliding filament theory is;

1. Calcium ions (Ca^{+2}) are released in the stimulated muscle cells. The Ca^{+2} bonds to troponin C allowing the myosin head to bind with the binding site.
2. Actin stimulates the complete hydrolysis of ATP to ADP. The release of ADP results in transition to a strong binding state.
3. Upon strong binding, myosin rotates at the myosin-actin interface. The extensible region in the myosin head pulls the filaments across each other and shortening occurs. Myosin remains attached to the actin.
4. The binding of ATP-Mg complex to the myosin head detaches myosin from actin. While detached, ATP hydrolysis occurs causing the myosin to straighten out. The split of ATP (into ADP and phosphate) stores energy in the myosin head and releases some heat.
5. The cycle starts again if the myosin head finds a new actin binding site.

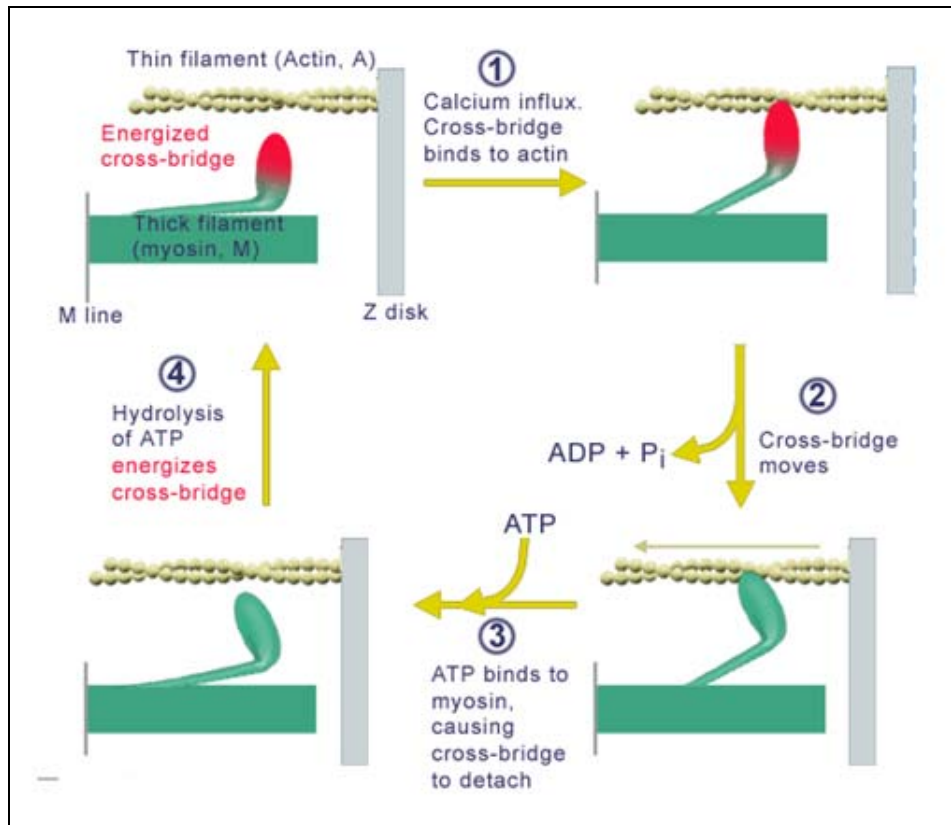


Figure 1.3 A schematic representation of sliding filament theory. Modified from [4]

The collective bending of numerous myosin heads (all in the same direction), combine to move the actin filament relative to the myosin filament. This results in muscle contraction.

The contraction force is proportional to the degree of overlap between actin and myosin filaments. After lengthening the muscle till there is no overlap, the sarcomere cannot develop any tension when stimulated. In the other extreme, after shortening the sarcomere till the myosin filament crumble up against the Z-discs; the myosin heads become out of alignment and, therefore, prevented from forming cross bridges.

1.1.2 The Length Dependence of Force

The development of sarcomere force can be determined by the length of the sarcomere since it represents the overlap between actin and myosin filaments. The

length-force relationship of a muscle can be determined by isometric measurements. The length of the sarcomere (and the muscle) is not altered during the contraction in isometric measurements. Collection of data at different muscle lengths gives the length-force relationship.

The length-force relationship has an ascending limb, a plateau and a descending limb (from short to long length). When the muscle is lengthened, the active force increases to maximum, which corresponds with the sarcomere length at which optimal cross bridge occurs.

When a muscle is maximally activated, the isometric force of the muscle is length dependent. If an active sarcomere is lengthened above the optimum length the number of cross-bridges and the exerted force will decrease. This reduction occurs in a linear fashion as a function of sarcomere length. If an active sarcomere is shortened below the optimum length the force will again reduce.

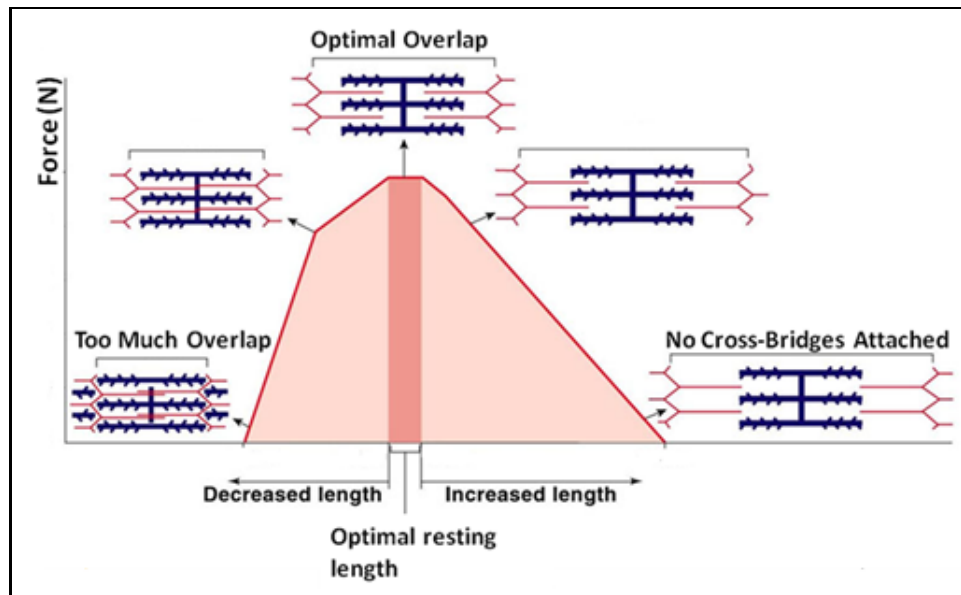


Figure 1.4 Active length-tension relationships during titanic activation. Idealized length tension relationship and the sarcomere position believed to produce it. Modified from[5]

Muscle length-force characteristics comprise important elements of muscular mechanics. Such characteristics have been widely studied using standardized experimental procedures in numerous animals [6][7]. On the other hand, direct measurements

of length-force characteristics in human muscles have been rarely studied compared to animal experiments.

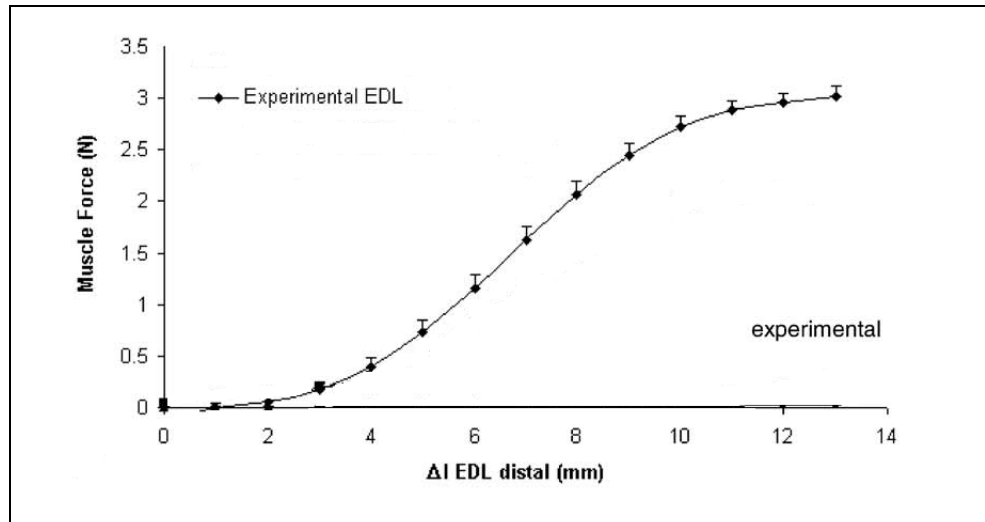


Figure 1.5 The isometric muscle length force curves of a rat's Extensor Digitorum Longus (EDL) muscle. Modified from[8]

1.1.3 History Dependence of Force

History dependence of force has been shown almost half a century ago by Abbott and Aubert[9]. This dependence is assessed by the difference of an isometric force at a given level of activation which is caused by a prior activity (such as shortening, lengthening or a combination of shortening and lengthening of the muscle). Recent studies in animals showed that previous activity at high muscle lengths causes substantial force changes at lower lengths [10][11]. Although the existence of history dependence of force production is shown for animal muscles, the mechanism of this effect remains unclear since it cannot be explained by the sliding filament theory alone. Additionally, this dependence has not been shown in human muscle.

1.2 Myofascial Force Transmission

Experiments performed in muscle mechanics were generally based on dissected muscles. Such classical approach considers each muscle as an independent unit excluding the interaction with its surroundings. However certain studies [12][13] demonstrate that although each muscle is innervated independently, the concept of muscles acting as independent components is not adequate to describe force transmission from muscle to bone: in addition to myotendinous force transmission, a second pathway referred to as myofascial force transmission (MFT) has been shown to exist[14]. This new pathway uses the muscle's cytoskeletal lattice and trans-sarcolemmal and basal lamina connecting molecules to transmit force onto connective tissue. Finite element modeling and experimental data of recent studies showed that myofascial force transmission has substantial effects on muscle mechanics such as; (i) proximal-distal force differences, (ii) altered distributions of sarcomere lengths, and (iii) relative position dependence of force exerted at origin and insertion[11]. Therefore the classical approach which considers muscles as independent units appears to be inadequate to explain the altered mechanics of healthy and spastic muscle. There are two potential paths for myofascial force transmission;

1.2.1 Intermuscular Myofascial Force Transmission

It is the force transmission between the linked intramuscular stroma of two adjacent (synergistic) muscles via direct collagenous connections and indirectly via collagen reinforced structures including neurovascular tracts.

1.2.2 Extramuscular Myofascial Force Transmission

It is the force transmission from a muscle onto extramuscular tissues via (a) neurovascular tracts (i.e., the collagen fiber reinforced protection of the blood and lymph vessels and nerves), and (b) the compartment delimiting connective tissues (intermus-

cular septa, interosseal membranes, periost and general fascia). Note that any MFT between antagonistic muscles or muscle groups by definition involves extramuscular myofascial connections since such muscles are separated by intermuscular septa or interosseal membranes.

1.3 Gracilis Muscle

The gracilis muscle is a thin and flattened skeletal muscle located on the medial side of the thigh. It is a biarticular muscle passing behind the medial condyle of the femur and curving around the medial condyle of the tibia. Gracilis muscle is the only

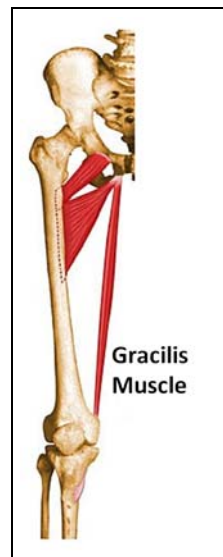


Figure 1.6 Gracilis Muscle. Modified from[15]

muscle in human body functioning as a both hip adductor and knee flexor [16]. It flexes the leg at the knee and medially rotates leg when knee is flexed. The tightness of this muscle results in knee flexion contracture as well as abnormal adduction of the hip. When the leg is in full extension (knee angle= 0°), the gracilis muscle is in its highest length. The muscle shortens with increasing knee angle and flexion.

1.4 Spastic Paresis

Spastic paresis is a neuromuscular disease which causes continuous stimulation of certain muscles, contractures and therefore a severe restriction in joint range of motion and movement disorders. In subjects having spastic paresis, the expectation is that spastic muscles are kept short due to excessive reflex activity and this shortness become permanent in time[11]. Hence, the affected muscles' length range of motion becomes limited and this limitation adversely affects the ability of movement. However the pathology of spasticity and its physiological effects on muscles are not sufficiently understood. It is known that spastic muscles have altered material properties, but since the muscle consists of muscle fibers and intramuscular connective tissues, the clinical and functional consequences of these altered properties are also not clear. On the other hand, recent studies [17][18] showed that active and passive length force characteristics of partially isolated spastic flexor carpi ulnaris (FCU) muscle could be similar to those predicted for healthy muscles.. Another study [19] also showed that although it is spastic, the FCU itself may not be the limiting factor for wrist extension in spastic patients. Hence, the apparent stiffness of the spastic muscle and the contractures in the limbs could not be explained sufficiently.

1.4.1 Spastic Paresis Patients with Hip Adduction and Knee Flexion Deformities

This thesis is concentrated on subjects having spastic paresis on their lower extremities, more specifically subjects with hip adduction and knee flexion deformity. Subjects having these deformities have scissor-type deformation on their gait and have difficulty on walking, sitting and standing. It is known that adduction deformities at the hips may be caused by; (i) Spasticity of adductors combined with flaccidity of abductors (ii) Spasticity of adductors combined with overstretching or weakness of abductors (iii) Spasticity of adductors and internal rotators (iv) Spasticity of the gracilis muscle [16]. For patients with adduction deformity of the hip associated with flexion deformity of the knee, gracilis muscle is targeted for treatment, since it is the

only muscle which is both knee flexor and adductor of the hip[16]. It is also well known that in children having knee flexion contracture, a secondary hip flexion contracture will frequently develop. Surgical correction of the hip deformity will not be beneficial since the deformity will reoccur rapidly unless the flexion contracture is operated[20]. Additionally, a clinical comparative study [20] showed that adduction deformity was considerably corrected by releasing the gracilis muscle and preserving the adductor muscles (adductor longus and adductor brevis) while the release of adductor muscles adversely affects the stabilization of hip joint and body upright position. It is concluded that adductor muscles are less hyperactive and so much related to adduction deformity than the Gracilis muscle.

1.4.2 Clinical Assessment of Spasticity

The severity of spasticity is hard to measure and it is an important parameter affecting the muscle's condition and the maximum force that can be generated. To quantify the severity of the disease and to detect the contracted muscle, non-invasive test methods are used in clinical assessments. Some of these clinical assessments are explained below.

Phelps Gracilis Test: This test detects the stiffness or increased muscle tone of gracilis muscle. In the presence of a tight gracilis muscle, adduction of the hip takes place when the knee is passively extended. The patient is placed faced down on the table with the knees flexed to 90°. The legs are then abducted as far as possible, and the knees are gradually extended. If the gracilis muscle is not spastic, there will be no tendency for the hips to adduct as the knees are extended. If the gracilis muscle is spastic and tight, there is some degree of knee flexion contracture which if forced out into extension, results in adduction of the hip [21].

Thomas Test: This test measures the hip flexion deformities. The patient lay supine on the examination table and brings one knee in direction to the chest, while the other leg remains extended. If the thigh is raised off the table, this test is considered

to be positive (meaning that the patient has hip flexion deformity), and the angle of the thigh is measured.

Abduction: Abduction of the hip is measured when the hips are kept constant at zero angle. If the limbs are away from the median (sagittal) plane, the test is considered to be positive and the angle of the limb is measured.

Popliteal Angle: This angle is measured to detect any possible abnormality in hamstring muscles. The hip is flexed at 90° and the degree of knee flexion gives the popliteal angle. Normal ranges of popliteal angle for children are given in literature [22]. A popliteal angle greater than 50° indicates abnormal hamstring tightness.

1.4.3 Aponeurotomy as a Treatment Method for Spasticity

Treatment methods for the disease range from physical and occupational therapies, injection of Botulinum toxin A to relax contracting muscles and surgical intervention to release tight muscles and fixed joints (aponeurotomy).

Aponeurotomy is a surgical technique used for the correction of movement problems caused by spasticity. The purpose of aponeurotomy is to lengthen the target muscle, decrease its force and increase the angle of motion. It includes the cutting of the intramuscular aponeurosis in the direction perpendicular to its longitudinal direction. After aponeurotomy, the muscle is expected to be lengthened and weakened. However the mechanism of biomechanical effects of aponeurotomy could not be understood substantially. A better functioning and lengthening of the target muscle is achieved in some clinical applications[23][24][25] whereas repairing and strengthening of the operated muscle is also observed [26][27]. Clinical applications of aponeurotomy in rat muscles also showed that aponeurotomy affects the force and length characteristics of muscles[28]. Studies on finite element analysis also underlined the effect of aponeurotomy on both the functioning of muscles and their force and length characteristics. Studies performed by using finite element analysis showed the dominating role of in-

tramuscular myofascial force transmission on complex sarcomere length distributions which determine the acute effects[29]. Although many studies underline the importance of aponeurotomy both in clinical applications and finite element analysis, the acute effects of aponeurotomy in human muscles have not been not studied sufficiently.

1.5 Intraoperative Force Measurements

The main goal of this study is to understand the mechanism of spastic human muscles. Available human muscle data are mostly obtained from indirect approaches such as cadaver studies [30], joint torque measurements [31][32], modeling [33] and use of dynamometry and ultrasound [34]. Direct in-vivo measurement of healthy human muscle force was performed only in limited studies [35]. Direct measurements of isometric length-force characteristics for human muscles are limited to upper extremities [36][37]. Moreover, Gracilis muscle isometric force is intraoperatively measured in healthy human subjects as a function of knee angle during their Anterior Cruciate Ligament constructions [38]. For a better understanding of human muscle functioning, collection of substantial data that relates isometric muscle force to length (or joint angle) is crucial. Hence, the importance of this study lies in the fact that isometric length (knee angle)-force characteristics for spastic Gracilis muscle will be intraoperatively measured for the first time in literature. Generating such an experimental data set and establishing a measurement technique is crucial for

- Understanding the mechanism of spasticity (in terms of comparing this data set with healthy human subjects data)
- Providing a milestone step for intraoperative measurements of human muscle force and future studies about spasticity (i.e., studies about the acute effects of aponeurotomy, studies comprising novel treatments and improving the convenient ones).

The goal of this study is to measure intraoperatively the previously unstudied isometric forces of activated spastic human Gracilis muscle as a function of knee joint angle. Experiments were conducted before aponeurotomy operations of subjects having spasticity.

2. METHODS

All intraoperative experimental was collected in Department of Orthopedics and Traumatology in School of Medicine, Istanbul University. All surgical operations were performed by the same surgeon; Prof. Dr. Yener Temelli. Experimental data was analyzed in Biomechanics Laboratory of Biomedical Engineering Institute in Bogazici University.

Prior to surgery, clinical assessments of the spastic Gracilis muscle were performed by the same surgeon and his group in Gait Analysis Laboratory in School of Medicine, Istanbul University. 10 knee angle-force characteristics data were measured from 7 subjects. Subjects' ages ranged from 5 to 18. Before the operation, subjects and their parents were informed about the study and their written consents were taken.

2.1 Surgical Procedures

During the operation subjects were under general anesthesia but no muscle relaxants were used. All intraoperative experiments were performed after routine incisions to reach the distal Gracilis tendon and before any other surgical procedures of aponeurotomy. A buckle force transducer (Teknofil, TURKEY) was mounted over the distal gracilis tendon. Prior to each experiment, the force transducer was (i) calibrated using bovine achilles tendon stripes and (ii) sterilized (using dry gas at maximally 50°C). Isometric Gracilis force was measured at various muscle lengths imposed by manipulating the knee joint angle (starting at 120° (i.e., knee joint at maximal experimentally attainable flexion, as limited by the surgery table), Gracilis length was increased progressively by extending the knee with 30° increments, until full knee extension (i.e., Gracilis force was measured at 120°, 90°, 60°, 30° and 0°). After each contraction, the muscle was allowed to recover for 2 minutes at a flexed knee posture. Subsequent to collection of a complete set of knee joint angle-force data, control mea-

surements were performed at lower Gracilis length (corresponding to 90° knee angle) in order to test if previous activity at high length (imposed by full knee extension) had any effect on the force exerted. To obtain the maximum muscle force, Gracilis muscle is maximally stimulated. A pair of gel-filled skin electrodes (EL501, BIOPAC Systems, CA, USA) were placed on the skin, over Gracilis muscle belly. Using a custom made constant current high voltage source (cccVBioS, TEKNOFIL, Turkey) the muscle was stimulated supra-maximally (transcutaneous electrical stimulation with a bipolar rectangular signal, 160 mA, 50Hz). The magnitude of the signal is determined to be adequate to supramaximally stimulate the muscle in a previous study [38]. Two twitches were evoked (100 ms apart) which after 300 ms were followed by a pulse train for 1000 ms to induce a titanic contraction and a subsequent twitch (see Figure 3.1 for superimposed examples of force-time traces for Gracilis muscle at five knee angles). An apparatus (see Figure 2.1) was designed to set the knee angle and restrain it during the contractions (Teknofil, TURKEY). The apparatus is designed specifically for the surgery tables in Istanbul University such that it can be mounted on the surgery table. The component includes (i) a leg holder, (ii) an ankle holder (iii) a knee angle adjustor and (iv) a height adjustor. A fixture with adjustable tightness fixes the position of the leg and the ankle. The height adjustor fits in the slot of the surgery table which allows a vertical position adjustment.

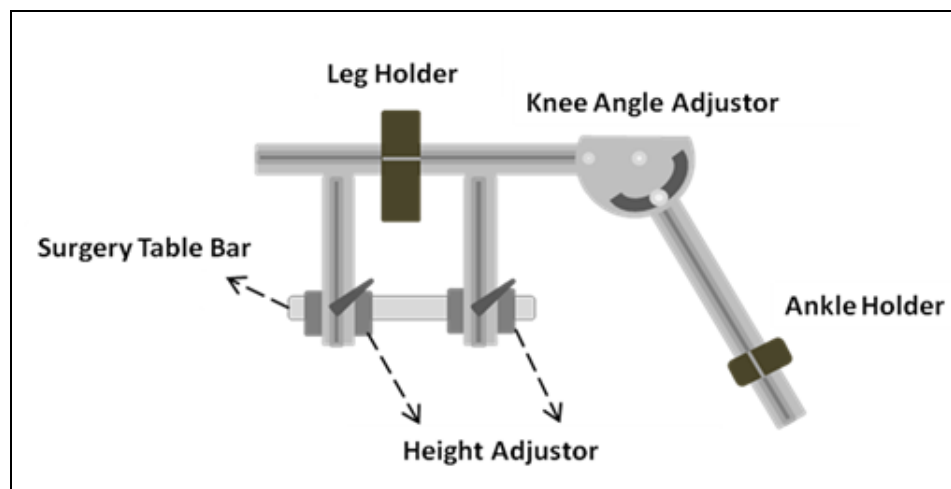


Figure 2.1 Knee angle fixation apparatus

2.2 Buckle Force Transducer

2.2.1 Buckle Force Transducer Design

Buckle force transducers (BFT) were revised from a direct in vivo force measurement study [39]. BFT was an S shaped stainless steel frame including four strain gages located on the side surfaces of the central beam. Four strain gages form a Wheat-stone bridge and this arrangement provides a higher resolution and compensates the effects of temperature changes which occur during the force measurement. The transducer works with torque principle (i.e., tensile force along the tendon generates a torque on the central beam). Torque is a function of the offset h which is the thickness of the tendon. The thicker the tendon the more sensitive the transducer is to measure the tendon [39].

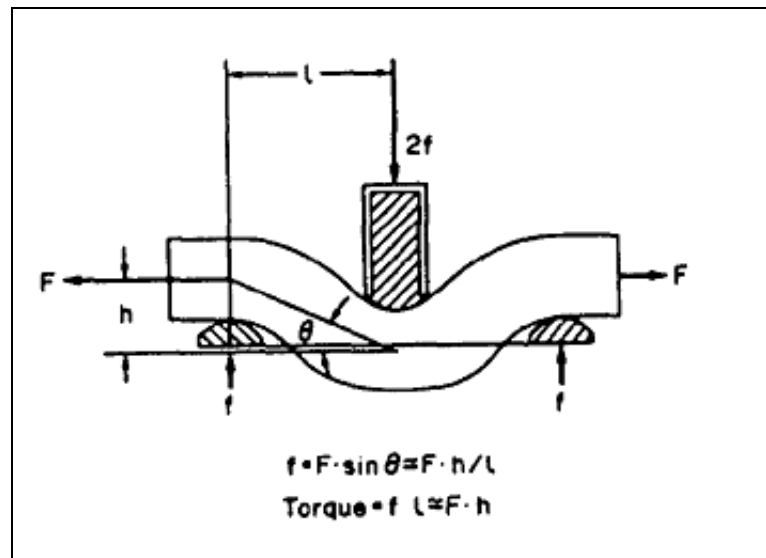


Figure 2.2 The cross section of an S shaped BFT. The sensitivity is dependent on the tendon thickness h [39].

2.2.2 Calibration of the Buckle Force Transducer

BFTs were calibrated by bovine Achilles tendon. The prepared and shaped tendons were stored in a freezer at -20°C . On the calibration day, tendons were thawed at the room temperature. Tendons were preconditioned by cyclic loading by means of a

material testing machine (MTS). Every tendon used in the calibration process is loaded 10 times with a rate of 0.167 mm/sec between 180 and 10 N. After preconditioning, BFT was attached to the tendon and the tendon was loaded with a rate of 250 N/min (the maximum safe loading rate of the MTS) to four different loading amplitudes (50, 100, 150 and 200 N). After the MTS reaches to the maximum loading amplitude and waits for one second at that force, the applied force becomes simulating the tetanus state of the muscle. BFT was calibrated by controlling the output data with the loading graphs obtained from the MTS.

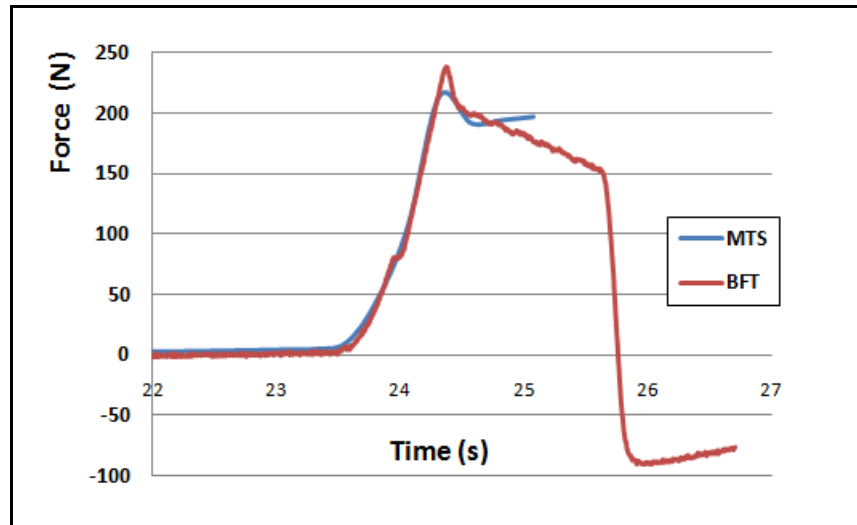


Figure 2.3 Comparison of BFT and MTS outputs

2.3 Optimization of Stimulation Signal Amplitude

The object of the study is to measure maximum Gracilis muscle isometric force as a function of knee angle. The amplitude of the stimulation should be strong enough to activate all fibers in the muscle and low enough to avoid skin burns and other complications. Hence a pre-study [40] is performed to optimize the signal amplitude. The pre-study had confirmed that 160mA ensures a maximal activation: randomized use of current amplitudes of 130, 140, 150 and 160 mA at 3 knee angles (90, 60 and 30°) yielded (i) no systematic force increase as a result of increasing current amplitude and (ii) no appreciable force variation.

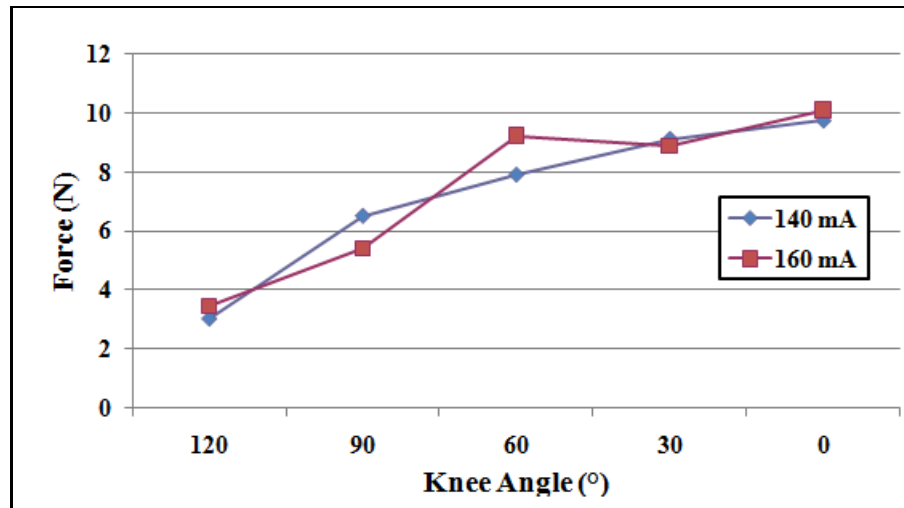


Figure 2.4 Gracilis muscle force after stimulating the muscle with 140 and 160 mA signals in a randomized order at five knee angles.

2.4 Treatment of Data

Active Gracilis muscle forces measured during a 500 ms period in the middle of the tetanus were averaged to obtain the muscle force. A data acquisition system (MP150WS, BIOPAC Systems, CA, USA, 16-bit A/D converter, sampling frequency 40 kHz) was used with an amplifier for each transducer (DA100C, BIOPAC Systems, CA, USA). In some subjects, moving average method ($n=20$) was used to eliminate the noise arising from the devices in the surgery room. An example for moving average method is given in Fig 2.5.

2.5 Processing of Data

1. Peak Gracilis forces and the corresponding optimal knee angles were studied. Pearson's correlation coefficient was calculated to quantify inter-subject variability between the peak force and subject (i) total leg length, (ii) mid-upper leg perimeter and (iii) upper leg length.
2. Knee joint angle-Gracilis muscle force characteristics were studied separately for each subject: operational portion of the length-force characteristics were charac-

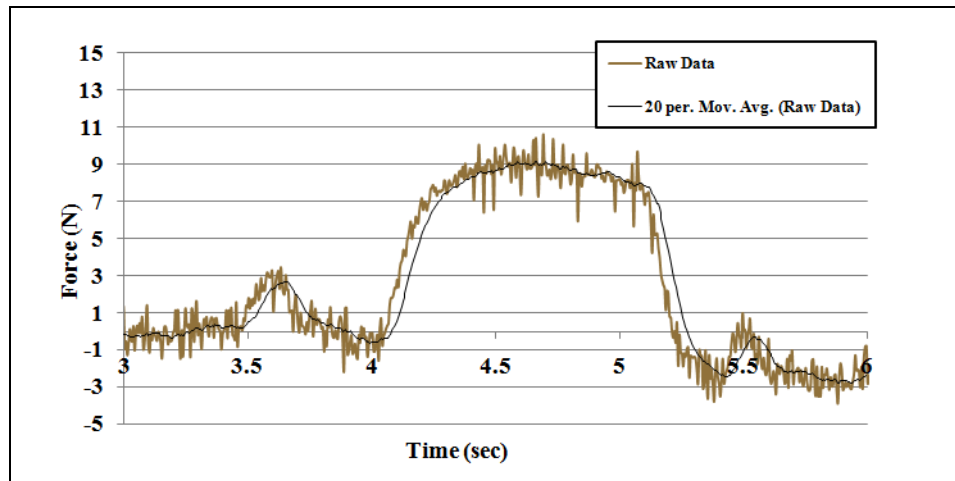


Figure 2.5 An example for moving average method ($n=20$, Subject C, knee angle = 60°).

terized.

3. Effects of previous activity at high length on muscle force exerted at lower lengths were assessed: (i) control force for each subject measured at 90° knee angle was compared to the force measured at identical knee angle during collection of knee angle-gracilis muscle force data. Absolute values of percentage force changes were used to quantify length history effects. (ii) Existence of a correlation between optimal knee angle (representative of high muscle length, activity above which is considered to lead to history effects at lower lengths) and such history effect was tested: Pearson's correlation coefficient was calculated using absolute values of % force changes for all subjects.

3. RESULTS

3.1 Tetanus State

The first success criteria of measurements is to reach the tetanus state in which the muscle exerts a constant maximal force after stimulating the muscle. For this purpose, skin electrodes are located by the surgeon carefully to achieve the maximum contraction of the muscle. Tetanus states are controlled during the measurements. An example of force-time traces is given In Figure 3.1.

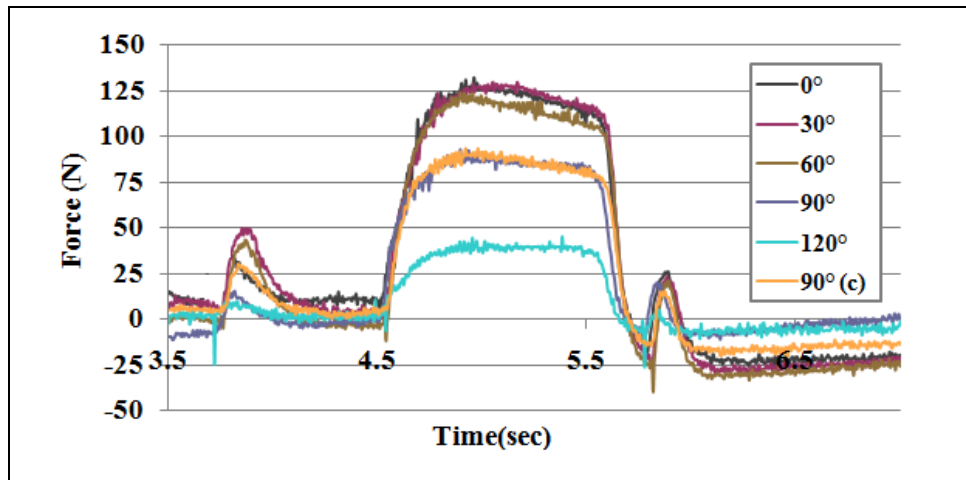


Figure 3.1 An example for force-time traces (Subject F, Right Leg)

3.2 Peak Gracilis Forces, Anthropometric and Physical Condition Data

Physical condition in terms of knee flexion and hip adduction was measured for each subject before the surgical operation. Table 3.1 shows the physical condition of each subject. In this table it can be seen that all of the subjects had both knee flexion and hip adduction deformity. Table 3.2 shows the peak Gracilis force at the corresponding knee joint angle and anthropometric data for each subject.

Peak Gracilis forces show a sizable inter-subject variability: peak force (mean=41.19 N) ranges between 9.74 and 125.14 N. Mean optimal knee angle is 30° (stdev=31.62°). No significant correlation is found between the anthropometric data and Gracilis muscle maximal force. Pearson's correlation coefficients for leg length, upper leg length and mid thigh perimeter with Gracilis muscle maximal force are calculated to be -0.27, -0.32 and -0.32 respectively.

Table 3.1
Physical data for each subject

Subject	Gender	Age	Leg	Thomas Test(°)	Abduction at 0 hip angle(°)	Popliteal Angle(°)	Anteversion(°)
A	M	9	Left	10	10	90	80-85
B	M	5	Right	10	35-40	75-80	55-60
C	F	5	Left	5	35	65	50-55
D	M	6	Left	5	30-35	80-85	50
E	F	18	Left	15	15-20	75-80	45-50
E	F	18	Right	15	15-20	60-65	55-60
F	F	6	Left	15	35-40	55-60	45-50
F	F	6	Right	10	40	40-45	45-50
G	M	7	Left	10	25	60	65
G	M	7	Right	10	25	90	50

Table 3.2
Anthropometric and peak Gracilis force data of subject

Subject	Gender	Age	Leg	Leg Length (cm)	Upper Leg Length (cm)	Mid Thigh Perimeter (cm)	Peak Gracilis Force (N)	Optimum Knee Angle(°)
A	M	9	Left	57.5	29	34	35.35	60
B	M	5	Right	45	24	28	9.74	0
C	F	5	Left	47	24	31	10.1	0
D	M	6	Left	56	28	30	80.2	30
E	F	18	Left	78	38	47	13.14	0
E	F	18	Right	77.5	38.5	49	13.34	30
F	F	6	Left	48	24	32.5	12.1	60
F	F	6	Right	48	24	33	125.14	30
G	M	7	Left	45	23	23.5	26.8	90
G	M	7	Right	47	23	23	85.95	0

3.3 Gracilis Muscle Force as a Function of Knee Angle

In Figure 3.2 maximal Gracilis muscle force is given for each subject as a function of knee angle. In three force vs. knee angle plots (B, E (Left) and G (Right)) Gracilis muscle operates in the ascending limb of its length-force characteristics. Note that in subjects C and D, Gracilis muscle force difference is negligible (0.3 and 0.7 N) when the muscle is lengthened from a knee angle of 60° to 30° and 30° to 0° respectively. Knee angle-force characteristics of these subjects can also be also considered to be in ascending limb. In rest of the graphs (A, E (Right), F (both legs), G (Left)) Gracilis muscle operate in both ascending and descending limbs.

3.4 Length History Effects

Figure 3.3 compares the control force measured at knee angle 90° with the force measured at identical knee angle during collection of Gracilis muscle knee angle-force data. Figure 3.3 shows that previous activity during measurement of a complete set of knee angle-force data caused Gracilis force at reference joint angle (90°) to change considerably. Gracilis muscle control forces were lower than those measured during collection of knee angle-force data in all data sets except two (C and D). In subjects C and D, force increments are negligible (0.3% and 0.5% respectively). The magnitude of force changes range from 0.3% (Subject C) to 11.6% (Subject g, Right Leg). No correlation was found between the % force change and anthropometric data.

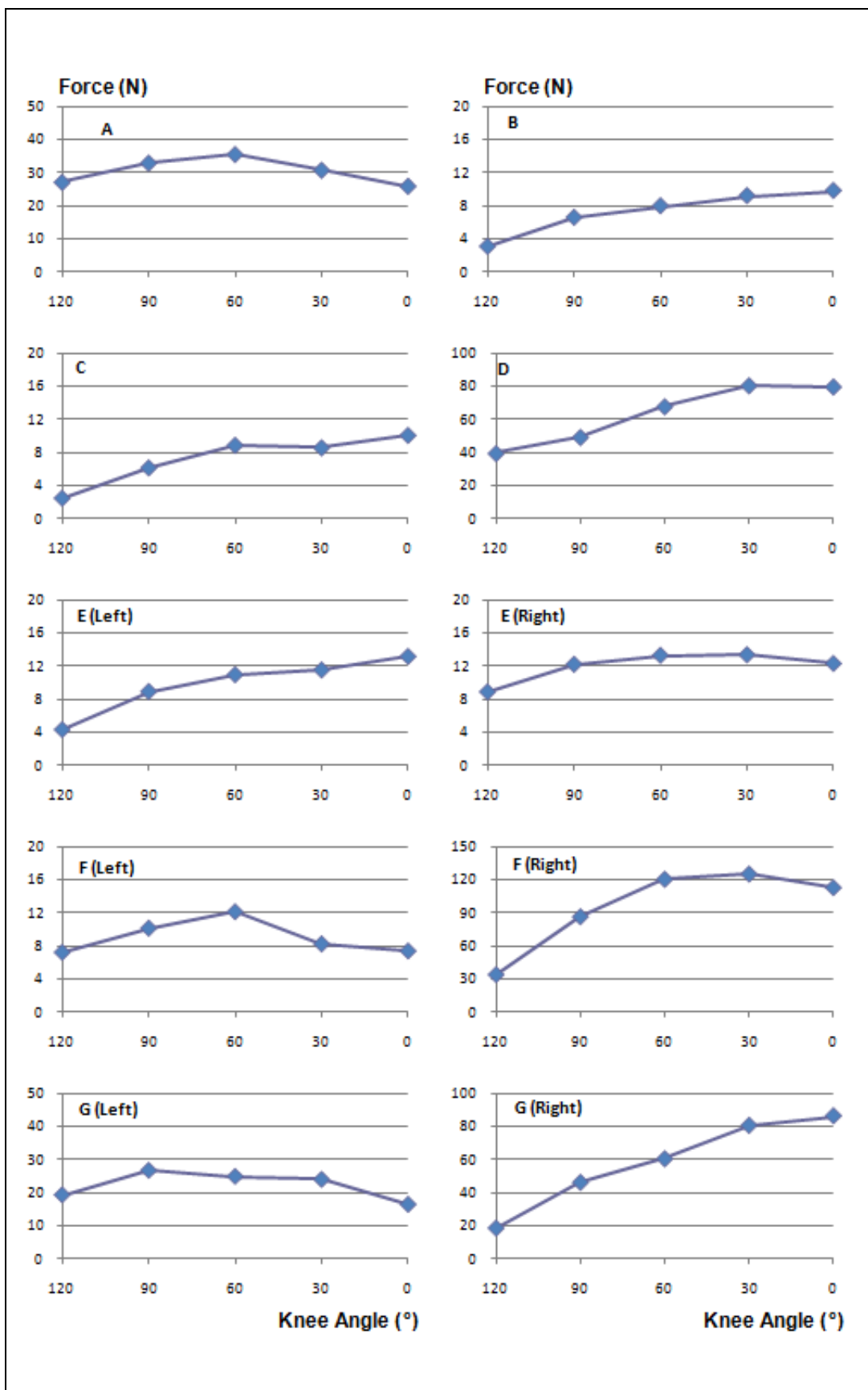


Figure 3.2 Gracilis muscle force as a function of knee angle for each subject

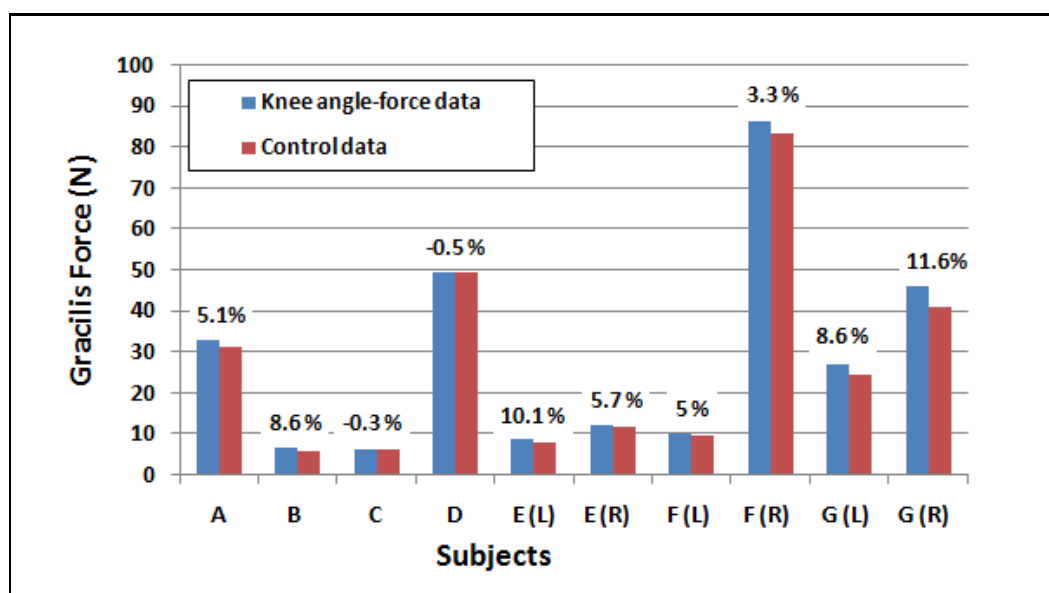


Figure 3.3 Comparison of control force measurements with the force measured at identical knee angles

4. DISCUSSION

4.1 Intraoperative Force Measurement Method

In this study a technique for intraoperative measurement of human muscle isometric force is established. Intraoperative measurement of human muscle force is very rare in literature due to invasiveness and limited access to target muscle. Former studies of human isometric muscle force [41][42][43] had some drawbacks such as movement artifacts. Recent intraoperative measurement studies [36][37] could remove the problems arising from limb movement by a standardized well established technique but they were limited to upper extremities (i.e., arm muscles) and the measurements were not performed in real environment of the muscle. Our direct force measurement approach of buckle force transducers provides a more in vivo representative situation due to the minimal tissue innervation. Hence, establishment of direct isometric force measurement technique in our study provides a milestone step in human muscle mechanics.

4.2 Functional Joint Range of Motion

Knee angle-force data of 7 subjects showed inter-subject variability and individual specific characteristics. Even in the same subject, knee angle-force characteristics and optimum muscle force were substantially different at different legs. In subjects E and G, one leg of the subject is in the ascending limb of the knee angle-force characteristic whereas the other leg belongs to both ascending and descending limbs. Similarly, in Subject F optimum muscle force varies substantially in different legs. Hence, knee angle-force characteristic of human spastic Gracilis muscle is not unique and leg specific. In all subjects Gracilis muscle could exert non-zero force at each knee angle. This data shows that spastic Gracilis muscle is capable of contributing to knee flexor moment for joint positions between 0° to 120° just like the healthy human Gracilis muscle [38]. Exerting non-zero force at all knee angles also show that spastic Gracilis muscle

does not operate within the entire range of force exertion at these knee angles. In 4 subjects and 5 leg data (A, E (Right), F (both legs) and G (Right)) Gracilis muscle clearly functions in both ascending and descending limbs. In 2 subjects, force difference between two successive knee angles are negligible, hence these subjects' data can also be considered to be operating in both ascending and descending limbs. Note that there is no data operating in descending limb of the knee angle-force characteristic. This data also suggests that the portion of the knee angle-force relationship operationalized is not unique but individual (and leg) specific.

4.3 Comparison of Spastic and Healthy Gracilis Data

The average spastic Gracilis muscle force (41.19 ± 41.07 N) is measured to be considerably less than the average healthy human gracilis force (178.5 ± 270.3) [38] although spastic muscles are expected to be stiffer [44]. One of the main reasons for this dramatic decrease in the mean force may be the difference in subject ages: in the present study, subjects' ages range from 5 to 18, whereas all subjects were adults in the previous one. On the other hand, knee angle-force characteristics of spastic Gracilis muscle is not found notably different than the ones of healthy human Gracilis muscle. Although, a limited joint range of motion was clearly detectable, the spastic gracilis muscles tested presently were not operating in a narrow length range. Although this statement is contradicting with the general expectation from a spastic muscle, it is in agreement with the suggestions of Smeulders and Kreulen [36] indicating that length-force characteristics of spastic muscles should not be necessarily different from non-spastic ones. Additionally, for all of the knee angles studied, spastic gracilis muscle exerts non-zero force indicating that active-slack length of the muscle corresponds to a knee flexion over 120° . Moreover, half of the knee angle-force data are on their ascending limb of knee angle-force characteristics and 80% of the collected data points represent muscle forces exerted at muscle lengths corresponding to optimum knee angle or lower. Hence, it can be concluded that the activity of the spastic Gracilis muscle is predominantly at its ascending limb of knee angle-force characteristics. This is in contradiction with the expectation of spastic muscle to exert high forces at low lengths

since the spastic muscle force predominantly increases with the increased muscle length in the present study. Hence it can be concluded that human spastic Gracilis muscle is not representative of the pathological condition occurring at the joint. Recently, Huijing [11] addressed this contradiction with the question "What could be the origin of high forces causing movement limitation at the joint?" and came up with a hypothesis which includes MFT. According to this hypothesis, the movement limitation occurring at the joint may arise from the high forces exerted by another muscle (rather than the targeted spastic muscle) and MFT may be the determining mechanism. If the synergistic muscles are considered, production of high forces is not plausible since these muscles are also at low length. On the other hand, antagonistic muscles which are at their high lengths because of the contracture can exert high amounts of force which leads to limited range of motion and this high amount of force can be transmitted by extramuscular MFT to the distal tendon of the target spastic muscle. In order to test this hypothesis, a future study in which an antagonist knee extensor muscle is simultaneously stimulated with the spastic Gracilis muscle can be performed. This new hypothesis will be confirmed if the spastic Gracilis muscle may exert higher amounts of force at knee angles corresponding to lower muscle lengths and if a narrower range of motion is found. Such achievement may make a major contribution to our understanding of the unclear mechanics of spastic muscle with many important implications for remedial surgery or conservative treatment techniques.

4.4 History Effects

Although its exact mechanism is still not apparent, history effects on animal [10] and human muscle [37] have been shown recently. Activity specifically over muscle optimum length has been argued to cause force reducing length history effects [10]. In our study, control force decreases substantially in most subjects (all subjects except C and D). Two subjects (C and D) did not show a force decrement in control group and they were not activated over their optimum muscle lengths (optimum muscle length is at 0° knee angle in subject C and the force decreases for only 0.7 N when the muscle is lengthened from 30° to 0° in subject D). On the other hand, in 3 subjects (B, E

(Left leg) and G (Right Leg) history effects can be seen clearly in the negative way although the muscles were not lengthened over the optimum muscle length. This data suggests that there will be a more complex mechanism behind the history effects of spastic human muscle.

4.5 Gender Effects

The mean peak Gracilis forces for male (A, B, D and G) and female subjects (C, E and F) are measured to be 34.76 ± 50.54 and 47.61 ± 33.73 N respectively. In female subjects, the huge standard deviation stems from the substantial difference between the right leg data of Subject F (125.14 N) and the rest of the data. Excluding this leg's data, one can see that the mean peak spastic Gracilis muscle force of female subjects (12.17 ± 1.48 N) is substantially less than the male subjects' data. This difference may be attributed to the differences in subcutaneous fat thicknesses. A recent study [45] has shown that female subjects have thicker subcutaneous fat and thinner muscles (rectus abdominis muscle) than male subjects in two different age groups. Consequently, this substantial difference in peak forces may stem from two possible reasons: (i) thinner Gracilis muscle in female subjects may result in lower peak Gracilis muscle force, (ii) due to the thicker subcutaneous fat thickness, the current amplitude (160 mA) may be inadequate to stimulate the Gracilis muscle maximally in female subjects.

4.6 Limitations

Measurement of passive force is not possible with the method developed in this study. After stimulation, the tendon buckles which result in negative force measurements since BFTs work on a principle of torque measurement. This negative force is not representative of the passive state. In future studies, this buckling can be eliminated by either increasing the twitch current amplitude before tetanus or by measuring a passive knee angle-force characteristic separately. Another limitation of the study

may be the usage of surface electrodes for maximal stimulation of the muscle. Several factors may interfere with the stimulation such as subcutaneous fat and temperature. Experimental [46] and computational [47] studies have shown that the higher subcutaneous fat thickness necessitates greater current amplitude to cause maximal muscle stimulation. Petrofsky [46] also showed the dependence of maximal stimulation on skin temperature which is related with skin blood flow stating that skin increased blood flow necessitates greater current amplitude.

5. CONCLUSION

In conclusion, mean peak Gracilis force and optimal knee angles are measured to be 41.19 ± 41.07 N and $30 \pm 31.6^\circ$ respectively. A substantial inter-subject variability was found between subjects (even in different legs of the same subject). No correlation was found between the anthropometric data and the peak Gracilis muscle force (and also optimum knee angles) of the subjects. Hence, anthropometric data cannot be used to predict the knee angle-force characteristics of human spastic Gracilis muscles. The functional joint range of motion for human spastic Gracilis muscle was measured to be at least as wide as from full knee extension (0°) to 120° of knee flexion. The knee angle-force characteristics of spastic Gracilis muscle are found to be comparable to those measured from healthy subjects. The spastic Gracilis muscle is determined to produce higher forces only at higher lengths. Additionally, the operationalized portion of the knee angle-force characteristics of the spastic Gracilis muscle is found to be not unique but individual specific. As history effects, previous isometric activity of human muscle was shown to cause a decrease in muscle force in most of the subjects.

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