MASTER THESIS

EFFECTS OF A 12-WEEK ECCENTRIC EXERCISE ON GASTROCNEMIUS MEDIALIS MUSCLE-TENDON COMPLEX LENGTH

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Masters in biomedical engineering, Medical Device Design

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Abstract

During a duration of 12 weeks, this study helped to investigate the effects of eccentric exercise on the force-generating capacity, length of the fascicle, and ankle positions of the Gastrocnemuis Medialis (GM) muscle. The aim of the study was to investigate the differences in generation of force with respect to the ankle joints and the position of the ankle joint with respect to the length of the muscle fascicle at weeks 0, 6, and 12. Six healthy volunteers (25+4.42 years) participated in this study and were provided with different sets of eccentric training. With the help of a 3D ultrasound and length tensile protocol, the fascicle length, pennation angle, active and passive Significant changes in the passive and active forces were measured at 5 different positions and 4 different positions, respectively. Similarly, changes in the passive and active positions were measured at four different lengths. No significant changes ($P > 0.05$) were observed at the different positions and lengths over the course of 3 measured weeks. The results of the study prove that more research that includes a larger number of participants and a more definitive way to monitor the training regime of the participants would improve

performance, thus leading to stable statistical data. In conclusion, the eccentric exercises did not have a significant impact on the force-generating capacity and ankle joint positions in this study.

I. Introduction

Exercise is a pivotal part of living a healthy life with a reduced risk of diseases, helps manage weight, strengthens bones and muscles, and overall leads to enhanced day-to-day performance. When a muscle experiences a force that is greater than the force the muscle produces itself (applied force), the muscle-tendon complex lengthens under tension [1]. In terms of cross-bridge theory, actin and myosin slide apart, which causes serial sarcomeres to elongate, and we observe an increase in the sarcomere length. A few examples that can be given for eccentric training in our daily lives would be climbing down the stairs (muscles lengthen), going down while performing squatting, or a basketball player landing from a jump [2]. The gastrocnemius medialis (GM) muscle-tendon complex is situated in the calf along with the gastrocnemius lateralis (GL), and the soleus helps in providing propulsive power generation during activities such as running, walking, and jumping [3], which is employed to start the push-off motion during the mid-stance phase (Fig. 1) and provides power over at a variety of ankle joint angles to regulate the centre of mass throughout the stance phase [4]. Knowing how eccentric exercise has an effect on the GM muscle-tendon complex will help provide a broader picture of how athletes and trainers can enhance their physical fitness [5]and prevent musculoskeletal injuries [6].

Eccentric training programmes in the past that have been studied have shown significant changes in the major morphological determinants such as muscle thickness, fascicle length, pennation angle (PA), and length-tension relationship[7]. The length of muscles is dependent upon the extent of the length of the fascicles, which is established by the number of sarcomeres organized in a series[8], [9]. The physiological cross-sectional area (PCSA) is the muscle cross-sectional area perpendicular to the line of axis of the muscle fibers and the factor that helps in ascertaining the muscle generating capacity and the maximal muscle force [9]. An example of a pennate muscle where we can pack more sarcomeres in parallel which leads to more contribution on a greater amount of force. The muscle's length and the range of active force generation are determined by its geometric organisation, which in turn affects the ability of the muscle to generate force, its contraction velocity, and the effective range of motion the muscle can contract and relax[8], [10], [11]. In a study done before, they reflect on how the longer fascicles directly have an influence on sprint performance and would muster greater shortening velocity of the muscle[12], [13]. Muscle length here is determined by the eccentric exercises, which have been shown to have the highest force-production capacity [14]and have resulted in showcasing the fact that they show more significant force gains than normal or concentric training[5]. Eccentric training has reportedly had strong implications and influence on the fascicle force-length relationship in older adults, showing an increase in fascicle length and PA [14]. Eccentric activities of the gastrocnemius muscles prove vital for one's body stability and control during processes such as slowing down or stopping, where they generate force that acts as a natural break against gravity [6], [15].

There is a lack of knowledge concerning the study of gastrocnemius muscles, even though there have been past studies that have studied the effects of eccentric training on muscle architecture in general. This can also be because of the variation that occurs in the results. Studies done in the past have seen an increase in the PA and muscle thickness during rest and contraction; however, a significant increase in the fascicle length was seen during rest but not during contraction after eccentric training[5], [16], [17]. Two other studies showed that the fascicle lengths of GM and GL increased from pre-training to post-training, and eccentric training also led to an increase in eccentric torques and force production [18], [19]. There were also other studies that proved there were no changes in the GM architecture [20] and another study where no significant difference was seen in the GM for male elite basketball players [9].

Eccentric exercises have been shown to have the highest force-production capacity [13] and have resulted in showcasing the fact that they show significant force gains compared to normal or concentric training[5]. The optimal muscle fibre length, along with the PA, has an impact on the overall length of the muscle and the range within which the muscle can generate force. The alignment of the sarcomere in series also determines the optimum muscle fibre length [9]. The muscle length at which the muscle generates its maximum active force is the optimal muscle fibre length. An increase in serial sarcomere numbers has been observed after stints of eccentric exercises in rats [21].

These morphological determinants are easily analysed using the ultrasound imaging technique during rest or when there is activity. Previous studies have compared the GM muscle volume, which was lower in spastic cerebral palsy children compared to a normal group, with the help of a 3D ultrasound [22]. The GM has been often addressed in interventional research aimed at enhancing biomechanical function due to its locomotor relevance and simplicity of identification of muscle architecture under ultrasonography [23]. Muscle thickness and fascicle length increased both at rest and during contraction, whereas fascicle length only increased after a 7-week eccentric training programme performed on the GM muscle morphological determinants with the help of 3D ultrasound[18] . Another application of the usage of 3DUS on the GM was studied, where the physiological cross-section area (PCSA) and the muscle fascicle length increased in healthy children aged between 5 and 12 years with no change observed in the PA[24] . The 3D Ultrasound Imaging Technique (3D-US) has been fast spreading over the past few years due to its low cost, non-invasive imaging without the use of radiation, and interactive feedback to obtain high-quality images and spatial data[25] . The few other benefits of a 3D-US technique would be the flexibility in terms of subject positioning during imaging, where the angle position is not fixed and can be moved over during imaging rather than fixing a particular angle during the whole imaging. It also provides quick and effective imaging, even in confined spaces[25].

Limited information is available regarding the GM muscle architecture for the duration of 12 weeks of eccentric exercise [17], [18], [26] which would help us observe long-term effects and adaptive changes in the GM muscle. However, there is inadequate knowledge and no comprehensive study or literature that talks about the insights that combine both the 3DUS protocol and the Length-Tension Protocol to determine and analyse the force-generating capacity at different angles of the different morphological determinants such as fascicle length and muscle volume, even though they can be individually found. The main aim of the study was to understand how eccentric exercises affect the Gastrocnemius muscle, which has a direct influence on rehab programs, training activities in sports, and the prevention of muscle strain injuries in athletes and to help enhancing the muscle function ([9]. The sort-out research question of the study was to observe 1) how the position-torque relationship and 2) the position of the fascicle-position relationship of the GM muscle-tendon complex length change after 12 weeks of eccentric training. The hypothesis was that the 12-week eccentric training program would lead to changes in the Gastrocnemius muscle-tendon complex, such as (i) an increase observed in the position of the muscle fascicle and (ii) an increase observed in the force-generating capacity post-training.

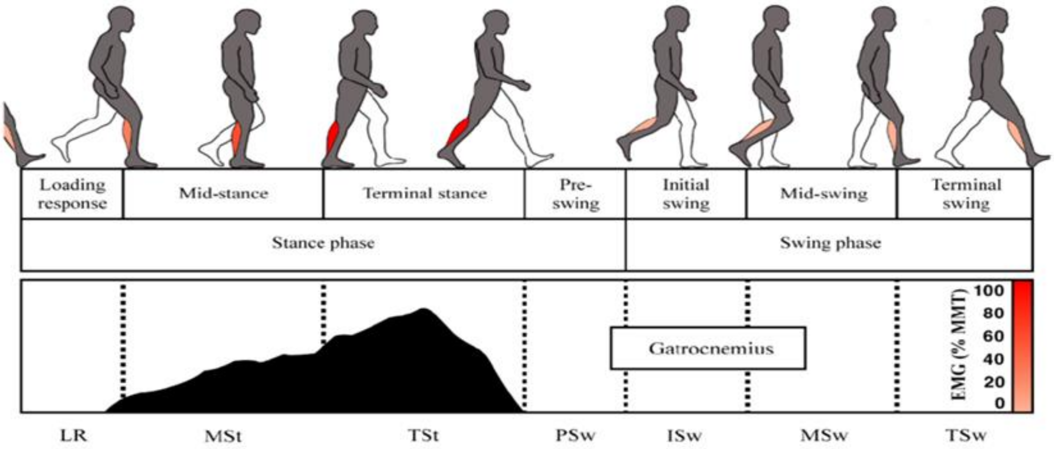

Fig 1: *Depicts the GM muscle activity for the different gait cycles. We can observe the activation of GM highest during the terminal stance phase where the plantar flexors contribute* [27].

II. Materials and Methods

A. (i) Subjects and Exclusion Criteria

6 healthy volunteers (3 males and 3 females) were initially enrolled to participate in the study. Subjects were excluded from the study if they were pregnant or had a history of any calf-related surgery in the past year, bone fracture, or muscle strain. All the participants were provided with a printed consent form before starting the trials and tests.

(ii) Anthropometrics

Height, weight, foot size, tibia, and fibula length were measured for all the participants. The tibia length was measured starting from the tibia (medial) plateau to the medial malleoli and the fibula length from the fibula to the lateral malleoli. A sole was designed for each subject taking into consideration the height of the navicularis bone[28]. This was done to address the issue of a drop of the navicular bone during weight bearing. This compensation is crucial to prevent an excessive inward rolling of the foot, known as foot eversion, when force is applied during weight-bearing activities[29]. Each participant's right leg sole was made, and it was later put in the footplate of the Biodex Multi-Joint System, ProCare (Fig. 2) (Biodex Medical Systems, Inc., New York). Certain muscles and muscle groups can be examined and trained using the Biodex System 4, an isokinetic device. This isokinetic equipment helps to gauge the range of motion of important muscles and joints, as well as strength, endurance, and power along with the help of straps and supports [30].

Fig 2: *The subject lying in a prone position on the Biodex Multi-Joint System 4 that helps in maintaining the position, balance, and transfer of weight* [31].

B. Eccentric Exercise Regime for Participants

For the scheduled duration of 12 weeks, experiments were planned for the participants. All exercises were asked to be repeated four times, 8–12 reps with a 2-minute break in between for each set and performed on both legs. When the required reps were met, weights were increased by 5kg until they felt fatigued. For the first 6 weeks and weeks 10–12, 3 types of exercises (Fig. 3A, B, and C) were prepared. The exercises were done, preferably on a stair or in the gym. For weeks 6–10, new types of exercises were introduced. Donkey calf raises with 1 leg, where they are asked to bend forward, flexing the hip joint, and try to keep the knees fully stretched (Fig. 3D). From this position, they had to fully lower the heel of the right leg till a full calf stretch was reached and use the left leg to raise the heels, reaching a full tiptoe standing position (Fig. 3E). Repeating this cycle in both legs for a straight knee and a bent knee were the two types of exercises.

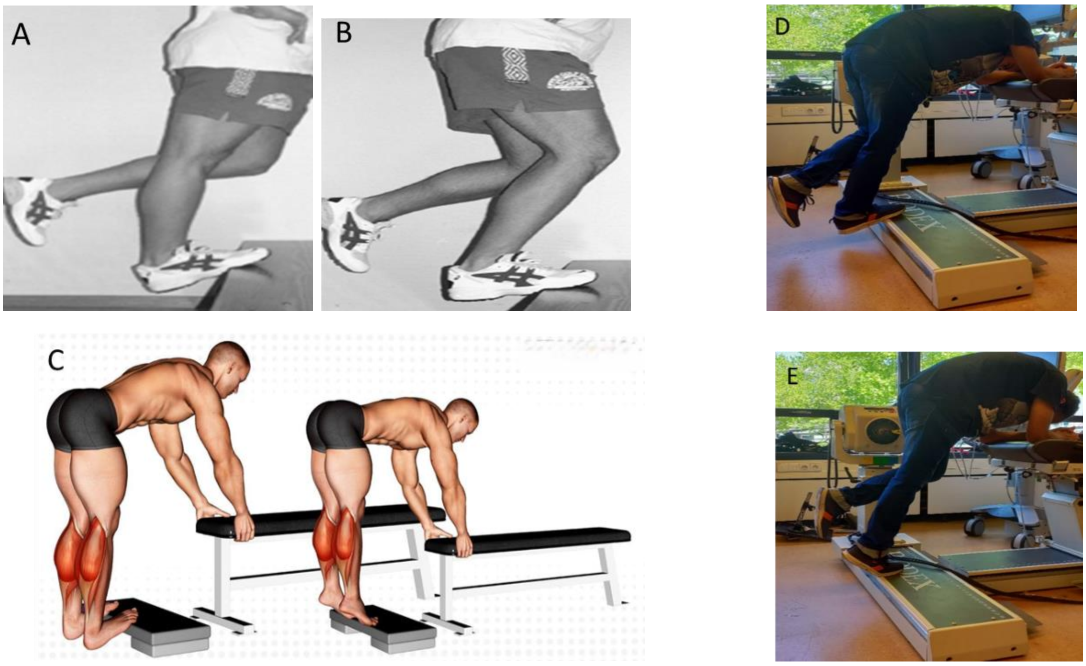

Fig 3: *Exercise regime for the participants from week 0-week 6 and from week 10-week 12.* ***(A)*** *Loading the calf muscle eccentrically by lowering the heel with a straight knee*[32]. ***(B)*** *Loading the calf muscle eccentrically by lowering the heel with a bent knee* [32]. ***(C)*** *The 2-leg donkey calf raises, lowering the heel and pushing the toes down to raise the heel. Exercise regime for the participants from week 6-week 10 the donkey calf raises with 1 leg which has to be repeated also with a bent knee.* ***(D)*** *Starting position of the exercise with a straight knee. (E) Finishing or final position of the exercise with a straight knee.*

C. Purpose of Dynamometer

Subjects were lying in a prone position on the Biodex with both their feet hanging free of any movements. Before fixing up the footplate, we measured the footplate angle with the help of a hand-held dynamometer [33] .The purpose of using the hand-held dynamometer was to control and adjust the angle between the bottom of the foot, the foot sole, and the shinbone, which is the tibia, which also helps to correct foot deformities where the foot at times deviates inwards or outwards away from the mid-line of the body [25]. By doing this, the dynamometer accounts for differences in foot shape and structure among subjects, and the goal of making these individual corrections is to standardise foot-plate angles and reduce variations in the movement of the subtalar joints performing plantar and dorsiflexion movements[25] .The corrections made to these limitations are:

(i) The mid-point of the foot was calculated and marked from the two malleoli.

(ii) Then a 10 cm measurement was taken from that midpoint to a distal part of the heel.

(iii) Footplate angle and torque were gathered at (a) an angle linked to 0 Nm and (b) an angle linked to 4 Nm (dorsiflexion) [34][24].

With the help of a goniometer that measures angles for the externally applied torque (Nm), which was measured with the dynamometer, we can get a reading of the angle between the footplate and the tibia, which is related to the neutral and dorsal movements in our case[25].

D. 3D-Ultrasound Calibration and Imaging Protocol

The required tools to perform calibration and imaging are present in the figures below (Fig. 5A-5G.) In order to ensure the images obtained through the ultrasound are related to the positions of the cross-wired water cube, we perform a spatial calibration to calculate the transformation matrix [35]. The protocol goes like this:

1) starting by filling the water cube that has a small plastic that acts as two crossing wires with warm water ranging from 22 to 37°[35], [36].

2) To negate discrepancies in the calibration, the water cube was unmoved and placed at a similar length from the camera to the subject's position during measurements (39).

3) A pointer is used to record the location and alignment of the centre of the phantom for 1-2 seconds [36].

4) Then, with the help of the screen grabber software (fcal), we simultaneously record the Polaris camera data acquisition and start the sync device (the flashbox with the piezo crystal) to identify the initiation of the acquisition.

5) Then the ultrasound probe's head was placed in the water cube, and recordings were done by hitting the foot switch for different translation motions in all directions [36].

6) Stop the data acquisition and save the data.

A 3-D ultrasound reconstruction of the GM to study the geometry of the muscle was performed at 0 Nm and 4 Nm, corresponding to their respective footplate angles. The first step in the 3D-Ultrasound protocol, which uses an Acuson S2000 Ultrasound System with 14L5 Probe (4-15MHz frequency) was for the subject to lie in a prone position on the biodex so that the ankle could move freely. After fixing the footplate angles, the right leg was attached to a strapped footplate to avoid any movement. Identifying the most proximal medial and lateral femur epicondyles using ultrasound to mark the border of the GM marks the start of the scanning process[34]. A sufficient amount of ultrasound gel was applied to the region to be scanned to limit probe pressure [34], [36]. Then, the screen grabber (epiphan capture tool) was opened on the computer, and the US acquisition was started by hitting a record button [36]. At 0 Nm, 2 scans of around 6–8 swipes (0.5cm overlapping) were made, followed by 2 scans at 4 Nm. During the scans, a Polaris Spectra Camera (Northern Digital Inc. (NDI), Waterloo, Ontario, Canada) was used to track the 4-marker frame that was attached to the probe. The swipes were made covering the region of interest starting from the border of the GM with the probe, from the proximal medial of the femur condyle to the medial distal calcaneal in a proximo-distal direction along and the proximal later femur of the condyle to the distal lateral calcaneus (Achilles tendon) [25].

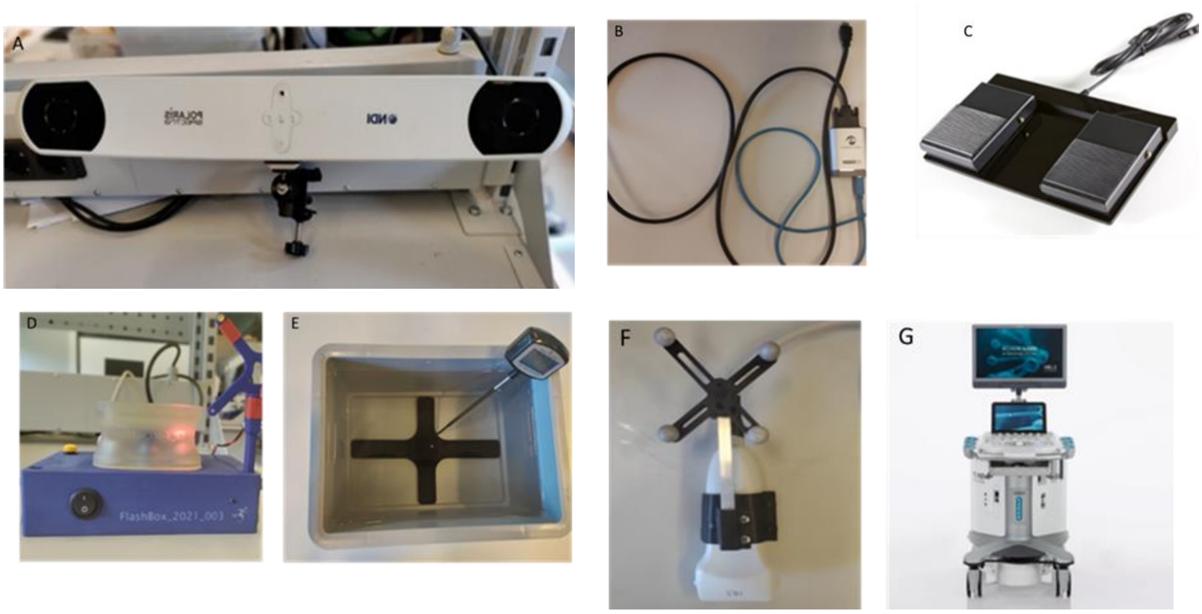

Fig 5: ***(a)*** *a Polaris Spectra Camera (Northern Digital Inc. (NDI), Waterloo, Ontario, Canada) that helps to track spatial positions of markers. The camera helps to track the 4-marker frame connected to the ultrasound probe.* ***(b)*** *an Epiphan Video DVI2USB 3.0 high-performance USB video or frame grab hardware captures images and records videos from HDMI, VGA, and other video sources and gives them in a computerized form to the laptop with the help of a connected USB 3.0 port.* ***(c)*** *Scythe USB Double Foot Switch II ((Model name USB Foot Switch 2 Double, Model Number USB-2FS-2 Scythe, Germany) acts as an alternate version of a performing keyboard or mouse which helps to operate the fcal program while recording.* ***(d)*** *flash box containing a piezo crystal, functions as a synchronization device and plays a major role in making sure that the camera data and US image data are aligned,* ***(e)*** *a water cube filled with water embedded with 2 submerged crosswires and a*

thermometer to measure the water temperature before the start. ***(f)*** *a Siemens Acuson 14L5(HELX Evolution, Siemens Medical Solutions USA, Inc., Mountain View, California, United States) Linear array Multi D-probe (5-14MHz frequency range) ultrasound probe transducer, with a pointer along with 4 markers, (g) ACUSON S2000 Ultrasound System, HELX Evolution with Touch Control helps in providing clear and sharp images for full body range coverage with a 30.7cm high-resolution display screen.*

E. Length-Tension Protocol

A B-Mode ultrasound scanner, an Acuson S2000 Ultrasound System with an 18L6 probe (HELX Evolution, Siemens Medical Solutions USA, Inc., Mountain View, California, United States) with touch control, was used to record ultrasound images. The subject lay in a prone position on the Biodex with their feet hanging outside. In performing this experiment, we had to find the muscle belly to observe the difference in the belly length and PA for different plantar flexion and dorsi flexion angles [24]. The area that was supposed to be scanned was spread with a sufficient amount of ultrasound gel to ensure better contact between the probe and the skin. The origin and insertion of the muscle-tendon junction of GM were marked on both the medial and lateral regions of the muscle with the help of the ultrasound probe. The lengths of both the origin and insertion were marked. Later, the midpoint of both lengths was calculated, and a region of interest was drawn upon identifying the muscle belly. The ultrasound probe was placed in such a way that it was held horizontally in a transverse orientation with the line of action of the muscle fascicles that appear in the muscle belly region[37] (Fig. 7). The orientation of the probe was placed in such a way that the line of action of the fascicles in the mid-belly region of the muscle and the midline of the muscle were aligned [22]. The length of the muscle fascicle (Fig.10) was measured between the deep and superficial aponeuroses, which run along the lines of collagenous tissue [22]. The PA is made between the two aponeuroses[38] and the direction of the fascicles of the muscles. Recordings were made in active and passive states with the subject’s right leg tightly strapped up in the footplate. The subject was asked to relax and then give maximal voluntary contraction (MVC) force for the different footplate angles within 2-4 seconds. Using an epiphan capture tool, a frame or screen grabber that helps capture images or videos, the captured images were later analysed by software known as the Fiji app. More about this will later be discussed in the data analysis part.

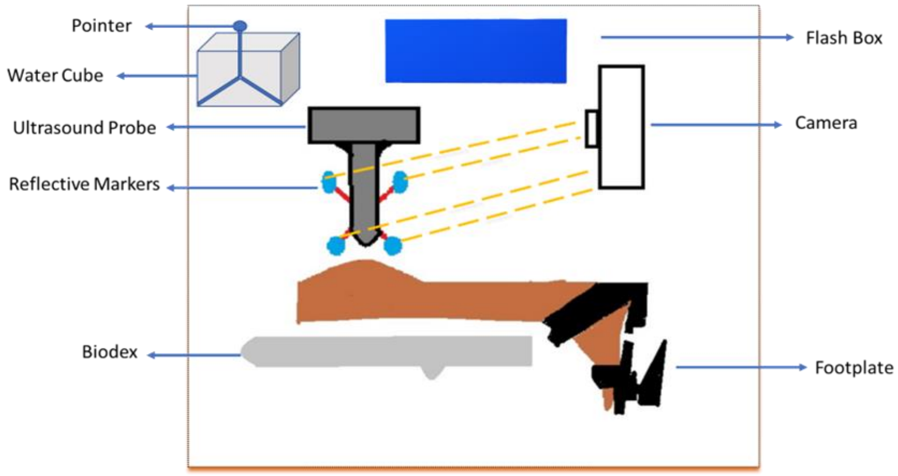

Fig 6: *This a diagrammatic representation of how the setup and the protocol being performed in the 3D-Ultrasound protocol*

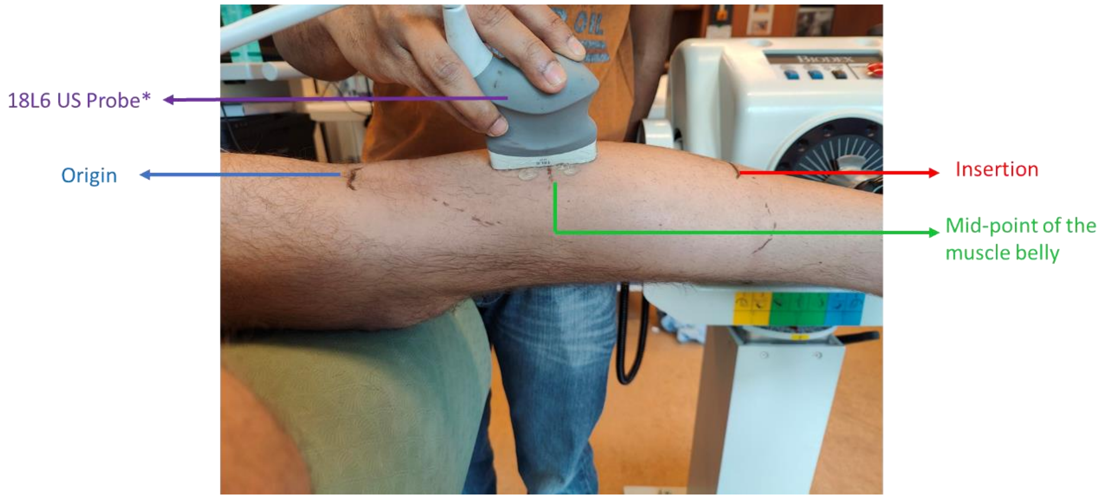

Fig 7*: An example of the experimental process of finding the origin (blue arrow) and insertion (red arrow) of the GM muscle with the help of ultrasound and calculating the mid-point of the muscle belly (green arrow) which later becomes marked and remains the region of interest for scans. The orientation or the direction (violet arrow) of holding the ultrasound transducer 18L6 becomes crucial for the alignment of muscle fascicles in the mid-belly region and if it was placed in the other way around the fascicle length had a reversed or negative value in cm.*

F. Data Analysis

a) 3DUS Muscle Volume Estimation

After the reconstruction of images (Fig. 8), we use a software known as Medical Interaction Toolkit (MITK) The Medical Imaging Interaction Toolkit (MITK) - mitk.org to load the 3DUS

Image to perform the segmentation and hence find the muscle volume. The slicing was set to 'Coupled crosshair rotation' after the image was loaded. To locate the proximal and distal end of the muscle belly borders the MITK proves to be a good help which in turn helps to estimate the muscle volume[36]. To segment (Fig. 9) the cross-sections of GM we use the segmentation toolkit to manually add labels. We can add more labels (at least 6) to the image in the axial, coronal, and sagittal planes to see a complete segmentation of the cross-section. Then the segmented regions of the muscle boundary were 'set to interpolate' which shows the borders of the segmented parts as greenish-yellow lines. The previous step of segmentation has to be repeated to the area muscle boundary and the interpolated region does not match along the muscle belly. We then confirm the interpolation for all the slices.

b) Estimation of Fascicle Length and Force with the Length-Tension Protocol

Once the length-tension protocol was done procedure images were obtained (Fig. 10), and we found the passive and active state of the muscle to calculate the active and passive PAs and fascicle length respectively. The FL was the length of the fascicle between the superficial and the deep aponeuroses (Fig. 10). The angle made between the muscle fascicle and the deep aponeurosis was the PA (Fig. 10). To obtain this we used a software called Fiji. app installed in Windows where we loaded the raw images and started to find the fascicle length and PA for all plantar (p40.raw for example) and dorsi flexion (d10.raw for example) angles of the subject. After loading the unprocessed image, we choose the option "Straight, segmented, or freehand lines" option. We then choose the 'analyze' tool and 'set scale' for entering the same distance as the depth of the ultrasound that the image possesses. With the help of the freehand line, we mark the two borders of the aponeurosis in the passive and as well as in the active state. As explained in the protocol above we measure the fascicle length and PA between the deep and the superficial aponeurosis. The mean and standard deviation of the plantar and dorsi flexion angles were calculated with the help of 'Excel' and graphs were plotted for the mean active and passive PAs and fascicles respectively.

c) Calculating Active and Passive Torque

Active and passive muscle forces combine to form the total tension that a muscle develops, and this tension is length-dependent[39]. A muscle's active force is determined by the quantity of cross-bridges it forms, which is influenced by the degree of myo-filamentary overlap. The optimal force of the muscle is generally in the middle of the muscle length and lower at shorter and longer relative lengths [27]. At longer lengths, passive forces have been proposed to be formed by weakly linked cross-bridges [40]. In order to obtain the passive torque of the subject, we first have to calculate the passive torque from the footplate, where the data file input consists of velocity, torque, trigger, position, and time. Then we obtain a curve of passive torque vs. footplate angle, where the footplate angle consists of values from Plantar 50 to Dorsi 40, that is, from 40 to 130 degrees, ascending in steps of 10 degrees. This curve helps to establish a relationship between the passive torque of the footplate (y-axis) with respect to the footplate angle (x-axis) without the subject. The next step would be to calculate passive torque from the subject, where the input would be the passive torque measurements obtained during the relaxing stage in the length-tension protocol. Then we create a similar curve to establish the relationship between the passive torque obtained from the subjects with respect to the ankle angle (40–130°). The next step is calculating the passive force difference by subtracting the footplate passive torque data from the subject's passive torque for the same angle and distance. In order to calculate the active torque, a similar curve-fit relationship between peak torque and passive torque is obtained, which is then later used to calculate the active torque by subtracting passive torque from the peak torque. I would later add all the separate graphs consisting of the passive torque from the footplate, passive torque, and peak torque of the foot for all the individual subjects in the supplementary*.

Eqn. (i) Passive Torque = Passive Torque of Subject – Passive Torque of Footplate

(ii) Active Torque = Peak Torque -Passive Torque

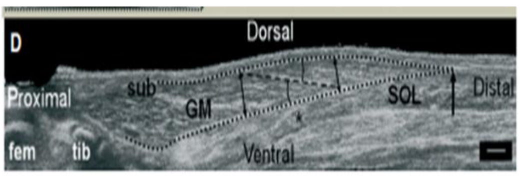

***Fig. 8:** Position of muscles in axial plane in a 3D-US image before segmentation* [28]

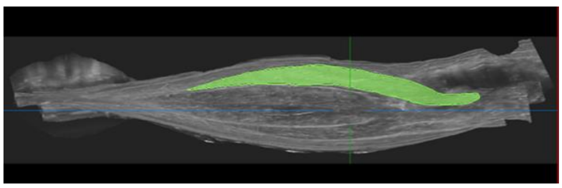

***Fig. 9**: Axial plane image after segmentation in the MITK tool*

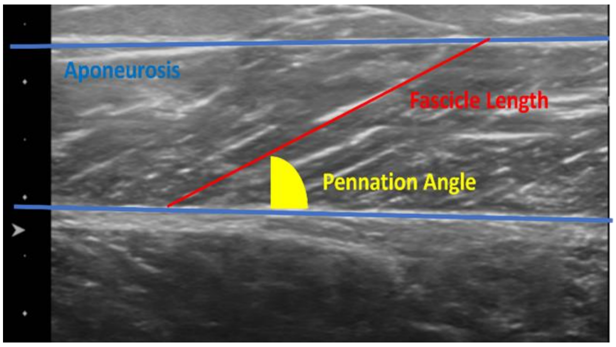

Fig. 10: *Example of a measurement of fascicle length(red), pennation angle(yellow), and the marked superficial and deep aponeuroses(blue) in the form of an image from the Fiji.app at a Passive State of the GM muscle at plantar flexion 40°*

d) Statistics

Statistics were tested for normality (Shapiro Wilk and Kolmogorov-Smirnov) and if the normality test failed data was transformed using log transformation, non-parametric tests were performed.

A dependent t-test was used to test the significant difference between the weights of six subjects for weeks 0 and 12. A Wilcoxon signed-rank test, a non-parametric test, was used to test the significant difference between the maximal forces for weeks 6 and 12. The dependent t-test was again used to test the significant difference between the normalised maximal forces for weeks 6 and 12. A two-way ANOVA with repeated measures was used to test the relationship between position (ankle angle) and absolute and normalised forces (passive force*weeks, active force*weeks). A two-way ANOVA with repeated measures was used again to test the relationship between fascicle length and passive and active positions (passive positions vs. weeks, active positions vs. weeks). The same methods were also followed for the normalised forces and lengths.

All statistical analyses are set to be performed using SPSS (IBM SPSS Statistics 29.0.1.0). Data are likely to be presented in the form of a mean ± standard deviation. For all statistics, the level of significance was set at $P < 0.05$.

G. Results

(i) Anthropometric variables

6 healthy young adults (3 males and 3 females) participated in this study. Their mean and standard deviation of different variables have been illustrated in Table 1.

Variables	Mean ± Standard Deviation
Age (years)	25±4.42
Height (cm)	178.41±9.85
Weight (kg)	75.25±16.15(week0), 75.33±15.09(week12)
Tibia Length (cm)	37.5±2.5
Fibula Length (cm)	38.01±2.93

Table 1: Mean and standard deviation of the variables for anthropometry

(ii) Weights:

No significant difference in weights was observed between week 0 (75.25±16.15) and week 12 (75.33±15.09), $t(5)= -0.103, p>0.05$ (Fig.11A).

(iii) Maximal Forces:

The non-parametric test of the dependent t-test the Wilcoxon signed-rank test showed no significant difference between the absolute maximal forces of week 6(40.44± 24.25Nm) and maximal forces of week 12(49.215±30.847Nm), $z=-1.461$, $p>0.05$ (Fig. 11B).

There was no significant difference when the forces were normalised by weights (Nm/kg) from week 6((0.60±0.34Nm/kg) compared to week 12 (0.71±0.42Nm/kg),$t(3)=-1.655, p>0.05$(Fig.11C).

(iv) Passive and Active Force-Position Properties:

There was no significant difference ($P>0.05$) observed between the active and passive force properties with respect to ankle angle from week 0 to week 6 to week 12 for the absolute (Fig. 12A) and as well as for the normalised curves (Fig. 12B) where the passive and active forces were normalised with regards to weights of the subjects in the particular week in which the passive forces were calculated at positions 55,65,75,85 and 95 degrees and active forces were calculated at 60,70,80 and 90 degrees.

(v) Passive and Active Position-Fascicle Length Relationship:

There was no significant difference ($P>0.05$) observed between the active and passive positions with respect to the fascicle length of the muscle from week 0 to week 6 to week 12 for the absolute (Fig. 12C) and as well as for the normalised curves (Fig. 12D) where the passive and active lengths were normalised with respect to tibia length in which the passive positions were calculated at lengths 3.6,4.46,5.32 and 6.18cm and active positions were calculated at 2.77,3.70,4.63,5.56.

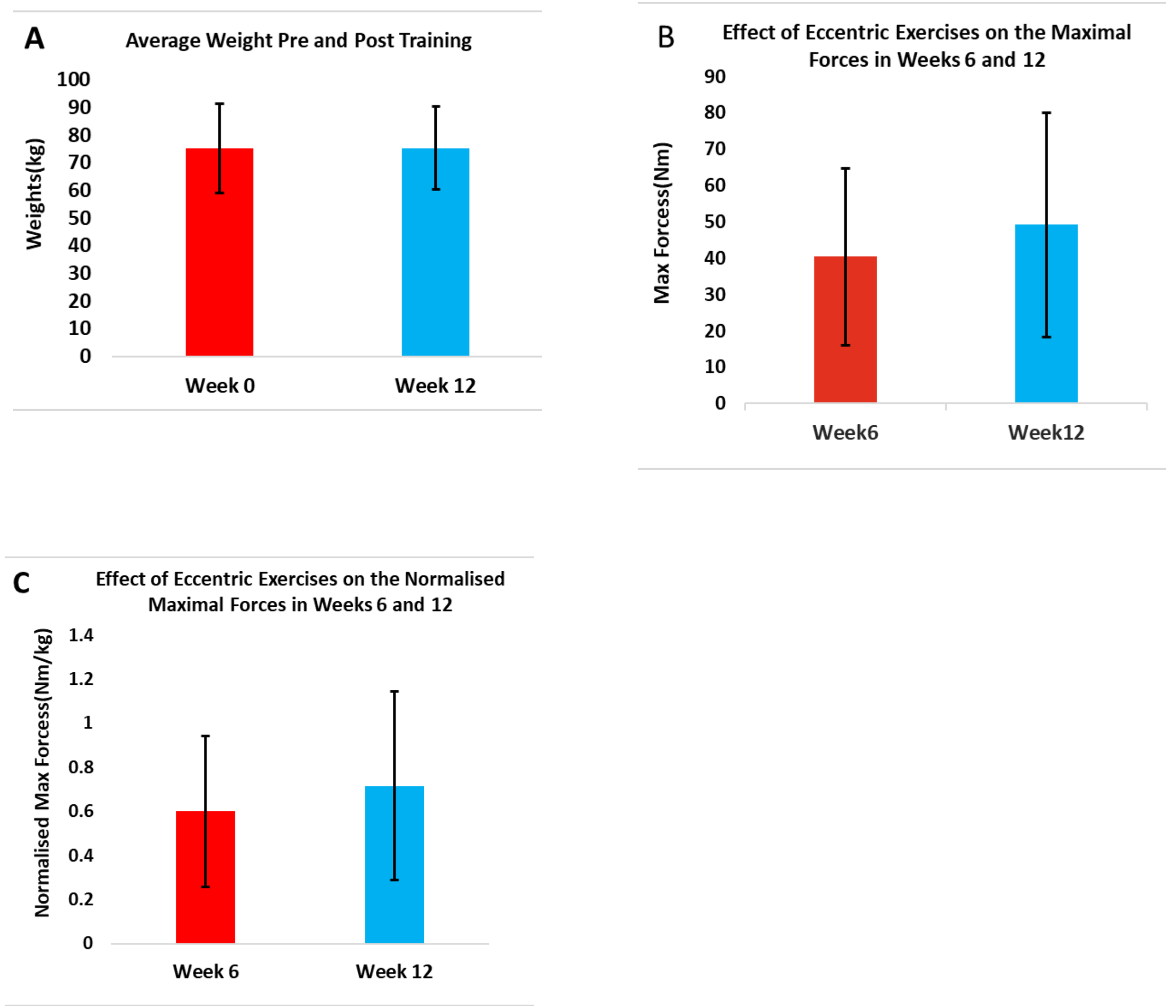

Figure 11*. *Effects of the eccentric exercises on body weight and forces pre-, mid, and post-training.* *(A) *Average body weight of all 6 subjects pre and post-training.* ***(B)*** *Maximal forces of 4 subjects (Subjects 13,15,16 and 18) at week 0 and week 12.* ***(C)*** *Normalized Maximal Forces of 4 subjects (Subjects 13,15,16 and 18) at week 0 and week 12. Data represented in the format mean±Sd.*

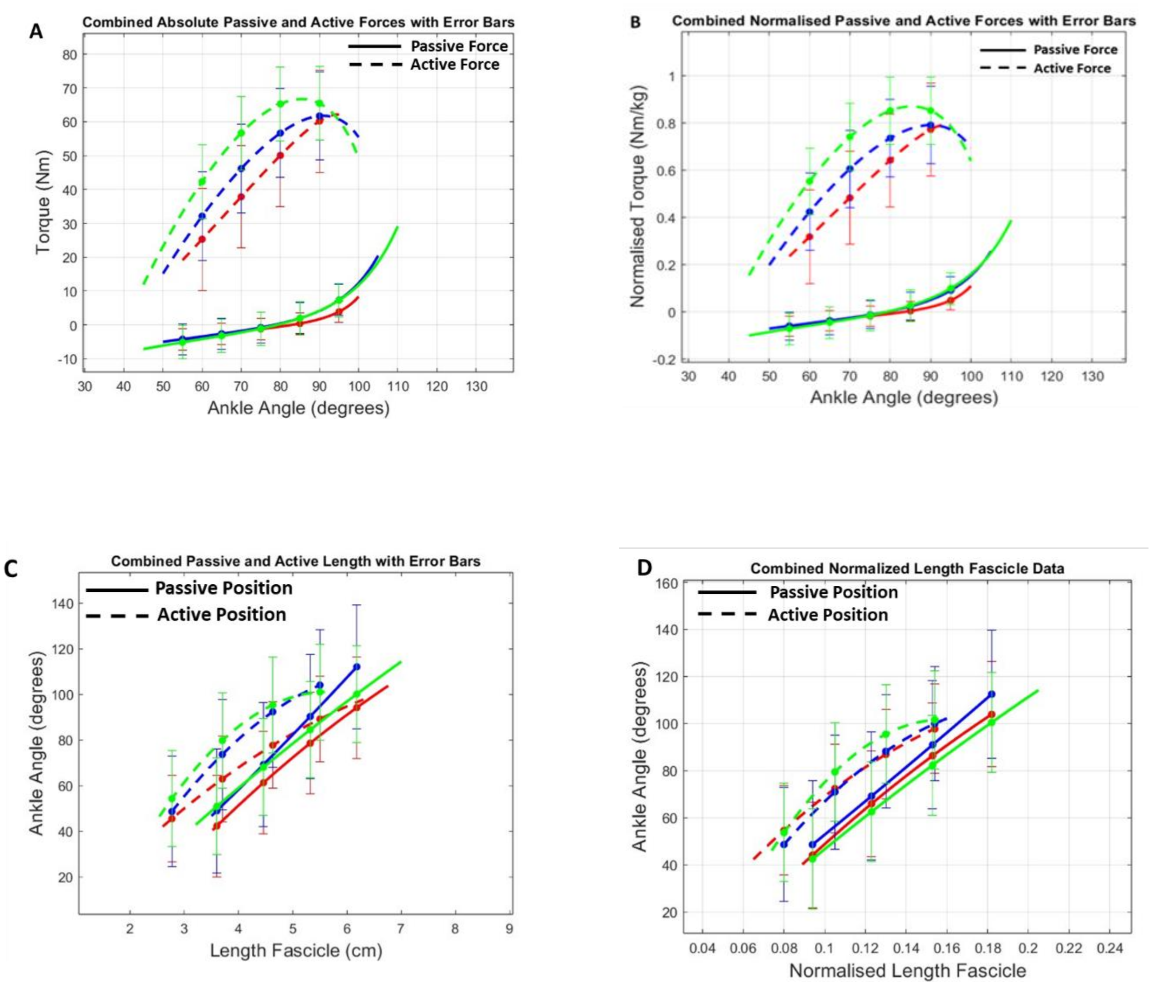

Figure 12: ***Effects of the eccentric exercises on forces and position pre, mid, and post-training where red lines represent the forces of week 0, blue lines represent week 6 and green lines represent week 12. (A)*** *Torque vs Ankle Angle relationship of 6 subjects combined,* ***(B)*** *Normalised Torque vs Ankle Angle relationship of 6 subjects combined,* ***(C)****Ankle Angle vs Fascicle Length of muscle relationship of 5 subjects*,* ***(D)*** *Ankle Angle vs Normalised Fascicle Length of muscle relationship of 5 subjects.*

**Subject 14 was considered as an outlier*

H. Discussion

In the present study, the main goal was to compare how the 12-week eccentric training had an impact on the force-generating capacity, changes observed in the fascicle length, and position of the muscle. To my knowledge, this is one of the first studies that discusses the force-generating capacity as well as the morphological determinant changes in the GM. The force value has been normalised to body weight (Fig. 12B), and length values to tibia length (Fig. 12D). The idea behind normalising the torque with respect to body weight was to try and understand the relative force generated by the muscles, considering individual variations in body size, and also to allow fair comparison among subjects. Similarly, normalising the length of the fascicle with respect to the tibia length ensures individual variations in the length of the leg do not cause trouble while performing analysis.

The findings from this study with respect to the force-generating capacity in both the absolute and normalised torques were that there is no significant impact of the eccentric exercise in terms of force-generating capacity and the length positions. However, the fascicle length appears to be slightly longer in the passive state from weeks 0 to weeks 6 and 12 (Fig 12C, D). The active length stayed relatively the same for all 3 weeks except for the fact that the normalised active length fascicle of week 0 started in a smaller normalised length (Duclay 2009).

The findings are not in agreement with the hypothesis above as there is no significant difference between the force-generating capacity from week 0 to week 6 to week 12 (Fig. 12 A, B) which contradicts some of the previous studies [16], [17] as the eccentric training had an impact as we observed an increase in the force-generating capacity from week 0 to week 6 to week 12 in both the absolute and normalised curves in increasing the force-generating capacity. From my understanding, the reason for the negative torques is that while the muscle lengthens during the eccentric concentration, an individual participates in the act of reducing weight or actively opposes an external force that seeks to contract the muscle. When the applied forces oppose the direction of motion along with the elastic properties of the muscles and tissues that store energy, this can lead to a negative torque. The passive force is represented in the form of an exponential curve, which is explained by the length of the sarcomeres pulling on the titin that acts as a spring. In obtaining the passive force the contributors were mainly the footplate, the weight of the foot, and titin in which the titin is length-dependent. If we just had the weight of the foot which is also position dependent, the relationship would have been a straight line and not an exponential curve that needs to be subtracted, but in this case, we also have the position of the footplate which would give more force at longer lengths and is not linear.

Even though there is no significant difference between the forces at the discovered positions we can still see that the force for example at 80 degrees in the active state seems to be higher for week 6(56±13.083Nm) and week 12(65.25±10.87Nm) when compared to week 0(50.04±15.114Nm). Similarly, the range of the curves has also lengthened, i.e., in the passive curve range increases from 55 to 100(Plantar flexion 35 to Dorsiflexion 10) to 45 to 110(Plantar 45 to Dorsi 20). The higher muscle force-generating capacity can be associated with an increase in the number of sarcomeres packed in parallel [9]. The elements that collectively contribute to the muscle's force-generating capacity are the number of activated muscle fibres, the sarcomere arrangement (parallel in this case) within the fibers, and the extent of actin and myosin overlap[9]. With more sarcomeres or a large number of contractile units in parallel, the muscle experiences an increase in muscle size, causing it to produce greater force. According to a few previous studies, an increase in parallel sarcomeres contributes to an increase in fascicle PA[7], [16], which I haven't discussed in this study for now and which can be a good step in the future to consider.

No significant change in positions with respect to the length of the fascicle has been observed which contradicts our hypothesis, where a few studies reinstates the fact that fascicle length increase is a commonly found trend with respect to eccentric training [16], [17], [41], but few studies that were earlier observed this kind of effect [42], [43]. There has also been a study conducted where an increase in fascicle length of the GM was observed after eccentric training, even at the expense of Achilles tendinosis injury [44]. The increase in the fascicle length after the 6th week of eccentric training, which can be evidently seen in week 12 at the passive state Figure 5d) indicates an increase of sarcomere in series. During eccentric exercises, the muscle is lengthened while producing tension. The lengthening of the muscle fibres causes an increase in the fascicle length and also means more sarcomeres are packed in series. The increase in fascicle length has also been studied to show an increase in the overall muscle mass in animals [13]. An increase in fascicle length and when the sarcomeres are stretched helps the muscle generate greater force at longer lengths, and the optimal muscle length, the length where the muscle produces maximum force also increases[9]. The increase in sarcomere is generally observed with a rightward shift in the length fascicle curve[41], [44] .

The muscle contraction velocity increases when more sarcomeres are arranged in series (more actin and myosin head binding). This leads to an increase in force production at longer muscle fibre lengths[45]. An increase in muscle thickness was also associated with eccentric training, and an increase in fascicle length but not with respect to PA change was observed in two other previous studies [17] [16] and some have shown no changes [7]. A study conducted with healthy children showed that the impact of an increase in fascicle length surpasses the consequences of a reduction in fascicle angle with regard to muscle thickness [24].

Consequently, this leads to an overall enhancement in muscle thickness. The increase in the fascicle length can also be associated with an increase in eccentric loads (loading 5kg every time after reps were met until they felt fatigued) and the intensity of the training (8-12 reps 4 times every alternate day) could have provided optimised conditions for large serial sarcomere additions [19].

Due to the increase in fascicle length and greater force and strength of the muscles, the subjects can jump a bit higher by storing more energy in eccentric contractions, taking longer strides during running, and jumping longer. In sports such as basketball, the movements are short sprints with high intensity, and the tendency to jump higher to dunk the ball is provided by a longer length of muscle. Sports injuries can also be avoided if we can predict how much optimum force one can deliver in order to produce optimal muscle length. The eccentric exercise could also be used as a training programme for the elderly, as they experience a decrease in muscle fibre length due to sarcopenia, which plays a significant role in the slower muscle shortening (nearly ½ of the speed) compared to young adults. [17], [19], [46]. Longer fascicles directly have an influence on sprint performance in relation to greater shortening velocity of the muscle[12].

I. Limitation, Future Work and Conclusion

There are some limitations with respect to this study. Firstly, the study involves a smaller number of participants, which can have an impact with respect to the results not being applicable to larger populations. Since only 6 subjects were considered for participation and from that at times only 4 subjects or 5 subjects could reach the maximal force the data represented became less powerful and led to no significant differences in force. Secondly, all the muscle architecture and its properties were solely focused on the GM, which is one of the plantar flexor muscles, among the 3 triceps surae muscles, including the GL and soleus, that are responsible for generating ankle torque[39]. That is the reason why a relationship was not found between fascicle length and torque values, as the GM is not the only muscle that contributes to generating the entire force associated with it. This could also be a good opening for an experiment in the future. A further limitation that this study had was that there was no way to ensure that the participants strictly followed their training regime. Even though they had been given smartwatches to track their daily activity, it was a bit challenging to follow whether they had imbibed the training regime every alternate day, which could affect the consistency of training and show variation in results. There were also a few technical hardware issues that we had to face throughout the study since it was dealing with equipment such as Biodex and ultrasound, which did cause problems in between weeks where we had to skip experiments on subjects that had been planned during that particular week.

In conclusion, this study did show slight changes in force generation and changes in position with respect to the length fascicle but not enough to be significant. However, in future, if more subjects are to be analysed and examined, there is a higher possibility that significant changes and changes reported in the morphological determinants could occur with the eccentric training that was prepared.

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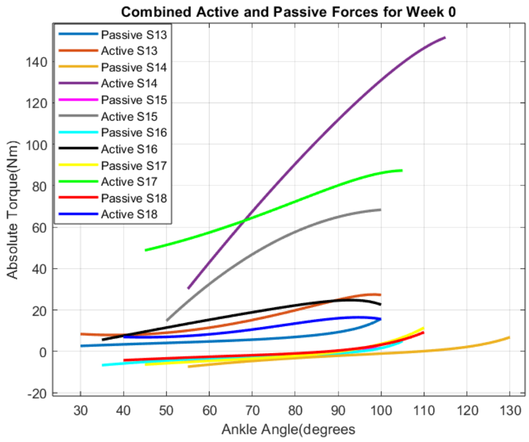
Appendix : Consists of all the different individual active and passive absolute and normalized lengths and torques combined together which would give an overall view of how the subjects have performed

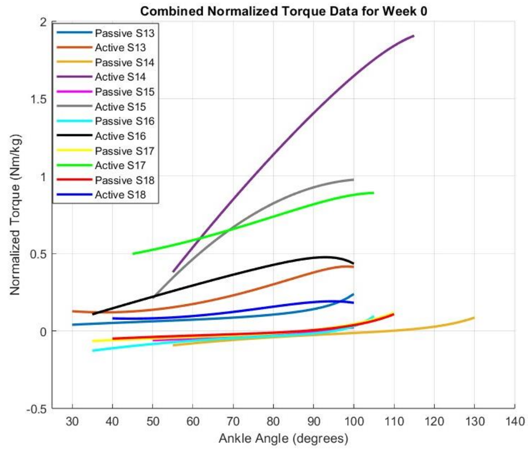
Figures a to f) represent the combined version of all the 6 subjects' individual graphs of week 0, week 6, and week 12 that discuss the position vs absolute and normalized torque.

Figures g to l) represent the combined version of all 6 subjects' individual graphs of week 0, week 6, and week 12, which discuss about the absolute and normalized length with respect to the position.

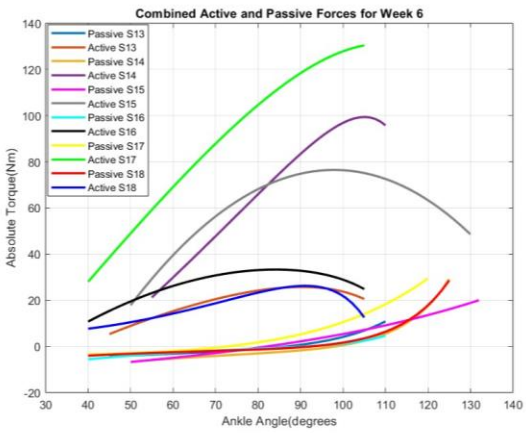
a)


Combined Active and Passive Forces for Week 0
Absolute Torque(Nm)
Ankle Angle(degrees
Passive S13
Active S13
Passive S14
Active S14
Passive S15
Active S15
Passive S16
Active S16
Passive S17
Active S17
Passive S18
Active S18

b)


Combined Normalized Torque Data for Week 0
Normalized Torque (Nm/kg)
Ankle Angle (degrees)
Passive S13
Active S13
Passive S14
Active S14
Passive S15
Active S15
Passive S16
Active S16
Passive S17
Active S17
Passive S18
Active S18

c)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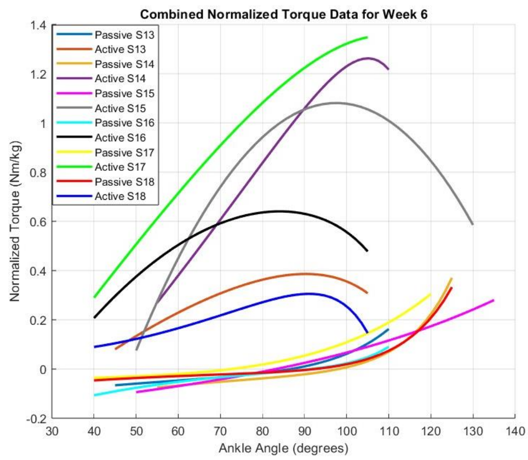
Absolute Torque(Nm)
Ankle Angle(degrees
Passive S13
Active S13
Passive S14
Active S14
Passive S15
Active S15
Passive S16
Active S16
Passive S17
Active S17
Passive S18
Active S18

d)


Combined Normalized Torque Data for Week 6
Normalized Torque (Nm/kg)
Ankle Angle (degrees)
Passive S13
Active S13
Passive S14
Active S14
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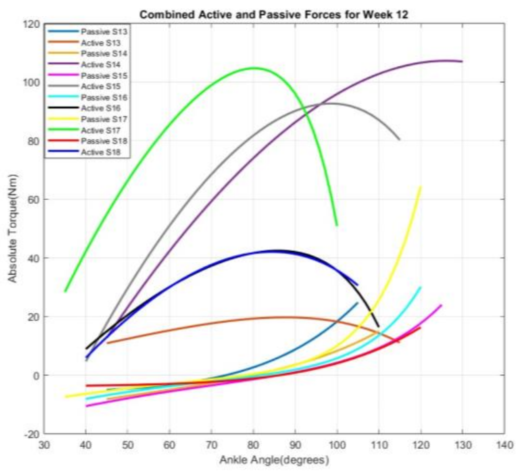
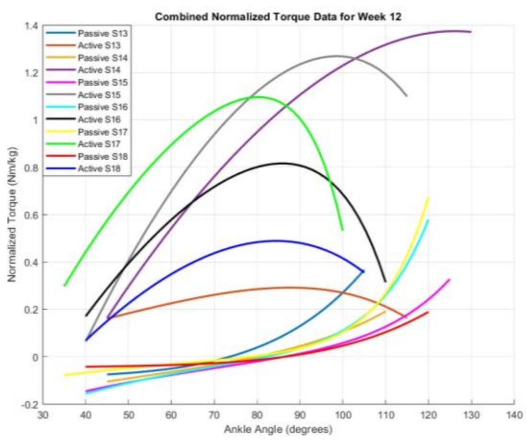
Active S15
Passive S16
Active S16
Passive S17
Active S17
Passive S18
Active S18

e)


Combined Active and Passive Forces for Week 12
Absolute Torque(Nm)
Ankle Angle(degrees)
Passive S13
Active S13
Passive S14
Active S14
Passive S15
Active S15
Passive S16
Active S16
Passive S17
Active S17
Passive S18
Active S18

f)


Combined Normalized Torque Data for Week 12
Normalized Torque (Nm/kg)
Ankle Angle (degrees)
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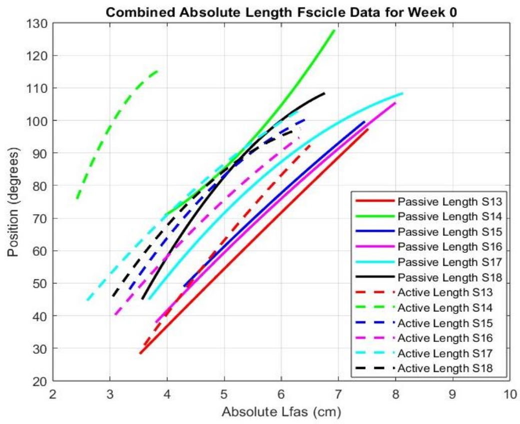
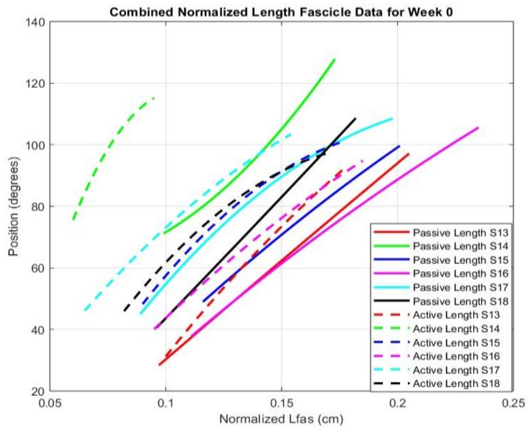
Active S13
Passive S14
Active S14
Passive S15
Active S15
Passive S16
Active S16
Passive S17
Active S17
Passive S18
Active S18

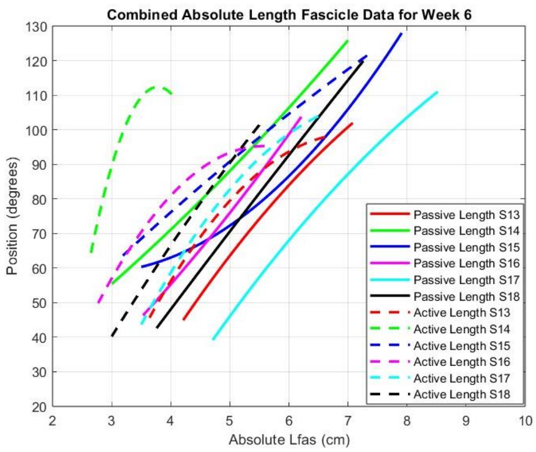
g)


Combined Absolute Length Fscicle Data for Week 0
Position (degrees)
Absolute Lfas (cm)
Passive Length S13
Passive Length S14
Passive Length S15
Passive Length S16
Passive Length S17
Passive Length S18
Active Length S13
Active Length S14
Active Length S15
Active Length S16
Active Length S17
Active Length S18

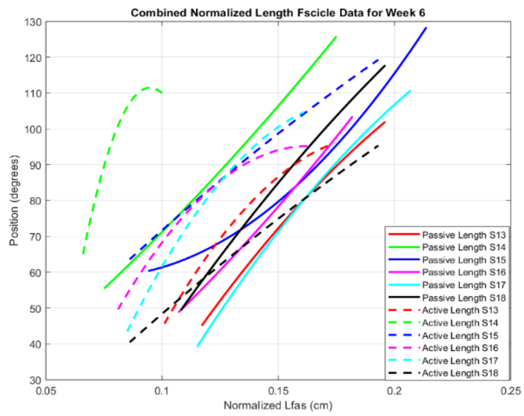
h)


Combined Normalized Length Fascicle Data for Week 0
Position (degrees)
Normalized Lfas (cm)
Passive Length S13
Passive Length S14
Passive Length S15
Passive Length S16
Passive Length S17
Passive Length S18
Active Length S13
Active Length S14
Active Length S15
Active Length S16
Active Length S17
Active Length S18

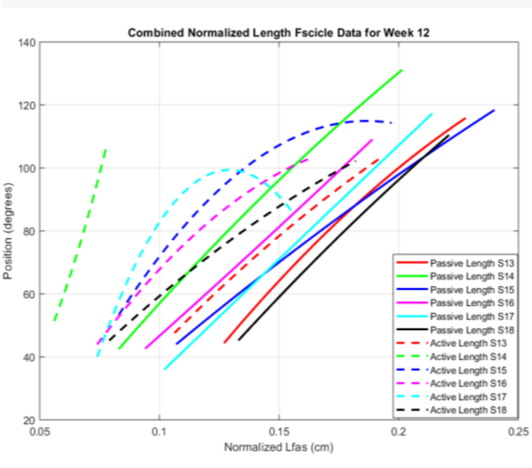
i)


Combined Absolute Length Fascicle Data for Week 6
Position (degrees)
Absolute Lfas (cm)
Passive Length S13
Passive Length S14
Passive Length S15
Passive Length S16
Passive Length S17
Passive Length S18
Active Length S13
Active Length S14
Active Length S15
Active Length S16
Active Length S17
Active Length S18

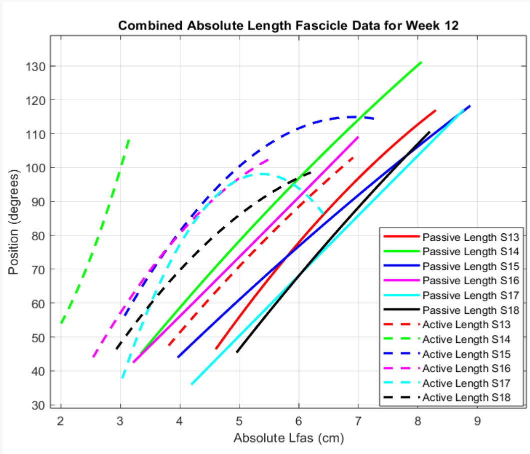
J)


Combined Normalized Length Fscicle Data for Week 6
Position (degrees)
Normalized Lfas (cm)
Passive Length S13
Passive Length S14
Passive Length S15
Passive Length S16
Passive Length S17
Passive Length S18
Active Length S13
Active Length S14
Active Length S15
Active Length S16
Active Length S17
Active Length S18

k)


Combined Normalized Length Fscicle Data for Week 12
Position (degrees)
Normalized Lfas (cm)
Passive Length S13
Passive Length S14
Passive Length S15
Passive Length S16
Passive Length S17
Passive Length S18
Active Length S13
Active Length S14
Active Length S15
Active Length S16
Active Length S17
Active Length S18

l)


Combined Absolute Length Fascicle Data for Week 12
Position (degrees)
Absolute Lfas (cm)
Passive Length S13
Passive Length S14
Passive Length S15
Passive Length S16
Passive Length S17
Passive Length S18
Active Length S13
Active Length S14
Active Length S15
Active Length S16
Active Length S17
Active Length S18