

## CHAPTER III

### METHODS

#### 1. Participants

The sixteen healthy participants (3 men and 13 women) were recruited from students in Chiang Mai University. The objective and the protocol of this study were fully explained and each participant signed an informed consent (see Appendix A and F). The study protocol was approved by the Research Ethics Committee, Faculty of Associated Medical Sciences, Chiang Mai University. During the study, all participants were asked to maintain normal activity.

##### **Inclusion criteria**

- Healthy young (man and women) participants.
- Age 18 to 30 years.
- Participants are free from musculoskeletal, cardiovascular, and neurological disorder.

**Exclusion criteria:** participants were excluded if they:

- Had history of ankle surgery or fractures of lower limbs in the past year.
- Participated in any types of resistance training program in their lower legs or play any sports which particularly used their lower legs e.g. foot ball for at least 1 year before testing.
- Had symptom of delayed onset muscle soreness after training.
- Can not follow an assigned exercise schedule at least 3 consecutive sessions or a total of greater than 6 sessions (20%) of the whole 30 sessions.

## 2. Study design

Pretest-posttest design and compare between an eccentric leg condition and control leg condition were used in the study. Before and after 6 weeks, all participants were evaluated for measured variables: maximal plantar flexion strength, tendon displacement and tendon moment arm length and calculated variables: Achilles tendon force and tendon stiffness. All measurements were taken on both legs and the non-dominant leg was defined as the eccentric leg condition (EL) and the dominant leg was defined as the control leg condition (CL). The participants of eccentric leg condition performed calf muscle eccentric training 15 repetitions per set, 6 sets, once a day, 5 days a week for 6 weeks at laboratory. The participants of control leg condition unintentionally performed concentric training and were not received an eccentric training program.

## 3. Equipments

1. 90 ° ankle bar with load cell (Load cell: ORIENTEC, LC 1205-k200, CAP. 2 kN, SER. E6308136)
2. Diagnostic ultrasound system: real-time ultrasonography (Famio: model SSA-530A, Toshiba, Fundamentals 2B730-670E\*D)
3. Magnetic resonance imaging (Philips, MR system Achieva 1.5T)
4. Step
5. Velcro
6. Metronome
7. Consent form
8. Weight scale

## 9. Ice bags and towels

**4. Outcome measures****Measured variables**

1. Maximal plantar flexion strength (Newtons·meter: Nm)
2. Tendon displacement (millimeter: mm)
3. Tendon moment arm length (millimeter: mm)

**Calculated variables**

1. Achilles tendon force (Newtons: N):  $\frac{\text{plantar flexor moment (Nm)}}{\text{tendon moment arm length (m)}}$
2. Tendon stiffness:  $\frac{\text{Tendon force (N)}}{\text{Tendon displacement (mm)}}$

**5. Procedures**

Participants were screened by the inclusion and exclusion criteria. Information sheet was given to participants to inform the purpose and procedures of this study. All participants signed the consent form before starting the data collection. All variables were evaluated by one tester and all baseline variables were measured in both legs. The non-dominant side of the leg as defines in the eccentric leg condition and the other leg defines as in control leg condition. In the eccentric leg condition, participants then performed the warm-up and stretching exercise of lower leg before eccentric training protocol as well as the cool-down exercise after the training protocol. In the control leg condition, participants also performed warm-up, stretching of lower leg and cool-down and unintentionally performed concentric training. With

eccentric training protocol, participants trained 15 repetitions per set, 6 sets, once a day, 5 days a week for 6 weeks at laboratory. Finally, all variables were measured after 6 weeks training (Fig. 3)

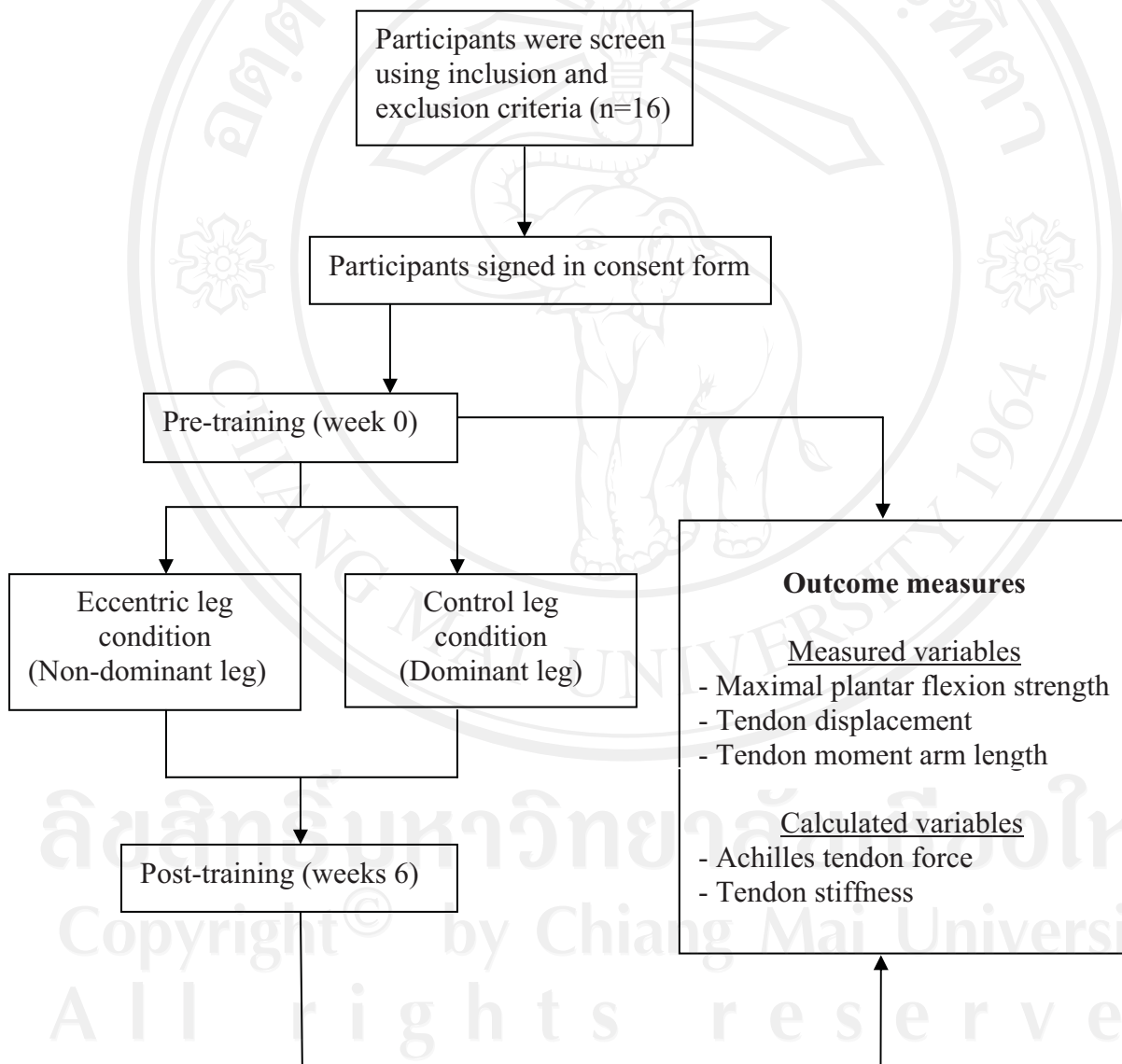


Figure 3 Diagram of experimental procedure

### **5.1 Measurement of maximal voluntary isometric contraction (MVC) of plantar flexor muscles**

Plantar flexor muscles force (N) was converted to plantar flexor moment (Nm) by multiplying the moment arm of load cell (0.215 m) that measured using the ankle leg bar attached with a strain-gauge load cell (capacity 20 kN). Participants were securely fixed in the prone lying on Physiotherapy bed with the knee in fully extension and the ankle in anatomical position against the ankle bar (Fig. 4). To prevent the ankle displacement, Velcro straps were securely applied to the participants' leg at the thigh and the forefoot. Before testing, participants performed 5 submaximal voluntary isometric contractions to familiar with the test. After that, they performed 3 MVC which each maximal effort lasted for 4 seconds separated by a 1 minute rest period (46). The highest MVC value was selected for the analysis and was used to calculate 25%, 50% and 75% MVC. The intra-tester ( $ICC_{(3,3)}$ ) reliability of maximal plantar flexor strength was reported in Table 5 (see Appendix B).

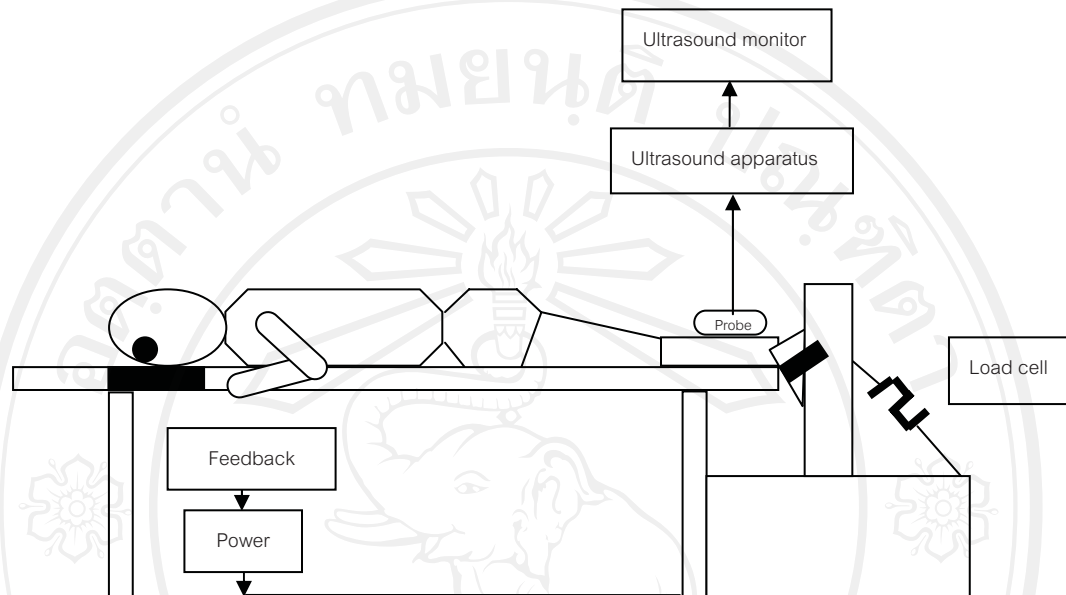
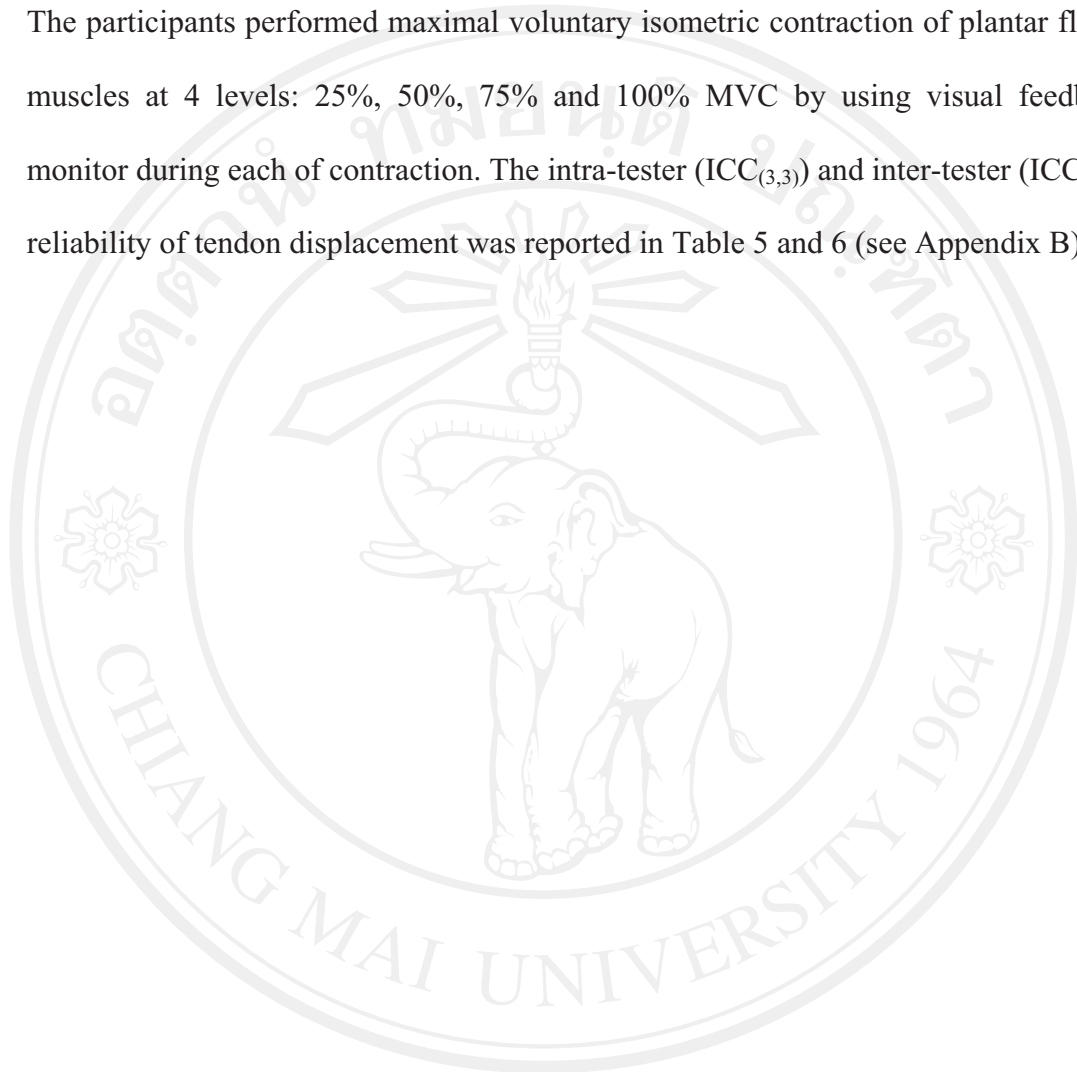


Figure 4 Experimental setup, ankle was set at neutral position and strap with ankle bar. The strain-gauge load cell connected to the ankle bar for measure maximal plantar flexion strength.

## 5.2 Measurement of tendon displacement

The tendon displacement during rest and during contraction was assessed using the real-time B-mode ultrasonography (SSA-530A, Famiio, Toshiba). An electronic linear-array probe of 8.5 MHz wave frequency was tightly fitted into a block of rigid polystyrene plastic and taped onto the skin of participants, overlying myotendinous junction of medial gastrocnemius (MG). At rest and during muscle contraction, the tendon displacement were measured the displacement of the myotendinous junction ( $\Delta$  mm) of the MG in the transition from rest (Fig.5a) to each

level contraction (an example during 50% and 100%MVC were shown in Fig.5b-5c). The participants performed maximal voluntary isometric contraction of plantar flexor muscles at 4 levels: 25%, 50%, 75% and 100% MVC by using visual feedback monitor during each of contraction. The intra-tester ( $ICC_{(3,3)}$ ) and inter-tester ( $ICC_{(2,3)}$ ) reliability of tendon displacement was reported in Table 5 and 6 (see Appendix B).



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่  
Copyright© by Chiang Mai University  
All rights reserved

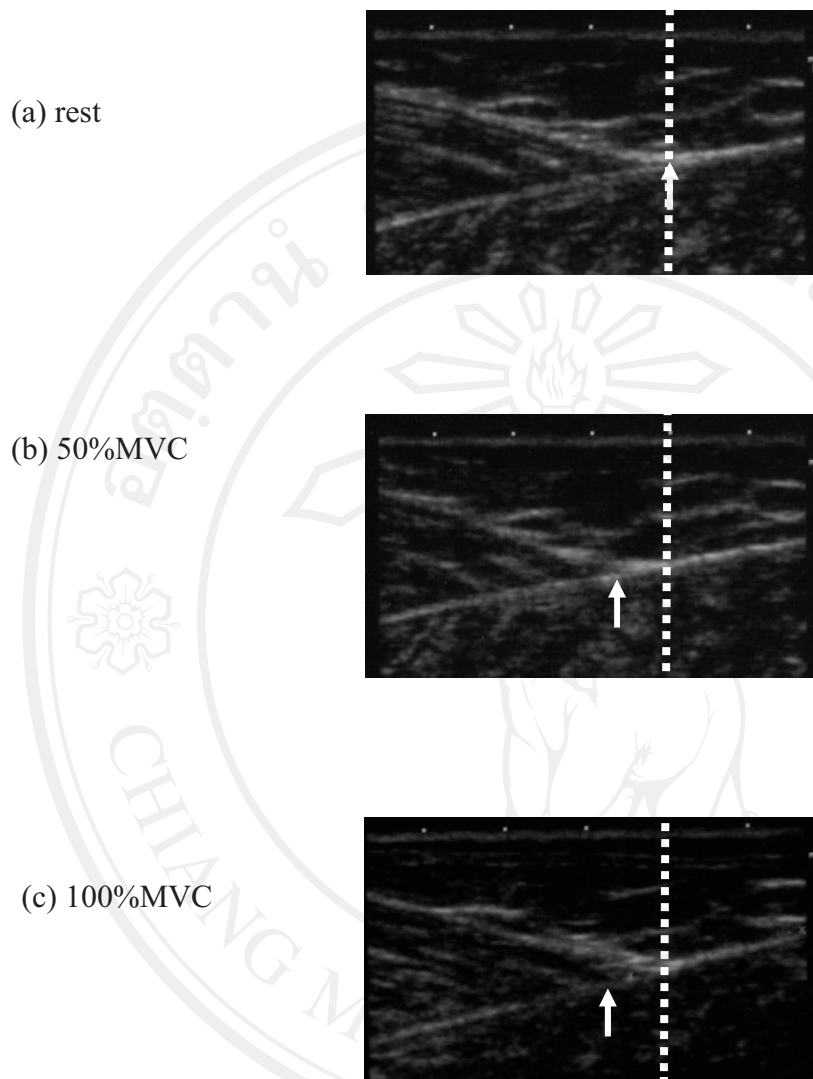


Figure 5 Sagittal plane ultrasound images over distal myotendinous junction of the medial gastrosoleus muscle during rest (a), 50 % (b), and 100 % MVC (c). White dashed line represents the myotendinous junction during rest as a reference point. White arrow represents the myotendinous junction during contractions.



### 5.3 Moment arm length of Achilles tendon and calculation of tendon force

The moment arm length of Achilles tendon was assessed using the magnetic resonance imaging (MRI) scanner by radiographer and was measured to calculate individual tendon force. The participants lied supine position with the knee fully extended and ankle in 90 degrees (Fig.4). Sagittal plane T1-weighted spin echo images (Philips, MR system Achieva 1.5 T) with parameters: repetition time/echo time (TR/TE): 500/20 ms, field of view (FOV) 219 mm, matrix 480 × 230, slice thickness 3 mm and spacing 0.3 mm. To estimate the Achilles tendon moment arm length we used a modified Reuleaux method (51, 52). This method was assumed that the joint surface of the talus was circular and using geometric rules obtained a center of rotation at a specific point distal to the joint surface of talus. The tendon moment arm length was obtained by measuring the perpendicular distance from the Achilles tendon to the center of rotation (Fig.6). The measurement of the moment arm from the MRI was performed 3 times for each participant and mean value was used for analysis. The intra-tester (ICC<sub>(3,3)</sub>) reliability of moment arm length of Achilles tendon was reported in Table 5 (see Appendix B).

Achilles tendon force was calculated by the following equation (33, 49):

$$\text{Achilles tendon force (N)} = \frac{\text{plantar flexor moment (Nm)}}{\text{tendon moment arm length (m)}}$$

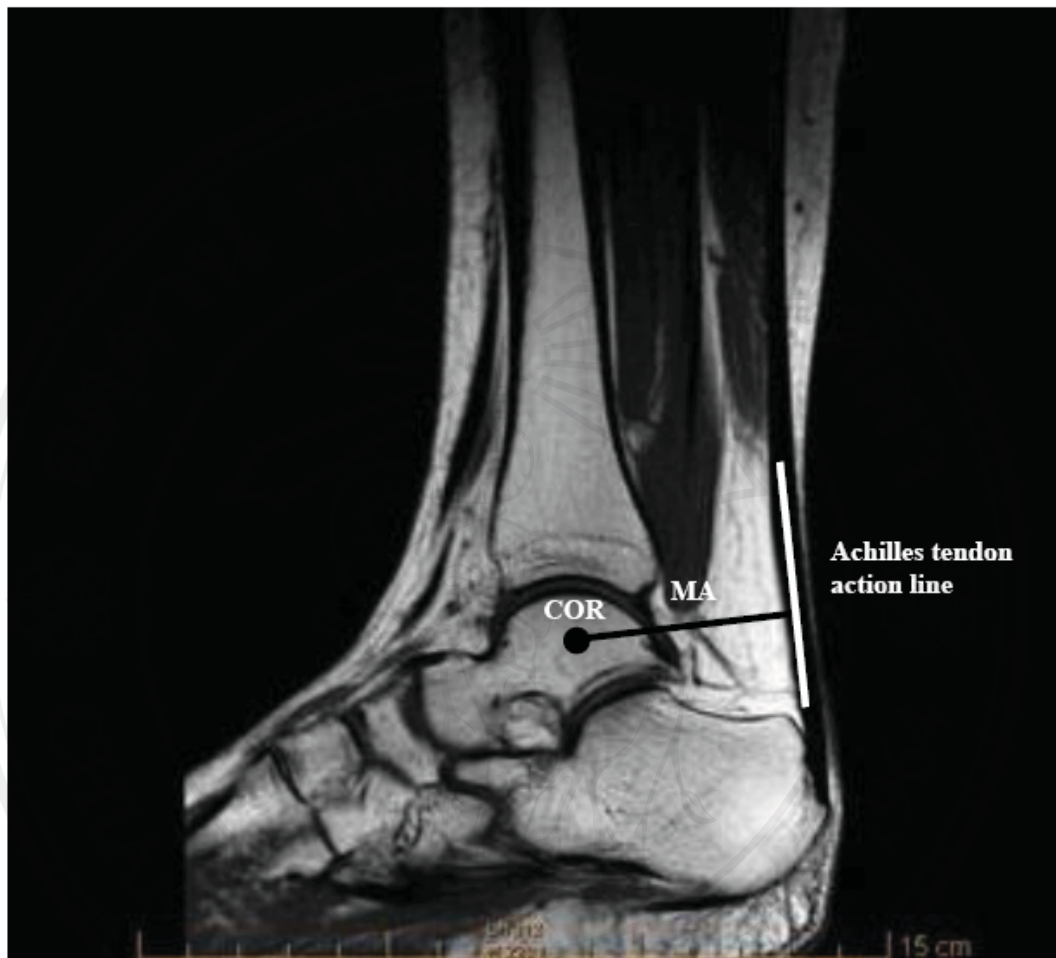


Figure 6 Sagittal plane magnetic resonance images of the foot at the neutral position at rest, the black circle on the talar bone is the instantaneous center of rotation in the tibiotalar joint, the white line from the middle of Achilles tendon represents Achilles tendon action line. The moment arm length (black line) represents perpendicular distance from center of rotation to Achilles tendon action line.

#### 5.4 Calculation of the stiffness of the Achilles tendon

Each tendon force-displacement curve was fitted with a quadratic model and tendon stiffness ( $\text{Nmm}^{-1}$ ) was calculated in the final 10% of the tendon force-displacement curve ( $\Delta F / \Delta \text{mm}$ ) (49, 53) (Fig.7).

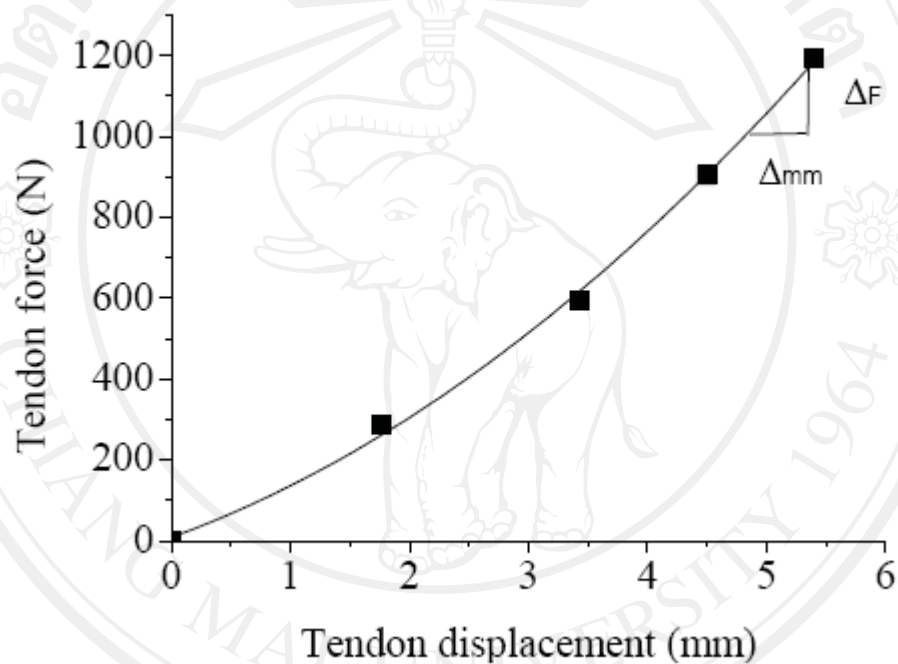


Figure 7 Tendon force-displacement relationship of one participant. Tendon stiffness ( $\Delta F / \Delta \text{mm}$ ) was calculated from the tendon force-displacement relationship in the final 10% of the tendon force range, i.e. from 90% to 100% of tendon force after a quadratic fitted.

### **5.5 Eccentric training protocol**

The eccentric training protocol was modified from a recently published studies (10, 11) on eccentric calf muscle training in patients with Achilles tendonopathy. Although the protocol was designed to train for eccentric calf muscle contraction, the opposite leg unintentionally performed concentric muscle contraction. In the beginning, the participants stand on the steps (9.3 cm in height in each step) on the non-dominant leg with the ankle in plantar flexed position and the forefoot was only placed on the step (Fig.8a). Then, the calf muscle was loaded eccentrically when the participants lower the heel beneath the level of forefoot as much as they can. After that the dominant leg was used to return to the starting position. Two types of exercise were used. The calf muscle was eccentrically loaded both with the knee straight (Fig.8b) and, to also maximize the activation of soleus muscle with knee slightly bent (Fig.8c). The cycle of exercise was monitored by audio feedback from metronome (4 seconds: 8 seconds for lift up to lower down) (modified from Mahieu et al, 17). Participants were instructed to regularly perform the exercise at the laboratory 5 days/week for 6 weeks. For the progression of the exercise, participants' maximal plantar flexion forces were assessed in every week to adjust proper number of sets and loads. If their maximal plantar flexion forces decreased, the number of sets would be decreased. If maximal plantar flexion forces were the same or increased, the numbers of sets would be firstly increased for a week and the load would be adjusted later. Therefore, either two sets or two kilograms were used to progress the exercise. Backpack was used to apply the load. Between the exercise sets, the participants rested for 2 minutes. The first week, participants performed exercise 10 repetitions

for 4-6 sets; in week 2, participants performed exercise 15 repetitions for 8 sets; in week 3, participants performed exercise 15 repetitions for 8 sets with load  $2.8 \pm 0.7$  kg (range 2-4 kg); in week 4, participants performed exercise 15 repetitions for 10 sets; in week 5, participants performed exercise 15 repetitions for 10 sets with load  $5.1 \pm 0.8$  kg (range 4-7 kg); in week 6, participants performed exercise 15 repetitions for 12 sets (see the exercise progression in Appendix H). Participants attended an average 90.71% of exercise session which was higher than a minimal criterion for participating in the study.



(a) Starting position

(b) Gastrosoleus loading

(c). Soleus loading

Figure 8 The difference position of eccentric training (a) starting position: ankle joint is in plantar flexion position, (b) eccentric loading of the calf muscle with the knee straight (gastrosoleus loading) and (c) eccentric loading of the calf muscle with the knee slightly bent (soleus loading).

## 6. Statistical analysis

Data analysis was undertaken using the statistical software package version 12. The nonparametric test was chosen for this study because of a small sample size. Wilcoxon Signed Rank test was for compare pre- post training variables and compare between eccentric training leg and control leg. A probability level of 0.05 was set to denote significance.

## 7. Location

The study was conducted in the laboratory room at 3<sup>rd</sup> and 4<sup>th</sup> floor at Department of Physical Therapy and Radiologic Technology, Faculty of Associated Medical Sciences in Chiang Mai University.