Investigating Sex Difference in 2-Dimensional Ankle Stiffness during Upright Standing Balance

by

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ABSTRACT

It has been repeatedly shown that females have lower stability and increased risk of ankle injury when compared to males participating in similar sports activities (e.g., basketball and soccer), yet sex differences in neuromuscular control of the ankle, including the modulation of ankle stiffness, and their contribution to stability remain unknown. To identify sex differences in human ankle stiffness, this study quantified 2-dimensional (2D) ankle stiffness in 20 young, healthy men and 20 young, healthy women during upright standing over a range of tasks, specifically, ankle muscle co-contraction tasks (4 levels up to 20% maximum voluntary co-contraction of ankle muscles), weight-bearing tasks (4 levels up to 90% of body weight), and ankle torque generation tasks accomplished by maintaining offset center-of-pressure (5 levels up to +6 cm to the center-of-pressure during quiet standing). A dual-axial robotic platform, capable of perturbing the ankle in both the sagittal and frontal planes and measuring the corresponding ankle torques, was used to reliably quantify the 2D ankle stiffness during upright standing. In all task conditions and in both planes of ankle motion, ankle stiffness in males was consistently greater than that in females. Among all 26 experimental conditions, all but 2 conditions in the frontal plane showed statistically significant sex differences. Further analysis on the normalized ankle stiffness scaled by weight times height suggests that while sex differences in ankle stiffness in the sagittal plane could be explained by sex differences in anthropometric factors as well as neuromuscular factors, the differences in the frontal plane could be mostly explained by anthropometric factors. This study also demonstrates that the sex differences in the sagittal plane were significantly higher as compared to those in the frontal plane. The results indicate that

females have lower ankle stiffness during upright standing thereby providing the neuromuscular basis for further investigations on the correlation of ankle stiffness and the higher risk of ankle injury in females.

Dedicated to my parents for their unconditional love and unwavering belief

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I acknowledge Dr. Hyunglae Lee’s guidance, patience and time invested in me. Thank you, Dr. Lee, for believing in me and for giving me the opportunity to work on this research project. This experience has opened many opportunities for my growth and success, and for that, I am grateful.

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TABLE OF CONTENTS

CHAPTER Page

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

Figure Page

Figure Page

CHAPTER 1

INTRODUCTION

Serving as the interface between the neuromechanical system and the physical environment, the human ankle is an essential joint which plays one of the most important roles in postural stability and locomotion [1], [2], [3]. It contributes to movement and stabilization of the entire human body in both static and dynamic conditions and allows for shock absorption, propulsion and smooth lower-limb joint coordination [4], [5]. This movement predominantly occurs in two planes; the sagittal plane, where most of the ankle movement is dorsiflexion and plantar flexion, and the frontal plane, where eversion and inversion are dominant [6].

Despite the crucial role the ankle plays in locomotion, the incidence of musculoskeletal injuries at the ankle joint is an ever-increasing problem. Lambers et al [7] reported that the incidence of ankle injury was greatest among lower extremity injuries. Notably, it has been reported that the incidence of ankle injuries in females is significantly higher than in males engaging in similar activities, such as basketball and soccer [8], [9]. According to Hosea et al [10], females are 25% more likely to sustain ankle injuries than their male counterparts. This study was consistent with an incidence report from Doherty [11], that concluded that females are at a higher risk of sustaining ankle injury as compared males.

This higher risk of musculoskeletal injuries in females has been attributed to anatomical, hormonal and neuromuscular factors that differentiate females from males. Anatomically, the increased rate of musculoskeletal injury is largely associated with the greater range of motion [12], [13], lower Young's modulus [14], [15] and higher joint and ligamentous laxity [16], [17], [18], [19] in females. It is also speculated that cyclic hormonal variations could cause a decrease in the strength of muscles and ligaments, and could increase ligamentous laxity and decrease stability [20]–[22], however, compared to the extensive research on knee injury [20]–[22], there is limited information regarding cyclic hormonal fluctuations and the risk of ankle injury. A few studies highlight that hormonal fluctuations do not affect ankle laxity or stability [23], [24], thus, the hormonal variations in females, is not sufficient to fully explain the sex difference in risk of ankle injury. Further, sex differences in neuromuscular control to properly resist external loading and stabilize the body could contribute to sex differences in musculoskeletal injuries [25]–[29].

Compared to the substantial research on sex differences in knee injuries [20], [21], [25] and their underlying mechanisms, there is very limited study investigating factors contributing to sex differences in ankle injuries, in particular the neuromuscular factor. In an effort to better understand the higher risk of ankle injury in females, this thesis investigates sex differences in ankle stiffness, one of the most important neuromuscular factors that resist external loading and hence prevent ankle injury [3], [6], [30]–[33].

Given the importance of the ankle stiffness in lower extremity function, ankle stiffness has been extensively studied under various task conditions, including seated, standing, and walking [34], [35]. Recent studies have also characterized 2D ankle stiffness in both the sagittal and frontal planes, since it contributes to not only dorsiflexion-plantarflexion (DP) movement in the sagittal plane but also inversion-eversion (IE) movement in the frontal plane [32], [36]–[40]. However, there is little information regarding the sex differences in ankle stiffness. One study has investigated sex differences in ankle stiffness in the IE-DP space, but it was limited to a static seated position [41]. It concluded that 2D ankle stiffness under various muscle activation conditions is significantly lower in females than males. While this study has provided an important baseline to understand sex differences in ankle stiffness, it is unknown if results obtained in the non-functional seated tasks would apply to functional tasks, such as standing balance and walking. As standing is fundamental in everyday activity and serves as a precursor to the initiation of other activities of daily living, identifying the sex differences in upright standing balance is significant.

Building upon the previous seated study, this thesis aimed at investigating sex differences in 2D ankle stiffness during upright standing balance. It also investigated sex differences of normalized ankle stiffness, scaled by weight times height, to determine how anthropometric factors influence the sex differences in 2D ankle stiffness. Inspired by a simple inverted pendulum model describing ankle stiffness during standing balance, this normalization factor was chosen [42], [43]

Purpose of Study

The purpose of this study is to quantify sex differences in 2D ankle stiffness during different tasks which include ankle muscle co-contraction tasks, weight-bearing tasks and ankle torque generation tasks (tasks which stimulate balance at different levels of ankle torque), during upright standing, and to investigate the mechanisms for the differences.

The specific aims of this thesis are in three folds;

Specific Aim 1:

To compare 2D ankle stiffness between males and females during different ankle muscle co-contraction tasks, weight-bearing tasks and ankle torque generation tasks.

Hypothesis:

From previous findings about the anatomical factors (greater range of motion, lower Young's modulus, and higher laxity) contributing to the higher incidence of ankle injury in females, as well as the recent study identifying sex differences in 2D ankle stiffness in a seated position [41] , it is hypothesized that ankle stiffness in females is significantly lower than in males in both the sagittal and frontal planes, for all the different conditions for ankle muscle co-contraction tasks, weight-bearing tasks and ankle torque generation tasks.

Specific Aim 2:

To determine the sex differences in ankle stiffness between the sagittal and frontal planes for all the three different tasks.

Hypothesis:

It is hypothesized that because the degree of ankle modulation in the sagittal plane is substantially higher than the degree of ankle modulation in the frontal plane [32], [40], [44], the sex difference in ankle stiffness in the sagittal plane is significantly higher than in the frontal plane.

Specific Aim 3:

To determine how weight and height influence sex differences in ankle stiffness.

Hypothesis:

It is hypothesized that based on previous findings of sex differences in active muscle mechanics [25], [45], [46], the sex difference in ankle stiffness in the sagittal plane, but not in the frontal plane, still persists even when normalized by body weight times height.

Significance of the hypotheses:

The results from this study will provide the basis for further research to determine whether the sex differences in ankle stiffness influences the sex differences in lower limb stability and correlates with the higher risk of injury in females. Consequently, this will shed more light on the understanding of the sex differences in risk of ankle injury.

Thesis Outline

This thesis investigates the sex difference in 2D ankle stiffness during three different tasks; ankle muscle co-contraction tasks, weight-bearing tasks, and ankle torque generation tasks, during upright standing. Chapter 2 explains how these different tasks influence ankle stiffness. Chapter 3 gives a detailed information about the methodology, and chapter 4 outlines the results from data collection. Chapter 5 discusses the results obtained, and chapter 6 gives the conclusion of the findings in the previous chapters and highlights the limitations in this study.

CHAPTER 2

CONTRIBUTION OF MUSCLE CO-CONTRACTION, WEIGHT, ANKLE TORQUE TO ANKLE STIFFNESS

Muscle Co-contraction

During upright standing, the ankle plays an important role in stabilizing the body by exerting a counteractive gravitational torque, known as ankle torque, to the ground. This torque is influenced by a passive mechanism provided by the mechanical properties of muscles fibers, ligaments, and tendons with zero delay and by an active mechanism provided by the modulation of ankle muscles by the central nervous system (CNS) [47].

When faced with small perturbations, such as different terrains, the initial response of the muscle is predominantly given by the passive mechanism and causes a rise in muscle contractile force to resist stretch, stabilize the lower limbs and prevent falls. This stretch resistance without control from the CNS, lasts for a short duration and is termed as short-range stiffness.

According to De Groote et al [48], although the short-range stiffness is not entirely sufficient to maintain stability over a long period, it can contribute to maintaining stability over a short period, due to its zero delay and thus, gives room for neural feedback control, which has a latency of 50ms – 100ms [49].

Furthermore, Hogan [50] highlighted that muscle stiffness increases linearly with the contractile force of the muscle, therefore an increase in muscle contraction results in a corresponding increase in stiffness. This finding has been proven by several other studies that have demonstrated that muscle contraction increases joint stiffness at the elbow [51] and ankle [31]. The reason for this conclusion is that muscle contraction is as a result of an increased number of activated cross-bridges, which generate tension within the muscle to resist stretch [52], [53]. Consequently, an increase in the number of activated muscle fibers will lead to greater tension within the muscle, hence greater resistance to muscle stretch (i.e.; muscle stiffness). Since muscle contraction increases stiffness, co-contraction will lead to a larger increase in joint stiffness as it involves activation of two groups or muscles: agonist and antagonist muscles [54], [55], whose stiffnesses add up [50]. This thesis exploits this correlation between muscle co-contraction and stiffness to investigate sex differences in ankle stiffness during muscle co-contractions.

Weight

Weight also plays a vital role in stability. According to Hue et al [56], there is a strong correlation between body weight and stability, with an increase in body weight corresponding to a decrease in stability. Anker et al [57] also investigated the relationship between weight distribution and postural stability during standing and concluded that increasing weight-bearing asymmetry increases postural instability.

The counteractive torques exerted by the ankle against the gravitational torques from the ground, during upright standing, stems from the weight of the entire body resting on the ankle joint. During weight bearing tasks involving loading or unloading one leg, the muscles of the loading leg generates stabilizing torques to compensate that of the unloading leg [57]. This leads to an increase in the torque on the loading leg, which in turn increases the stiffness to combat the instability.

As the load on the ankle increases, there is a corresponding increase in ankle torque exerted. This document exploits the correlation between weight and ankle torque to understand the sex differences in ankle stiffness during different weight-bearing conditions.

Ankle Torque

The ability to ensure postural control, stability and balance of the human body during upright standing, can be challenging, as the human body must regulate the center of mass to ensure that the center of pressure is within the base of support. This can be modeled as an inverted pendulum, as it involves controlling a large mass at a substantial height above the ankle, over a small base of support [58]. This inevitably leads to instabilities, involving slight movements about the center of mass, termed as postural sways.

Postural sways, which indicate the amount of torque generated at the ankle, shift the center-of-pressure (CoP) of the human body during quiet standing and leads to minimal instabilities during stance. Thus, the CoP of a neuromechanical system can serve as a measure of postural sway, and hence, the ankle torque generated. The amount of ankle torque generated as a result of a shift in CoP can therefore serve as an indicator of neuromuscular control [59] in maintaining postural stability. Since ankle stiffness is a measure of ankle torque with respect to position, ankle stiffness may also be influenced during changes in ankle torque. For this reason, investigating the sex differences in ankle stiffness during different ankle torque generation tasks can provide information about the ability of the both sexes to control instabilities, and hence, and provide information to help understand the risk of injuries at the ankle joint.

CHAPTER 3

METHODOLOGY

Subjects

Recruitment for the study was carried out under the approval of the Arizona State University Institutional Review Board - STUDY00010123 (Appendix A) and was based on the following inclusion and exclusion criteria:

Inclusion criteria: Subjects between the ages of 18 and 32, with no reported history of musculoskeletal or connective tissue disorders that could affect ankle stiffness, and who understood the instructions and were willing to sign the informed consent to participate in the study, were included.

Exclusion criteria: Subjects with neurological or orthopedic disorders that would affect their balance, upright standing and stiffness were excluded. Subjects were also excluded if they had any of the following conditions: Nerve injury or broken bones in the back or lower extremity twelve months prior to participation, any surgeries to the back or lower extremities twelve months prior to participation, regular dizziness or fainting, hearing sensitivity or visual impairments, excessive soreness of joints, injuries related to fractures, or joint dislocation or torn ligaments. Subjects who weighed 90kg or higher were also excluded from the study due to the limitations of the robotic platform used for this study. As the robotic platform was designed for the right leg, subjects who had left leg dominance were excluded from the study.

A total of forty subjects were recruited; twenty young men (age: 22.6 (1.7), 20 – 27; weight: 68.2 (9.0), 60.7 – 88.0 kg; height: 170.3 (6.2), 154.5 – 179.2 cm) and twenty young women (age: 22.6 (3.0), 18 – 32; weight: 55.9 (8.2), 44.0 – 82.1 kg; height: 163.7

(5.3), 155.5 – 177.7 cm) with no reported history of musculoskeletal or connective tissue disorders that could affect ankle stiffness. Subjects gave written informed consent before participation.

Experimental Setup

To quantify 2D ankle stiffness, specifically stiffness in both sagittal and frontal planes, during upright standing, a novel dual-axial robotic platform was used. This was capable of providing rapid position perturbations to the ankle in the sagittal and frontal planes and measuring the corresponding ankle torques using a force plate (9260AA3, Kistler, NY) embedded in the platform.

Subjects stood upright with their right foot on the robotic platform, recessed into an elevated floor, and their left foot on the elevated floor to the left of the recessed platform (Figure 1A). The right foot was placed in a fashion to ensure that the axis of rotation of the robotic platform for DP was in line with that of the ankle [6]. This was done to guarantee a harmonized movement of the platform and the foot. The axis of rotation of the robotic platform for IE was about 10 cm below that of the ankle, but a previous study confirmed that this offset has minimal impact on IE ankle stiffness estimation [60]. The foot position on the platform and the elevated floor was marked out to remove inconsistencies associated with different standing positions.

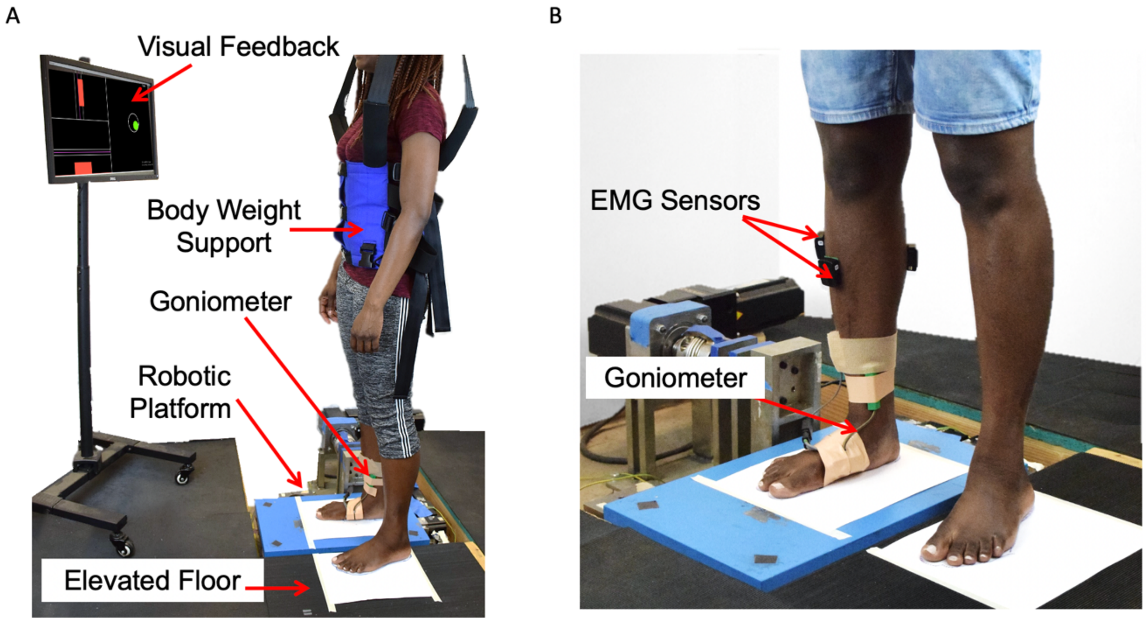

Figure 1: Experimental Setup to quantify 2-dimensional ankle stiffness
A: The total view of the experimental set-up. B: A close view of the goniometer and EMG sensors placed on the subject

In addition to the robotic platform, the experimental setup consisted of a dual-axis goniometer, surface electromyography (EMG) sensors, a safety harness, and a visual feedback display.

Ankle angles in both the sagittal and frontal planes, i.e., DP and IE angles, were measured using a goniometer (SG 110, Biometrics, Ltd, UK) attached to the ankle-foot complex. Depending on the direction of the perturbation, the goniometer was placed at different parts of the foot-shank complex (Figure 2), to ensure the best reading of angular displacements.

Muscle activation of major ankle muscles, specifically, tibialis anterior (TA), soleus (SL), medial gastrocnemius (GA), and peroneus longus (PL), was measured using wireless surface EMG sensors (Trigno EMG systems, Delsys, MA). To ensure safety, each subject wore a safety harness attached to a bodyweight support system (LiteGait, AZ), but no body weight support was provided.

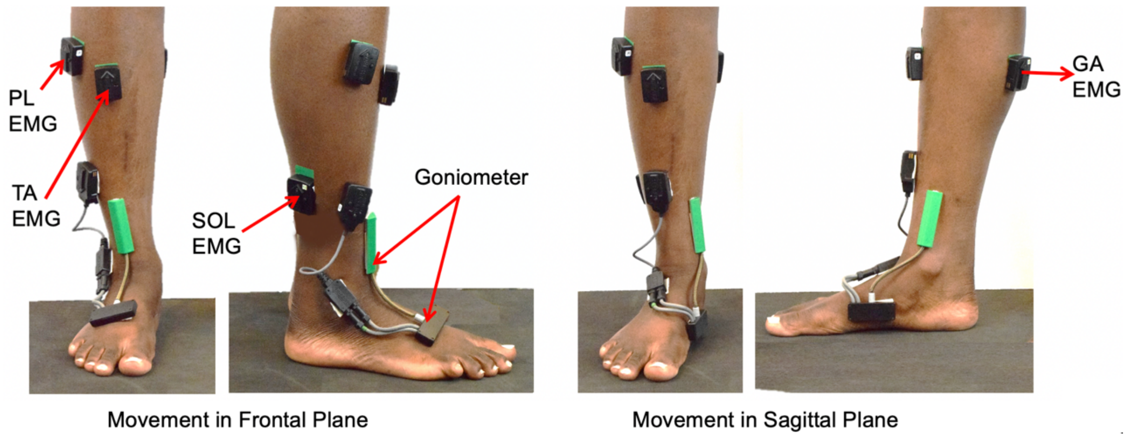

Figure 2: Sensor Placement for DP and IE directions

The visual feedback (Figure 3) was placed in front of the subject at the eye level. It displayed the target, acceptable limits of the target and the current levels of the three parameters to be controlled by the subjects during the standing balance tasks: weight distribution between the legs, CoP displacements in both DP and IE directions, and TA muscle activation. Controlling CoP alone allowed the subjects to maintain ankle torque at a given target level. Controlling both CoP and TA activation allowed the subjects to effectively control the level of co-contraction of ankle muscles.

The acceptable limits of the target level for each parameter were: ±0.5 cm from the target for CoP, ±2.5% of MVC from the target for muscle activation and ±1kg of body weight for weight distribution.

A single board computer (PCM 3356, Advantech, CA) was used to control the setup and acquire data using a real-time Simulink model at 2Hz.

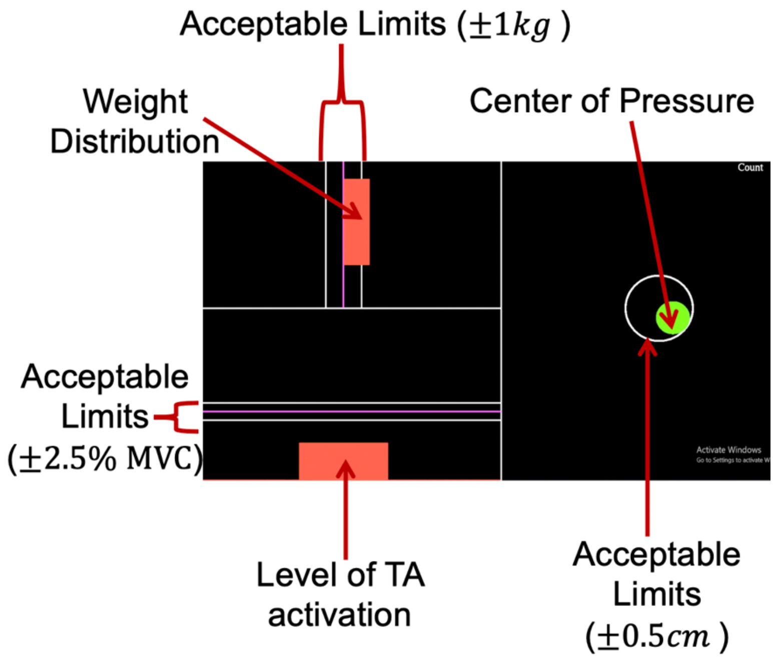

Figure 3: The visual feedback displays all the three parameters with their acceptable limits

Experimental Protocol

Before the experiment, weight and CoP during quiet standing (neutral CoP) were recorded. In addition, maximum voluntary contraction (MVC) of each of the 4 selected ankle muscles was measured according to the standard muscle testing procedures [61]. These measurements were used as references to determine target levels for 3 tasks in main experiments. In addition, the recordings from the goniometer was evaluated by applying a sinusoidal perturbation to the platform while the movement of the ankle was observed in Simulink Real-time. This was done to calculate the gain of the goniometer and to ensure the platform and foot moved harmoniously.

A practice session (5 – 10 minutes) was conducted to allow subjects to familiarize with the experimental setup. The main experiments began once subjects felt confident in changing levels of muscle co-contraction, weight-bearing, and CoP in the right leg. No perturbations were applied to the platform during the practice session.

All subjects participated in two sets of experiments, one for the quantification of stiffness in the sagittal plane ($K_{sagittal}$) and the other for stiffness in the frontal plane ($K_{frontal}$). Each experiment consisted of 3 distinct tasks, namely, muscle co-contraction tasks, weight-bearing tasks, and ankle torque generation tasks.

For the muscle co-contraction task, ankle stiffness was quantified at 4 different levels of ankle muscle co-contraction: 0% (relaxed), 10%, 15%, and 20% of the maximum voluntary co-contraction (MVCC). Subjects were instructed to maintain 0% (relaxed), 10%, 15%, and 20% MVCC of TA while keeping the neutral CoP. Controlling the neutral CoP during TA (dorsiflexor and inverter) activation essentially requires proper activation of plantarflexors and evertors, and thus this instruction could properly change the level of overall ankle muscle co-contraction. During this task, subjects were instructed to load 50% of body weight in one leg, which was defined as the neutral weight-bearing.

For the weight-bearing task, ankle stiffness was quantified at 4 different levels of weight-bearing: 30%, 50% (neutral weight-bearing), 70% and 90% of the total body weight in the right leg. Subjects were instructed to maintain the neutral CoP during this task, but no instruction was given regarding muscle activation.

For the ankle torque generation task, this was achieved by instructing subjects to maintain different offsets of the neutral CoP to target different levels ankle torque. Ankle stiffness was quantified at 5 different levels of CoP offset. In the sagittal plane, -2, 0 (neutral CoP), +2, +4, and +6 cm were tested. In the frontal plane, -1.5, -0.75, 0, +0.75,

+1.5 cm were tested. Subjects were instructed to maintain the neutral weight-bearing condition this task, but no instruction was given regarding muscle activation.

Each of the 26 experimental conditions ((4 muscle co-contraction levels + 4 weight-bearing levels + 5 CoP or ankle torque levels) × 2 planes of ankle motion) was repeated 15 times. For each trial, subjects were instructed to reach and control the selected target levels displayed on the visual feedback display. Feedback indicators for each task changed from red to green when acceptable limits of the target levels were maintained. During the weight-bearing and ankle torque generation tasks, visual feedback of muscle activation was disabled.

Once acceptable limits of the target levels were maintained at a random period of 0.5 – 0.7 seconds, a rapid ramp-and-hold perturbation lasting for 100 ms with an amplitude of 3° was applied to the ankle. Dorsiflexion and eversion perturbations were used to quantify $K_{sagittal}$ and $K_{frontal}$, respectively. Plantarflexion and inversion perturbations were not used because of possible loss of contact between the robotic platform and the foot during perturbations.

For each set of experiment, a total of 195 trials were split into 13 blocks (15 trials/block). Each block contained 15 trials of one of the 3 tasks, and the order of the target levels within the block was fully randomized. To prevent fatigue during the experiment, a 1-minute rest period was provided between blocks and a 3-minute rest period between tasks for each plane of movement. A 5-minute rest period was also provided between the two sets of experiment during which, the position of the goniometer was changed.

Data Analysis

Data from the experiments (ankle kinematics, ankle torques, and EMG data) were collected using a data acquisition board (DX-32 AT DAQ; Diamond Systems, CA) at a sampling rate of 2 kHz. Ankle kinematics and torque data were filtered using a 2nd order Butterworth low-pass filter having a cut-off frequency of 20 Hz, while the EMG data was demeaned, rectified, and filtered using a 2nd order Butterworth low-pass filter with a cut-off frequency of 5 Hz. Filtered EMG signals during the experiment were normalized to the pre-recorded MVC and calculated over an interval of 100ms just before the perturbation.

Torques due to platform dynamics were identified under no loading condition (no human subject on the platform) and subtracted from each subject's measured dynamics to obtain the ankle torques for each subject.

Ankle stiffness was calculated by fitting a 2nd order model, consisting of ankle stiffness, K, ankle damping, B, and foot inertia, I, Eq (1), to the measured ankle kinematics, θ, and torques, τ.

$$K\dot{\theta} = \tau - B\theta - I\ddot{\theta} \quad (1)$$

This was achieved by running a linear regression over an interval of 75ms starting from the onset of the perturbation. In addition, to check the reliability of parameter estimation, the percentage variance accounted for (%VAF) between the estimated ankle torque calculated from the estimated stiffness, damping, and inertia and the measured ankle torque was calculated [32], [40].

Statistical Analysis

To test sex differences in ankle stiffness in the sagittal and frontal planes, the following statistical analyses were performed.

First, statistical analysis was conducted to determine if there exist any significant sex differences in 2D ankle stiffness ($K_{sagittal}$ and $K_{frontal}$) and normalized 2D ankle stiffness ($K_{normalized\_sagittal}$ and $K_{normalized\_frontal}$) for the 3 task conditions (muscle co-contraction, weight-bearing and ankle torque generation tasks). Normalized ankle stiffness was calculated by dividing the ankle stiffness by the total body weight of the subject times height of the subject.

For each stiffness and each normalized stiffness, a separate mixed-design analysis of variance (mixed ANOVA) was performed, with task level as the within-subject factor and sex as the between-subject factor. Following the mixed ANOVA, post-hoc analyses were performed by running unpaired, independent, two-tailed t-tests to identify sex differences at each task level. Further, post-hoc analyses using the Bonferroni correction for pairwise comparisons among different task levels, was conducted.

Next, statistical analysis was conducted to determine if the sex difference in ankle stiffness and normalized ankle stiffness in the sagittal plane is significantly higher as compared to that in the frontal plane. For each task, a separate mixed ANOVA was performed, with plane of ankle motion as the within-subject factor and sex as the between-subject factor and the significance of interaction between these two factors was investigated.

To compare the strategies both sexes used in performing all three tasks, for each task, the calculated baseline ankle stiffness (ankle stiffness during 0%MVC, 50% weight and 0 CoP offset for muscle co-contraction tasks, weight-bearing tasks and ankle torque generation tasks respectively for both sagittal and frontal planes) was subtracted from the ankle stiffness during the other experimental conditions to obtain the offset-adjusted stiffness. The slope of the offset-adjusted stiffness for each subject was calculated using linear regression and an unpaired, independent, two-tailed t-test was performed for each task, to determine if there is exists any statistical difference in the strategies both sexes used during each task, to influence ankle stiffness.

In all statistical analyses, the normality of the data and equal variance (homogeneity of variance) across data sets were verified by running Shapiro–Wilk and Levene's tests respectively. If the null hypothesis was rejected in the Levene's tests, equal variance was not assumed in the subsequent statistical analyses. In addition, Mauchly's test of sphericity was used to formally test the assumption of sphericity. If the assumption was violated, the degrees-of-freedom were adjusted using the Greenhouse–Geisser correction before calculating the p-value. All statistical tests were made using the SPSS statistical package at a significance level of $p < 0.05$.

CHAPTER 4

RESULTS

Based on precise ankle torque and kinematic measurements, ankle stiffness was quantified with a high reliability in both the sagittal and frontal planes. Figure 4 illustrates the reliability of the regression used in calculating the ankle stiffness. It shows both the position and torque profiles of a representative male and female subject, during natural stance with equal weight distribution between both legs and in a relaxed state. The %VAF for this condition was 99.7% and 99.6% for the male (Figure 4A) and female (Figure 4B) subject respectively. Appendix B highlights the average %VAF (Goodness) of all 20 male subjects and 20 female subjects, for each of the three different tasks, in both sagittal and frontal planes.

A. B.

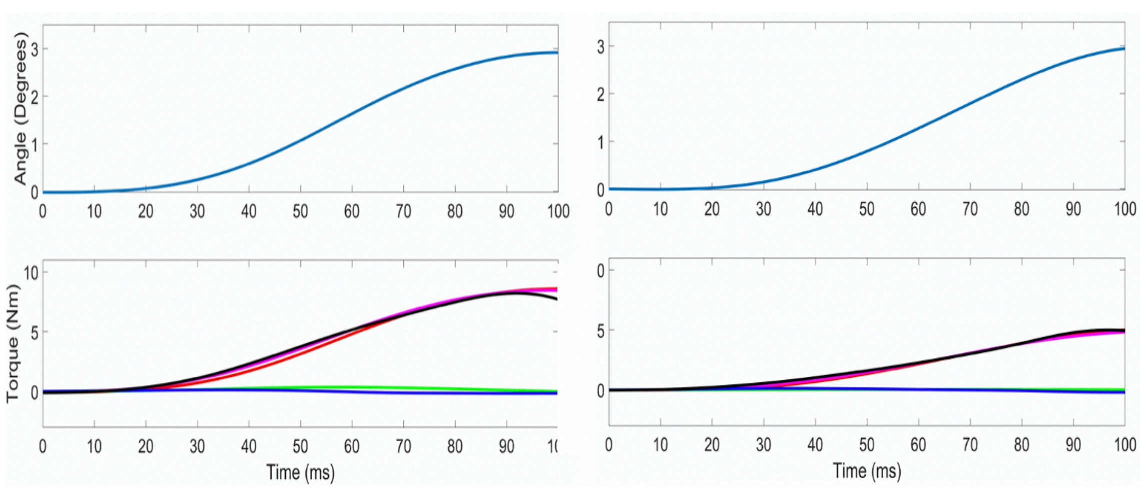

Figure 4: A representative quantification of ankle stiffness obtained by regression. (Top) The position profile of the perturbation. (Bottom) The torque responses. Red, green and blue denote the contribution of stiffness, damping, and inertia. Measured torque (black) matched well with the estimated torque (magenta) by summing the torque contributions of three ankle parameters. A: Male example B: Female example.

Subsequent sections of this chapter highlight the results obtained during each of the three different tasks.

Results for the Muscle Co-contraction Task

Each subject completed all four experimental conditions for the muscle co-contraction task: 0%, 10%, 15% and 20% MVC, in both the sagittal and frontal planes. The figures below illustrate the position profile and the torque response of a representative female and male subject, for the sagittal and frontal planes.

A. B.

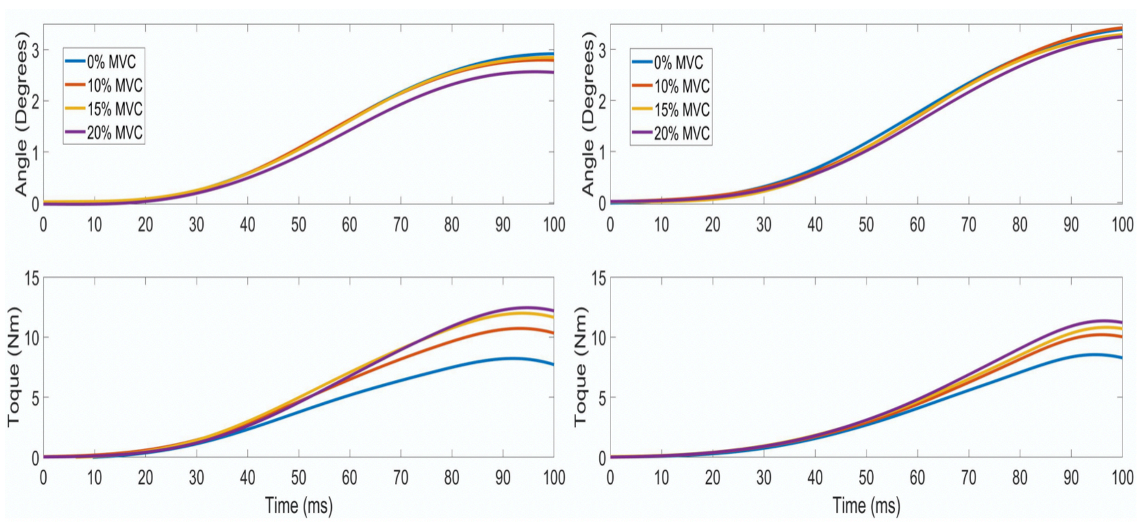

Figure 5: Torque and position measurements in response to the ramp-and-hold perturbations for each experimental condition in the sagittal plane.
The position profile of the foot (Top). The torque responses (Bottom). A: Male example B: Female example

A. B.

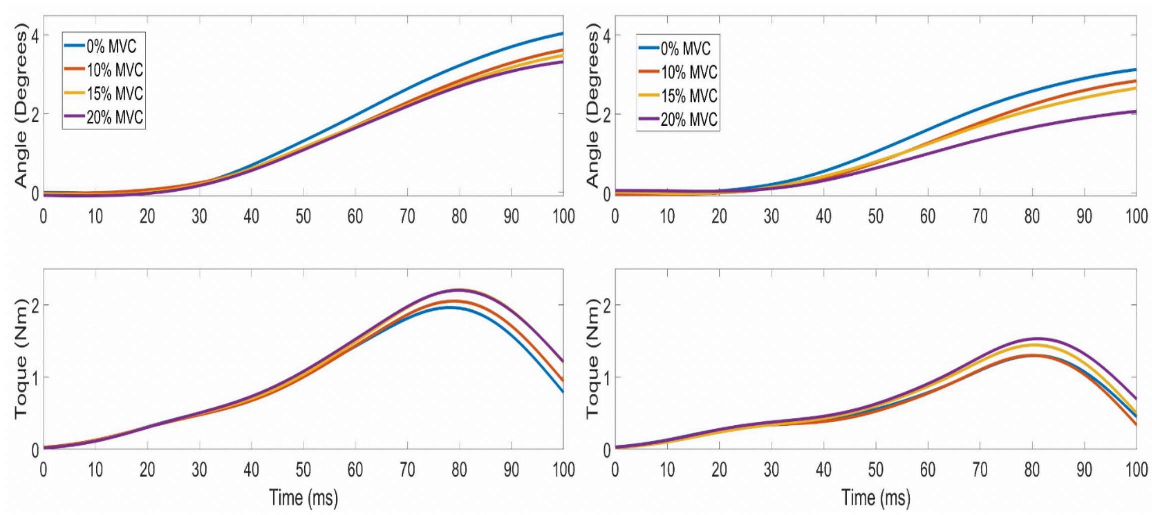

Figure 6: Torque and position measurements in response to the ramp-and-hold perturbation for each experimental condition in frontal plane.
The position profile of the foot (Top). The torque responses (Bottom). A: Male example B: Female example

As evident from Figure 5 and Figure 6, the torque response increased as the experimental conditions increased from 0% to 20% MVC in the two planes. In calculating the ankle stiffness, the results showed a similar trend, with ankle stiffness increasing with increasing co-contraction of ankle muscles in both the sagittal and frontal planes, and in all male and female subjects.

Results from statistical analysis revealed that co-contraction of ankle muscles significantly increased $K_{sagittal}$ and $K_{frontal}$ in both male and female subjects, and there was a significant sex difference in all muscle activation levels, with males having higher stiffness than females (Figure 9).

For $K_{sagittal}$, a significant main effect of the within-subjects factor of muscle activation was identified ($F (1.6, 59.9) = 111.7$, $p < 0.001$). Post-hoc multiple comparisons with the Bonferroni correction confirmed that $K_{sagittal}$ with a higher level of ankle muscle co-contraction was always significantly higher than that in the lower level in both males ($p < 0.001$) and females ($p < 0.01$), refer to Appendix C.

In addition, a significant main effect of the between-subjects factor of sex was identified ($F (1, 38) = 64.3$, $p < 0.001$). Post-hoc tests revealed that sex differences were statistically significant across all muscle co-contraction levels: $\Delta = 61.6$ Nm/rad ($p < 0.001$), $\Delta = 74.7$ Nm/rad ($p < 0.001$), $\Delta = 84.2$ Nm/rad ($p < 0.001$), and $\Delta = 87.9$ Nm/rad ($p < 0.001$) for 0, 10, 15 and 20 %MVCC, respectively, with females having lower stiffness than males (Figure 9A). While a trend was observed that the sex difference in $K_{sagittal}$ increased with increasing ankle muscle co-contraction (Figure 7), interaction between ankle muscle activation and sex did not reach the statistical significance ($F (1.6, 59.9) = 2.6$, $p = 0.10$).

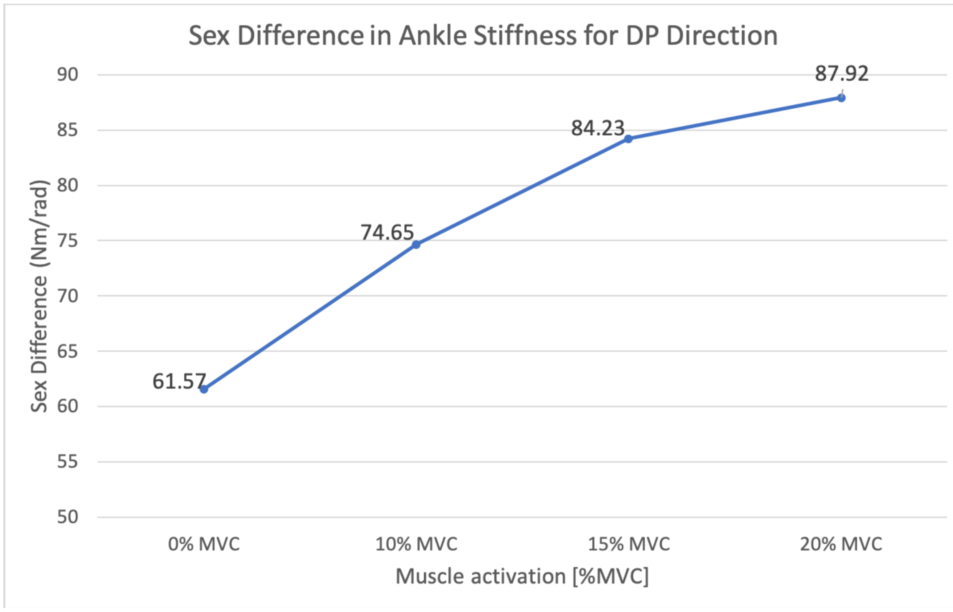

Figure 7: Trend of sex differences in ankle stiffness during the muscle co-contraction task in the sagittal plane.

For $K_{frontal}$, a significant main effect of muscle co-contraction was identified ($F(2.0, 73.9) = 33.9$, $p < 0.001$). Post-hoc multiple comparisons with the Bonferroni correction confirmed that $K_{frontal}$ with a higher level of ankle muscle co-contraction was always significantly higher than that in the lower levels in both males and females ($p < 0.05$), except the comparison condition of 10% vs. 15% MVCC ($p = 0.42$) for males (Appendix C).

A significant main effect of sex was also identified ($F(1, 38) = 8.0$, $p < 0.05$). Post-hoc tests revealed that sex differences were statistically significant across all muscle co-contraction levels except 20% MVCC: $\Delta = 9.5$ Nm/rad ($p < 0.01$), $\Delta = 9.2$ Nm/rad ($p < 0.01$), $\Delta = 7.1$ Nm/rad ($p < 0.05$) and $\Delta = 7.6$ Nm/rad ($p = 0.10$) for 0, 10, 15 and 20 %MVCC, respectively) with females having lower stiffness than males (Figure 9B).

There was no significant interaction between ankle muscle activation and sex (F (2.0, 73.9) = 0.31, p = 0.73), Figure 8.

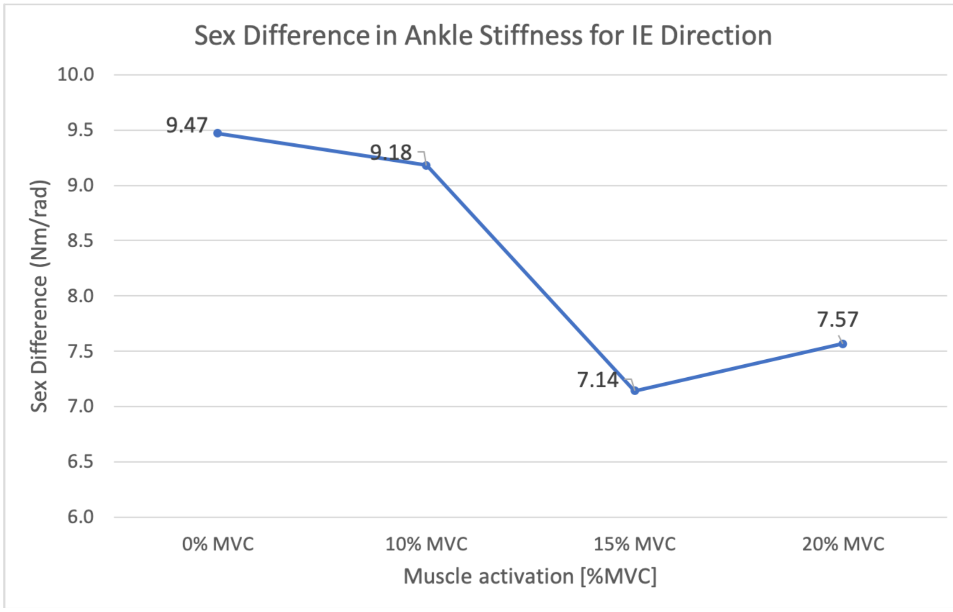

Figure 8: Trend of sex differences in ankle stiffness during the muscle co-contraction task in the frontal plane

While the sex difference in ankle stiffness was observed in both planes of ankle motion in all muscle activation levels, the difference was significantly greater in the sagittal plane (Δ = 77.1 Nm/rad ($p < 0.001$)) than the frontal plane (Δ = 8.3 Nm/rad ($p < 0.001$)) (Figure 9C). This was evidenced by significant interaction between the within-subjects factor of plane of ankle motion and the between-subjects factor of sex (F (1, 158) = 93.1, $p < 0.001$).

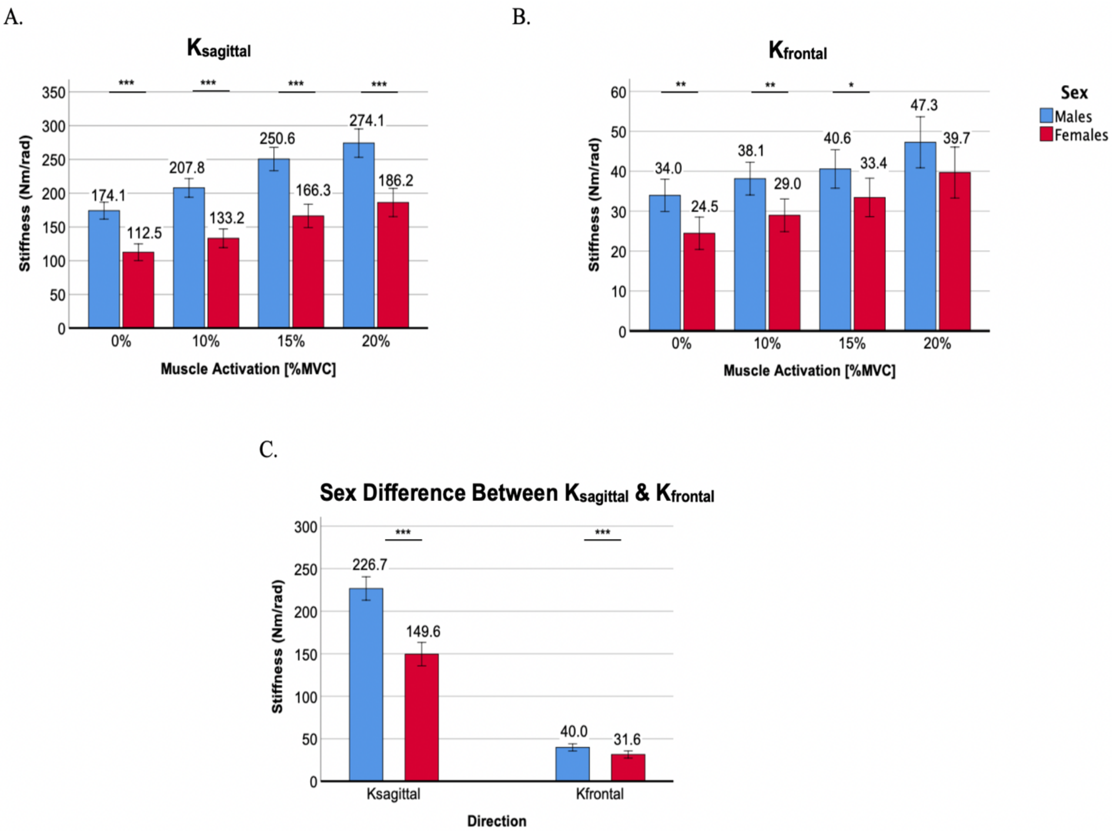

Figure 9: Ankle stiffness in both the sagittal and frontal plane for Muscle Activation task. A) $K_{sagittal}$ B) $K_{frontal}$. C compares ankle stiffness in both planes

Results for the Weight task

Each subject completed all four experimental conditions for the weight task: 30%, 50%, 70% and 90% total body weight, in the sagittal and frontal planes. Figure 10 and Figure 11 illustrate the position profile and the torque response of a representative male and female subject, for the sagittal and frontal planes respectively.

A.   B.

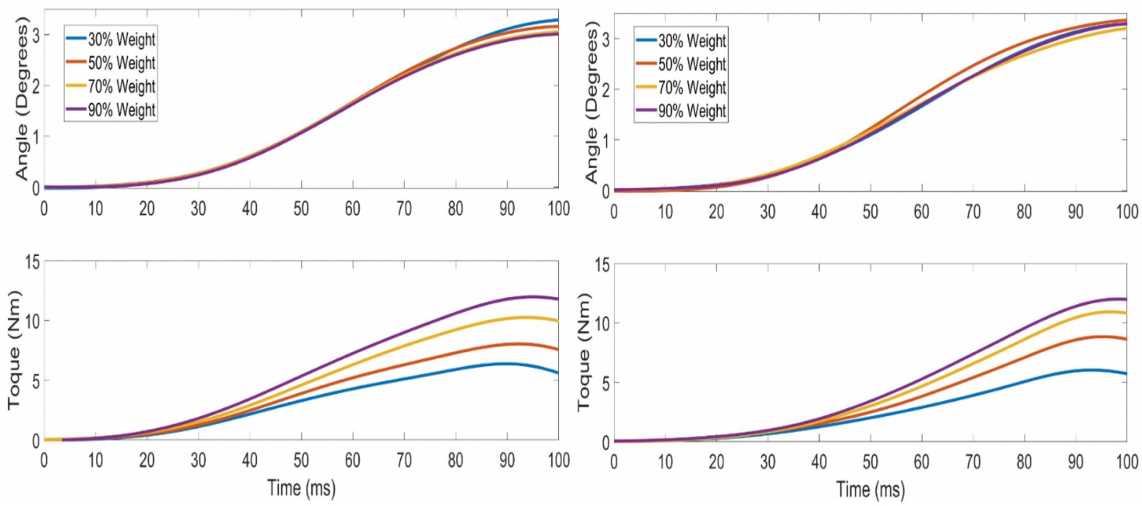

Figure 10: Torque and position measurements in response to the ramp-and-hold perturbation for each experimental condition in the sagittal plane.
The position profile of the foot (Top). The torque responses (Bottom). A: Male example
B: Female example

A.   B.

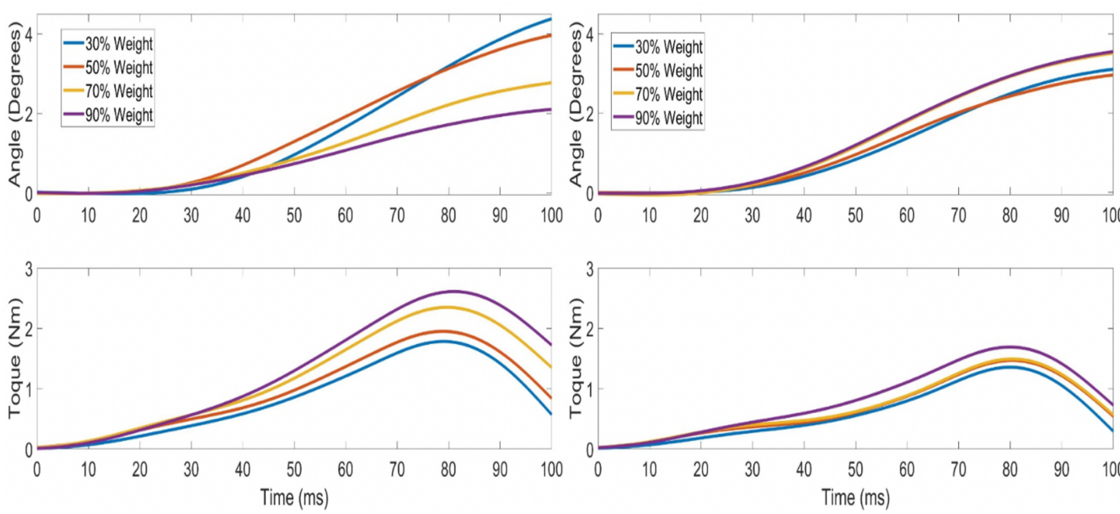

Figure 11: Torque and position measurements in response to the ramp-and-hold perturbation for each experimental condition in the frontal plane.
The position profile of the foot (Top). The torque responses (Bottom). A: Male example
B: Female example

As evident from Figure 10 and Figure 11, the torque response increased as the experimental conditions increased from 30% to 90% total body weight for both sagittal and frontal planes. In calculating the ankle stiffness, the results showed a similar trend, with ankle stiffness increasing with increasing weight on the robotic platform in both sagittal and frontal planes, for all male and female subjects.

Furthermore, results from statistical analysis revealed that weight-bearing at the ankle significantly increased $K_{sagittal}$ and $K_{frontal}$ in both male and female subjects, and there was a significant sex difference in all weight levels, with males having higher stiffness than females (Figure 14).

For $K_{sagittal}$, a significant main effect of the within-subjects factor of weight was identified ($F(1.6, 61.4) = 215.7$, $p < 0.001$). Post-hoc multiple comparisons with the Bonferroni correction confirmed that $K_{sagittal}$ with a higher level of weight-bearing was always significantly higher than that in the lower levels in both males ($p < 0.001$) and females ($p < 0.001$).

In addition, a significant main effect of the between-subjects factor of sex was identified ($F(1, 38) = 52.8$, $p < 0.001$). Post-hoc tests further revealed that sex differences were statistically significant across all weight levels in the sagittal plane: Δ = 41.3 Nm/rad ($p < 0.001$), Δ = 59.0 Nm/rad ($p < 0.001$), Δ = 76.8 Nm/rad ($p < 0.001$) and Δ = 81.3 Nm/rad ($p < 0.001$) for 30, 50, 70, and 90% of the total body weight, respectively) with females having lower stiffness than males (Figure 14A). There was a significant interaction between weight-bearing and sex ($F(1.6, 61.4) = 6.8$, $p < 0.05$) which supported the increasing trend in sex difference in ankle stiffness as weight conditions increased from 30% to 90% total body weight (Figure 12).

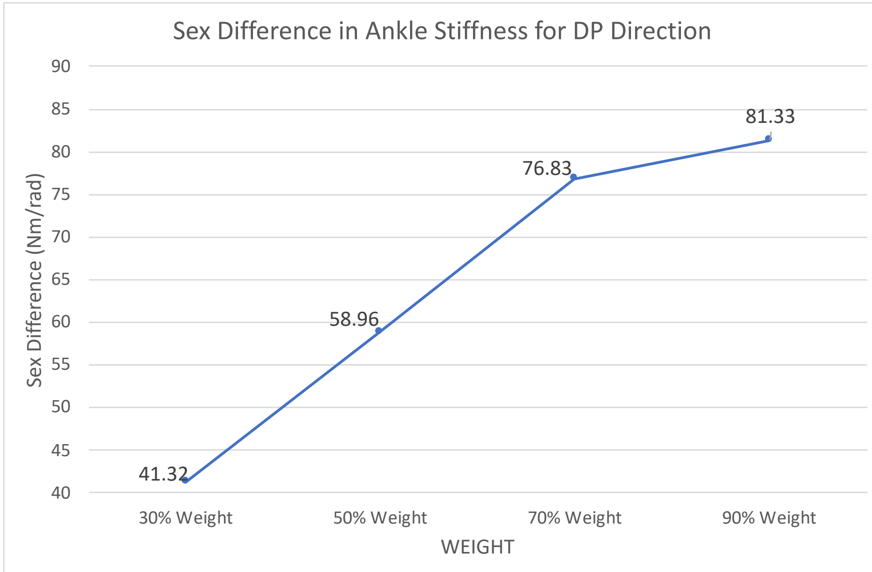

Figure 12: Trend of sex difference in ankle stiffness during weight-bearing conditions in the sagittal plane

For $K_{frontal}$, while a significant main effect of weight was identified ($F(1.7, 65.2) = 21.9$, $p < 0.001$), this effect was mainly due to the significant effect in males. Post-hoc multiple comparisons with the Bonferroni correction confirmed that $K_{frontal}$ with a higher level of weight-bearing was always significantly higher than that in the lower levels in males ($p = 0.02$ for 30% vs. 50% and $p < 0.001$ otherwise). However, no statistical difference was identified in any multiple comparisons ($p = 1.00$) in females. See Appendix C.

In addition, a significant main effect of sex was identified ($F(1,38) = 12.6$, $p < 0.01$). Post-hoc tests further revealed that sex differences were statistically significant across all weight levels except 30% body weight: $\Delta = 5.0$ Nm/rad ($p = 0.10$), $\Delta = 8.4$ Nm/rad ($p < 0.01$), $\Delta = 15.0$ Nm/rad ($p < 0.01$) and $\Delta = 25.1$ Nm/rad ($p < 0.01$) for 30, 50, 70, and 90 % of total body weight, respectively, with females having lower stiffness than males (Figure 14B). There was a statistically significant interaction between weight-bearing and sex ($F(1.7, 65.2) = 9.8$, $p < 0.001$) which supported the increasing trend in sex difference in ankle stiffness, as weight conditions increased from 30% to 90% total body weight (Figure 13**Error! Reference source not found.**).

While the sex difference in ankle stiffness was observed in both planes of ankle motion in all weight levels, the difference was significantly greater in the sagittal plane ($\Delta = 64.6$ Nm/rad ($p < 0.001$)) than in the frontal plane ($\Delta = 13.4$ Nm/rad ($p < 0.001$)) (Figure 14C). This was evidenced by significant interaction between the within-subjects factor of plane of ankle motion and the between-subjects factor of sex ($F(1, 158) = 40.1$, $p < 0.001$).

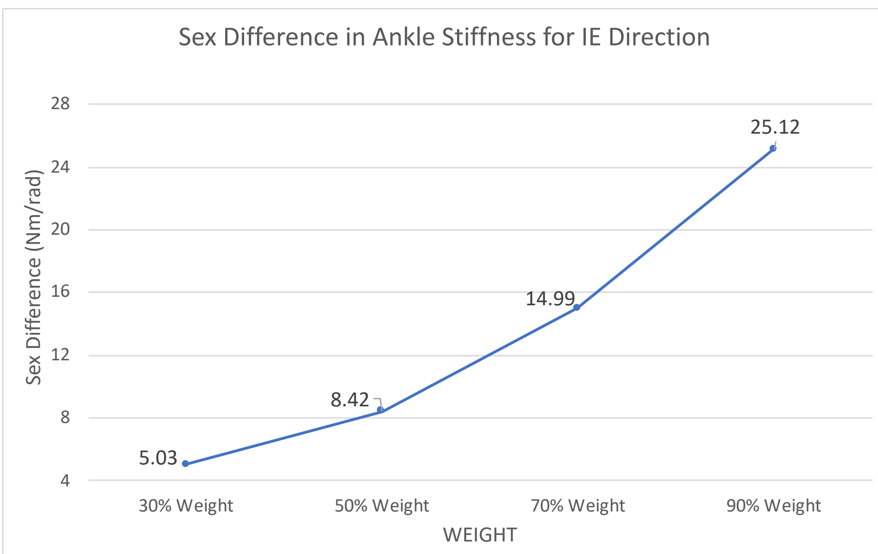

Figure 13: Trend of sex difference in ankle stiffness during weight-bearing conditions in the frontal plane.

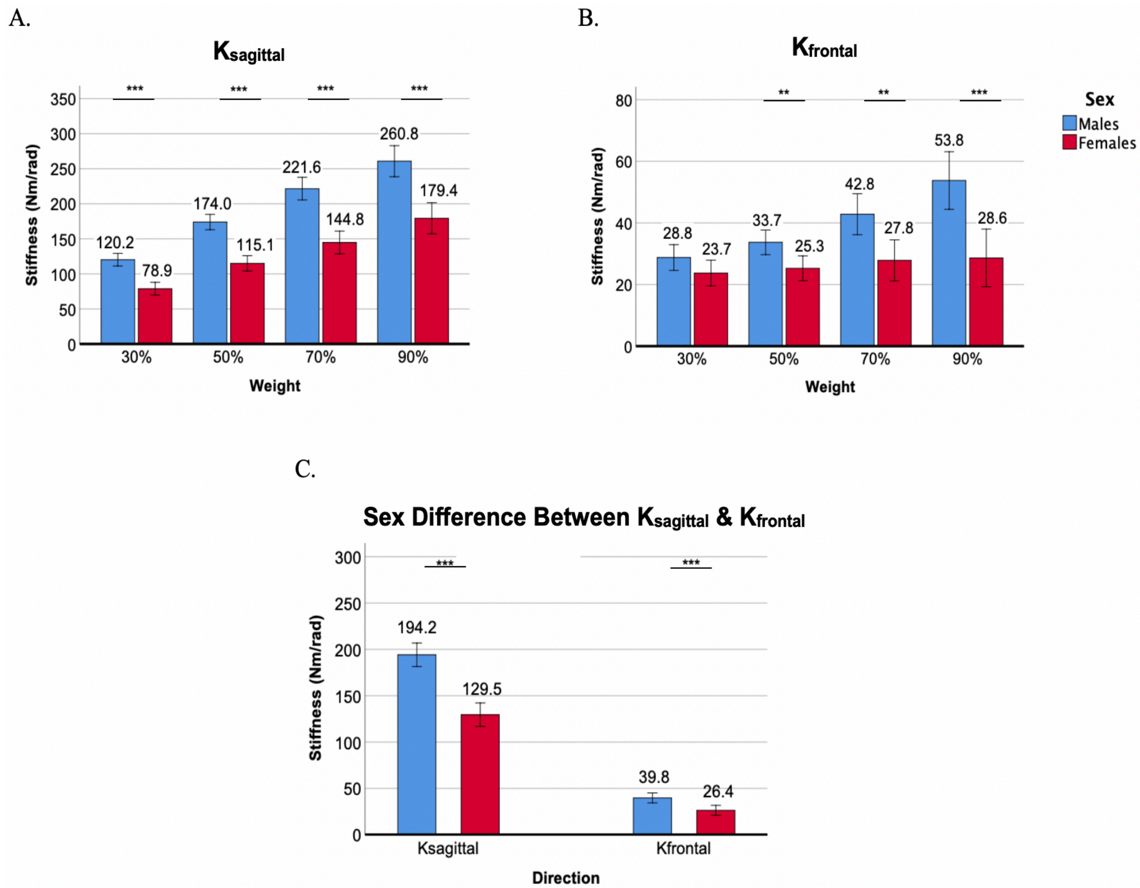

Figure 14: Ankle stiffness in both the sagittal and frontal plane for Weight-Bearing task level. A) $K_{sagittal}$ B) $K_{frontal}$. C compares ankle stiffness in both planes.

Results for the Ankle Torque Generation Task

Each subject completed all five experimental conditions for the ankle torque generation task: -2cm, 0cm, +2cm, +4cm and +6cm offsets to the neutral CoP for the sagittal plane and -1.5cm, -0.75cm, 0cm, +0.75cm, and +1.5cm offsets to the neutral CoP for the frontal plane. Figure 15 and Figure 16 illustrate the position profile and the torque response of a representative female and male subject, in both the sagittal and frontal planes respectively.

A. B.

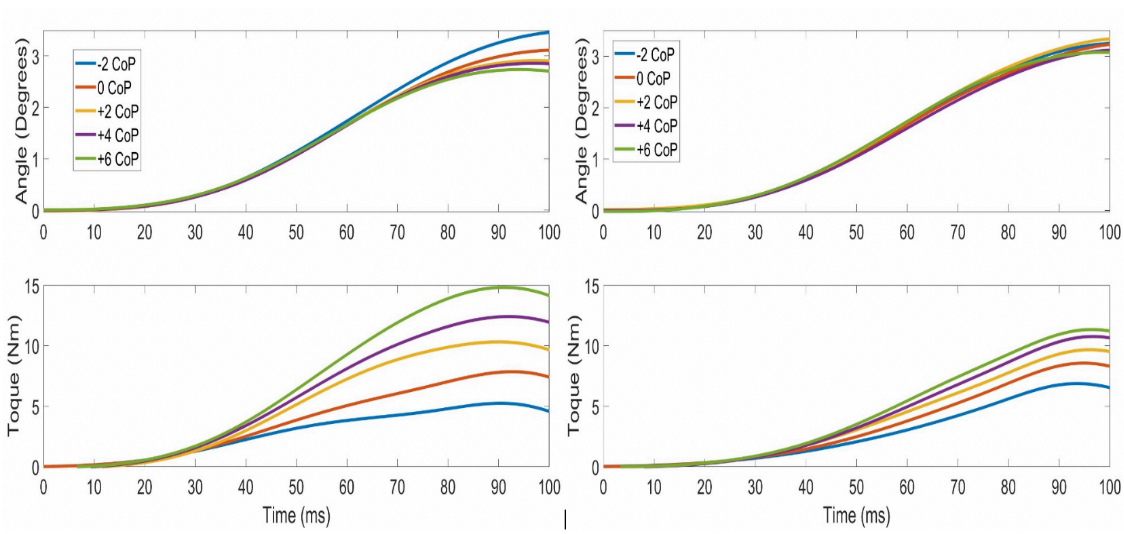

Figure 15: Torque and position measurements in response to the ramp-and-hold perturbation for each experimental condition in the sagittal plane.
The position profile of the foot (Top). The torque responses (Bottom). A: Male example
B: Female example

A. B.

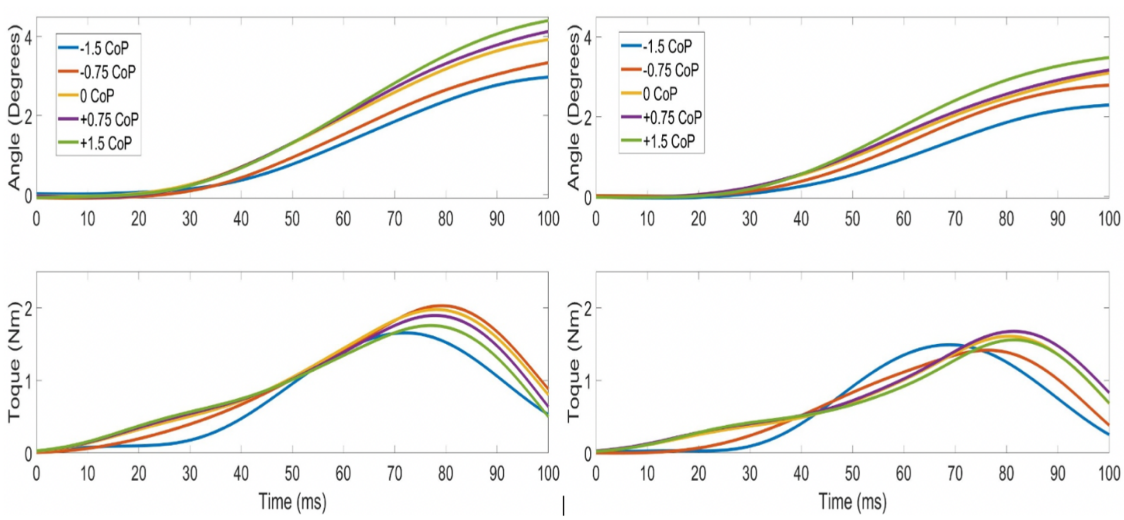

Figure 16: Torque and position measurements in response to the ramp-and-hold perturbation for each experimental condition in the frontal plane.
The position profile of the foot (Top). The torque responses (Bottom). A: Male example
B: Female example

As evident from Figure 15, the torque response increased as the experimental conditions increased from -2 cm to +6 cm in the sagittal plane while in the frontal plane, the position profile increased as the experimental conditions increased (Figure 16), from -1.5cm to +1.5cm. In calculating the ankle stiffness, the results showed a similar trend for the sagittal plane, with ankle stiffness increasing with increasing experimental conditions from -2 cm to +6 cm, in all subjects. In the frontal plane, however, the ankle stiffness decreased with increasing experimental conditions, from -1.5cm to +1.5cm (Figure 19).

Furthermore, results from statistical analysis revealed that significant sex differences in $K_{sagittal}$ and $K_{frontal}$ were observed in all ankle torque generation (CoP displacement) conditions, with stiffness in males being greater than females.

For $K_{sagittal}$, a significant main effect of the within-subjects factor of ankle torque was identified ($F(2.2, 81.8) = 384.5$, $p < 0.001$). Post-hoc multiple comparisons with the Bonferroni correction confirmed that $K_{sagittal}$ with a higher level of ankle torque (note that ankle torque at the neutral CoP, i.e., CoP = 0 cm, was higher than that at CoP = -2 cm) was always significantly higher than that in the lower ankle torque levels in both males ($p < 0.001$) and females ($p < 0.01$).

In addition, a significant main effect of the between-subjects factor of sex was identified ($F(1, 38) = 43.1$, $p < 0.001$). Post-hoc tests further revealed that sex differences were statistically significant across all torque conditions: $\Delta = 25.6$ Nm/rad ($p < 0.01$), $\Delta = 58.6$ Nm/rad ($p < 0.001$), $\Delta = 62.5$ Nm/rad ($p < 0.001$), $\Delta = 78.0$ Nm/rad ($p < 0.001$), and $\Delta = 91.8$ Nm/rad ($p < 0.001$) for -2, 0, +2, +4, and +6cm offsets to the neutral CoP, respectively, with females having lower stiffness than males (Figure 19*A*). There was a significant interaction between ankle torque generation in the sagittal plane and sex

(F (2.2, 81.8) = 14.7, p < 0.05) which supported the increasing trend in sex difference in ankle stiffness, as the experimental conditions increased from -2cm to +6cm. (Figure 17).

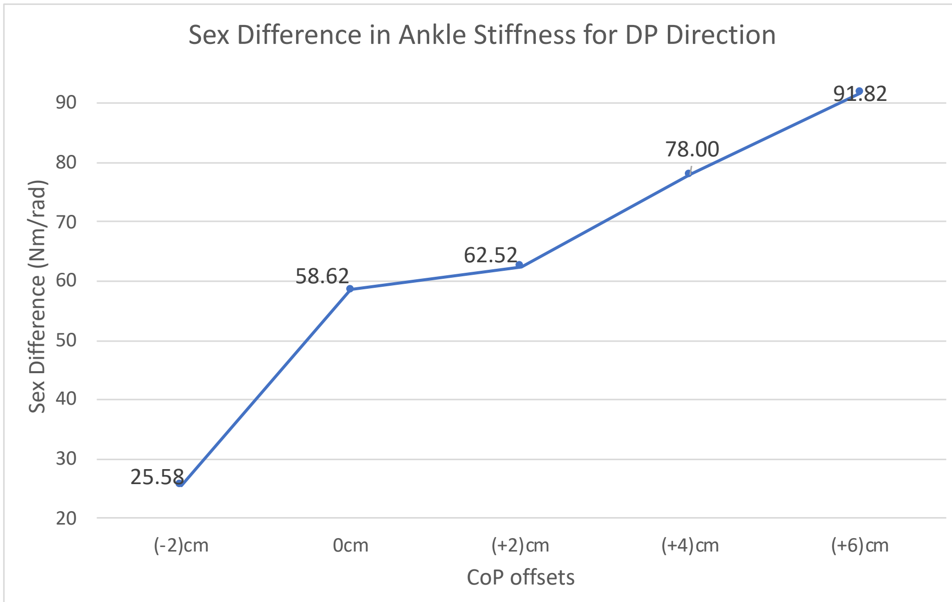

Figure 17: Trend of sex difference in ankle stiffness during the ankle torque generation task in the sagittal plane.

For $K_{frontal}$, while a significant main effect of ankle torque was identified (F (2.4, 91.9) = 34.3, p < 0.001), this effect was mainly due to the significant difference in eversion torque generation conditions. Post-hoc multiple comparisons with the Bonferroni correction confirmed that $K_{frontal}$ in males was significantly different among -1.5, -0.75, and 0 conditions (p < 0.05), but not different among 0, +0.75, and +1.5 conditions (p = 1.0). The same trend was observed in females, but the difference was smaller than males. There was no statistical difference among 0, +0.75, and +1.5 conditions (p = 1.0). Further even in the eversion torque generation conditions, two comparison conditions (-1.5 vs. -0.75 ($p = 0.50$) and -0.75 vs. 0 ($p = 0.09$)) did not reach the statistical significance. See Appendix C.

A significant main effect of the between-subjects factor of sex was identified ($F (1, 38) = 13.9$, $p < 0.001$). Post-hoc tests further revealed that sex differences were statistically significant across all torque conditions: $\Delta = 14.9$ Nm/rad ($p < 0.01$), $\Delta = 11.6$ Nm/rad ($p < 0.01$), $\Delta = 8.0$ Nm/rad ($p < 0.01$), $\Delta = 8.1$ Nm/rad ($p < 0.01$) and $\Delta = 8.1$ Nm/rad ($p < 0.05$) for –1.5, -0.75, 0, +0.75, and +1.5cm offsets to the neutral CoP, respectively, with females having lower stiffness than males (Figure 19B). Unlike $K_{sagittal}$, interaction between ankle torque generation in the frontal plane and sex was not significant ($F (2.4, 91.9) = 1.9$, $p = 0.15$), see Figure 18.

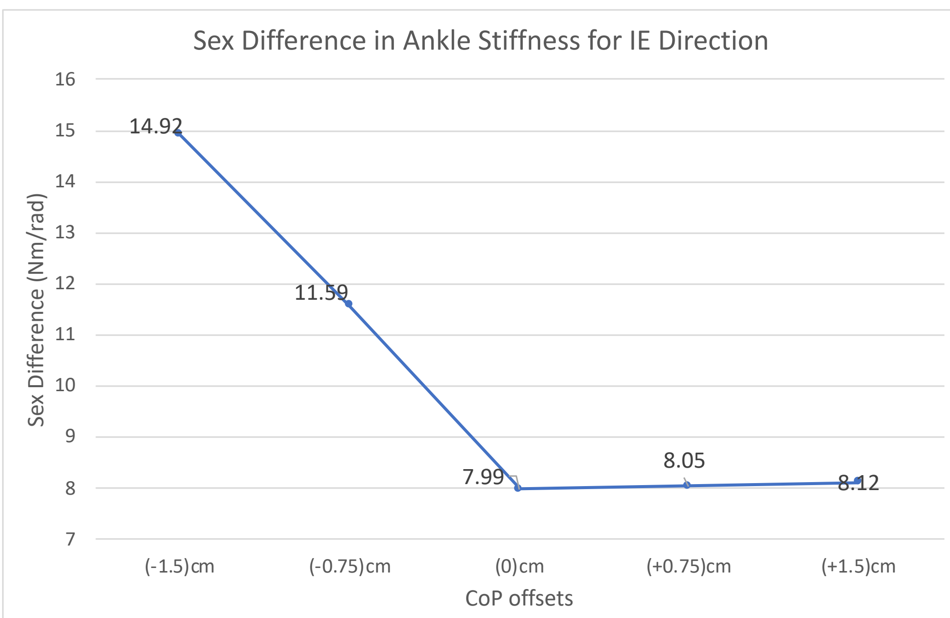

Figure 18: Trend of sex differences in ankle stiffness during the ankle torque generation task in the frontal plane.

While the sex difference in ankle stiffness was observed in both planes of ankle motion in all ankle generation levels, the difference was significantly greater in the sagittal plane (Δ = 63.3 Nm/rad (p < 0.001)) than the frontal plane (Δ = 10.1 Nm/rad (p < 0.001)). This was evidenced by significant interaction between the within-subjects factor of plane of ankle motion and the between-subjects factor of sex (F (1, 198) = 26.5, p < 0.001) (Figure 19*C*).

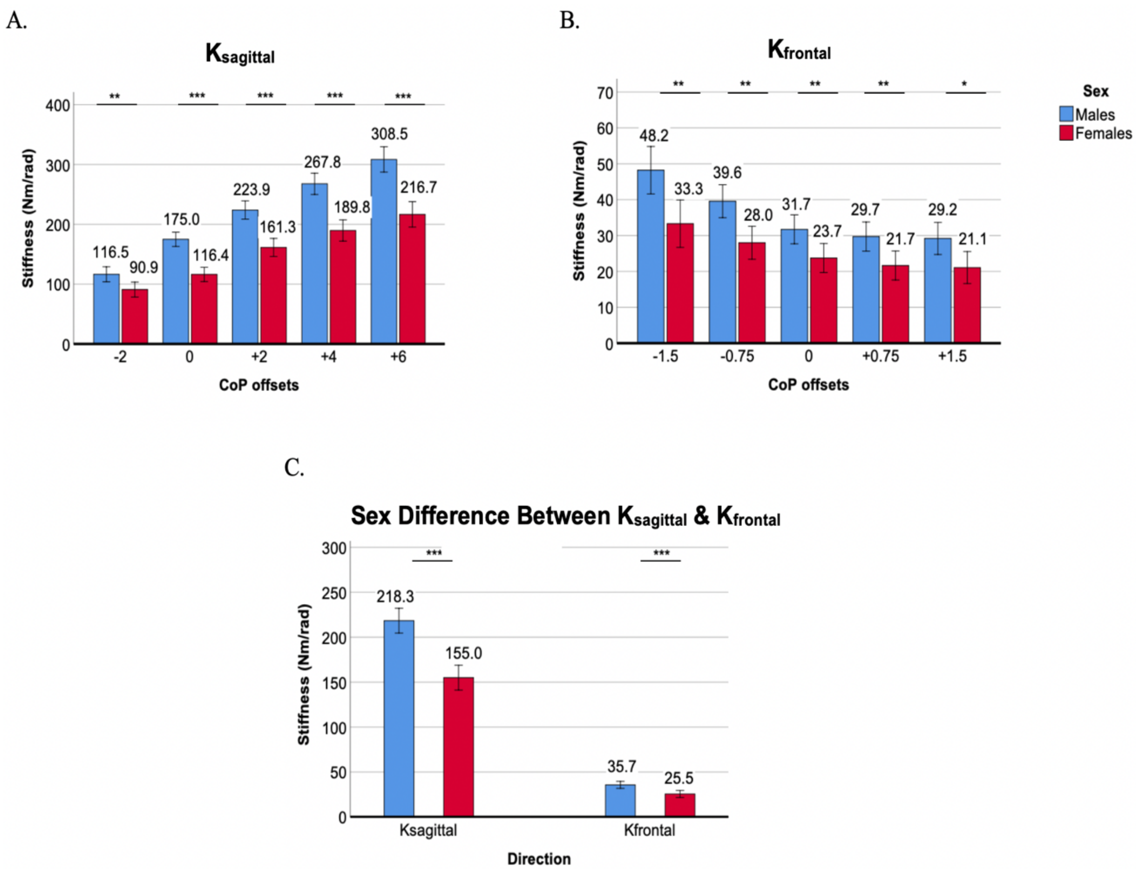

Figure 19: Ankle stiffness in both the sagittal and frontal plane for ankle torque generation task level. A) $K_{sagittal}$ B) $K_{frontal}$. C compares ankle stiffness in both planes.

Sex Differences in Normalized 2D Ankle Stiffness

Muscle Co-contraction Task

Co-contraction of ankle muscles increased normalized ankle stiffness in both male and female subjects, but there was a significant sex difference only in the sagittal plane (Figure 20). For $K_{normalized\_sagittal}$, a significant main effect of the between-subjects factor of sex was identified ($F(1, 38) = 11.4$, $p < 0.01$). Post-hoc t-tests revealed that sex differences were statistically significant across all muscle co-contraction conditions: Δ = 0.25 Nm/rad.kg.m ($p < 0.01$), Δ = 0.32 Nm/rad.kg.m ($p < 0.01$), Δ = 0.35 Nm/rad.kg.m ($p < 0.01$), and Δ = 0.34 Nm/rad.kg.m ($p < 0.05$) for 0, 10, 15 and 20 %MVCC, respectively, with females having lower normalized stiffness than males (Figure 20A).

For $K_{normalized\_frontal}$, there was no significant main effect of the between-subjects factor of sex ($F(1, 38) = 0.2$, $p = 0.64$). In addition, post-hoc t-tests revealed no statistical sex differences across all muscle co-contraction conditions: Δ = 0.03 Nm/rad.kg.m ($p = 0.259$), Δ = 0.025 Nm/rad.kg.m ($p = 0.363$), Δ = 0.002 Nm/rad.kg.m ($p = 0.950$), and Δ = -0.004 Nm/rad.kg.m ($p = 0.938$) for 0, 10, 15 and 20 %MVCC respectively (Figure 20B).

Sex difference observed in the sagittal plane was significantly greater than in the frontal plane, evidenced by significant interaction between the within-subjects factor of plane of ankle motion and the between-subjects factor of sex ($F(1, 158) = 19.5$, $p < 0.001$) (Figure 20 C).

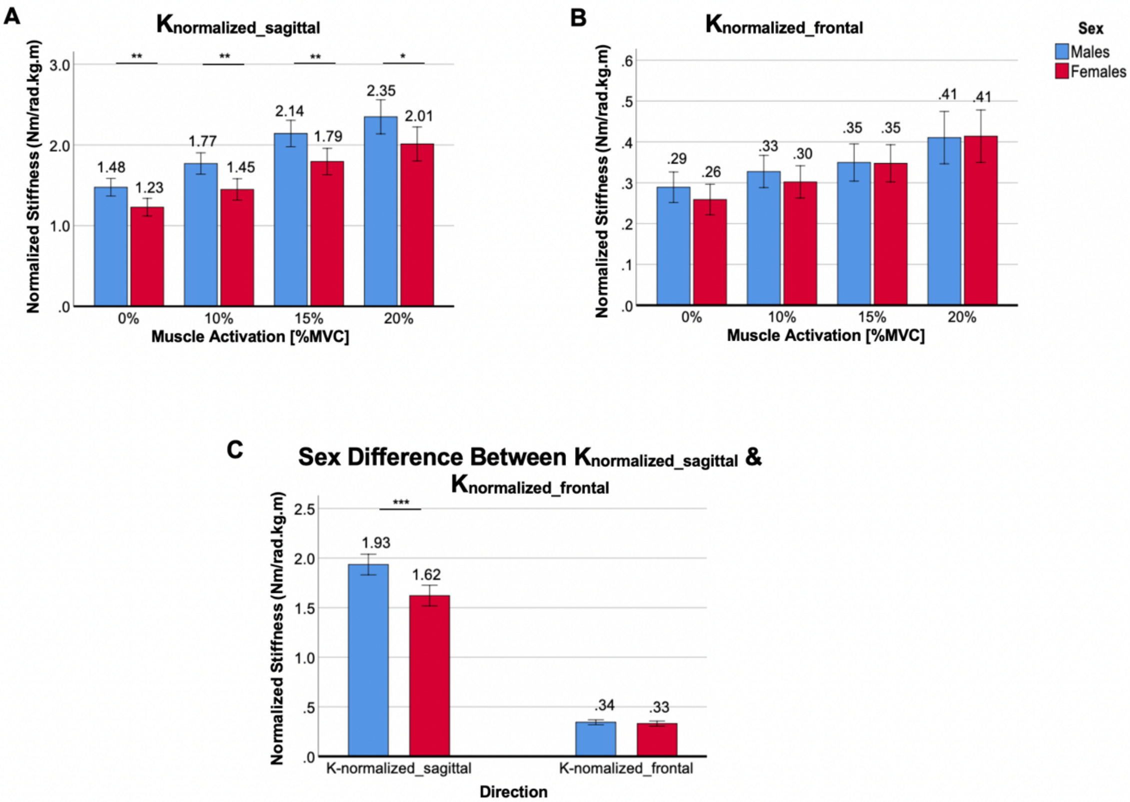

Figure 20: Normalized ankle stiffness in both the sagittal and frontal plane for muscle co-contraction task level. A) $K_{normalized\_sagittal}$ B) $K_{normalized\_frontal}$. C compares normalized ankle stiffness in both planes.

Weight-Bearing Task

Increasing weight-bearing at the ankle joint increased $K_{normalized\_sagittal}$ in both male and female subjects, but there was a significant sex difference in all weight-bearing conditions. A significant main effect of the between-subjects factor of sex was identified ($F (1, 38) = 10.5$, $p < 0.01$), and post-hoc tests revealed that sex differences were statistically significant across all weight-bearing conditions: $\Delta = 0.17$ Nm/rad.kg.m ($p < 0.05$), $\Delta = 0.227$ Nm/rad.kg.m ($p < 0.01$), $\Delta = 0.288$ Nm/rad.kg.m ($p < 0.01$), and $\Delta = 0.243$ Nm/rad.kg.m ($p < 0.05$) for 30%, 50% 70% and 90% weight, respectively, with females having lower normalized stiffness than males (Figure 21A).

For $K_{normalized\_frontal}$, there was no significant main effect of the between-subjects factor of sex ($F(1, 38) = 2.9$, $p = 0.10$). In addition, post-hoc t-tests revealed that no statistically significant sex differences were identified across all weight-bearing levels with the exception of 90% weight: $\Delta = -0.010$ Nm/rad.kg.m ($p = 0.681$), $\Delta = 0.019$ Nm/rad.kg.m ($p = 0.464$), $\Delta = 0.069$ Nm/rad.kg.m ($p = 0.121$), and $\Delta = 0.151$ Nm/rad.kg.m ($p = 0.013$) for 30%, 50% 70% and 90% weight, respectively (Figure 21B).

Sex difference observed in the sagittal plane was significantly greater than in the frontal plane, evidenced by the significant interaction between the plane of ankle motion and sex ($F(1, 158) = 5.6$, $p < 0.05$) (Figure 21C).

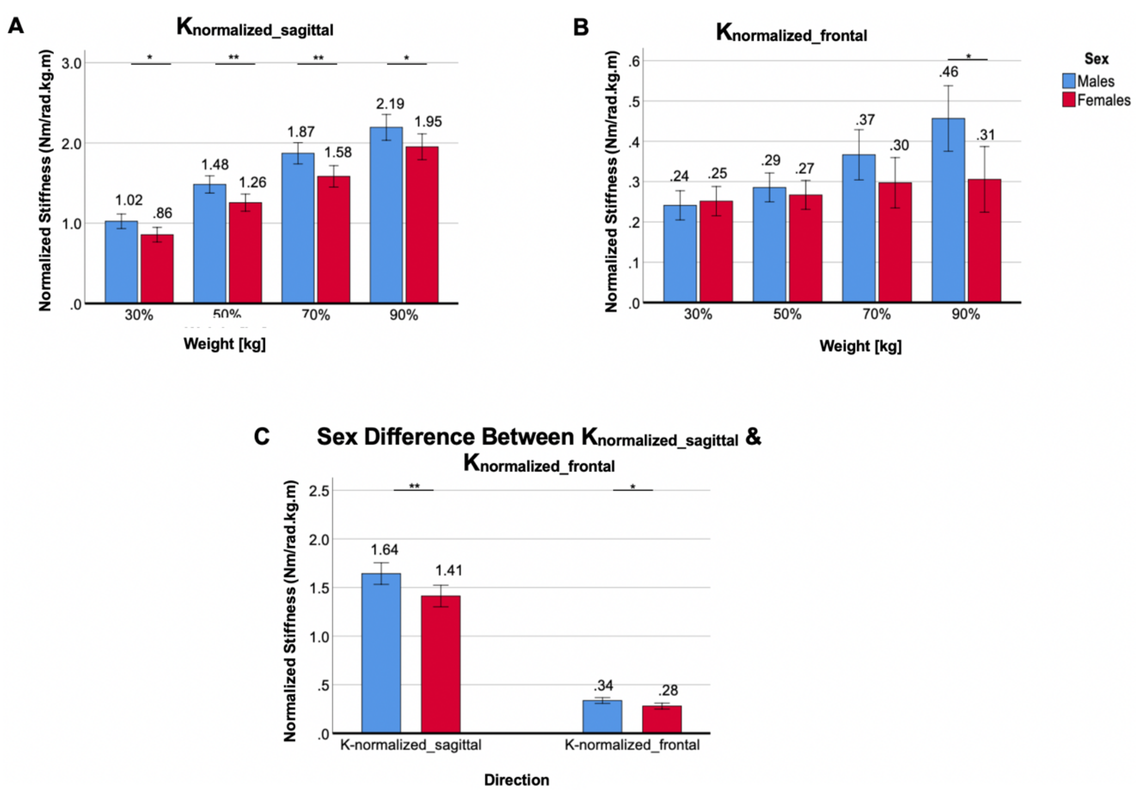

Figure 21: Normalized ankle stiffness in both the sagittal and frontal plane for weight-bearing task level. A) $K_{normalized\_sagittal}$ B) $K_{normalized\_frontal}$. C compares normalized ankle stiffness in both planes.

Ankle Torque Generation Task

For the ankle torque generation tasks, similar trends were observed in normalized ankle stiffness when compared to absolute ankle stiffness. For $K_{normalized\_sagittal}$, a significant main effect of the between-subjects factor of sex was identified ($F(1, 38) = 5.1$, $p < 0.05$). Post-hoc tests revealed significant sex differences for only two conditions, namely 0 and +6 CoP offsets, and one marginal condition for +4 CoP with females having lower normalized stiffness than males: Δ = 0.004 Nm/rad.kg.m ($p = 0.958$), Δ = 0.225 Nm/rad.kg.m ($p = 0.003$), Δ = 0.15 Nm/rad.kg.m ($p = 0.095$), Δ = 0.209 Nm/rad.kg.m ($p = 0.052$) and Δ = 0.254 Nm/rad.kg.m ($p = 0.034$) for -2, 0, +2, +4, and +6cm offsets to the neutral CoP respectively (Figure 22A).

For $K_{normalized\_frontal}$, there was no significant main effect of the between-subjects factor of sex ($F(1, 38) = 1.5$, $p = 0.22$). Post-hoc tests revealed no statistically significant sex difference across all ankle torque generation conditions: Δ = 0.058 Nm/rad.kg.m ($p = 0.214$), Δ = 0.046 Nm/rad.kg.m ($p = 0.150$), Δ = 0.017 Nm/rad.kg.m ($p = 0.541$), Δ = 0.021 Nm/rad.kg.m ($p = 0.444$) and Δ = 0.022 Nm/rad.kg.m ($p = 0.455$) for -1.5, -0.75, 0, +0.75, and +1.5cm offsets to the neutral CoP respectively (Figure 22B).

Although sex differences in the sagittal plane was greater than in the frontal plane, there was no significant interaction between the plane of ankle motion and sex ($F(1, 198) = 2.05$, $p = 0.15$) (Figure 22C).

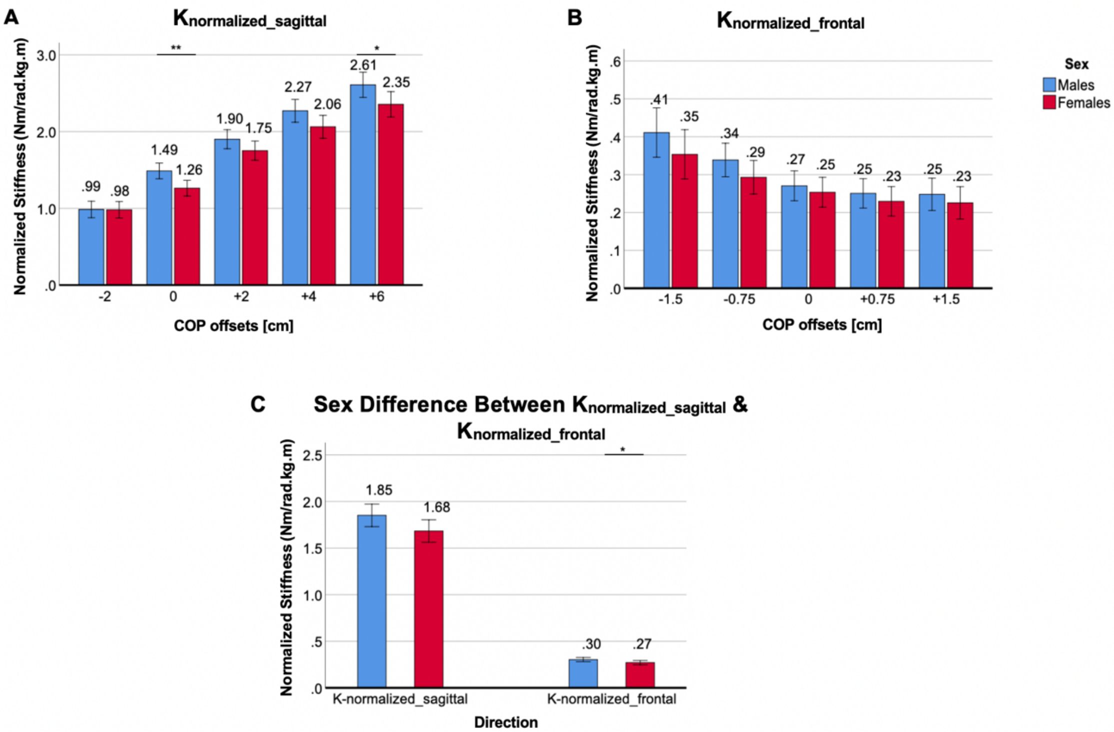

Figure 22: Normalized ankle stiffness in both the sagittal and frontal plane for ankle torque generation task level. A) $K_{normalized\_sagittal}$ B) $K_{normalized\_frontal}$. C compares normalized ankle stiffness in both planes.

Sex Differences in the Slope of the Offset-Adjusted Stiffness

In the sagittal plane, post-hoc tests revealed that sex differences were statistically significant in both weight-bearing and ankle torque generation tasks: $\Delta = 1.72$ Nm/rad/%MVC ($p = 0.088$), $\Delta = 0.728$ Nm/rad/%weight ($p < 0.01$), and $\Delta = 7.94$ Nm/rad/cm ($p < 0.01$), for muscle co-contraction, weight-bearing and ankle torque generation task respectively, with females having lower slope as compared to males (Figure 23A, B and C).

In the frontal plane, post-hoc tests revealed that sex differences were statistically significant in only the weight-bearing tasks: $\Delta = -0.029$ Nm/rad/%MVC ($p = 0.918$), $\Delta = 0.351$ Nm/rad/%weight ($p < 0.01$), and $\Delta = -2.258$ Nm/rad/cm ($p = 0.163$) for muscle co-contraction, weight-bearing and ankle torque generation task respectively, with males having lower slope as compared to females in all but the weight-bearing task (Figure 23D, E and F).

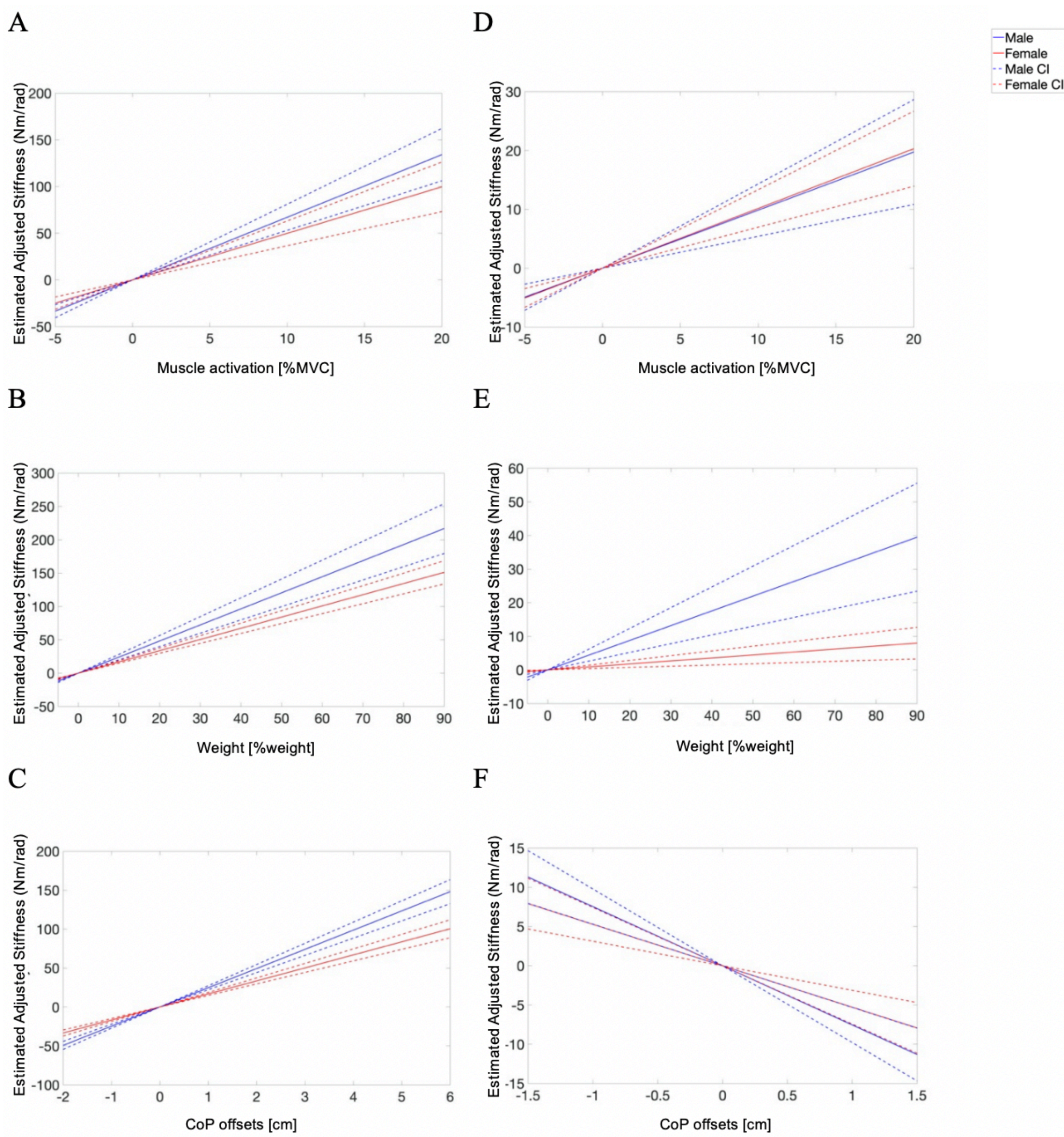

Figure 23: The fit of the mean adjusted stiffness along with the lower and upper bound confidence intervals for both males and females, for all the three different tasks in both the sagittal and frontal plane. A and D = fit for muscle activation task in the sagittal and frontal plane respectively. B and E = fit for weight-bearing task in the sagittal and frontal plane respectively. C and F = fit for ankle torque generation task in the sagittal and frontal plane respectively

CHAPTER 5

DISCUSSION

Previous studies have demonstrated that the incidence of musculoskeletal injuries in females is significantly higher than in males participating in similar sports activities [8], [62], [63]. Among the risk factors contributing to this higher incidence of musculoskeletal injuries in females, the neuromuscular control of stability has been identified as one of the potential factors contributing to the sex difference in risk of injury [25]–[27]. With ankle stiffness recognized as a measure of neuromuscular control [33], [38], [64], this study investigated the sex differences in 2D ankle stiffness during upright standing, to shed more light on the understanding of the sex differences in risk of ankle injury.

Extending on a previous study [41], sex differences in 2D ankle stiffness was investigated during different levels of ankle muscle co-contraction tasks, weight-bearing tasks and ankle torque generation tasks. The following sections discuss the results obtained for each task.

Muscle Activation Task

The sex differences in 2D ankle stiffness was studied during different levels of muscle co-contractions. The results showed that as the level of muscle co-contraction increased from 0% to 20% MVC, ankle stiffness significantly increased for both males and females, in both sagittal and frontal planes. This increase in stiffness is consistent with the previous study in seated conditions [41], and other findings [52], [65] and can be attributed to passive and active muscle stiffness.

Passive stiffness is mainly derived from the parallel elastic components of the muscle structure surrounding the joint [45], [66]. Since in the relaxed state, there is minimum muscle activation, at the onset of a perturbation, resistance to joint motion is derived from the elastic characteristics of the muscle fibers. This predominantly explains the stiffness in 0%MVCC.

Active muscle stiffness on the other hand arises as a result of the formation of cross-bridges in muscle fibers [67], which produces tension within the muscle. The number of cross-bridges formed, determine the amount of tension generated by a muscle fiber, and the level of muscle activation. Consequently, as the level of co-contraction increases from 10% to 20%MVCC, there is an increase in the number of cross-bridges formed, leading to an increase in active muscle stiffness [50], [68]. While the increasing trend was observed in both sexes, there were clear sex differences in both relaxed (quiet standing) and contracted muscle conditions.

During quiet standing (0% MVCC), females exhibited lower ankle stiffness in both sagittal and frontal planes. This could be mostly explained by the sex differences in passive resistance to joint motion and in anatomical factors, such as greater range of motion, lower Young's modulus, and the higher ligamentous laxity [12]–[19], in females than in males. These results are congruent with existing literature that report lower knee stiffness [65], [69] and ankle stiffness [70] in females than in males.

The lower stiffness in females during co-contraction (10-20% MVCC) could be mostly explained by sex differences in active muscle mechanics. It has been confirmed that active muscle stiffness increases with muscle contraction [68], which arises as a result of the formation of cross-bridges [71]. Previous studies have demonstrated that females have less than 57% of active muscle stiffness as compared to males [25], mainly because males have more fast-twitch fibers [72], more leg muscle mass [71], and a higher cross-sectional area [73] compared to females.

Even after normalizing ankle stiffness by weight times height, $K_{normalized\_sagittal}$ still showed significant sex differences in all muscle co-contraction levels. It is worth noting that the overall $K_{normalized\_sagittal}$ in females was 19.1% lower than that in males, which is significantly lower than the difference of $K_{sagittal}$ between sexes (50.0%). Contrary to the results in the sagittal plane, $K_{normalized\_frontal}$ showed no statistical difference in all muscle co-contraction tasks.

Based on these results, sex differences in ankle stiffness in the sagittal plane during muscle co-contraction tasks could be explained by both anthropometric factors and sex differences in neuromuscular factors. On the other hand, sex differences in ankle stiffness in the frontal plane could be mostly explained by anthropometric factors but not the neuromuscular factors.

Weight - Bearing Task

For this task level, sex differences in 2D ankle stiffness was studied under different weight-bearing conditions. The results indicated that ankle stiffness increased with increasing load at the ankle, from 30% to 90% of the total body weight, for both males and females, in both sagittal and frontal planes.

During weight-bearing tasks, ankle muscles of the loaded leg generate increased stabilizing torques [57] which translate to an increase in ankle stiffness [47], [74]. This explains the increasing stiffness with increasing weight-bearing from 30% to 90% of the total body weight.

Clear sex differences were identified for all weight-bearing conditions in both the sagittal and frontal planes. This could be primarily due to higher weight and height of males than females which translates to higher ankle torque and ankle stiffness in males than females [71]. In this study, the average female weight and height was 85.6% and 96.1% of those in males.

Indeed, when normalized by weight times height, the sex differences significantly decreased compared to absolute ankle stiffness. In the frontal plane, there was no statistical sex difference in $K_{normalized\_frontal}$ except the highest loading condition (90%). In the sagittal plane, while there were sex differences in $K_{normalized\_sagittal}$ in all weight-bearing conditions, the level of statistical differences decreased compared to that in $K_{sagittal}$. This suggests that while anthropometric factors contribute to the modulation of 2D ankle stiffness during weight bearing tasks, there is another significant factor that account for the sex differences in the sagittal plane, for example, active ankle mechanics.

The increase in ankle stiffness was statistically significant for both males and females in the sagittal plane. However, unlike males, females showed no statistical difference in frontal plane stiffness across all weight-bearing conditions. This implies that loading the ankle in females is not as effective as co-contracting ankle muscles to increase ankle torque and resist external perturbations.

Ankle Torque Generation Task

In ankle torque generation tasks, subjects maintained CoP offsets to their neutral CoP to control the amount of ankle torque generated. In the sagittal plane, increasing CoP offsets from -2 cm to +6 cm correlates to an increase in the moment arm of the applied force. This leads to an increase in ankle torque, and consequently, an increase in ankle stiffness from -2 cm to +6 cm. These results are consistent with Casadio et al.'s observation that stiffness increases with increase in active ankle torque during quiet standing [74].

In the frontal plane, increasing the magnitude of CoP offsets from 0 cm to 1.5 cm, increases the moment arm of the applied force, thus, leading to an increase in ankle torque. With the increase in ankle torque generation, a U-shaped trend in ankle stiffness from -1.5 cm to +1.5 cm is expected. However, this trend is only observed for -1.5 cm to 0 cm (eversion torque required), but not for 0 cm to +1.5 cm (inversion torque required). Further investigation on the ankle kinematics showed that ankle displacement increased with increasing CoP from 0 cm to +1.5 cm. This trend was consistent in both males and females, implying that generating inversion torque during standing balance is not an effective strategy to increase frontal plane ankle stiffness.

Clear sex differences were identified in all ankle torque generation conditions in both the sagittal and frontal planes. This is primarily due to different target levels of ankle torque generation between males and females. Even for the same level of CoP offset, heavier males require more ankle torque than females.

When normalized by weight times height, the sex differences in ankle stiffness were reduced significantly. Although the ANOVA analysis confirmed a significant main effect of sex and there was a consistent trend of greater $K_{normalized\_sagittal}$ in males than in females except the -2 cm CoP condition, pairwise comparison for each ankle torque generation condition showed that only two conditions in $K_{normalized\_sagittal}$ reached the statistical significance. None of the conditions in $K_{normalized\_frontal}$ were statistically different. This is somewhat expected as ankle torque is proportional to the normalization factor of weight times height in the inverted pendulum model that incorporate postural sway for standing balance [42], [43]

Thus, sex differences in ankle stiffness in the sagittal plane during ankle torque generation via sway angle changes could be largely explained by anthropometric factors while there still exist non-negligible contribution of neuromuscular factors. The differences in the frontal plane could be mostly explained by anthropometric factors but not the neuromuscular factors.

Direction Dependent Sex differences in Ankle stiffness:

Sagittal Plane vs. Frontal Plane

For all the three different tasks, i.e., ankle muscle co-contraction tasks, weight-bearing tasks, and ankle torque generation tasks, ankle stiffness in the sagittal plane was significantly higher than in the frontal plane, which indicates that the ankle is relatively vulnerable to perturbations in the frontal plane. This is consistent with observations that musculoskeletal injuries at the ankle joint occur mostly in the frontal plane than in the sagittal plane [9], [11].

This is not surprising because ankle movement predominantly occurs in the sagittal plane, with most of the ankle muscles contributing to movement in the sagittal plane whereas a few contribute to movement in the frontal plane [6]. Consequently, the range of ankle torque generation and ankle stiffness modulation is significantly higher in the sagittal plane than in the frontal plane [32], [40], [44], and sex differences in ankle stiffness are more amplified in the sagittal plane than the frontal plane. Even when ankle stiffness was normalized by the anthropometric factor, the sex differences in the sagittal plane was greater than in the frontal plane, implying that neuromuscular factors contribute to the direction dependent sex differences in ankle stiffness.

Sex Differences in the Slope of the Offset-Adjusted Stiffness

From the linear regression of the offset-adjusted stiffness, there was a significant difference between male and female slopes for both ankle torque generation and weight-bearing task in the sagittal plane. In the frontal, significant difference was only evident in the weight -bearing task. The lack of significant difference in the muscle co-contraction task in both the sagittal and frontal planes implies that the ability to increase resistance to perturbations by increasing the level of co-contraction may not be different between both sexes. This suggests that males and females might be using similar strategies in increasing ankle stiffness during co-contraction. The significant difference observed in both weight-bearing and ankle torque generation tasks in the sagittal plane, suggests that males and females might be using different strategies in increasing ankle stiffness during these tasks.

However, in the frontal plane the significant difference observed during weight-bearing tasks is largely due to the fact that, females showed no statistical difference in $K_{frontal}$ across all weight-bearing conditions thus, making the change in stiffness with increasing weight-bearing conditions significantly higher in males. For ankle torque generation tasks, the results are not surprising because females did not show any significant change in ankle stiffness with increasing COP offsets and in males, there were also less clear changes in ankle stiffness across all conditions. This implies that the ability to generate ankle torque in the frontal plane may not be so different between both sexes.

CHAPTER 6

CONCLUSION AND LIMITATION

This study investigated the sex differences in 2D ankle stiffness during standing under three different tasks: ankle muscle co-contraction tasks, weight-bearing tasks and ankle torque generation tasks. Experimental results confirmed that sex influences ankle stiffness with respect to muscle activation, weight-bearing, and ankle torque generation. These results will provide the foundation for further investigations on sex differences in 2D ankle stiffness during dynamic tasks such as walking and running.

Outcomes from the studies during static and dynamic tasks could be utilized to design a future study to determine whether sex difference in ankle stiffness prospectively influences the sex difference in lower body stability and risk of ankle injury. In addition, the outcomes will serve as a basis to develop a risk assessment tool and sex-specific training programs for efficient ankle injury prevention or rehabilitation.

However, there are a few limitations to this study that are worth noting. First, the investigation of sex differences in ankle stiffness was limited to upright standing, which prevents translation to real-world dynamic tasks such as locomotion. Future studies investigating sex differences in ankle stiffness during walking and running, can complement findings in this study and would better shed light on our understanding of the neuromuscular basis for sex differences in risk of ankle injuries.

Second, as the experimental set-up was designed for the right leg, the investigation of sex differences in ankle stiffness was strictly limited to people with right leg dominance, which may be slightly different when comparing ankle stiffness in the non-dominant leg. This limitation may be addressed in future studies, using a robotic platform designed for the left leg.

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APPENDIX A

IRB APPROVAL LETTER

APPROVAL: EXPEDITED REVIEW

Hyunglae Lee
SEMTE: Engineering of Matter, Transport and Energy, School for
-
Hyunglae.Lee@asu.edu

Dear Hyunglae Lee:

On 5/8/2019 the ASU IRB reviewed the following protocol:

Type of Review:	Initial Study
Title:	Quantitative characterization of intrinsic, reflexive, and voluntary mechanics of human limbs and joints
Investigator:	Hyunglae Lee
IRB ID:	STUDY00010123
Category of review:	(4) Noninvasive procedures, (7)(a) Behavioral research
Funding:	None
Grant Title:	None
Grant ID:	None
Documents Reviewed:	• Consent Bioscience.pdf, Category: Consent Form; • IRB Submission.docx, Category: IRB Protocol; • Recruitment Letter.pdf, Category: Recruitment Materials;

The IRB approved the protocol from 5/8/2019 to 5/7/2020 inclusive. Three weeks before 5/7/2020 you are to submit a completed Continuing Review application and required attachments to request continuing approval or closure.

If continuing review approval is not granted before the expiration date of 5/7/2020 approval of this protocol expires on that date. When consent is appropriate, you must use final, watermarked versions available under the “Documents” tab in ERA-IRB.

In conducting this protocol you are required to follow the requirements listed in the INVESTIGATOR MANUAL (HRP-103).

APPENDIX B

THE AVERAGE OF ALL DATA COLLECTED FROM ALL MALE AND FEMALE SUBJECTS

Table 1: Results from Data Collection for Males in Sagittal Plane

Average for DP									
	Stiffness	Damping	Inertia	Goodness	TA	PL	SOL	GCA	COP
-2cm	109.71	0.08	0.01	94.14	3.69	4.55	6.34	4.58	0.12
0cm	166.50	0.05	0.01	94.69	2.42	4.33	7.80	6.84	0.05
2cm	210.19	0.02	0.01	94.71	2.59	6.14	10.19	10.31	-0.02
4 cm	250.36	0.03	0.01	94.80	2.77	7.97	12.93	12.30	-0.06
6cm	284.20	0.00	0.01	94.76	3.32	10.10	15.22	13.72	-0.13
0% MVC	166.51	0.01	0.01	94.69	2.54	4.45	8.15	6.55	-0.01
10% MVC	195.95	0.01	0.01	94.65	8.63	6.75	10.05	8.61	0.02
15% MVC	232.43	0.00	0.01	94.64	12.65	9.04	12.12	10.29	0.00
20% MVC	251.93	0.05	0.01	93.62	17.27	11.17	12.42	9.26	0.01
30% weight	114.84	0.09	0.01	94.34	2.57	4.28	6.60	4.82	0.11
50% weight	162.49	0.04	0.01	94.54	2.57	4.75	7.66	6.76	0.07
70% weight	205.64	0.05	0.01	94.83	2.78	5.26	9.47	10.67	0.05
90% weight	238.49	0.04	0.01	94.85	3.07	6.34	10.32	16.54	0.10

Table 2: Results from Data Collection for Males in Frontal Plane

Average for IE									
	Stiffness	Damping	Inertia	Goodness	TA	PL	SOL	GCA	COP
(-)1.5 cm	48.57	0.89	0.00	98.88	3.59	13.15	11.54	4.87	0.25
(-)0.75 cm	39.84	0.72	0.00	98.35	2.69	7.24	8.25	5.63	0.16
0 cm	31.48	0.63	0.00	97.20	2.63	3.93	6.87	6.07	0.04
0.75 cm	29.83	0.66	0.00	96.57	2.55	3.83	7.02	5.92	-0.10
1.5 cm	29.27	0.75	0.00	96.97	2.84	3.99	6.88	5.59	-0.15
0% MVC	33.65	0.66	0.00	97.55	2.48	4.10	7.49	7.15	0.00
10% MVC	38.13	0.65	0.00	97.29	7.95	5.80	8.78	6.93	0.07
15% MVC	41.31	0.66	0.00	97.77	12.23	7.65	10.12	7.85	0.08
20% MVC	47.77	0.65	0.00	97.76	16.08	10.09	11.50	8.58	0.04
30% weight	28.82	0.58	0.00	97.25	2.40	4.04	5.89	4.00	-0.02
50% weight	33.38	0.60	0.00	97.17	2.32	4.04	7.00	5.93	-0.03
70% weight	42.13	0.69	0.00	97.70	3.19	4.93	8.30	9.84	0.09
90% weight	52.89	0.81	0.00	98.10	3.06	6.92	9.24	12.62	0.07

Table 3: Results from Data Collection for Females in Sagittal Plane

Average for DP									
	Stiffness	Damping	Inertia	Goodness	TA	PL	SOL	GCA	COP
-2 cm	93.21	0.02	0.01	98.50	7.29	6.88	7.65	4.21	0.13
0 cm	120.20	0.00	0.01	98.78	3.81	6.16	9.00	5.31	0.00
2 cm	165.25	0.01	0.01	99.00	3.49	7.46	12.09	7.89	-0.06
4 cm	193.54	0.00	0.01	98.87	4.51	11.07	15.59	10.34	-0.08
6cm	218.86	0.00	0.01	99.09	4.05	14.28	17.06	12.38	-0.14
0% MVC	115.25	0.00	0.01	98.67	3.30	5.86	7.90	5.16	-0.03
10% MVC	136.56	0.00	0.01	98.92	9.01	10.01	11.95	5.93	-0.06
15% MVC	169.06	0.00	0.01	98.99	14.25	11.26	14.48	6.48	-0.03
20% MVC	187.67	0.00	0.01	99.15	18.30	13.40	18.57	8.20	0.00
30% weight	81.49	0.03	0.01	98.35	4.17	5.74	7.47	4.32	0.07
50% weight	119.08	0.00	0.01	98.77	3.61	6.06	9.06	5.52	0.04
70% weight	150.07	0.00	0.01	98.91	4.37	6.84	9.75	9.17	0.03
90% weight	187.66	0.00	0.01	99.32	6.05	9.29	11.75	11.85	0.09

Table 4: Results from Data Collection for Females in Frontal Plane

Average for IE									
	Stiffness	Damping	Inertia	Goodness	TA	PL	SOL	GCA	COP
(-)1.5 cm	34.86	0.66	0.00	96.63	4.14	16.40	12.59	4.47	0.31
(-)0.75 cm	29.28	0.44	0.00	97.62	3.54	11.12	10.18	5.03	0.18
0 cm	25.18	0.24	0.00	94.45	3.31	5.77	7.94	5.04	0.03
0.75 cm	22.93	0.27	0.00	92.49	3.40	5.57	7.97	5.08	-0.10
1.5 cm	21.51	0.35	0.00	92.68	4.33	5.96	8.12	5.16	-0.16
0% MVC	25.78	0.28	0.00	94.89	3.16	5.65	7.78	4.75	0.03
10% MVC	30.26	0.30	0.00	93.53	8.50	8.14	11.85	4.82	0.05
15% MVC	35.18	0.31	0.00	93.95	13.01	9.34	13.93	6.00	0.05
20% MVC	41.37	0.29	0.00	93.60	17.23	11.83	15.01	7.22	0.07
30% weight	24.75	0.29	0.00	96.34	3.37	5.20	7.04	4.05	-0.03
50% weight	26.67	0.24	0.00	95.09	3.05	5.38	8.38	4.56	0.02
70% weight	28.95	0.29	0.00	94.74	3.17	6.43	9.33	6.73	0.15
90% weight	29.51	0.47	0.00	95.60	4.11	8.58	11.12	8.66	0.17

APPENDIX C

DIFFERENCES IN ANKLE STIFFNESS BETWEEN EXPERIMENTAL CONDITIONS FOR EACH TASK

Table 5: Differences in ankle stiffness between different levels of muscle co-contraction in Sagittal Plane

Pairwise Comparisons

Measure: Stiffness

Sex	(I) Activation	(J) Activation	Mean Difference (I–J)	Sig.
Males	0%MVC	10%MVC	-33.709*	.000
		15%MVC	-76.474*	.000
		20%MVC	-99.996*	.000
	10%MVC	0%MVC	33.709*	.000
		15%MVC	-42.765*	.000
		20%MVC	-66.287*	.000
	15%MVC	0%MVC	76.474*	.000
		10%MVC	42.765*	.000
		20%MVC	-23.522*	.000
	20%MVC	0%MVC	99.996*	.000
		10%MVC	66.287*	.000
		15%MVC	23.522*	.000
Females	0%MVC	10%MVC	-20.629*	.003
		15%MVC	-53.811*	.000
		20%MVC	-73.646*	.000
	10%MVC	0%MVC	20.629*	.003
		15%MVC	-33.183*	.000
		20%MVC	-53.017*	.000
	15%MVC	0%MVC	53.811*	.000
		10%MVC	33.183*	.000
		20%MVC	-19.834*	.001
	20%MVC	0%MVC	73.646*	.000
		10%MVC	53.017*	.000
		15%MVC	19.834*	.001

Based on estimated marginal means

*. The mean difference is significant at the

Table 6: Differences in ankle stiffness between different levels of muscle co-contraction in Frontal Plane

Pairwise Comparisons

Measure: Stiffness

Sex	(I) Activation	(J) Activation	Mean Difference (I–J)	Sig.
Males	0%MVC	10%MVC	-4.193*	.025
		15%MVC	-6.623*	.026
		20%MVC	-13.295*	.000
	10%MVC	0%MVC	4.193*	.025
		15%MVC	-2.430	.417
		20%MVC	-9.102*	.001
	15%MVC	0%MVC	6.624*	.026
		10%MVC	2.430	.417
		20%MVC	-6.672*	.018
	20%MVC	0%MVC	13.295*	.000
		10%MVC	9.102*	.001
		15%MVC	6.672*	.018
Females	0%MVC	10%MVC	-4.483*	.014
		15%MVC	-8.954*	.001
		20%MVC	-15.201*	.000
	10%MVC	0%MVC	4.483*	.014
		15%MVC	-4.471*	.009
		20%MVC	-10.718*	.000
	15%MVC	0%MVC	8.954*	.001
		10%MVC	4.471*	.009
		20%MVC	-6.247*	.031
	20%MVC	0%MVC	15.201*	.000
		10%MVC	10.718*	.000
		15%MVC	6.247*	.031

Based on estimated marginal means

*. The mean difference is significant at the

Weight-Bearing Tasks

Table 7: Differences in ankle stiffness between different weight-bearing conditions in Sagittal Plane

Pairwise Comparisons

Measure: Stiffness

Sex	(I) Weight	(J) Weight	Mean Difference (I–J)	Sig.
Males	30% Weight	50% Weight	-53.817*	.000
		70% Weight	-101.354*	.000
		90% Weight	-140.523*	.000
	50% Weight	30% Weight	53.817*	.000
		70% Weight	-47.536*	.000
		90% Weight	-86.705*	.000
	70% Weight	30% Weight	101.354*	.000
		50% Weight	47.536*	.000
		90% Weight	-39.169*	.000
	90% Weight	30% Weight	140.523*	.000
		50% Weight	86.706*	.000
		70% Weight	39.169*	.000
Females	30% Weight	50% Weight	-36.176*	.000
		70% Weight	-65.841*	.000
		90% Weight	-100.507*	.000
	50% Weight	30% Weight	36.176*	.000
		70% Weight	-29.665*	.000
		90% Weight	-64.331*	.000
	70% Weight	30% Weight	65.841*	.000
		50% Weight	29.665*	.000
		90% Weight	-34.666*	.000
	90% Weight	30% Weight	100.507*	.000
		50% Weight	64.331*	.000
		70% Weight	34.666*	.000

Based on estimated marginal means

*. The mean difference is significant at the

Table 8: Differences in ankle stiffness between different weight-bearing conditions in Frontal Plane

Pairwise Comparisons

Measure: Stiffness

Sex	(I) Weight	(J) Weight	Mean Difference (I–J)	Sig.
Males	30% Weight	50% Weight	-4.925*	.021
		70% Weight	-14.056*	.000
		90% Weight	-24.990*	.000
	50% Weight	30% Weight	4.925*	.021
		70% Weight	-9.131*	.001
		90% Weight	-20.066*	.000
	70% Weight	30% Weight	14.056*	.000
		50% Weight	9.131*	.001
		90% Weight	-10.934*	.000
	90% Weight	30% Weight	24.990*	.000
		50% Weight	20.066*	.000
		70% Weight	10.935*	.000
Females	30% Weight	50% Weight	-1.534	1.000
		70% Weight	-4.102	1.000
		90% Weight	-4.907	1.000
	50% Weight	30% Weight	1.534	1.000
		70% Weight	-2.568	1.000
		90% Weight	-3.374	1.000
	70% Weight	30% Weight	4.102	1.000
		50% Weight	2.568	1.000
		90% Weight	-.805	1.000
	90% Weight	30% Weight	4.907	1.000
		50% Weight	3.374	1.000
		70% Weight	.805	1.000

Based on estimated marginal means

*. The mean difference is significant at the

Ankle Torque Generation Tasks

Table 9: Differences in ankle stiffness between different ankle torque generation conditions in Sagittal Plane

Pairwise Comparisons

Measure: MEASURE_1

Sex	(I) COP	(J) COP	Mean Difference (I–J)	Sig.
Males	-2 cm	0cm	-58.511*	.000
		+2cm	-107.360*	.000
		+4 cm	-151.293*	.000
		+6 cm	-191.995*	.000
	0cm	1	58.511*	.000
		+2cm	-48.849*	.000
		+4 cm	-92.782*	.000
		+6 cm	-133.484*	.000
	+2 cm	1	107.360*	.000
		0cm	48.849*	.000
		+4 cm	-43.934*	.000
		+6 cm	-84.635*	.000
	+2 cm	1	151.294*	.000
		0cm	92.782*	.000
		+2cm	43.934*	.000
		+6 cm	-40.701*	.000
	+6 cm	1	191.995*	.000
		0cm	133.484*	.000
		+2cm	84.635*	.000
		+4 cm	40.701*	.000

Table 9: Differences in ankle stiffness between different ankle torque generation conditions in Sagittal Plane, continued

Females	-2 cm	0cm	-25.471*	.000
		+2cm	-70.420*	.000
		+4 cm	-98.866*	.000
		+6 cm	-125.754*	.000
	0cm	1	25.471*	.000
		+2cm	-44.949*	.000
		+4 cm	-73.396*	.000
		+6 cm	-100.283*	.000
	+2 cm	1	70.420*	.000
		0cm	44.949*	.000
		+4 cm	-28.446*	.000
		+6 cm	-55.334*	.000
	+2 cm	1	98.866*	.000
		0cm	73.396*	.000
		+2cm	28.446*	.000
		+6 cm	-26.888*	.000
	+6 cm	1	125.754*	.000
		0cm	100.283*	.000
		+2cm	55.334*	.000
		+4 cm	26.888*	.000

Based on estimated marginal means

*. The mean difference is significant at the

Table 10: Differences in ankle stiffness between different ankle torque generation conditions in Frontal Plane

Pairwise Comparisons

Measure: MEASURE_1

Sex	(I) COP	(J) COP	Mean Difference (I–J)	Sig.
Males	-1.5cm	-0.75cm	8.644*	.021
		0cm	16.493*	.000
		+0.75cm	18.514*	.000
		+1.5cm	19.027*	.000
	-0.75 cm	-1.5cm	-8.644*	.021
		0cm	7.849*	.000
		+0.75cm	9.870*	.000
		+1.5cm	10.382*	.001
	0cm	-1.5cm	-16.493*	.000
		-0.75cm	-7.849*	.000
		+0.75cm	2.021	1.000
		+1.5cm	2.533	1.000
	+0. 75cm	-1.5cm	-18.514*	.000
		-0.75cm	-9.870*	.000
		0cm	-2.021	1.000
		+1.5cm	.512	1.000
	+1.5cm	-1.5cm	-19.027*	.000
		-0.75cm	-10.382*	.001
		0cm	-2.533	1.000
		+0.75cm	-.512	1.000

Table 10: Differences in ankle stiffness between different ankle torque generation conditions in Frontal Plane, continued

Females	-1.5cm	-0.75cm	5.314	.495
		0cm	9.567*	.006
		+0.75cm	11.644*	.002
		+1.5cm	12.228*	.004
	-0.75 cm	-1.5cm	-5.314	.495
		0cm	4.253	.093
		+0.75cm	6.330*	.018
		+1.5cm	6.914	.073
	0cm	-1.5cm	-9.567*	.006
		-0.75cm	-4.253	.093
		+0.75cm	2.077	1.000
		+1.5cm	2.661	1.000
	+0. 75cm	-1.5cm	-11.644*	.002
		-0.75cm	-6.330*	.018
		0cm	-2.077	1.000
		+1.5cm	.584	1.000
	+1.5cm	-1.5cm	-12.228*	.004
		-0.75cm	-6.914	.073
		0cm	-2.661	1.000
		+0.75cm	-.584	1.000

Based on estimated marginal means

*. The mean difference is significant at the