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Sex Differences in 2-DOF Human Ankle Stiffness in Relaxed and Contracted Muscles

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Abstract—Ankle stiffness has been known as one of the most important components contributing to the maintenance of lower body stability during postural balance and locomotion. It has been repeatedly shown that women have lower stability and increased risk of injury when compared to men participating in similar sports activities, 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 multi-dimensional ankle stiffness in 20 young, healthy men and 20 young, healthy women over a range of ankle muscle contractions, from relaxed to 20% of maximum voluntary co-contraction of ankle muscles. A wearable ankle robot and a system identification method were used to reliably quantify ankle stiffness in a 2-dimensional space spanning the sagittal plane and the frontal plane. In all muscle activation levels, significant sex differences in ankle stiffness were identified in both the sagittal and frontal planes. In the given experimental conditions, ankle stiffness in males was higher than females up to 15.1 and 8.3 Nm/rad in the sagittal plane and the frontal plane, respectively. In addition, sex differences in the spatial structure of ankle stiffness were investigated by quantifying three parameters defining the stiffness ellipse of the ankle: area, aspect ratio, and orientation. In all muscle activation levels, a significant sex difference was identified in the area of stiffness ellipse as expected from the sex difference in the sagittal and frontal planes. However, no statistical sex difference was observed in the aspect ratio and orientation, which would be due to little differences in major anatomical configurations of the ankle joint between sexes. This study, in combination with future studies investigating sex differences during dynamic tasks (e.g. postural balance and locomotion) would serve as a basis to develop a risk assessment tool and sex-specific training programs for efficient ankle injury prevention or rehabilitation.

Keywords—Human ankle, Ankle stiffness, Ankle injury, Gender difference, Sex difference.

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INTRODUCTION

The human ankle is an essential joint in many lower extremity functions at the interface between the lower extremity and the physical world.[11,41,51,52] It plays significant roles in the maintenance of mediolateral stability in the frontal plane as well as anteroposterior stability in the sagittal plane.[11,52] It also contributes to shock absorption, propulsion, lower-limb joint coordination, and adaptation to environmental changes during locomotion.[40,44]

It has been demonstrated that incidence of musculoskeletal injury at the ankle joint in females is significantly higher than in males participating in similar sports activities such as basketball and soccer.[1,8,20,43] Sex differences in risk of ankle injury are likely multifactorial, i.e., multiple causative factors contribute to the increased rate of musculoskeletal injury in women, including anatomical, hormonal, and neuromuscular factors. Anatomical risk factors include higher joint and ligamentous laxity,[22,42,50] lower Young's modulus,[27] and greater range of motion[4,26] in females. Cyclic hormonal variations in females may contribute to decreased ligament strength, altered strength, or modified muscle recruitment. However, compared to extensive studies on knee injury,[16,17,48] experimental findings regarding hormones and risk of ankle injury are limited and require further investigation.[3] Neuromuscular and biomechanical factors also have been speculated to contribute to the risk of injury.[13,14,53] The National Research Council concluded that musculoskeletal injury occurs when neuromuscular control fails to stabilize applied biomechanical load.[7] If the neuromuscular system does not sufficiently support the applied load, then strain is applied to the joint structure, thereby resulting in injury.

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Ankle stiffness, an integral component of ankle mechanics, resists external loading and plays an essential role to maintain postural stability and prevent ankle injury during lower extremity function.[15,30,49,51] Thus, investigation of sex differences in ankle stiffness would shed lights on our understanding of sex differences in risk of ankle injury.

Given the importance of ankle stiffness in lower extremity function, it has been studied over the past few decades.[6,15,21,24,33,34,36,46,47,52] Many studies have been performed in a traditional gait lab setting consisting of a motion capture system and a force plate, which allows for the quantification of the torque–angle relationship at the ankle, known as quasi-stiffness.[15,47] However, quasi-stiffness and stiffness are distinct concepts,[28,45] except when the equilibrium point/trajectory of the joint is constant, which is not likely during various motor tasks, especially dynamic tasks. When the equilibrium point/trajectory of the joint is varying, for example through antagonistic muscle activation, there is no causal relationship between joint torque and joint stiffness and they can be independently controlled. Without exact information on the equilibrium point/trajectory, quasi-stiffness can significantly mislead our understanding on joint mechanics. Perturbations, i.e., external energy input, are required to directly quantify joint stiffness.

Simple devices consisting of a servo-controlled motor and a cast supporting the leg have been widely utilized to quantify ankle stiffness.[21,24,36] While these studies characterized ankle stiffness in various task conditions under different ankle positions and muscle activations, the characterizations were strictly limited to a single degree-of-freedom (DOF) of the ankle in the sagittal plane. As the ankle plays a critical role in the maintenance of mediolateral stability in the frontal plane as well as anteroposterior stability in the sagittal plane,[11,51] it is crucial to characterize multi-dimensional ankle stiffness in both the sagittal and frontal planes.

A few recent studies have characterized ankle stiffness in the sagittal and frontal planes by utilizing robotic devices capable of actuating 2-DOF of the ankle.[9,30–32] These studies have provided invaluable information on the directional characteristics of ankle stiffness, not achievable with single-DOF ankle studies.

In this paper, we built upon our previous multi-dimensional ankle studies[31,32] and aimed to investigate sex differences in ankle stiffness by quantifying its magnitude in both the sagittal and frontal planes over a range of ankle muscle contractions. We also aimed to investigate a spatial structure of ankle stiffness in a 2D space formed by dorsiflexion-plantarflexion (DP) and inversion-eversion (IE) axes by quantifying 3 parameters defining the stiffness ellipse of the ankle: area, aspect ratio (anisotropy), and orientation.

Based on previous findings on higher incidence of musculoskeletal injury at the ankle joint in females as well as the presence of additional mechanical and anatomical risk factors in females (higher joint laxity, lower Young's modulus, and greater range of motion) in females, we hypothesized that ankle stiffness in females is significantly lower than males in both the sagittal and frontal planes and in all muscle activation levels. This hypothesis further leads to our secondary hypothesis that the area of stiffness ellipse is significantly smaller in females than males. However, based on the conjecture that females might have consistently lower ankle stiffness than males in any movement directions in the 2D space, we also hypothesized that there is no significant difference in the aspect ratio and orientation of stiffness ellipse.

MATERIALS AND METHODS

Subjects

Twenty young men (age: 20–27, weight: 53.4–108.9 kg, height: 157.5–188.0 cm) and 20 young women (age: 21–28, weight: 46.0–96.3 kg, height: 149.9–172.7 cm) with no reported history of musculoskeletal or connective tissue disorders that could affect ankle stiffness were recruited among students at the Arizona State University (ASU). All protocols were approved by the ASU Institutional Review Board. Subjects gave written informed consent prior to participation.

Experimental Setup

To quantify multi-dimensional ankle stiffness over a range of muscle activation levels, we used a wearable ankle robot, Anklebot (Bionik Laboratories, Canada), an electromyography (EMG) system (Trigno Wireless EMG, Delsys, MA), and a visual feedback display. The same experimental setup has been successfully used in the author's previous studies to quantify multi-dimensional ankle stiffness.[31,32]

The wearable ankle robot consists of a main body having two actuators, a knee brace, and a shoe (Fig. 1a). The top part of the main body was attached to the knee brace and two end effectors of the actuators were connected to a U-shaped metal bracket attached to the bottom of the shoe. The knee brace was attached to the right leg approximately from the mid-shank to mid-thigh. This setup allows for actuation of the ankle in 2 DOFs, i.e., DP and IE. The third DOF along axial rotation was passive to prevent any inadvertent kinematic constraints at the ankle. During experiments, subjects sat in a chair with their ankle held by the ankle robot in a neutral position, defined as zero DP and IE angles from the horizontal plane (Fig. 1b).

Multiple shoe sizes were available to ensure both proper fit and prevention of foot slippage inside the shoe. The foot was further securely fastened with shoelaces and a wide Velcro strap. Multiple knee braces were also available to ensure proper fit on the leg and six Velcro straps were used to securely attach the knee brace to the leg. Weight of the entire setup and the leg was supported through elastic bands. The use of compliant elastic bands also helped to eliminate possible artifacts due to the coupling of the leg and the robot to surrounding structures, including the chair.[31]

For the quantification of multi-dimensional ankle stiffness, the robot applied torque perturbations and measured the corresponding angular displacements at the ankle in 2 DOFs. The torque resolution was 0.35 Nm and 0.24 Nm and for DP and IE, respectively. The displacement resolution was $1.0 \times 10^{-3\circ}$ and $1.4 \times 10^{-3\circ}$ for DP and IE, respectively. Both data were measured at 1 kHz.

This study quantified ankle stiffness at different muscle co-contraction levels. To control the muscle activation level during measurements, surface electromyographic (EMG) signals of tibialis anterior (TA) and soleus (SOL) were recorded. Wireless, differential surface electrodes with built-in amplifiers (Trigno, Delsys, MA) were attached to the skin over the bellies of TA and SOL. Prior to electrode placement, the skin was rubbed with alcohol swabs to reduce the impedance of skin–electrode interface.

EMG signals were band-pass filtered (20–450 Hz), also sampled at 1 kHz, and synchronized with ankle torque and kinematic data. Amplitudes of EMG signals were estimated using a root-mean-square average with a moving window of 200 ms after removing the DC bias offsets. Amplitudes of each muscle were normalized with respect to its maximum voluntary contraction (MVC).

The visual feedback display was used to inform subjects of real-time muscle activation of TA in %MVC and current ankle positions in the 2D space, i.e., IE and DP displacements with respect to the neutral position of the ankle (Fig. 1c). Simultaneous control of TA (dorsiflexor and inverter) and the neutral position of the ankle in the 2D space could effectively co-contract ankle muscles contributing to both DP and IE. A monitor display was placed at eye level in front of the subjects.

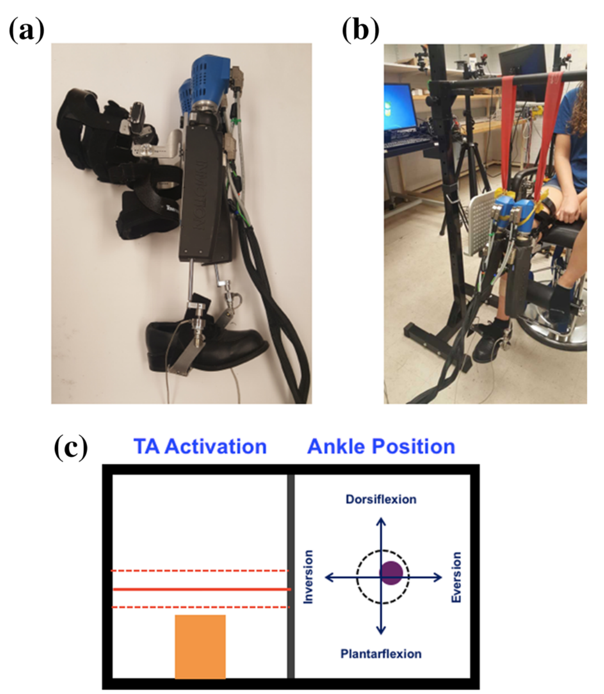

FIGURE 1. Experimental Setup. (a) A wearable ankle robot, Anklebot, connected to the custom designed shoe and the knee brace. (b) Quantification of ankle stiffness in a seated position, while weight of the entire setup and the leg was supported through elastic bands. (c) A visual feedback display for the control of target muscle co-contraction levels. Real-time muscle activation of TA in %MVC and current ankle positions in the 2D space (IE and DP displacements) are provided.

Experimental Protocol

First, the MVC of each muscle was measured following a standard muscle testing procedure.[37] Amplitudes of EMG signals were normalized with respect to MVC measurements.

Prior to the main experiment, each subject participated in a practice session to familiarize with the experimental setup and to practice maintaining different muscle co-contraction levels, i.e., simultaneously maintaining different target activation levels of TA and the neutral ankle position. The practice session lasted about 5 min until the subject felt confident and comfortable at maintaining the target level. During this practice, no perturbations were applied to the ankle.

In the main experiment, ankle stiffness was quantified at 3 different muscle activation levels: 0% (relaxed), 10%, and 20% of maximum voluntary co-contraction (MVCC) of ankle muscles. To prevent any effects of muscle fatigue, contractions were limited to no more than 20% of the estimated MVCC, and a minimum rest period of 3 min was provided between trials. A total of 3 repeated trials were performed for each muscle activation level. The order of target muscle activation was randomized.

In each trial, mild random torque perturbations were applied to the ankle for 20 s, and the corresponding ankle angles in 2 DOFs (DP and IE) were recorded. Band-limited white noise with a spectrum flat up to 100 Hz was used to reliably quantify ankle stiffness in all muscle activation conditions.[31] For the given torque perturbations, the root-mean-square (RMS) values of ankle angles in any muscle activation conditions were less than 2.1° and 3.2° for DP and IE, respectively.

Data Analysis

A linear time-invariant, multi-input multi-output system identification method based on spectral analysis was utilized to quantify multi-dimensional ankle stiffness in the IE-DP space.[2,32] The author's previous ankle studies[31,32] have demonstrated that this method could reliably quantify multi-dimensional ankle stiffness over a wide range of muscle contractions.

Briefly, the system identification method first quantified frequency response of ankle dynamics. Next, stiffness component of ankle dynamics was calculated by averaging the magnitude frequency response in a low frequency region below 5 Hz, where stiffness dominates the dynamic response (Fig. 2a).[24,31]

By repeating this identification process for different movement directions in the IE-DP space, a spatial structure of ankle stiffness was identified. The spatial structure was represented as a stiffness ellipse, which could be described by the following three parameters: area ($\pi\lambda_{max}\lambda_{min}$), aspect ratio ($\lambda_{max}/\lambda_{min}$), and orientation ($\alpha$) (Fig. 2b). The area encompasses the entire stiffness ellipse, which is proportional to the determinant of the symmetric stiffness matrix. The larger the area, the larger overall ankle stiffness in the IE-DP space. The aspect ratio is the ratio between the maximum and minimum stiffness values defined by the major and minor principal axes (or the larger and smaller eigenvalues), respectively. The higher aspect ratio implies significant direction-dependence of ankle stiffness in the IE-DP space. The orientation is measured between the major principal axis and the axis defining the DP direction. The smaller the angle, the smaller the coupling between the IE and DP axes.

Statistical Analysis

To test sex differences in multi-dimensional ankle stiffness, specifically, stiffness in the sagittal plane (DP stiffness [Nm/rad]), stiffness in the frontal plane [IE stiffness (Nm/rad)], area [Nm/rad]^2, aspect ratio, and orientation [°] of stiffness ellipse, we performed the following statistical analyses.

First, we tested if quantification of ankle stiffness was made while both male and female subjects maintained comparable levels of co-contraction of ankle muscles. We tested if there exist any sex differences in controlling TA activation levels and the neutral ankle position. Unpaired, independent, two-tailed t tests were performed.

Next, we tested if sex differences exist in the major directions of ankle stiffness (DP stiffness and IE stiffness) and the 3 parameters (area, aspect ratio, and orientation) defining the spatial structure of ankle stiffness. For each of 5 dependent variables, we performed a separate analysis by running a mixed-design analysis of variance (mixed ANOVA). Here the within-subjects factor was muscle activation and the between-subjects factor was sex. Following the mixed ANOVA, we performed post hoc analyses by running unpaired, independent, two-tailed t tests to identify sex differences at each muscle activation level.

In all statistical analyses, we checked normality of data by running Shapiro–Wilk tests, and evaluated equal variance (homogeneity of variance) across data sets by running Levene's tests. If the null hypothesis is 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 $p < 0.05$.

RESULTS

Maintenance of Co-contraction of Ankle Muscles

All subjects could successfully maintain three different target co-contraction levels (0, 10, 20 %MVCC) by simultaneously controlling both the target TA activation levels and the neutral ankle position. For each target level, minimal or no significant sex difference was observed (Table 1).

For TA activation, no statistical difference was observed in the 10 %MVCC ($p = 0.79$) and 20 %MVCC ($p = 0.74$) levels. While statistical significance was reached in the 0 %MVCC level ($p = 0.02$), the difference was minimal ($\Delta = 1.5$ %MVC). Although SOL activation, which counteracts TA activation, was not directly controlled during the study, we performed a secondary analysis to investigate if SOL activation levels were also comparable in male and female subjects. No statistical difference was observed in all activation levels ($p = 0.53$, $p = 0.36$, $p = 0.40$ for 0, 10, and 20 %MVCC, respectively).

For the ankle position in the sagittal plane (DP position), no statistical difference was observed in the 10 %MVCC ($p = 0.11$) and 20 %MVCC ($p = 0.65$)

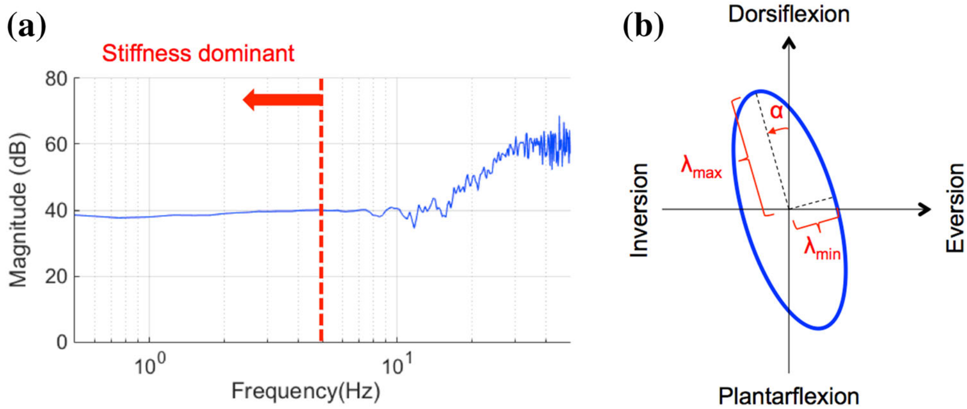

FIGURE 2. Quantification of ankle stiffness. (a) Ankle stiffness was quantified by averaging the magnitude frequency response of ankle mechanical impedance in a low frequency region below 5 Hz. (b) Three parameters defining the stiffness ellipse of the ankle: area, aspect ratio, and orientation.

TABLE 1. Minimal or No Significant Gender Difference was Observed in the Maintenance of the Target Co-contraction Levels (TA Activation Levels and the Neutral Ankle Position).

	Target co-contraction (% MVCC)	Male	Female
TA (%MVC)	0	2.6 (1.6)	4.1 (2.2)
	10	11.3 (2.0)	11.1 (2.4)
	20	21.0 (1.9)	21.3 (3.4)
DP position (deg.)	0	− 0.8 (0.7)	− 1.4 (0.8)
	10	0.4 (1.3)	− 0.2 (1.2)
	20	0.7 (2.0)	0.5 (1.2)
IE position (deg.)	0	0.4 (0.3)	0.1 (0.4)
	10	0.0 (0.9)	0.2 (0.7)
	20	− 0.2 (1.3)	0.3 (0.9)

levels. While statistical significance was reached in the 0 %MVCC level ($p = 0.01$), the difference was minimal ($\Delta = 0.6°$). For the ankle position in the frontal plane (IE position), no statistical difference was observed in all activation levels ($p = 0.22$, $p = 0.21$, $p = 0.31$ for 0, 10, and 20 %MVCC, respectively).

Ankle Stiffness in the Sagittal and Frontal Planes

Co-contraction of ankle muscles significantly increased DP stiffness and IE stiffness in both male and female subjects. However, significant sex difference was observed in all muscle activation levels, being greater in males than females (Fig. 3).

For DP stiffness in the sagittal plane, a significant main effect of the within-subjects factor of muscle activation was identified ($F(1.59,60.30) = 334.2$, $p \ll 0.001$). In addition, a significant main effect of the between-subjects factor of sex was identified ($F(1,38) = 14.17$, $p = 0.001$). Post hoc independent t tests further revealed that sex differences were statistically significant across all muscle activation levels: $\Delta = 6.3$ Nm/rad ($p = 0.001$), $\Delta = 14.2$ Nm/rad ($p = 0.001$), and $\Delta = 15.1$ Nm/rad ($p = 0.004$) for 0, 10, and 20 %MVCC, respectively) (Fig. 3a).

For IE stiffness in the frontal plane, a significant main effect of the within-subjects factor of muscle activation was identified ($F(1.49,50.71) = 192.4$, $p \ll 0.001$). In addition, a significant main effect of the between-subjects factor of sex was identified ($F(1,38) = 11.18$, $p = 0.002$). Post hoc independent t tests further revealed that sex differences were statistically significant across all muscle activation levels: $\Delta = 5.2$ Nm/rad ($p = 0.001$), $\Delta = 6.9$ Nm/rad ($p = 0.003$), and $\Delta = 8.3$ Nm/rad ($p = 0.008$) for 0, 10, and 20 %MVCC, respectively) (Fig. 3b).

Although no statistically significant interaction between the within- and between-subjects factors was reached ($p > 0.05$) in both DP stiffness and IE stiffness, a trend was observed that the sex difference in ankle stiffness increased with the increase of muscle activation level in both planes.

Spatial Structure of Ankle Stiffness

Co-contraction of ankle muscles significantly changed the area, aspect ratio, and orientation of stiffness ellipse in both male and female subjects. While significant sex difference was observed in the area of stiffness ellipse in all muscle activation levels, no statistical difference was reached in the aspect ratio and orientation in all muscle activation levels (Fig. 4).

For the area of stiffness ellipse, a significant main effect of the within-subjects factor of muscle activation was identified ($F(1.34,51.07) = 200.1$, $p \ll 0.001$). Muscle activation significantly increased the area in both male and female subjects. In addition, a significant main effect of the between-subjects factor of sex was identified ($F(1,38) = 15.80$, $p \ll 0.001$). Post hoc independent t tests further revealed that sex differences were statistically significant across all muscle activation levels ($p \ll 0.001$, $p = 0.003$, and $p = 0.008$ for 0, 10, and 20 %MVCC, respectively) (Fig. 4a).

For the aspect ratio of stiffness ellipse, a significant main effect of the within-subjects factor of muscle activation was identified ($F(1.39,52.67) = 48.4$, $p \ll 0.001$). Thus, muscle activation significantly alters the aspect ratio in both male and female subjects. Specifically, the aspect ratio in relaxed muscles (0 %MVCC) was significantly lower than that in active muscle conditions ($p \ll 0.001$). However, no statistical difference was observed between two active muscle conditions, i.e., 10 and 20 %MVCC ($p = 1.00$). No significant main effect of the between-subjects factor of sex was observed ($F(1,38) = 0.014$, $p = 0.91$) (Fig. 4b).

For the orientation of stiffness ellipse, a significant main effect of the within-subjects factor of muscle activation was identified ($F(1.20,45.71) = 12.91$, $p \ll 0.001$). Thus, muscle activation significantly alters the orientation in both male and female subjects. Specifically, the orientation in relaxed muscles (0 %MVCC) was significantly tilted from the sagittal plane than that in active muscle conditions ($p \ll 0.001$). While statistical significance was reached, the difference between the two active muscle conditions was small ($\Delta = 1.9°$; $p = 0.04$). No significant main effect of the between-subjects factor of sex was observed ($F(1,38) = 0.48$, $p = 0.50$) (Fig. 4c).

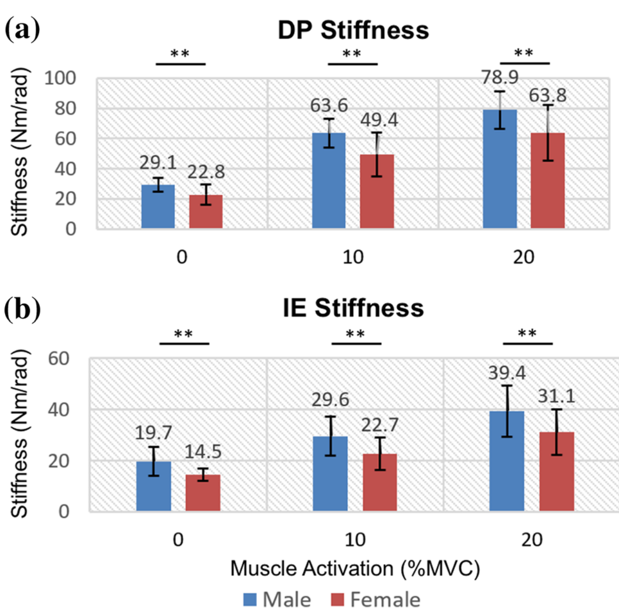

FIGURE 3. Ankle stiffness in the sagittal and frontal planes. A significant sex difference was observed in all muscle activation levels ($p < 0.01$). (a) DP stiffness, (b) IE stiffness.**

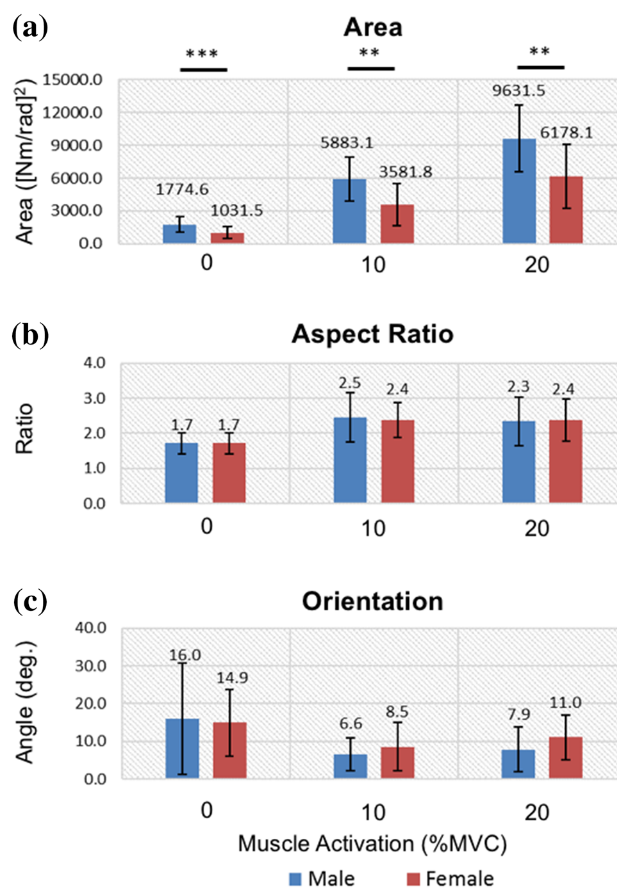

FIGURE 4. Spatial structure of ankle stiffness. (a) Area, (b) Aspect ratio, and (c) Orientation. A significant sex difference was observed in the area of stiffness ellipse in all muscle activation levels ($p < 0.01$, ***$p < 0.001$). No statistical sex difference was reached in the aspect ratio and orientation in any muscle activation levels.**

DISCUSSION

Previous studies have demonstrated that incidence of musculoskeletal injury at the ankle joint in females is significantly higher than in males participating in similar sports activities.[1,8,20,43] Among multiple risk factors contributing to the increased rate of musculoskeletal injury in women, neuromuscular control of stability has been identified as one of the most important potential contributors to sex differences in risk of injury.[13,14,53]

This study investigated sex differences in ankle stiffness, which plays significant roles in maintaining postural stability and preventing ankle injury during lower extremity function.[15,49,51] The use of a wearable ankle robot actuating 2-DOF of the ankle allowed for the reliable characterization of ankle stiffness in a 2D space spanning the sagittal and frontal planes, not achievable in previous single-DOF ankle studies.

Sex Differences in Multi-dimensional Ankle Stiffness

In both males and females, co-contraction of ankle muscles could significantly increase ankle stiffness in all movement directions in the 2D space. This result is consistent with previous findings that muscle co-contraction effectively increases joint/limb stiffness, which enhances robustness to external perturbations and compensates environmental instability.[5,10,19,29] While all subjects exhibited this increasing trend, clear sex differences were identified in both relaxed and active muscle conditions.

Lower ankle stiffness in females during relaxed muscles (0% MVCC) could be explained by sex differences in passive ankle mechanics including higher joint and ligamentous laxity.[22,42,50] and lower Young's modulus.[27]

On the other hand, lower ankle stiffness in females during muscle co-contraction (10 and 20 %MVCC) could be predominantly explained by sex differences in active muscle mechanics. It has been demonstrated that male muscles have more fast twitch fibers (type II),[12] have more muscle mass,[23] have higher cross-sectional area,[35] and have a higher capacity of anaerobic metabolism.[18] All these factors contribute to the rendering of higher maximum power output in male muscles than female muscles.[12,25] Since target co-contraction levels were scaled to the maximum co-contraction levels or maximum power output, for the same target level, we would expect lower absolute muscle activation in females than males and lower ankle stiffness accordingly.

In all muscle activation conditions, the sex difference in ankle stiffness was evident in both DP in the sagittal plane and IE in the frontal plane. This also resulted in significantly smaller area of stiffness ellipse in females than males implying that overall ankle stiffness in the IE-DP space is significantly lower in females than males.

Sex-Invariant Features of Multi-dimensional Ankle Stiffness

While the magnitude of ankle stiffness was significantly higher in males than females, no statistical differences were observed in two features defining the spatial structure of ankle stiffness in the 2D space, specifically, the aspect ratio and orientation of stiffness ellipse. This would be mainly because anatomical configurations, such as the number, attachment points, and types of ankle muscles, tendons, and ligaments, have no significant differences between sexes. Thus, for similar activation of ankle muscles, it is probable that geometrical features of multi-dimensional ankle stiffness would be rather invariant between sexes although its magnitude could be significantly different due to the sex differences in passive and active ankle mechanics as explained above.

The aspect ratio was significantly higher during muscle co-contraction than relaxed in both males and females. In other words, stiffness along the major axis (roughly aligned with DP in the sagittal plane) was relatively higher than the minor axis (roughly aligned with IE in the sagittal plane) during muscle co-contraction. However, no statistical difference was observed between two co-contraction levels. This observation could be explained by the integrated contribution of passive and active ankle mechanics to multi-dimensional ankle stiffness. While passive ankle mechanics determine ankle stiffness during relaxed muscles, both passive and active ankle mechanics contribute to the modulation of ankle stiffness during co-contraction. The significant difference between relaxed and co-contraction conditions explained that the contribution of active mechanics to multi-dimensional ankle stiffness is significantly different from that of passive mechanics. In addition, no difference between two co-contraction conditions (10 vs. 20 %MVCC) further confirmed that the contribution of active mechanics is significantly higher than that of passive mechanics when muscle co-contraction levels are higher than a certain level.

Co-contraction of ankle muscles significantly altered the orientation of multi-dimensional ankle stiffness in both males and females. Specifically, muscle co-contraction changed the orientation of the major principal axis of the stiffness ellipse towards the sagittal plane. This would be because co-contraction of ankle muscles increases ankle stiffness more along DP in the sagittal plane than IE in the frontal plane.

Since the aspect ratio and orientation of ankle stiffness ellipse are sex-invariant, we can simply model 2-DOF ankle stiffness in males as a scaled-up version of that in females. This invariant property is beneficial to simplifying the design of sex-specific training/exercise for ankle injury prevention or rehabilitation by having the magnitude or strength of training/exercise as a major variable between sexes.

Implications for Future Research

This study, for the first time, investigated sex differences in multi-dimensional ankle stiffness over a wide range of muscle contraction levels. Results of this study, performed in a static seated position, will serve as an important baseline to investigate sex differences during dynamic tasks such as postural balance and locomotion.

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

Limitations

There are a few limitations of this study that should be noted. First, investigation of sex differences in ankle stiffness was strictly limited to a static seated position, which prevents its direct translation to dynamic functional tasks such as postural balance and locomotion. In fact, we have recently extended the current study to these dynamic tasks using a multi-axis robotic platform developed for various lower extremity functions,[38,39] which will be a topic of future publication.

In addition, while we speculated that passive and active muscle mechanics contribute to sex differences in ankle stiffness over a range of muscle activation, it is important to investigate neuromuscular mechanisms underlying sex differences in ankle stiffness (e.g. using muscle force testing and anatomical measurement) to provide strong evidence for our speculation. It is also worth to investigate the effect of other important factors (e.g. physique variables), which might be closely correlated with sex.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in this study.

REFERENCES

[1]Almeida, S. A., D. W. Trone, D. M. Leone, R. A. Shaffer, S. L. Patheal, and K. Long. Gender differences in musculoskeletal injury rates: a function of symptom reporting? *Med. Sci. Sports Exerc*. 31(12):1807–1812, 1999.

[2]Bendat, J., and A. Piersol. Random Data: Analysis and Measurement Process (4th ed.). New York: Wiley, 2010.

[3]Beynnon, B. D., I. M. Bernstein, A. Belisle, B. Brattbakk, P. Devanny, R. Risinger, and D. Durant. The effect of estradiol and progesterone on knee and ankle joint laxity. *Am. J. Sports Med*. 33(9):1298–1304, 2005.

[4]Beynnon, B. D., P. A. Renstrom, D. M. Alosa, J. F. Baumhauer, and P. M. Vacek. Ankle ligament injury risk factors: a prospective study of college athletes. *J. Orthop. Res*. 19(2):213–220, 2001.

[5]Burdet, E., R. Osu, D. W. Franklin, T. E. Milner, and M. Kawato. The central nervous system stabilizes unstable dynamics by learning optimal impedance. *Nature* 414(6862):446–449, 2001.

[6]Casadio, M., P. G. Morasso, and V. Sanguineti. Direct measurement of ankle stiffness during quiet standing: implications for control modelling and clinical application. *Gait Posture* 21(4):410–424, 2005.

[7]Council, N. R. Musculoskeletal disorders and the workplace. Washington, DC: National Academy Press, 2001.

[8]Elias, S. R. 10-year trend in USA Cup soccer injuries: 1988-1997. *Med. Sci. Sports Exerc*. 33(3):359–367, 2001.

[9]Ficanha, E. M., G. Ribeiro, and M. A. Rastgaar. Design and evaluation of a 2-DOF instrumented platform for estimation of the ankle mechanical impedance in the sagittal and frontal planes. *IEEE/ASME Trans. Mechatron*. 21(5):2531–2542, 2016.

[10]Franklin, D. W., G. Liaw, T. E. Milner, R. Osu, E. Burdet, and M. Kawato. Endpoint stiffness of the arm is directionally tuned to instability in the environment. *J. Neurosci*. 27(29):7705–7716, 2007.

[11]Gatev, P., S. Thomas, T. Kepple, and M. Hallett. Feedforward ankle strategy of balance during quiet stance in adults. *J. Physiol*. 514(Pt 3):915–928, 1999.

[12]Glenmark, B., M. Nilsson, H. Gao, J. A. Gustafsson, K. Dahlman-Wright, and H. Westerblad. Difference in skeletal muscle function in males vs. females: role of estrogen receptor-beta. *Am. J. Physiol. Endocrinol. Metab*. 287(6):E1125–E1131, 2004.

[13]Granata, K. P., D. A. Padua, and S. E. Wilson. Gender differences in active musculoskeletal stiffness. Part II. Quantification of leg stiffness during functional hopping tasks. *J. Electromyogr. Kinesiol*. 12(2):127–135, 2002.

[14]Granata, K. P., S. E. Wilson, and D. A. Padua. Gender differences in active musculoskeletal stiffness. Part I. Quantification in controlled measurements of knee joint dynamics. *J. Electromyogr. Kinesiol*. 12(2):119–126, 2002.

[15]Hansen, A. H., D. S. Childress, S. C. Miff, S. A. Gard, and K. P. Mesplay. The human ankle during walking: implications for design of biomimetic ankle prostheses. *J. Biomech*. 37(10):1467–1474, 2004.

[16]Heitz, N. A., P. A. Eisenman, C. L. Beck, and J. A. Walker. Hormonal changes throughout the menstrual cycle and

increased anterior cruciate ligament laxity in females. *J. Athl. Train*. 34(2):144–149, 1999.
[17]Hewett, T. E. Neuromuscular and hormonal factors associated with knee injuries in female athletes. Strategies for intervention. *Sports Med*. 29(5):313–327, 2000.
[18]Hill, D. W., and J. C. Smith. Gender difference in anaerobic capacity: role of aerobic contribution. *Br. J. Sports Med*. 27(1):45–48, 1993.
[19]Hogan, N. Adaptive-control of mechanical impedance by coactivation of antagonist muscles. *IEEE Trans. Autom. Control* 29(8):681–690, 1984.
[20]Hosea, T. M., C. C. Carey, and M. F. Harrer. The gender issue: epidemiology of ankle injuries in athletes who participate in basketball. *Clin. Orthop. Relat. Res*. 372:45–49, 2000.
[21]Hunter, I. W., and R. E. Kearney. Dynamics of human ankle stiffness—variation with mean ankle torque. *J. Biomech*. 15(10):747–752, 1982.
[22]Huston, L. J., and E. M. Wojtys. Neuromuscular performance characteristics in elite female athletes. *Am. J. Sports Med*. 24(4):427–436, 1996.
[23]Janssen, I., S. B. Heymsfield, Z. M. Wang, and R. Ross. Skeletal muscle mass and distribution in 468 men and women aged 18-88 yr. *J. Appl. Physiol. (1985)* 89(1):81–88, 2000.
[24]Kearney, R. E., R. B. Stein, and L. Parameswaran. Identification of intrinsic and reflex contributions to human ankle stiffness dynamics. *IEEE Trans. Biomed. Eng*. 44(6):493–504, 1997.
[25]Kent-Braun, J. A., and A. V. Ng. Specific strength and voluntary muscle activation in young and elderly women and men. *J. Appl. Physiol. (1985)* 87(1):22–29, 1999.
[26]Kerrigan, D., M. Todd, and C. U. Della. Gender differences in joint biomechanics during walking: normative study in young adults. *Am. J. Med. Rehabil*. 77(1):2–7, 1998.
[27]Kubo, K., H. Kanehisa, and T. Fukunaga. Gender differences in the viscoelastic properties of tendon structures. *Eur. J. Appl. Physiol*. 88(6):520–526, 2003.
[28]Latash, M. L., and V. M. Zatsiorsky. Joint stiffness—myth or reality. *Human Mov. Sci*. 12(6):653–692, 1993.
[29]Lee, H., P. Ho, M. Rastgaar, H. I. Krebs, and N. Hogan. Multivariable static ankle mechanical impedance with active muscles. *IEEE Trans. Neural Syst. Rehabil. Eng*. 22(1):44–52, 2014.
[30]Lee, H., and N. Hogan. Time-varying ankle mechanical impedance during human locomotion. *IEEE Trans. Neural Syst. Rehabil. Eng*. 23(5):755–764, 2015.
[31]Lee, H., H. I. Krebs, and N. Hogan. Multivariable dynamic ankle mechanical impedance with active muscles. *IEEE Trans. Neural Syst. Rehabil*. 22(5):971–981, 2014.
[32]Lee, H., H. I. Krebs, and N. Hogan. Multivariable dynamic ankle mechanical impedance with relaxed muscles. *IEEE Trans. Neural Syst. Rehabil. Eng*. 22(6):1104–1114, 2014.
[33]Lee, H., E. Rouse, and H. I. Krebs. Summary of human ankle mechanical impedance during walking. *IEEE J. Transl. Eng. Health Med*. 4:1–7, 2016.
[34]Loram, I. D., and M. Lakie. Direct measurement of human ankle stiffness during quiet standing: the intrinsic mechanical stiffness is insufficient for stability. *J. Physiol. Lond*. 545(3):1041–1053, 2002.
[35]Miller, A. E., J. D. MacDougall, M. A. Tarnopolsky, and D. G. Sale. Gender differences in strength and muscle fiber characteristics. *Eur. J. Appl. Physiol. Occup. Physiol*. 66(3):254–262, 1993.
[36]Mirbagheri, M. M., H. Barbeau, and R. E. Kearney. Intrinsic and reflex contributions to human ankle stiffness: variation with activation level and position. *Exp. Brain Res*. 135(4):423–436, 2000.
[37]Montgomery, J., and D. Avers. Daniels and Worthingham's Muscle Testing: Techniques of Manual Examination (8th ed.). Philadelphia: Saunders, 2007.
[38]Nalam, V., H. Lee. Design and validation of a multi-axis robotic platform for the characterization of ankle neuromechanics. In: Proceedings of the IEEE International Conference on Robotics and Automation 2017 (ICRA 2017); Singapore.
[39]Nalam, V., H. Lee. A new robotic approach to characterize mechanical impedance and energetic passivity of the human ankle during standing. Paper presented at: In: Proceedings of the 39th Annual International Conference of IEEE Engineering Medicine and Biology Society, South Korea, 2017.
[40]Neptune, R. R., S. A. Kautz, and F. E. Zajac. Contributions of the individual ankle plantar flexors to support, forward progression and swing initiation during walking. *J. Biomech*. 34(11):1387–1398, 2001.
[41]Perry, J. Gait Analysis: Normal and Pathologic Functions. New Jersey: Slack Inc., 1992.
[42]Quatman, C. E., K. R. Ford, G. D. Myer, M. V. Paterno, and T. E. Hewett. The effects of gender and pubertal status on generalized joint laxity in young athletes. *J. Sci. Med. Sport*. 11(3):257–263, 2008.
[43]Ristolainen, L., A. Heinonen, B. Waller, U. M. Kujala, and J. A. Kettunen. Gender differences in sport injury risk and types of inju-ries: a retrospective twelve-month study on cross-country skiers, swimmers, long-distance runners and soccer players. *J. Sports Sci. Med*. 8(3):443–451, 2009.
[44]Robertson, D. G., and D. A. Winter. Mechanical energy generation, absorption and transfer amongst segments during walking. *J. Biomech*. 13(10):845–854, 1980.
[45]Rouse, E. J., R. D. Gregg, L. J. Hargrove, and J. W. Sensinger. The difference between stiffness and quasi-stiffness in the context of biomechanical modeling. *IEEE Trans. Biomed. Eng*. 60(2):562–568, 2013.
[46]Rouse, E. J., L. J. Hargrove, E. J. Perreault, and T. A. Kuiken. Estimation of human ankle impedance during the stance phase of walking. *IEEE Trans. Neural Syst. Rehabil. Eng*. 22(4):870–878, 2014.
[47]Shamaei, K., G. S. Sawicki, and A. M. Dollar. Estimation of quasi-stiffness and propulsive work of the human ankle in the stance phase of walking. *PLoS ONE* 8(3):e59935, 2013.
[48]Slauterbeck, J., C. Clevenger, W. Lundberg, and D. M. Burchfield. Estrogen level alters the failure load of the rabbit anterior cruciate ligament. *J. Orthop. Res*. 17(3):405–408, 1999.
[49]Stefanyshyn, D. J., and B. M. Nigg. Dynamic angular stiffness of the ankle joint during running and sprinting. *J. Appl. Biomech*. 14(3):292–299, 1998.
[50]Wilkerson, R. D., and M. A. Mason. Differences in men's and women's mean ankle ligamentous laxity. *Iowa Orthop. J*. 20:46–48, 2000.
[51]Winter, D. A. Human balance and posture control during standing and walking. *Gait Posture* 3:193–214, 1995.
[52]Winter, D. A., A. E. Patla, S. Rietdyk, and M. G. Ishac. Ankle muscle stiffness in the control of balance during quiet standing. *J. Neurophysiol*. 85(6):2630–2633, 2001.
[53]Zeller, B. L., J. L. McCrory, W. B. Kibler, and T. L. Uhl. Differences in kinematics and electromyographic activity between men and women during the single-legged squat. *Am. J. Sports Med*. 31(3):449–456, 2003.