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**Sex Differences in Heel Pad Stiffness during In-Vivo Loading and Unloading**

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## ABSTRACT

Due to conflicting data from previous studies a new methodological approach to evaluate heel pad stiffness and soft tissue deformation has been developed. The purpose of this study was to compare heel pad (HP) stiffness in both limbs between males and females during a dynamic unloading and loading activity. Ten males and ten females volunteered to perform three dynamic trials to unload and load the HP. The dynamic protocol consisted of three continuous phases: foot flat (baseline phase), bilateral heel raise (unloading phase) and foot flat (loading phase) with each phase lasting two seconds. Six retroreflective markers (3 mm) were attached to the skin of the left and right heels using a customised marker set. Three-dimensional motion analysis cameras synchronised with force plates collected the kinematic and kinetic data throughout the trials. Three-way repeated measures ANOVA together with a Bonferroni post hoc test were applied to the stiffness and marker displacement data sets. On average HP stiffness was higher in males than females during the loading and unloading phases. ANOVA results revealed no significant differences for the stiffness and displacement outputs with respect to sex, sidedness and phases interactions ( $P > 0.05$ ) in the X, Y and Z directions. Irrespective of direction, there were significant differences in stiffness between the baseline and unloading conditions ( $P < 0.001$ ) but no significant differences between the baseline and loaded conditions ( $P = 1.000$ ). Post hoc analyses for the marker displacement showed significant differences between phases for the X and Z directions ( $P < 0.032$ ) but no significant differences in the Y direction ( $P > 0.116$ ). Finally, females portrayed lower levels of mean HP stiffness while males had stiffer heels particularly in the vertical direction (Z) when the HP was both unloaded and loaded. High HP stiffness values and very small marker displacements could be valuable indicators for the risk of pathological foot conditions.

## KEYWORDS

marker displacement, foot flat, bilateral heel raise, plantar flexion, kinematics and kinetics

## INTRODUCTION

The calcaneal fat pad is comprised of fibro-adipose tissue that is designed to protect the lower limbs by bearing stress and dissipating impact shock during human locomotion (Wearing et al., 2014). Previous studies have shown that factors such as obesity and age may alter the elasticity, thickness and stiffness of the fat pads fibrous structure (Kwan et al., 2010, Pai and Ledoux, 2010). Pathology may affect heel pad stiffness and thickness. However, it is unclear whether sex may influence heel pad (HP) stiffness with research remaining equivocal within this area (Matteoli et al., 2012, Teoh and Lee, 2016). A lower maximal stiffness and higher elasticity within the HP has been indicated in young and adult females (Alcantara et al., 2002). On the other hand, young males have shown a significantly higher thickness in the midfoot fat pad when compared to young females (Mickle et al., 2008). Also, a recent study by Tas and colleagues suggests males have a significantly greater plantar fascia and heel fat pad thickness compared with females (Tas, 2018).

Contrasting soft tissue properties between sexes may predispose males and females to different diseases and injuries (Ozdemir et al., 2004). Stiffer heels have been associated with pathological foot conditions like plantar heel pain (PHP) which can have a detrimental impact on health by making it harder to perform the simple tasks that are needed for everyday living (League, 2008, Lin et al., 2015). Studying HP stiffness might provide further implications towards injury management and prevention between populations.

Clinically, HP stiffness has been commonly examined using in vitro analysis and equipment such as ultrasound and mechanical evaluation (Aerts et al., 1995, Egwu et al., 2012). Motion analysis systems have been used in the past to analyse functional and dysfunctional human gait patterns (Cappozzo et al., 2005, Chi and Schmitt, 2005) but are yet to be applied as a useful tool to investigate stiffness of the HP. A preliminary study by Santana and colleagues demonstrated that HP thickness, peak vertical force and HP stiffness were lower in females when compared with male counterparts (Santana et al., 2010). At present, no other study has investigated the deformation of the HP using motion analysis systems in conjunction with kinetic analysis systems. With the rise in the use of three-dimensional motion systems together with force plate technology within the biomechanics community, it is prudent that other measurement derivatives and outcome measures within the laboratory experimental environment can be obtained. One such measure is the deformation of the HP between sexes and differences between the dominant and non-dominant limbs in healthy participants or diseased participants. Although determining the mechanical and structural properties of the HP using unconventional techniques associated with motion analysis and force plate technology remain a key challenge, efforts have been focused on developing a method to determine the deformation of the soft tissues of the HP during the bodyweight loading and unloading phases of the dorsiflexion and plantar flexion movement. This methodological approach is considered robust as other biomechanical derivatives can be obtained from one single kinetic and kinematic data capture session.

The aim of this study was to compare the structural properties (stiffness) of the calcaneal fat pad in males and females during a dynamic loading and unloading task. The objectives were: (a) to measure the secant stiffness in the vertical (Z), anterior/posterior(X) and medial/lateral (Y) planes when both heels were unloaded and loaded; and (b) to determine the X, Y and Z displacements on the medial, central and lateral sides of the heel. The hypotheses for this study were; 1) HP stiffness will be higher in males compared to females, and 2) HP displacement will be less in males compared to females.

## METHODS

Ten healthy males (age  $26.3 \pm 11.7$  years, height  $180.2 \pm 4.5$  cm, body mass  $78.7 \pm 10.3$ kg; mean  $\pm$  SD) and ten healthy females (age  $22.2 \pm 11.6$  years, height  $164.3 \pm 6.0$  cm, body mass  $57.5 \pm 10.1$  kg; mean  $\pm$  SD) were recruited to take part in this study. Participants with a history of Achilles injury or PHP were excluded. Prior to testing, ethical approval was obtained from the University of the West of Scotland ethics committee and each participant provided written informed consent.

Kinetic data were measured and sampled at a frequency of 1000Hz using two force plates (AMTI, Watertown, US) embedded in concrete. Eight Vicon Nexus Bonita Motion Analysis (Oxford Metrics Ltd, UK) cameras sampled kinematic data at a rate of 250Hz and were placed on tripods positioned in a semi-circle surrounding the force plates. The positioning and height (44-77cm) of the cameras was labelled with tape to standardise the view of the retroreflective markers across participants. Both kinetic and kinematic output data were synchronised via the Vicon Motion Analysis system (Vicon Nexus 2.7.1, Oxford Metrics Ltd, UK).

Twelve retroreflective markers (3mm) were attached to the left and right heel (six markers on each heel). In accordance with Santana et al. (2010), the markers were positioned on the participants' skin using Double-sided Toupee Tape (30 m, Loughborough, United Kingdom) and were cut into 2mm individual squares. A customised template was used to standardise the placement of the markers on the skin. Participants were asked to stand barefoot with their weight distributed equally on both feet while the template was placed on the posterior aspect of the heel. The location of the template was marked with a Surgical Marking Pen (Medisave UK Ltd, UK) and the cut 2mm sized Toupee Tape was transferred to the marked areas on the heel. 3mm retroreflective markers were placed at two levels: middle and lower layers (Figure 1). Three retroreflective markers were placed along the lower circumference of the fat pad, while another three retroreflective markers were positioned on the middle section (upper surface of the calcaneus). The middle (MID\_1, MID\_2 and MID\_3) and lower (LOW\_1, LOW\_2 and LOW\_3) layer retroreflective markers from each heel were evaluated (Figure 1). These retroreflective markers represented the lateral, central and medial locations of the HP and upper surface of the calcaneus.

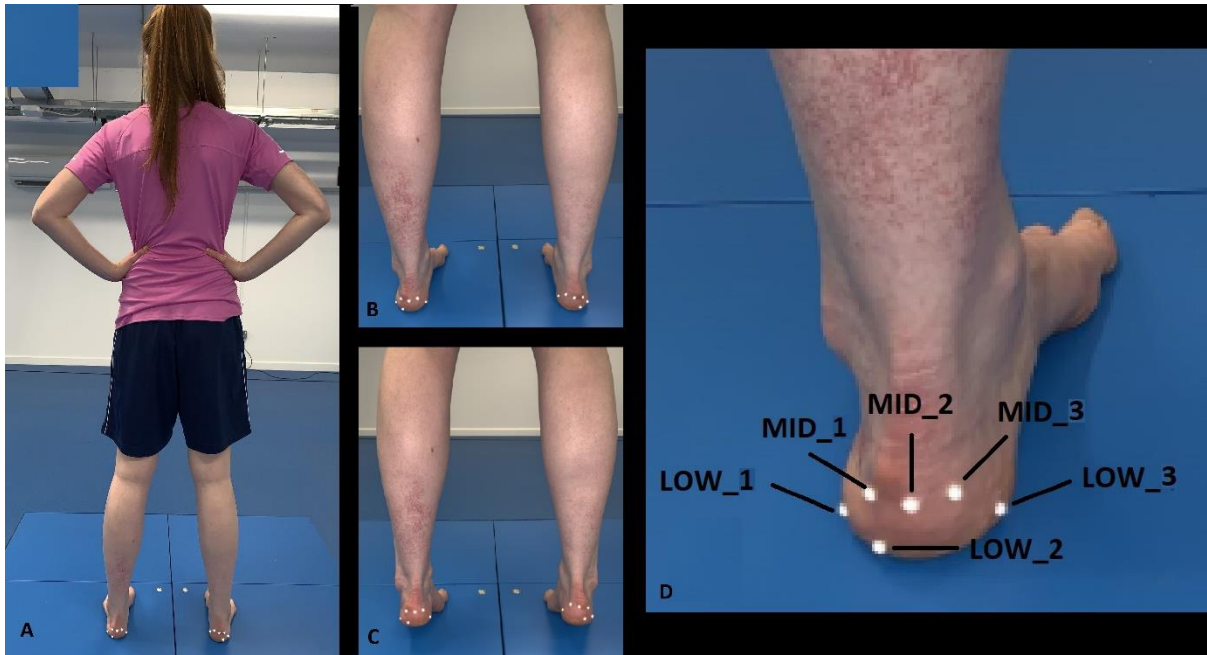


Figure 1: (A) Participant in static posture showing a set of six retroreflective markers positioned on two levels of both heels: middle and lower. (B) Heel pad position during foot flat, (C) Heel pad position during heel raise, and (D) Zoomed version of the 3mm retroreflective marker positioned on the heel pad. The central marker was randomly positioned anywhere within the middle of the heel provided this pattern matched both left and right heels.

Prior to testing, each participant was given a ten-minute familiarisation period to practise performing two-footed heel raises at a self-generated controlled speed. Participants were asked to stand on two force plates facing away from the cameras with hands on their hips. To account for sidedness all participants stood on two force plates positioned adjacent to each other; the left foot was positioned in the centre of the left force plate and the right foot was positioned in the centre of the right force plate (Ugbolue et al., 2019). The dynamic protocol involved three continuous phases: bilateral foot flat (baseline phase), bilateral heel raise (unloading phase) and bilateral foot flat (loading phase). This required participants to stand still then unload both heels by performing a two-footed heel raise. Participants then loaded the HP by placing both heels back on the ground. During the bodyweight unloading and loading process, forces on the HP were not isolated from forces on the ball of the foot. Each phase lasted two seconds and was verbally counted by a practitioner with the aid of a metronome. Three dynamic trials were recorded and analysed. The stiffness of the heel was evaluated based on the position of the HP during dynamic activity with respect to each phase (baseline, unloading and loading). Stiffness was calculated as the mean load of each phase divided by the corresponding displacement value (Hsu et al., 1998). The mean load in the anterior/posterior (X), medial/lateral (Y) and vertical (Z) directions were divided by their corresponding directional displacements. Displacement was defined as the difference between the heel positional phases (i.e. baseline, unloading and loading conditions) based on the marker orientation (i.e. X, Y, Z) in relation to marker position (i.e. lateral, central and medial) and biomechanical measure (i.e. vertical and horizontal directions). Regarding the lateral, central and medial marker positions, the vertical marker orientation gap was calculated as the difference between the mid markers (MID\_1, MID\_2 & MID\_3) and the low markers (LOW\_1, LOW\_2 & LOW\_3) i.e. (MID\_1 – LOW\_1); (MID\_2 – LOW\_2); (MID\_3 – LOW\_3). Similarly the horizontal marker orientation gap was calculated as the difference between (a)

the lateral and central mid markers (i.e. MID\_1 – MID\_2); (b) the central and medial mid markers (i.e. MID\_2 – MID\_3); (c) the lateral and central low markers (i.e. LOW\_1 – LOW\_2); and (d) the central and medial low markers (i.e. LOW\_2 – LOW\_3).

Kinematic and kinetic data were exported from the Vicon Nexus Bonita Motion System (Oxford Metrics Ltd, UK) as a .csv file and analysed using Microsoft Excel 2017 version 16.10 (Microsoft Corporation, Redmond, Washington). A 3-way repeated measures ANOVA was applied to the data recorded using IBM SPSS Statistics for Windows, Version 25.0. (IBM Corp., Armonk, New York). The within subject variable (dependent variable) was the marker position in the X, Y and Z directions. The between subject factors (independent variable) included sex, sidedness and phases. To determine the effect size, the Partial eta squared statistic ( $\eta_p^2$ ) in relation to an ANOVA was calculated. The values of 0.0099, 0.0588, and 0.1379 were considered small, medium, and large effect sizes respectively (Richardson, 2011). A Bonferroni post hoc test was applied to test for multiple comparisons in heel stiffness and marker displacements for observed means with respect to sex, sidedness and phases. Age and body mass index statistical differences between males and females were also examined. A P-value of <0.05 was considered significant.

## RESULTS

The descriptive statistics associated with the marker position for the females and males at different loading phases for the left and right limbs are illustrated in Figures 2 – 5. There were no significant differences between males and females regarding age ( $P=0.452$ ) and body mass index ( $P=0.060$ ). The ANOVA HP stiffness results indicate that there was a significant main effect for the marker positions (X:  $F=5.098$ ,  $P=0.016$ ,  $\eta_p^2=0.045$ , small; Y:  $F=315.318$ ,  $P<0.001$ ,  $\eta_p^2=0.747$ , large; Z:  $F=58.892$ ,  $P<0.001$ ,  $\eta_p^2=0.355$ , large). Apart from the marker position and sex interaction in the Y direction ( $F=5.673$ ,  $P=0.018$ ,  $\eta_p^2=0.050$ , small), no significant differences were observed for interactions between marker position and sex (X:  $F=0.721$ ,  $P=0.436$ ,  $\eta_p^2=0.007$ , small; Z:  $F=2.596$ ,  $P=0.094$ ,  $\eta_p^2=0.024$ , small). The interaction effect for marker position and sidedness was not significant (X:  $F=0.403$ ,  $P=0.587$ ,  $\eta_p^2=0.004$ , small; Y:  $F=0.077$ ,  $P=0.787$ ,  $\eta_p^2=0.001$ , small; Z:  $F=1.100$ ,  $P=0.320$ ,  $\eta_p^2=0.010$ , small). The marker position and phases interactions were significant (X:  $F=7.113$ ,  $P<0.001$ ,  $\eta_p^2=0.117$ , medium; Y:  $F=11.235$ ,  $P<0.001$ ,  $\eta_p^2=0.174$ , large; Z:  $F=15.548$ ,  $P<0.001$ ,  $\eta_p^2=0.225$ , large). The interaction effect between marker position, sex and sidedness was not significant (X:  $F=0.534$ ,  $P=0.517$ ,  $\eta_p^2=0.005$ , small; Y:  $F=2.940$ ,  $P=0.088$ ,  $\eta_p^2=0.027$ , small; Z:  $F=0.747$ ,  $P=0.437$ ,  $\eta_p^2=0.007$ , small). No significant difference was observed for interactions between marker position, sex and phases (X:  $F=1.746$ ,  $P=0.166$ ,  $\eta_p^2=0.032$ , small; Y:  $F=1.356$ ,  $P=0.262$ ,  $\eta_p^2=0.025$ , small; Z:  $F=0.790$ ,  $P=0.499$ ,  $\eta_p^2=0.015$ , small). No significant difference was observed for interactions between marker position, sidedness and phases (X:  $F=0.884$ ,  $P=0.442$ ,  $\eta_p^2=0.016$ , small; Y:  $F=0.080$ ,  $P=0.926$ ,  $\eta_p^2=0.002$ , small; Z:  $F=0.271$ ,  $P=0.841$ ,  $\eta_p^2=0.005$ , small). The interactions between marker position, sex, sidedness and phases was not significant (X:  $F=0.482$ ,  $P=0.674$ ,  $\eta_p^2=0.009$ , small; Y:  $F=0.369$ ,  $P=0.697$ ,  $\eta_p^2=0.007$ , small; Z:  $F=0.151$ ,  $P=0.925$ ,  $\eta_p^2=0.003$ , small).

The between subjects ANOVA yielded a significant effect for sex in the Y ( $F=8.403$ ,  $P=0.005$ ,  $\eta_p^2=0.073$ , medium) and Z directions ( $F=63.675$ ,  $P<0.001$ ,  $\eta_p^2=0.373$ , large) but no significant effect for sex in the X direction ( $F=1.519$ ,  $P=0.220$ ,  $\eta_p^2=0.014$ , small). In terms of sidedness no significant differences between subjects effects was observed (X:  $F=0.580$ ,  $P=0.448$ ,  $\eta_p^2=0.005$ , small; Y:  $F=0.183$ ,  $P=0.670$ ,  $\eta_p^2=0.002$ , small; Z:  $F=0.843$ ,  $P=0.361$ ,  $\eta_p^2=0.008$ , small). However, a significant effect was observed for phases in all directions (X:  $F=9.783$ ,  $P<0.001$ ,  $\eta_p^2=0.155$ , large; Y:  $F=10.161$ ,  $P<0.001$ ,  $\eta_p^2=0.160$ , large; Z:  $F=211.725$ ,  $P<0.001$ ,  $\eta_p^2=0.798$ , large). Although significant between subjects, effects were

observed for interactions between sex and phases in the Z ( $F=5.983$ ,  $P=0.003$ ,  $\eta_p^2=0.101$ , medium) direction, no significant interactions between the subjects effects for sex and sidedness (X:  $F=0.936$ ,  $P=0.336$ ,  $\eta_p^2=0.009$ , small; Y:  $F=1.726$ ,  $P=0.192$ ,  $\eta_p^2=0.016$ , small; Z:  $F=0.402$ ,  $P=0.527$ ,  $\eta_p^2=0.004$ , small), sex and phases (X:  $F=2.746$ ,  $P=0.069$ ,  $\eta_p^2=0.049$ , small; Y:  $F=0.653$ ,  $P=0.523$ ,  $\eta_p^2=0.012$ ), sidedness and phases (X:  $F=0.827$ ,  $P=0.440$ ,  $\eta_p^2=0.015$ , small; Y:  $F=0.016$ ,  $P=0.984$ ,  $\eta_p^2=0.0003$ , small; Z:  $F=0.443$ ,  $P=0.643$ ,  $\eta_p^2=0.008$ ) and sex, sidedness and phases (X:  $F=0.196$ ,  $P=0.823$ ,  $\eta_p^2=0.004$ , small; Y:  $F=0.123$ ,  $P=0.885$ ,  $\eta_p^2=0.002$ , small; Z:  $F=0.007$ ,  $P=0.993$ ,  $\eta_p^2=0.0001$ , small) were observed. The post hoc analysis showed similar results in all measured stiffness directions. Irrespective of direction, there were significant differences between the baseline and unloading conditions ( $P<0.001$ ) but no significant differences between the baseline and loaded conditions ( $P=1.000$ ).

The descriptive statistical outputs for the vertical marker and horizontal marker displacements are shown in Table 1. The within subjects effects for the vertical displacement indicate there was a significant main effect (X:  $F=532.927$ ,  $P<0.001$ ,  $\eta_p^2=0.831$ , large; Y:  $F=5.261$ ,  $P=0.016$ ,  $\eta_p^2=0.046$ , small; Z:  $F=26.906$ ,  $P<0.001$ ,  $\eta_p^2=0.199$ , large). All vertical displacement and sex interactions in the X, Y and Z directions (X:  $F=1.223$ ,  $P=0.292$ ,  $\eta_p^2=0.011$ , small; Y:  $F=0.963$ ,  $P=0.348$ ,  $\eta_p^2=0.009$ , small; Z:  $F=0.615$ ,  $P=0.520$ ,  $\eta_p^2=0.006$ , small) showed no significant differences. The vertical displacement and sidedness interactions produced significant differences in both X and Y directions (X:  $F=5.894$ ,  $P=0.005$ ,  $\eta_p^2=0.052$ , small; Y:  $F=430.446$ ,  $P<0.001$ ,  $\eta_p^2=0.799$ , large) whereas no significant differences were observed in the Z direction (Z:  $F=0.067$ ;  $P=0.915$ ,  $\eta_p^2=0.001$ , small). Vertical displacement and phases interactions produced only one significant difference in the Z direction (Z:  $F=14.578$ ,  $P<0.001$ ,  $\eta_p^2=0.213$ , large). All other vertical displacement outputs including interactions between vertical displacement with sex and phases; sidedness and phases; sex, sidedness and phases; all showed no significant differences in their interactions ( $F>0.058$ ,  $P>0.05$ ,  $\eta_p^2<0.0588$ , small) in the X, Y and Z directions.

The between subjects' effects produced significant differences for sex in the X direction (X:  $F=4.966$ ,  $P=0.028$ ,  $\eta_p^2=0.044$ , small). Significant differences in the Y direction were observed for sidedness (Y:  $F=272.762$ ,  $P<0.001$ ,  $\eta_p^2=0.716$  large) and interactions between sidedness and phases (Y:  $F=14.809$ ,  $P<0.001$ ,  $\eta_p^2=0.215$ , large). All other interactions in the X, Y and Z directions for sex and sidedness; sex and phases; and sex, sidedness and phases all produced no significant differences ( $F>0.021$ ,  $P>0.05$ ,  $\eta_p^2<0.0588$ , small) with respect to the between subjects' effects. The post hoc analyses showed significant differences between phases for the X and Z directions ( $P<0.001$ ) but no significant differences for the Y direction ( $P>0.116$ ).

The ANOVA HP horizontal displacement results for the within subjects effects indicate that there were significant differences in the X and Z directions (X:  $F=2673.273$ ,  $P<0.001$ ,  $\eta_p^2=0.961$ , large; Z:  $F=137.796$ ,  $P<0.001$ ,  $\eta_p^2=0.561$ , large) but not in the Y direction (Y:  $F=0.962$ ,  $P=0.385$ ,  $\eta_p^2=0.009$ , small). No significant differences were observed for the horizontal displacement and sex interactions in the Z direction (Z:  $F=0.876$ ,  $P=0.406$ ,  $\eta_p^2=0.008$ , small), however, significant differences were observed for the horizontal displacement and sex interactions in the X and Y directions (X:  $F=3.381$ ,  $P=0.039$ ,  $\eta_p^2=0.030$ , small; Y:  $F=3.496$ ,  $P=0.031$ ,  $\eta_p^2=0.031$ , small). The horizontal displacement and sidedness interactions showed significant differences in the Y direction (Y:  $F=819.838$ ,  $P<0.001$ ) but no significant differences in the X and Z directions (X:  $F=1.936$ ,  $P=0.150$ ,  $\eta_p^2=0.018$ , small; Z:  $F=0.450$ ,  $P=0.612$ ,  $\eta_p^2=0.004$ , small). The horizontal displacement and phases interactions produced no significant differences in the Y direction (Y:  $F=0.303$ ,  $P=0.880$ ,  $\eta_p^2=0.006$ , small) but significant differences in the X and Z directions (X:  $F=3.223$ ,  $P=0.015$ ,  $\eta_p^2=0.056$ , small; Z:  $F=75.190$ ,  $P<0.001$ ,  $\eta_p^2=0.582$ , large). All other combined interactions with horizontal displacement such as sex and sidedness; sex and phases;



sidedness and phases; and sex, sidedness and phases; produced no significant differences in the X Y and Z directions ( $F > 0.361$ ,  $P > 0.095$ ,  $\eta_p^2 < 0.0588$ , small).

The between subjects effects for the horizontal displacement yielded no significant differences in the Y and Z directions (Y:  $F = 0.805$ ,  $P = 0.372$ ,  $\eta_p^2 = 0.007$ , small; Z:  $F = 0.639$ ,  $P = 0.426$ ,  $\eta_p^2 = 0.006$ , small) with respect to sex but significant differences in the X direction (X:  $F = 26.300$ ,  $P < 0.001$ ,  $\eta_p^2 = 0.196$ , large). Significant differences were observed in the Y and Z directions (Y:  $F = 24137.971$ ,  $P < 0.001$ ,  $\eta_p^2 = 0.996$ , large; Z:  $F = 4.335$ ,  $P = 0.040$ ,  $\eta_p^2 = 0.039$ , small) for the horizontal displacement with respect to sidedness, however, no significant differences were observed for X direction (X:  $F = 0.786$ ,  $P = 0.377$ ,  $\eta_p^2 = 0.007$ , small). Regarding the phases, both X and Z directions (X:  $F = 5.651$ ,  $P = 0.005$ ,  $\eta_p^2 = 0.095$ , medium; Z:  $F = 37.075$ ,  $P < 0.001$ ,  $\eta_p^2 = 0.407$ , large) produced significant differences but no significant difference in the Y direction (Y:  $F = 0.227$ ,  $P = 0.797$ ,  $\eta_p^2 = 0.004$ , small) was observed. The interactions between sex and sidedness produced significant differences for the X, Y and Z directions (X:  $F = 7.582$ ,  $P = 0.007$ ,  $\eta_p^2 = 0.066$ , medium; Y:  $F = 20.382$ ,  $P < 0.001$ ,  $\eta_p^2 = 0.159$ , large; Z:  $F = 7.706$ ,  $P = 0.006$ ,  $\eta_p^2 = 0.067$ , medium). All other interactions with respect to sex and phases; sidedness and phases; and sex, sidedness and phases produced no significant differences in the X, Y and Z directions ( $F > 0.005$ ,  $P > 0.05$ ,  $\eta_p^2 < 0.0588$ , small). The post hoc tests between the phases showed significant differences in the X direction ( $P < 0.032$ ) and Z direction ( $P < 0.001$ ), however, no significant differences were observed in the Y direction ( $P > 0.05$ ).

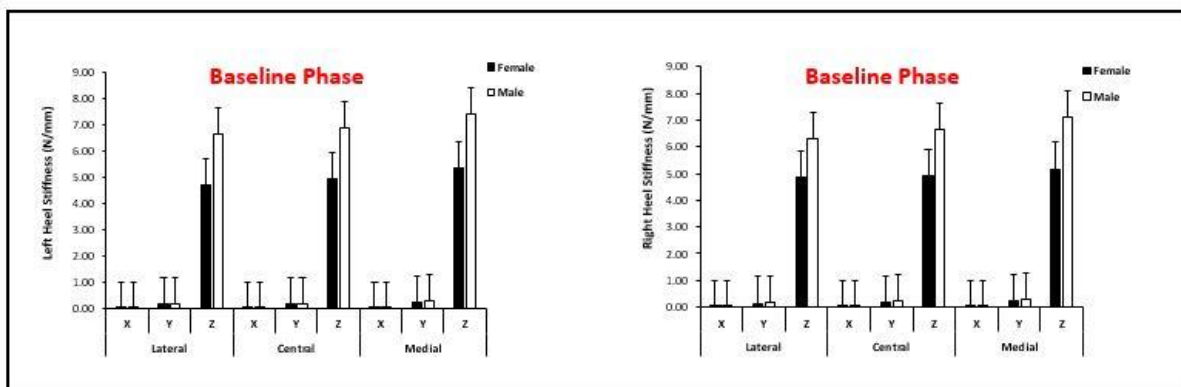


Figure 2: Mean sex differences in the left heel pad stiffness and right heel pad stiffness with respect to the anterior/posterior (X), medial/lateral (Y) and vertical (Z) planes at each location (lateral, central and medial) during the baseline phase with error bars ( $\pm$  standard deviation) ( $n = 20$ ).

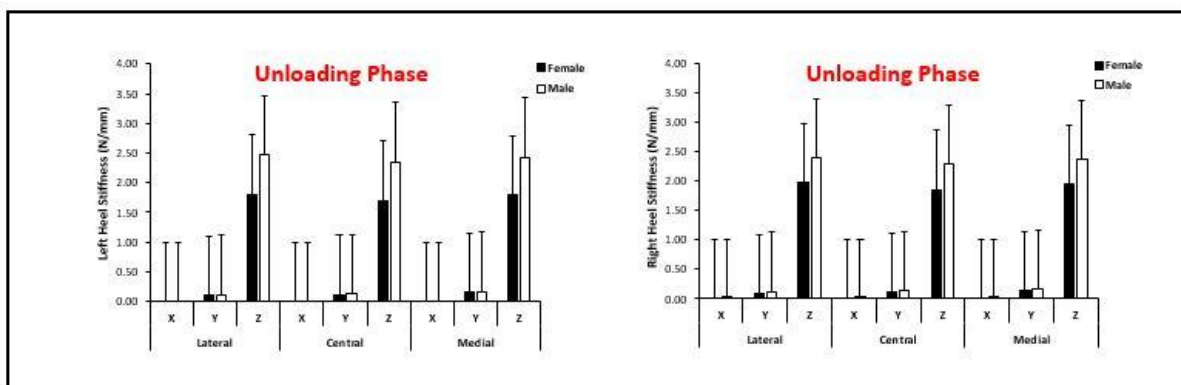


Figure 3: Mean sex differences in the left heel pad stiffness and right heel pad stiffness with respect to the anterior/posterior (X), medial/lateral (Y) and vertical (Z) planes at each location (lateral, central and medial) during the bodyweight unloading phase with error bars ( $\pm$  standard deviation) (n=20).

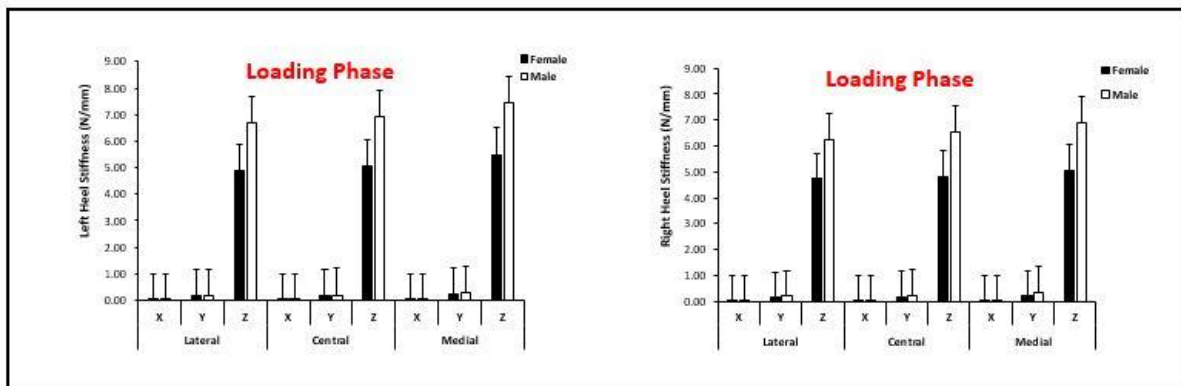


Figure 4: Mean sex differences in the left heel pad stiffness and right heel pad stiffness with respect to the anterior/posterior (X), medial/lateral (Y) and vertical (Z) planes at each location (lateral, central and medial) during the bodyweight loading phase with error bars ( $\pm$  standard deviation) (n=20).

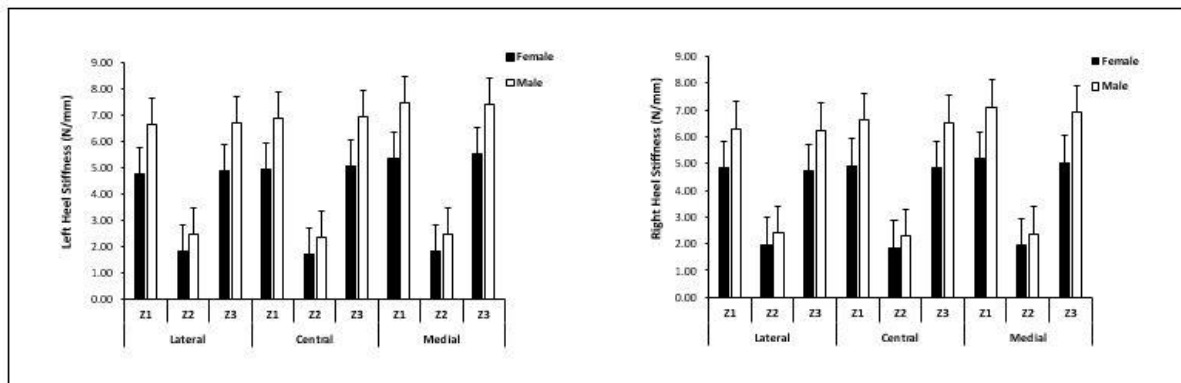








Figure 5: Mean sex differences in the vertical (Z) plane showing the left heel pad stiffness and right heel pad stiffness during the bodyweight loading phases (baseline (Z1), unloading (Z2) and Loading (Z3)) at each location (lateral, central and medial) with error bars ( $\pm$  standard deviation) (n=20).

Table 1: Descriptive results showing the displacements in terms of heel positional phase, marker position and marker orientation gap for the left and right limbs

Heel Positional Phase	Marker Position	Biomechanical Measure	Left Limb Marker Orientation Gap (Mean (SD))			Right Limb Marker Orientation Gap (Mean (SD))			
			X	Y	Z	X	Y	Z	
	Baseline	Lateral	Vertical Marker Displacement (mm)	10.29 (4.27)	13.38 (2.88)	17.17 (2.68)	9.06 (3.04)	-12.94 (2.64)	17.87 (2.51)
		Central	Vertical Marker Displacement (mm)	1.03 (3.48)	12.90 (2.59)	18.03 (3.01)	0.81 (2.26)	-12.02 (3.70)	18.78 (1.95)
		Medial	Vertical Marker Displacement (mm)	12.47 (3.85)	-5.89 (3.19)	19.79 (2.80)	13.35 (4.80)	5.86 (2.85)	20.71 (2.92)
	Unloading	Lateral	Vertical Marker Displacement (mm)	0.11 (3.70)	8.86 (3.38)	21.75 (1.80)	-1.28 (3.23)	-7.78 (2.92)	21.68 (1.58)
		Central	Vertical Marker Displacement (mm)	-8.06 (2.38)	8.45 (2.14)	17.93 (1.92)	-8.47 (2.13)	-7.30 (3.31)	18.11 (1.84)
		Medial	Vertical Marker Displacement (mm)	2.44 (4.33)	-8.45 (2.45)	21.32 (1.97)	4.22 (4.10)	7.48 (7.53)	21.04 (4.06)
	Loading	Lateral	Vertical Marker Displacement (mm)	10.24 (4.42)	13.33 (2.94)	17.13 (2.71)	8.28 (3.24)	-7.30 (11.78)	17.26 (3.06)
		Central	Vertical Marker Displacement (mm)	1.18 (2.99)	12.74 (2.32)	18.26 (2.43)	0.58 (2.13)	-6.55 (10.92)	18.65 (2.51)
		Medial	Vertical Marker Displacement (mm)	12.69 (3.87)	-5.91 (3.14)	19.80 (2.81)	13.12 (4.33)	3.85 (6.08)	19.86 (2.78)
	Baseline	Middle Row: From Lateral to Central	Horizontal Marker Displacement (mm)	-4.78 (2.15)	-15.48 (1.30)	1.37 (1.74)	-5.59 (3.40)	15.42 (1.77)	0.69 (1.93)
		Middle Row: From Central to Medial	Horizontal Marker Displacement (mm)	7.65 (1.68)	-14.52 (1.67)	2.58 (1.50)	7.34 (2.26)	14.60 (0.97)	1.39 (1.83)
		Lower Row: From Lateral to Central	Horizontal Marker Displacement (mm)	-14.04 (3.00)	-15.97 (3.36)	2.23 (3.09)	-13.84 (3.12)	16.34 (4.03)	1.61 (3.43)
		Lower Row: From Central to Medial	Horizontal Marker Displacement (mm)	19.09 (4.77)	-33.30 (4.05)	4.31 (4.42)	19.87 (5.20)	32.48 (4.57)	3.32 (4.54)
	Unloading	Middle Row: From Lateral to Central	Horizontal Marker Displacement (mm)	-2.86 (1.60)	-15.60 (1.21)	-4.23 (1.53)	-3.13 (3.41)	15.52 (1.37)	-4.72 (1.81)
		Middle Row: From Central to Medial	Horizontal Marker Displacement (mm)	7.79 (1.52)	-14.54 (1.58)	3.52 (1.63)	7.51 (1.75)	14.32 (1.29)	2.69 (1.75)
		Lower Row: From Lateral to Central	Horizontal Marker Displacement (mm)	-11.03 (3.09)	-16.01 (2.85)	-8.05 (1.76)	-10.32 (3.15)	16.00 (3.84)	-8.30 (2.65)
		Lower Row: From Central to Medial	Horizontal Marker Displacement (mm)	18.30 (3.48)	-31.45 (3.34)	6.90 (3.80)	20.70 (5.45)	30.11 (3.72)	7.46 (3.12)
	Loading	Middle Row: From Lateral to Central	Horizontal Marker Displacement (mm)	-4.82 (2.07)	-15.51 (1.34)	1.28 (1.71)	-4.74 (4.37)	13.78 (6.73)	1.50 (4.08)
		Middle Row: From Central to Medial	Horizontal Marker Displacement (mm)	7.65 (1.60)	-14.58 (1.77)	2.55 (1.46)	7.47 (2.20)	14.49 (1.07)	1.32 (1.87)
		Lower Row: From Lateral to Central	Horizontal Marker Displacement (mm)	-13.88 (3.30)	-16.09 (3.26)	2.41 (3.08)	-12.83 (3.62)	16.69 (4.11)	1.77 (3.18)
		Lower Row: From Central to Medial	Horizontal Marker Displacement (mm)	19.16 (4.24)	-33.24 (3.78)	4.09 (3.56)	19.68 (5.69)	32.44 (4.65)	2.60 (4.47)

± signs suggest marker direction of movement. X: anterior (+), posterior (-); Y: medial (-), lateral (+) and Z: vertical directions (±)

## DISCUSSION

This study examined the heel pad (HP) stiffness between males and females during a dynamic bodyweight unloading and loading activity. The findings of the study indicated that females portrayed lower levels of mean HP stiffness whereas, males had stiffer heels particularly at the vertical direction (Z) when the HP was unloaded and loaded. Likewise, an experimental study by Matteoli et al. (2012) indicated that HP stiffness was significantly reduced in females compared to males when the dominant heel was loaded using a compression instrument. Similarly, using an ultrasonography device, the results reported by Tas and associates showed that the plantar fascia and HP stiffness were similar in both sexes; however, females had a lower plantar fascia and HP thickness compared with males (Tas, 2018). One possible reason for this outcome could be that research suggests that females may be more susceptible to softer heels because of higher levels of oestrogen in comparison to males (Rome, 1998). Additionally, potent levels of oestrogen within the female body during different phases of the menstrual cycle have been linked with reduced stiffness in other soft tissues like muscles and tendons (Bell et al., 2012, Eiling et al., 2007). In contrast, a small participant study by Boros and Challis (2003) found that females had a greater HP stiffness ( $3.13 \pm 0.7$  N/mm) compared to males ( $2.58 \pm 0.5$  N/mm) when the right HP was examined using an indentation device.

This present study found that left HP stiffness was significantly lower in females at the anterior/posterior (X) direction during the baseline phase and that right HP stiffness was significantly reduced in females than males at the medial/lateral (Y) direction when the heel was loaded. These results highlight the viscoelastic behaviour of the fat pad and shows that HP stiffness follows a non-linear pattern (Declercq et al., 1994). Despite this, the stiffness within the HP is commonly tested by equipment such as ballistic pendulum and indentation which often analyse the HP in a vertical loading direction (Aerts et al., 1995). As a result of this, the literature seems to be controversial when determining the influence of sex on HP stiffness (Alcantara et al., 2002, Borros and Challis, 2003, Matteoli et al., 2012, Teoh and Lee, 2016).

Stiffness was significantly higher in male participants in the vertical direction (Z) when the left and right HP was unloaded. In our study, there were no significant differences between males and females for age and body mass index anthropometry. The inability of the HP to recover to its natural form after deformation has also been demonstrated in aged heels (Hsu et al., 1998). Furthermore, research by Kinoshita and associates suggests that a higher degree of stiffness in an unloaded HP may be because of disorganised fibro-adipose tissue inhibiting the capability of the HP to re-coil after compression (Kinoshita et al., 1996). However, there is a lack of research investigating the HP during an unloaded state with the majority of research using compression and indentation devices which measure stiffness by applying small loads to the surface of the fat pad (Challis et al., 2008, Rome et al., 2001). This may not represent the true characteristics of the fat pad when the heel is unloaded and off the ground.

The HP vertical and horizontal displacements disclosed a trend in the measurement outputs. Sex, sidedness and phases for both the vertical and horizontal displacements all showed no significant differences in their interactions in the X, Y and Z directions with respect to within and between subject effects. Furthermore, regarding the phases, post hoc analyses revealed that there were no significant differences in the Y direction but significant differences in the X and Z directions for both the vertical and horizontal marker displacements. During the bodyweight unloading and loading conditions the vertical marker displacement produced larger displacements in the X direction compared to the Y and Z directions, while the horizontal marker displacement produced larger displacements in the Z direction and larger horizontal marker displacements in the lower row compared to the middle row. These findings may be due to the changes in soft tissue mechanics of the HP and Achilles tendon during

ankle plantarflexion (i.e. from baseline to the unloading phase) and ankle dorsiflexion (i.e. from unloading to the loading phase).

Aside from the HP medial marker position which showed a larger Y directional vertical displacement among the males, all females produced a larger X and Z directional vertical displacement at the lateral and central HP marker positions. This outcome partially supports our hypotheses which infers that less displacement may suggest stiffer heels. The horizontal displacement in the Y direction for both the middle and lower rows were larger in males but inconsistent and variable in the X and Z directions. This outcome measure also suggests that the HP horizontal marker displacements in the X and Z directions appear unclear due to the variability in the viscoelastic properties of the HP. Our study is unique and thus cannot be accurately compared to previous work. The distribution of the structural and mechanical properties from a three-dimensional perspective warrants further discussion.

It is worth considering the implications of stiffer and softer heels between sexes and how these properties may be linked to different pathological conditions. Several studies have demonstrated that individuals with PHP have significantly stiffer heels (Lin et al., 2015, Prichasuk, 1994, Prichasuk et al., 1994, Tong et al., 2003). Despite this, there were contradictory results from other studies showing that a softer HP was associated with PHP in runners (Rome et al., 2001). This suggests that the different HP properties between males and females may result in one sex being more likely to be predisposed to musculoskeletal injury or pathological foot conditions. Therefore, future research should investigate the difference in HP stiffness and HP marker displacement between a healthy cohort compared with patients diagnosed with PHP. Also, measurements of strain caused by changes in the unloading or loading heel positional phase with respect to the marker orientation, marker position and biomechanical measure need further investigation in both healthy and patient cohorts. In addition, based on the marker orientation and marker position, Poisson Ratio expressed as the ratio of the horizontal strain to the vertical strain with respect to the heel positional phase (i.e. unloading and loading conditions) are research areas the group are currently working on.

This current study has some limitations. The thickness of the HP is an important component that may influence the biomechanical response of the HP in males and females during dynamic activity which involves loading. However, due to this study focusing solely on HP stiffness and HP marker displacement, parameters of HP thickness were not measured. Menstrual cycle status within female participants was not taken into consideration during this study. Therefore, menstrual cycle fluctuations could have affected the properties of the HP which may have influenced the results when comparing the stiffness between males and females. In addition, foot posture and gait were not accounted for when evaluating HP stiffness and marker displacements. Also, there was no control for skin marker artefacts by fixing two markers on the ankle bony landmark. This may have affected the results by altering the dynamic loading of the HP. Lastly, this study only recruited twenty healthy participants. It is expected that a larger scale study with different sexes, ages, physical activity levels as well as pathologies would provide additional insights that broaden our understanding of heel pad stiffness.

## CONCLUSION

To conclude, the findings from the study indicate that mean HP stiffness was higher in males than females in the vertical plane (Z) during the unloading and loading of both heels. Examining HP stiffness using motion analysis may provide important information on the physical properties of the underlying soft tissues and will benefit patients by being non-invasive. Thus, higher stiffness and low vertical and

horizontal marker displacements may be useful indicators for the risk of pathological foot conditions. However, further research is required before definitive conclusions can be made.

#### AUTHOR CONTRIBUTIONS

All authors contributed to the development of this manuscript.

UU, EY, and SW were involved in the experimental design. UU and EY were involved in the data collection. UU, EY, SW and WL were involved with the data processing and analyses. UU, EY, SW, YG, WL, JSB, SV, FD and NS were involved in the writing and proof reading of the manuscript.

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