

Foot morphology influences the change in arch index between standing and walking conditions

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Abstract

Human foot morphology has been of interest to anatomists, clinicians, and paleontologists for a century due to its importance in bipedal walking. Foot shape changes as forces move through it from the body to the substrate. Although the arch of the foot has been extensively evaluated, the role of foot morphology in the change of the arch height in walking is less explored. To remedy this lacuna, the Arch Indices (AIs) of the left and right feet of 77 people were calculated in double and single stance standing and walking (dynamic) conditions. The feet were categorized into clinical foot types (cavus, normal, planus). The change in static AI between double and single stance was used to predict dynamic AI and the difference between predicted and observed dynamic AI was examined. As expected, AIs increased (i.e., arch height decreased) with increasing load on the foot for the entire sample and each foot type (p 's > .001), but the ability of change in static AIs to predict dynamic AI varied among foot types, implicating the possibility of variability in foot mechanics among foot types. While planus feet change stiffness during walking, presumably due to muscular action, cavus feet are more variable in their response to load. Static and dynamic AIs are effective in reflecting the changes in foot stiffness that occur in walking and future work should examine the role of extrinsic muscle activation in this stiffness change.

KEYWORDS

footprints, loading, pedal shape

1 | INTRODUCTION

The human foot has been understood as a complex structure for decades (e.g., Morton, 1924a, 1924b, 1924c) that is based on longitudinal and transverse arches and created and maintained by interlocking tarsals (e.g., the wedge-shaped cuneiforms) that are connected by ligaments (e.g., the spring ligament) with reinforcement via muscular action (e.g., adductor hallucis). During bipedal locomotion, the foot is the interface between the rest of the body and the substrate and vertical forces in the foot when moving at comfortable walking gaits exceed 1.1 times body weight

(e.g., Winter, 1987) with faster walking velocities and burdened movement producing higher ground reaction forces (e.g., Inman et al., 1981; Winter, 1987). The ground reaction force for humans walking in a straight path is well-characterized and consistent among people with a peak force in the braking portion of stance and another in the propulsion portion (e.g., Inman et al., 1981; Sylvester et al., 2021; Winter, 1987). For comfortable walking velocity, the braking peak occurs ~20% of stance while the propulsion peak occurs ~80% of stance, both of which peaks occur when the entire foot is in contact with the substrate (e.g., Sylvester et al., 2021). The triceps surae muscle

complex is responsible for propulsion in bipedal walking (Morton, 1924b) and the muscle force produced by the complex peaks at ~80% of stance (e.g., Sylvester et al., 2021).

In any structure, the application of a load produces displacement that is related to stiffness, which is determined by the material and geometric properties of the structure (Budynas & Sadegh, 2020). In the foot, the vertical component of the ground reaction force of walking, which is the reaction to the vertical force produced by gravity, serves to flatten the longitudinal arch (e.g., Sichting & Ebrecht, 2021). The stiffness of the foot in an individual can vary due to the activation of muscles because resistance to that flattening is influenced by the efficacy of the bony and ligamentous structures and by muscular action (e.g., Kelly et al., 2014; Kelly et al., 2015; Venkadesan et al., 2020; Welte et al., 2018). When the muscles are less active, such as in sitting and quiet standing, the arch is at its most compliant because only bony interaction and ligamentous connection hold the structure together (Welte et al., 2018). In walking, contraction of the triceps surae complex serves both to propel the body forward (e.g., Inman et al., 1981; Perry et al., 2010; Sylvester et al., 2021) and to reinforce the longitudinal arch and activation of the intrinsic muscles of the foot parallels that of triceps surae (Kelly et al., 2015, 2019). This reinforcement of triceps surae occurs due to the Achilles tendon pulling on the distal calcaneal tuber, which produces a moment that tends to rotate the calcaneus about the subtalar joint in an opposite direction from the rotation caused by the intrinsic muscles, thereby providing support for the pull from the windlass mechanism and the intrinsic muscles of the foot that reinforces the arch (Figure 1; Morton, 1924b). The Achilles tendon resists the passive pull of the plantar fascia and contraction of the intrinsic muscles of the foot. For example, the triceps surae muscle complex anchors the actions of the windlass mechanism and the intrinsic muscles, which are an important reinforcement of the longitudinal arch (Kelly et al., 2014; Figure 1). Consequently, the arch should be stiffer when the triceps surae complex and the intrinsic muscles of the foot are active (in midstance through toe-off phases of walking) than it is when the muscles are inactive. This enhanced rigidity is particularly critical once the heel has lifted off the ground (heel rise), making the foot a cantilevered beam (Welte et al., 2018). This facultative aspect of arch shape has been understood as critical to foot function and, hence, bipedal walking and as the result of selective pressures over millions of years of hominin evolution (e.g., DeSilva & Throckmorton, 2010; Farris et al., 2019; Lovejoy et al., 2009).

Despite the long-appreciated importance of the arch to bipedalism (Le Gros Clark, 1960; Morton, 1924a,

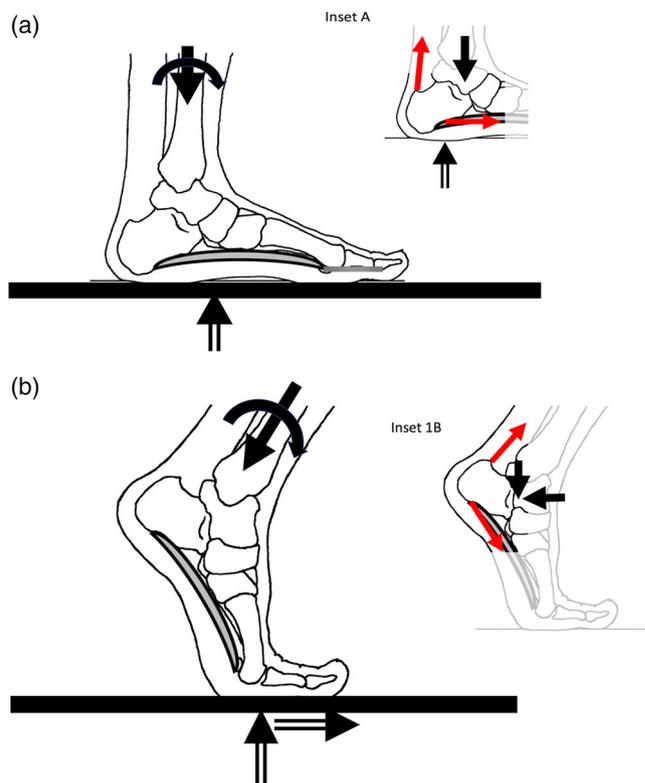


FIGURE 1 Freebody diagram of a normal foot at midstance (a) and after heel rise (b). Insets represent the freebody diagram of the calcaneus shown in its anatomical context. The ground reaction force (parallel black arrow) acts at the center of pressure on the contact surface of the foot and is balanced by a force and moment (solid black arrows). When the entire sole of the foot is in contact with the substrate (e.g., inset at midstance), the calcaneus is loaded by some portion of the ground reaction force pressure (shown as smaller parallel arrows), the pull of the intrinsic soft tissues and Achilles tendon (red arrows), and the subtalar joint (black arrows). Note that the portion of the ground reaction force pressure that the calcaneus experiences diminishes as the stance phase progresses and the center of pressure moves distally on the foot. When the foot contact is limited to the ball of the foot (e.g., inset B in terminal stance), the calcaneus is loaded by the soft tissues and Achilles tendon (red arrows), and the subtalar joint (black arrows). Note that although B is drawn late in the stance phase to clearly distinguish it from foot flat, the calcaneal freebody obtains for any point after heel rise (assuming no midtarsal break). (In all cases the influence of the cuboid on calcaneal loading is ignored.) illustration by M. Brynne

1924b), human feet vary in their shape and rigidity. Generally, this variation has been trichotomized into foot types (i.e., high arch (cavus), low arch (planus), and “normal” arch (rectus) feet (Cavanagh & Rodgers, 1987; Morton, 1924b), but the variation is continuous. Many ways to assess arch shape directly have been used, including calcaneal inclination angle (e.g., Agoada & Kramer, 2020; Lautzenheiser & Kramer, 2013; Sanner &

Whitney, 2015), talocalcaneal angles (e.g., Sanner & Whitney, 2015), Méary's angle (e.g., Bourdet et al., 2013), and navicular height (e.g., Agoada & Kramer, 2020; Butler et al., 2006; Zifchock et al., 2019). Angular measurements of the orientation of the tarsals assess the orientation of the tarsals to the substrate and each other, but require invasive imaging and do not provide information about arch stiffness. Consequently, externally accessible proxies are used. Navicular height (measured to the superior surface of the dorsum of the foot) can be measured on radiographs and through external means such as with the AHIMS (Zifchock et al., 2006). Foot stiffness can be assessed by applying an external force to the leg and measuring the change in navicular height, which is typically done with the subject seated and with force applied through the knee (e.g., Kelly et al., 2014; Welte et al., 2018; Zhao et al., 2020). This method of assessing navicular height and, through its change, arch stiffness is not feasible for dynamic conditions like walking (Bjelopetrovich & Barrios, 2016). Measuring dynamic navicular height requires access to a gait lab (e.g., Kelly et al., 2014, 2019; Sichting & Ebrecht, 2021; Welte et al., 2018), which is impractical for most clinical applications (e.g., Franettovich et al., 2007).

In addition to navicular height, the shape of the foot can also be observed externally by examining the shape of the footprint, the outline of the contact surface between the substrate and the sole of the foot. Feet with “normal” arches have a distinctive footprint shape with contiguous contact between the sole of the foot and the substrate from hindfoot (under the calcaneus), through the lateral midfoot (under the cuboid and fifth metatarsal), and across the forefoot (under the metatarsal heads) (Figure 2). The “arch” is seen on a normal footprint by the lack of contact on the medial side of the foot. Cavus feet have contact under the hindfoot and metatarsal heads, but little or no contact in the (lateral) midfoot. Planus feet are characterized by broad contact in the midfoot, which in more extreme cases fills in the medial “void” (Figure 2c). Consequently, one way to assess the shape of the arch is to evaluate the amount of sole-to-substrate contact in the midfoot. Multiple methods of assessing the amount of midfoot contact have been proposed, including the arch index (AI), a ratio of midfoot to total foot area (Cavanagh & Rodgers, 1987), and the footprints for individuals from many different groups (e.g., Igbigbi, 2005; D'Aout et al., 2009; Stolwijk et al., 2013; Gurney et al., 2012; Mukhra et al., 2020) have been evaluated through the calculation of the Arch Index. Factors other than arch height can impact AI, such as obesity (e.g., Rosende-Bautista et al., 2021; Wearing et al., 2012), repeated high loading (Maslon et al., 2017), and footwear type (D'Aout et al., 2009), so a critical component of the AI—and perhaps its greatest limitation—is the need to

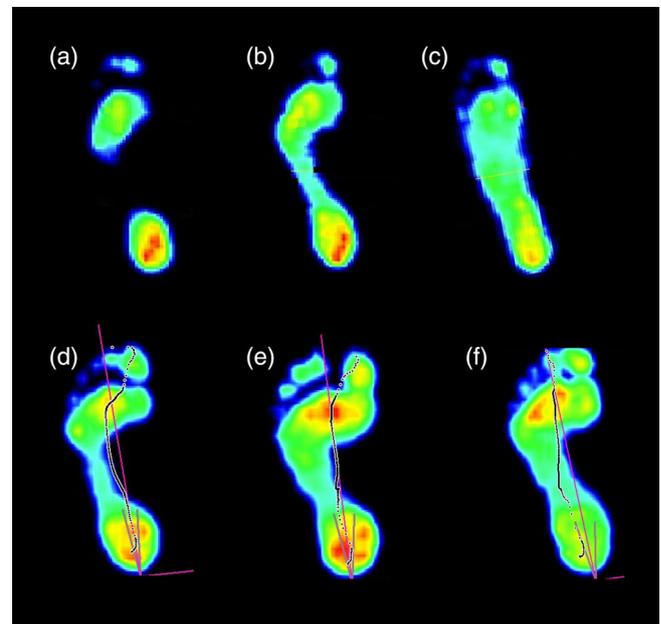


FIGURE 2 Double stance static and dynamic (walking) footprints of a cavus (a and d), normal (b and e), and planus (c and f) foot type. Note that the area of the foot in contact with the ground increases in walking over that of double stance standing and that while the cavus and normal feet increase AI between double stance and dynamic conditions, the AI of planus the foot decreases

categorize feet through metrics determined from data for the population. For example, the AIs of habitually shod people may differ from those of habitually unshod in the absence of underlying bony differences. Nonetheless, among loading conditions in a person, changes in AI are, presumably, an indication of foot stiffness because the increase in the midfoot area occurs due to displacement of the peak of the arch toward the substrate (Cen et al., 2020). Interestingly, foot type is associated with arch stiffness. Cavus feet tend to be stiffer than planus feet, but some variation in flexibility within foot type exists (Zifchock et al., 2017).

The application of an external load to produce changes in navicular height in order to determine foot stiffness is well established (e.g., Kelly et al., 2014). Arch height and, therefore, AI should also vary under different standing conditions. For instance, quiet standing on both feet (double stance, DS) distributes the forces (more or less) evenly between the feet, resulting in a force on each foot of approximately one half of body weight, but standing on one foot (single stance, SS) introduces full body weight through the foot. Theoretically, even though the foot is an irregular structure, it can be approximated as a curved, shallow shell or dome (Yu et al., 2020) and a shell's displacement is dependent on its stiffness (i.e., material properties and geometry) and is proportional to the load applied to it (Budynas & Sadegh, 2020,

table 13.3). For example, higher applied forces increase displacement. Empirically in the foot, this displacement flattens the arch and, at least up to 125% of body weight, the increasing load increases the vertical displacement of the arch (Kelly et al., 2015). For a given stiffness, the arch height decreases in proportion to the force applied to it (Bjelopetrovich & Barrios, 2016; Kelly et al., 2015) although static and dynamic conditions can produce different responses (Sichting & Ebrecht, 2021). The relationship between loading and the complexity of changing stiffness should, nonetheless, produce less contact area in DS than in SS or walking AI. For the condition of increased arch stiffness due to stimulation of the intrinsic muscles of the foot and concomitant support by triceps surae, the change of arch height should be less than it would have been without the activation of the intrinsic muscles. Our expectation, then, is that, in the absence of stiffness change, the conditions in which the foot experiences higher forces will exhibit more contact area in the midfoot, resulting in a higher AI. Consequently, in the single stance portion of walking, although greater than full body weight is experienced by the foot, the AI may not increase—or increase as much as it would—without the reinforcement.

From these lines of evidence, we expect that the AI will demonstrate arch shape changes due to foot loading comparable to those changes known to occur in navicular height. Our motivation to use footprints rather than navicular height as a proxy for arch stiffness stems from the ease of obtaining footprints for research or clinical use. Specifically, we hypothesize that AI will not change as much as one would expect due to the change in force alone. Further, categorizing foot type, as assessed by double stance static AI, will reveal different responses to load among foot types, that is, cavus feet will respond to increased forces differently from planus feet because cavus feet are stiffer. To evaluate these hypotheses, we captured the left and right footprints of participants during quiet double stance, quiet single stance, and walking at their comfortable walking velocity.

2 | METHODS AND MATERIALS

A pressure-sensitive mat and associated software (Footscan USB plate, RSScan International, Olen, Belgium) were used to obtain the double and single stance static and dynamic footprints of 77 people (57 females and 20 males assessed as a binary variable). The dynamic footprints were obtained with the participants walking at their self-selected normal velocity. The 0.5×0.4 m pressure-sensitive mat sends readings taken

at 300 Hz from 4,096 sensor areas to software that produces the footprint images. The RSScan mat was calibrated using the measured body weight of each participant. Informed consent was obtained from all participants and all study protocols were approved by the appropriate Institutional Review Board (IRB #52038).

Participants were recruited through flyers and word-of-mouth recommendation on a university campus. We included in the study those people who volunteered and were free of obvious gait abnormality and who denied lower limb pain, trauma, or surgery within the last year. The data were acquired anonymously and age (24 years; 18–52 years), sex, body mass (70 kg, 39–105 kg), and stature (1.70, 1.50–1.95 m) were recorded.

All footprints were obtained unshod. For the double stance static test, participants stood quietly on the mat with their weight evenly distributed between their feet. For the single stance static test, participants balanced on one foot with the other foot raised off the mat. To establish balance, the participants were allowed to touch the top of a table located immediately adjacent to the mat with the tip of their index finger. Once balance was achieved the finger was raised and the pressure data was recorded by the mat. This process was repeated for both left and right feet. For the dynamic test, participants walked at their normal velocity, taking three steps before contacting the mat. They were instructed to focus on the wall in front of them and not to attempt to change their gait in order to position their foot on the mat. Trials in which the subject appeared to take a “stutter-step” or those where an incomplete footprint was obtained were immediately discarded and the subject was asked to redo the trial. All participants were eventually able to provide a complete set of footprints.

Images of the static and dynamic footprints from the RSScan software were imported into ImageJ (ImageJ 1.29x, Wayne Rasband, National Institutes of Health, Bethesda, MD). The foot was divided into three areas based on the length of the foot from the proximal heel to the distal ball of the foot. The area of the entire footprint, not including the toes, and of the midfoot was determined. The arch index (AI) for each footprint (left and right single and double stance static and dynamic) for each participant was calculated as: $AI = \text{midfoot area} / \text{total area}$ consistent with others (e.g., Cavanagh & Rodgers, 1987; Cen et al., 2020). Interclass correlation estimates and their 95% confidence intervals were calculated based on a mean-rating ($k = 2$ absolute-agreement, 2-way random-effects model: ICC: 0.96; CI: 0.97–0.99) similar to others (Kirmizi et al., 2020).

Each footprint was categorized as a foot type by using the quartiles of AI of double stance for each side

(Cavanagh & Rodgers, 1987): cavus (smallest quartile of AI of the sample), normal (middle two quartiles of AI of the sample), or planus (largest quartile of AI of the sample). We did not use the values of the quartiles specified by Cavanagh and Rodgers (1987) because our data collection method (a pressure-sensitive mat) could have a different sensitivity to pressure than an inked footprint. Peak vertical ground reaction force was also obtained from the RScan software. Because ground reaction force varies with body mass, a normalized force was calculated: normalized force = peak vertical ground reaction force/body weight.

2.1 | Statistical analysis

Normality was assessed with the Shapiro-Wilks *W* test which indicated that the AIs for all conditions are not normally distributed (all p 's < .001); therefore, Mann-Whitney/Wilcoxon sign rank tests, a nonparametric form of Student's *t*-test, were used to detect potential differences between the side of the foot and foot types (both of which are matched data) and Mann-Whitney/Wilcoxon rank-sum tests, which uses the same distribution as the sign rank test but is for unmatched data, were used to detect differences between males and females.

We predicted the AI of the dynamic condition by extrapolating the change in normalized force and AI between the double and single stance conditions. This approach depends on the assumption that the impact of the change in AI from the normalized force is linear, that is, that the change in condition between static double and single stances is the same as that between static and dynamic single stances. We examine this assumption with linear regression analysis for the entire sample and the sample separated into foot types.

An increase in the stiffness of the foot was taken as an indication of the presence of a counteraction to the displacement caused by the force on the foot and was assessed by calculating the difference between the predicted and the measured dynamic AI. This difference was then predicted using the double stance AI and the participant's demographic characteristics (age, body mass, and stature).

Statistical analyses were performed using STATA (Statacorp, College Station, TX, V15) with statistical significance set at alpha = 0.05 and consideration of multiple comparisons.

3 | RESULTS

We found that the AIs of left and right feet for all conditions did not systematically vary (all p 's \geq .37), a result

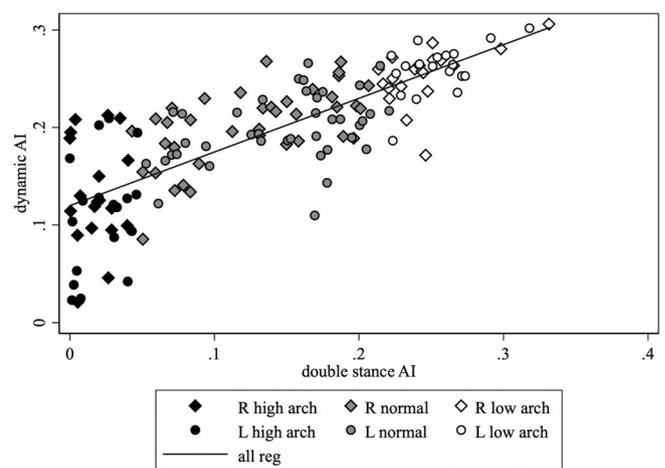


FIGURE 3 Double stance AI versus dynamic AI. The solid line represents the regression line including all foot types

similar to others (e.g., Yu et al., 2020). Consequently, left and right feet are combined in all analyses. Males are taller ($p < .001$), weigh more ($p < .001$), have higher vertical ground reaction force ($p = .006$), and larger total footprint areas across all conditions than females (all p 's > .001). The normalized forces of males and females did not systematically vary ($p = .78$), nor did AIs when mass or force was included in the statistical model ($p = .55$). Consequently, male and female feet are combined in all analyses. The dynamic normalized force is greater than 1 for all participants, indicating that walking forces are higher than those of single stance quiet standing, and averages 1.4. Double stance AI predicts 62% of the variation in dynamic AI ($r^2 = 0.62$, $p < .001$).

The AIs from double stance footprints are lower (higher arch) than those from single stance static footprints ($p < .001$) and both double stance and single stance static footprints are lower (higher arch) than the AIs from dynamic footprints (both p 's < .001). For planus and normal feet categories, variation in the AIs due to condition is as expected: double stance static AI > single stance static AI > dynamic AI (all p 's > .001; Figure 3). For cavus feet, however, dynamic AI is not less than single stance static AI ($p = .29$), although dynamic AI is less than double stance AI ($p < .001$).

The relationship between predicted and actual AI is weak for the entire sample ($p < .001$, $r^2 = 0.017$) and disappears when the sample is categorized by foot type (p 's > .21, $r^2 < 0.05$) (Figure 4).

A significant relationship between double stance AI and the difference between predicted and measured AI is apparent and foot type influences this relationship (Figure 5). Low AI footprints (those with higher arches) exhibit more difference between the predicted and measured AI and less predictability associated with double

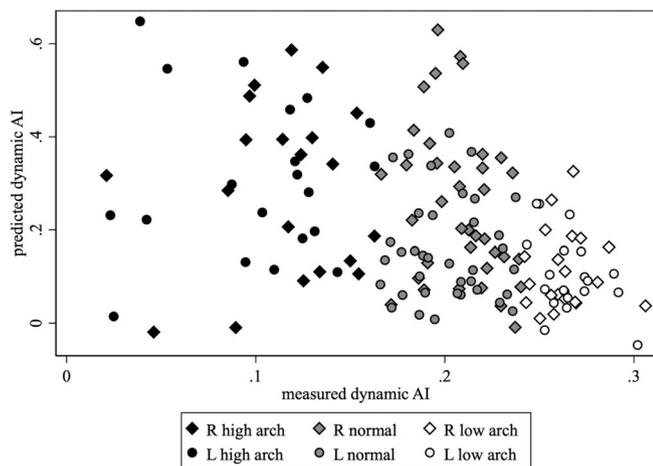


FIGURE 4 Measured dynamic AI versus predicted dynamic AI

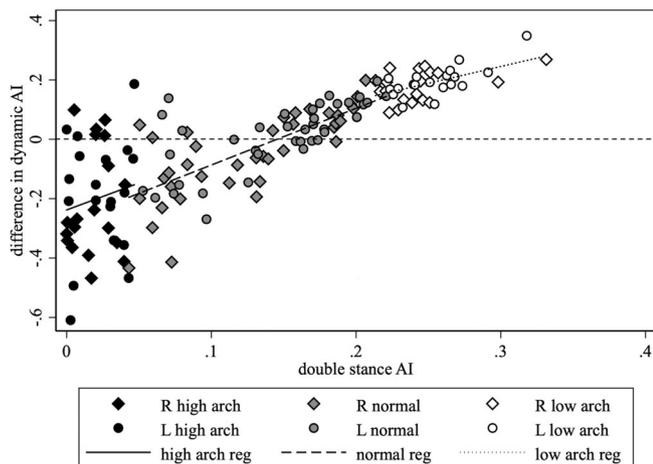


FIGURE 5 Double stance AI versus the difference between measured and predicted dynamic AI. Solid lines represent the regression by each foot type. Points that fall above the horizontal dashed line indicate feet with more change than expected while those below this line indicate feet with less change than expected

stance AI ($r^2 = 0.02$, $p = .43$). High and intermediate AI footprints (normal and low arches) exhibit less difference between predicted and measured values and greater predictability (r^2 's > 0.33 , p 's $> .001$).

4 | DISCUSSION

We aimed to determine whether or not changes in AI would provide the same evidence of change in foot stiffness as arch height measurements do. We did this because dynamic footprints are simple to collect in the clinical, as well as in research, context and hence might form a foundation to develop a protocol that is useful in diagnosis. We chose to use AI because substantial

literature exists with which to compare our results, but other simplifications that use footprints (such as mid-foot area) might provide similar results.

We found no differences between the AIs of left and right feet, consistent with previous work (e.g., Mukhra et al., 2020; Yu et al., 2020). We also did not find a statistically significant difference between the males and females, so we analyzed the combined sample. Our sample was, however, biased toward feet from females and a larger sample of males has produced a difference. Others have found small differences in dynamic AIs using large samples, for example, Mukhra et al. (2020) found a 0.01 difference in AI between 230 males and 231 females with $p = 0.048$. We included sex as a covariate in regression analyses and it did not improve the fit of the models used to test our hypotheses. While many other conditions contribute to sample- or population-based differences, such as obesity (e.g., Rosende-Bautista et al., 2021; Wearing et al., 2012), repeated high loading (Maslon et al., 2017), and footwear type (D'Aout et al., 2009), our approach evaluated changes within a person among conditions, that is, our participants were their own controls.

Unsurprisingly, higher ground reaction force produced higher AIs as has been previously demonstrated (e.g., Yu et al., 2020). Because we explicitly expected the muscles of the triceps surae complex to be important contributors to stiffening the arch, we did not evaluate the arch in the sitting position, as knee flexion minimizes the ability of gastrocnemius to contribute to Achilles tendon force. Our static conditions, double and single stance, produced loading conditions on the feet of ~50% and 100% of body weight and consistent changes in AI. We found that double stance AI predicts 62% of the variation in dynamic AI, consistent with others who have related static measurements to dynamic ones (e.g., Franettovich et al., 2007; Teyhen et al., 2009).

Predicting the dynamic variable from a static one is not, however, the same as predicting the change from static to dynamic conditions and dynamic AI is not predictable from the change between double and single stance for the entire sample or any foot type (Figure 4) that is expected if the only difference between conditions is a change in load. Our data suggest that a change in stiffness between our static and dynamic conditions occurs. We suspect, in agreement with other research (Farris et al., 2019; Kelly et al., 2014), that the foot intrinsic muscles, which pull the edges of the shell of the osseous foot toward the center of the foot dome, and triceps surae, which grounds or supports the posterior arch (via the calcaneus), is responsible. The action of the windlass due to extension of the metatarsal phalangeal joints potentially also plays a role but the extension may not be sufficient to contribute until late stance (Welte

et al., 2021). Consequently, changes in AI seem to parallel changes in arch height.

We found that the difference between our predicted and measured dynamic AI is dependent on foot type as assessed from double stance AI. Feet that we categorized using the static double stance AI as cavus showed a variable response to loading (Figure 5) but feet that we categorized as planus showed more consistency in their response. In the normal arch range, the difference in the change in AI between the double stance and dynamic conditions was predictable for lower end (higher AI) feet but not as much for the higher end. This prompts us to question if the categorization of foot types into three groups obscures functional differences, particularly with regard to stiffness changes. Zifchock et al. (2017) suggest five foot types, but perhaps two types are sufficient. The feet in the lower half of the arch height sample act similarly with load, while those in the higher arch half of the sample are more variable. Figure 5 might indicate that flatter arches require more muscular action to maintain their shape. Quiet standing flattens the arch, but muscle activation “facultatively” reinforces their arches. Higher arches seem less consistently responsive to muscular action, potentially because some cavus feet are stiff due to passive foot elements while other cavus feet can respond to muscular action. Planus feet all seem to respond to muscular activation potentially because they do not have passive elements that maintain the arch. Other factors may be at work, though, such as different muscle architecture or firing patterns. Future work needs to combine a clinical assessment that includes imaging of feet with analysis of footprints and EMG to tease apart this apparent heterogeneity of response in higher arched feet.

Our study was limited in several ways. We did not measure or control velocity, other than asking our participants to walk at their self-selected comfortable pace. We also only asked them to walk at one speed. Future work should measure participant velocity and include multiple velocities to provide several dynamic conditions for evaluation. Multiple dynamic conditions would provide data to assess whether or not dynamic conditions respond to increased load similarly. We also did not measure triceps surae activation directly with EMG, but rather inferred it from the extensive literature on muscle activation. This weakens our association of extrinsic muscle activation as a contributor to arch stiffness to a supposition. Finally, we did not directly measure the navicular height (or any kinematic variables), principally because we did not want to depend on access to motion capture. We perceived this work as an initial test case to see if footprints had the potential to detect stiffness changes. Given that footprints do provide evidence of changes in stiffness, future work to develop diagnostic routines would benefit from

establishing an explicit connection between dynamic foot shape and footprint changes.

5 | CONCLUSION

We found that footprint shape changes from static to dynamic loading conditions similar to other measures of arch height or stiffness, making footprints a viable choice for clinical use. We also found that foot type affects change in footprint shape (and hence arch stiffness) with feet categorized as cavus responding to the increased load of walking differently than planus feet: the AIs of cavus feet do not change in predictable ways while dynamic conditions, presumably through intrinsic and extrinsic muscle activation, appear to enhance the arch of planus feet.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest and no funding was obtained for this research.

AUTHOR CONTRIBUTIONS

Patricia Ann Kramer: Conceptualization (equal); data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); project administration (lead); resources (lead); supervision (equal); validation (equal); visualization (equal); writing – original draft (lead); writing – review and editing (equal). **Steven G Lautzenheiser:** Conceptualization (equal); data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); supervision (equal); validation (equal); visualization (equal); writing – review and editing (equal).

INFORMED CONSENT

Informed consent was obtained from all participants and all study protocols were approved by the appropriate Institutional Review Board (IRB #52038).

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