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RESEARCH PAPER

In vivo operating lengths of the gastrocnemius muscle during gait in children who idiopathically toe-walk

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Abstract

Children who idiopathically toe-walk (ITW) habitually operate at greater plantarflexion angles than typically developing (TD) children, which might result in shorter, sub-optimal gastrocnemius fascicle lengths. However, currently no experimental evidence exists to substantiate this notion. Five children who ITW and 14 TD children completed a gait analysis, whilst gastrocnemius fascicle behaviour was simultaneously quantified using ultrasound. The moment–angle (hip, knee and ankle) and moment–length (gastrocnemius) relationships were determined from isometric maximum voluntary contractions (MVC) on an isokinetic dynamometer combined with ultrasound. During gait, children who ITW operated at more plantarflexed angles ($\Delta = 20°$; $P = 0.013$) and longer muscle fascicle lengths ($\Delta = 12$ mm; $P = 0.008$) than TD children. During MVC, no differences in the peak moment of any joint were found. However, peak plantarflexor moment occurred at significantly more plantarflexed angles (-16 *vs.* $1°$; $P = 0.010$) and at longer muscle fascicle lengths (44 *vs.* 37 mm; $P = 0.001$) in children who ITW than TD children. Observed alterations in the moment–angle and moment–length relationships of children who ITW coincided with the ranges used during gait. Therefore, the gastrocnemius muscle in children who ITW operates close to the peak of the force–length relationship, similarly to TD children. Thus, this study indicates that idiopathic toe-walking is truly an ankle joint pathology, and children who ITW present with substantial alterations in the gastrocnemius muscle functional properties, which appear well adapted to the characteristic demands of equinus gait. These findings should be considered when prescribing clinical treatments to restore typical gait.

KEYWORDS
equinus, idiopathic toe-walking, muscle, ultrasound

1 | INTRODUCTION

Locomotion relies on joint moments to propel the body forwards, and so adequate contractile muscle force must be produced. The maximal contractile force is limited by the length and velocity of these muscles during contraction, according to the force–length–velocity relationship (Gordon et al., 1966). The plantarflexor muscles, a vital source of the required mechanical power for gait (Winter, 1983), have been shown to operate quasi-isometrically and close to the region of optimal sarcomere length during gait (Fukunaga et al., 2001), thereby maximising the potential muscle force and promoting the economical production of this force. However, this relies on gait kinematics that are matched to the underlying muscle–tendon architecture and functional properties.

Children who idiopathically toe-walk (ITW) present with an atypical gait pattern, despite no detected orthopaedic or neurological cause (Pomarino et al., 2016). Whilst the main pathology presents at the ankle joint, more proximal joints must also be considered, as weakness of the hip and knee joint muscles have previously been suggested to contribute to equinus gait (Hampton et al., 2003; Kennedy et al., 2020; Morozova et al., 2017; Wiley & Damiano, 1998). For children who ITW, there has been an indication that there may be some small effects of ankle joint weakness (De Oliveira et al., 2021); however, to our knowledge, muscle strength around the hip or knee joint has never been objectively studied in children who ITW. Therefore, to improve our understanding of how the altered gait kinematics may affect the ability of children who ITW to produce force economically, it is important to establish whether muscle strength, determined by the *in vivo* force–length and moment–angle relationships, may be contributing to the toe-walking gait characteristic in these children.

Equinus gait also presents in many children with cerebral palsy (CP), which is associated with alterations in contractile muscle properties, such as a greater muscle stiffness (Barber et al., 2011), longer sarcomere lengths (Lieber et al., 2017; Ponten et al., 2007), muscle weakness around the hip, knee and ankle joints (Eek & Beckung, 2008), and differences in the muscle lengthening characteristics (Kalkman et al., 2018) in the affected lower-limb muscles. However, although the gait kinematics in children with CP are similar to those of children who ITW, the pathway of cause-and-effect is far more complex in CP (Gage & Novacheck, 2001). Therefore, we should not assume that the muscle functional properties are similarly affected in children who ITW. Indeed recently, we showed that children who ITW had longer resting absolute and normalised gastrocnemius medialis muscle belly and fascicle lengths than typically developing (TD) children (Harkness-Armstrong et al., 2021). This is the exact opposite effect to that found in children with CP. Consequently, the more plantarflexed angles during gait and resulting shorter muscle fascicle lengths may suggest that the sarcomeres of children who ITW operate at shorter lengths than optimal (Arnold & Delp, 2011). However, plantarflexor muscle fascicle lengths in children who ITW have never been measured during gait. Thus, it remains unknown how the altered muscle lengths relate to dynamic muscle function.

New Findings

- **What is the central question of this study?**
  What are the *in vivo* operating lengths of the gastrocnemius muscle in children who idiopathically toe-walk?
- **What is the main finding and its importance?**
  Children who idiopathically toe-walk operate at more plantarflexed positions but at longer fascicle lengths than typically developing children during gait. However, these ranges utilised during gait correspond to where children who idiopathically toe-walk are optimally strong. This should be considered when prescribing clinical treatments to restore typical gait.

Current treatments for children who ITW primarily target the ankle joint because persistent, untreated, equinus gait can lead to muscle contracture, fixed deformity and/or worsening of symptoms (Dietz & Khunsree, 2012; Sobel et al., 1997; Solan et al., 2010). Therefore, interventions to lengthen the gastrocnemius muscle–tendon unit (MTU), such as casting with or without botulinum toxin-A injections and/or surgery (Engström et al., 2013; Fox et al., 2006; Jahn et al., 2009), are prescribed with the intention of restoring dorsiflexion range of motion (ROM) and a typical gait pattern. Therefore, there is a clinical assumption that achieving typical angles through intervention will restore optimal muscle length in children who ITW. However, as muscle function mirrors habitual use (Matano et al., 1994), it is possible that the MTU of children who ITW may have remodelled to match the altered gait kinematics. If true, then current treatments may negatively impact muscle function for these children, rather than improve it.

While it is somewhat rare for children who ITW to remain in equinus beyond early childhood (Engström & Tedroff , 2012), the gait pathology does continue in a small number of children who often require clinical treatments. Therefore, although this makes children who ITW a difficult population to recruit experimentally, it is vital to gain an understanding of the muscle strength and functional properties, relative to the demands of gait in these children. This will allow us to better understand the pathology and thus better inform clinical interventions for these children, which often have poor medium-long term success rates (Dietz & Khunsree, 2012; van Kuijk et al., 2014). The aims of this study were to (1) measure the moment–angle relationship of the ankle, knee and hip extensors of children who ITW and TD children relative to the demands of gait, and (2) to measure the moment–fascicle length relationship of the plantarflexors relative to the demands of gait. We hypothesised that children who ITW would operate lower down on the ascending limb of the force–length relationship and thus operate at joint angles that are further away from optimal than typical. We also hypothesised that there would be no difference in the moment–angle relationships of the hip and knee extensors between groups.

2 | METHODS

2.1 | Ethical approval

This study was completed in accordance with the recommendations of both the institutional and National Health Service (UK) ethics committees (18/NW/0526). Written informed consent was obtained from parent/guardians and written assent given by children, in accordance with the *Declaration of Helsinki*, except for registration in a database.

2.2 | Participants

Five children who bilaterally ITW (male $n = 2$; female $n = 3$; age 8 ± 2 years; height 1.38 ± 0.15 m; body mass 45.2 ± 26.7 kg) and 14 TD children (male $n = 5$; female $n = 9$; age 10 ± 2 years; height 1.39 ± 0.11 m; body mass 37.8 ± 17.5 kg) participated in this study. Children who ITW were recruited from outpatient lists at a children's hospital gait laboratory and orthopaedic clinics. All children had a confirmed diagnosis of idiopathic toe-walking based on an exclusion of all other causes. Children who ITW had not undergone any orthopaedic intervention (surgery or casting) within 2 years prior to the study and had not been given botulinum toxin A injections within 6 months prior to the study. Three children who ITW had significant fixed equinus contracture (range: 12 –30° of plantarflexion from ankle anatomical position with knee fully extended). All TD children were free from neuromuscular and skeletal disorders and were free from lower limb injuries within the 6 months prior to the study.

2.3 | Measurement protocol

Data were collected over two testing sessions to first collect gait data and then collect muscle function data in the second session at a university laboratory, separated by no more than 14 days. Measurements were obtained from the right leg of TD children, and the most affected leg of children who ITW, defined by the observed degree of plantarflexion angle during gait.

2.4 | Gait measurements

Children completed a gait analysis on an instrumented split-belt treadmill (Motek Medical, Amsterdam, The Netherlands). Prior to data collection, there was a 5—10-min period of familiarisation to identify the participants' self-selected walking speed and to ensure that they could walk comfortably in their preferred gait pattern. Following familiarisation, passive retro-reflective markers were positioned on the lower body in accordance with the 6-degrees-of-freedom marker set (37 total markers: anterior superior iliac spines, posterior superior iliac spines, sacrum, medial/lateral femoral epicondyles, medial/lateral malleoli, calcaneus, and first, second and fifth metatarsal heads, with rigid clusters of four tracking markers positioned on the thigh and shank segments). Participants walked barefoot in their preferred gait pattern and at their self-selected walking speed, whilst secured in an upper body fall-arrest harness for safety. Participants walked continuously until five consecutive successful gait cycles were collected on the measured side. Three-dimensional (3D) kinematics were collected using a 12-camera Vicon Vero system (Vicon, Oxford, UK) at a sample rate of 120 Hz. Kinetic data from the treadmill were also recorded in Vicon, at a sample rate of 1200 Hz.

To track fascicle lengthening during gait for the assessment of the moment–length relationship, a 60 mm linear B-mode ultrasound transducer (LV8-5L60N-2; Telemed Medical Systems, Milan, Italy) was securely fastened over the mid-belly of the gastrocnemius medialis muscle using a custom-made probe holder. To minimise measurement error, guidance on probe alignment was followed (Bénard et al., 2009). Ultrasound data were recorded at 30 Hz, synchronised to motion data by a 5 V digital signal captured in Vicon.

2.5 | Muscle functional properties and strength measurements

The active moment–angle relationships of the ankle, knee and hip extensors were determined on an isokinetic dynamometer (Humac Norm, Computer Sports Medicine Inc., Stoughton, MA, USA). Children performed two isometric maximum voluntary contractions (MVC) at five individualised joint positions (randomised order) across their full ROM. Joint positions included maximum hip and knee flexion and ankle dorsiflexion (0%), maximum hip and knee extension and ankle plantarflexion (100%), and at individual 25% intervals between (rounded to nearest degree). For the ankle, an additional measurement was obtained at 12.5% of ROM, to increase sensitivity to detect optimum joint angle, which is often towards extreme dorsiflexion (Gravel et al., 1990). Joint ROM was determined by manually rotating the joint until either (1) no further joint rotation was achieved with the application of increased joint moment or (2) the participant indicated their stretch threshold. Joint angle and moment were sampled from the dynamometer analogue output at 1600 Hz in AcqKnowledge software (Biopac Systems, Palgrave, UK). Net joint moment was corrected for the gravitational moment caused by the weight of both the dynamometer arm and the participant's limb.

2.5.1 | Ankle joint set-up

To measure the plantarflexor moment–angle relationship, participants lay prone on an isokinetic dynamometer bed with their hip in full extension and lower leg supported on a cushion so that the knee was 20° flexed. The axis of rotation of the dynamometer arm was aligned with the lateral malleolus throughout joint rotation before the participant's foot was securely fastened to the footplate. A custom-made arch support ensured that heel contact was maintained with the footplate across full ROM.

To determine the moment–fascicle length relationship of the plantarflexors, a linear B-mode ultrasound transducer (Phillips EPIQ7, Amsterdam, The Netherlands) was securely fastened in the same position as in the gait session, over the mid-portion of the gastrocnemius medialis muscle, using a custom-made probe holder and recorded at 15–30 Hz, depending on ultrasound settings. A 5 V trigger switch recorded on the ultrasound video and in AcqKnowledge was used to synchronise data. To measure muscle activity, surface EMG electrodes (Biopac Systems Inc.) were placed on the mid-portion of the tibialis anterior and the gastrocnemius lateralis and recorded synchronously with angle and moment data at 1600 Hz in AcqKnowledge.

2.5.2 | Knee joint set-up

To measure the moment–angle relationship of the knee extensors, participants were seated and securely strapped onto an isokinetic dynamometer chair so that their hip was 90° flexed. The axis of rotation of the dynamometer arm was aligned with the lateral femoral condyle during contraction before the distal end of the shank (proximal to malleoli) was securely fastened to the dynamometer arm.

2.5.3 | Hip joint set-up

To measure the moment–angle relationship of the hip extensors, participants lay supine and were securely strapped to the dynamometer bed. The axis of rotation of the dynamometer arm was aligned with the greater trochanter of the femur during contraction, before the participants distal end of the thigh (proximal to the knee joint) was securely fastened to the dynamometer arm.

2.6 | Data processing

Gait data were processed in Visual 3D software (C-Motion, Rockville, MD, USA) using a custom-made pipeline. All data were low-pass filtered with a cut-off frequency of 6 Hz. Initial contact and toe-off events were defined using a force plate threshold of 10 N. All gait data were averaged for five gait cycles per participant and exported to MATLAB (R2019a; The MathWorks Inc. Natick, MS, USA) for subsequent analyses to determine the moment–angle relationship of the hip, knee and ankle joints during stance.

Dynamometer data were processed and analysed in MATLAB, to determine the moment–angle relationship of the hip, knee and ankle joints across the full ROM. EMG from the ankle joint muscles were also analysed in MATLAB. At each joint angle, EMG at peak moment were extracted. Raw EMG signals were band-pass filtered from 20 to 400 Hz. The root mean square envelope of the EMG was then extracted before EMG was averaged across the plateau of peak moment. All MVC data were averaged for two trials at each joint angle.

Ultrasound videos of the gastrocnemius medialis fascicles during gait and at peak MVC at each joint angle on the dynamometer were manually tracked in ImageJ software (1.51j8; NIH, Bethesda, MD, USA) to determine the ankle moment–fascicle length relationship during stance, and across the full ROM, respectively. Each ultrasound video was analysed twice, and data averaged.

2.7 | Statistical analysis

All statistical analyses were completed in MATLAB. All variables were checked for normal distribution using the Shapiro–Wilk test and visual inspection of the *Q*–*Q* plots. Gait data were compared between groups using either statistical parametric mapping (SPM) for normally distributed variables or statistical non-parametric mapping (SnPM) for non-normally distributed variables. All MVC variables were normally distributed, and therefore MVC data across ROM were compared between groups using a multi-variate analysis of variance (MANOVA). Follow up *post hoc* tests (Tukey) were performed where appropriate. Statistical significance was set at $P < 0.05$. All results are presented as the mean ± standard deviation (SD).

3 | RESULTS

3.1 | Moment–angle relationship

During gait, SPM analysis indicated that the ROM used by children who ITW was significantly more plantarflexed than TD children between 0% and 6% ($P = 0.049$) and 43% and 100% ($P = 0.013$) of stance and at peak plantarflexion moment during propulsion (−11° vs. 10°; $P = 0.002$) (Figure 1a, g). Additionally, children who ITW produced a significantly greater plantarflexion moment between 0% and 39% of stance ($P = 0.001$), and a significantly smaller plantarflexion moment between 80% and 93% of stance ($P = 0.011$) than TD children (Figure 1d). There were no significant differences in knee angle or moment, nor hip angle or moment at any point throughout stance between groups ($P > 0.05$) (Figure 1b, c, e, f).

The MVC moment–angle relationship of the ankle plantarflexors in children who ITW was displaced to more plantarflexed angles compared to TD children, with a significant difference in the angle of peak moment (−16° vs. 1°; $P = 0.010$) (Figure 1g). Children who ITW produced a significantly greater plantarflexion moment than TD children at individual maximum plantarflexion angle (0.58 vs. 0.32 N m kg^{-1}; $P = 0.005$) (Figure 1g), but there were no other differences in MVC between groups at any other relative joint position, or in the peak MVC moment ($P > 0.05$). There were no differences in the MVC moment–angle relationship of the knee or hip extensors between groups (Figure 1h, i). Therefore, when compared at similar relative

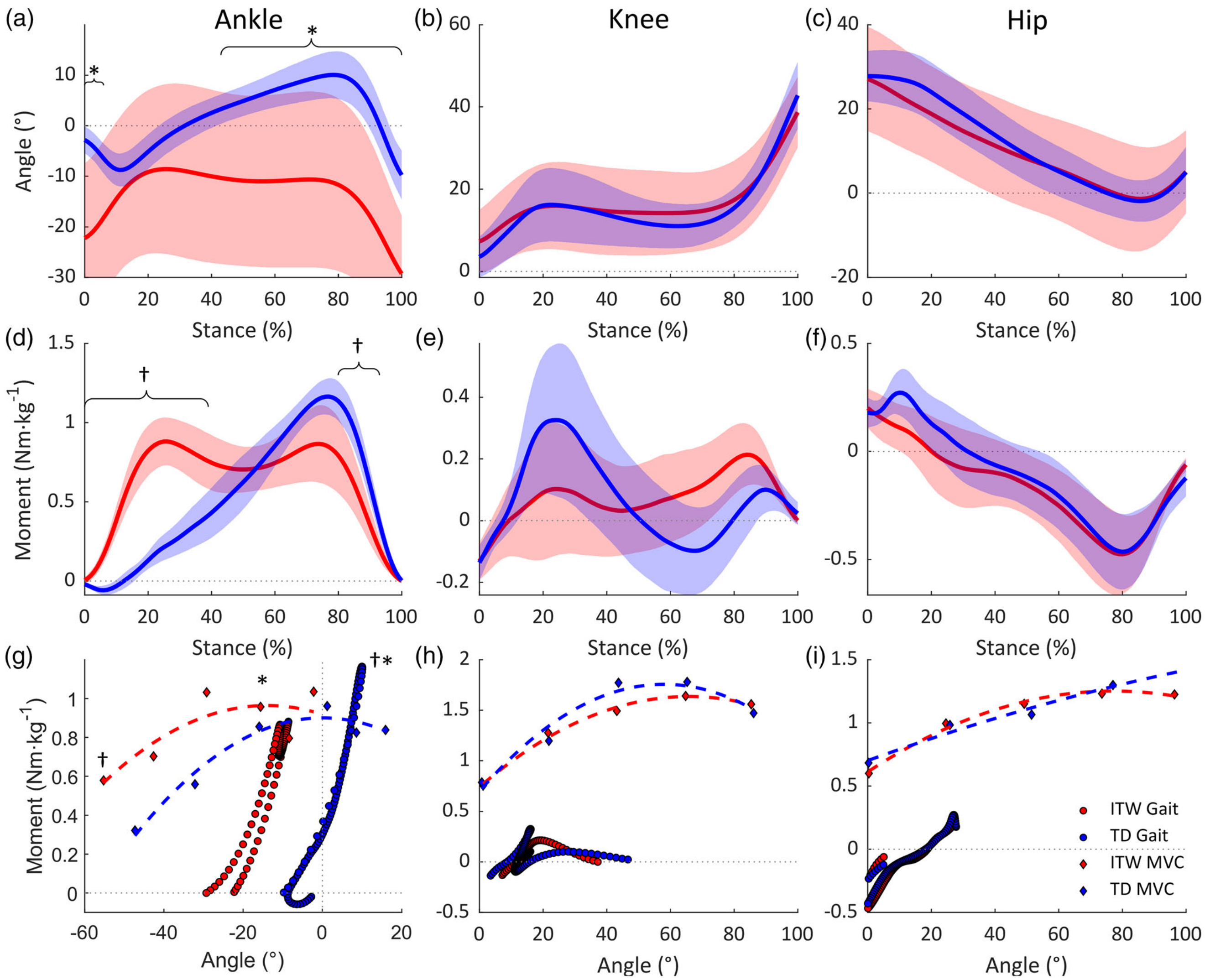

FIGURE 1 (a–f) Joint angles (a–c) and joint moments (d–f) (normalised to body mass) throughout stance. (g–i) Moment–angle relationship of the ankle plantarflexors (g), knee extensors (h), and hip extensors (i) measured during gait (circles) and during maximum voluntary isometric contractions across full range of motion (diamonds). *Significant difference in joint angle between groups ($P < 0.05$). †Significant difference in joint moment between groups ($P < 0.05$). Abbreviations: ITW, children who idiopathically toe-walk; MVC, maximum voluntary contraction; TD, typically developing children

joint positions across ROM, there was no difference in the knee or hip extensor MVC moment between groups ($P > 0.05$).

3.2 | Ankle moment–fascicle length relationship

During gait, medial gastrocnemius fascicle lengths were longer in children who ITW than TD children, between 9% and 70% of stance ($P = 0.008$) (Figure 2a, b). During plantarflexion MVC, the moment–fascicle length relationship was also at longer fascicle lengths in children who ITW, with peak moment occurring at a significantly longer optimal fascicle length (44 mm *vs.* 37 mm; $P = 0.001$) (Figure 2b) than TD children. When compared at similar relative joint positions, fascicle length at MVC was significantly longer in children who ITW at 25% ($P = 0.007$), 50% ($P = 0.020$) and 75% ($P = 0.025$) of individual ROM than TD children. In both groups, gastrocnemius lateralis muscle activity decreased (60%) in dorsiflexed positions compared to the peak EMG recorded at maximum plantarflexion (Figure 3).

4 | DISCUSSION

This study is the first to assess ankle, knee and hip extensor muscle strength, and the *in vivo* gastrocnemius medialis functional properties, relative to the demands of gait in children who ITW. During gait, children who ITW operated at more plantarflexed angles, but at longer fascicle lengths than TD children. This coincided with observed differences in the peak of the moment–angle and moment–length relationships, measured from MVCs, which showed that children who ITW had the greatest strength at more plantarflexed angles and an

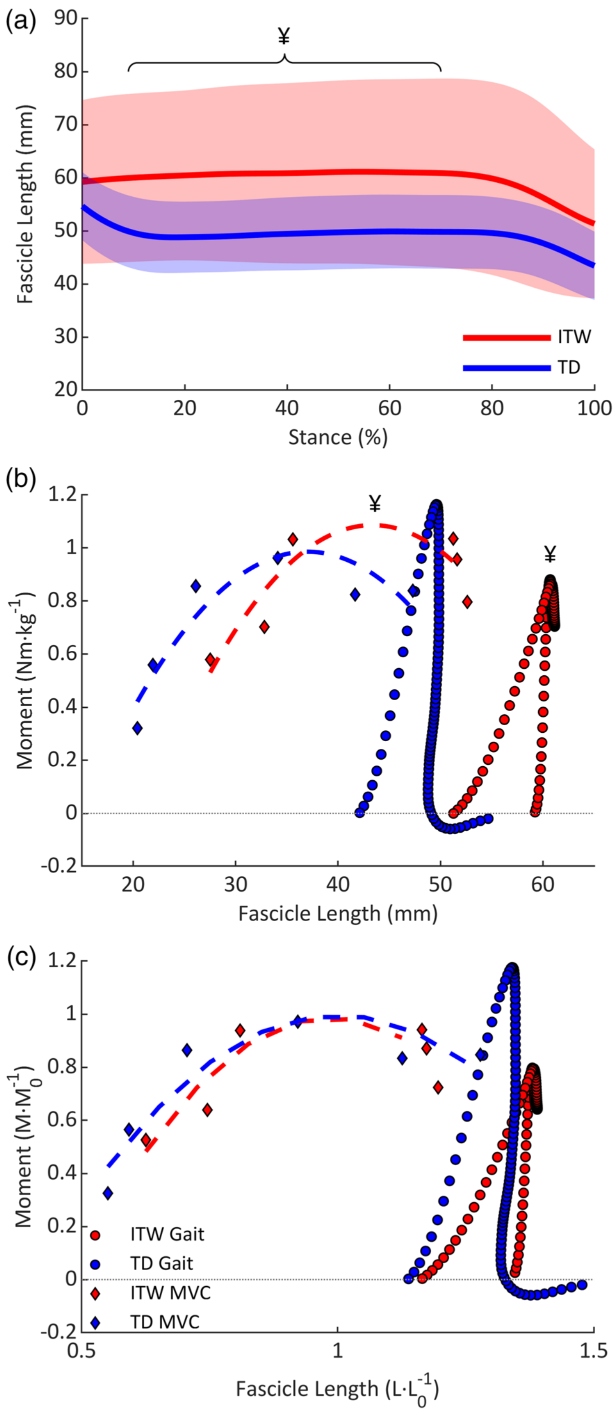

FIGURE 2 (a) Gastrocnemius medialis fascicle lengths throughout stance. (b, c) Absolute (b) and normalised to optimum (c) moment–fascicle length relationship of the plantarflexors during gait (circles), and during maximum voluntary isometric contractions across full range of motion (diamonds). *Significant difference in fascicle length between groups ($P < 0.05$). Abbreviations: ITW, children who idiopathically toe-walk; MVC, maximum voluntary contraction; TD, typically developing children

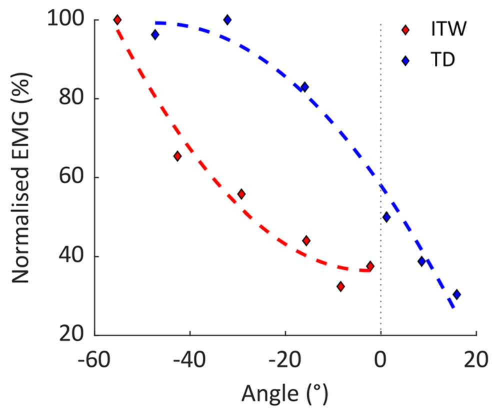

FIGURE 3 Normalised EMG at peak moment during maximum voluntary isometric contractions performed across full range of motion. Abbreviations: ITW, children who idiopathically toe-walk; TD, typically developing children

optimum fascicle length at longer lengths than TD children. Thus, data from the current study indicate that children who ITW present with substantial alterations in the gastrocnemius medialis functional properties, which appear well adapted to the characteristic demands of equinus gait. These should be considered when prescribing clinical treatments to restore typical gait.

Equinus gait has previously been linked with ankle, knee and hip extensor weakness in other clinical populations (Hampton et al., 2003; Kennedy et al., 2020; Morozova et al., 2017; Wiley & Damiano, 1998). However, we found no differences in the peak moment of any joint during MVC. Therefore, underlying weakness did not present in these children who ITW, and a lack of proximal joint weakness supports the notion that idiopathic toe-walking is truly an ankle joint pathology. Thus, clinical interventions should, as currently prescribed, target the ankle joint in isolation. During gait, there were no differences in the hip or knee joint moments between groups throughout stance; however, children who ITW produced a significantly smaller plantarflexor moment during propulsion than TD children. As there were no differences in plantarflexor strength between groups, this suggests that the reduction of plantarflexor moment may be due to changes in moment arm length, but this should be studied.

A significant shift was observed in the moment–angle relationship of the plantarflexors in children who ITW, with peak moment occurring at a significantly more plantarflexed position than TD children (Figure 1g). Despite being more plantarflexed, the moment–fascicle length relationship shifted significantly towards longer fascicle lengths in children who ITW (Figure 2b). This is consistent with recent findings showing that the passive gastrocnemius fascicle lengths were longer in children who ITW than TD children (Harkness-Armstrong et al., 2021), and corresponded with the ranges used during gait. Consequently, children who ITW and TD children both operated at similar joint angles and fascicle lengths relative to their respective measured optimum. Whilst both groups utilised their optimum joint angle, they both appear to operate at sub-optimal fascicle lengths, on the descending limb of both the absolute and normalised moment–fascicle length relationships. However, this apparent discrepancy may be explained by a deficit in muscle activation during the isometric contractions towards dorsiflexed positions (~60%; Figure 3), which would have shifted the measured peak moment to shorter fascicle lengths in both groups. Alternatively, between-group differences in Achilles tendon stiffness, moment arm length or role of the gastrocnemius medialis within the overall plantarflexor group may exist (Lichtwark & Wilson, 2008). Nonetheless, the data in this study demonstrate that children who ITW and TD children operate at similar portions of the moment–angle and moment–length profiles during gait. This suggests that for these children who ITW, equinus gait combined with the changes in functional muscle properties enables utilisation of their optimum joint angle, which may allow the economical production of high contractile force similarly to typically developing children, and as is the case in typically developed adults (Fukunaga et al., 2001).

Current treatments for children who ITW aim to restore a typical heel–toe gait pattern by lengthening the gastrocnemius MTU (Engström et al., 2013; Fox et al., 2006; Jahn et al., 2009). However, here we have shown the presence of substantial alterations in the gastrocnemius medialis functional properties in children who ITW. Consequently, clinical interventions may compromise the force producing capabilities of the gastrocnemius medialis in the short-term, with unknown consequences. For example, if the primary aim is to restore a typical gait pattern, and thus to shift the functional moment–angle loop of children who ITW to overlay that of a TD child (Figure 1g), the gastrocnemius may no longer operate at optimum lengths post-intervention, until the contractile elements have remodelled to match the surgically altered MTU lengths and new gait pattern. If true, this may contribute to the low medium- to long-term success rates of these interventions for children who ITW (Dietz & Khunsree, 2012; van Kuijk et al., 2014). Thus, substantial changes in the muscle functional properties are required. However, we currently cannot distinguish a causal relationship between the muscle properties and altered gait kinematics. It is unclear whether an underlying neuro-structural alteration is present that causes the children to adapt functionally, or whether muscle remodelling has occurred in response to persistent equinus gait. Therefore, longitudinal studies of the development of muscle in children who ITW are needed. Additionally, whether these functional properties are also altered through clinical intervention is not known. Therefore, further work is also required to assess the gastrocnemius muscle functional properties pre- and post-intervention.

Some limitations in our tests should be acknowledged. Firstly, we did not account for co-contraction in our measurements of joint moments. This would cause an underestimation of moments produced by the agonist muscle groups on the dynamometer and during gait. For the hip and knee extensors, EMG was not recorded. For the plantarflexors, this was because of the difficulty for most children to perform the necessary dorsiflexion contractions, which would have introduced errors in itself (Billot et al., 2010). However, to our knowledge, there is no evidence to suggest that co-contraction differs between children who ITW and TD children. Secondly, to avoid excessive time demands on the children, we only measured fascicle behaviour from the gastrocnemius medialis yet reported joint moments of the whole plantarflexor muscle group. Therefore, it is unclear as to the potential mechanical contributions of the remaining plantarflexor muscles to overall MVC and gait performance. Finally, our sample size of children who ITW may appear small ($n = 5$). However, this is representative of the small population of children who remain in equinus beyond early childhood (Engström & Tedroff , 2012), and have not undergone any recent orthopaedic intervention. Although small, our sample includes children with good variability in participant characteristics and equinus severity, yet we have still detected statistically significant and functionally meaningful differences.

To conclude, data from the present study indicate that children who ITW have substantial alterations in the gastrocnemius medialis functional properties, which correspond to the functional operating ranges during gait. Therefore, by restoring a typical gait pattern, clinical interventions may, in the short-term, shift children who ITW away from their 'optimum' joint angle. However, further work is required to assess how these muscle functional properties are altered post-intervention. Longitudinal studies of the development of muscle in children who ITW are also needed to determine the causal relationship of such alterations.

COMPETING INTERESTS

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

C.H.-A., T.O., C.M. and V.B. contributed to conception and design of the research. C.H.-A. data acquisition and analysis. All authors to the interpretation of the results. C.H.-A. drafted the manuscript. All authors edited and revised the manuscript and agreed to its submission for publication. All authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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