

Intrinsic versus Extrinsic Muscle Fatigue on Spatiotemporal Gait Parameters in Women with Flexible Flatfoot

ARTICLEINFO

Article Type Original Article

Authors

Mahsa Mohammadi¹, MSc Hashem Piri^{1*}, PhD Rahman Sheikhhoseini¹ PhD Ebrahim Ebrahimi¹, MSc

How to cite this article

Mohammadi M, Piri H, Sheikhhoseini R, Ebrahimi E. Intrinsic versus Extrinsic Muscle Fatigue on Spatiotemporal Gait Parameters in Women with Flexible Flatfoot. Int.J. Musculoskelet. Pain. Prev. 2025;10(3): 1281-1286.

- ¹ Department of Corrective Exercise and Sport Injury, Faculty of Physical Education and Sport Sciences, Allameh Tabataba'i University, Tehran, Iran.
- ² Department of Sport Injuries and Biomechanics, Faculty of Sport Sciences and Health, University of Tehran, Tehran, Iran



* Correspondence

Department of Corrective Exercise and Sport Injury, Faculty of Physical Education and Sport Sciences, Allameh Tabataba'I University, Tehran, Iran. P.O. Box: 1485643449

Fax: 44737526 Tel: +98 9199033390 E-mail hpiry63@gmail.com

Article History

Received: Agu 27, 2025 Accepted: Oct 10, 2025 E Published: Oct 15, 2025

ABSTRACT

Aims: Flatfoot is associated with altered knee kinematics and knee pain. Yet, there is a lack of evidence about gait parameters after fatigue in flatfoot individuals. Therefore, this study aims to compare the effect of intrinsic and extrinsic muscles' fatigue on spatiotemporal parameters during walking in women with flexible flatfoot.

Method and Materials: This study included 20 women with flexible flatfoot. They were divided into intrinsic muscles fatigue (N=10) and extrinsic muscles fatigue groups (N=10). The Foot Posture Index and Zebris FDM-T Treadmill were used to assess flatfoot and spatiotemporal parameters, respectively. Also, fatigue was applied with paper grip and heel-rise endurance tests. Analysis of Covariance (ANCOVA) and Quade's tests were utilized for inferential statistics. Data analysis was conducted at a significance level of 0.05.

Findings: The findings showed there is no significant difference between intrinsic and extrinsic muscle fatigue on spatiotemporal gait parameters in women with flatfoot during treadmill walking (P>0.05).

Conclusion: This study suggests that intrinsic and extrinsic muscle fatigue may not significantly impact spatiotemporal parameters in individuals with flexible flatfoot. These findings highlight the robustness and adaptability of gait mechanics under fatigue, even in the presence of postural foot abnormalities.

Keywords: Patellofemoral Pain Syndrome, Muscular Synergy, Gait

Introduction

The foot, a complex structure that supports various functions, provides a base of support during standing and must absorb shock and remain stable at foot strike and push off during walking(1). Anatomically, it consists of the hindfoot, midfoot, and forefoot (2). Passive and active tissues in the foot region form three arches: medial and lateral the longitudinal arches. and the arch. which transverse are essential for shock absorption, weight transfer, and propulsion (3). The Medial Longitudinal Arch (MLA) is higher than the lateral longitudinal arch, and when bearing weight, its curvature may vary to variable degrees(4). Pes planus, commonly referred to as flatfoot, is characterized by a reduced height of the MLA, resulting in the sole making near or complete contact with the ground(5). This condition typically categorized into two forms: flexible and rigid flatfoot (6, 7).

In a flexible flatfoot, the arch appears normal when the foot is not bearing weight (open kinetic chain) but flattens upon weightbearing (closed kinetic chain)(8). In contrast, a rigid flatfoot exhibits a consistently diminished regardless of whether the kinematic chain is open or closed (9). The prevalence of flatfoot among students aged 18-25 years was 11.25%. and all affected individuals had bilateral flatfoot (8). It is also associated with intricate several structural alterations, including calcaneal valgus and adduction, forefoot increased forefoot abduction. pronation, subluxation of the talonavicular joint, and abduction and dorsiflexion of the first metatarsal (10, 11).

While ligaments and fascia contribute to the support of the MLA, muscles play a crucial role in maintaining its structure ⁽¹²⁾. Intrinsic foot muscles like the Abductor Hallucis (ABH) and Flexor Digitorum Brevis(FDB)

are situated with both origins and insertions near the foot bones (13).

In contrast, extrinsic muscles such as the Tibialis Anterior (TA), Tibialis Posterior (TP), and Flexor Digit rum Longus (FDL) play a significant role in reinforcing the MLA due to their mechanical advantage and capacity to generate movement and propulsion during gait⁽¹⁾. It has been demonstrated that the early activation of intrinsic foot muscles may help stabilize the foot, allowing the extrinsic muscles generate torque more effectively. Additionally, intrinsic muscles may assist in the final phase of push-off, continuing to function the extrinsic muscles have deactivated(14).

Fatigue is a common condition characterized by depleted physical and mental energy levels. It impairs motor performance and can negatively impact an individual's gait (15). A study indicated that fatigue diminishes the body's capacity to absorb impact forces during walking, which can lead to discomfort in the lower limb muscles and joints (16). Previous study showed that fatigue resulted in a decrease in the knee flexion and abduction moments and an increase in knee ROM in sagittal and frontal planes in the flatfoot group (17). Another study by previous study demonstrated that calf muscle fatigue caused medialization of the maximum force, and the midfoot was affected differently by fatigue in flexible flatfoot and normal feet (18). In addition, previous study demonstrated that fatigue changes ankle plantar flexor moment and spatiotemporal gait variables Although fatigue is known to affect gait mechanics and motor performance, there is a notable lack of research comparing the specific effects of intrinsic and extrinsic muscle fatigue on spatiotemporal parameters during walking, particularly in individuals with flexible flatfoot. Given the importance of walking for functional mobility and the potential for increased injury risk in this population, examining how fatigue in these two muscle groups specifically influences walking dvnamics and spatiotemporal crucial. This research parameters is could offer critical insights for targeted rehabilitation and injury prevention strategies

for women with flexible flatfoot.

Method and Materials

This study included 20 women with flexible flatfoot. Thev were selected convenience sampling and divided into intrinsic muscles (N=10) and muscles' fatigue groups (N=10). According to a previous study (20) and considering an effect size of 0.68, a statistical power of 0.80, and a significance level of 0.05, as determined using G*Power Ver. 3.1 software, the sample size was calculated to be 10 in each group. The inclusion criteria for the study were as follows: being female, aged between 20 and 35 years, having flatfoot, and having a score of six or higher based on the Foot Posture Index (FPI)⁽²¹⁾. Also, flatfoot was considered flexible if the MLA height was restored and hind foot valgus was correctable with standing on toes (tiptoe test) (22). Exclusion criteria included any clinical condition that restricted physical activity, failure to complete the test, a previous history of lower extremity surgery, experiencing any lower limb pain at the time of the test, and taking medication that may affect vigilance and attention. At first, for ethical considerations based on the Declaration of Helsinki, all stages of the study were discussed with the subjects. Then, written informed consent was obtained. Additionally, individuals were informed that in the event of any issues during the tests, the examiner would take all necessary actions to address them. The subjects were instructed on how to perform each test. All steps were explained to the participants. Before starting the tests, the procedure was presented to them. It is worth mentioning that all tests were conducted in the Sport Sciences Research Institute laboratory in Tehran, Iran. granted by the Approval was Ethics Committee of the Research Institute of Movement Science of Kharazmi University in Iran (No. IR-KHU.KRC.1000.273).

Foot posture was evaluated by measuring the FPI. At the same time, subjects were barefoot and in a relaxed standing position, allowing for visual and manual inspection. The FPI comprises six components about the positioning of the forefoot, midfoot, and

hindfoot across three planes of motion. This index includes: 1) Palpation of the talar head; 2) Assessment of symmetry in the supra and infra lateral malleolar curvature; 3) Evaluation of inversion and eversion of the calcaneus; 4) Identification of prominence talonavicular joint region; Measurement of the height of the MLA; 6) Analysis of forefoot abduction and adduction. The obtained FPI ranges from -12, indicating high supination, to +12, indicating high pronation (23). A greater positive value indicates a more pronated foot (21). Interobserver reliability was found to be good, with an Intraclass Correlation Coefficient (ICC) ranging from 0.852 to $0.895^{(24,25)}$.

gait **Spatiotemporal** parameters were obtained using the Zebris FDM-T Treadmill (Zebris Medical GmbH, Germany) fitted with an electronic mat of 10,240 miniature force sensors, each approximately 0.85 cm × 0.85 cm, embedded underneath the belt. The treadmill's contact surface measures 150 cm x 50 cm, and its speed can be adjusted from 0.2 to 22 km/h at 0.1 km/h intervals. When the subject walked on the treadmill, the force exerted by her feet was recorded by the sensors at a sampling rate of 120 Hz ⁽²⁶⁾. Due to the high density of the sensors, the foot is mapped with a high resolution, facilitating even the most subtle changes in force distribution. Primary spatiotemporal data, including stride time (s), right and left step time (s), right and left single support phases (% of gait cycle), double support phases (% of gait cycle), and stride width, were evaluated during a barefoot gait cycle in this study. Participants walked on a treadmill for five minutes to ensure familiarization and warmup. Gait data were collected one minute before and immediately after the fatigue protocol. To minimize the influence of potential noise, the first and last 10 seconds of each recording were excluded from statistical analysis, and the middle 40 seconds were used for further

evaluation. The treadmill speed for gait parameter measurement was set at 5 km/h. A study reported that the Zebris treadmill had excellent reliability for most gait characteristics (ICC: .91–1.00) (27).

The Borg scale assessed localized perceived fatigue (28). At the beginning and end of the fatigue protocol, participants were asked to rate their perceived exertion of the activity using the Borg scale to ensure fatigue was achieved. For this, participants instructed to rate the level of fatigue in their muscles on a scale from 0 to 10. A score of 0 indicated no fatigue, while a score of 10 represented the maximum imaginable fatigue. In this study, a score of 8 or above indicated muscle fatigue. Also, a fatigue protocol involving a heel-rise endurance test (targeting extrinsic foot muscles) and a paper-grip endurance test (targeting intrinsic foot muscles) was employed. Each participant performed a minimum of five consecutive repetitions⁽²⁹⁾. The rationale for using this protocol was that these tests effectively induced fatigue in intrinsic and extrinsic foot muscles while representing functional activities. This research used descriptive statistics to describe the variables and inferential statistics for data analysis. The normality of the data distribution was assessed using the Shapiro-Wilk test. If the data were normally distributed, the Analysis of Covariance (ANCOVA) was used for inferential statistics. Also, the Quade's test was utilized for nonparametric data analysis. Data analysis was conducted using SPSS software version 27, with a significance level of 0.05 (corresponding to a 95% confidence level).

Findings

Table 1 displays the demographic characteristics of participants in both groups. The results of the independent t-test showed no significant difference between the groups (Table 1).

Table 1) Demographic characteristics of participants

Table 2/ Belliographic characteristics of participants								
Variable	Intrinsic	Extrinsic	p-value					
	Mean ± SD	Mean ±SD						
Age (year)	29.20±4.49	27.30±4.11	0.33					
Weight (kg)	69±7.87	65.50±6.94	0.30					
Height (m)	1.63±0.07	1.67±0.10	0.32					
BMI (Kg/m ²)	25.94±3.84	22.97±3.14	0.07					

BMI: Body Mass Index; SD: Standard Deviation

Table 2) Quade's test results

Variable	Stage	Intrinsic	Extrinsic	F	Mean	P-value
		Mean ± SD	Mean ± SD		square	
Left step time (sec)	Pre-test	0.47 ± 0.02	0.46±0.03	0.067	0.697	0.79
	Post-test	0.48 ± 0.03	0.48±0.02			
Stride time (sec)	Pre-test	0.94±0.05	0.92±0.07	2.196	9.474	0.15
	Post-test	0.96±0.07	0.95±0.04			
Right single support phase (%)	Pre-test	38.90±1.44	39.13±1.35	1.843	43.117	0.19
	Post-test	38.84±1.24	39.76±1.29			
Left single support phase (%)	Pre-test	39.06±0.87	39.58±1.86	0.036	0.448	0.85
	Post-test	38.41±1.21	39.42±1.58			
double support phase (%)	Pre-test	22.06±1.69	21.26±3.20	2.82	26.406	0.11
	Post-test	22.78±1.96	20.84±2.67			
Stride Width (cm)	Pre-test	20.70±3.56	16.00±2.44	1.22	25.01	0.28
	Post-test	17.50±3.34	16.44±2.40			
Right step time (sec) +	Pre-test	0.47±0.02	0.46±0.03	0.148	7.83	0.70
	Post-test	0.48±0.03	0.47±0.02			

sec: Seconds; cm: Centimeter; SD: Standard Deviation

The results of Table 2 showed there is no significant difference between intrinsic and extrinsic muscle fatigue on spatiotemporal gait parameters in women with flatfoot during treadmill walking (P>0.05).

Discussion

This study found no significant effect of intrinsic and extrinsic muscle fatigue on spatiotemporal gait parameters during walking in women with flexible flatfoot. Previous studies exploring fatigue-related gait changes have yielded mixed findings. For instance, Sanjari et al. (2016) reported fatigue may not alter the biomechanical behavior of individuals with flatfoot compared to those with normal arches (30), a finding that is consistent with our results. In contrast, a study by Dorrtaj et al. (2020) reported that fatigue may affect several spatial-temporal parameters in normal and flatfooted adolescents. increasing step width various temporal parameters, with strong interaction effects in the flatfoot group (31). However, their study induced lower-limb fatigue using intensive protocols, rather than focusing on intrinsic versus extrinsic foot muscles, which may explain the discrepancy with our findings. Barbieri et al. (2013) that fatigue demonstrated appears modulate spatiotemporal gait parameters;

however, the effects of fatigue seem to be dependent on the muscles that are fatigued ⁽³²⁾. In another study by Keklicek et al. (2023), it was demonstrated that extrinsic and intrinsic foot core fatigue led to increases in the single support and terminal stance durations ⁽³³⁾. A key difference lies in the methodology that they studied general adult volunteers, whereas ours focused on female flexible flatfoot subjects.

Furthermore, a review supports that flexible flatfoot primarily affects joint kinematics and gait speed, but not necessarily in relation to states (34). Our findings reassuring insight for clinicians and physical therapists: individuals with flexible flatfoot may maintain stable walking patterns even when fatigued. This suggests that standardized walking tasks may not reveal functional deficits under fatigue, and more challenging or varied environments may be needed in rehabilitation to detect or isolate impairments. Given that interventions like intrinsic muscle strengthening have proven to bolster arch morphology and reduce navicular drop (35), future research might explore improved muscle whether endurance translates into gait resilience under variable conditions. This study has several limitations. Firstly, our protocol may not have induced sufficient fatigue in target muscle groups to

⁺ Analysis of Covariance was used.

affect spatiotemporal parameters. Validating with electromyography dynamometry could provide deeper insights. Secondly, the study focused on women with flexible flatfoot. Moreover, it appears difficult to distinctly separate the fatigue effects of intrinsic and extrinsic foot muscles, as both groups often work synergistically during gait and postural control. Results may not generalize to men, rigid flatfoot cases, or individuals with symptomatic flatfoot pain. Lastly, our focus was limited to basic spatiotemporal variables. Additionally, a small sample size was another limitation that should be taken into consideration. Future work should include muscle activation patterns (via EMG) and measures of dynamic stability or balance.

Conclusion

This study indicated that fatigue may not affect the spatiotemporal parameters of gait in individuals with flexible flatfoot. These findings highlight the robustness and adaptability of gait mechanics under fatigue, even in the presence of postural foot abnormalities. For both researchers and clinicians, this suggests that assessing gait under more demanding conditions may be necessary to make evident the influence of muscle fatigue on foot posture and movement control.

Acknowledgments

We thank all the participants who participated in this study.

Authors' Contribution

All authors contributed to the methodology, conceptualization, project administration, formal resources, and authors analysis. All helped the investigation. All authors contributed to data curation. All authors approved the final version of the manuscript.

Conflicts of Interest

The authors have no conflicts of interest.

Ethical Permission

This research was approved by the Ethics Committee of the Research Institute of Movement Science at Kharazmi University in Iran (No. IR-KHU.KRC.1000.273).

Funding/Supports

No funding

References

- McKeon PO, Hertel J, Bramble D, Davis I. The foot core system: a new paradigm for understanding intrinsic foot muscle function. Br J Sports Med. 2015;49(5):290-. DOI: 10.1136/bjsports-2013-092690.
- 2. Brijwasi T, Borkar P. A comprehensive exercise program improves foot alignment in people with flexible flat foot: a randomised trial. J Physiother. 2023;69(1):42–6.
- 3. Patel M, Shah P, Ravaliya S, Patel M. Relationship of anterior knee pain and flat foot: A cross-sectional study. Int J Health Sci Res. 2021;11:86–92.
- 4. Aenumulapalli A, Kulkarni MM, Gandotra AR. Prevalence of Flexible Flat Foot in Adults: A Cross-sectional Study. J Clin Diagn Res. 2017;11(6):AC17-AC20. doi: 10.7860/JCDR/2017/26566.10059.
- 5. Raj MA, Tafti D, Kiel J. Pes Planus 2023. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025. PMID: 28613553.
- Atik A, Ozyurek S. Flexible flatfoot. North Clin Istanb. 2014 Aug 3;1(1):57-64. doi: 10.14744/ nci.2014.29292.
- Kalahrodi OG, Piri H, Ebrahimi E. The Effect of Eight Weeks of Corrective Games on the Functional Assessment, Navicular Drop Index, and Balance in Male Students Aged 9 to 12 with Flexible Flatfoot. J Clin Res Paramed Sci. 2025;14(14). https://doi.org/10.5812/jcrps-157990.
- 8. Bhoir T, Anap DB, Diwate A. Prevalence of flat foot among 18-25 years old physiotherapy students: cross sectional study. Indian J Basic Appl Med Res. 2014;3(4):272-8.
- 9. Sivachandiran S, Kumar G. Effect of corrective exercises programme among athletes with flat feet on foot alignment factors. Int J Phys Educ Sport Health. 2016;3(6):193–6.
- 10. Cai Y, Zhao Z, Huang J, Yu Z, Jiang M, Kang S, et al. Morphological changes in flatfoot: a 3D analysis using weight-bearing CT scans. BMC Med Imaging. 2024;24(1):219. https://doi.org/10.1186/s12880-024-01396-0
- 11. Fallon Verbruggen F, Killen BA, Burssens A, Boey H, Vander Sloten J, Jonkers I. Unique shape variations of hind and midfoot bones in flatfoot subjects—A statistical shape modeling approach. Clin Anat. 2023;36(6):848–57.
- 12. McKeon PO, Fourchet F. Freeing the foot: integrating the foot core system into rehabilitation for lower extremity injuries. Clin Sports Med. 2015; 34(2):347-61.
- 13. Kelly LA, Cresswell AG, Racinais S, Whiteley R, Lichtwark G. Intrinsic foot muscles have the capacity to control deformation of the longitudinal arch. J R Soc Interface. 2014 Jan 29;11(93): 20131188. doi: 10.1098/rsif.2013.1188.

- 14. A Akuzawa H, Morito T, Oshikawa T, Kumai T, Kaneoka K. Functional relationship between the foot intrinsic and extrinsic muscles in walking. J Electromyogr Kinesiol. 2023;71:102781. doi: 10.1016/j.jelekin.2023.102781.
- 15. Jin Y, Sano Y, Shogenji M, Watanabe T. Fatigue Effect on Minimal Toe Clearance and Toe Activity during Walking. Sensors (Basel). 2022;22(23): 9300. doi: 10.3390/s22239300.
- 16. Weist R, Eils E, Rosenbaum D. The influence of muscle fatigue on electromyogram and plantar pressure patterns as an explanation for the incidence of metatarsal stress fractures. Am J Sports Med. 2004;32(8):1893–8.
- 17. Farahpour N, Sharifmoradi K, Azizi S. Effect of Fatigue on Knee Kinematics and Kinetics During Walking in Individuals With Flat Feet. Phys Treat Spec Phys Ther. 2017;7(3):141–8.
- 18. Kirmizi M, Sengul YS, Angin S. The effects of calf muscles fatigue on dynamic plantar pressure distribution in normal foot posture and flexible flatfoot: A case-control study. J Back Musculoskelet Rehabil. 2022;35(3):649–57.
- Sharifmoradi K, Azizi S. Effect of Fatigue on Spatiotemporal and Moment Variables during Walking in Flatfeet. J Rehab Med. 2018;7(3):183–9.
- 20. Heydari M, Mousavi Sadati SK, Daneshjoo A. Short-term Effects of Using Arch Supporting Insoles on Balance and Symptoms of Flexible Flat Foot in Children. Sci J Rehabil Med. 2022;10(6):1244–57.
- 21. Lee JS, Kim KB, Jeong JO, Kwon NY, Jeong SM. Correlation of foot posture index with plantar pressure and radiographic measurements in pediatric flatfoot. Ann Rehabil Med. 2015;39(1):10–7.
- 22. Hegazy FA, Aboelnasr EA, Salem Y, Zaghloul AA. Validity and diagnostic accuracy of foot posture Index-6 using radiographic findings as the gold standard to determine paediatric flexible flatfoot between ages of 6-18 years: A cross-sectional study. Musculoskelet Sci Pract. 2020;46:102107. doi: 10.1016/j.msksp.2020.102107.
- 23. Gijon-Nogueron G, Montes-Alguacil J, Alfageme-Garcia P, Cervera-Marin JA, Morales-Asencio JM, Martinez-Nova A. Establishing normative foot posture index values for the paediatric population: a cross-sectional study. J Foot Ankle Res. 2016;9:24. DOI 10.1186/s13047-016-0156-3.
- 24. Redmond AC, Crosbie J, Ouvrier RA. Development and validation of a novel rating system for scoring standing foot posture: the Foot Posture Index. Clin Biomech (Bristol, Avon). 2006;21(1):89–98.

- 25. Redmond A. The Foot Posture Index: user guide and manual. Retrieved September. 2005;29 (29):2014.
- 26. Kalron A. Association between perceived fatigue and gait parameters measured by an instrumented treadmill in people with multiple sclerosis: a cross-sectional study. J Neuroeng Rehabil. 2015;12:doi: 10.1186/s12984-015-0028-2.
- 27. Donath L, Faude O, Lichtenstein E, Nüesch C, Mündermann A. Validity and reliability of a portable gait analysis system for measuring spatiotemporal gait characteristics: comparison to an instrumented treadmill. J Neuroeng Rehabil. 2016;13:6. doi: 10.1186/s12984-016-0115-z.
- 28. Williams N. The Borg rating of perceived exertion (RPE) scale. Occup Med (Lond). 2017;67(5):404–5.
- 29. Keklicek H, Selcuk H, Yilmaz A. Fatigue of the intrinsic foot core muscles had a greater effect on gait than extrinsic foot core muscles: A time-series based analyze. Foot (Edinb). 2024;59:102088. doi: 10.1016/j.foot.2024.102088.
- 30. Sanjari MA, Boozari S, Ashraf Jamshidi A, Nikmaram MR. Fatigue Effect on Linear Center of Pressure Measures during Gait in People with Flat Feet. Asian J Sports Med. 2016 Sep 18;7(4):e34832. doi: 10.5812/asjsm.34832.
- 31. 31. dorr taj e, alizadeh m, abbasi h, Shirzad Iraqi E. effect of fatigue on spatial and temporal parameters of gait in normal and flexible flat foot. Stud Sport Med. 2020;12(27):17–34.
- 32. Barbieri FA, Dos Santos PCR, Lirani-Silva E, Vitório R, Gobbi LTB, Van Diëen JH. Systematic review of the effects of fatigue on spatiotemporal gait parameters. J Back Musculoskelet Rehabil. 2013;26(2):125–31.
- 33. Keklicek H, Selcuk H, Yilmaz A. Fatigue of the intrinsic foot core muscles had a greater effect on gait than extrinsic foot core muscles: A time-series based analyze. Foot (Edinb). 2024;59:102088. doi: 10.1016/j.foot.2024.102088.
- 34. Nourbakhsh, SA., Sheikhhoseini, R., Piri, H. Soltani F, Ebrahimi E. Spatiotemporal and kinematic gait changes in flexible flatfoot: a systematic review and meta-analysis. J Orthop Surg Res 2025;20(223) https://doi.org/10.1186/s13018-025-05649-8
- 35. Ketabchi J, Seidi F, Haghighat S, Falsone S, Moghadas-Tabrizi Y, Khoshroo F. Differential effects of intrinsic-versus extrinsic-first corrective exercise programs on morphometric outcomes and navicular drop in pediatric flatfoot. Scientific Reports. 2024;14(1): doi.org/10.1038/s41598-024 82970-y.