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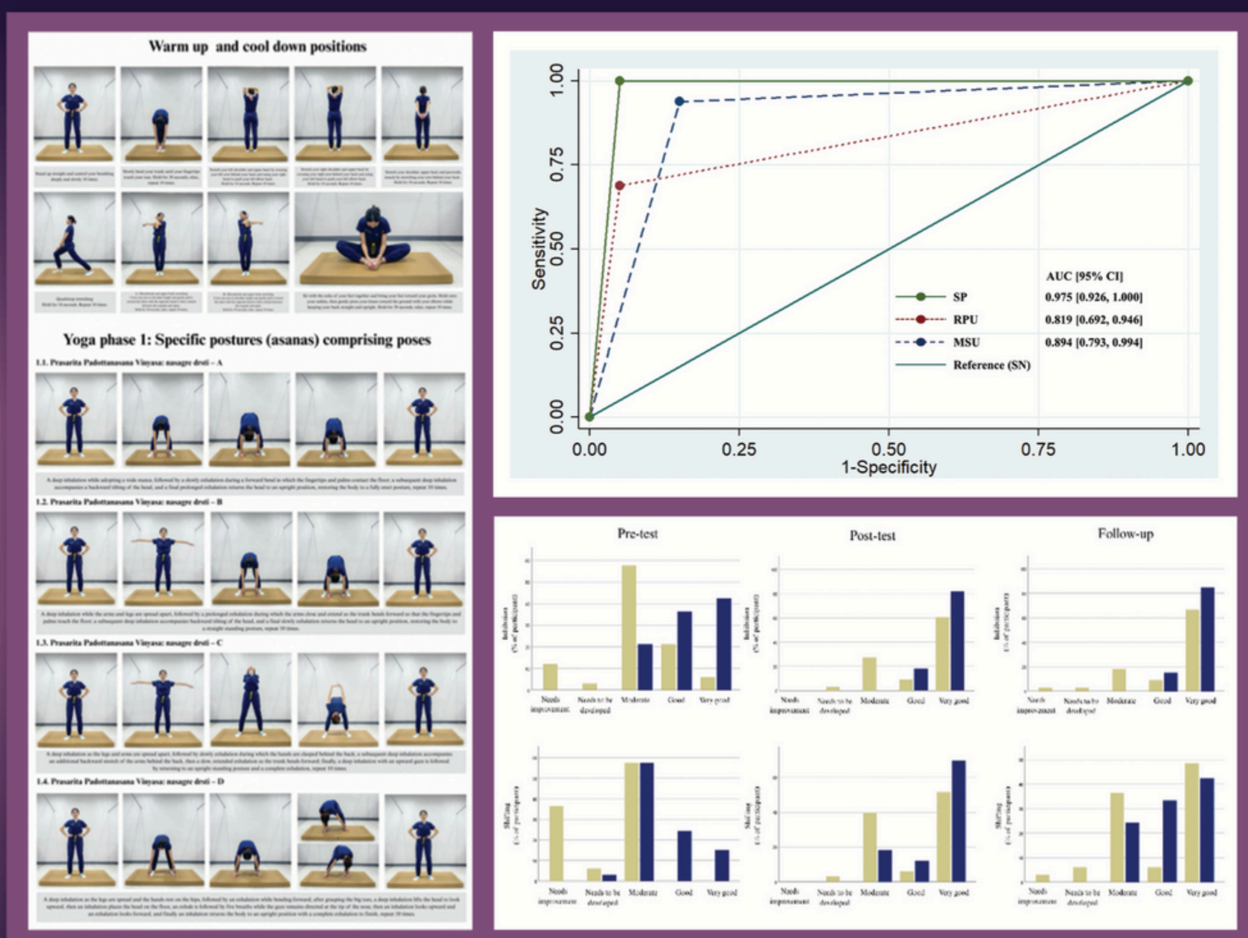

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ORIGINAL ARTICLE

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Effects of 4-week balance training program on executive function in preschool children: A randomized controlled trial

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KEYWORDS
Balance;
Emotional control;
Working memory;
Planning;
Preschool children.

ABSTRACT

Executive function delays have been steadily increasing in early childhood, influencing both academic achievement and life success. Effective interventions to promote executive function development are therefore urgently needed. This study aimed to investigate the effects of a four-week balance training program on executive function in 66 preschool children aged 4–6 years. Participants were randomly assigned to either a balance training group or a control group. The control group continued with the standard preschool curriculum, while the balance training group participated in four structured balance exercises, including double-leg stance, single-leg stance, balance path, and forward hopping on marking sheets for 45 minutes per day, three days per week, over four weeks. Executive function, including inhibition, shifting, emotional control, working memory, and planning, was assessed by teachers using the Executive Function Development Assessment at three time points: before training, after the four-week program, and at an eight-week follow-up. Data were analyzed using the Friedman test and the Mann–Whitney U test, with statistical significance set at p-value < 0.05. Results revealed significant improvements in all executive function domains in both groups after training. Consistent with the hypothesis, the balance training group showed greater gains in emotional control than the control group following four weeks of training. At the eight-week follow-up, the balance training group demonstrated significantly higher levels of emotional control (p-value = 0.036), working memory (p-value = 0.016), and planning (p-value = 0.039) compared to the control group. Improvements in inhibition, emotional control, and planning were particularly pronounced among children in the balance training group. In conclusion, both the standard preschool curriculum and balance training for at least four weeks can enhance executive function development in preschool children. However, the findings suggest that a structured balance exercise program may be more effective in promoting executive function development than the standard curriculum alone.

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Introduction

Executive function (EF) refers to the regulation of cognition through top-down, higher-order mental processes that facilitate goal-directed behavior throughout life. EF comprises three fundamental components, including inhibition, working memory, and shifting. The development of more complex and higher-level EF skills occurs during the preschool years, characterized by the emergence and refinement of these three core components[1]. Preschool children aged 2 to 6 years represent a critical period for EF development[2-4], coinciding with peak myelination and synaptogenesis in the prefrontal cortex[1,4]. Therefore, the preschool years are essential for fostering EF skills across five domains namely inhibition, shifting, emotional control, working memory, and planning[2]. Previous research has suggested that EF skills are stronger predictors of future success than intelligence quotient (IQ) or emotional quotient (EQ)[5]. The level of EF in early childhood plays a crucial role in shaping a child's developmental trajectory, influencing academic achievement and overall life success through mechanisms such as attention, decision-making, and self-regulation[1,2]. Rapid EF development occurs between the ages of 3.5 and 6 years and is strongly correlated with later academic performance in both primary and secondary education [6]. EF can be enhanced through age-appropriate activities and play-based learning. Conversely, deficits in EF skills are associated with learning and behavioral difficulties that may persist into adolescence and adulthood, potentially leading to academic underachievement and diminished life outcomes[2-4]. In Thailand, a previous study reported that more than 30% of 2,965 children aged 2 to 6 years exhibited EF dysfunction. Furthermore, 18.5% of 243 children aged 3 to 6 years demonstrated EF-related difficulties, particularly in inhibition[2]. These findings highlight the urgent need for effective interventions to promote EF development in preschool children.

In recent years, researchers have explored various activities to enhance EF in young children, including aerobic exercise, physical activity programs, dance training, and exergaming[7-9]. For example, a five-week creative dance program conducted twice weekly for 45 minutes per session led to significant improvements in EF compared with baseline measurements[7]. Similarly, an eight-week street dance training program, performed three times weekly for 40 minutes per session, produced notable EF enhancements[8]. These findings suggest that programs integrating movement, rhythm, and aerobic activity engage multiple EF components by requiring children to coordinate sensory and motor systems[7,8]. A recent systematic review reported that motor skills and EF develop concurrently in preschool- and school-aged children[10,11]. Motor skill development is closely associated with EF, with balance and manual dexterity playing key roles in coordination[11,12], which are essential for maintaining movement during various daily activities in childhood[13]. Balance, defined as the ability to maintain the body's center of mass within its base of support through multisensory and cognitive regulation[14-16], consists of both static and dynamic components[14,15]. Balance skills typically mature around six years of age[17], and both static and dynamic balance in children aged 3 to 6 years are positively correlated with gross motor development[18]. All fundamental movement skills arise from postural control and balance, which form the foundation for both basic and complex motor abilities throughout life[16].

Previous research has suggested that multitask balance training programs including static, dynamic, and dual-task training can enhance balance abilities in children[19,20], with an effective training duration of at least 4–6 weeks or 240–360 minutes[21]. However, the effect of balance training on children's EF has not yet been fully elucidated. Therefore, the objective of this study was to investigate the effects of a structured balance exercise program on EF in preschool children, aiming to determine whether such training can effectively promote all EF domains and serve as an optimal early intervention strategy.

Materials and methods

Study design and participants

This study was a single-blind, randomized controlled trial conducted at Klongbangnamjued School in Samut Prakan Province, Thailand. Participants were selected using convenience sampling from one kindergarten. Ethical approval for the study was obtained from the Human Research Ethics Committee of Huachiew Chalermprakiet University (Approval No. 1307/2566). Based on previous research in which executive function (EF) task scores served as the primary outcome[8], the sample size was estimated using G*Power version 3.1.9.6. The calculation was based on the difference between two independent means, with an effect size of 0.92, an alpha level of 0.05, and a power of 0.95. A total of 66 preschool children from second- and third-year classes were randomly assigned according to classroom to either the balance training group or the control group, with 33 participants in each group. The inclusion criteria were children aged 4 to 6 years who were able to communicate and follow verbal instructions. Each participant's height and weight were assessed according to the age reference chart for children aged 2–7 years developed by the Bureau of Nutrition, Department of Health, Ministry of Public Health, Thailand. Children whose body mass index exceeded +2 standard deviations (SD) were excluded. Moreover, children with a history of neurological or musculoskeletal disorders that could affect balance or executive function (EF), as identified through parent reports and school health screenings, were excluded from the study. Written informed consent was obtained from the parents of all participants prior to enrollment.

Balance exercise program

The balance exercise program was developed based on previous research[20] and is summarized in Table 1. The program replaced the standard preschool physical activity component and consisted of static balance training, dynamic balance training, and dual-task training. Exercise progression was designed to individually challenge each child's balance ability, with the level of difficulty increased progressively according to individual performance. The training program comprised four levels of four tasks: (1) double-leg stance, (2) single-leg stance, (3) balance path, and (4) forward hop on marking sheets. Participants rotated through the four tasks under the supervision of four physical therapy students, who determined each child's appropriate progression level. If a participant was unable to complete a given level, they remained at that level for the duration of the session rather than advancing to the next one. The balance exercise program was conducted three times per week for 45 minutes per session, including a 10-minute warm-up and a 5-minute cool-down, for a total of 12 sessions over four weeks.

Control group

The control group continued the standard preschool curriculum during the four-week study period, which primarily consisted of art-based activities, including drawing and painting.

Table 1 Balance exercise program[20]

Balance exercise	Exercise progression level
Double leg stance 10 seconds, 3 sets	Level 1: double leg stance, eyes closed Level 2: stand on toes, eyes open Level 3: stand on toes, eyes closed Level 4: stand on toes, eyes closed, object in hands
Single leg stance (Both legs) 10 seconds on each leg, 3 sets	Level 1: single leg stance on firm, eyes open Level 2: single leg stance on firm, eyes closed Level 3: single leg stance on form, eyes open Level 4: single leg stance on form, eyes closed
Balance path 4.5 meters, 3 round trips	Level 1: walk on toes in straight line Level 2: walk on toes in curved path Level 3: heel-toe walk in straight line Level 4: heel-toe walk in curved path
Forward hop on marking sheets 10 repetitions on each leg, 3 sets	Level 1: jump on marking sheets Level 2: hop on marking sheets Level 3: jump on marking sheets, object in hands Level 4: hop on marking sheets, object in hands

Executive Function Development Assessment

The primary outcome of this study was the five domains of executive function (EF), assessed using the Executive Function Development Assessment (MU.EF-101), developed by Chutabhakdikul et al[2]. The MU.EF-101 demonstrated acceptable internal consistency, with a reported Cronbach's alpha coefficient of 0.77[2]. This assessment evaluates the frequency of EF behaviors in preschool children aged 2–6 years, based on reports from teachers who had been familiar with the participants for at least three months. Before using the MU.EF-101, teachers received workshop training from a physical therapist. The tool demonstrated good reliability, with intra-rater correlation (ICC = 0.84) and inter-rater correlation (ICC = 0.90). The assessment measures five EF domains: inhibition, shifting, emotional control, working memory, and planning. Responses are rated on a 5-point scale: 0 (never), 1 (1–2 times per month), 2 (1–2 times per week), 3 (3–4 times per week), and 4 (every day), with a total possible raw score of 128 points. The total score allocation is as follows: 40 points for inhibition, 20 points for shifting, 20 points for emotional control, 24 points for working memory, and 24 points for planning. EF behavior is interpreted according to T-scores: >60, much higher than average (very good); 56–60, higher than average (good); 45–55, average (moderate); 40–44, slightly lower than average (needs development); and <40, much lower than average (needs improvement).

Data collection

Data collection is presented in the CONSORT flow diagram of participants (Figure 1). Executive function (EF) behaviors were assessed at three time points: before training (pre-test, T0), after four weeks of training (post-test, T1), and at an eight-week follow-up (T2). Assessments were conducted by four teachers (one teacher per classroom) who were blinded to the participants' group assignments.

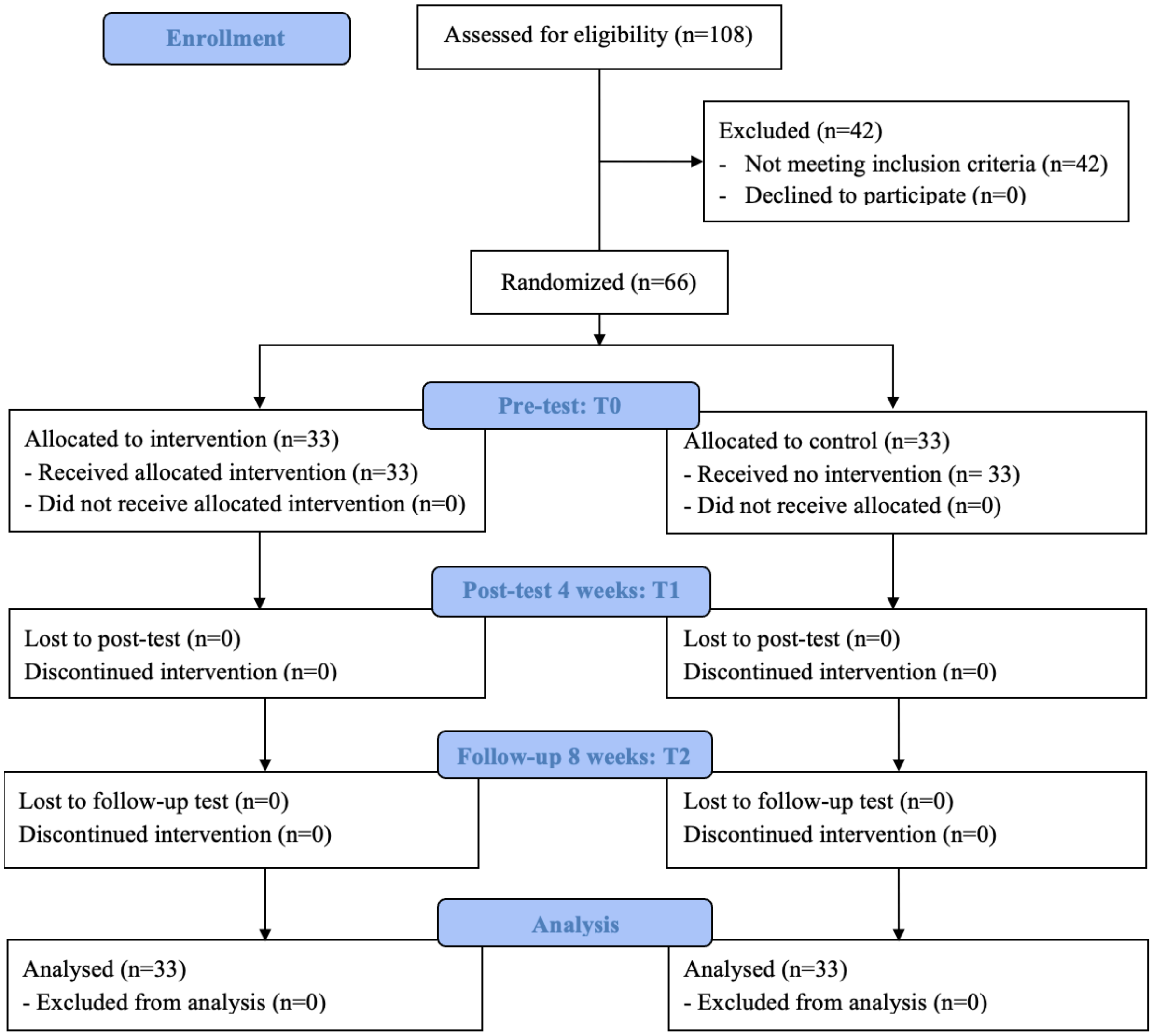

Figure 1 The CONSORT flow diagram of the participants.

Data analysis

Demographic data and EF T-scores were summarized using descriptive statistics, including means ± standard deviations (SD) and frequencies (percentages). The Kolmogorov-Smirnov test indicated that the data were not normally distributed; therefore, non-parametric statistical methods were employed to analyze EF outcomes. Within-group comparisons across the pre-test (T0), post-test (T1), and follow-up (T2) time points were conducted using the Friedman test and the Wilcoxon signed-rank test. Between-group differences were assessed using the Mann-Whitney U test. Additionally, one-way analysis of covariance (ANCOVA) was performed to examine potential baseline differences between groups at pre-test. All statistical analyses were conducted with a significance level set at p-value < 0.05.

Results

The demographic characteristics of the participants are presented in Table 2. There were no significant differences between the groups in any of the demographic variables.

Table 2 Demographic characteristics of the participants

Variable	Control group (n=33)	Balance group (n=33)	*p-value*
Age (year); (mean ± SD)	5.01±0.56	4.82±0.48	0.14
Weight (kilogram); (mean ± SD)	17.18±2.43	17.52±3.14	0.53
Height (centimeter); (mean ± SD)	108.03±5.31	104.42±16.47	0.39
Weight-height for age; (n/%)			0.54
Chubby (+1.5 SD to +2 SD)	1/3.00	1/3.00	
Slender (-1.5 SD to +1.5 SD)	27/81.80	29/87.90	
Underweight (-2 SD to -1.5 SD)	3/9.10	1/3.00	
Thin (< -2 SD)	2/6.10	2/6.10	
Sex; (n/%)			0.45
Male	11/33.30	14/42.40	
Female	22/66.70	19/57.60	

The EF raw scores of all participants are summarized in Table 3. Within-group analyses revealed significant improvements in EF behaviors after four weeks of training and at the eight-week follow-up for both groups compared with pre-test scores (T0–T1 and T0–T2, *p*-value < 0.001). However, comparisons between post-test and follow-up (T1–T2) showed some declines. In the control group, working memory (*p*-value = 0.042), planning (*p*-value = 0.004), and total EF score (*p*-value = 0.024) significantly decreased. In the balance training group, shifting (*p*-value = 0.042) and emotional control (*p*-value = 0.007) showed a significant decline between post-test and follow-up. Between-group analysis indicated that the balance group demonstrated significantly higher emotional control than the control group at post-test (*p*-value = 0.002). At the eight-week follow-up, the balance group exhibited significantly better emotional control (*p*-value = 0.036), working memory (*p*-value = 0.016), and planning (*p*-value = 0.039) compared to the control group. Nevertheless, one-way analysis of covariance (ANCOVA) revealed no significant differences between the groups in any EF domain.

The EF T-score distribution, presented as percentages of participants, is shown in Figure 2. After the four-week balance training program, 100% of participants in the balance group demonstrated moderate to very good EF behaviors across all five domains at both post-test and follow-up. In contrast, some participants in the control group continued to display slightly lower than average or much lower than average EF T-scores, indicating areas in need of development. At post-test, participants in the control group who required EF development were: inhibition (3%), shifting (3%), emotional control (6.1%), working memory (6.1%), planning (6.1%), and total EF (3%). At follow-up, participants needing EF development in the control group included inhibition (3%), shifting (6.1%), working memory (9.1%), and planning (15.2%). Additionally, at follow-up, participants requiring improvement in EF behaviors were: inhibition (3%), shifting (3%), emotional control (3%), working memory (6.1%), planning (3%), and total EF (3%).

Table 3 The raw scores of the executive function behavior

Executive function		**Groups of subjects**		***p*-value[a]**	***p*-value[b] (ANCOVA)**
		Control group (n=33)	**Balance group (n=33)**		
Inhibition	Pre-test (T0)	26.15 ± 8.28	32.45 ± 5.78	0.001*	0.001
	Post-test (T1)	35.00 ± 6.69	37.18 ± 3.36	0.754	0.138
	Follow-up (T2)	35.21 ± 7.44	37.33 ± 3.06	0.822	0.055
	p-value[c]	<0.001	<0.001		
	T0-T1	<0.001	<0.001		
	T0-T2	<0.001	<0.001		
	T1-T2	0.753	0.608		
Shifting	Pre-test (T0)	12.61 ± 3.12	15.70 ± 2.60	<0.001	<0.001
	Post-test (T1)	17.24 ± 3.21	18.73 ± 1.91	0.830	0.568
	Follow-up (T2)	16.70 ± 3.96	17.79 ± 1.96	0.677	0.067
	p-value[c]	<0.001	<0.001		
	T0-T1	<0.001	<0.001		
	T0-T2	<0.001	<0.001		
	T1-T2	0.141	0.042*		
Emotion control	Pre-test (T0)	12.45 ± 3.07	16.67 ± 2.62	<0.001	<0.001
	Post-test (T1)	16.85 ± 3.17	19.24 ± 1.66	0.002*	0.907
	Follow-up (T2)	16.39 ± 3.82	18.58 ± 1.62	0.036*	0.068
	p-value[c]	<0.001	<0.001		
	T0-T1	<0.001	<0.001		
	T0-T2	<0.001	<0.001		
	T1-T2	0.264	0.007*		
Working memory	Pre-test (T0)	14.36 ± 4.66	18.81 ± 3.35	<0.001	<0.001
	Post-test (T1)	20.64 ± 4.28	22.67 ± 2.25	0.051	0.494
	Follow-up (T2)	19.55 ± 5.23	22.82 ± 1.67	0.016*	0.776
	p-value[c]	<0.001	<0.001		
	T0-T1	<0.001	<0.001		
	T0-T2	<0.001	<0.001		
	T1-T2	0.042*	0.775		
Planning	Pre-test (T0)	14.97 ± 4.23	19.39 ± 3.26	<0.001	<0.001
	Post-test (T1)	21.67 ± 3.76	23.12 ± 1.88	0.293	0.144
	Follow-up (T2)	19.67 ± 4.97	22.70 ± 1.45	0.039*	0.993
	p-value[c]	<0.001	<0.001		
	T0-T1	<0.001	<0.001		
	T0-T2	<0.001	<0.001		
	T1-T2	0.004*	0.139		

Table 3 The raw scores of the executive function behavior (Cont.)

Executive function		Groups of subjects		*p*-value[a]	*p*-value[b] (ANCOVA)
		Control group (n=33)	Balance group (n=33)		
Total	Pre-test (T0)	80.55 ± 22.79	103.03 ± 15.35	<0.001	<0.001
	Post-test (T1)	111.39 ± 20.08	120.94 ± 7.32	0.419	0.100
	Follow-up (T2)	107.52 ± 24.41	119.21 ± 7.67	0.419	0.061
	p-value[c]	<0.001	<0.001		
	T0-T1	<0.001	<0.001		
	T0-T2	<0.001	<0.001		
	T1-T2	**0.024***	0.083		

Note: Data reported as mean ± standard deviation, the unit of measurement is points, T0-T1; within-group differences between pre-test to pos-test, T0-T2; within-group differences between pre-test to follow-up, T1-T2; within-group differences post-test to follow-up, *significance tested at <0.05, *p*-value[a] Mann-Whitney test, *p*-value[b] ANCOVA, *p*-value[c] Friedman test

Discussion

Our study indicated that a four-week balance exercise program can enhance executive function (EF) development in preschool children aged 4–6 years, particularly in the balance training group. Notably, significant differences in EF raw scores between groups at baseline may have influenced post-test outcomes due to potential ceiling effects. However, when examining EF development using T-scores, most participants in the balance exercise group exhibited moderate to very good EF skills across all domains at both post-test and follow-up. In contrast, some participants in the control group continued to show slightly below average to well below average EF T-scores across all domains, indicating a need for further development and support in EF skills. These findings suggest that children who participate in the balance exercise program may have greater opportunities to enhance their EF capabilities.

Previous research has emphasized that Thai children aged 5–6 years require interventions to improve inhibition and emotional control[22]. This aligns with our findings, as children in the control group demonstrated less improvement in shifting and emotional control compared with those in the balance exercise group. Emotional control and planning are critical components of higher-order EF regulation during early childhood, developing from foundational EF skills such as inhibition and cognitive flexibility, and evolving throughout development[23,24]. Numerous studies[24] have highlighted the importance of EF in school-aged children, particularly in supporting academic performance, social functioning, and emotional regulation. As children enter structured social and educational settings, the demands for self-control and EF regulation increase.

Diamond et al[9] suggested that a variety of activities, including physical activity and school curricula, can enhance executive function (EF). Classroom curricula led by regular teachers, such as reading, mathematics, and drawing, have been shown to improve EF skills in children aged 4–5 years. In addition, diverse physical activities, including martial arts, yoga, aerobics, and mindfulness, can promote EF development and may be incorporated into school curricula[9]. Therefore, a comprehensive approach that integrates emotional, social, and physical development rather than focusing exclusively on either physical activity or classroom instruction may be an effective strategy for enhancing EF and, consequently, academic achievement. Our findings indicate that children in the balance group, who participated in both physical activities and routine school tasks, exhibited significant improvements in EF.

Consistent with our results, previous studies[25,26] have demonstrated that various physical activity programs positively influence EF during early childhood. These interventions were designed to be active, enjoyable, and socially engaging[25,26]. Best[25] reported that coordinating multiple movements in dynamic, goal-directed tasks is effective for EF development. Similarly, Nejati[26] found that dual-task balance and cognitive activities can enhance EF and reduce symptoms in children with attention deficit hyperactivity disorder (ADHD). Although balance is often considered an automatic activity, it still requires attention, cognitive flexibility, inhibition, and working memory, particularly under unpredictable conditions or dual-task scenarios. Likewise, the balance program in the present study included unstable surfaces, interrupted sensory input, and dual-task challenges, all based on goal-directed balance tasks. In contrast, Şendil et al[27] reported no significant effects on inhibitory control following an 8-week structured coordination exercise program, which included locomotor and balance skills training. Differences in study outcomes may be attributable to sample characteristics and context: Şendil et al's study included a higher proportion of boys recruited from forty-one kindergartens with potentially varying curricula and physical education schedules. In comparison, the present study was conducted in a single kindergarten with a greater proportion of girls.

Despite these findings, our study demonstrates that incorporating a balance exercise program into the classroom curriculum significantly enhances the frequency of positive EF-related behaviors, resulting in overall improved EF development in the balance group. Balance exercises require the coordination of multisensory systems and cognitive processes, including attention and executive function[28]. Attention, a core EF component, enables children to focus on environmental stimuli, thereby supporting EF performance[29]. The relationship between EF and balance is mediated by the cortical-ponto-cerebellar pathway[30,31]. Previous research has shown that cerebellar damage is a risk factor for dysexecutive function and social-emotional difficulties in children[32]. Similarly, damage to the cerebral cortex, basal ganglia, or cerebellum can impair cognitive functions related to balance. The prefrontal cortex plays a critical role in regulating EF skills, such as planning and attention, which are essential for goal-directed skilled movement[30,31]. Moreover, the dorsolateral prefrontal cortex is particularly important for EF regulation, and brain maturation in children aged 4–6 years significantly contributes to cognitive development, especially inhibition[33,34]. Therefore, balance and EF are interconnected through multiple components of the nervous system, particularly the prefrontal cortex. Children who practice balance tasks simultaneously engage their EF skills, thereby promoting EF development.

However, this study has several limitations. Conducted in a single school, the findings may have limited generalizability, and the study did not include comparisons with other interventions, such as aerobic exercise or dance training. Additionally, EF assessments completed by teachers may have been subject to observer bias. Future research should address these limitations, including determining the optimal comprehensive balance training protocol in larger and more diverse populations. Moreover, outcome measurements for investigating EF development should consider both EF domains and associated neuronal adaptations.

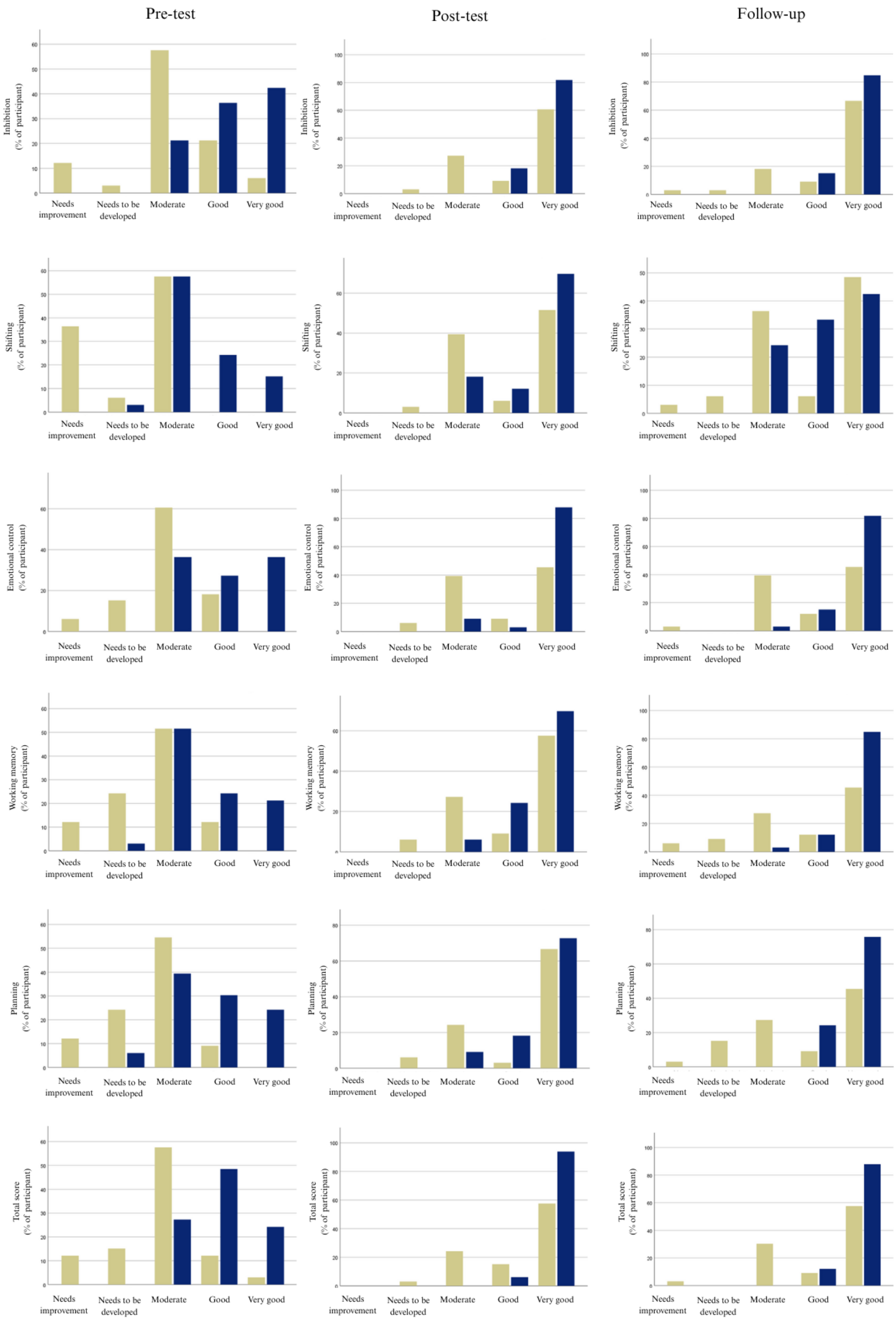

Figure 2 Executive function T-score interpretation.
Note: Data are presented as the percentage (%) of participations; gray bars indicate the control group, and blue bars indicate the balance training group

Conclusion

A four-week balance exercise program can effectively enhance executive function (EF) development in preschool children more than a standard preschool curriculum. These findings provide guidance for educators to integrate balance exercises into regular preschool activities, such as physical education classes, to support and enhance children's EF development.

Take home messages

The four-week balance exercise program is a feasible and effective strategy for promoting executive function development in preschool children aged 4–6 years. Incorporating this program into a standard preschool curriculum can optimize developmental outcomes in EF skills.

Conflicts of interest

The authors declare no conflict of interest.

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Author contributions

Supanee Chounchay: Conceptualization, Methodology, Formal analysis, Writing – Original draft, Review & Editing.

Jenjira Assapun: Conceptualization, Methodology, Data collection, Formal analysis.

Boonrat Ngowtrakul: Conceptualization, Data collection, Formal analysis.

Wannisa Saenphan: Conceptualization, Data collection, Formal analysis.

Data availability

Data available on request due to privacy/ethical restrictions

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Supplementary

Supplementary table 1 The executive function T-score interpretation.

Executive function	T-score interpretation	Control group (n=33)						Balance group (n=33)					
		Pre-test		Post-test		Follow-up		Pre-test		Post-test		Follow-up	
		N	%	N	%	N	%	N	%	N	%	N	%
Inhibition	Very good	2	6.1	20	60.6	22	66.7	14	42.4	27	81.8	28	84.8
	Good	7	21.2	3	9.1	3	9.1	12	36.4	6	18.2	5	15.2
	Moderate	19	57.6	9	27.3	6	18.2	7	21.2	0	0.0	0	0.0
	Needs to be developed	**1**	**3.0**	**1**	**3.0**	**1**	**3.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**
	Needs improvement	4	12.1	0	**0.0**	**1**	**3.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**
Shifting	Very good	0	0.0	17	51.5	16	48.5	5	15.2	23	69.7	14	42.4
	Good	0	0.0	2	6.1	2	6.1	8	24.2	4	12.1	11	33.3
	Moderate	19	57.6	13	39.4	12	36.4	19	57.6	6	18.2	8	24.2
	Needs to be developed	2	6.1	1	3.0	2	6.1	1	3.0	0	0.0	0	0.0
	Needs improvement	**12**	**36.4**	**0**	**0.0**	**1**	**3.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**
Emotional control	Very good	0	0.0	15	45.5	15	45.5	12	36.4	29	87.9	27	81.8
	Good	6	18.2	3	9.1	4	12.1	9	27.3	1	3.0	5	15.2
	Moderate	20	60.6	13	39.4	13	39.4	12	36.4	3	9.1	1	3.0
	Needs to be developed	5	15.2	2	6.1	0	0.0	0	0.0	0	0.0	0	0.0
	Needs improvement	2	6.1	0	0.0	1	3.0	0	0.0	0	0.0	0	0.0
Working memory	Very good	0	0.0	19	57.6	15	45.5	7	21.2	23	69.7	28	84.8
	Good	4	12.1	3	9.1	4	12.1	8	24.2	8	24.2	4	12.1

Supplementary table 1 The executive function T-score interpretation. (Cont.)

Executive function	T-score interpretation	Control group						Balance group					
		Pre-test		Post-test		Follow-up		Pre-test		Post-test		Follow-up	
		N	%	N	%	N	%	N	%	N	%	N	%
	Moderate	17	51.5	9	27.3	9	27.3	17	51.5	2	6.1	1	3.0
	Needs to be developed	8	24.2	2	6.1	3	9.1	1	3.0	0	0.0	0	0.0
	Needs improvement	4	12.1	0	0.0	2	6.1	0	0.0	0	0.0	0	0.0
Planning	Very good	0	0.0	22	66.7	15	45.5	8	24.2	24	72.7	25	75.8
	Good	3	9.1	1	3.0	3	9.1	10	30.3	6	18.2	8	24.2
	Moderate	18	54.5	8	24.2	9	27.3	13	39.4	3	9.1	0	0.0
	Needs to be developed	8	24.2	2	6.1	5	15.2	**2**	**6.1**	**0**	**0.0**	**0**	**0.0**
	Needs improvement	**4**	**12.1**	**0**	**0.0**	**1**	**3.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**
Total EF	Very good	1	3.0	19	57.6	19	57.6	8	24.2	31	93.9	29	87.9
	Good	4	12.1	5	15.2	3	9.1	16	48.5	2	6.1	4	12.1
	Moderate	19	57.6	8	24.2	10	30.3	9	27.3	0	0.0	0	0.0
	Needs to be developed	**5**	**15.2**	**1**	**3.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**
	Needs improvement	4	12.1	0	0.0	1	3.0	0	0.0	0	0.0	0	0.0

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Stone culturing: a more effective approach to diagnosing bacterial infections in kidney stone formers

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KEYWORDS
Kidney stones;
Urinary tract infection;
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ABSTRACT
According to the bacteria found in stone niduses, these bacteria may be responsible for lithogenesis. Therefore, we considered culturing stone niduses (SN) as the gold standard for comparing bacterial culture results from stone peripheries (SP), renal pelvic urine (RPU), and midstream urine (MSU), including an evaluation of performance. Data from 36 kidney stone formers were collected, including demographics, imaging diagnostics, urinalysis, and preoperative midstream urine culture. The samples of SN, SP, and RPU were cultured to identify microorganisms. SN were also analyzed for their chemical composition. Diagnostic testing, including sensitivity, specificity, positive likelihood ratio (LR+), negative likelihood ratio (LR-), positive predictive value (PPV), negative predictive value (NPV), and area under the curve (AUC) with 95% confidence intervals (CI), was performed. The results showed that 16 (44.44%) SN, 17 (47.22%) SP, 12 (33.33%) RPU, and 18 (50.00%) MSU were positive for bacterial culture. For the performance testing that compares SN and the other three specimens, the sensitivity, LR+, PPV, NPV, and AUC of SP culture (sensitivity = 100%, LR+ = 20.00, PPV = 94.10%, NPV = 100%, AUC = 0.975) demonstrated a high level, exceeding that of RPU and MSU cultures. The level of agreement between SN and SP cultures was almost perfect (0.94). *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* were the most commonly isolated bacteria from stone and urine cultures. Moreover, *P. mirabilis* and *E. coli* were the most common bacteria isolated from struvite and calcium oxalate monohydrate (COM) stone compositions, respectively. Our data indicate that culturing SN exhibited higher concordance with SP than the urine culture. *P. mirabilis* and *E. coli* were the most commonly isolated from infection-induced (i.e., struvite) and non infection-induced (i.e., COM) stones, respectively. Integrating stone and urine cultures into the diagnostic workflow for bacterial infections in KSFs is recommended.

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Introduction

Nephrolithiasis and urinary tract infections (UTIs) are often associated, but the etiological relationship remains unclear[1,2]. A dilemma arises: (i) infection-induced stones result from UTIs, commonly caused by urease-producing bacteria such as *Proteus* spp., which are typically composed of struvite (SV), carbonate apatite (CA), or ammonium urate[1-5]. UTIs caused by certain secretory products and/or virulence factors of microorganisms can also induce stone formation; (ii) for stones with subsequent infection,[2] the occurrence of uroliths can lead to subsequent UTIs by urease or non-urease producing bacteria, which have metabolic chemical components such as calcium oxalate (CaOx), and uric acid (UA) [2-3,5-6]. In a 2012 study, 45 bacteria were isolated from the urine and/or stones of 36 patients with nephrolithiasis[7]. Subsequently, a 2017 study revealed that *Escherichia coli* within the stone may adapt to survive in the microenvironment and subsequently cause recurrent UTIs in kidney stone formers (KSFs)[8]. Consequently, bacterial identification within the stone and urine is crucial for completely eradicating these bacteria. Nevertheless, conventional midstream urine cultures, the most routine procedure for evaluating UTIs in KSFs, may often provide insufficiently reliable results[2,9-10]. Previous studies in urosepsis[11-12] have recommended culturing kidney stone and renal pelvic urine samples collected during nephrectomy for bacterial infections. Additionally, the diagnostic performance of these samples for bacterial infections in KSFs remains limited. Based on previous studies conducted in 2012 and 2017, the bacteria present in the stone sample, particularly within the stone nidus, may be the causative agents involved in stone formation and pathogenesis[7-8]. Herein, we selected the culture results from the stone niduses (SN) as the gold standard. Moreover, the stone peripheries (SP) and the renal pelvic urine (RPU) were also collected during the perioperative period. Accordingly, this study was conducted to compare the results of bacterial culture from midstream urine (MSU), RPU, and kidney stone samples in KSFs, aiming to identify the most appropriate sampling culture for accurately detecting the bacterial infection, including determining the concordance between stone and urine culturing results. The types of microorganisms from SN, SP, and RPU were identified. SN were also analyzed for their chemical composition.

Materials and methods

Data and specimen collection

This study represents a diagnostic investigation for bacterial infections in KSFs. The Khon Kaen University Ethics Committee approved this study for Human Research based on the Declaration of Helsinki (HE611387 and HE651324). Informed consent was obtained from all patients. A total of 63 KSFs who underwent percutaneous nephrolithotomy or open nephrectomy between 2018 and 2021 were included. Seven of the 63 KSFs without RPU were excluded. Data from 20 KSFs' midstream urine cultures with mixed microorganisms and incomplete data were also excluded. Therefore, 36 KSFs were enrolled in this study. Patient data were collected as follows: (i) demographic, (ii) imaging diagnostics, and (iii) urinalysis and MSU culture data. Samples of SN, SP, and RPU were collected aseptically during PCNL or open nephrectomy, cultured, and identified for the presence of microorganisms. In this study, the stone nidus culture was considered the gold standard for evaluating the characteristics of the remaining sample culture. SN were also analyzed for their chemical composition.

Identification of bacteria isolated from stone and renal-pelvic urine samples

The samples of SN, SP, and RPU were processed and cultured on blood and MacConkey agar at 35-37 °C for 18-24 h[7]. All microorganisms were identified by morphology (e.g., colony shape, size, and color), microscopic examination of cell morphology and Gram staining, and standard biochemical methods that assess bacterial enzymatic activity and metabolic capabilities, further refining the identification process[13].

Analysis of the chemical composition of kidney stones

After drying, the SN were ground into powder. Subsequently, the chemical compositions were analyzed using an Attenuated Total Reflection Fourier-transform infrared (ATR-FTIR) spectrometer (model Tensor-II; Bruker Optics, Germany) with a resolution of 4 cm^{-1}, co-add scans at 32 cm^{-1}, and a spectral range of 4,000-400 cm^{-1}. The FTIR spectral data of each sample were analyzed using Bruker's spectral libraries to determine the chemical composition of uroliths. The chemical compositions of stone samples were obtained by matching the RENAL, RENAL01, and RENAL02 spectral BLG libraries with the hit quality of the main components[14]. Based on the main chemical compositions of the stone niduses, they were categorized into two groups: infection-induced stones (IIS) and non infection-induced stones (non-IIS)[5]. IIS mainly comprises SV, CA, whitlockite (Wk), amorphous carbonated calcium phosphate, or ammonium urate stones[5,15]. Others were considered to be non-IIS or metabolic stones.

Statistical analysis

Statistical significance was defined as a *p*-value of less than 0.05. Statistical analyses were conducted using STATA version 18.0 (College Station, Texas, USA). Descriptive statistics summarized the data, and results were reported as means with standard deviations (mean ± SD) or medians with interquartile ranges (IQR) for continuous variables. For the categorical data section, frequencies and percentages were reported.The independent t-test was used to compare the continuous data. Pearson's chi-squared or Fisher's exact test was used to compare categorical data outcomes. The diagnostic testing of each sample culture, including sensitivity, specificity, positive likelihood ratio (LR+), negative likelihood ratio (LR-), positive predictive value (PPV), and negative predictive value (NPV) with 95% confidence intervals (CI), was performed. The areas under the curve (AUC) were also calculated for each sample culture, and the results were compared to identify the most suitable sample for optimal performance.

Results

Demographic data of kidney stone formers

A total of 36 KSFs (Table 1) were divided into two groups: males (n=23) and females (n=13). The average ages of males and females were 56.83 ± 9.14 and 55.77 ± 11.56 years, respectively. Among the underlying diseases, including hypertension (HT), dyslipidemia, chronic kidney disease, and gout, there were no statistically significant differences between the two groups. Only diabetes mellitus (DM) was statistically significantly more frequent in the female group (30.77%) compared to the male group (4.35%) (p-value < 0.05). Furthermore, HT showed a high frequency in KSFs. The average stone size in females (4.52 ± 1.66 cm) was greater than in males (4.15 ± 1.12 cm). Females exhibited a significantly higher frequency in IIS compositions than males (*p*-value < 0.05). In the urinalysis, urinary nitrite positivity and urinary pH showed higher frequencies and values in females compared to males (all *p*-values < 0.05). Furthermore, the culture results from all four specimens in females demonstrated a notably higher positivity rate than in males (all *p*-values < 0.05).

Characteristics of bacterial culturing results from SN, SP, RPU, and MSU

Bacterial positivity culture results from SN, SP, RPU, MSU were evaluated (Table 2). SN and SP cultures consistently showed better results than the urine sample cultures. Considering the SN culture as the gold standard, the sensitivity, specificity, LR+, LR-, PPV, and NPV with 95% CI of SP, RPU, and MSU cultures were also calculated (Table 3). SP culture demonstrated high sensitivity and specificity, with values of 95% or higher. In urine culture samples, RPU exhibited high specificity, whereas MSU revealed high sensitivity. Notably, the PPVs of SP (94.10%) and RPU (91.70%) cultures were higher than that of MSU (83.30%).

Table 1 Characteristics and clinical laboratory data of kidney stone formers

Parameters		Kidney stone formers (n=36)	Male (n=23)	Female (n=13)	*p*-value
Demographic data					
Age (years) [a]		56.44 ± 9.93	56.83 ± 9.14	55.77 ± 11.56	0.764
Age group [c]					
≤ 40 yrs		3 (8.33%)	2 (8.70%)	1 (7.69%)	1.000
> 40 yrs		33 (91.67%)	21 (91.30%)	12 (92.31%)	
Underlying diseases					
Hypertension (HT) [b]	Yes	15 (41.67%)	9 (39.13%)	6 (46.15%)	0.681
	No	21 (58.33%)	14 (60.87%)	7 (53.85%)	
Diabetes mellitus (DM) [c]	Yes	5 (13.89%)	1 (4.35%)	4 (30.77%)	**0.047**
	No	31 (86.11%)	22 (95.65%)	9 (69.23%)	
Dyslipidemia [c]	Yes	6 (16.67%)	2 (8.70%)	4 (30.77%)	0.161
	No	30 (83.33%)	21 (91.30%)	9 (69.23%)	
Chronic kidney disease (CKD) [c]	Yes	3 (8.33%)	3 (13.04%)	0	0.288
	No	33 (91.67%)	20 (86.96%)	13 (100%)	
Gout [c]	Yes	2 (5.56%)	2 (8.70%)	0	0.525
	No	34 (94.44%)	21 (91.30%)	13 (100%)	
Imaging diagnostics and chemical compositions of kidney stones					
Location of stone in kidney [b]					
Left		16 (44.44%)	12 (52.17%)	4 (30.77%)	0.721
Right		20 (55.56%)	11 (47.83%)	9 (69.23%)	
Stone sizes					
Stone sizes; longest length* (cm) [a]		4.28 ± 1.33	4.15 ± 1.12	4.52 ± 1.66	0.428
Chemical compositions [c]					
IIS		9 (25.00%)	3 (13.04%)	6 (46.15%)	**0.046**
Non-IIS		27 (75.00%)	20 (86.96%)	7 (53.85%)	
Urinalysis					
Leukocyte esterase [c]	Positive	29 (80.56%)	17 (73.91%)	12 (92.31%)	0.382
	Negative	7 (19.44%)	6 (26.09%)	1 (7.69%)	
Nitrite [c]	Positive	11 (30.56%)	3 (13.04%)	8 (61.54%)	**0.006**
	Negative	25 (69.44%)	20 (86.96%)	5 (38.46%)	
Pyuria [b]	Yes	21 (58.33%)	11 (47.83%)	10 (76.92%)	0.089
(WBCs in urine >10 cells/HPF)	No	15 (41.67%)	12 (52.17%)	3 (23.08%)	
Urinary pH [a]		6.17 ± 0.85	5.96 ± 0.69	6.54 ± 1.00	**0.048**
Urinary pH ≥7: <7 [c]		7: 29	2: 21	5: 8	0.073

Table 1 Characteristics and clinical laboratory data of kidney stone formers (Cont.)

Parameters		Kidney stone formers (n=36)	Male (n=23)	Female (n=13)	*p*-value
Culturing results of stone and urine samples					
SN [b]	Positive	16 (44.44%)	5 (21.74%)	11 (84.62%)	**<0.001**
	Negative	20 (55.56%)	18 (78.26%)	2 (15.38%)	
SP [b]	Positive	17 (47.22%)	6 (26.09%)	11 (84.62%)	**0.001**
	Negative	19 (52.78%)	17 (73.91%)	2 (15.38%)	
RPU [c]	Positive	12 (33.33%)	4 (17.39%)	8 (61.54%)	**0.011**
	Negative	24 (66.67%)	19 (82.61%)	5 (38.46%)	
MSU [c]	Positive	18 (50.00%)	7 (30.43%)	11 (84.62%)	**0.002**
	Negative	18 (50.00%)	16 (69.57%)	2 (15.38%)	

Note: Values are mean±SD; n or n (%), Analyzed by [a] Independent t-test; [b] Pearson's chi-squared; [c] Fisher's exact test, [*] stone sizes were evaluated from plain KUB (kidney, ureter, and bladder) films.
Abbreviations: IIS, infection-induced stones; non-IIS, non infection-induced stones; WBCs, white blood cells; SN, stone niduses; SP, stone peripheries; RPU, renal pelvic urine; MSU, midstream urine.

Table 2 Simultaneous bacterial culturing results from SN, SP, RPU, and MSU

Parameters	Test status of culture	Results of SN	
		Positive	Negative
SP	Positive	16 (100%)	1 (5.00%)
	Negative	0	19 (95.00%)
RPU	Positive	11 (68.75%)	1 (5.00%)
	Negative	5 (31.25%)	19 (95.00%)
MSU	Positive	15 (93.75%)	3 (15.00%)
	Negative	1 (6.25%)	17 (85.00%)
Total		16	20

Abbreviations: SN, stone niduses; SP, stone peripheries; RPU, renal pelvic urine; MSU, midstream urine.

Table 3 Diagnostic testing with 95% CI of bacterial infections in KSFs by bacterial culturing results from SP, RPU, and MSU

Parameters	SP	RPU	MSU
Sensitivity	100% [79.40%, 100%]	68.80% [41.30%, 89.00%]	93.80% [69.80%, 99.80%]
Specificity	95.00% [75.10%, 99.90%]	95.00% [75.10%, 99.90%]	85.00% [62.10%, 96.80%]
LR+	20.00 [2.96, 135.00]	13.80 [1.98, 95.60]	6.25 [2.19, 17.90]
LR-	NA	0.33 [0.16, 0.68]	0.07 [0.01, 0.50]
PPV	94.10% [71.30%, 99.90%]	91.70% [61.50%, 99.80%]	83.30% [58.60%, 96.40%]
NPV	100% [82.40%, 100%]	79.20% [57.80%, 92.90%]	94.40% [72.70%, 99.90%]

Abbreviations: SP, stone peripheries; RPU, renal pelvic urine; MSU, midstream urine; LR+, positive likelihood ratio; LR-, negative likelihood ratio; PPV, positive predictive value; NPV, negative predictive value; NA, not applicable.

Comparison of three sample culturing

SP, RPU, and MSU cultures were evaluated using the receiver operating characteristic (ROC)-derived AUC. The AUC for the three-sample culture demonstrated a significant difference (p-value < 0.05) (Figure 1). The AUC of the SP culture was higher than both the RPU and MSU cultures. Notably, SP culture exhibited a significantly higher AUC than RPU culture alone (p-value < 0.05). On the other hand, no significant difference was found between the AUC of SP versus MSU (p-value = 0.080) and RPU versus MSU (p-value = 0.348).

The agreement levels among the stone and urine cultures

Cohen's Kappa levels of agreement in SN, SP, RPU, and MSU cultures were performed (Figure 2). According to McHugh.[16], on the interpretation of Cohen's Kappa, only SN and SP revealed an almost perfect agreement level (0.94), indicating the strongest concordance in bacterial detection between SN and SP. Meanwhile, the remaining pairs (e.g., SP with MSU or SP with RPU) exhibit varying levels of agreement, ranging from weak to strong.

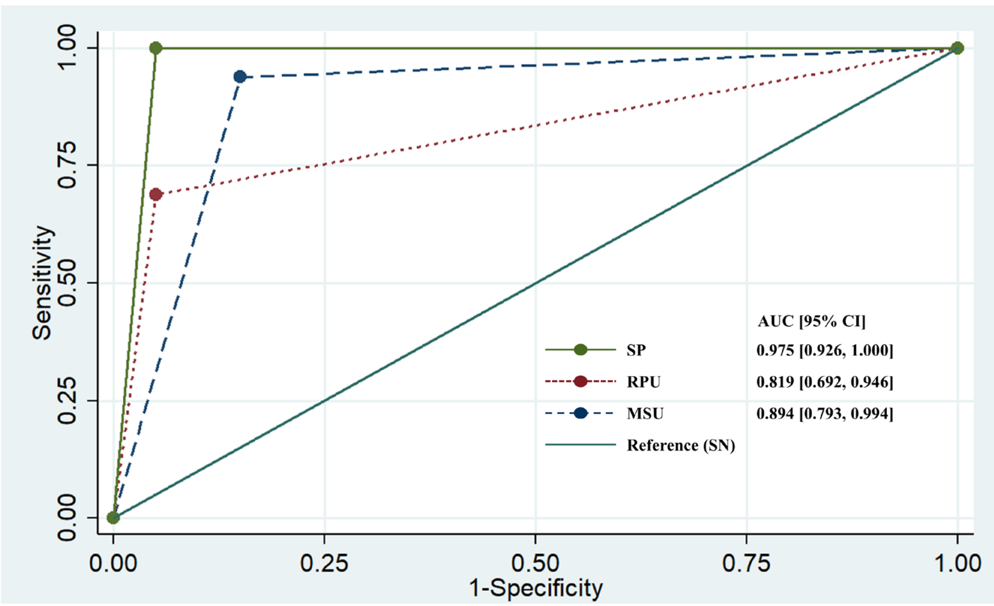

Figure 1 Receiver operating characteristic (ROC) curves for stone peripheries (SP), renal pelvis urine (RPU), and midstream urine (MSU) cultures with the area under the curve (AUC) and its 95% confidence intervals (CI)

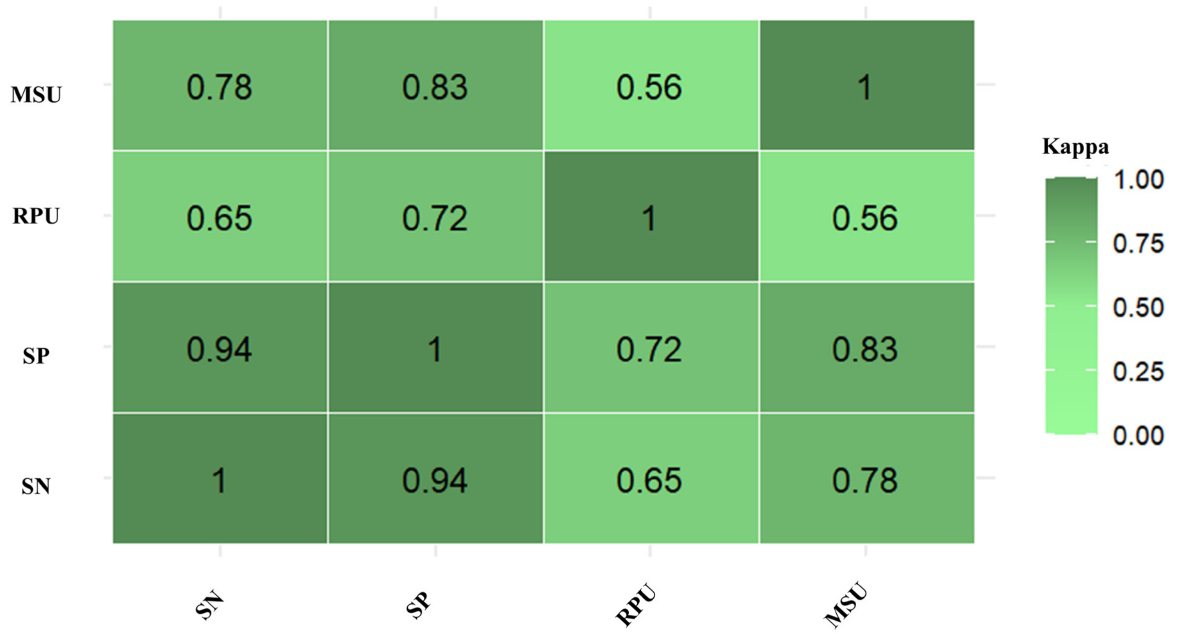

Figure 2 Cohen's Kappa agreement between stone niduses (SN), stone peripheries(SP), renal pelvic urine (RPU), and midstream urine (MSU)
The level of agreement is interpreted based on the Kappa level as > 0.90 (Almost perfect), 0.80-0.90 (Strong), 0.60-0.79 (Moderate), 0.40-0.59 (Weak), 0.21-0.39 (Minimal), < 0.20 (None)[16]

Concordance between bacteria isolated from the stone and urine samples

According to the strongest levels of agreement in SN and SP cultures, the similarity between bacterial isolates from SN and SP, as well as the chemical compositions of SN, was demonstrated (Table 4). Of the 36 KSFs recruited, 16 (44.44%) and 17 (47.22%) patients had bacteria isolated from their SN and SP, respectively. The most commonly isolated bacteria from SN and SP specimens were *E. coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis*. Of the 16 cases out of 17 KSFs with bacterial isolates from both SN and SP, one remaining case had *Pseudomonas* spp. isolated from SP, RPU, and MSU. In a comprehensive analysis of the bacterial types and SN chemical compositions, *P. mirabilis* and *E. coli* were the most commonly isolated bacteria from SV and COM stones, respectively.

Table 4 Comparative analysis of bacteria isolated from SN and SP samples

Bacteria	Bacteria isolated from SN (cases)	Bacteria isolated from SP (cases)	Chemical compositions of SN
Pure microorganism			
Gram-negative bacteria			
Escherichia coli	5	5	COM
Klebsiella pneumoniae	3	3	COM or UA or CA or Wk
Proteus mirabilis	3	3	SV or CA
Escherichia fergusonii	1	1	SU
Pseudomonas spp.	0	1	COM
Gram-positive bacteria			
Enterococcus faecalis	1	1	COM
Staphylococcus saprophyticus	1	1	SU
Mixed microorganisms			
E. coli & *P. mirabilis*	1	1	SV
E. coli & *K. pneumoniae*	1	1	COM
Total	16	17	

Abbreviations: SN, stone niduses; SP, stone peripheries; COM, calcium oxalate monohydrate; UA, uric acid; CA, carbonate apatite; Wk, whitlockite; SV, struvite; SU, sodium urate

Discussion

High prevalence rates of nephrolithiasis have been reported globally and in northeastern Thailand[17-19]. According to sex and underlying diseases in KSFs, previous studies have shown high frequencies of males[20] and HT[21], which align with our findings. Notably, DM was found more frequently in females than in males, consistent with a previous study[22]. In part of the chemical compositions, non-IIS and IIS were observed in most males and females, respectively, consistent with a previous study[23]. Interestingly, our urinalysis results (leukocyte esterase, nitrite, pyuria, and urinary pH) and the culturing of stone and urine samples were consistent in the cases of IIS. A risk of UTIs in females is attributed to the anatomy of their urinary system, such as the shorter urethra compared to males, which may contribute to ascending infections and IIS formation[4,15,23]. Considering that SN culture is the gold standard, the sensitivity, LR+, PPV, NPV, and AUC of SP were high, exceeding those of RPU and MSU. These findings support and reinforce a previous study from 2012, which emphasized the importance of culturing stones rather than relying solely on urine-based diagnostics[7]. MSU culture may not represent the infection status of the upper tract, especially in the presence of obstruction[24]. According to PPV results, intraoperative stones and RPU provided better diagnostic accuracy for bacterial infections in KSFs than MSU, particularly when culture results were positive, consistent with previous studies[9-12,24]. Additionally, SP, RPU, and MSU cultures were evaluated using the receiver operating characteristic (ROC)-derived AUC. SP culture showed the best performance in discriminating against bacterial infections in KSFs. The comprehensive evaluation of the cultures of four specimens (i.e., SN, SP, RPU, and MSU) in KSFs regarding the association between nephrolithiasis and UTIs is still limited. Previous studies have emphasized the correlation among MSU, RPU, and stone cultures in KSFs with post-operative sepsis or recurrence complications, in which stone culture is consistently regarded as the most accurate diagnostic method[11-12, 24]. As part of the agreement analysis of four specimens, SN and SP revealed an almost perfect level of agreement, indicating the strongest concordance in bacterial detection between SN and SP. These findings suggest that collecting stone fragments, including SN and SP, could be used to assess the bacterial infection. Meanwhile, the remaining pairs (e.g., SP with MSU or SP with RPU cultures) exhibit varying levels of agreement, ranging from weak to strong. Therefore, SP may prioritize diagnostic bacterial infections in KSFs. The implications for perioperative antimicrobial management and infection control strategies in patients undergoing procedures for kidney stone removal may affect urine samples, potentially influencing the agreement between stone and urine cultures, which can range from weak to strong. To our knowledge, we hypothesized that a bidirectional relationship between nephrolithiasis and UTIs could be elucidated using the culture results from SN specimens, which are regarded as the gold standard in our study. The bacterial isolates from SN and SP were related to the chemical composition of SN. Stone culture may be associated with bacterial infections related to lithogenesis, while urine culture may be involved in distributing stones that lead to subsequent infections. Therefore, incorporating stone and urine cultures into KSFs may be beneficial for elucidating the temporal sequence of challenges between nephrolithiasis and UTIs. Herein, the bacteria isolated from the SN and SP samples were observed. *E. coli*, *K. pneumoniae*, and *P. mirabilis* were the most common bacterial isolates from the stone samples, consistent with a previous study[7]. In a comprehensive analysis of the bacterial types and SN's chemical compositions, almost all bacterial isolates from SN and SP samples were related to the SN compositions. *P. mirabilis* and *E. coli* were the most common bacterial isolates from SV and COM compositions, respectively. These findings were consistent with previous studies[1-3,7,25]. Urea-splitting bacteria, such as *P. mirabilis* and *K. pneumoniae*, use the urease enzyme to break down urea into ammonia and carbon dioxide. As a result, this breakdown produces ammonium and bicarbonate ions, increasing urine alkalinity. When ammonium ions and bicarbonate attach to available ions, they promote the formation of SV or CA. Additionally, some SV crystals may accumulate, damage the urothelium, and potentially lead to the development of massive staghorn SV stones[3-4,26-27]. In contrast to the association between *E. coli* and COM stones, a 2013 study revealed that *E. coli* promoted the growth and aggregation of CaOx crystals in *in vitro* experiments[28]. Following a 2015 *in vivo* murine investigation, a study indicated that *E. coli* could increase CaOx deposition in the kidney[29]. Moreover, previous *in vitro* studies have revealed that certain components of *E. coli* (e.g., elongation factor Tu (EF-Tu), outer membrane vesicles (OMVs), and flagellum) could play significant roles in promoting CaOx crystal growth and aggregation[30-31]. The stone culture diagnostic is highly effective, enabling the identification of bacterial infections in KSFs. At both the PPV and agreement levels, we also identified SP culture positivity as a potential diagnostic marker of bacterial infections in KSFs. Our findings facilitate the development of a diagnostic protocal for bacterial infections in KSFs.

Conclusion

Comparing SN with the other three culture specimens, the sensitivity, LR+, PPV, NPV, and AUC of SP culture were higher, exceeding those of RPU and MSU. The agreement level between SN and SP cultures was almost perfect. *E. coli*, *K. pneumoniae*, and *P. mirabilis* were the most frequently isolated bacteria from stone samples. A thorough analysis of the types of bacterial isolates and SN chemical compositions revealed that *P. mirabilis* and *E. coli* were the most isolated bacteria from IIS (i.e., SV) and non-IIS (i.e., COM). Stone culture may be associated with bacterial infections related to lithogenesis, while urine culture may be involved in distributing stones that lead to subsequent infections. Herein, integrating stone and urine cultures into the diagnostic workflow for bacterial infections in KSFs is recommended.

Take home messages

Stone cultures demonstrate superior efficacy in detecting bacteria in kidney stones compared to urine cultures. Bacteria, including *E. coli* and *P. mirabilis*, are frequently associated with specific types of stones. Integrating stone cultures with urine cultures significantly enhances the diagnosis for bacterial infections in KSFs.

Conflict of interest statement

The authors declare no conflict of interest.

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Data availability

Data available on request due to privacy/ethical restrictions

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Tactile sensation is needed for challenging walking ability among ambulatory individuals with stroke

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KEYWORDS
Cerebrovascular accident; Gait, Neurology; Proprioception; Rehabilitation.

ABSTRACT
The improvement of walking ability is a key goal of stroke rehabilitation. The exploration of the contribution of sensorimotor functions to walking ability specifically among ambulatory individuals with stroke may provide data to enhance patient-centered care planning for this population. This study investigated the relationship between the lower extremity (LE) sensorimotor functions and walking ability as measured using the 10-meter walk test (10MWT) among 75 ambulatory individuals with stroke. Participants with stroke who could walk independently with or without a walking device were assessed for their LE sensorimotor functions, and 10MWT while walking at their preferred and fastest speeds. The correlations were analyzed using the Spearman's rank correlation coefficient (r_s). The findings indicated that walking ability of the participants correlated predominantly with LE motor scores (r_s = 0.628–0.660; *p*-value < 0.01), followed by LE proprioception scores (r_s = 0.449-0.473, *p*-value < 0.01), while the LE tactile scores significantly correlated only with the fastest walking speed, and the differences between preferred and fastest speed) (r_s = 0.254–0.279, *p*-value < 0.01). The fastest walking speed is important for community participation, and the ability to voluntarily increase walking speed could indicate the remaining capacity for a community challenge as well as indicate and quantify how well the individuals could modify their walking pattern to varying demands during daily life. The present findings suggest contribution of sensorimotor scores on walking ability of the participants, whereby the tactile sensation is needed for challenging ability. Therefore, apart from motor functions and proprioception, tactile sensation is also required to thoroughly promote walking ability of ambulatory individuals with stroke.

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Introduction

Gait restoration is accounted as a key goal in stroke rehabilitation[1]. However, current rehabilitation therapy likely emphasizes on motor functions, while sensory deterioration is frequently overlooked[2,3]. Previous studies reported that sensory impairments can be commonly found in up to 64% of all individuals with stroke, especially the tactile and proprioception senses of the lower extremity (LE), and are closely related to their functional recovery and mobility[2-4]. The LE somatosensory impairment also attributes significant impacts on independence in daily activities[5], as well as prolongs hospital stays, and decreases the possibility of discharge after stroke[4]. As a key goal of rehabilitation treatments, the exploration on the contribution of sensorimotor functions to walking ability specifically among ambulatory individuals with stroke may provide data to enhance patient-centered care planning for this population.

Previous studies reported that the 10-meter walk test (10MWT) is a vital outcome that is widely used to indicate overall quality of gait, independence in daily living, and community participation[6,7]. However, existing evidence likely involves the data of preferred walking speed[8,9]. In fact, obtaining sufficient information on adequate walking ability requires measurements of both preferred and maximum walking speed, as the latter may be important for community involvement, such as crossing a street, walking with others, and catching a bus. In addition, the ability to voluntarily increase walking speed could reflect the remaining capacity for a community challenge as well as indicate and quantify how well the individuals could modify their walking pattern to varying demands during daily life[10]. Therefore, this study explored the correlation between LE sensorimotor functions and outcomes of the 10MWT (preferred, fastest, and the differences between preferred and fastest speed) in ambulatory individuals with stroke. The researchers hypothesized that both proprioception and tactile sensations significantly correlated to walking ability, especially the challenging ability, i.e., fastest walking speed and their ability to modify their walking pattern. The findings would thoroughly suggest clinical contribution of LE somatosensory functions on walking ability of ambulatory individuals with stroke.

Materials and Methods

Study design and participants

This cross-sectional study was conducted among ambulatory individuals with the first stroke episode from hospitals and rural communities of the northeast areas of a developing country. The eligible participants required the ability of independent walking with or without a walking device for at least 10m, aged between 30 to 75 years old, had stable medical conditions, and were able to understand and follow the instructions of this study[11,12]. The individuals with a condition that might affect participation or the outcomes of the study were excluded from the study, such as aphasia, neurogenic or musculoskeletal pain with a score of at least 5 out of 10 on a visual analog scale, severe spasticity (modified Ashworth scale score of > 2)[13], joint limitation and/or deformity affecting mobility, and LE discrepancy (>1.4 cm)[14].

The sample size calculation for a correlation coefficient (r) using data from 14 pilot cases indicated that the study required at least 75 participants. The study protocol was approved by the Khon Kaen University Ethics Committees for Human Research (HE671050). All eligible participants signed a written informed consent form prior to participation in the study.

Research protocol

The eligible participants were interviewed and assessed for their demographics (i.e., age, sex, body mass index), stroke characteristics (i.e., post-stroke onset, type of stroke, and hemiplegic side), and walking ability (i.e., the ability of walking with or without a walking device). Subsequently, participants were assessed for the outcomes of the study, including LE somatosensory functions using the sensory section of the Fugl-Meyer assessment (FMA), and the motor functions using the Motricity Index (MI). Then, the participants were assessed for their walking ability using the 10MWT. Details of the assessments are described below.

Fugl-Meyer Assessment

The sensory section of the FMA is one of the most widely used as a standard reliable scale to quantify somatosensory functions of individuals with stroke (Kappa = 0.3–0.55 for tactile and 0.71–0.99 for proprioception)[15]. The outcomes were assessed with the eye-closed and compared with the non-affected side. The LE tactile sensation was assessed in the areas of thigh, and sole of the affected foot. The outcomes of each area were scored as 0 for absent, 1 for impaired, and 2 for intact, and the total score ranging from 0 to 4. The proprioception was tested in the joints of the LE, including the hip, knee, ankle, and great toe. The score of each joint was given as 0 for absent, 1 for impaired, and 2 for intact. Hence, the total LE proprioception score ranged from 0 to 8, and the maximum FMA sensory score of LE is 12[16,17].

Motricity Index

The MI is a feasible, brief, and reliable (intraclass correlation coefficient [ICC] = 0.93) measure of the general motor strength among individuals with stroke, with the outcomes predicting post-stroke mobility. Participants were assessed in a sitting position for the three movements for the LE, including the hip flexion, knee extension, and ankle dorsiflexion. The scores were assigned as 0 for no movement, 9 for palpable contraction in muscle but no movement, 14 for visible movement but not full range against gravity, 19 for full range of movement against gravity but not against resistance, 25 for full movement against gravity but weaker than the other side, and 33 for normal strength. The total LE-MI score is 99[18,19].

10-Meter Walk Test

The 10MWT is a standard and excellent reliability measure (ICC = 0.99)[7,9] with the outcomes reflecting overall walking ability, the ability to perform daily activities, and community participation[6,7]. Participants walked at a preferred and fastest speed, with or without a walking device according to their daily walking, along a 10m walkway with the time recording during the middle 4m of the walkway, and the average value of the two trials was converted to their walking speed (in m/s)[7,21].

The aforementioned tests were executed by two excellent rater reliability examiners (ICC = 0.96–0.98). During the tests, participants wore proper-sized sandal sport shoes that were prepared by the researchers, and were given a practice session in order for them to familiarize with the shoes. An examiner walked alongside or was beside the participant without interruption to ensure their safety and accuracy of the outcomes. Participants were able to rest between the trials and the tests as required.

Statistical analysis

The Kolmogorov-Smirnov test was used to assess the normality of the data distribution. As the data were non-normally distributed, the descriptive statistics (number [percent], and median with interquartile range [Q1:Q3]) were applied to explain demographics and stroke characteristics of the participants, as well as the findings of the study. Then the correlations between the sensorimotor outcomes and the 10MWT data were analyzed using the Spearman's rank correlation coefficient (r_s). The correlation strength was designated as weak (< 0.3), moderate (0.3-0.6), strong (> 0.6)[22]. A level of statistical significance was set at p-value < 0.05.

Results

Seventy-five ambulatory individuals with stroke (average age of approximately 59 years and normal body mass index) completed the study (Table 1). More than half of the participants were male with equal number of those with right and left hemiplegia. Most participants had ischemic stroke and were at a chronic stage (72%). About one-fourth of the participants walked with a walking device (28%), primarily using a tripod cane.

Table 1 Demographics and stroke characteristics of the participants, and outcomes of the study

Variable	n = 75
Sex; male[a]	43 (57.3)
Types of stroke; ischemic[a]	65 (86.7)
Hemiplegia side; left[a]	38 (50.7)
Use of walking aid; yes[a]	21 (28.0)
Age (years)[b]	59.0 (51.0:68.0)
Body mass index (kg/m^2)[b]	23.0 (21.3:25.3)
Post-stroke onset (months)[b]	12.0 (6.0:26.5)
Fugl-Meyer Assessment of the lower extremity[b]	
Total (12 score)	11.0 (9.0:12.0)
Tactile (4 scores)	4.0 (2.5:4.0)
Proprioception (8 scores)	7.0 (6.0:8.0)
Motricity index of the lower extremity (99 scores)[b]	72.0 (53.0:85.0)
10-Meter walk test[b] (m/s)	
Preferred speed	0.63 (0.39:0.81)
Fastest speed	0.85 (0.49:1.13)
Difference between preferred and fastest speed	0.18 (0.08:0.3)

Note: [a]The data are presented using the number of participants (%).
[b]The data are presented using median (interquartile range, Q1:Q3).

Most participants had intact tactile sensation (n = 52), but impaired proprioception with the median FMA scores of 11.0 (Table 1). Their walking speed data, including preferred, fastest, and the difference between preferred and fastest walking speed are presented in table 1.

The findings further indicate a significant correlation between the LE sensorimotor scores and data of the 10MWT, especially the motor scores (r_s = 0.628–0.660, *p*-value < 0.01; Table 2). In addition, the proprioception scores showed moderate correlation with the 10MWT data (r_s = 0.449–0.473, *p*-value < 0.01), while the tactile scores had weak but significant correlation with the fastest, and the difference between preferred and fastest walking speed of the participants (r_s = 0.254–0.279, *p*-value < 0.05, Table 2).

Table 2 Correlation between the data of the 10-meter walk test and lower extremity sensorimotor scores

10-Meter walk test	Motor	Proprioception	Tactile
Comfortable speed	0.628**	0.449**	0.226
Fastest speed	0.660**	0.473**	0.254*
Difference between preferred and fastest speed	0.649**	0.461**	0.279*

Note: Motor scores were derived from the Motricity index. Sensory scores were gathered using the Fugl Meyer (sensory) assessment. The data were analyzed using the Spearman's rank correlation coefficients. *, **indicate significant correlation with a *p*-value of < 0.05 and < 0.01, respectively.

Discussion

The efficient and functional walking is a prerequisite for independent living. This study explored the correlation between walking ability as measured using the 10MWT and the LE sensorimotor scores. The findings indicated that the 10MWT data correlated predominantly with the motor scores, followed by the proprioception scores, while the tactile scores had weak correlation with the fastest, and the difference between the preferred and fastest speed (p-value < 0.05, Table 2).

Several studies have reported the vital role of motor ability on walking activity of many individuals[23,24]. After stroke, a large proportion of the patients reduced their motor strength. In this study, the motor scores were measured using the MI for the three LE muscles, including hip flexor, knee extensor, and ankle dorsiflexor muscles, that are the key components for both stance and swing phases of walking[18]. The present findings support the close relation between motor strength and gait performance of the participants (Table 2). Thus, the improvement in motor strength is crucially needed in stroke rehabilitation that subsequently improves walking ability and facilitates participation in everyday activities of individuals with stroke[24,25].

However, the ability to perceive information is needed for the adequate utilization and control of motor functions. Thus, the findings additionally indicated the significant correlation between walking ability and proprioception scores (r_s = 0.473, Table 2). Wirz et al[23] reported that an increase in motor scores is the factor associating with functional improvement. Proprioceptive feedback plays vital roles for an extra-activation of LE muscles[26]. The proprioception allows the body to sense its position and movement in space; thus it is crucial for enabling smooth, coordinated, and automatic walking and balance adjustments without consciously thought[27,28]. Lee et al[29] reported that intact proprioception from the affected ankle enabled individuals to position and load the foot during movements. Proprioceptive feedback is also needed for weight-bearing information, underscoring their roles in movement stability while walking[29,30].

Interestingly, a weak but significant correlation also suggested the contribution of tactile sensation on challenging walking ability, fastest speed, and the differences between preferred and fastest speed (r_s = 0.254-0.279, Table 2). Although it has low attention in stroke rehabilitation, tactile sensation is important for individuals to perceive their feet placement and weight-bearing during locomotion. Cutaneous afferent information from the foot contributes to the adequate upright mobility and postural sway. Thus, the decreased tactile sensation is frequently associated with stability and mobility of the individuals[31]. Previously, post-stroke tactile deficits have also been reported to relate to the reduction in balance ability and increased postural sway. Furthermore, tactile sensation in combination with proprioception provide accurate intrinsic feedback essential for movement adjustments, weight-bearing information and stability, underscoring their roles for demanding tasks[29,30]. However, the small proportion of participants with impaired tactile sensation (31%) with high tactile scores may reflect natural characteristics of those who could walk independently. These particular characteristics of the participants may result in a low correlation of the tactile scores, and the significant correlation with the tactile scores was found only for challenged walking ability (Table 2).

Without clear evidence on the sensorimotor contributions on walking ability, the present findings confirm the need of not only motor functions but also proprioception and tactile sensations on walking ability of ambulatory individuals with stroke. However, the findings contain some limitations. This study assessed sensorimotor functions of only the lower extremity as they are directly linked to walking ability. However, walking ability also required the involvement of the trunk and upper extremities. Therefore, the findings cannot clearly reflect the roles and contributions of sensorimotor functions of the trunk and upper extremities during walking. Second, a majority of participants walked without a walking device (72%) and were in the chronic stage of stroke (72%) with mild somatosensory impairments (total FMA scores = 11.0 [Q1:Q3 = 9.0:12.0]). Therefore, the findings may not thoroughly reflect all ambulatory individuals in the various stages of stroke, types of walking device used, and severity of stroke. A further study may need to additionally explore the relationship between overall sensorimotor scores on walking ability of ambulatory individuals with stroke who are at various stages of stroke and walking ability.

Conclusion

The improvement of walking ability is one of the optimal goals of stroke rehabilitation. This study explored the correlation between walking ability as measured using the 10MWT and the LE sensorimotor scores. The finding indicated that walking ability correlated predominantly with motor strength, followed by proprioception, and tactile sensation, respectively. The findings suggest the roles and importance of these sensorimotor functions for rehabilitation practice among ambulatory individuals with stroke.

Take home messages

Motor functions, proprioceptive sensation, and tactile sensation are needed to promote walking ability of ambulatory individuals with stroke.

Conflicts of interest

The authors declare no conflict of interest.

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Author contributions

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Data availability

Data available on request due to privacy/ethical restrictions

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The association between mobility limitations and physical performance measures in community-dwelling elderly: a cross-sectional study

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ABSTRACT
Effective management of mobility limitations in older adults requires two key components: early identification of at-risk individuals and the ability to target the most critical underlying physical deficits. While numerous clinical tests for balance and strength exist, there is a lack of clarity on which measure best serves both these screening and prescriptive purposes. This study aimed to (1) describe age-group differences, (2) examine bivariate associations between SPPB and flexibility/balance/strength measures, and (3) identify independent predictors and explained variance via stepwise multiple linear regression with age and sex as covariates. This cross-sectional study included 108 older adults (mean age 71.0 ± 5.0 years; 67 women, 41 men) with mobility limitations (SPPB score ≤ 9). Flexibility was measured with the sit-and-reach test; balance by the functional reach test (FRT), single-leg stance test (SLST), timed up and go test (TUGT), and y-balance test (YBT); and strength by handgrip dynamometry. Pearson correlations identified bivariate associations with SPPB score. Stepwise multiple regression was used to determine which measures were most strongly and independently associated with mobility limitation. Although no significant differences were observed between sexes, SPPB scores were significantly lower among the older age groups (75–84 and ≥85 years) compared with the youngest group (*p*-value < 0.01). The FRT demonstrated the strongest correlation with SPPB (r = 0.37–0.67, *p*-value < 0.01). In Model 1, FRT alone accounted for 48.3% of the variance in SPPB (β = 0.695, *p*-value < 0.01). When age was added in Model 2, the explained variance increased slightly to 49.3% ($\Delta R^2 = 0.01$; β = 0.143, *p*-value < 0.05). Functional reach emerged as a strong, independent predictor of mobility limitation, explaining nearly half the variance in SPPB score. Its simplicity and feasibility make it a valuable tool for routine geriatric screening to identify older adults at greatest risk of mobility decline. Age provides a modest but significant contribution. These findings support the incorporation of quick, practical balance assessments (FRT) in both community and clinical practice. The cross-sectional design limits causal inference, and findings may not be applicable to institutionalized populations.

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Introduction

The World Health Organization defines older adults as those aged 60 years and above. A significant concern arising from an aging population is the prevention of mobility impairment(1). Approximately one-third to one-half of adults aged 65 and older report experiencing difficulty with ambulation or climbing stairs(2,3), which are common indicators of mobility capabilities(2,4). Webber et al. (2010) defines mobility "as the ability to move oneself (either independently or by using assistive devices or transportation) within environments that expand from one's home to the neighborhood and to regions beyond"(5). Mobility limitations correlate with heightened risks of falls, disability, hospitalization, and mortality risk as well as decreased quality of life(5,6), and poor psychosocial health(7). The prevalence increases with age, affecting 35% of persons aged 70 and the majority of persons over 85 years(8). Age-related declines in physical function(9), including flexibility, balance, and muscle strength(10), which contribute to mobility limitations(8,9).

Sex and age are critical factors that influence multiple domains of physical function. Women typically exhibit lower muscle strength and poorer balance than men, disparities attributed in part to hormonal changes, such as postmenopausal estrogen decline, and age-related differences in body composition(8, 11-12). These physiological differences contribute to an earlier onset and higher frequency of mobility limitations in women. Furthermore, epidemiological studies confirm that women experience more falls and functional dependence, spending a greater proportion of their later life with mobility limitations compared to men of the same age. Concurrently, advancing age is strongly associated with progressive declines in flexibility, balance, and overall mobility, as increasing stiffness in muscles and connective tissues further restricts daily function (13, 14). Thus, both sex and age are crucial determinants of mobility and independence in activities of daily living.

Objective clinical tests commonly used to characterize physical function include measures of flexibility (Sit-and-Reach Test: SRT), balance (static: Single-Leg Stand Test (SLST); dynamic: Timed Up and Go Test (TUGT), Functional Reach Test (FRT), and Y-Balance Test (YBT), and muscle strength (handgrip dynamometry). Lower sit-and-reach performance reflects reduced hamstring and lumbar flexibility, which can alter gait mechanics and compromise balance(15). The SLST assesses postural stability under static conditions (16), while the TUGT captures functional mobility and dynamic postural control(17). The FRT and YBT provide insight into dynamic balance, reach control, and neuromuscular coordination (18-20). Impairments on these tests are associated with increased fall risk and loss of independence. Handgrip strength is a well-established indicator of overall functional status and health outcomes(21). Therefore, these assessments cover the major domains of physical function most relevant to understanding mobility limitation in community-dwelling older adults.

Although many studies have examined mobility in relation to a single domain—such as flexibility, balance, or strength—most have focused on outcomes like gait or endurance in older adults without mobility limitations(14). Furthermore, few studies have compared the static and dynamic components of these functional domains within a single cohort. The identified gaps are significant, as epidemiological data indicate a higher prevalence of mobility limitations in women, with a decline in mobility associated with aging(22-23). Nonetheless, limited research utilizes multivariate methods to assess the independent and relative contributions of flexibility, balance, and strength while adjusted for age and sex. Consequently, clinicians still lack clear evidence regarding which clinical tests explain unique variance in mobility status, necessitating prioritization for screening. Therefore, the aims of this study were (i) to describe age-group differences in participant characteristics (65–74, 75–84, ≥85 years); (ii) to examine bivariate associations between mobility limitation—indexed by the Short Physical Performance Battery (SPPB)—and objectively measured flexibility, static and dynamic balance, and muscle strength; and (iii) to determine the independent contributions of age, sex, and these physical components to variance in mobility limitation using stepwise multiple linear regression

Materials and methods

Study design and participants

This cross-sectional study was conducted between July 2022 and June 2023 in the northern areas of Thailand. A total of 276 individuals were initially screened for eligibility. *The sample size was determined based on a correlation analysis between the SPPB and balance ability in older adults. A two-tailed test with an alpha level (a) of 0.05, a power (1−β) of 0.80, and an anticipated moderate correlation coefficient (r = 0.26), based on previous literature, was used to calculate the required sample size[28]. Thus, the required sample size was 108 participants.*

Inclusion criteria were (1) age ≥ 65 years; (2) ability to ambulate independently for 10 m without an assistive device; (3) Short Physical Performance Battery (SPPB) score ≤ 9; (4) no acute cardiovascular, pulmonary, or renal conditions; (5) no prior diagnosis of Parkinson's disease or neurological symptoms; and (7) no diagnosed psychiatric disorder and no use of sedative medications. The exclusion criteria included (1) substantial auditory or visual impairment; (2) lower-limb pain > 5 on the visual analogue scale; (3); and (4) inability to follow instructions. The study protocol was approved by the Human Research Ethics Committee at the University of Phayao review board, following the Declaration of Helsinki (protocol code: UP-HEC 1.3/035/64). Informed written consent was acquired from all participants following eligibility screening and before participation in any study procedures.

Data collection

Demographic data, disease history, medication use, and fall history were obtained via questionnaire. Fall history was classified as no falls, one fall, or ≥2 falls within the past 12 months[25]. Disease status was categorized into nine conditions: none, hypercholesterolemia, hypertension, diabetes, osteoarthritis, heart disease, low back pain, thyroid disease, and others. Medication use was classified into five categories. Physical examination included measurement of height (to the nearest 0.1 cm; TSCALE M301-200), weight (to the nearest 0.1 kg; TANITA UM-051), and calculation of body mass index (BMI, kg/m^2). Blood pressure (systolic and diastolic) was measured three times using a digital sphygmomanometer (Omron HEM-7156A), with 10-minute rest intervals between measurements; the average data of the three readings were recorded. Physical performance was evaluated using a battery of tests, including the SPPB, SRT, SLST, FRT, TUGT, YBT, and a handgrip strength test. To mitigate the effects of test order and fatigue, the sequence of these tests was randomized for each participant by drawing lots, and a sufficient rest period was provided between each test until the participant felt recovered. Detailed procedures for each test are described below

Short physical performance battery

The SPPB quantifies lower-extremity physical performance and indicates mobility limitation, using three standardized subtests, and has excellent results in older populations ICC = 0.88–0.92[24]: a **three-stage balance test** (side-by-side, semi-tandem, and tandem stances held up to 10 s each); **gait speed** over 4 meters at their normal, comfortable pace while their time was recorded (a single trial is acceptable and has demonstrated reliability); and the five-times sit**-to-stand** (time to rise from a chair and sit down five times as quickly as possible). Each subtest is scored from **0** (unable) to **4** (best performance) according to published cut-points, yielding a **total score of 0–12** (higher scores indicate better function).

Sit-and-reach test

The SRT was used to assess hamstring flexibility and spinal range of motion during forward flexion, demonstrating acceptable reproducibility (ICC = 0.92)[15]. Participants sat barefoot on the floor with legs extended and heels positioned against a standardized sit-and-reach box (5×30.5 cm) equipped with a measuring ruler extending 15 cm beyond the box edge. With knees fully extended, participants placed their right hand over their left (fingertips aligned) and reached forward maximally along the measuring board without knee flexion. The score represented the furthest distance (cm) reached beyond the toes by the fingertips. Following two 10-second warm-up stretches, three trials were performed and averaged for analysis.

Single-leg stand test

For an SLST, participants stand on one foot with the knee of the other leg bent and not contacting the opposite leg. Single-leg balance results demonstrated acceptable intertrial reliability (ICC = 0.60-0.81)[16]. Participants were placed in the testing position and instructed to maintain balance for 30 seconds. During testing, if the raised leg touched the limb being tested or if movements such as hopping on one leg or using external support to assist with balance occurred, the participant was disqualified, and the researcher immediately stopped the test. The assessment was performed with both eyes open and closed, and the times from three trials were recorded and averaged to determine the mean value.

Functional reach test

The FRT assesses standing dynamic balance and demonstrates good test–retest reliability (ICC = 0.83–0.87)[18]. Participants stood near a wall without touching it. For the forward and backward directions, the participant extended the arm closest to the wall to 90° of shoulder flexion, and for the lateral directions, they abducted the arm to 90°, keeping the shoulder parallel to the measurement scale and the hand in a fist. Without moving their feet, they reached as far as possible, and the distance from the initial to the final position of the third metacarpal was measured in centimeters. Each participant completed three trials.

Timed up and go test

TUGT was used to assess functional mobility and fall risk. This test demonstrates excellent intertrial reliability, with a reported Intraclass Correlation Coefficient (ICC) of 0.96 [17]. For the procedure, a standard chair with a 43 cm seat height and armrests was used, and a cone was positioned 3 meters away. Participants were instructed to rise from the chair, walk to and around the cone, return to the chair, and sit down completely, all while walking at their normal, safe pace. On the command 'Go', a stopwatch was used to record the duration, starting as the participant's back lifted from the backrest and stopping as their buttocks made contact with the seat upon their return. Each participant completed three trials, and the average time in seconds was calculated for the final analysis.

Y balance test

The YBT assessed dynamic balance in three directions (anterior, posteromedial, and postero-lateral) positioned at 135° angles to each other. The test demonstrates intertrial reliability (ICC = 0.89–0.97)[26]. Participants stood on their preferred leg at the center of a Y-shaped grid with the great toe positioned at the line intersection. The non-stance leg reached maximally along each of the three directional lines while maintaining single-leg balance. Two practice trials per leg in each direction preceded testing. Trials were invalidated if the stance foot moved or the reaching leg failed to return to the starting position. Three trials were performed in each direction for both legs. Maximum reach distances were normalized to anatomical leg length, and YBT composite scores were calculated by dividing the sum of maximal reach distances in each direction by three times the leg length.

Grip strength test

Hand and forearm strength were assessed using a hand grip dynamometer (Takei Scientific Instruments Co. Ltd., Niigata, Japan). Test-retest reliability for measurements of grip strength was excellent for the dominant hand (ICC = 0.97; *p*-value = 0.001). Participants were instructed to stand with their arms extended straight down by their sides while the measurement was obtained with the dominant hand. Before the test, the grip device was adjusted to fit each participant's hand. The participants were then asked to squeeze the grip dynamometer using maximum force. Squeeze the dynamometer as hard as possible for 3-5 seconds[21]. Each participant was asked to perform this action twice with a one-minute interval between attempts. Their best performance was recorded.

Statistical analysis

All analyses were conducted using the IBM Statistical Package for Social Sciences (SPSS, version 29.0, IBM Corp, Chicago, IL, USA) for the statistical analysis, and the data were presented as averages, standard deviations (SD), and the number (percentage) for categorical variables. To address objective 1 (describing group differences), characteristics were compared across age groups and by sex. An independent samples t-test was performed for continuous variables, while a chi-square test was applied for categorical variables, provided the data met the assumption of normality. For subgroups where data were not normally distributed, the appropriate non-parametric equivalents (Mann-Whitney U test and Kruskal-Wallis test) were employed. Pearson product-moment correlation coefficients were used to examine bivariate relationships between continuous variables. Stepwise multiple linear regression analysis was used to investigate multivariate relationships among predictors of mobility limitations. The potential covariates (univariate analysis, *p*-value < 0.1) were included for the multiple regression model[27]. Results were reported as unstandardized coefficients (B) along with the standard error (SE). The statistical significance level was set at *p*-value < 0.05.

Results

The study included 108 community-dwelling older adults with mobility limitations (67 females, 41 males) aged 65–86 years. The baseline characteristic, vital sign, number of diseases, number of medications, and history of falls exhibited no significant differences between males and females (*p*-value > 0.05). In comparison to the 65–74-year-old group, participants aged 75–84 years and >85 years exhibited significantly reduced weight, height, BMI, and leg length (*p*-value < 0.01). The majority of participants indicated no falls in the previous year, with polypharmacy (defined as the use of three or more medications) being most prevalent in the 65–74 age group (Table 1).

Table 2 indicates that there was no significant difference in total SPPB and TUGT between males and females, demonstrating comparable overall mobility performance. However, men demonstrated significantly greater upper limb strength and balance performance—specifically in the YBT—when compared to women (*p*-value < 0.01). Participants aged 75–84 years and over 85 years exhibited significantly lower SPPB scores, flexibility, balance performance, and muscle strength compared with the 65–74-year-old group (*p*-value < 0.05–0.01). Dynamic balance (FRT and YBT scores) decreased consistently with advancing age (Table 2).

Correlation analysis revealed that SPPB scores were moderately and positively correlated with static balance (SLST, *p*-value < 0.01), dynamic balance as measured by the FRT in all directions (r = 0.402–0.695, *p*-value < 0.01), and grip strength (*p*-value < 0.01). In contrast, a significant negative correlation was observed between SPPB and TUGT performance (r = −0.253, p-value < 0.01). No significant correlations were found between SPPB and age or gender (Table 3).

Table 1 Characteristics of sex and age-groups differences in elderly with mobility limitation (aged 65-86 years)

Variable	Sex		Age groups		
	Female (n=67)	**Male (n=41)**	**65-74 y (n=86)**	**75-84 y (n=19)**	**>85 y (n=3)**
Age (year)†	71.07±4.90	70.90±5.22	68.98±2.82	77.95±2.52^{a**}	85.33±0.57^{a**b**}
Weight (kg)†	50.98±7.86	59.90±9.24	56.04±0.95	47.47±7.79^{a**}	50.13±11.58
Height (cm)†	151.70±7.97	162.12±6.52	157.13±8.43	149.74±8.49^{a**}	151.00±14.17
BMI (kg/m)^{2†}	22.13±2.84	22.67±3.02	22.67±2.94	21.12±2.69^{a*}	21.67±0.85
Leg length (cm)	68.27±3.58	72.95±2.93	70.71±3.79	67.38±3.82^{a**}	67.95±6.38
SBP (mmHg)†	131.90±19.39	133.15±17.66	131.84±17.33	135.74±23.60	126.33±26.63
DBP (mmHg)†	73.69±11.89	73.41±10.74	74.36±10.64	72.68±13.72	57.00±5.19^{a**}
Heart rate (beats/min)†	78.45±9.96	75.88±13.63	77.52±11.74	75.68±9.00	87.33±17.78
Oxygen saturation (%)†	98.24±0.97	98.27±0.89	98.21±0.95	98.42±0.83	98.33±1.15
Number of diseases n, (%)$^{\#}$					
No disease	15 (22.4)	14 (34.1)	22 (25.6)	5 (26.3)	2 (66.7)
Cholesterol	21 (31.1)	9 (22)	28 (32.6)	2 (10.5)	0 (0)
Hypertension	29 (43.3)	14 (34.1)	36 (41.9)	6 (31.6)	1 (33.3)
Diabetes mellitus	9 (13.4)	5 (12.2)	13 (15.1)	1 (5.3)	0 (0)
Osteoarthritis	7 (10.4)	3 (7.3)	6 (7)	3 (15.8)	1 (33.3)
Heart disease	3 (4.5)	4 (9.8)	4 (4.7)	2 (10.5)	1 (33.3)
Low back pain	2 (3)	1 (2.4)	3 (3.5)	0 (0)	0 (3)
Thyroid disease	2 (3)	1 (2.4)	3 (3.5)	0 (0)	0 (3)
Others	12 (17.9)	9 (22)	18 (20.9)	3 (15.8)	3 (100)
Number of medication n, (%)$^{\#}$					
0 type	24 (35.8)	16 (39)	32 (37.2)	6 (31.6)	2 (66.7)
1 type	15 (22.4)	7 (17.1)	18 (20.9)	4 (21.1)	0 (0)
2 type	13 (19.4)	8 (19.5)	18 (20.9)	3 (15.8)	0 (0)
3 type	6 (9)	4 (9.8)	8 (9.3)	2 (10.5)	0 (0)
≥4 type	9 (13.4)	6 (14.6)	10 (11.6)	4 (21.1)	1 (33.3)

Table 1 Characteristics of sex and age-groups differences in elderly with mobility limitation (aged 65-86 years) (Cont.)

Variable	Sex		Age groups		
	Female (n=67)	**Male (n=41)**	**65-74 y (n=86)**	**75-84 y (n=19)**	**>85 y (n=3)**
History of falls n, (%)[#]					
0 falls	59 (88.1)	39 (95.1)	78 (90.7)	17 (89.5)	3 (100)
1 fall	7 (4.9)	2 (10.4)	7 (8.1)	2 (10.5)	0 (0)
≥2 falls	1(1.5)	0 (0)	1 (1.2)	0 (0)	0 (0)

Note: † The data are presented as mean ± standard deviation (95% confidence interval). Comparisons among the three age groups were performed using the Mann-Whitney U test, while comparisons between male and female participants were conducted using the independent t-test. # The data are presented as the number (percent of total participants), and group comparisons were performed using the Chi-square test. [a] indicates a statistically significant difference compared to the 65-74 y group. [b] indicates a statistically significant difference compared to the 75-84 y group. * significant difference (p-value < 0.05), ** (p-value < 0.01).

Abbreviations: Y = years, Kg = kilogram, cm = centimeter, BMI = body mass index, SBP = systolic blood pressure, and DBP = diastolic blood pressure.

Table 2 Clinical balance assessment, upper limb muscle strength test, and flexibility test in elderly with mobility limitation group (n = 108)

Characteristics	Sex		Age groups		
	Female (n=67)	**Male (n=41)**	**65-74 y (n=86)**	**75-84 y (n=19)**	**>85 y (n=3)**
SPPB (score)	7.51±1.61	7.74±1.57	8.26±0.99	5.47±1.07 [b**]	4.00±0.00 [b** c*]
SRT (cm)	8.02 ± 7.46	2.66 ± 5.93[a**]	6.05 ± 7.27	6.51 ± 7.71	0.63 ± 8.60 [b*]
SLST (s)	10.33 ± 7.35	13.71 ± 7.26[a*]	12.84 ± 7.29	7.28 ± 6.53[b**]	3.91 ± 2.77[b*]
FRT forward (cm)	18.57 ± 7.62	21.23 ± 8.43	20.53 ± 7.94	17.08 ± 6.78	8.33 ± 6.68[b*]
FRT backward (cm)	12.47 ± 8.06	13.93 ± 6.48	13.75 ± 7.54	11.34 ± 6.55	2.92 ± 2.83[b* c*]
FRT right side (cm)	12.97 ± 5.29	16.81 ± 5.29[a**]	15.21 ± 5.90	12.04 ± 4.53[b*]	7.10 ± 1.51[b*]
FRT left side (cm)	12.70 ± 4.42	15.93 ± 4.43[a**]	14.44 ± 4.86	11.86 ± 3.37[b*]	12.21 ± 2.80
TUGT (s)	12.03 ± 3.09	12.07 ± 2.98	11.81 ± 2.88	12.68 ± 3.60	15.00 ± 2.28 [b*]
YBT anterior (cm)	30.41 ±10.88	37.73 ± 13.55[a**]	35.11 ± 12.42	26.66 ± 8.66[b*]	19.57 ± 13.71
YBT posteromedial (cm)	54.05 ± 9.98	60.62 ± 11.26[a**]	57.95 ± 10.95	51.86 ± 8.19[b*]	45.78 ± 14.27
YBT posterolateral (cm)	43.54 ±10.06	49.75±13.0[a**]	47.38 ± 11.59	41.25 ± 8.72[a**]	32.73 ± 16.35
Grip strength (kg)	16.10 ± 4.41	23.71 ± 6.99[a**]	20.08 ± 6.67	15.01 ± 4.69[b**]	13.05 ± 3.07 [b*]

Note: The data are presented by mean ± SD. Comparisons among the three age groups were performed using the Mann-Whitney U test, while comparisons between male and female participants were conducted using the independent t-test. [a] indicates a statistically significant difference compared to the female group. [b] indicates a statistically significant difference compared to the 65-74 y group. [c] indicates a statistically significant difference compared to the 75-84 y group * significant difference (p-value < 0.05), ** (p-value < 0.01).
Abbreviations: TUGT, Timed up and go test; SRT, Sit and reach test; SLST, Single leg stand test; FRT, Functional reach test; YBT, Y balance test; s, second; kg, kilogram; cm, centimeter.

Table 3 Correlation analysis between SPPB, static and dynamic balance ability, flexibility test and muscle strength participants (aged 65-86 years)

	SRT	SLST	FRT forward	FRT backward	FRT right side	FRT left side	TUGT	YBT postero-medial	YBT postero lateral	YBT anterior	Grip strength	SPPB
Age	-0.063	-0.277**	-0.246*	-0.232*	-0.320**	-0.142	0.308**	-0.276**	-0.267**	-0.317**	-0.329**	-0.037
Gender	0.354**	-0.220*	-0.162	-0.095	-0.179	-0.336**	-0.006	-0.293**	-0.261**	-0.288**	-0.559**	-0.083
BMI	-0.119	-0.111	0.078	0.032	0.223*	0.140	-0.039	-0.203*	-0.204*	-0.115	0.107	0.075
SPPB	0.129	0.326**	0.695**	0.402**	0.522**	0.527**	-0.253**	0.218*	0.158	0.283**	0.259**	1.000
SRT	1.000	0.132	0.215*	0.145	0.079	-0.029	-0.151	0.107	0.131	0.074	0.020	0.129
SLST		1.000	0.367**	0.325**	0.279**	0.281**	-0.199*	0.344**	0.429**	0.367**	0.251**	0.326**
FRT forward			1.000	0.600**	0.718**	0.648**	-0.274**	0.443**	0.316**	0.477**	0.365**	0.695**
FRT backward				1.000	0.549**	0.479**	-0.355**	0.397**	0.311**	0.434**	0.370**	0.402**
FRT right side					1.000	0.754**	-0.190*	0.273**	0.235*	0.389**	0.414**	0.552**
FRT left side						1.000	-0.075	0.289**	0.205*	0.352**	0.282**	0.527**
TUGT							1.000	-0.292**	-0.325**	-0.424**	-0.169	-0.253**
YBT- posteromedial								1.000	0.799**	0.809**	0.442**	0.218*
YBT- posterolateral									1.000	0.798**	0.354**	0.158
YBT- anterior										1.000	0.426**	0.283**
Grip strength											1.000	0.259**

Note: Correlations were analyzed using Pearson correlation coefficient. * Indicated significant differences (*p*-value < 0.05), ** (*p*-value < 0.01).

Abbreviations: BMI, body mass index; SPPB, Short Physical Performance Battery; TUGT, Timed up and go test; SRT, Sit and reach test; SLST, single legs stand test; FRT, Functional reaching test; YBT, Y-balance test.

Table 4 Multiple linear regression model predicting mobility limitation

Predictor	Unstandardized β	SE	Standardized Beta	R^2 change	95%CI
Model 1					
FRT forward	0.217	0.022	0.695**	0.483	0.174-0.260
Model 2					
FRT forward	0.228	0.022	0.730**	0.493	0.184-0.272
Age	0.071	0.036	0.143*		0.001-0.142

Note: * Indicates significant differences (*p*-value < 0.05), ** (*p*-value < 0.01).
Abbreviations: CI, Confidence Intervals represent the range for unstandardized; β, coefficients; FRT, functional reach test.

Based on Table 4, the multiple linear regression analysis (stepwise) was conducted to predict mobility limitation using FRT forward and age as predictor variables. In Model 1, FRT forward was a significant predictor of mobility limitation (β = 0.695, *p*-value < 0.01), explaining 48.3% of the variance (R^2 change = 0.483) in the outcome variable. The 95% confidence interval (CI) for the unstandardized coefficient ranged from 0.174 to 0.260. In Model 2, age was added as an additional predictor. Both FRT forward (β = 0.730, *p*-value < 0.01) and age (β = 0.143, *p*-value < 0.05) remained significant contributors to the model. The inclusion of age led to a slight improvement in model performance, increasing the R^2 change to 0.493, with the 95% CI for age ranging from 0.001 to 0.142, highlighting its additional impact on mobility decline.

Discussion

This study aimed to examine the relationship between mobility limitations and various parameters of physical function in older adults. This study found significant sex differences in physical function, with men exhibiting greater muscle strength and balance than women. Mobility limitation was moderately to strongly associated with balance and flexibility, with forward reach on the FRT emerging as the strongest predictor, explaining 48.3% of the variance. Adding age to the model increased the explained variance by 1%, highlighting the combined influence of balance performance and aging on mobility limitation in older adults.

In this study, significant differences in balance, flexibility, and muscle strength were observed across age groups and between sexes; however, SPPB and TUGT did not show significant differences. These findings are consistent with previous research demonstrating that balance and strength decline progressively with advancing age due to reductions in muscle mass, tendon stiffness, and neuromuscular coordination associated with sarcopenia and age-related sensory degradation[14,29]. Men generally outperform women in muscle strength and static balance tasks, which may be attributed to greater muscle mass, higher lower-limb strength, and superior postural control strategies[30]. Conversely, women exhibit higher flexibility due to differences in joint range, pelvic tilt, and connective tissue properties, which indicate sex-specific patterns of physical function decline in aging.

Several factors may explain no significant differences in SPPB and TUGT scores between groups. First, all participants in the present study were classified as having mobility limitations (SPPB ≤ 9), resulting in a restricted performance range and potential floor effects, thereby limiting the sensitivity of these global mobility measures to detect between-group variation. Second, both SPPB and TUGT are composite functional assessments that integrate multiple domains—including balance, gait, and strength—and may thus obscure domain-specific differences observed in isolated balance or strength tests. Similar findings have been reported by previous studies showing that global mobility scores may remain stable despite domain-specific impairments, as individuals compensate for deficits in one system (e.g., balance) with preserved function in another (e.g., strength or gait speed)[31,32]. Third, the relatively small sample size in the oldest age group (>85 years) may have reduced the statistical power to detect true group differences. Overall, these results suggest that while static balance and muscle strength decline more sharply and are sensitive indicators of age- and sex-related functional differences, global mobility tests such as SPPB and TUGT may lack sufficient granularity to distinguish these variations in populations already exhibiting mobility limitations.

The present study found that SPPB scores were significantly and positively correlated with SLST and all directions of the FRT, with the strongest association observed in the forward reach direction. This finding suggests that forward reaching ability, a measure of dynamic balance and postural control, is a key determinant of overall mobility performance in older adults with mobility limitations. The importance of forward reach is consistent with previous studies reporting that reduced anterior reaching distance is associated with impaired balance, limited functional mobility, and increased fall risk in older adults[33]. Moreover, the observed positive association between SLST and SPPB supports evidence that single-leg stance performance reflects the integration of sensory, neuromuscular, and balance control systems that are critical for safe ambulation and functional independence[34]. Conversely, the negative correlation between SPPB and TUGT performance aligns with prior research showing that prolonged TUGT completion time is indicative of slower gait speed, poorer mobility, and higher disability risk[35]. Collectively, these findings emphasize that specific dynamic balance measures, particularly forward reach may serve as sensitive indicators of mobility performance and could be prioritized in geriatric screening and intervention programs.

In the multiple linear regression analysis, forward reach distance on the FRT emerged as the strongest predictor of mobility, accounting for 48.3% of the variance ($\beta = 0.695$, *p*-value < 0.01). This result aligns with previous evidence that dynamic balance measured by the FRT, is a critical determinant of functional mobility in older adults. Rosa et al. (2019) reported normative values of 26.6 cm for community-dwelling older adults and emphasized the FRT's strong association with fall risk, underscoring its clinical relevance[36]. The addition of age in Model 2 increased the explained variance only marginally to 49.3%, with age demonstrating a smaller yet statistically significant effect ($\beta = 0.143$, *p*-value < 0.05), consistent with findings by Trombetti et al. (2016) linking advancing age to mobility decline[37]. Similarly, Duncan et al. (1990) demonstrated that the FRT effectively measures limits of stability during functional tasks, while Springer et al. (2007) confirmed its reliability and predictive validity for falls in older populations[33,34]. Conversely, some studies, such as Thomas and Lane (2005), have reported weaker or non-significant associations between FRT and mobility outcomes, particularly in cohorts with high baseline functional capacity, suggesting that its predictive value may be reduced in more active populations[38]. The comparatively larger effect size of FRT relative to age in the present study indicates that functional performance measures may offer more immediate and actionable insights into mobility status than chronological age alone. Moreover, the narrow 95% confidence intervals for both predictors (FRT: 0.184-0.272; Age: 0.001-0.142) reflect the precision and stability of these estimates, reinforcing the value of incorporating the FRT as a central component in mobility assessment and targeted interventions for older adults.

A key strength of this study is its comprehensive assessment of balance, strength, flexibility, and mobility, along with sex- and age-based comparisons that provide valuable insights into factors influencing functional capacity in older adults. However, certain limitations should be noted. The cross-sectional design precludes causal inferences regarding the observed associations. Potential confounding factors, such as habitual physical activity, nutrition, and lifestyle behaviors, were not fully controlled. In addition, the oldest age group (>85 years) was underrepresented, limiting statistical power and the generalizability of findings for this segment.

Conclusion

This study identified significant sex- and age-related differences in physical performance among older adults. Forward reach performance on the FRT was the strongest predictor of mobility limitation, with age providing an additional but smaller contribution. These findings highlight the FRT as a practical and effective tool for detecting mobility limitations and emphasize the need for targeted strategies to maintain functional capacity in older populations.

Take home messages

The FRT is a quick, practical balance measure that strongly predicts mobility limitation in older adults. Incorporating FRT into routine screening may help identify individuals at high risk of mobility decline, supporting early intervention to preserve independence and prevent falls.

Conflict of interest statement

The authors declare no conflict of interest.

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Author contributions

Chonticha Kaewjoho: Conceptualization, Methodology, Writing review and editing, Formal analysis, Investigation, Data curation, and Writing original draft preparation.

Narongsak Khamnon: Validation, Software, Resources, Visualization.

Sinthuporn Maharan: Supervision, Project administration.

All authors have read and agreed to the published version of the manuscript.

Data availability

Data available on request due to privacy/ethical restrictions.

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Comparative study on the effects of yoga on soft surface on sleep, strength, flexibility, and balance in senior university students with mild insomnia

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KEYWORDS
Pittsburgh Sleep Quality Index;
Unstable surface;
Exercise;
Functional ability.

ABSTRACT

This study aimed to investigate the influence of yoga exercises performed on soft versus hard surfaces and to compare these effects with those of general exercise on sleep quality and isometric muscle strength, flexibility, and dynamic balance outcomes in senior university students with mild insomnia. An assessor-blind randomized controlled trial was conducted with 13 participants per group aged 18 years and older randomly assigned to practice yoga on a hard surface (YH), a soft surface (YS), or a control group that received a guidebook, in 45-minute sessions, three days a week, for four weeks. Sleep quality, the main outcome, improved markedly from baseline, most notably among those who practiced yoga on a soft surface, with a mean PSQI reduction of 7.11 points (95% CI: 3.81–10.41). In addition, muscle strength and flexibility also showed significant improvements. Thus, yoga, particularly on a soft surface, could effectively enhance sleep quality, muscle strength, and flexibility in senior university students facing sleep problems.

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Introduction

Senior university students reported experiencing sleep quality issues and excessive daytime sleepiness related to insomnia caused by academic workload, night shifts, and stress[1]. Previous research found that individuals who exhibited clinically significant insomnia at the assessment level, as defined by a Pittsburgh Sleep Quality Index (PSQI) score of ≥5, were associated with subthreshold (mild) insomnia[2,3]. Insufficient and poor-quality sleep due to insomnia is pervasive in contemporary society, serving as both a predictive indicator and a symptom of various health conditions[4]. Some studies reported that poor sleep quality was linked to impaired balance, primarily through its effects on the vestibular and visual systems[5]. A study among Chinese university students found a positive association between good sleep quality and muscle strength. Men with sleep durations of less than six hours exhibited poorer muscle strength than those with adequate sleep[6]. In addition, poor sleep quality was linked to sarcopenia, a condition characterized by loss of muscle mass and function, which could affect muscle flexibility and overall physical performance[7]. Furthermore, disrupted sleep patterns have been associated with notable declines in muscle strength, flexibility, and balance, consequently leading to a decrease in overall well-being[4]. Therefore, the exploration for an intervention helping to manage these problems is important.

Existing evidence reported benefit of mild-moderate aerobic exercise among young adults when performed approximately 30 minutes, three times a week[8,9]. Moreover, mind-body practices could reduce sleep onset latency, and increase total sleep time, flexibility[10-12], and resistance training enhanced sleep efficiency and reduced wake after sleep onset[13,14], potentially offering added benefits when combined with aerobic exercise. In addition, previous studies found that mind-body practices improved sleep quality by promoting relaxation, and reducing arousal, anxiety, and depression, which were often comorbid with insomnia[10]. These findings may suggest the combination of physical and mental exercises to augment sleep quality in people with insomnia.

Recent studies have reported the challenging effects of unstable surfaces after 3 to 8 weeks on various physical aspects, including proprioception, muscular co-contraction, and energy expenditure, in diverse populations, such as healthy young adults, older adults, and individuals with spinal cord injuries[15,16]. Yoga has been identified as a highly beneficial exercise for mental relaxation and flexibility[10,12,17,18]. However, yoga has not been determined as sufficient to improve strength and balance in young people under stress[19-21]. Thus, the researchers hypothesized that the combination of yoga exercise performed on a soft surface may attribute challenging effects on muscle strength, balance, flexibility, and mental wellbeing of stressed students. Therefore, this study compared the effects of practicing yoga on soft and hard surfaces on the sleep quality and isometric muscle strength, flexibility, and dynamic balance outcomes in senior university students with mild insomnia. The present findings may suggest that practicing yoga on a soft surface could improve sleep quality, muscle strength, flexibility, and dynamic balance in this group, potentially offering additional benefits compared with yoga on a hard surface, and supporting the value of integrating mind body practices with aerobic or resistance training on soft surface to enhance overall well-being in this population. Further research is needed to confirm causality, understand mechanisms, and determine optimal protocols.

Materials and methods

Participants

This assessor-blind randomized controlled study involved senior university students from Mae fah luang university, both males and females, had a normal body mass index (18.5-22.9 kg/m^2), were aged 18 years and older, had a Pittsburgh Sleep Quality Index (PSQI) score of ≥ 5 points[22], and had not recently engaged in regular exercise. The study excluded individuals taking medications associated with heart disease and sleeping pills, or those with conditions affecting participation, such as unstable medical conditions, lower extremity joint inflammation with significant pain indicated by a visual analog scale of ≥ 5 points, or neurological deficits. The sample size calculation was performed in G*Power (version 3.1) to detect a standardized effect size three groups with d = 0.46 for the PSQI at week 4, using a baseline-adjusted analysis (ANCOVA) with 80% power, and α = 0.05. The findings indicated that 13 participants per group were needed for the study. All eligible participants were required to sign an informed consent form that was approved by the Human Research Ethics Committees of Human Research Ethics Committees of Mae Fah Luang University (EC 23098 - 25) prior to participation in the study, and their ID numbers were used as identifiers, all personal information was kept confidential.

Research protocols

The eligible participants underwent interviews regarding their demographics. Then, they were randomly assigned to one of the three groups: yoga on a hard surface (YH), yoga on a soft surface (YS), or a control group receiving a general exercise guidebook. On the next day, outcomes were measured at baseline and reassessed at four weeks. The program in each group was led by primary instructor with an additional instructor assisting who was one of the three researchers (Figure 1).

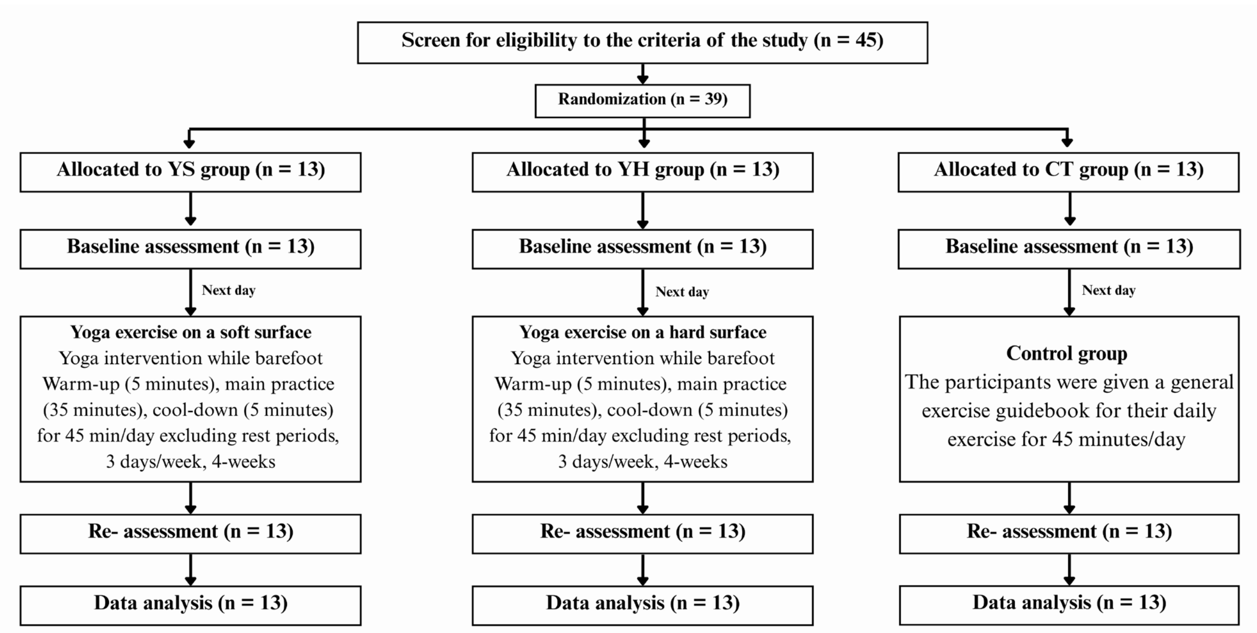

Figure 1 Participation flowchart.
Abbreviations: YS, Yoga exercise on a soft surface; YH, Yoga exercise on a hard surface; CT, Control group.

- Yoga exercise on a soft surface group (YS group): The participants performed yoga exercise program on a soft surface, which was developed from a compressed flexible 3-inch-thick sponge foam yoga mat with the dimensions of 1 meter in width and 2 meters in length (Figure 2)[15].

- Yoga exercise on a hard surface group (YH group): The participants practiced yoga exercise program on a flat, hard, smooth surface using a mat with the same poses as in the YS group.

- Control group (CT): The participants were given a general exercise guidebook for their daily exercise for 45 minutes/day[17].

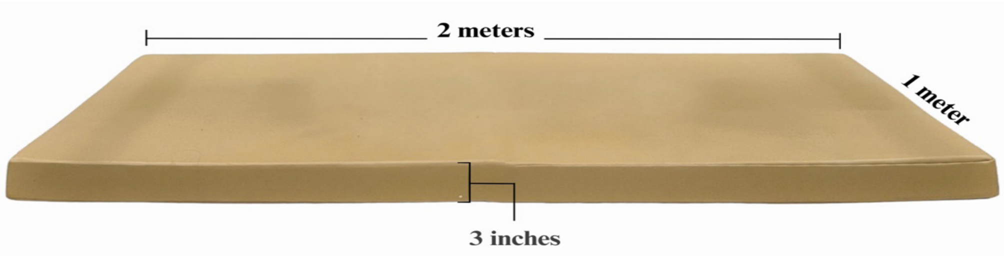

Figure 2 Characteristics of a soft surface used in this study

Yoga training programs

Yoga training sessions least 45 minutes (excluding rest periods) which were divided into 3 parts, including warm-up (5 minutes), main practice (35 minutes), and cool-down (5 minutes) (Figure 3). The yoga postures which were the main practice divided into two phases[17,21,23] with the following details:

Phase 1: Specific postures (asanas) comprising six poses: Prasarita Padottanasana Vinyasa, Parivrtta Trikonasana, Utkatasana, Garudasana, Vrikshasana, and Ushtrasana were performed five times/session over three sessions (Figure 3).

Phase 2: Breathing exercises (Pranayama) comprising two poses: Anulom-vilom and Omkar Chanting (Figure 3).

The yoga program was led by primary instructor with an additional instructor assisting who was one of the three researchers. The participants performed the yoga exercise programs while barefoot for three days/week over four weeks with the intensity controlled by heart rate to maintain no more than approximately 75% of age-predicted maximum [24,25]. Each position was performed 10 times with 30–60 seconds of rest, and both phases were repeated at least twice. Supervision consisted of one primary instructor with an additional instructor assisting who was one of the three researchers. Adverse events were recorded, and intention-to-treat analyses were planned for missing adherence data. However, there were no missing data in the study.

Outcome measures

Participants' functional mobility was assessed using the PSQI, muscle strength, sit-and-reach, and timed up and go (TUG) tests in random order to balance test sequence across participants and minimize fatigue and learning effects. The randomization schedule was independent of data collection and not shared with assessors before testing. Three trained raters with excellent inter-rater reliability (intraclass correlation or ICC ranging from 0.90 to 0.99) conducted the outcomes assessments. The details of each assessment are as follows.

- Pittsburgh Sleep Quality Index: The PSQI (Thai version), a primary measure of the study, is a 19-item questionnaire with seven subscales scores, ranging from 0 to 3, to assess sleep efficiency, disturbances, duration, latency, quality, and daytime dysfunction [26]. The PSQI global score ranges from 0 to 21, with the higher scores indicating poorer sleep quality. Scores of 5 or more significantly denoted poor sleep[2,3,26].

- Isometric muscle strength testing: This study used the Takei 5002 (digital model) portable dynamometer to assess leg-back muscle strength (ICC > 0.99). Participants placed their feet on the dynamometer and held their arms at the sides of their bodies, with the trunk positioned on their backs and slightly forward, avoid hip flexion/rotation. They were then instructed to pull the dynamometer bar with their hands at maximum speed, using only their legs and not their backs, until their knees were fully extended, hold no more than 3 seconds. Three trials were conducted, with a two-minute rest between each trial. Maximal muscle strength (in kilograms) was recorded for each trial[27].

- The sit-and-reach test: This validated method for assessing hamstring and low back flexibility was executed while participants seated with legs close together and feet against a support (ICC = 0.98)[28]. They were then instructed to lean forward as far as possible with straight arms, holding the position briefly, with the farthest reach recorded in centimeters in three trials and the highest value was reported[28].
- Timed Up and Go test: The TUG test, a high reliability measure (ICC = 0.76-0.99), was used to assess dynamic balance and mobility[29,30]. Participants were timed their ability of rising from a chair with armrests, walking around a traffic cone placed three meters ahead, and returning to a seated position on the chair quickly and securely in three trials, with the average time was reported[29,30].

An assessor monitored the participants to ensure safety and measurement accuracy, by either standing or walking nearby. If the participants experienced any condition or abnormal vital signs during the test or training, they could stop immediately.

All assessments were conducted by a blinded assessor at baseline and after four weeks (Figure 1). Additionally, to preserve blinding, a new test-record form was used for each assessment, and participants provided their ID number to the blinded assessor for data entry on the new form.

Statistical analyses

Data were analyzed using SPSS version 31. Descriptive statistics were used to explain the demographic data of the participants and the findings of the study. The dependent sample t-test was used to analyze the different findings between baseline and 4-week measurement of each outcome (within-group comparisons). The one-way analysis of covariance (ANCOVA) with the use of baseline data as covariate and the group as a fixed factor was used to compare 4-week data between the groups. The significance level was set at p-value < 0.05.

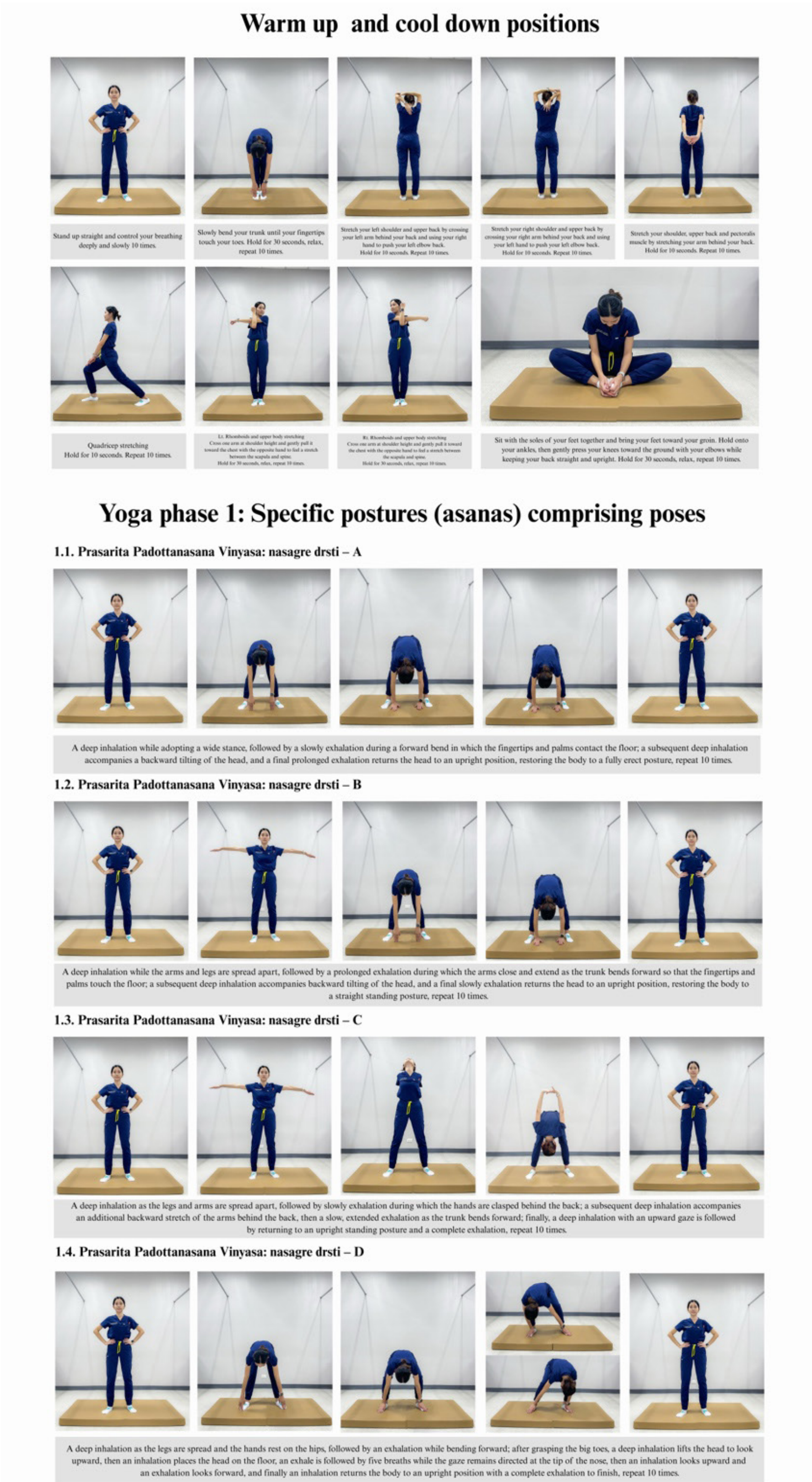

Figure 3 Detailed postures of yoga program

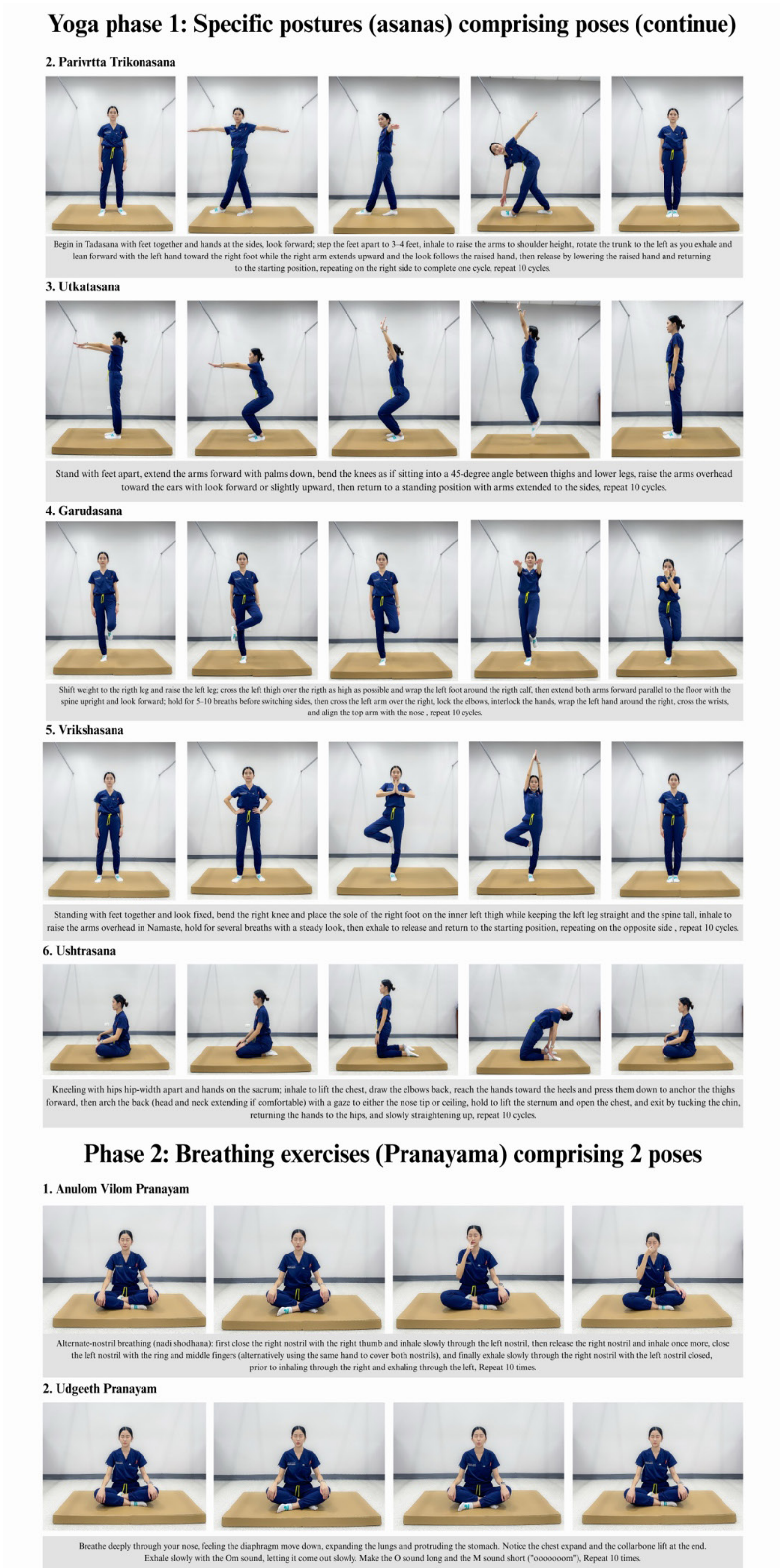

Figure 3 Detailed postures of yoga program (continue)

Results

Thirty-nine participants, with an average age of 21 years and normal body mass index, who did not perform regularly exercise, were included in the study. Most were female, and all participants reported sleep disturbances with the PSQI scores ≥ 5. Baseline demographics showed no significant differences between the groups (*p*-value > 0.05) (Table 1).

Table 1 Baseline demographics of the participants

Variables	Total (n=39)	CT (n=13)	YH (n=13)	YS (n= 13)	p-value[b]
Gender: male, n (%)	8 (20.51)	3 (23.08)	2 (15.38)	3 (23.08)	0.382
Age: year [a]	21.67 ± 1.00	21.03 ± 0.24	21.62 ± 0.65	21.92 ± 0.99	0.890
Body mass index: kg/m^{2} [a]	20.03 ± 2.20	20.80 ± 2.10	20.80 ± 2.70	21.22 ± 2.27	0.530
PSQI score [a]	11.32 ± 2.43	10.69 ± 1.70	11.67 ± 3.05	11.56 ± 3.24	0.140
PSQI score reduction ≥ 3 points from baseline, n (%)	22 (56.41)	3 (23.07)	7 (53.85)	12 (92.30)	0.012*
PSQI < 5 at 4-week, n (%)	13 (33.33)	2 (15.38)	4 (30.77)	7 (53.85)	0.042*
Frequency of exercise per week	1.74 ± 1.04	1.62±0.50	1.92±1.44	1.67±0.70	0.660

Note: [a] The data are presented using mean ± standard deviation, [b] *p*-values from one-way ANOVA. * The superscripts indicated significant differences with the *p*-value < 0.05.
Abbreviations: n, number; PSQI, Pittsburgh Sleep Quality Index; YH, Yoga exercise on a hard surface; YS, Yoga exercise on a soft surface; CT, Control group.

Quality of sleep outcomes

Sleep quality was determined using the PSQI. In the within-group analysis, participants in the YH and YS groups demonstrated highly significant improvement in PSQI scores, while participants in the control group did not show any improvement. The improvements were seen in all sub-score components for both yoga groups (*p*-value < 0.001, Table 2), with the YS group showing significantly better PSQI scores than the YH group. In contrast, the control group demonstrated significant changes only in the subjective quality and latency of sleep (*p*-value < 0.05, Table 2). Moreover, significant differences between the groups were observed in all sub-score components, except for sleep latency (Table 2).

In addition, at week 4, a greater proportion of participants in the YS group achieved a clinically meaningful improvement (≥ 3-point reduction in PSQI) compared with YH and CT (Table 1). The proportion with PSQI <5 at week 4 was higher in YS than in YH and CT (Table 1). These findings were interpreted against prespecified thresholds, defining a minimum clinically important difference of 3 points on the PSQI and PSQI < 5 as indicating good sleep.

Isometric muscle strength, flexibility, and dynamic balance outcomes

All participants completed the program safely. After four weeks, significant improvements were observed in isometric muscle strength and flexibility in both yoga groups (YS and YH) (*p*-value < 0.01, Table 3). Dynamic balance did not show a significant improvement in either yoga group. Between-group comparisons revealed that the YS group showed greater gains in isometric muscle strength and in the sit-and-reach flexibility test than both the YH and control groups (*p*-value < 0.01, Table 4). No significant differences were found between YH and control for these outcomes.

Table 2 Pittsburgh Sleep Quality Index and sub-scores in each group (n = 13 per group)

Variables	Group	Baseline	4-week assessments	Mean change from baseline data (Baseline - 4-week assessments)[a]	*p-value*[b]
PSQI score	CT	10.69 ± 1.70 (8.54, 11.84)	7.46 ± 2.69 (5.64, 9.29)	2.23 ± 3.06 $^{YS***, YH***}$ (0.38, 4.08)	0.055
	YH	11.67 ± 3.05 (9.46, 13.87)	6.33 ± 2.67 (3.40, 8.26)	5.33 ± 3.28 ^{CT***} (4.25, 8.42)	0.001***
	YS	11.56 ± 3.24 (8.50, 14.61)	3.44 ± 3.53 (1.11, 7.78)	7.11 ± 4.28 $^{CT***, YH***}$ (3.81, 10.41)	0.001***
Subjective sleep quality	CT	1.85 ± 0.37 (1.59, 2.10)	1.38 ± 0.50 (1.04, 1.73)	0.46 ± 0.52 ^{YS***} (0.15, 0.76)	0.008**
	YH	1.83 ± 0.38 (1.55, 2.11)	0.95 ± 0.62 (0.98, 1.80)	0.83 ± 0.67 ^{YS**} (0.66, 1.51)	0.001***
	YS	1.89 ± 0.33 (1.57, 2.20)	0.34 ± 0.52 (-0.09, 0.98)	1.44 ± 0.53 $^{CT***, YH**}$ (1.04, 1.85)	0.001***
Sleep latency	CT	1.92 ± 0.64 (1.49, 2.36)	1.31 ± 0.85 (0.73, 1.89)	0.61 ± 0.87 (0.09, 1.14)	0.025*
	YH	2.33 ± 1.07 (1.56, 3.11)	1.25 ± 0.86 (0.62, 1.88)	1.08 ± 1.24 (0.18, 2.95)	0.012*
	YS	2.56 ± 0.72 (1.87, 3.24)	0.89 ± 0.78 (0.15, 1.63)	1.67 ± 1.00 (0.89, 2.44)	0.001***
Sleep duration	CT	2.45 ± 0.80 (1.30, 2.79)	1.69 ± 0.75 (1.18, 2.20)	0.15 ± 1.06 ^{YS***} (-0.49, 0.79)	0.613
	YH	2.33 ± 0.49 (1.98, 2.69)	1.08 ± 0.90 (0.43, 1.73)	1.25 ± 0.96 ^{YS**} (0.64, 1.86)	0.005**
	YS	2.22 ± 0.66 (1.16, 2.85)	0.58 ± 0.97 (-0.19, 1.89)	1.44 ± 1.13 $^{CT***, YH**}$ (0.58, 2.31)	0.001***
Sleep efficiency	CT	1.26 ± 0.87 (1.03, 2.20)	1.38 ± 0.76 (-0.24, 1.10)	-0.23 ± 0.73 $^{YH*, YS**}$ (-0.21, 0.67)	0.273
	YH	1.17 ± 1.19 (0.31, 2.03)	0.62 ± 0.79 (-0.26, 1.12)	0.55 ± 0.96 $^{CT*, YS**}$ (-0.22, 1.78)	0.111
	YS	1.11 ± 1.45 (-0.26, 2.48)	0.33 ± 1.00 (-0.61, 1.28)	0.77 ± 0.85 $^{CT**, YH**}$ (0.14, 1.36)	0.021*

Note: The data were presented using mean ± SD (95% confidence intervals). Superscripts CT, YH, YS, and denote the groups that differed significantly from the indicated groups, as determined by the Bonferroni post-hoc comparisons: ^{CT} = Control group, ^{YH} = Yoga exercise on hard surface, and ^{YS} = Yoga exercise on soft surface, [a] The one-way analysis of covariance (ANCOVA) was used to compare 4-week data between the groups. [b] A paired-samples t-test was used to compare baseline and 4-week outcomes within each group. * The superscripts indicated significant differences with the **p*-value < 0.05, ** *p*-value < 0.01, ****p*-value < 0.001.

Abbreviations: n, number; PSQI= Pittsburgh Sleep Quality Index; YH, Yoga exercise on a hard surface; YS, Yoga exercise on a soft surface; CT, Control group.

Table 3 Isometric muscle strength, flexibility, and dynamic balance outcomes of the participants in each group (n= 13 per group)

Variables	Group	Baseline	4-week assessments	Mean change from baseline data (4-week assessments - Baseline) [a]	*p*-value [b]
Isometric Muscle Strength Testing (kg)	CT	65.34 ± 28.72 (45.90, 84.79)	61.03 ± 31.63 (49.61, 92.44)	-5.67 ± 7.12 ^{YS**} (-9.98, -1.38)	0.086
	YH	62.34 ± 23.56 (45.34, 89.36)	69.75 ± 29.48 (49.47, 92.03)	7.40 ± 9.69 ^{YS*} (-1.25, 14.56)	0.014*
	YS	63.72 ± 21.38 (43.58, 83.86)	75.06 ± 25.57 (50.97, 99.14)	11.33 ± 7.36 ^{YH*} (-2.00, 17.67)	0.012*
Sit-and-reach test (cm)	CT	5.84 ± 12.26 (-7.09, 16.52)	3.22 ± 4.28 (-9.89, 16.44)	-1.44 ± 4.66 ^{YS**} (-4.25, -1.37)	0.288
	YH	6.88 ± 10.04 (-0.38, 14.13)	8.59 ± 8.46 (2.49, 14.70)	1.72 ± 2.07 ^{YS*} (0.40, 3.03)	0.015*
	YS	6.02 ± 7.70 (-3.23, 11.28)	10.82 ± 6.66 (1.55, 14.09)	4.80 ± 2.72 ^{YH*} (1.71, 5.89)	0.003**
Timed up and go test (s)	CT	6.16 ± 0.62 (5.74, 6.58)	5.55 ± 0.39 (5.29, 5.82)	-0.60 ± 0.47 (-0.32 to 0.89)	0.819
	YH	6.14 ±0.86 (5.52, 6.77)	6.55 ± 4.16 (3.55, 9.55)	0.41 ± 4.32 (-3.15 to -2.34)	0.784
	YS	6.67 ± 1.17 (5.56, 7.77)	5.52 ± 0.67 (4.88, 6.15)	-1.15 ± 0.98 (-2.15 to -0.15)	0.510

Note: The data were presented using mean ± SD (95% confidence intervals). Superscripts CT, YH, YS, and denote the groups that differed significantly from the indicated groups, as determined by the Bonferroni post-hoc comparisons: ^{CT} = Control group, ^{YH} = Yoga exercise on hard surface, and ^{YS} = Yoga exercise on soft surface, [a] The one-way analysis of covariance (ANCOVA) was used to compare 4-week data between the groups. [b] A paired-samples t-test was used to compare baseline and 4-week outcomes within each group. * The superscripts indicated significant differences with the **p*-value < 0.05, ** *p*-value < 0.01, ****p*-value < 0.001.

Abbreviation: The data were presented using kg, kilograms; cm, centimeters; s, second; n, number; YS, Yoga exercise on soft surface; YH, Yoga exercise on hard surface; CT, Control group.

Table 4 Difference between groups regarding the mean scores of isometric muscle strength, flexibility, and dynamic balance outcomes in each group (n= 13 per group)

Dependent Variable	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	95% Confidence Interval		*p*-value
					Lower Bound	Upper Bound	
Isometric Muscle Strength Testing (kg)	CT	YH	-5.65	4.96	-15.76	4.45	0.263
		YS	-6.72	2.58	-12.05	6.61	0.006**
	YH	YS	4.93	1.04	-9.35	13.21	0.043*
Sit-and-reach test (cm)	CT	YH	2.08	1.51	-1.01	5.18	0.179
		YS	-5.24	1.49	-8.28	-2.20	0.001***
	YH	YS	-3.16	1.38	-5.96	-0.35	0.029*
Timed up and go test (s)	CT	YH	0.54	1.16	-1.83	2.90	0.645
		YS	-1.01	1.07	-3.20	1.17	0.351
	YH	YS	-1.55	1.18	-3.96	0.85	0.197

Note: * The superscripts indicated significant differences with the *p-value < 0.05, **p-value < 0.01, ***p-value < 0.001.

Abbreviation: The data were presented using kg, kilograms; cm, centimeters; s, second; n= number; YS, Yoga exercise on soft surface; YH, Yoga exercise on hard surface; CT, Control group.

Discussion

The participants in our study showed significant improvements before and after participation in YH and YS on sleep quality (all sub-scores of PSQI; p-value < 0.001, Table 2), flexibility, and muscle strength outcomes (Table 3), particularly in the YS group (p-value < 0.001, Table 2, 3). Compared with the control group, the 4-week outcomes for both yoga groups differed significantly (p-value < 0.001, Table 4) in muscle flexibility and strength. No significant differences were observed in sleep latency and balance ability (TUG; p-value > 0.05, Table 2, 3, 4).

The yoga practice performed in this study involved stretching to stimulate blood vessels and coordinated body movements and breathing to induce relaxation(29,31), leading to improve quality and reduced latency of sleep and fewer sleep disorders, as indicated by various studies in other populations(18,19,31,32). Participants in the intervention groups of previous research typically showed shorter average sleep duration yet better habitual sleep efficiency compared to the control groups. The synchronized body movements and breathing techniques employed in yoga practices can help alleviate functional disability and anxiety(33). Studies have consistently shown that sleep quality in various group is improved by yoga(10,19,21,23). For instance, a 6-month program involving postures, relaxation, breathing, and philosophy enhanced sleep latency, duration, and morning refreshment(31). Yoga was also shown to improve overall sleep quality among elderly nursing home residents. Moreover, previous studies also demonstrated that gentle yoga postures and controlled breathing decreased sympathetic activity, thereby promoting parasympathetic dominance and reducing stress responses in healthy individuals and those with psychosomatic conditions(34). Although it focused on young students with study-related sleep issues without other health problems, this research showed that practicing yoga significantly improved sleep quality, even in individuals without additional health concerns. Therefore, both groups that practiced yoga exercises experienced significantly improved sleep (p-value < 0.001, Table 2).

These changes may have increased the metabolic rate and energy expenditure through harder yoga exercise on soft surfaces, potentially enhancing sleep quality[35]. Furthermore, exercising on a soft surface promoted muscle co-contraction and proprioceptive input due to the unstable nature of the surface, which led to muscle fatigue and soreness, influencing sleep onset and quality[36]. The condition could have resulted in unstable positions during yoga exercise, increasing energy expenditure leading to the intensity beyond the intended moderate level of the soft surface yoga intervention. Consistent with previous studies reporting that high-intensity exercise is often associated with psychological stress, which may predispose individuals to insomnia and impair sleep performance. Thus, this study demonstrated that soft-surface yoga can be performed at a moderate intensity and is appropriate for improving sleep[25]. The significant enhancements in isometric muscle strength, flexibility, and dynamic balance outcomes seen following participation in the YS training are linked to the utilization of a soft surface. Research indicates that exercising on an unstable sand surface decreases muscle mechanical work, leading to increased muscle co-contraction and greater challenges for leg and back muscle strength compared to exercising on a hard surface[37].

Thus, the participants showed significantly dramatic improvement in the isometric muscle strength testing (6% on the soft surface, 3% on the hard surface; Table 3), which is commonly used to reflect functional leg and back strength [27], in comparison with those who trained on a firm surface. Although statistically significant, the changes observed in the yoga group that practiced on a hard surface were not clinically meaningful. Previous research suggests that isometric outcomes in healthy students require a 5% minimal clinically important difference to be significant[27]. Similarly, prior research has demonstrated that walking on sand increases electromyographic activation, leading to a 13% average improvement in lower extremity motor strength among participants, while those walking on a solid surface did not show any enhancements[37,38].

Furthermore, yoga poses not only improved muscle strength but also served as a joint and muscle stretching regimen emphasizing controlled breathing and posture, especially on soft surfaces. The study results indicate significant gains in flexibility (4.8 cm, p-value < 0.001) after four weeks. Previous research also indicated that yoga improved flexibility in women, with increases of 3.5-10 cm after 8-16 weeks[39,40].

Hence, the practice of rigorous yoga on a soft surface led to significant improvements in sleep quality, flexibility, and strength measures within four weeks (Table 2, 3). However, reliance on subjective measures such as the PSQI limited accuracy; thus, future research should incorporate objective assessments for more precise sleep quality evaluation.

Conclusion

The effect 4-weeks of yoga, especially on a soft or unstable surface, may enhance sleep quality and increased isometric strength and flexibility with no between-group difference in dynamic balance in stress with poor sleep students. However, longer-term follow-up with larger samples and more advanced assessments is needed to confirm durability and clarify the underlying mechanisms.

Take home messages

This finding suggests that stressed students with mild insomnia may benefit from 4-weeks of yoga on either a hard or soft (unstable) surface, as both improve sleep quality, flexibility, and muscle strength, with soft-surface yoga yielding superior and more pronounced improvements than hard surface yoga.

Conflicts of interest

The author declares no conflict of interest.

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Author contributions

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Data availability

Author elects to not share data

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