

The effects of ankle joint muscle strengthening and proprioceptive exercise programs accompanied by functional electrical stimulation on stroke patients' balance

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Abstract. [Purpose] The purpose of the present study was to examine the effects of ankle joint muscle strengthening and proprioceptive exercises accompanied by functional electrical stimulation on stroke patients' balance ability. [Methods] For six weeks beginning in April 2015, 22 stroke patients receiving physical therapy at K Hospital located in Gyeonggi-do were divided into a functional electrical stimulation (FES), ankle proprioceptive exercise and ankle joint muscle strengthening exercise group (FPS group) of 11 patients and an FES and stretching exercise group (FS group) of 11 patients. The stimulation and exercises were conducted for 30 min per day, five days per week for six weeks. Balance ability was measured using a BioRescue and the Berg balance scale, functional reach test, and the timed up-and-go test were also used as clinical evaluation indices. Repeated measures ANOVA was conducted to examine differences between before the exercises and at three and six weeks after beginning the exercises within each group, and the amounts of change between the two groups were compared. [Results] In the comparison within each group, both groups showed significant differences between before and after the experiment in all the tests and comparison between the groups showed that greater improvement was seen in all values in the FPS group. [Conclusion] In the present study, implementing FES and stretching exercises plus ankle joint muscle strengthening and proprioceptive exercises was more effective at improving stroke patients balance ability than implementing only FES and stretching exercises.

Key words: Proprioceptive, Strengthening, Stroke

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INTRODUCTION

Due to the loss of the central nervous system's ability to control the paretic side, stroke patients show excessive muscle tone, such as spasticity and loss of proprioceptive and equilibrium senses¹⁾. In particular, Carr and Shepherd²⁾ reported that stroke patients have disorders in standing and gait due to asymmetric postures, abnormal body balance, and the loss of certain motor abilities.

In the human body, the hip joints and ankle joints play important roles in maintaining stability. In particular, the hip joints mainly act when the range of body sway is large, and the ankle joints mainly act when the range of body sway is small³⁾. The most important roles of the ankle joints and the feet are in controlling body sway and forward movements of the lower extremities, and these roles require a sufficient

range of motion, muscle strength, and proprioceptive sense in the ankle joints⁴⁾.

Regarding the effects of functional electrical stimulation treatment, Achache et al.⁵⁾ reported that when functional electrical stimulation was applied to the dorsal flexor muscle of the ankle joints, increased muscle activity improved muscle strength. This led to the enhancement of the stability of the knee joints, which had positive effects on balance. In addition, Sabut et al.⁶⁾ reported that when functional electrical stimulation was applied for 12 weeks, the activity of the anterior tibial muscle increased and energy consumption decreased by 34.6%.

Regarding the reason that ankle muscle strength is necessary to maintain balance, Macrae et al.⁷⁾ stated that according to the results of tests of seven lower extremity muscles, the ankle dorsal flexor muscle was the best predictive factor for the prediction of falls. In addition, Lord et al.⁸⁾ advised that one of differences between no-fall groups and those who experienced falls was the muscle strength of the ankle dorsal flexor muscle.

The retraining of the movements of the ankle joints that control body balance plays as important role in solutions to problems in balance control, and it mitigates problems such as abnormal forms of muscle control of the ankle joint, joint

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contracture over a long period, and proprioceptive sense disorders⁹). To improve stroke patients' balance ability that has deteriorated due to ankle instability and proprioceptive sense declines, the stability of the ankles needs to be restored through ankle muscle strengthening¹⁰). However, studies that have simultaneously applied functional electrical stimulation treatment, proprioceptive sense treatment, and ankle muscle strengthening exercises in an ankle training program for stroke patients are rare.

As noted above, to improve stroke patients' balance ability, the ankle muscles need to be strengthened and the proprioceptive sense needs to be recovered. Therefore, the purpose of the present study was to examine the effects of approaches to ankle joint treatment on balance ability. The study focused on that the factors affecting stroke patients' balance, the loss of the ankles' proprioceptive sense, and ankle muscle weakening, in order to determine diverse protocols for stroke patients' balance improvement and functional enhancement.

SUBJECTS AND METHODS

The subjects were 22 stroke patients in the rehabilitation center of a general hospital located in Korea. The selection criteria were: first-time stroke, at least 6 weeks post-stroke, a Mini-Mental State Examination score ≥ 24 , and no serious visual disorder, visual field defect, or hearing impairment based on the opinion of the doctor in charge. All subjects understood the content of the study and voluntarily participated in the study. The exclusion criteria were: severe hemineglect, pregnancy, or prior adverse reaction to Functional electrical stimulation (FES). This study was approved by the Institutional Human Research Review Board of Sahmyook University.

To minimize the selection bias of group division, the subjects were randomly assigned to two groups. The subjects drew O-X lots and those who drew O were assigned to the group receiving the application of FES, ankle muscle strengthening exercises, and ankle proprioceptive exercises, the FPS group, while those who drew X were assigned to the FP group. A preliminary evaluation was conducted before the experiment and an ex post facto evaluation was conducted at three weeks and six weeks after the start of the experiment.

FES group received FES for 30 min, and performed ankle proprioceptive exercises for 15 min, and an ankle muscle strengthening exercise program for 15 min in 60-minute sessions, five times per week for six weeks.

FES was applied as a square waveform with a pulse width of 400 μ s and a stimulation frequency of 40 Hz. To induce accurate movements of the muscles, the stimulation intensity was adjusted to 30–70 mA at the common peroneal nerve through observations by the therapist. The subjects performed ankle dorsiflexion movement during functional electrical stimulation producing muscle contraction delivered via surface electrodes. The stimulation increase and decrease times were set to 0.3 s, and to minimize muscle contraction and fatigue due to the subjects' excessive efforts, the electrical stimulation given to patients was restricted to below the threshold value¹¹). The ankle proprioception exercise program was designed by modifying and supplementing

the method proposed by Lynch and Grison¹²) and the details are as follows: ankle joint mobility was promoted in a sitting position, an exercise to move the weight toward the paretic side and get up was repeatedly performed, the weight was moved in various directions in a standing position, and upper extremity task training was performed in a standing position. This program was implemented for a total of 15 min. The ankle exercise program used was created by modifying the method proposed by Chaitow¹³). The subjects lay in a supine position on a bed and performed dorsal flexion of the ankle joint with 20–30% of the total muscle strength that could be mobilized by the subject. In addition, the therapist provided counter-pressure equal to the pressure of the subject's ankle joint dorsal flexion using manual resistance so isometric contraction could occur in the muscle anterior to the tibia. The subjects took a rest for 10 s after the contraction. This exercise was performed three times repeatedly with a rest of 10 s after each trial. During the trials, the therapist helped the subject so that accurate movements could be made without the occurrence of compensation by the ankle joint¹⁴). Ankle dorsiflexion, ankle plantarflexion, ankle eversion, and ankle inversion were performed for a total of 15 min.

The FS group received FES for 30 min and performed ankle stretching exercises for 30 min in 60-minute sessions, five times per week for six weeks.

For the stretching exercises, the subjects were instructed to apply force toward the sole in a prone position and the therapist applied the same level of force in the opposite direction to maintain isometric contraction for 6 s. While the subject relaxed the muscle for 2–3 s after the contraction, the therapist extended the length of the muscle for further stretching. At this time, the therapist's hands moved to the point where the hands were stopped by the muscle, and the position was maintained for 15–16 seconds. The subjects were instructed to maintain the relaxed state and to take a rest for 10 s. This exercise was performed repeatedly for a total of 15 min¹⁵). Thereafter, the subjects held the therapist's hand in a standing position, leaned against a wall, stood on a tripod, and performed stretching for 15 min with the ankle joints at 15–20°. A rest was allowed when the patient felt pain or needed a rest and the exercise was performed again thereafter.

In the present study, a balance ability measuring and training system (Analysis Systems by Biofeedback, AP1153 BioRescue, France) was used to measure subjects' balance ability. The measuring method was demonstrated to the subjects before measuring their balance ability. With their eyes open and eyes closed, the Romberg test, the measured the migration length of the center of gravity (COG) and the surface area while standing on the force plate with both feet was measured for 60 s. The unit of the migration length of the COP was cm and that of the unit of surface area was mm². In this evaluation, smaller values mean less sway, which indicates a better balance ability. In the limit of stability (LOS) test, the COP was moved in eight directions (forward, backward, leftward, rightward, and diagonally) using a BioRescue software program, and the movement area was recorded in cm². In this test, direction arrows randomly appear on the computer screen and subjects move their COP in the arrow direction. Both feet have to be placed on the force

plate at all times, and When subjects lifted a foot off the force plate, the measurement was conducted again from the beginning. Larger measured values indicate better balance ability. The forward and backward (FB) test measures the length of migration of the COP while subjects move the non-paretic side foot to the front of the force plate in a standing position supported by two feet and then move it back to the original position; smaller measured values indicate better balance ability. The FB test was conducted three times for each study subject and the average values were used in the analysis.

For the functional reach test (FRT), the subjects adopted a standing position on a fixed surface with the feet shoulder width apart and maximally stretched out an arm while maintaining the arm horizontal to a bar ruler installed horizontally. With the first clenched and the elbow joint extended subjects bent forward as far as they could without losing balance. Then, the distance between the first point and the last point of the distal part of the third metacarpal bone was measured. The measurement was conducted three times for each study subject and the average values were used in the analysis. For this test an intra-rater reliability of $r = 0.98$ and an inter-rater reliability of $r = 0.99$ have been reported¹⁶⁾.

The Berg balance scale (BBS) is a tool for measuring the balance of elderly persons with damaged balance functions. It evaluates their ability to perform functional tasks and the tasks are largely divided into three areas: sitting, standing, and postural changes. Fourteen items are scored from 0 to 4 and thus, the maximum possible score is 56 points. This test is used to measure the ability to maintain dynamic balance and it is widely used to measure the balance ability in movements or standing positions of patients with hemiplegia due to a senile disease or stroke. This test is highly reliable for the measurement of balance and it has been reported to have an intra-rater reliability of $r = 0.98$ and an inter-rater reliability of $r = 0.97$ ¹⁷⁾.

The timed up-and-go (TUG) test is used to evaluate simple functions by measuring the time it takes to get up from a chair, walk to a turning point at a distance of 3 m from the chair, and return, and sit on the chair. Each subject completed the TUG test three times, and the average value was calculated. The test-retest reliability of the TUG test for stroke patients is reported to be high, $r = 0.95$ ¹⁸⁾.

All statistical analyses in this study were conducted using PASW 18.0 for Windows. For the general characteristics of the two groups, the χ^2 test was used to analyze ankylosis, sex, diagnoses, and the side of brain lesions. The patients' heights, weights, Mini-Mental State Examination scores, and time since the onset of stroke were analyzed using the independent samples t-test. Normality was tested using the Shapiro-Wilk test. Repeated measures ANOVA was conducted to examine differences within the two groups among the treatment times (zero, three, and six weeks of training) and to examine differences between the two groups resulting from the therapeutic exercise methods before the training, after three weeks' training, and after six weeks' training. A statistical significance level of $\alpha = 0.05$ was used in all tests.

Table 1. The general characteristics of the subjects (N=22)

Group variable	FS (n=11)	FPS (n=11)
Age (years)	54.9 ± 14.0	49.0 ± 13.1
Height (cm)	165.2 ± 5.2	166.3 ± 2.8
Weight (kg)	70.0 ± 11.0	66.1 ± 9.6
MMSE-K (score)	27.1 ± 1.6	27.5 ± 1.9
Gender		
Male	8 (72.7%)	6 (54.5%)
Female	3 (27.3%)	5 (45.5%)
Brain lesion location		
Cortex level	2 (18.2%)	2 (18.2%)
Subcortex level	5 (45.5%)	4 (36.4%)
Mixed	4 (36.4%)	5 (45.5%)
Diagnosis		
Infarction	7 (63.6%)	7 (63.6%)
Hemorrhage	4 (36.4%)	4 (36.4%)
Affected side		
Left	6 (54.5%)	8 (72.7%)
Right	5 (45.5%)	3 (27.3%)

Values are N (%) or Mean ± standard deviation, ns: not significant, FS: FES with Stretching exercise group, FPS: FES with Proprioception and Strengthening exercise group, MMSE-K: Mini-mental state examination-Korea, MAS: Modified Ashworth scale

General characteristics and dependent variables were calculated using the χ^2 test and the independent t-test

RESULTS

The general characteristics of the study subjects are shown in Table 1.

In the balance ability test, COP migration lengths and surface areas showed significant differences from before the training, at three and six weeks after the beginning of the training in both groups ($p < 0.05$). LOS tests showed significant increases from before the training at three and six weeks after the start of the training in both groups ($p < 0.05$). The FB tests showed significant increases from before the training at three and six weeks after the start of the training in both groups ($p < 0.05$). The BBS results showed significant increases from before the training at three and six weeks after the start of the training in both groups. The FRT results also showed significant increases from before the training at three and six weeks after the start of the training in both groups. The FPS group showed significantly greater improvements in all variables of balance ability than the FP group ($p < 0.05$) (Table 2).

DISCUSSION

In the present study, the effects of ankle joint muscle strengthening and proprioceptive exercise programs on stroke patients' balance ability were examined. According to the results, balance ability improved in both groups and in the comparison of the two groups, the FPS group was revealed to have improved more.

Regarding the changes in balance ability of the two

Table 2. Comparison of the test results between the two groups (N=22)

Group variable		FS (n=11)	FPS (n=11)
REO (cm)	Pre-	55.4 ± 16.8	57.3 ± 18.7
	3 weeks	50.0 ± 16.8*	46.0 ± 14.1*,†
	6 weeks	46.3 ± 16.3*	38.6 ± 14.0*,‡
REC (cm)	Pre-	82.1 ± 19.9	84.5 ± 16.2
	3 weeks	78.2 ± 18.6*	74.6 ± 10.8*,†
	6 weeks	74.9 ± 19.6*	68.2 ± 11.3*,‡
SA (cm)	Pre-	97.7 ± 22.3	111.6 ± 26.3
	3 weeks	89.0 ± 20.4*	95.5 ± 24.4*,†
	6 weeks	72.5 ± 16.5*	72.9 ± 18.3*,‡
LOS (mm)	Pre-	4,165.5 ± 1,016.0	4,199.7 ± 1,289.2
	3 weeks	4,333.5 ± 992.0*	5,216.5 ± 1,879.4*,†
	6 weeks	4,564.9 ± 1,088.1*	6,286.5 ± 2,201.0*,‡
FB (cm)	Pre-	50.7 ± 5.0	49.9 ± 5.00
	3 weeks	49.0 ± 4.6*	47.3 ± 4.9*,†
	6 weeks	48.9 ± 5.3*	45.1 ± 4.9*,‡
FRT (cm)	Pre-	18.6 ± 5.3	18.0 ± 7.9
	3 weeks	22.5 ± 4.8*	23.6 ± 7.5*,†
	6 weeks	24.1 ± 4.6*	26.6 ± 7.0*,‡
BBS (score)	Pre-	48.9 ± 4.1	47.3 ± 3.0
	3 weeks	49.8 ± 3.7*	49.4 ± 2.4*,†
	6 weeks	50.8 ± 3.5*	52.2 ± 2.1*,‡
TUG (sec)	Pre-	19.2 ± 4.1	19.3 ± 4.3
	3 weeks	15.2 ± 1.9*	12.9 ± 2.5*,†
	6 weeks	12.8 ± 2.7*	11.7 ± 2.7*,‡

*p<0.05, Values are Mean ± standard deviation, †significantly different between 0–3 weeks between the two groups, ‡significantly different between 3–6 weeks between the two groups by repeated ANOVA, FS: FES with Stretching exercise group, FPS: FES with Proprioception and Strengthening exercise group, REO: Romberg's eyes open test, REC: Romberg's eyes closed test, SA: eyes open Surface Area, LOS: Limit of Stability test, FB: Forward and Back test, FRT: Functional Reach Test, BBS: Berg Balance Scale, TUG: Timed Up and Go test

groups, the FPS group showed significant differences in the COP migration length, surface area, LOS test, and FB test. Though the results were inconsistent with the results of a previous study indicating that ankle strengthening training did not have any effect on stroke patients' postural sway¹⁹⁾, in a study conducted by Niam et al.²⁰⁾ of 13 stroke patients, it was reported that compared to subjects with intact ankle proprioceptive sense, subjects with damaged ankle proprioceptive sense showed increased sway speed and decreased balance ability. The results of the present study are consistent with the results of the study conducted by Lisinski et al.²¹⁾, who divided 26 stroke patients into a balance training group and a no training group, and examined the effects of balance training on motor skills. The group that received balance training showed reductions in the asymmetry of bilateral weight-bearing and could perform selective movements. The results of the present study are also consistent with the results of a study conducted of 16 stroke patients which examined the effects of task-oriented exercise programs on postural stability, The study reported that the experimental group that performed task-oriented exercises and received ankle joint proprioceptive sense inputs showed

significant improvements in COP movements²²⁾. Therefore, to improve balance ability, the muscles around the ankles need to be activated and ankle joint proprioceptive sense inputs should accompany the activation. The diverse inputs derived from ankle joint muscle strengthening and functional electrical stimulation applied together with repetitive ankle movements appeared to have great effects on the promotion of motor learning, and balance control ability improved because the body perception regarding changes in the position of the body increased in an environment in which these elements were provided, so the environment and proprioceptive sense interacted with each other and they were integrated.

In the present study, BBS, FRT, TUG, and balance ability tests showed significant differences after the intervention in both groups and in the comparison of the groups, and improvements the FPS group were significantly greater than in the FS group. These results are consistent with the study conducted by Geiger et al.²³⁾ of 13 stroke patients. They reported that the TUG result improved from 23.08 s to 14.62 s in the group that performed an additional exercise program using a balance master. Our present results are also consistent with a study conducted by Park et al.²⁴⁾ who

reported that when an ankle joint proprioceptive movement control program was conducted for 13 stroke patients, sway speed and distance decreased, balance ability improved, and TUG values decreased from 20.5 s before the experiment to 17.9 s four weeks after the start of the experiment and to 15.3 s eight weeks after the start of the experiment. Given these results, it can be seen that the ROM was increased by the ankle muscle strengthening exercises, so the stability limit increase and the feed-back and feed-forward control mechanisms were improved thanks to proprioceptive sense inputs. Thus, the ability to establish and maintain correct posture improved through the provision of information on the movement and location of individual parts of the body. In addition, the ability to appropriately control balance during sway was improved through better coordination and mobilization of the senses and muscle functions.

Limitations of the present study include the possibility of interference of balance factors could not be excluded because the subjects' daily lives could not be completely controlled, and whether or not ankle muscle strengthening exercises and proprioceptive sense training have continuous effects was not determined through follow-up.

In conclusion, the two groups showed differences in balance ability between each other at three and six weeks after the start of the experiment, and the FES, ankle proprioception exercise, and ankle muscle strengthening group showed greater improvements. The treatment method that added a program centered on ankle muscle strengthening to FES and ankle proprioceptive exercises was more effective at enhancing balance ability.

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REFERENCES

- Kerrigan DC, Karvosky ME, Riley PO: Spastic paretic stiff-legged gait: joint kinetics. *Am J Phys Med Rehabil*, 2001, 80: 244–249. [[Medline](#)] [[CrossRef](#)]
- Carr JH, Shepherd RB: *Stroke Rehabilitation: Guidelines for Exercise and Training to Optimize Motor Skill*. Oxford: Butterworth Heinemann, 2003, p 426.
- Runge CF, Shupert CL, Horak FB, et al.: Ankle and hip postural strategies defined by joint torques. *Gait Posture*, 1999, 10: 161–170. [[Medline](#)] [[CrossRef](#)]
- Neumann DA: *Kinesiology of the Musculoskeletal System: Foundations for Physical Rehabilitation*. Mosby, 2002, pp 528–529.
- Achache V, Mazevet D, Iglesias C, et al.: Enhanced spinal excitation from ankle flexors to knee extensors during walking in stroke patients. *Clin Neurophysiol*, 2010, 121: 930–938. [[Medline](#)] [[CrossRef](#)]
- Sabut SK, Lenka PK, Kumar R, et al.: Effect of functional electrical stimulation on the effort and walking speed, surface electromyography activity, and metabolic responses in stroke subjects. *J Electromyogr Kinesiol*, 2010, 20: 1170–1177. [[Medline](#)] [[CrossRef](#)]
- Macrae PG, Lacourse M, Moldavon R: Physical performance measures that predict faller status in community-dwelling older adults. *J Orthop Sports Phys Ther*, 1992, 16: 123–128. [[Medline](#)] [[CrossRef](#)]
- Lord SR, Clark RD, Webster IW: Physiological factors associated with falls in an elderly population. *J Am Geriatr Soc*, 1991, 39: 1194–1200. [[Medline](#)] [[CrossRef](#)]
- Cheng PT, Wu SH, Liaw MY, et al.: Symmetrical body-weight distribution training in stroke patients and its effect on fall prevention. *Arch Phys Med Rehabil*, 2001, 82: 1650–1654. [[Medline](#)] [[CrossRef](#)]
- Matjacić Z, Johannesen IL, Sinkjaer T: A multi-purpose rehabilitation frame: a novel apparatus for balance training during standing of neurologically impaired individuals. *J Rehabil Res Dev*, 2000, 37: 681–691. [[Medline](#)]
- Tong RK, Ng MF, Li LS, et al.: Gait training of patients after stroke using an electromechanical gait trainer combined with simultaneous functional electrical stimulation. *Phys Ther*, 2006, 86: 1282–1294. [[Medline](#)] [[CrossRef](#)]
- Lynch M, Grisogono V: *Stroke and head injuries*. London: John Murray, 1999, pp 113–136.
- Kim S, Park J, Hwang S: *Muscle energy technique*. Chaitow L. Seoul: Yeongmunsu, 2001, pp 74–75.
- Dean CM, Richards CL, Malouin F: Task-related circuit training improves performance of locomotor tasks in chronic stroke: a randomized, controlled pilot trial. *Arch Phys Med Rehabil*, 2000, 81: 409–417. [[Medline](#)] [[CrossRef](#)]
- Evjenth O, Hamberg J: *Muscle stretching in manual therapy: a clinical manual*, Alfta Sweden, 1994.
- Duncan PW, Weiner DK, Chandler J, et al.: Functional reach: a new clinical measure of balance. *J Gerontol*, 1990, 45: M192–M197. [[Medline](#)] [[CrossRef](#)]
- Berg K, Wood-Dauphinee S, Williams JI, et al.: Measuring balance in the elderly: preliminary development of an instrument. *Physiother Can*, 1989, 41: 304–311. [[CrossRef](#)]
- Ng SS, Hui-Chan CW: The timed up & go test: its reliability and association with lower-limb impairments and locomotor capacities in people with chronic stroke. *Arch Phys Med Rehabil*, 2005, 86: 1641–1647. [[Medline](#)] [[CrossRef](#)]
- Kaminski TW, Buckley BD, Powers ME, et al.: Effect of strength and proprioception training on eversion to inversion strength ratios in subjects with unilateral functional ankle instability. *Br J Sports Med*, 2003, 37: 410–415, discussion 415. [[Medline](#)] [[CrossRef](#)]
- Niam S, Cheung W, Sullivan PE, et al.: Balance and physical impairments after stroke. *Arch Phys Med Rehabil*, 1999, 80: 1227–1233. [[Medline](#)] [[CrossRef](#)]
- Lisiński P, Huber J, Gajewska E, et al.: The body balance training effect on improvement of motor functions in paretic extremities in patients after stroke. A randomized, single blinded trial. *Clin Neurol Neurosurg*, 2012, 114: 31–36. [[Medline](#)] [[CrossRef](#)]
- Bayouk JF, Boucher JP, Leroux A: Balance training following stroke: effects of task-oriented exercises with and without altered sensory input. *Int J Rehabil Res*, 2006, 29: 51–59. [[Medline](#)] [[CrossRef](#)]
- Geiger RA, Allen JB, O'Keefe J, et al.: Balance and mobility following stroke: effects of physical therapy interventions with and without biofeedback/forceplate training. *Phys Ther*, 2001, 81: 995–1005. [[Medline](#)]
- Park YH, Kim YM, Lee BH: An ankle proprioceptive control program improves balance, gait ability of chronic stroke patients. *J Phys Ther Sci*, 2013, 25: 1321–1324. [[Medline](#)] [[CrossRef](#)]