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# Reliability of Surface Electromyography From the Lower-limb Muscles During Maximal and Submaximal Voluntary Isometric Contractions in In-bed Healthy Individuals and Patients With Subacute Stroke

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## HIGHLIGHTS

- Maximal and submaximal contraction methods are feasible in in-bed stroke patients.
- In most muscles, maximal and submaximal contraction methods show good reliability.
- The submaximal contraction method is a useful technique in in-bed stroke patients.

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# Reliability of Surface Electromyography From the Lower-limb Muscles During Maximal and Submaximal Voluntary Isometric Contractions in In-bed Healthy Individuals and Patients With Subacute Stroke

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## ABSTRACT

This study aims to develop maximal voluntary isometric contraction (MVIC) and submaximal voluntary isometric contraction (subMVIC) methods and to assess the reliability of the developed methods for in-bed healthy individuals and patients with subacute stroke. The electromyography (EMG) activities from the lower-limb muscles including the tensor fascia lata (TFL), rectus femoris (RF), tibialis anterior (TA), and gastrocnemius (GC) on both sides were recorded during MVIC and subMVIC using surface EMG sensors in 20 healthy individuals and 20 subacute stroke patients. In inter-trial reliability, both MVIC and subMVIC methods demonstrated excellent reliability for all the measured muscles at baseline and follow-up evaluations in both healthy individuals and stroke patients. In inter-day reliability, MVIC showed good reliability for the TFL and moderate reliability for the RF, TA, and GC, while subMVIC showed good reliability for the TFL, RF, and GC and poor reliability for the TA in healthy individuals. In conclusion, the MVIC and subMVIC methods of EMG activities were feasible in in-bed healthy individuals and patients with subacute stroke. The results can serve as a basis for the clinical evaluation of muscular activities using quantitative EMG signals on the lower-limb muscles in stroke patients with impaired mobility.

**Keywords:** Electromyography; Lower Limb; Reliability; Submaximal Contraction; Stroke

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**Conflict of Interest**
The authors have no potential conflicts of interest to disclose.

**Author Contributions**
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# INTRODUCTION

Recent advances in wearable sensing technologies have led to a significant impact on post-stroke rehabilitation research [1,2]. Specifically, quantitative measurement of muscular activities in the paretic limbs in stroke patients has been regarded as a valuable approach for evaluating muscle strength, monitoring functional behaviors, and tailoring treatment strategies [3-5]. Surface electromyography (sEMG) is a major method for measuring muscular activities in a non-obtrusive, objective, and quantitative manner [6]. This method enables the detection and tracking of residual muscular activities in paralyzed limbs, and facilitates motor training based on visualized sEMG signals in patients with stroke [7].

Normalization is a critical aspect of quantitative analyses when dealing with sEMG signals, as it ensures the reliability of data [8-10]. It is a crucial process to mitigate the inherent variability of sEMG signals and helps to interpret results, especially in patients with neurological impairment [11,12]. The most commonly utilized standardized technique for normalization is maximal voluntary isometric contraction (MVIC) [8]. In patients with chronic stroke, both conventional and modified methods of MVIC have been shown to yield reliable results in previous studies [11,13]. However, it should be noted that the reliability of the MVIC method for in-bed patients with stroke has not yet been fully demonstrated. Moreover, it may not be an optimal option for patients with recent onset weakness, such as the acute or subacute phase of stroke recovery, because these patients often have several contributing factors including advanced age, susceptibility to fatigue, and lack of motivation, which may affect the effectiveness of normalization using an MVIC method [14,15].

As an alternative for the normalization of sEMG signals, the submaximal voluntary isometric contraction (subMVIC) method has been proposed in pathological populations [16]. This normalization method has been inspired by the isometric grade 3 of manual muscle testing (MMT), which is the ability of a muscle to maintain a position against gravity and showed greater within-day and between-day reliability than the MVIC method. However, even though the proposed subMVIC method proved to be highly reliable, its applicability in patients with stroke remains to be established. This would be challenging in stroke patients, as the exercises in the subMVIC method require lower-limb movements in seated or standing positions, as outlined in previous studies [16,17]. These types of exercises may be limited in general stroke populations with impaired functional mobility and increased fall risks associated with hemiparesis or decreased sensation. Although in-bed environments can be relatively safe for patients with stroke, there has been a lack of studies exploring normalization methods of sEMG signals for in-bed environments, which are highly restricted to performing static or dynamic postures.

To address the challenges of obtaining reliable sEMG measurements in patients with subacute stroke, this study aims to develop MVIC and subMVIC methods for in-bed environments and to assess the reliability of the developed methods for in-bed healthy individuals and patients with subacute stroke.

# MATERIALS AND METHODS

## Participants

From March 2021 to December 2021, 20 healthy individuals and 20 stroke patients were enrolled in the present study. Inclusion criteria were as follows: healthy individuals aged 18 years or older who voluntarily consented to participate in this study; stroke patients with ≥ 18 years of age, stroke onset of fewer than 6 months, ≤ 4 grades of muscle strength at the affected lower limbs according to MMT, and able to follow the inspector's instruction. Stroke patients were excluded if they had a recurrent stroke during the study period, other underlying neurologic disorders (e.g., Parkinson's disease, dementia), decreased consciousness, inability to comprehend task instructions, ongoing medical conditions (e.g., acute infection, active cancer), or implanted electrical stimulators which substantially interfere EMG measurements. Clinical information including age, sex, duration from stroke onset to measurement, lesion type, lesion laterality, lesion location, muscle strength in terms of MMT, Berg Balance Scale score, Fugl-Meyer Assessment score, Functional Ambulation Category, 10-Meter Walk Test, Mini-Mental State Examination, and results of motor evoked potential were obtained from patients with subacute stroke. This study was approved by the Institutional Review Board of the Seoul National University Hospital (2012-129-1183) and National Traffic Injury Rehabilitation Hospital (NTRH-20030). Written informed consent was obtained from all participants. The procedures were performed in accordance with the principles of the Declarations of Helsinki and all relevant guidelines and regulations.

### Assessments of EMG activities during MVIC and subMVIC

The test maneuvers for body positions and joint angles during MVIC and subMVIC were standardized at each lower-limb muscle including tensor fascia lata (TFL), rectus femoris (RF), tibialis anterior (TA), and gastrocnemius (GC), which are illustrated in **Fig. 1**. MVIC was conducted against gravity and manual resistance for 4 seconds, and subMVIC was conducted against only gravity and to maintain body positions and joint angles for 4 seconds. The body positions and joint angles in measurements of the lower-limb muscles during MVIC were as follows: (i) hip abduction at 30° in a side-lying position for the TFL; (ii) knee extension at 30° of knee flexion in the supine position for the RF; (iii) ankle dorsiflexion at neutral position of the ankle joint in the supine position for the TA; (iv) ankle plantar flexion at neutral position of the ankle joint in the supine position for the GC. The body positions and joint angles in measurements of the lower-limb muscles during subMVIC were identical except for the GC: maintenance of ankle plantar flexion with the metatarsal area on the edge of a plastic box during subMVIC (**Fig. 1H**). We excluded data obtained from muscles that had MMT grade zero since they did not accurately represent the MVIC value. In this study, a single physical therapist assessed MMT grade and instructed test maneuvers during MVIC and subMVIC for the TFL, TF, TA, and GC.

### Acquisition and analyses of EMG activity

EMG activities for the lower-limb muscles during MVICs and subMVICs were recorded using wireless sEMG sensors (Trigno Wireless EMG System; Delsys Inc., Boston, MA, USA). The sEMG signals were acquired from the 4 lower-limb muscles including the TFL, RF, TA, and GC on both sides. Skin preparation and electrode placement were standardized according to previous studies [18,19]. A surface electrode was placed on the muscle belly along a line parallel to the direction of the muscle fibers and attached to the skin with a strap. The attachment points of the surface electrodes were as follows: at 2 finger breadths anterior to the greater trochanter for the TFL; at half of the line from the anterior superior iliac spine to the superior part of the patella for the RF; at one third of the line from the head of the fibula to the medial malleolus for the TA; one fourth of the line from the medial popliteal fossa to the medial border of Achilles tendon for the GC [20-22]. All participants performed 3 repetitions of MVICs and subMVICs, respectively, while maintaining the lying position and sensor attachments. EMG signals were obtained for healthy individuals and stroke patients at baseline and 3 weeks after baseline measurements.

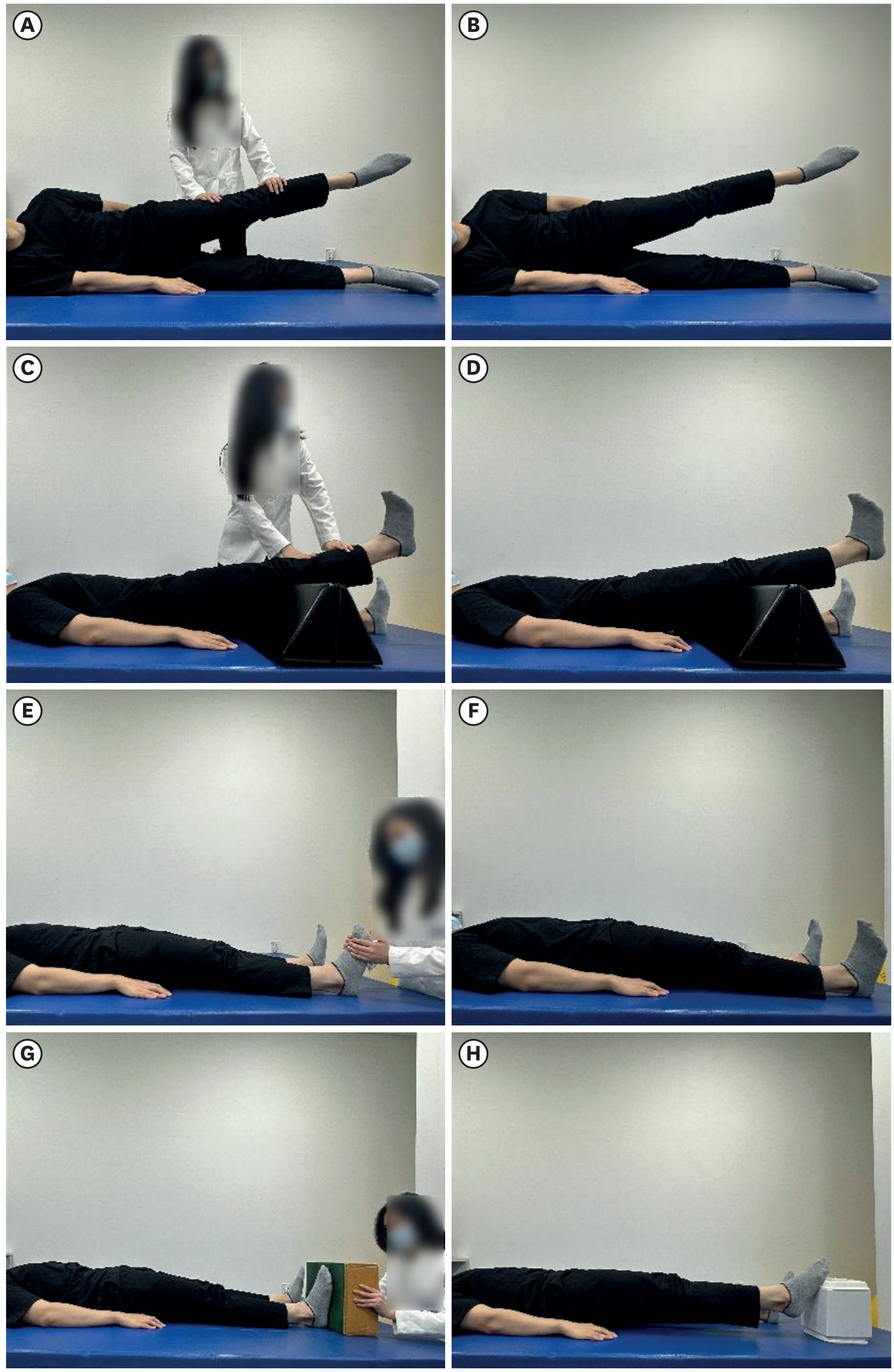

**Fig. 1.** Test positions during maximal and submaximal voluntary contractions for the tensor fascia lata (A, B), rectus femoris (C, D), tibialis anterior (E, F), and gastrocnemius (G, H).

In this study, sEMG data were collected at a sampling rate of 1,259 Hz and a bandwidth of 20–450 Hz using the EMGworks Acquisition software program (Delsys EMGworks 4.0; Delsys Inc.). The onset and offset of sEMG signals were identified based on visual inspection in each trial of MVICs and subMVICs. The captured sEMG signals were then normalized by subtracting the mean background EMG activities and filtered using a Hampel filter to eliminate any outlier signals [23]. In each trial, the root mean square of the EMG amplitudes was computed using a window of 500 ms in the lower-limb muscles [24,25]. The peak amplitude was determined as the greatest value of the root mean square of the sEMG signals.

### Statistical analysis

To assess inter-trial reliability in healthy individuals and stroke patients, interclass correlation coefficients (ICCs) were analyzed among the amplitudes of the EMG signals from the 4 lower-limb muscles in 3 repeated trials of MVICs and subMVICs at the baseline and follow-up, respectively. To assess inter-day reliability in healthy individuals, ICCs were analyzed between the mean amplitudes of the EMG signals from the 4 lower-limb muscles in 3 repetitive trials of MVICs and subMVICs at the baseline and follow-up. Inter-day comparison of sEMG signals in stroke patients was not conducted owing to the fact that the muscle strength of the patients changes as a natural course of neural recovery in subacute stroke. Based on the 95% confidence interval of the ICC estimates, ICC of less than 0.5 indicates poor reliability, ICC of 0.5 to 0.75 indicates moderate reliability, ICC of 0.75 to 0.9 indicates good reliability, and ICC greater than 0.9 indicates excellent reliability, respectively [26]. The Kolmogorov-Smirnov test was used to determine the distribution of the data. The standard error of the measurement was calculated for absolute reliability. The inter-trial and inter-day standard error of measurement was also computed. Pearson correlation coefficients were analyzed to evaluate correlations between EMG activities during MVICs and subMVICs. All statistical analyses were performed using SPSS software (version 25; SPSS Inc., Chicago, IL, USA), and the significance level was set at $p < 0.05$.

## RESULTS

### Clinical characteristics

The mean age of the participants was 39.4 ± 14.7 years among healthy individuals and 60.1 ± 13.3 years in patients with subacute stroke. There were 10 (50.0%) females among healthy individuals and 9 (45.0%) among patients with subacute stroke. **Table 1** shows clinical characteristics in patients with subacute stroke. The 10-Meter Walk Test was measured in 10 patients with subacute stroke, and motor evoked potentials at the affected lower limb were measured in 8 patients with subacute stroke. The lower extremity subscore of the Fugl-Meyer Assessment at the affected limb was 18.1 ± 10.8. The mean values of the modified Rankin Scale and Functional Ambulation Category were 3.7 ± 1.0 and 2.4 ± 1.2.

### Inter-trial and inter-day reliability

Illustrations of EMG activities at the lower limbs during MVIC and subMVIC in representative cases of a healthy individual and a patient with subacute stroke are shown in **Fig. 2**. In inter-trial reliability, both MVIC and subMVIC methods demonstrated excellent reliability for all of the measured lower-limb muscles ranging from 0.954 to 0.992 in the healthy individuals and from 0.904 to 0.990 in the stroke patients at baseline (**Table 2**); ranging from 0.878 to 0.991 in the healthy individuals and from 0.881 to 0.991 in the stroke patients at follow-up (**Table 3**). In inter-day reliability, MVIC methods demonstrated good reliability at the TFL and moderate reliability at the RF, TA, and GC, and subMVIC methods demonstrated good reliability at the TFL, RF, and GC and poor reliability at the TA in the healthy individuals (**Table 4**). **Supplementary Tables 2** and **3** showed the peak amplitudes of EMG activities at the


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**Table 1.** Clinical characteristics in stroke patients (n = 20)

| Characteristics | Stroke patients |
|---|---|
| Age (yr) | 60.1 ± 13.3 |
| Sex (female) | 9 (45.0) |
| Duration from stroke onset to measurement (day) | 72.9 ± 52.0 |
| Lesion type | |
| Infarction | 14 (70.0) |
| Hemorrhage | 6 (30.0) |
| Lesion laterality | |
| Right | 9 (45.0) |
| Left | 11 (55.0) |
| Lesion location | |
| Supra-tentorial | 19 (95.0) |
| Infra-tentorial | 1 (5.0) |
| Muscle strength of affected side at baseline (manual muscle test grade) | |
| Hip abductor | 2.45 ± 1.19 |
| Knee extensor | 2.55 ± 1.28 |
| Ankle dorsiflexor | 1.95 ± 1.57 |
| Ankle plantar flexor | 2.05 ± 1.64 |
| Modified Rankin Scale | |
| 0–2 | 3 (15.0) |
| 3–5 | 17 (85.0) |
| Berg Balance Scale | 27.1 ± 16.8 |
| Lower extremity subscore of Fugl-Meyer Assessment | 18.1 ± 10.8 |
| Functional Ambulation Category (3–5) | 7 (35.0) |
| 10-Meter Walk Test* (sec) | 38.5 ± 30.1 |
| Motor evoked potentials† | |
| Response | 4 (50.0) |
| Amplitude (μV) | 1,974.0 ± 2,273.9 |
| Mini-Mental State Examination | 24.5 ± 6.9 |

Values are presented as mean ± standard deviation or number (percentage).
*10-Meter Walk Test was performed in 10 stroke patients.
†Motor evoked potentials were measured in 8 stroke patients at the affected lower extremities at the baseline.

lower limbs during MVICs and subMVICs in healthy individuals and patients with subacute stroke, respectively.

### Correlations between the EMG activities during MVIC and subMVIC

Pearson correlation coefficients between EMG amplitudes at baseline during MVIC and subMVIC methods are shown in **Supplementary Table 1**. In the healthy individuals, there were significant correlations between the EMG amplitudes of each muscle during MVIC and subMVIC. In patients with subacute stroke, there were significant correlations between the EMG amplitudes of each unaffected muscle during MVIC and subMVIC.

## DISCUSSION

The objective of this study was to develop MVIC and subMVIC methods based on sEMG and evaluate their inter-trial and inter-day reliability in healthy individuals and stroke patients. The results of inter-trial reliability indicated that both MVIC and subMVIC methods showed excellent reliability in all lower-limb muscles of the healthy individuals and patients with subacute stroke. The results of inter-day reliability exhibited that the TFL shows good reliability during both MVIC and subMVIC and that the RF and GC show good reliability during only subMVIC in healthy individuals. Additionally, the EMG amplitudes of the lower-limb muscles during MVIC were correlated with those during subMVIC in both healthy individuals and stroke patients except for the GC in the stroke patients.


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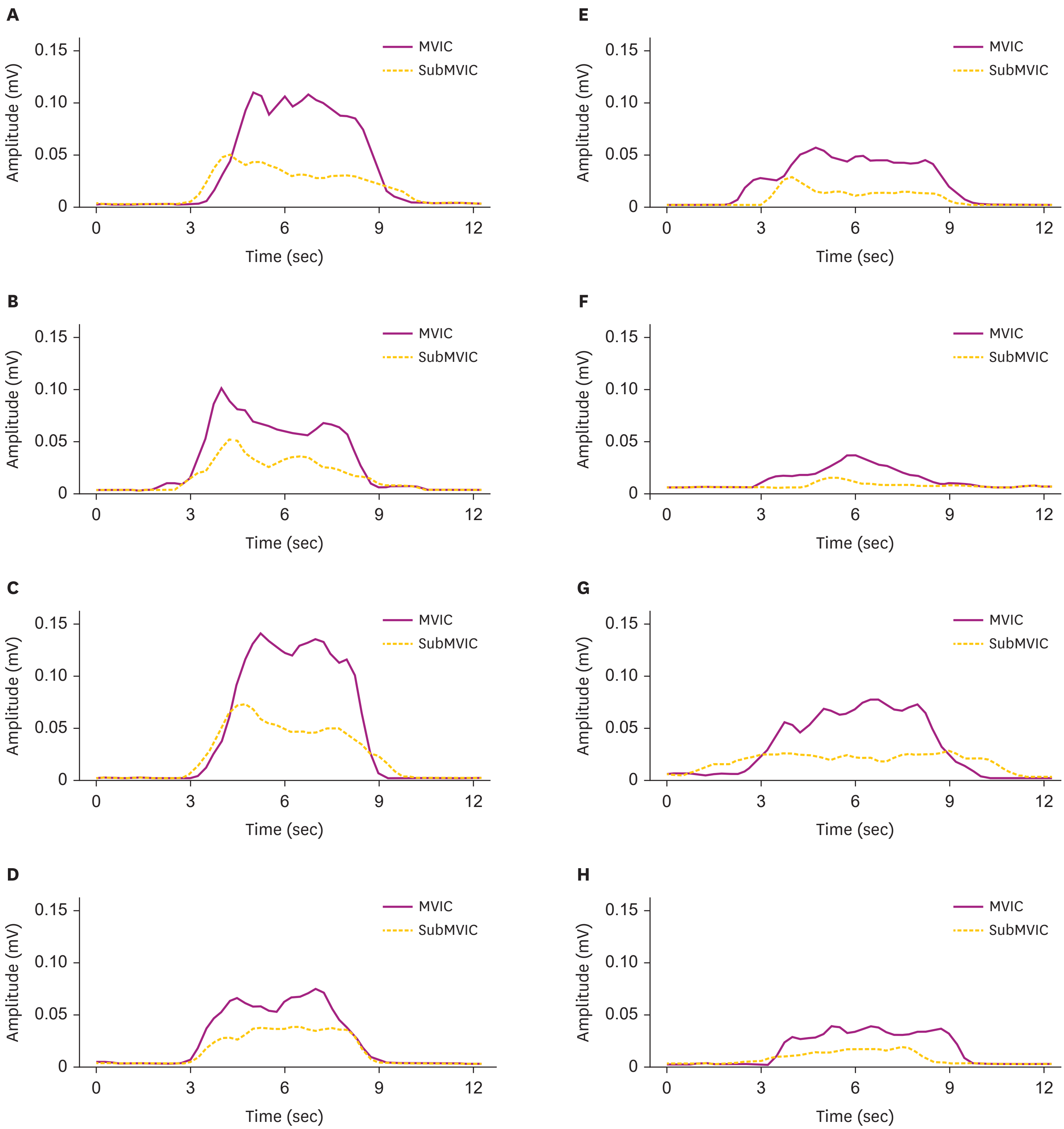


**Fig. 2.** Representative cases of EMG activities at the tensor fascia lata, rectus femoris, tibialis anterior, and gastrocnemius medialis in a healthy individual (A-D) and a stroke patient (E-H). The black lines refer to the EMG activities at the right limb and the affected lower limb during MVICs in the healthy individual and the stroke patient, respectively; the gray lines refer to the EMG activities at the right limb and the affected lower limb during subMVICs) in the healthy individual and the stroke patient, respectively. The peak amplitudes of EMG activities were utilized to calculate interclass correlation coefficients in this study.
EMG, electromyography; MVIC, maximal voluntary isometric contraction; subMVIC, submaximal voluntary isometric contraction.

The normalization methods which can be performed in in-bed settings can be clinically meaningful in patients with true paralysis in that they are real candidates to be assessed for muscle functions or to be provided treatments based on EMG signals [27]. Notably, there

**Table 2.** Inter-trial reliability of electromyography activities at the baseline during MVICs and subMVICs in healthy individuals and stroke patients

| Lower-limb muscles | Healthy individuals | Stroke patients | |
|---|---|---|---|
| | | Affected | Unaffected |
| Tensor fascia lata | | | |
| MVIC | | | |
| ICC (95% CI) | 0.992 (0.982–0.996) | 0.980 (0.957–0.992) | 0.992 (0.983–0.997) |
| %SEM | 7.1 | 12.6 | 7.0 |
| subMVIC | | | |
| ICC (95% CI) | 0.980 (0.958–0.991) | 0.978 (0.917–0.996) | 0.963 (0.918–0.985) |
| %SEM | 8.6 | 8.0 | 10.8 |
| Rectus femoris | | | |
| MVIC | | | |
| ICC (95% CI) | 0.977 (0.952–0.990) | 0.912 (0.810–0.963) | 0.987 (0.973–0.995) |
| %SEM | 5.6 | 22.2 | 7.3 |
| subMVIC | | | |
| ICC (95% CI) | 0.958 (0.912–0.982) | 0.978 (0.937–0.994) | 0.987 (0.972–0.994) |
| %SEM | 8.6 | 8.9 | 8.2 |
| Tibialis anterior | | | |
| MVIC | | | |
| ICC (95% CI) | 0.987 (0.973–0.995) | 0.990 (0.977–0.996) | 0.972 (0.940–0.998) |
| %SEM | 6.2 | 9.8 | 8.9 |
| subMVIC | | | |
| ICC (95% CI) | 0.982 (0.962–0.992) | 0.915 (0.638–0.987) | 0.911 (0.813–0.962) |
| %SEM | 11.1 | 9.6 | 18.6 |
| Gastrocnemius | | | |
| MVIC | | | |
| ICC (95% CI) | 0.954 (0.904–0.981) | 0.988 (0.973–0.996) | 0.977 (0.952–0.990) |
| %SEM | 14.2 | 7.6 | 12.1 |
| subMVIC | | | |
| ICC (95% CI) | 0.960 (0.915–0.983) | 0.985 (0.943–0.997) | 0.904 (0.798–0.959) |
| %SEM | 15.4 | 10.4 | 23.4 |

MVIC, maximal voluntary isometric contraction; subMVIC, submaximal voluntary isometric contraction;
ICC, intraclass correlation coefficient; CI, confidence interval; SEM, standard error of the mean.

have been few studies to develop and assess the reliability of the subMVIC method in patients with impaired sitting or standing due to pathologic conditions including stroke. The current study suggested the MVIC and subMVIC methods as feasible normalization methods in stroke patients with in-bed position during muscle contractions. Even though in-bed settings should be considered applicable for patients with true paralysis, it can be difficult to provide gravity and resistance for subMVIC and MVIC, respectively, particularly in the RF and GC. In this study, simple tools such as wedge cushions or plastic boxes were utilized to induce consistent positions or joint angles of the lower limbs in participants during contractions of the RF and GC. The results indicated that the MVIC and subMVIC methods for lower-limb muscles were feasible for both healthy individuals and subacute stroke patients at in-bed position. Both MVIC and subMVIC methods demonstrated excellent inter-trial reliability, and the subMVIC method showed comparable inter-day reliability with the MVIC method in healthy individuals.

There have been previous attempts to investigate the inter-trial or inter-day reliability of MVIC and subMVIC methods for normalization of EMG signals in various muscles and settings [16,17,28]. As a functional task, single leg stance was employed as the subMVIC method based on the rationale that this static posture may enable to measure the coordinated function of the lower-limb muscles in response to a standardized demand, while minimizing variations in joint positions among participants [17]. During single leg stance, participants are instructed to stand on the dominant leg with the hands on the hips and the non-dominant knee which are flexed to 90°. Both the single leg stance and MVIC methods demonstrated


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**Table 3.** Inter-trial reliability of electromyography activities at the follow-up during MVICs and subMVICs in healthy individuals and stroke patients

| Lower-limb muscles | Healthy individuals | Stroke patients | |
|---|---|---|---|
| | | Affected | Unaffected |
| Tensor fascia lata | | | |
| MVIC | | | |
| ICC (95% CI) | 0.984 (0.967–0.993) | 0.988 (0.973–0.996) | 0.989 (0.974–0.996) |
| %SEM | 8.4 | 14.6 | 8.2 |
| subMVIC | | | |
| ICC (95% CI) | 0.934 (0.861–0.972) | 0.985 (0.926–0.998) | 0.963 (0.910–0.987) |
| %SEM | 14.1 | 11.0 | 12.4 |
| Rectus femoris | | | |
| MVIC | | | |
| ICC (95% CI) | 0.980 (0.958–0.991) | 0.935 (0.850–0.975) | 0.958 (0.906–0.984) |
| %SEM | 6.5 | 16.4 | 10.2 |
| subMVIC | | | |
| ICC (95% CI) | 0.878 (0.743–0.948) | 0.958 (0.888–0.987) | 0.948 (0.877–0.981) |
| %SEM | 16.8 | 7.4 | 11.0 |
| Tibialis anterior | | | |
| MVIC | | | |
| ICC (95% CI) | 0.991 (0.981–0.996) | 0.991 (0.978–0.997) | 0.979 (0.952–0.992) |
| %SEM | 4.0 | 10.7 | 7.2 |
| subMVIC | | | |
| ICC (95% CI) | 0.987 (0.972–0.994) | 0.881 (0.215–0.992) | 0.957 (0.897–0.984) |
| %SEM | 7.7 | 15.8 | 13.5 |
| Gastrocnemius | | | |
| MVIC | | | |
| ICC (95% CI) | 0.907 (0.803–0.960) | 0.961 (0.905–0.987) | 0.957 (0.903–0.983) |
| %SEM | 13.3 | 13.7 | 14.7 |
| subMVIC | | | |
| ICC (95% CI) | 0.957 (0.910–0.982) | 0.968 (0.790–0.998) | 0.928 (0.829–0.974) |
| %SEM | 13.3 | 15.6 | 18.9 |

MVIC, maximal voluntary isometric contraction; subMVIC, submaximal voluntary isometric contraction; ICC, intraclass correlation coefficient; CI, confidence interval; SEM, standard error of the mean.

**Table 4.** Inter-day reliability of the electromyography activities during MVICs and subMVICs in healthy individuals

| Lower-limb muscles | ICC (95% CI) | %SEM |
|---|---|---|
| Tensor fascia lata | | |
| MVIC | 0.773 (0.427–0.910) | 33.6 |
| subMVIC | 0.854 (0.631–0.942) | 22.9 |
| Rectus femoris | | |
| MVIC | 0.736 (0.333–0.895) | 21.5 |
| subMVIC | 0.821 (0.547–0.929) | 17.9 |
| Tibialis anterior | | |
| MVIC | 0.541 (−0.161–0.818) | 32.4 |
| subMVIC | 0.489 (−0.291–0.798) | 52.0 |
| Gastrocnemius | | |
| MVIC | 0.558 (−0.117–0.825) | 32.8 |
| subMVIC | 0.786 (0.459–0.915) | 30.8 |

MVIC, maximal voluntary isometric contraction; subMVIC, submaximal voluntary isometric contraction; ICC, intraclass correlation coefficient; CI, confidence interval; SEM, standard error of the mean.

good-to-excellent inter-trial reliability in healthy individuals for the gluteus maximus, gluteus medius, RF, vastus lateralis, hip adductor, and biceps femoris muscles. Trunk musculatures including several abdominal and back muscles were also investigated to compare the inter-trial and inter-day reliability between MVIC and subMVIC methods in healthy individuals and patients with chronic low back pain [28]. The previous study reported that both MVIC and subMVIC methods showed excellent inter-trial reliability in healthy individuals and patients with chronic low back pain. Interestingly, the subMVIC method showed trends towards higher


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inter-day reliability compared with that of the MVIC method in both healthy individuals and patients with chronic low back pain. Another research study attempted to develop novel subMVIC methods in side-lying, sitting, and standing positions using the isometric grade 3 of MMT, which is partially similar to the subMVIC method used in the current study [16]. The results showed that both inter-trial and inter-day reliability were comparable between the MVIC and subMVIC methods in the gluteus medius, RF, TA, and semitendinosus muscles. The results of this previous research are generally consistent with those of the current study in that the inter-trial or inter-day reliability of a subMVIC method in healthy individuals and patients with subacute stroke were not inferior or rather partially superior to that of a MVIC method in measuring EMG activities in lower-limb muscles. Additionally, the EMG amplitudes of most lower-limb muscles during MVIC were significantly correlated with those during subMVIC in both healthy individuals and stroke patients, which was consistent with the previous study [17]. However, the relatively low correlation of the EMG amplitudes of the GC during MVIC and subMVIC was observed in healthy individuals, and a poor correlation of those during MVIC and subMVIC was found in patients with stroke. This might be explained by different muscle activation strategies and suggests that the interpretation of the subMVIC method for the GC should be approached with caution.

The relative low inter-day reliability when compared with the inter-trial reliability in MVIC and subMVIC methods might be explained by potential sources of variability of EMG signals such as the use of manual resistance [28], surface electrode positioning [19], and respiratory conditions [29]. Although this study established standardized protocols including the test maneuvers for body positions and joint angles during muscle contractions and the attachment sites of sEMG electrodes for the lower-limb muscles to acquire consistent EMG signals of the targeted muscles, the between-day variation in sEMG signals is probably inevitable in both healthy individuals and stroke patients. The poor inter-day reliability of the subMVIC method in the TA might be due to the difficulty in maintaining neutral position of the ankle during the contraction, since participants rely on their own proprioception without any tools in this study.

There are several limitations in this study. First, the sample size was relatively small, including 20 healthy individuals and 20 stroke patients. A further study with a large population of healthy individuals and stroke patients is needed. Second, this study did not analyze the inter-day reliability of patients with subacute stroke. The muscle strength of stroke patients can be markedly changed during the natural course of motor recovery or active rehabilitation, and it can be necessary to analyze inter-day reliability between the initial and short-term follow-up measurements in further studies. Third, only patients with subacute stroke who did not have decreased consciousness or inability to comprehend task instructions were included in the current study. Considering that impaired consciousness or comprehension is a common symptom in stroke patients, the developed MVIC and subMVIC methods can be applied in the relatively limited population. Fourth, this study adopted the peak amplitude of the sEMG signals as the only parameter for reliability analysis. In future studies, it may be helpful to utilize other various features of EMG signals, including mean absolute value, zero crossing rate, wavelength, and average power.

We were able to demonstrate that the MVIC and subMVIC methods for lower-limb muscles were feasible in healthy individuals and patients with subacute stroke at in-bed position. The inter-trial reliability of both MVIC and subMVIC methods demonstrated excellent reliability in all lower-limb muscles of healthy individuals and stroke patients. The subMVIC method showed


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comparable inter-day reliability with the MVIC method in healthy individuals. The results of this study can serve as a basis for clinical evaluation of muscular activities using quantitative EMG signals on the lower-limb muscles in paralytic patients with impaired mobility.

## SUPPLEMENTARY MATERIALS

### Supplementary Table 1

Results of Pearson correlation coefficients between baseline electromyography activities during maximal and submaximal voluntary isometric contractions in healthy individuals and patients with subacute stroke

### Supplementary Table 2

The peak amplitudes (μV) of electromyography activities at the lower limbs during MVICs and subMVICs in healthy individuals

### Supplementary Table 3

The peak amplitudes (μV) of electromyography activities at the lower limbs during MVICs and subMVICs in patients with stroke

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