

Original research papers

TIME-OF-DAY EFFECTS ON CALF-MUSCLE ELECTROMYOGRAPHY DURING STATIC SINGLE-LEG BALANCE IN YOUNG ACTIVE MALES

SAMAR IZEDI FARD¹, FARZANEH MOVASEGHI², NASIBE KAZEMI¹, SARA GHANE³

¹Islamic Azad University, Department of Physical Education and Sport Sciences, Marvdasht Branch, Fars, Iran

²Islamic Azad University, Department of Physical Education and Sport Sciences, Sepidan Branch, Sepidan, Iran

³Persian Gulf University, Department of Engineering, Bushehr, Iran

Mailing address: Sara Ghane, Department of Engineering, Persian Gulf University, Persian Gulf Street, Bushehr 7516913817, Iran, e-mail: eng.saraghane1999@gmail.com

Abstract

Introduction. Circadian rhythms influence neuromuscular performance, yet their effect on postural muscle activation during balance tasks remains unclear. This study aimed to determine time-of-day differences in calf-muscle EMG activity during static single-leg stance. **Material and Methods.** Ten healthy, physically active males (24.8 ± 0.8 years) performed three 15-s single-leg balance tests at 07:00-07:30 and 17:00-17:30 on the same day. EMG signals were recorded according to SENIAM standards, normalized to maximal voluntary isometric contractions (MVICs), and evaluated as a percentage of MVIC (%MVC). Paired t-tests and effect sizes (Cohen's *dz*) were used to compare sessions. **Results.** EMG amplitudes were consistent in two time points. Mean differences between morning and evening were minimal (Soleus: $-0.12 \mu\text{V}$; Medial gastrocnemius: $0.13 \mu\text{V}$; Lateral gastrocnemius: $0.83 \mu\text{V}$), with 95% confidence intervals crossing zero and small effect sizes ($dz < 0.30$). **Conclusions.** The EMG activity of the calf muscles does not change with the time of day in young, physically active men. These findings support the physiological stability of tonic postural muscles under low-intensity conditions and suggest that balance training or rehabilitation tasks of this type can be performed flexibly throughout the day without affecting neuromuscular engagement.

Keywords: electromyography, static single-leg balance, circadian rhythm, calf-muscle activation, postural control

Introduction

Circadian rhythms are the internal clock of the human body, regulating biological behaviors and physiological functions throughout the light-dark cycle [1]. The effect of time of day on biological processes has been well established through regular 24-hour periodic changes in central body temperature, hormone secretion such as cortisol and melatonin, nerve conduction velocity, and cellular metabolism [2, 3]. Regardless of these rhythmic fluctuations, it has been suggested that central and peripheral mechanisms help to maintain vital functions, like balance, in a stable range to provide safety and coordination throughout the day [4]. It remains unclear whether these mechanisms are sufficient to strengthen neuromuscular stability at different times of a day.

Physical balance is a fundamental component of human motor ability, which underlies daily activities such as standing, walking, and coordination of the movements of the upper and lower limbs during different activities [5]. For athletes, accurate postural control is especially crucial: it not only serves to enhance performance but also reduces injury risk by effectively transforming forces and ensuring safe performance of fast or complex movements [6]. Swift direction changes, consistent landings, and maximum kinetic chain usage all rest on great balance ability [7]. The postural control requirements of different sports vary; gymnasts and boat racers, for instance, possess greater postural control than swimmers, and the accuracy of archers or shooters is less dependent on reducing sway [8, 9]. A reduction

in balance capacity has always been linked with elevated lower-limb injury risk, especially for young male athletes [6].

Surface electromyography (sEMG) is recognized as a reliable and noninvasive approach to monitor neuromuscular activity [10]. It assists in investigating neural activation patterns, motor unit recruitment, and the fluctuation of muscle activity. However, the evidence on whether EMG parameters follow a daily rhythm remains fairly inconsistent. For example, Stawarska et al. found higher EMG activity in the evening [11], while Sedliak and Häkkinen reported no clear differences across the day [12]. In children, Souissi and colleagues observed evening increases in muscle power and EMG frequency indices, though the RMS amplitude showed little change [13]. Only a few studies have actually examined EMG activity of the calf muscles in balance tasks, and even fewer have considered how time of day might play a role. For instance, Castaingts et al. reported signs of better neuromuscular efficiency in the evening [14], while Tamm et al. found that EMG amplitudes were higher later in the day compared to morning or midday sessions [15]. Yet most of this research was carried out under conditions of high muscular demand, such as isometric strength tasks or jumping, and much less attention was given to low-intensity, stable postural activities like single-leg stance.

The purpose of the present study is to examine whether EMG amplitude of the triceps surae varies across the day when measured during a single-leg stance on a balance board. To investigate this, young, healthy, and physically active men were assessed twice, once in the morning and once in the evening. To reduce inter-individual variability and allow meaningful com-

parisons between the two time points, EMG signals were normalized to maximum voluntary isometric contraction (MVIC). The findings of this study could help identify more effective times of day for balance-oriented training in athletes and offer deeper insight into how circadian rhythms influence the regulation of balance.

Material and Methods

Design and participants

The population for this quasi-experimental study included thirty-five physical education students at Kharazmi University, Tehran. They were all young, active males involved in sports training as part of their curriculum, typically training 5–7 times per week. This selection was made to control for differences in neuromuscular response based on sex and to ensure a relatively matched level of athletic background. Participants were disqualified based on the following criteria: 1) deformities of the lower extremities were observed; 2) lower limb injury or surgery occurred within the last twelve months; 3) experienced with balance boards or BOSU balls.

The a priori power analysis was conducted using G*Power v3.1.9.7 at $\alpha = 0.05$ (two-tailed) and power = 0.80 based on a large effect size ($d_z = 1.13$). This estimate of effect size was taken from pilot findings and earlier reports of EMG variation with balance tasks with morning–evening session differences indicating the possibility for large effects within homogeneous, small groups. While the assumption undoubtedly exaggerates time-of-day distinctions relative to larger populations, it kept the study manageable within available subjects. Consequently, power analysis suggested that 10 participants would be adequate. Ten participants were randomly recruited from a computer-generated participant list, and their demographic profiles are shown in Table 1. Ethics approval was received from the Ethics Committee of Marvdasht Azad University (IR.IAU.M.REC.1402.134), and all participants signed the informed consent form before the test. The Declaration of Helsinki and STROBE Guidelines were applied in this research.

Table 1. Demographic characteristics of the participants (n = 10).

Variable	Active Young Males
Age (year) ¹	24.8 ± 0.8
Height (cm) ¹	174 ± 4.3
Weight (kg) ¹	69.6 ± 2.4
BMI (kg/m ²) ¹	22.9 ± 0.9

Note: ¹ = Data are presented as mean ± standard deviation; BMI = Body Mass Index.

Research procedure & EMG preparation

Participants had practiced the single-leg balance on the instability board until they were comfortable, one week before data collection. To minimize confounding variables, they were asked to sleep enough the night before the test and to refrain from caffeine, intense exercise, and large meals two hours before each experiment. During the testing session, the skin overlying the location of the soleus (SOL), medial gastrocnemius (GastM), and lateral gastrocnemius (GastL) was shaved and cleaned with alcohol. Surface Ag/AgCl electrodes (20 mm diameter, 20 mm electrode-to-electrode distance) were positioned to SENIAM standards [16]: SOL on the lower third of the calf (laterally to midline), GastM between the knee and ankle in mid-position of the inner calf, and GastL perpendicular to the fibers on the late-

ral calf (Fig. 1). Electrodes were affixed with adhesive tape, and placement was consistently performed by a single experienced investigator trained according to SENIAM guidelines.

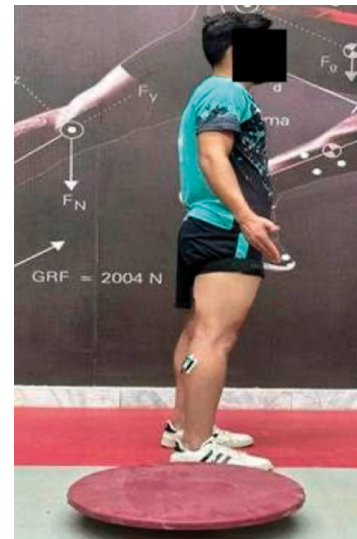


Fig. 1. Surface electrode placement on leg muscles according to SENIAM protocols.

MVIC Testing Protocol

Before balance trials of EMG signal correction, MVICs for soleus (SOL), medial gastrocnemius (GastM), and lateral gastrocnemius (GastL) were conducted. Participants were seated with around 90° of knee flexion and at neutral position of the ankle. A trained investigator applied manual resistance to each participant with the same fixed strap tethered to a consistent anchor point, establishing the same amount of resistance given to every participant during each trial. To combat any discrepancies due to learning effects, two submaximal practice contractions were performed prior to each MVIC trial with 1 minute of rest between each contraction to prevent fatigue.

Single-leg balance trials

Participants completed three 15s trials of single-leg stance on their non-dominant leg on a 10-cm balance board. The knee was flexed at 90° and to constrain upper body movement, participants were encouraged to keep their knees in place with hands on hips. There was a 2-minute seated rest between trials. Testing occurred at two different times on the same day (07:00–07:30 and 17:00–17:30) to account for diurnal variation. Participants focused on a visual target placed 1 meter in front of them during trials and placed the foot with the black mark located in the center of the balance board, attempting to maintain the hip and knee angles for the duration of the maneuver (Fig. 2). This was selected to allow for a low-level postural challenge of the triceps surae in a controlled visual and standing orientation.

EMG data collection

Surface electromyography (EMG) signals were recorded using a Biometric Ltd. system (UK) with a sampling rate of 1,000 Hz. The signals were amplified based on the manufacturer's default settings and then processed using a zero-lag, fourth-order Butterworth band-pass filter (20–500 Hz), following recommended procedures for EMG signal conditioning [17]. For normalization, the root mean square (RMS) value was calculated from the middle 3 seconds of each MVIC trial for each muscle. These RMS values were used as reference points to express EMG



Fig. 2. Single-leg static balance test.

amplitudes during the balance task as a percentage of maximal voluntary contraction (%MVC). This method helps reduce individual differences and supports consistent comparisons between morning and evening measurements.

Statistical analysis

Descriptive statistics for RMS and %MVC values were reported as mean $\pm$ standard deviation (SD). The Shapiro-Wilk test was applied to each muscle-time dataset to assess normality. The data were normally distributed, so paired two-tailed t-tests were used to compare morning and evening EMG amplitudes within subjects. Effect sizes were calculated using Cohen's d_z ($t/\sqrt{n}$), appropriate for within-subject designs with repeated measures. The sample size ($n = 10$) was determined via a priori power analysis based on pilot data and prior literature suggesting large diurnal effects in EMG amplitude during balance tasks [18]. All statistical procedures were conducted in Python (v3.10) using NumPy (v1.23) and SciPy (v1.9).

Results

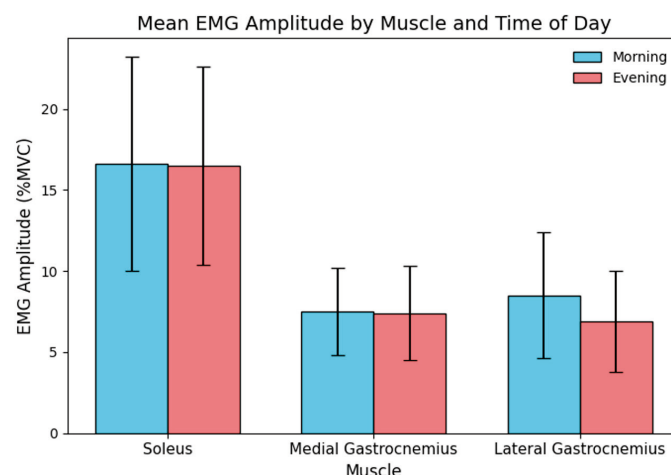
Table 2 summarizes the descriptive EMG results of the soleus, medial, and lateral gastrocnemius regions. Mean RMS and %MVC scores were stable throughout the time points (morning and night), with differences generally $<1 \mu\text{V}$ for RMS and $<2\%$ for %MVC. Standard deviations were similar among muscle groups during each time point. Figure 3 displays the mean %MVC with error bars ($\pm\text{SD}$) to show the morning and evening time point amplitude consistency between the two sessions.

Statistical tests verified that EMG amplitudes from morning to evening were not significantly different between any of the muscle groups (Tab. 3). The variations in averages were minimal (Soleus: $-0.12 \mu\text{V}$; Medial gastrocnemius: $0.13 \mu\text{V}$; Lateral gastrocnemius: $0.83 \mu\text{V}$), and the 95% confidence intervals encompassed zero for all, indicating that there was no significant change related to the time of day. The effect sizes (Cohen's d_z) were small (<0.30), further supporting the lack of meaningful differences in muscle activation between sessions.

Table 2. EMG descriptive statistics (RMS and %MVC) by muscle group ($n = 10$).

Muscle Group	Time	RMS1 (μV)	%MVC 1
Soleus	Morning	21.58 ± 8.54	16.6 ± 6.6
	Evening	21.46 ± 7.95	16.5 ± 6.1
Gastrocnemius Medial	Morning	8.98 ± 3.24	7.5 ± 2.7
	Evening	8.85 ± 3.44	7.4 ± 2.9
Gastrocnemius Lateral	Morning	9.76 ± 4.48	8.5 ± 3.9
	Evening	7.93 ± 3.60	6.9 ± 3.1

Abbreviations: RMS = root mean square, %MVC = percentage of maximum voluntary contraction. ¹ = Data are presented as mean $\pm$ standard deviation. ² = MVIC (mean $\pm$ SD, μV): Soleus 130 ± 15 ; Medial GCM 120 ± 12 ; Lateral GCM 115 ± 10 .



Bars represent mean $\pm$ standard deviation (SD). No significant differences were observed between sessions (paired t-tests, $p > 0.05$).

Fig. 3. Mean EMG amplitude (%MVC) for soleus, medial gastrocnemius, and lateral gastrocnemius during morning and evening sessions ($n = 10$).

Table 3. Paired t-test results by muscle group ($n = 10$).

Muscle Group	MD* (μV)	95% CI (μV)	t-value	p-value	d_z
Soleus	-0.12	[-2.10, 1.86]	-0.10	0.93	-0.03
Gastrocnemius Medial	0.13	[-0.18, 0.44]	0.93	0.38	0.29
Gastrocnemius Lateral	0.83	[-1.10, 2.76]	0.90	0.39	0.29

No significant differences ($p > 0.05$). Abbreviations: MD = mean differences, CI = confidence interval, p-value = Probability value, t-value = Paired t-test statistic, d_z = Cohen's d_z (effect size for paired differences). * = Mean differences calculated as Evening - Morning.

Discussion

This study specifically focused on postural muscles under low-intensity, ecologically valid balance conditions, an area that has received relatively little attention in research on circadian rhythms. The aim was to examine whether activation of the calf postural muscles during a low-intensity single-leg balance task differs between morning and evening in young, active men. The findings showed that EMG amplitudes of the soleus, medial gastrocnemius, and lateral gastrocnemius did not differ significantly across the two testing sessions. Small effect sizes and con-

fidence intervals including zero indicated a relative consistency in the activation patterns of the triceps surae throughout the day.

Previous studies indicate that the response of muscles to time of day is highly dependent on the type and intensity of the task, aligning with the present findings. For instance, in the Wingate test, Souissi et al. demonstrated that although peak mechanical power increases in the evening, the RMS EMG amplitude remains unchanged [13]. Similarly, Sedliak et al. reported no significant difference in EMG amplitude during submaximal tasks, despite diurnal variations in torque and neuromuscular efficiency [12]. Furthermore, while frequency parameters and neuromuscular efficiency indices such as MPF tend to increase in the afternoon during explosive or fatiguing tasks, these changes are not reflected in submaximal activities. This pattern is entirely consistent with the present study's findings, where the EMG amplitude of the soleus and both medial and lateral gastrocnemius muscles in the single-leg balance task (less than 10% MVC) showed no significant difference between morning and evening sessions. In contrast, high-intensity tasks, as highlighted in the systematic review by Chtourou and Souissi, exhibit a clear afternoon increase [19].

The findings are clinically applicable, and low-intensity postural rehabilitation training can be prescribed at any time of day with negligible impact on muscle activation. There are, however, several limitations to consider when evaluating larger groups. The sample was homogeneous and small, consisting only of active young males, and therefore does not lend itself to generalization to females, older individuals, or less active individuals. Sampling was limited to only two time points in close windows during morning and evening, leaving scope for potential variability at other times to remain untested. Sleep time and quality were not objectively measured, and other variables that have been previously known to influence neuromuscular control – i.e., body temperature, chronotype, caffeine consumption, and activity in the run-up to the experiment – remained uncontrolled. These are all variables that can introduce modest variability that was not detectable under these test conditions.

Future studies should aim to increase sample size, expand time intervals, diversify the study population, and integrate EMG with kinematic, kinetic, and behavioral and physiological metrics to provide a more comprehensive and multidimensional interpretation of the time-of-day effects on neuromuscular performance and balance.

Conclusions

In conclusion, the present study demonstrates that calf-muscle activation during a low-intensity single-leg balance task remains largely unaffected by the time of day in young, physically active men. These findings align with the physiological expectation that tonic, feedback-driven muscles operate stably under typical waking conditions, highlight the task-dependent nature of temporal effects in neuromuscular performance, and support the practical notion that low-intensity postural training can be scheduled flexibly without compromising muscle engagement.

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Submitted: August 30, 2025

Accepted: June 8, 2026