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RESEARCH ARTICLE

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Time-of-day effects on motor unit firing and muscle contractile properties in humans

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Abstract

Intrinsic factors related to neuromuscular function are time-of-day dependent, but diurnal rhythms in neural and muscular components of the human neuromuscular system remain unclear. The present study aimed to investigate the time-of-day effects on neural excitability and muscle contractile properties by assessing the firing properties of tracked motor units and electrically evoked twitch muscle contraction. In 15 young adults (22.9 ± 4.7 yr), neuromuscular function was measured in the morning (10:00), at noon (13:30), in the evening (17:00), and at night (20:30). Four measurements were completed within 24 h. The measurements consisted of maximal voluntary contraction (MVC) strength of knee extension, recording of high-density surface electromyography (HDsEMG) from the vastus lateralis during ramp-up contraction to 50% of MVC, and evoked twitch torque of knee extensors by electrical stimulation. Recorded HDsEMG signals were decomposed to individual motor unit firing behaviors and the same motor units were tracked among the times of day, and recruitment thresholds and firing rates were calculated. The number of detected and tracked motor units was 127. Motor unit firing rates significantly increased from morning to noon, evening, and night ($P < 0.01$), but there were no significant differences in recruitment thresholds among the times of day ($P > 0.05$). Also, there were no significant effects of time of day on evoked twitch torque ($P > 0.05$). Changes in the motor unit firing rate and evoked twitch torque were not significantly correlated ($P > 0.05$). These findings suggest that neural excitability may be affected by the time of day, but it did not accompany changes in peripheral contractile properties in a diurnal manner.

NEW & NOTEWORTHY We investigated the variations of tracked motor unit firing properties and electrically evoked twitch contraction during the day within 24 h. The variation of motor unit firing rate was observed, and tracked motor unit firing rate increased at noon, in the evening, and at night compared with that in the morning. The variation in motor unit firing rate was independent of changes in twitch contraction. Motor unit firing rate may be affected by diurnal rhythms.

diurnal rhythm; electrical stimulation; high-density surface electromyography; motor unit; performance

INTRODUCTION

In our body, multiple intrinsic physiological responses fluctuate and are linked to each other. For example, the core body temperature (1), heart rate, and blood pressure (2, 3) fluctuate in circadian or daily manners. These are well-known as circadian or diurnal rhythms, which are important body systems to maintain physiological regulation (4). Physical performance (5, 6), such as endurance exercise (7), strength (8, 9), and maximal power output (10, 11), also exhibits diurnal variations, which could influence long- and short-duration training improvements (12, 13), as well as submaximal force exertion performances (9, 11). The time-of-day variations in these physical performances may be due to diurnal variations in motor control. Circadian physiological responses such as body temperature may relate to muscle conditions and motor control

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mechanisms, which may cause diurnal variations of performances (14), but the intrinsic physiological mechanisms of adaptation remain unclear.

Voluntary muscle contraction force is determined by the conditions of central and peripheral components of the neuromuscular system, i.e., central nervous excitability and muscle contractile properties. The time-of-day effects on neural activation were investigated using electromyography (EMG) in previous studies (8, 9). One previous study reported that the root mean square of EMG signals was not significantly different between morning and evening (13), but another study indicated significantly greater neuromuscular activation assessed by EMG in the evening compared with morning (15). As surface EMG signals are affected by various factors, such as nonphysiological factors (skin condition or noise), the number of active motor units, motor unit recruitment pattern, and motor unit amplitude shape and duration (16), the details of neurophysiological diurnal changes, such as motor unit activation, have remained unclear. Although it is important to consider central nervous excitability to generate muscle force when clarifying motor control mechanisms, peripheral muscle contractile properties should also be considered as major components of the neuromuscular system. The diurnal changes in contractile properties can be attributed to intracellular variation of calcium kinetics, excitation-contraction coupling mechanisms, and the core temperature (17, 18). Therefore, both central nervous excitability and peripheral contractile properties can exhibit variation as time-of-day effects. Also, these motor unit firing properties are adapted to peripheral muscular contractile properties, i.e., a decrease in the firing rate during a continuous contraction task with acute muscular fatigue (19, 20). As glucose uptake in active skeletal muscle during contraction (21) and body temperature affect muscle conditions (22), these circadian physiological factors can relate to motor unit firing or contractile properties. We thus considered that neural and muscular components of the neuromuscular system should be associated with each other in terms of circadian or diurnal rhythms.

The purpose of the present study was to identify the time-of-day effects on central nervous activation and muscle contractile properties in humans based on evaluations of motor unit firing properties by decomposing HDsEMG signals using the tracking method (23) and evoked twitch torque by electrical stimulation. We also tested the association between the time-of-day effects on neural and muscular components and other related variables.

METHODS

Participants

Fifteen healthy young men (age: 22.9 ± 4.9 yr: height: 170.6 ± 3.7 cm, body mass: 63.5 ± 5.0 kg) participated in the present study. According to the Morningness-Eveningness Questionnaire (MEQ), the participants comprised the intermediate type ($n = 10$), eveningness ($n = 1$), and morningness ($n = 4$), and the average score was 53.5 ± 10.0 (range: 35–73). They did not have any problems involving their right leg. The experimental procedures were explained to the participants before they provided informed written consent to participate in the present study. This study was approved by the Research Ethics Committee of Chukyo University (2022-001) and conducted in accordance with the Declaration of Helsinki.

Study Protocol

The study protocol is described in Fig. 1. The measurements were conducted four continuous times of day with an interval of 3.5 h within 24 h. The times of the measurements were: morning (10:00), noon (13:30), evening (17:00), and night (20:30). The start times for participants were randomly determined. If they started the experiment at noon, in the evening, or at night, they visited the laboratory the next day again to be measured for morning and any subsequent data. To control intake of energy and nutrition, they were given the same meals: lunch box including rice, grilled salmon, and some root vegetables (697 kcal) at 9:00 as breakfast; rice, fried minced meat, and fried fish (1,006 kcal) at 12:30 as lunch; and rice, sauteed meat, fish, and some vegetables (813 kcal) at 19:30 as dinner during the experiment. The participants were instructed not to exercise more than necessary to perform usual daily activities from 24 h before the beginning of and during the experiment.

Maximum Knee Extension Strength

The participants were seated in a custom-made chair to perform maximum voluntary isometric knee extension with hip and knee angles at 90°. A dynamometer (Takei Scientific Instruments Co., Ltd., Niigata, Japan) and force transducer (LU-100KSE; Kyowa Electronic Instruments, Aichi, Japan) were fixed on the leg of the chair. The participants performed maximum voluntary isometric contractions (MVCs) of the knee extensor twice after several familiarization sessions at 50 to 90% of MVC. MVC torque was calculated by multiplying the force value and distance between the knee joint axis and force transducer. The largest MVC value on each measurement was selected for further analysis as the MVC torque at a given time.

High-Density Surface Electromyography

The participants performed submaximal isometric ramp-up contraction to 50% of MVC, consisting of a 17-s increasing phase (∼3% of MVC per second), a 10-s sustained phase at 50% of MVC, and HDsEMG from the vastus lateralis, and exerted force signals were obtained during the submaximal ramp-up contraction, using an EMG acquisition device (Sessantaquatro, OT Bioelettronica, Torino, Italy). The target level was determined as the MVC value at the first visit (the start time), and the performed torque was equal among the four times of day in each participant. The exerted and target levels were presented on a monitor in real-time as visual feedback using software (OTBio Lab + , OT Bioelettronica). A 64-channel electrode grid (13 × 5 with one missing electrode in the corner; a 1-mm diameter and 8-mm interelectrode interval; GR08MM1305, OT Bioelettronica) was attached on the skin over the vastus lateralis with a biadhesive sheet (KITAD064, OT Bioelettronica) and conductive paste (Elefix Z-181BE, Nihonkohden, Tokyo, Japan). The grid electrodes were attached along the line between the head of the greater trochanter and superior and lateral edge of the patella, and the center of the grid was placed at the midpoint of the line

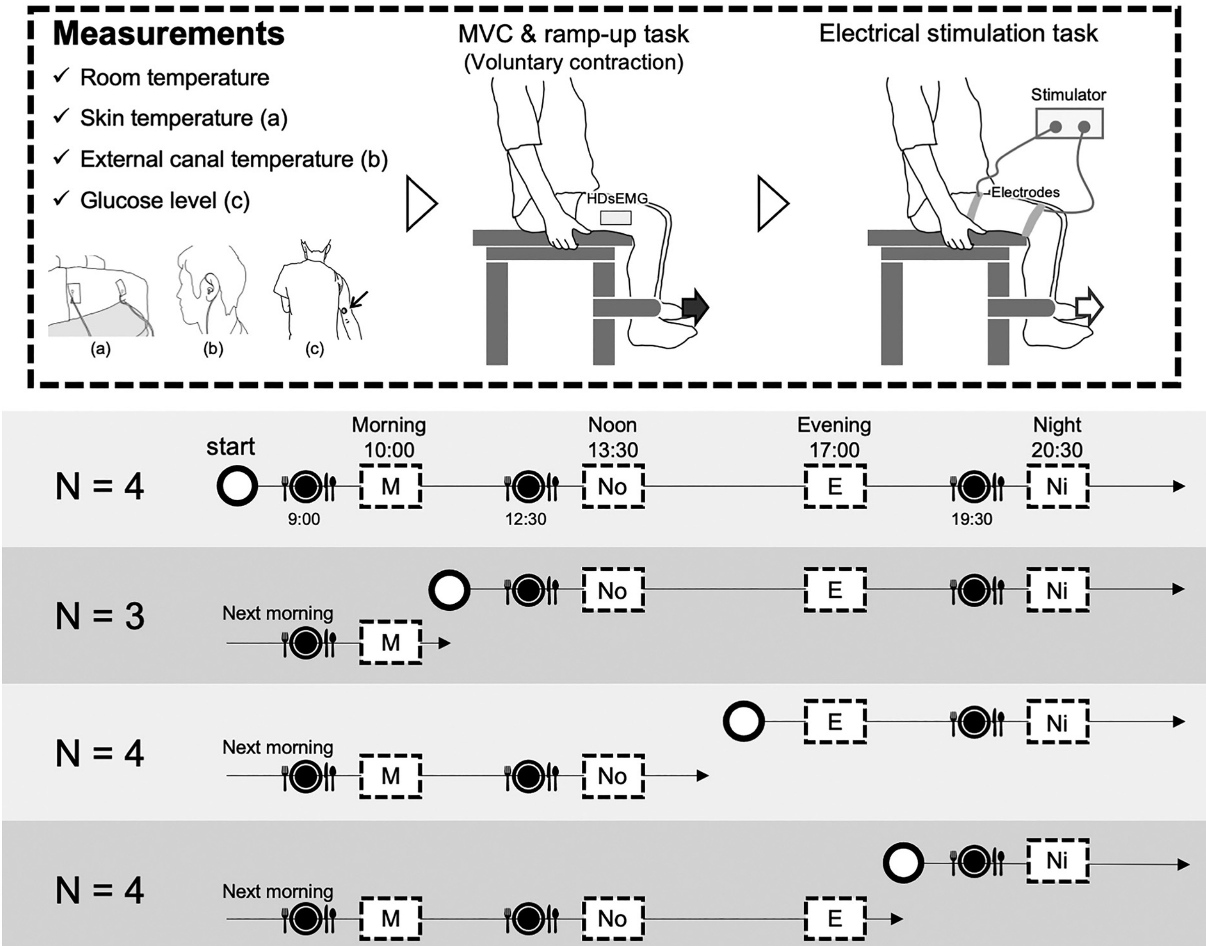

Figure 1. Study protocol. Measurements were conducted four times with an interval of 3.5 h from 10:00 during the daytime: 10:00 (Morning, M), 13:30 (Noon, No), 17:00 (Evening, E), and 20:30 (Night, Ni). Breakfast, lunch, and dinner were given and participants ate them at 9:00, 12:30, and 19:30, respectively. The starting time for each participant was randomly determined. If they started the experiment at noon, in the evening, or at night, they visited the laboratory the next day again to be measured for morning and any subsequent data. *N* represents the number of participants who started in the morning, at noon, in the evening, and at night.

after the skin was cleaned with alcohol and water. The electrode location was marked with an ink marker, to ensure the same positioning in subjects also performing experiments on the next day or subjects who had to undergo reattachment when action potentials could not be recorded well due to the influence of various factors (noise or paste oxidation). Some subjects completed the experiment using the same 64-channel electrode grid without reattachment. A reference electrode (WS2, OT Bioelettronica) was attached to the tibial tuberosity. Monopolar EMG signals were filtered with a bandpass filter between 10 and 500 Hz, amplified by a factor of 150, and sampled at 2,000 Hz. Recorded EMG signals were transferred to analysis software (MATLAB R2019a, MathWorks GK, MA), and the signals were differentiated between adjacent electrodes in the longitudinal direction. The differentiated EMG signals were decomposed and individual motor units were identified by the Convolution Kernel Compensation (CKC) technique using Decomposition of Motor Unit Surface EMG (DEMUSE) software (ver. 6.1; The University of Maribor, Slovenia) (24, 25). The detected motor unit firings were tracked four times of day using DEMUSE software (26), and the same motor unit firings were compared four times. A single experimental investigator (KI) manually inspected all decomposition results. Any physiologically irregular motor unit discharges (less than 4 or more than 50 Hz) were discarded (27). The recruitment threshold of individual motor units was defined as the force level when the motor unit first discharged. The individual motor units firing rate was calculated from the interspike intervals and defined as the median during the plateau phase of ramp-up task. We have defined the analysis interval as 1-s period occurring 1 s after reaching 50% of MVC. Motor units with recruitment thresholds over 45% of MVC were discarded. To investigate the global activities of the vastus lateralis, average rectified values (ARVs) of 59 differentiated EMG signals were calculated during MVC and 50% of MVC. After dividing the EMG signals into 0.5-s epochs using software (OTBio Lab + , OT Bioelettronica), the analyzed interval of MVC and 50% of MVC were defined as the epoch where peak torque was obtained and as 1-s period occurring 1 s after reaching 50% of MVC, respectively.

Evoked Knee Extension Torque by Electrical Stimulation

Electrical stimulation was used to evoke knee extensor contraction, which was measured to estimate muscle contractile properties using a constant current stimulator (DS7AH, Digitimer Ltd., Hertfordshire, UK). Two carbon rubber electrodes (4.5 × 28 cm) were attached in proximal and distal regions of the quadriceps femoris according to a previous study (28). The electrodes covered proximal regions of the rectus femoris and vastus lateralis and distal regions of the rectus femoris, vastus lateralis, and medialis. Electrical stimulation was applied via these electrodes with a 200-µs pulse width, and the intensity was increased by 50 mA evoked torque until reaching a plateau to evaluate the maximal twitch torque using an analog to digital converter (PowerLab16/35, AD Instruments, Australia). The supramaximal current intensity was used to measure singlet twitch torque, whereas 30% of the maximal intensity was used to measure subtetanic and tetanic torques. The maximal current intensity was redetermined at each measurement. The electrodes were reattached in the same region, referring to an ink marker on the thigh. The singlet twitch was measured twice and average values were calculated. In addition, time to twitch peak (TTP) during singlet twitch, which is one of the peripheral muscle contractile properties, was evaluated by computing the time between the onset of stimulation and time of force peak using software (LabChart 8, AD Instruments, Australia). To investigate the muscle contractile properties during subtetanic- and tetanic-evoked contraction, 10- and 100-Hz electrical stimulations were applied. The different frequencies of electrical stimulation induced incomplete or complete tetanus (29, 30). Because we confirmed in a pilot study that the stimulation necessary for incomplete or complete tetanic contraction with supramaximal intensity was too painful, 30% of the maximal intensity was used to evoke subtetanic and tetanic torque in the present study. The peak torque during subtetanic- and tetanic-evoked contraction was used for subsequent analysis.

Body and Room Temperatures

External canal temperature was assessed using continuous-measurement (NIPRO CE Thermo 2, NIPRO, Osaka, Japan). In addition, skin temperature was also measured using a data-collecting handheld thermometer (LT-8 Series, Gram Corp., Saitama, Japan). After 15 min of rest in a chair and before voluntary contraction tasks, the external canal and skin temperatures were sampled every 1 min, and the average values over 3 min were calculated. Skin temperature was further defined as the average between two points: proximal and distal regions of the vastus lateralis. Between the points, the grid of HDsEMG was attached. Room temperature was also measured using the same device as for skin temperature by hanging the probe ∼1 m above the floor.

Glucose Level

We used a glucose-monitoring system (FreeStyle Libre, Abbott Japan, Chiba, Japan). A sensor probe was inserted into the subcutaneous tissue of the back of the right upper arm. The subcutaneous interstitial glucose level was measured immediately before assessing neuromuscular properties.

Statistical Analysis

Statistical analyses were performed using the Statistical Package for the Social Science (SPSS ver. 25.0; IBM Japan Inc., Tokyo, Japan). Shapiro–Wilk tests were performed to check the normality of all variables. Based on the results, repeated one-way analyses of variance (ANOVAs) or Friedman tests were performed. When a main effect was identified as significant, paired *t* tests or Wilcoxon signed-rank tests with Bonferroni corrections were used. To consider conditional dependence, linear mixed model ANOVAs as a random factor and time as a fixed factor, with unstructured variance-covariance structure, were used for the changes in motor unit firing rate and recruitment threshold. To investigate the relationship between the time-of-day effects on neural and muscular components, the average firing rate in each participant was calculated. Based on a previous report (31), repeated-measures correlations were performed using rmcorrShiny (https://lmarusich.shinyapps.io/shiny_rmcorr/). Significance was set as $P < 0.05$.

RESULTS

Neuromuscular Properties

Repeated one-way ANOVA for maximal knee extension torque did not reveal significance for time of day ($P = 0.681$, Table 1).

The number of detected motor units at each time was 142 in the morning, 144 at noon, 147 in the evening, and 154 at night, and cumulative total number of motor units was 587, including duplicates. After tracking procedure, the total number of the same tracked motor units among the four times was 127. Regarding tracking methods, the average cross-correlation coefficient was 0.81 ± 0.06 between two pairs for four times. The linear mixed model ANOVA considering the time of day revealed no significant effect for recruitment threshold ($F = 0.30$, degrees of freedom = 126, $P = 0.823$), but a significant one for the firing rate ($F = 35.0$,

Table 1. *Diurnal changes in neuromuscular parameters*

	Morning (10:00)	Noon (13:30)	Evening (17:00)	Night (20:30)	*P* Value
Knee extension strength, Nm	203.2 ± 31.0	205.1 ± 35.1	207.9 ± 27.1	204.3 ± 31.4	0.681
Singlet twitch torque, Nm	42.0 ± 7.4	43.2 ± 10.1	44.5 ± 8.6	45.8 ± 9.0	0.135
Time to twitch peak, ms	88.1 ± 7.3	93.2 ± 16.9	86.7 ± 12.7	86.2 ± 8.6	0.408
Subtetanic- and tetanic-evoked torque					
with 10 Hz, Nm	40.6 ± 15.2	43.9 ± 15.6	41.4 ± 13.7	44.1 ± 12.4	0.418
with 100 Hz, Nm	110.1 ± 49.6	122.2 ± 43.9	104.1 ± 41.2	118.3 ± 46.0	0.055
ARV during MVC, mV	127.7 ± 27.4	121.7 ± 25.0	125.0 ± 24.8	122.9 ± 27.9	0.692
ARV at 50% of MVC, mV	72.3 ± 27.0	79.8 ± 48.0	66.4 ± 37.3	76.9 ± 40.9	0.444

Data presented as means ± SD. *P* value calculated by repeated one-way ANOVA or Friedman test. ARV, average rectified value; MVC, maximal voluntary contraction.

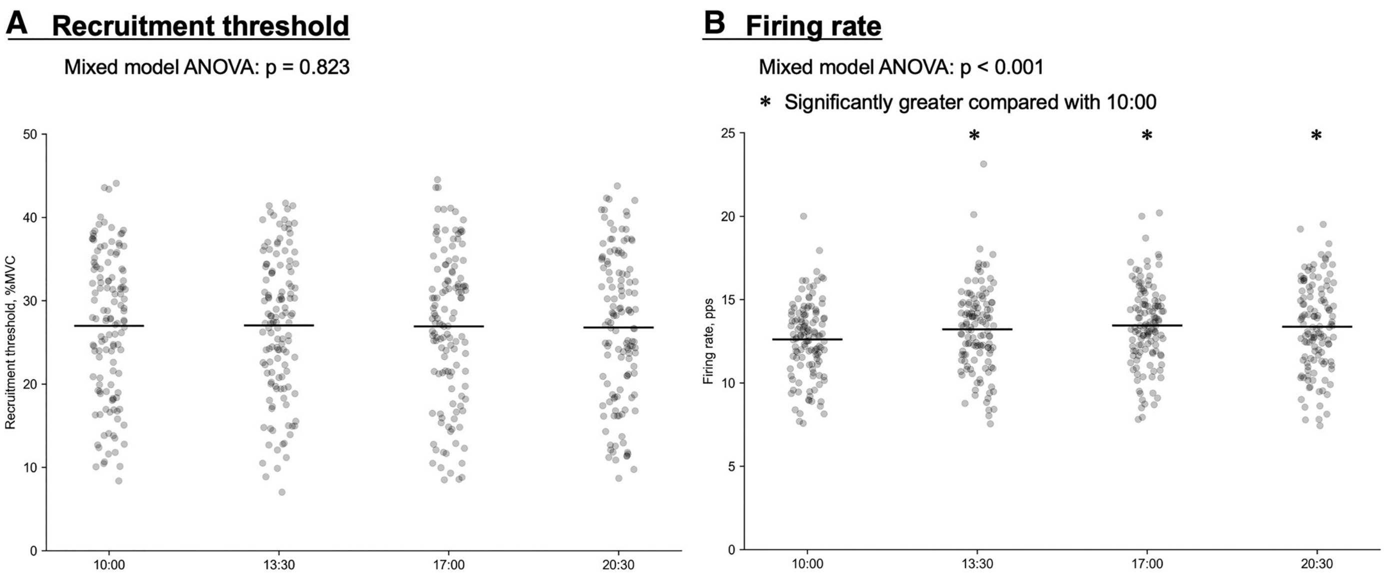

Figure 2. Motor unit recruitment threshold (*A*) and firing rate (*B*). *Significant differences compared with data in the morning (10:00).

degrees of freedom = 124.4, $P < 0.001$) (Fig. 2). Post hoc tests revealed that the firing rate at noon, in the evening, and at night was significantly greater than that in the morning (all, $P < 0.001$).

ARVs during MVC and during 50% of MVC in ramp-up contraction were not significantly changed among the times of day ($P = 0.692$ and $P = 0.444$, Table 1) based on repeated ANOVA and Friedman tests, respectively.

Singlet twitch torque and subtetanic- and tetanic-evoked torques with 10 and 100 Hz were not significantly changed among the times of day ($P = 0.135$, 0.418, and 0.055, respectively) based on repeated ANOVA (Table 1). TTP was not significantly different among the times of day ($P = 0.408$).

Temperatures and Glucose Levels

Table 2 presents data on temperatures and subcutaneous interstitial glucose levels. Repeated ANOVA for room temperature indicated nonsignificance ($P = 0.443$). ANOVA for skin temperature indicated a main effect for time of day ($P = 0.004$). Post hoc tests revealed that the skin temperature in the evening was significantly greater than that in the morning ($P = 0.030$). The Friedman test for external canal temperature did not reveal significance ($P = 0.068$). The Friedman test for glucose revealed a main effect for time of day ($P < 0.001$), and post hoc tests showed that the glucose level in the evening was significantly lower than those in the morning, at noon, and at night ($P = 0.001$, $P = 0.002$, and $P < 0.001$, respectively).

Relationships between the Variables

Table 3 shows the results of repeated-measures correlation analyses. There were significant correlations between the skin temperature and the external canal temperature (95% CI [0.033, 0.558], $P = 0.030$), between the skin temperature and the motor unit firing rate (95% CI [0.24, 0.687], $P < 0.001$), and between the external canal temperature and the motor unit firing rate (95% CI [0.004, 0.538], $P = 0.048$). The test did not reveal a significant correlation between singlet twitch torque and the motor unit firing rate (95% CI [−0.291, 0.29], $P = 0.996$).

DISCUSSION

The present study measured the motor unit firing rate, recruitment thresholds, and contractile properties at four different times of day within 24 h to investigate the time-of-day effects on the neuromuscular system. Our results showed that the motor unit firing rate was higher at noon, in the evening, and at night than in the morning, while time-of-day differences in recruitment thresholds or contractile properties were not observed. These findings suggest the existence of diurnal variations of motor unit firing properties. Fluctuation of motor unit firing properties was not associated with peripheral muscular contractile properties (Table 3). This is the first study to investigate the time-of-day effects on the neuromuscular system.

Table 2. *Diurnal changes in temperatures and glucose concentration*

	Morning (10:00)	Noon (13:30)	Evening (17:00)	Night (20:30)	*P* Value
Room temperature, °C	18.9 ± 1.1	18.8 ± 1.3	19.0 ± 1.2	19.2 ± 0.9	0.443
Skin temperature, °C	28.7 ± 1.1	29.4 ± 1.2	29.6 ± 1.1[a]	29.5 ± 1.1	0.004
External canal temperature, °C	36.2 ± 0.4	36.4 ± 0.3	36.4 ± 0.3	36.3 ± 0.4	0.068
Glucose, mg/dL	119.4 ± 22.8	110.4 ± 21.4	87.1 ± 14.4[a,b]	120.8 ± 22.6[c]	< 0.001

Data presented as means ± SD. *P* value calculated by repeated one-way ANOVA or Friedman test. Lowercase letters indicate significant differences by the post hoc test. [a]Significant difference compared with data in the morning, [b]significant difference compared with data at noon, and [c]significant difference compared with data in the evening.

Table 3. *Repeated-measures correlations among body temperature, twitch torque, motor unit firing rate, knee extension torque, and glucose*

	External Canal Temperature	Singlet Twitch Torque	Motor Unit Firing Rate	Knee Extension Torque	Glucose
Skin temperature	$r_{rm} = 0.32$* $P = 0.030$	$r_{rm} = 0.24$ $P = 0.111$	$r_{rm} = 0.50$* $P < 0.001$	$r_{rm} = 0.13$ $P = 0.405$	$r_{rm} = -0.14$ $P = 0.367$
External canal temperature		$r_{rm} = 0.13$ $P = 0.376$	$r_{rm} = 0.29$* $P = 0.048$	$r_{rm} = 0.08$ $P = 0.575$	$r_{rm} = 0.03$ $P = 0.844$
Singlet twitch torque			$r_{rm} = 0.00$ $P = 0.996$	$r_{rm} = -0.07$ $P = 0.667$	$r_{rm} = -0.02$ $P = 0.887$
Motor unit firing rate				$r_{rm} = 0.05$ $P = 0.755$	$r_{rm} = 0.04$ $P = 0.81$
Knee extension torque					$r_{rm} = 0.08$ $P = 0.618$

r_{rm}, Correlation coefficients calculated by repeated-measures correlation. *Significance ($P < 0.05$).

In the present study, the motor unit firing rate and recruitment thresholds during the same submaximal force exertion were assessed at four different times of the day. The results indicate that motor unit firing rates at noon, in the evening, and at night were higher (Fig. 2). Callard et al. (32) reported that EMG amplitudes of quadriceps muscles during maximal voluntary knee extension were higher in the evening and at night compared with those in the morning and at noon. EMG amplitudes are influenced by many various factors (16), including the motor unit firing rate and number of recruitments; thus, our results supported the previous study regarding the increased motor unit firing rate. The present study revealed a time-of-day effect on motor unit firing properties based on detailed neuromuscular measurements.

One of the reasons for the increase in the motor unit firing rate may be the presence of a strategy to compensate for the attenuation of excitation-contraction coupling or a physiological response to improve motor unit recruitment. The increase in motor unit firing rate could indicate marked excitability of central nervous activation, which can contribute to greater neuromuscular excitability. These increases in neuromuscular excitabilities are controlled by the central nervous system and primary motor cortex. However, our ARV results during MVC and submaximal ramp-up contraction showed no significant differences among the four times of day (Table 1). Considering the increase in the motor unit firing rate at noon, in the evening, and at night (Fig. 2), other factors related to EMG amplitude (16) might contribute to offset ARV. The presence of diurnal differences in the EMG amplitude was reported in a previous study (15) but not in others (13, 33). Indeed, EMG amplitude is confounded by various factors (16). These findings suggest that while motor unit firing rates could be considered a sensitive variable, ARV could not at least in these experimental conditions. In addition, the findings of an increase in motor unit firing rate and unchanged recruitment thresholds and ARV suggest that derecruitment of motor units might have occurred at noon, in the evening, and at night, and fewer motor units may have discharged to generate the same muscle strength. Lagerquist et al. (34) measured spinal reflex excitability of the soleus, which relates to motoneuronal pool excitability in the morning and evening, revealing that the H-reflex amplitude was significantly greater in the evening than in the morning. Although we did not measure spinal reflex excitability, we suggest that diurnal fluctuations of motor unit firing properties are associated with spinal motoneuronal excitability. Our results also showed body temperatures could be linked to motor unit firing rate in the positive relationship (Table 3), but a previous study investigating the effect of hot water on neuromuscular properties reported a decrease in motor unit firing rate, whereas an increase in twitch torque when muscle temperature increased (35). The increase of muscle and core temperatures in the previous study (35) was markedly greater than the diurnal changes in the current study, Thus, the positive relationship between body temperatures and motor unit firing rate could be specifically caused by diurnal rhythm.

Neuromuscular properties are determined by not only central nervous excitability but also peripheral muscle contractile properties (36–38). Pearson and Onambele (39) compared muscle and tendon compliances of the quadriceps between the morning and evening and reported that evoked strength was not different between the morning and evening, but that the time to twitch peak was significantly increased in the evening compared with morning. The reason for the different result may be because of a different joint angle (70° knee joint was used in the previous study) (39), which affects muscle-tendon slack. In a previous study (39), changing muscle-tendon slack within a day was considered a factor in the diurnal variation of TTP. Another previous study reported that muscle-tendon unit length varies with the joint angle (40), suggesting that muscle-tendon properties may vary with this angle. The joint angles used in the present study might have less influence on muscle-tendon slack. Therefore, as both TTP and evoked twitch torque results were not significantly different among the four times in the present study (Table 1), muscle contractile property as a peripheral component of the neuromuscular system may be less affected by time of effect. In addition, we measured subtetanic- and tetanic-evoked torques by electrical stimulation with 10 and 100 Hz. The different frequencies of electrical stimulation induced incomplete or complete tetanus (29, 30). However, time-of-day effects on evoked torque by continuous electrical stimulation were not observed (Table 1). Although diurnal changes in the motor unit firing pattern were noted in the present study (Fig. 2), peripheral components of muscle contractile properties did not show significant fluctuation with a diurnal rhythm. In addition, diurnal fluctuations of the motor unit firing rate and evoked twitch torque were not related (Table 3). These findings suggest that central nervous excitability and peripheral contractile properties are independent within a single day.

Many previous studies investigated time-of-day effects on muscle strength (11, 12), and most of them reported that muscle strength in the evening or at night was significantly greater than that in the morning (9, 11, 18). The contradictory results whereby diurnal variations in muscle strength were not observed in the present study (Table 1) might be due to several reasons. One is that the protocols of previous studies differed from that of the present study, especially number of measurements (2 times in the morning vs. evening in most studies) and the interval between measurements was different. Another possible reason involves the characteristics of participants. The difference in muscle strength between the morning and evening faded, especially in the morning strength training group (13). Most participants were categorized as the morningness or intermediate type according to MEQ. The test score does not directly indicate a morning-specific adaptation like that reported in previous studies (13, 41, 42). The sample size was too small to conclude the effect of the chronotype on diurnal neuromuscular properties in the present study. Future studies are needed to investigate the effect of the chronotype on neuromuscular properties. In addition, a low glucose level in the evening can affect muscle strength. In the evening, the glucose level was significantly lower than at other times (Table 2). As glucose is taken up during skeletal muscle contraction (21, 43) and is necessary to promote muscle strength and growth (44), the glucose level in the evening would cause a nonsignificant improvement in muscle strength at this time. Future studies are needed to investigate the effects of the glucose level and the number of measurements conducted per day.

There were some limitations of the present study. There are other physiological circadian rhythms such as those linked to some hormones or neuromodulators, such as serotonin, which increase during the evening and at night (45), and another previous study reported that the availability of receptors related to serotonin follows a circadian rhythm (46). Serotonin has been reported to be directly related to the amplitude of persistent inward current (PIC) (47); thus, it is possible that the diurnal fluctuation in serotonin affects PIC and motor unit firing behaviors. However, the physiological relationship between serotonin and motor unit behaviors was unclear in the present study for the following two reasons. First, PIC cannot be calculated because our method did not include a ramp-down phase, which is needed to calculate PIC (48). Second, as serotonin was not measured in the present study, the relationship between the diurnal variation in serotonin secretion and motor unit behavior was unclear. Future studies should investigate how physiological responses including serotonin could influence motor unit behaviors. Another limitation was the use of 30% maximal current intensity when assessing complete and incomplete tetanic contractile properties because the stimulation with supramaximal current intensity was too painful for subjects. The amount of current stimulation was unified across the four periods in each subject. Therefore, we consider that this method could detect trends of diurnal changes in the subtetanic and tetanic contractile properties in each subject. However, it is also possible that we might not have estimated the subtetanic and tetanic contractile properties sufficiently with this method.

In summary, the present study investigated the time-of-day effects on motor unit firing and muscle contractile properties in humans. The motor unit firing rate may be affected by diurnal rhythms, but not contractile properties. Also, the motor unit firing rate changed within 24 h independent of muscle contractile properties. These findings indicate the existence of diurnal fluctuation of the motor unit firing rate and suggested that motor performances and resistance exercises could indicate time-of-day effects of motor unit firing properties.

DATA AVAILABILITY

Data will be made available upon reasonable request.

ACKNOWLEDGMENTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

T.H., K.I., and K.W. conceived and designed research; T.H., K.I., and T.N. performed experiments; T.H., K.I., and M.O. analyzed data; T.H., K.I., M.O., R.T., T.N., and K.W. interpreted results of experiments; T.H. prepared figures; T.H., K.I., and K.W. drafted manuscript; T.H., K.I., M.O., R.T., T.N., and K.W. edited and revised manuscript; T.H., K.I., M.O., R.T., T.N., and K.W. approved final version of manuscript.

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