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Effects of time-of-day on neuromuscular function in untrained men: specific responses of high morning performers and high evening performers

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ABSTRACT

It has been clearly established that maximal force varies during the day in human muscles but the exact mechanisms behind the diurnal rhythms are still not fully clarified. Therefore, the aim of this study was to examine the diurnal rhythms in maximal isometric force production in a large group of participants and also by separating the high morning performance types (n=8) and the high evening performance types (n=19) from the neutral types (n=45) based on their actual maximal isometric force levels. Measurements were performed in the morning (7:26 h ± 63 min) and in the evening (17:57 h ± 74 min) for maximal bilateral isometric leg press force (MVC_{LP}) together with myoelectric activity (EMG_{LP}), maximal unilateral isometric knee extension force (MVC_{KE}) and maximal voluntary activation level (VA%) during maximal unilateral isometric knee extension force (MVC_{VA}) together with myoelectric activity (EMG_{VA}). In addition, venous blood samples were drawn four times a day and serum testosterone and cortisol concentrations were analyzed. None of the participants belonged to the extreme morning or evening chronotype according to the Munich Chronotype Questionnaire. In the total group of participants MVC_{LP} and MVC_{KE} were 4.4 ± 12.9% ($p<0.01$) and 4.3 ± 10.6% ($p<0.01$) higher in the evening compared to the morning. MVC_{VA} and VA% did not show significant diurnal variation. The high morning performance types showed lower force values in the evening compared to the morning for MVC_{LP} (10.8 ± 9.1%; $p<0.05$) and MVC_{KE} (5.7 ± 4.9%; $p<0.05$). No significant diurnal variation was observed for MVC_{VA} and VA%. The high evening performance types showed higher force values in the evening for MVC_{LP} (16.1 ± 15.9%; $p<0.001$), MVC_{KE} (13.5 ± 11.3%; $p<0.001$) and MVC_{VA} (6.2 ± 9.9%; $p<0.05$) with a concomitant higher VA% in the evening ($p<0.05$). The neutral types showed significantly higher evening force values for the MVC_{LP} (2.1 ± 6.7%; $p<0.05$). All the other neuromuscular variables did not show significant diurnal variations. $\mathrm{EMG}_{\mathrm{LP}}$ and $\mathrm{EMG}_{\mathrm{VA}}$ did not show significant diurnal fluctuations in any group. Serum testosterone and cortisol concentrations showed normal daily rhythms with higher values observed in the morning in all of the groups ($p<0.001$). Between-group differences were observed for $\mathrm{MVC}_{\mathrm{LP}}$ ($p<0.001$) and $\mathrm{MVC}_{\mathrm{KE}}$ ($p<0.001$) between all of the three groups. Diurnal changes in VA% differed between the high evening performance types and the neutral types ($p<0.05$) and the testosterone/cortisol ratio ($p<0.05$) as well as VL $\mathrm{EMG}_{\mathrm{VA}}$ ($p<0.05$) differed between the high morning and high evening performance types. In conclusion, we were able to identify the high morning performance types, the high evening performance types and the neutral types who showed significantly different diurnal rhythms in force production, irrespective of their actual chronotype. Therefore, the questionnaires designed to determine the chronotype may not always be sensitive enough to determine the "morningness" or "eveningness" in maximal neuromuscular performance. In general, central factors could partially explain the diurnal fluctuations in maximal strength performance, but peripheral mechanisms were also possibly involved.

Key words: diurnal variation; maximal isometric strength; neuromuscular performance; knee extensor muscles; electromyography; voluntary activation level; testosterone; cortisol

INTRODUCTION

It has been clearly established that functional capacities during maximal isometric voluntary contraction (MVC) of human muscle varies during the day. Diurnal rhythms in MVC production have been shown for a variety of muscles including quadriceps femoris (Callard et al., 2000; Giacomoni et al., 2006; Guette et al., 2005a; Nicolas et al., 2005; Onambele-Pearson & Pearson, 2007; Racinais et al., 2005; Sedliak et al., 2008). The acrophase of the muscle capacity to develop maximal force has been found to occur in the early evening between 16:00 and 19:30 (Callard et al., 2000; Giacomoni et al., 2005; Guette et al., 2005a; Nicolas et al., 2005; Teo et al., 2011). Peak-to-trough variation of maximum strength has been reported to range from 5% up to 21% (Coldwells et al., 1994; Guette et al., 2005b), depending on the population and the muscle groups tested as well as on the experimental design (Nicolas et al., 2007). However, the previous literature has been unable to determine the exact mechanisms behind the diurnal rhythms. The ability to generate force depends on both peripheral and central mechanisms. Peripheral mechanisms include muscle contractility and metabolism, morphology of muscle fibers and local muscle temperature (Araujo et al., 2011; Edwards et al., 2013; Reilly and Waterhouse, 2009). Central mechanisms include central nervous system command, alertness and motivation (Castaingts et al., 2004; Giacomoni et al., 2005; Racinais et al., 2005; Tamm et al., 2009). Peripheral (Edwards et al., 2013; Guette et al., 2005a; Martin et al., 1999; Racinais et al., 2005; Sedliak et al., 2008;), central (Tamm et al., 2009), or combination of both mechanisms (Castaingts et al., 2004; Gauthier et al., 1996; Sedliak et al., 2011) have been proposed to explain the diurnal rhythms in force production. Electromyography (EMG) and twitch interpolation technique have been widely used to discriminate the involvement of peripheral and central mechanisms in the diurnal variations (Chtourou & Souissi, 2012). Though diurnal rhythm in muscle force has been well established during maximal isometric contraction (Callard et al., 2000; Gauthier et al., 1996; Martin et al., 1999), the results concerning EMG data are more controversial. Previous studies have shown no significant daily changes in EMG activity (Giacomoni et al., 2006; Guette et al., 2005a; Martin et al., 1999; Nicolas et al., 2005; Onambele-Pearson & Pearson, 2007; Racinais et al., 2005; Sedliak et al., 2008), higher EMG in the morning

(Gauthier et al., 1996; Guette et al., 2005b) or higher in the evening (Callard et al., 2000; Sedliak et al., 2011) when compared to the rest of the day. These controversial results can be partly explained by the fact that EMG data as such can be affected by several methodological factors and by the muscle groups examined, as calf muscles seem to have higher EMG in the morning (Guette et al., 2005b; Guette et al 2006).

Moreover, it has been suggested that diurnal fluctuations in physical performance may be partly controlled by the day-time level of hormonal fluctuations of some hormones (Giacomoni et al., 2005; Martin et al., 1999). Both testosterone (T) and cortisol (C) exhibit circadian rhythmicity with morning peaks and evening nadirs (Hayes et al., 2012; Kraemer et al., 2001). T and C are considered as important biomarkers in exercise science, because in addition to circadian rhythmicity they are known to correlate with athletic performance (Crewther et al., 2009). The T/C ratio indicates the anabolic/catabolic environment of an organism due to their roles in protein synthesis and degradation. Higher T/C ratio in the evening may provide a more desirable physiological condition (more anabolic environment with less physiological stress), which prepares participants for the physical performance (Hayes et al., 2013).

In addition to that, person’s chronotype may be a confounding variable in diurnal rhythms of force production (Tamm et al., 2009). Individual circadian rhythm differences in activity, termed “morningness-eveningness”, may modify performance at particular times of day (Brown et al., 2008). Only few studies have examined the temporal fluctuation of performance while considering the individual’s circadian preference. Performance tests administered at optimal times of day are more efficient compared to the tests at non-optimal time of day, as inferred for each subject by the score obtained at morningness-eveningness questionnaire (Schmidt et al., 2007).

The identification of exact mechanism is difficult, because there are many underlying factors that occur at the same time and are thereby contributing to diurnal rhythms in physical performance. The purpose of the present study was twofold. Firstly, to examine the diurnal rhythms of maximal isometric force of the lower limbs along with surface EMG and maximal voluntary activation level as well as with serum hormone concentrations. Secondly, the large number of participants in this study gave us the opportunity to separate the high morning performance types and high evening performance types from the neutral types based on their maximal isometric strength performance of the lower limbs. This allowed us to investigate the neuromuscular mechanisms that underlie diurnal patterns in isometric knee extension force production and determine whether they are different for high morning performers, high evening performers and the neutral types.

METHODS

Participants

72 men volunteered to participate in the study. Their mean ± SD age, height and weight were 32 ± 6 years, 1.8 ± 0.1 m and 80.9 ± 10.8 kg, respectively. These individuals were previously untrained but physically active men with similar backgrounds of both health status and physical condition. The target group was free of acute and chronic illnesses. Participants reported to be free of any medications that could influence on their hormonal and neuromuscular systems. In addition, a cardiologist reviewed each participant’s health questionnaire and ECG. Participants’ morningness and eveningness was assessed. According to Munich Chronotype Questionnaire (Roenneberg et al., 2003), participants did not belong to an extreme chronotype. Neutral types

(n=27), slight late (n=5) and moderate late (n=1) as well as slight early types (n=30) and moderate early types (n=9) were selected. Shift and night workers were excluded. After participants were informed of the purpose and risks of the study, the participants provided written consent before participation. This study was conducted in accordance with the ethical standards of the journal (Portaluppi et al., 2010), complied with the Declaration of Helsinki and was approved by the Ethics Committee in the University of Jyväskylä.

Experimental design

All the participants performed the present strength measurements twice: once in the morning and once in the evening. The strength measurements lasted about 45 min and were carried out in the morning between 6:00-10:00 h and in the evening between 16:00-20:00 h. The average testing time in the morning was 7:26 h ± 63 min and in the evening 17:57 h ± 73 min. Test times did not statistically differ between the groups. The morning and evening tests were carried out in a randomized order. At least 24 h separated the morning and evening tests and participants were asked to keep two days of rest before the testing and follow their normal sleep rhythm. They were also requested to choose the least physically demanding option when coming to the measurements (preferably car or public transport). In the case it was not possible, they were asked to arrive 15 min earlier and rest before the measurements. Participants were asked to refrain from alcohol for 24 h and from coffee for 12 h before the measurements.

Venous blood samples and intra-aural body temperature were measured on a separate day from the strength measurements and were collected 4 times per day at 7:30 h ± 30 min, 9:30 h ± 30 min, 16:30 h ± 30 min and 18:30 h ± 30 min. Participants had to arrive to the lab 10-15 min before the scheduled time and then sit quietly on a chair until the blood samples and body temperature were collected. Participants were asked to fast 12 hours before the first blood sample, after which everybody were provided with a standardized breakfast. They were asked to eat lunch which had to follow previously given instructions about the relative content of protein (10-20%), fat (25-35%) and carbohydrate (50-60%) at around 12:00-13:00 h. After that, participants were asked to avoid food until the last two blood samples (16:30 h ± 30 min and 18:30 h ± 30 min) were drawn. For possible correlations between the serum hormone concentration or body temperature and force production as well as for the between-group comparison the mean of the two morning or two of evening measurements was used for the serum hormone concentration or body temperature values.

Force measurements

A familiarization session was carried out before the true strength measurement on a non-testing-specific time of day. All the force measurements were performed in the same testing session in the following order described below. All the subjects were allowed to have three warm-up trials for the isometric leg press, which also served as a short warm-up.

Bilateral isometric leg press force (MVC_{LP}): Maximal bilateral isometric strength (N) was measured using a horizontal dynamometer (designed and manufactured by the Department of Biology of Physical Activity, University of Jyväskylä, Finland) at the knee angle of 107° (180 ° = knee fully extended) (Häkkinen et al., 1998). Participants were instructed to generate maximum force as rapidly as possible against the force plate for a duration of 2-4 sec. Participants were verbally encouraged to perform their maximal. A minimum of three up to five trails were used to determine the maximal isometric leg extension with one minute break separating the trials. Isometric force signals were passed in real-time to an analog-to-digital (AD) converter (Micro

1401, Cambridge Electronic Design, UK). The trial with the highest peak force was selected for further analysis. Force signals were sampled at 2000Hz and low-pass filtered (20 Hz). Muscle activity was recorded through surface electromyography (EMG_{LP}) during MVC_{LP} from vastus lateralis (VL) and vastus medialis (VM) muscles of the right leg. During a familiarization session the VL and VM motor points of the right leg were measured according to Seniam guidelines, and marked with indelible ink tattoos to endure electrode position. The raw EMG signal from the measurement was amplified by 1000 and sampled at 3000 Hz. The signals were passed from a portable transmitter (Telemyo 2400R, Noraxon, Scottsdale, AZ, USA) from which the signal was relayed to a desktop computer via an AD converter (Micro 1401, Cambridge Electronic Design, UK). Analysis of the isometric EMG was performed using a customized script (Signal 4.10, Cambridge Electronic Design Ltd., Cambridge, UK) and converted to integrated EMG. Maximum EMG was determined from the 500-1500 ms time period of contraction representing the peak force phase.

Unilateral isometric knee extension force (MVC_{KE}): Maximum voluntary isometric force of knee extensors was tested using unilateral knee extension of the right leg at the knee angle of 107° (180˚= knee fully extended). The participants were secured to a sitting position in the modified knee extension device (David 200; David Health Solutions Ltd., Helsinki, Finland) with a safety belt in the hip area. Correct position was endured by the adjustable back support, lever arm and ankle pad. Left leg rested in horizontal position on a support. The upper extremities were placed next to the body holding handgrips. Participants were instructed to generate maximum force rapidly against the ankle pad and maintain it for 2-4 sec. A minimum of three up to five trails were used to determine the maximal isometric leg extension with a one-minute resting period separating the trials. The trial with the highest peak force was taken for further analysis. The force signal was low-pass filtered (20 Hz) and analyzed (Signal 4.10, Cambridge Electronic Design Ltd., Cambridge, UK).

Maximal voluntary muscle activation level (VA%): Maximal unilateral knee extension force (MVC_{VA}) was measured using a device designed and manufactured in the Department of Biology of Physical Activity (University of Jyväskylä, Finland). During the knee extension action muscle stimulation was performed to the right quadriceps femoris muscles. The participant was seated with knee angle 107° in the right leg and left leg rested in horizontal position on a chair. Hip and knee angles were firmly secured by a seatbelt at the hip, strapped pad over the right knee and with a Velcro strap above the right ankle. Participants were asked to perform three maximal trials by increasing force gradually over 3 seconds. The trial with the highest force was used for further analysis. The force signal was sampled at 2000 Hz and low-pass filtered (20 Hz). Maximal force was manually analyzed on Signal 4.04 (Cambridge Electronic Design, UK).

To assess the maximal voluntary activation level (%) of quadriceps femoris muscle interpolated twitch technique was used to give a supramaximal stimulus during the isometric knee extension action. Four self-adhesive electrodes (7 cm PolarTrode; Polar Frost USA; Anaheim, CA; USA) were placed on the proximal and mid-region of the quadriceps muscle belly of the right thigh. The current of single 1-ms rectangular pulses were increased progressively by a constant-current stimulator (Model DS7AH, Digitimer Ltd, UK) in 5mA steps until a plateau was observed. 25% of the stimulation current was added to ensure maximal effect for the knee extension trials. This supramaximal single-pulse stimulation was delivered during the plateau of peak knee extension force and 5 seconds after the cessation of contraction (Merton, 1954). Voluntary activation level was calculated according to the formula by Bellemare & Bigland-Ritchie (1984):

$$\text{Activation level (\%)} = [1-(P_{ts}/P_t)] \cdot 100,$$

where P_{ts} is the amplitude of the twitch elicited by the electrical stimulation on top of the maximal voluntary contraction and P_t is the amplitude of the twitch delivered to the muscle 5 seconds after the voluntary contraction. EMG_{VA} was collected from VL and VM from the maximum force level over the 500 ms time period, immediately before the superimposed twitch. EMG was multiplied by 1000 by a preamplifier (NeuroLog Systems NL844, Digitimer Ltd, UK) and sampled at a frequency of 2000 Hz. The raw EMG signal was band-bass filtered (20-350 Hz) and, due to technical reasons, converted to root mean square manually on Signal 4.04 software (Cambridge Electronic Design, UK).

Serum hormone concentrations and body temperature

Venous blood samples and body temperature were measured with the participant in a sitting position. Venous blood samples (~10 ml) for the determination of serum hormone concentration were collected by a qualified laboratory technician from an antecubital vein with a vacutainer and test tubes (Vacuette, GEiner Bio-OneGmbH, Kremsmünster, Austria) containing appropriate preservatives. Samples were centrifuged at 3.500 rpm (Heraus Megafuge 1.0 R, Gendro Laboratory Products, Hanau, Germany) for 10 min, plasma harvested and all samples were stored at −80°C until assayed. Analysis of total serum testosterone (T) and cortisol (C) was performed using chemical luminescence techniques (Immunlite 2000, Simens Healthcare, Diagnostics Products Ltd., Llanberies, UK) and hormone specific immunoassay kits (Siemens, New York, USA). The sensitivity for serum hormones was: T 0.5 $nmol/l^{-1}$ and C 5.5 $nmol/l^{-1}$. The intra-assay coefficient of variation was 8.3% and 5.3% for T and C, respectively. The interassay coefficient of variation was 9.1% for T and 7.2% for C. In addition, T/C ratio was calculated.

Intra-aural temperature was collected by a qualified laboratory technician by digital thermometer (ThermoScan PRO, BRAUN, Southborough, MA, USA). Two trials were required. The mean of two values were used for further statistical analyses.

Separation to high morning performance types and high evening performance types

The mean of MVC_{LP} and MVC_{KE} morning to evening differences (expressed as percentage of morning values) was used to classify the high morning performance types, high evening performance types and the neutral types. The criteria for determining the different types was set based on the whole group mean morning to evening differences, which was observed to be 4% for both, MVC_{LP} and MVC_{KE}. Morning types were expected to show similar 4% morning to evening difference, with higher values observed in the morning. For the high evening performance types the criterion was set so, that double of the morning to evening difference observed in the large group exists (8%), with higher values observed in the evening. Based on that, any participant that demonstrated morning performance (mean of MVC_{LP} and MVC_{KE} morning to evening difference) at least 4% greater than his evening performance was classified as “high morning performance type”. Any participant that demonstrated evening performance (mean of MVC_{LP} and MVC_{KE} morning to evening difference) at least 8% greater than his morning performance was classified as “high evening performance type”. All the remaining participants were determined as neutral types. None of the participants included to the high morning performance group showed higher evening force values for either MVC_{LP} or MVC_{KE} compared to the morning values and none of the participants in the high evening performance group showed higher morning force values for either MVC_{LP} or MVC_{KE} compared to the evening values. Out of all of the participants, 8 were classified as high morning performance types, 19 as high evening performance types and 45 as neutral types.

Statistical analysis

Descriptive data were generated for all variables and expressed as mean ± SD. Normality of the data was checked and subsequently confirmed using the Shapiro-Wilk test. For normally distributed data Paired-Samples T-Test was used to compare the values from the morning and evening tests. EMG, body temperature and hormonal data was not normally distributed and for that reason the non-parametric tests were used. For morning and evening comparison Wilcoxon-Signed Rank Test was used for the not normally distributed data. For diurnal rhythms of testosterone, cortisol and T/C ratio Friedman test was performed. Between-group analyses were performed with relative values from morning to evening differences. For normally distributed data One-Way ANOVA was used when all three groups were compared and Independent Samples T-test when only extreme groups (high morning performance group vs. high evening performance group) were analyzed for the between-group differences. Mann-Whitney U-test or Kruskal-Wallis one-way analysis of variance was performed, respectively, for not normally distributed data. For correlations between daily relative changes in strength performance and serum hormone concentration Pearson correlation coefficients were calculated. Statistical significance was accepted when $p<0.05$, whereas values $p\leq0.07$ were accepted as a significant trend. Analysis was performed using the Statistical Package for Social Sciences (SPSS verion 22, Chicago, IL).

RESULTS

Body temperature and serum hormone concentrations

Body temperature significantly increased between 7:30 h ± 30 min and 16:30 h ± 30 min with a mean morning to evening difference of 0.2 ± 0.4℃ ($p<0.05$). Serum T and C as well as T/C ratio showed a regular diurnal variation. T and C demonstrated the highest concentrations in the morning (7:30 h ± 30 min) and decreasing concentrations throughout the day ($p<0.001$), whereas T/C ratio showed lowest values in the morning and increasing concentrations throughout the day ($p<0.001$) (Table 1).

Neuromuscular performance

MVC_{LP} was significantly higher when tested in the evening as compared with the morning. The mean difference between the morning and evening measurements was 4.4 ± 12.9% ($p<0.01$). EMG_{LP} measured from MVC_{LP} did not show any significant morning to evening difference. In MVC_{KE} evening values were also 4.3 ± 10.6% ($p<0.01$) higher compared to the morning values. MVC_{VA} was higher in the evening compared to the morning, although just a trend to reach the statistical significance level was observed (2.8 ± 10.8%; $p=0.07$). No significant morning to evening difference was observed for the absolute EMG_{VA} or VA%. Absolute values (mean ± SD) for the morning and evening neuromuscular performance are presented in Table 2.

High morning performance types

The high morning performance types showed 10.8 ± 9.1% lower force values in the evening compared to morning for MVC_{LP} (2329 ± 474 N vs. 2068 ± 728 N; $p<0.05$) and 5.7 ± 4.9% for

MVC_{KE} (730 ± 171 vs. 682 ± 129 N; $p<0.05$). The corresponding differences in the relative scale (Δ%) are presented in the lower part of Figure 1. No significant morning to evening difference was observed for the MVC_{VA} (532 ± 73 vs. 538 ± 65 N). EMG_{LP}, EMG_{VA} and VA% did not show any significant morning to evening difference.

High evening performance types

The high evening performance types showed 16.1 ± 15.9% higher force values in the evening compared to the morning for MVC_{LP} (2587 ± 680 vs. 2968 ± 728 N; $p<0.001$), 13.5 ± 11.3% for MVC_{KE} (682 ± 144 vs. 770 ± 154 N; $p<0.001$) and 6.2 ± 9.9% for MVC_{VA} (569 ± 110 vs. 599 ± 101 N; $p<0.05$). This difference in MVC_{VA} was accompanied by neural changes so that VA% was significantly higher in the evening (90 ± 6 %) compared to the morning (87 ± 6%) ($p<0.05$). The corresponding differences in the relative scale (Δ%) are presented in the upper part of Figure 1. No significant morning to evening differences were observed for EMG_{LP} and EMG_{VA} in the high evening performance group.

Neutral types

Neutral types showed 2.1 ± 6.7% higher force values in the evening compared to the morning for MVC_{LP} (2804 ± 734 vs. 2859 ± 751 N; $p<0.05$). No significant morning to evening difference was observed for the MVC_{KE} (777 ± 138 vs. 790 ± 135 N) or for MVC_{VA} (599 ± 108 vs. 607 ± 113 N). EMG_{LP}, EMG_{VA} and VA% did not show any significant morning to evening difference.

No significant increase in body temperature was observed for the high morning performance types (36.0 ± 0.6℃ vs. 36.1 ± 0.6℃) or for the neutral types (36.3 ± 0.3℃ vs. 36.3 ± 0.4℃). Whereas in the high evening performance group, body temperature significantly increased between 7:30 h ± 30 min and 16:30 h ± 30 min from 36.0 ± 0.5℃ to 36.3 ± 0.6℃ ($p<0.01$). All three groups showed significantly higher serum testosterone and cortisol concentrations in the morning compared to the evening ($p<0.001$) and significantly lower T/C ratio values in the morning compared to the evening values (Table 3). Pearson correlation did not reveal any significant correlations between the daily variations in maximal force and serum hormone levels in any of the groups.

Between-group differences

Significant between-group differences were observed for morning to evening changes in MVC_{LP} for the high evening performance types and the high morning performance types ($p<0.001$), the high morning performance types and the neutral types ($p<0.01$) as well as for the high evening performance types and the neutral types ($p<0.001$). Morning to evening changes in MVC_{KE} were significantly different between the high morning performance types and the high evening performance types ($p<0.001$) and between the high evening performance types and the neutral types ($p<0.001$) with the morning types showing significantly higher morning values and the evening and neutral types higher evening values (Figure 1). High evening performance types showed significantly different morning to evening change in VA% compared to the neutral types ($p<0.05$), while high performance evening types showed significantly higher values in the evening and neutral type group did not show any significant diurnal variations in VA%. However, when the two extreme groups (high morning performance group vs. high evening performance group) were compared, then in addition to significant between-group differences in diurnal variations in force production, morning to evening changes in VL $\mathrm{EMG}_{\mathrm{VA}}$ reached a significant between-group difference with higher morning values in the high morning performance types by 24 ± 27% and higher evening values in the high evening performance types by 7 ± 23% ($p<0.05$). There was a trend for statistical significance between the high evening performance group and the high morning performance group in the morning to evening differences in VL $\mathrm{EMG}_{\mathrm{LP}}$ (10.2 ± 13.9% vs. 8.4 ± 24.8%; $p=0.060$), in the high morning performance types with higher morning values and in the high evening performance type with higher evening values, respectively. Morning to evening variation in T/C ratio showed a significant difference between the high morning performance and high evening performance types (67 ± 57% vs. 168 ± 166%; $p<0.05$), respectively (Table 3).

DISCUSSION

The present study was designed to investigate the effects of the time of day on the neuromuscular performance of thigh muscles and possible mechanisms behind the inter-individual differences in the diurnal fluctuations in strength performance. In the total group of participants, so far the largest experiment sample in this area of research, our results were consistent with the previous studies with smaller sample sizes investigating knee extensor muscles (Araujo et al., 2011; Callard et al., 2000; Deschenes et al., 1998; Guette et al., 2005a; Nicolas et al., 2005; Onambele-Pearson & Pearson, 2007; Racinais et al., 2005; Sedliak et al., 2008; Sedliak et al., 2011; Teo et al., 2011), since maximal force was significantly greater (~4%) in the evening compared to the morning. The uniqueness of the present study including a large group of participants, however, lays in the fact that the high morning performance and high evening performance types were separated from the neutral types and compared with each other. To the best of our knowledge, this is the first study to divide the morning types, evening types and neutral types into the groups according to their lower limb strength performance, since based on the chronotype questionnaire (Roenneberg et al., 2003) none of our participants belonged to the extreme morning or evening chronotype. We were able to allocate the high morning performance types who showed significantly higher force values in the morning (~6-11%), neutral types who showed slightly higher evening performance or no morning to evening differences in maximal isometric force production and the high evening performance types who showed high morning to evening fluctuations (~6-16%), with significantly higher force values observed in the evening. Moreover, the higher evening force values observed in the high evening performance group could at least partly be explained by the increased central nervous system drive to the thigh muscles since VA% was concomitantly higher in the evening.

Diurnal fluctuations in maximal voluntary force have been explained by (i) changes in the central nervous system drive or (ii) changes at the peripheral level (Guette et al., 2006). In the present study myoelectrical activity of VL and VM muscles did not significantly differ between the morning and the evening hours in the large group of participants. This was in line with previous research results studying the diurnal rhythms in knee extensors (Guette et al., 2005a; Nicolas et al., 2005; Sedliak et al., 2008), showing no fluctuation in EMG recordings over the day. These authors have concluded that daily variations in maximal force can be explained by changes at the muscle tissue level rather than by the failure in the central motor command to activate the knee extensor muscles in the morning. Castaingts et al. (2004) have suggested that the diurnal changes in force are influenced by the changes in the contractile and elastic properties of the muscle, which seem to favor the evening hours. However, others (Callard et al., 2000; Sedliak et al., 2011) have reported maximal EMG activity accompanying with higher force values in the evening for knee extensor muscles and proposed that both neural and muscular factors are involved in the circadian rhythms of MVC. In the present study, EMG mirrored maximal force production as it increased over the day for the high evening performance types and decreased over the day for the high morning performance types, although did not reach the significance level due to large inter-individual differences. Moreover, a significant between-group difference was found for VL $EMG_{VA,}$ showing that the maximal activation of knee extensors took place on different times of the day for the high morning performance types and the high evening performance types.

The possible influence of central factors on diurnal fluctuation in maximal strength performance can also be evaluated by the maximal voluntary activation level (VA%). In the present study maximal VA% did not show any significant difference between the morning and evening in the total group of participants. This suggests that the capacity to activate the knee extensor muscles was not affected by the time of day and that central mechanisms could not explain the diurnal rhythms observed in MVC as supported also by previous research results (Guette et al., 2005a; Martin et al., 1999; Onambele-Pearson & Pearson, 2007). However, the present high evening performance group showed significantly higher VA% in the evening compared to the morning. These results suggest that the higher evening force values in the high evening performance group might be also due to the increased central nervous system drive to the quadriceps femoris muscles. To express this in other words, our evening types may have experienced reduced central motor drive in the morning associated with lower maximal activation of the muscle (Guette et al., 2006). This can be a result of a failure to recruit all motor units or a reduction in maximal discharge rate (Kent-Braun & Le Blanc, 1996). Therefore, the force measured in the morning may not have been a representative of maximal force in the evening type group (Kent-Braun & Le Blanc, 1996). However, this was not the case for the high morning performance types and in the neutral types, who showed similar VA% both in the morning and in the evening. However, the between-group comparisons showed that daily fluctuation in VA% significantly differed between the high evening performance types and neutral types. Although both groups showed higher force values in the evening, the diurnal fluctuations in the high evening performers seem to result from the diurnal fluctuations in the central mechanisms. It is possible that the type II error, caused by a small number of participants in the high morning performance group may have led to false negative results and restricted to find significant between-group differences.

One recent study (Tamm et al., 2009) have investigated differences in strength performance in the morning types and evening types by grouping the participants based on the morningness-eveningness questionnaire (Horne & Ostberg, 1976). However, when Tamm et al. (2009) studied the extreme morning and evening chronotypes, only the evening types showed significant diurnal rhythm in force production. EMG recording was mimicking force showing higher values in the evening for the evening types and no morning to evening differences for the morning types. Based on the EMG data, they proposed that the increased central nervous system drive to the triceps surae could explain the higher force values in the evening recorded in the evening type group. No diurnal variations were observed in VA% for the morning or evening types. This difference between our study and the investigation by Tamm et al. (2009) may come from the fact that small muscle groups such as triceps surae may be more easily maximally activated than larger muscle groups such as the quadriceps (Behm et al., 2002). In addition to that it needs to be kept in mind that in the study by Tamm et al. (2009) the morning and evening types were selected based on the chronotype questionnaire. In addition, it is possible that the calf muscles which are frequently used during daily activities may show dissimilar diurnal patterns compared to the knee extensors muscles (Guette et al., 2006). We propose that in the present study, simultaneous modifications in the neural and muscular mechanisms in the evening led to increased ability to generate force during MVC in the high evening performance group. It seems that in the high morning performance types and neutral types peripheral rather than central factors affect diurnal variations as central motor drive did not show significant time of day effect. However, the mechanisms explaining the diurnal fluctuations in maximal isometric force in these groups need further investigation.

In addition to central mechanisms, many studies have proposed that diurnal rhythms in body temperature can explain the fluctuation in force (Coldwells et al., 1994; Teo et al., 2011), since the acrophases of these two rhythms are simultaneous (Racinais et al., 2005). As listed in Bambaeichi et al. (2004) higher body temperature enhances conduction velocity of action potentials, enzymatic activity and extensibility of connective tissue but reduces muscle viscosity and antagonistic co-contraction. Although others have found positive correlations between muscle torque and body temperature, it cannot be concluded that these two variables depend on a common synchronizer or influence each other (Callard et al., 2000). Similar to Callard et al. (2000), the amplitudes of the rhythm in body temperature were too low in this study to account for the changes in maximal force production. Reilly & Waterhouse (2009) proposed that intra-aural body temperature may not be reliable because of temperature changes produced at the measurement site by air flow past the recording site. However, also other studies have used intra-aural body temperature measurements (Atkinson et al., 2005; Castaingts et al., 2004) and found the results to be similar to those by aural measurements. In the present study the diurnal changes in the body temperature were smaller compared to the previous studies (Castaingts et al., 2004; Gauthier et al., 2001). This may refer that the intra-aural temperature measurement technique used in the present study may have been not reliable enough to assess the diurnal fluctuations in the body temperature.

In addition to changes in body temperature, the central nervous system arousal associated with the sleep-wake cycle may influence force production over a day (Birch & Reilly, 2002). The amount of sleep on the night before the measurements and the time between awakening and tests was not documented in the present study. It has been previously shown that the sleep deprivation might temporally change performance patterns, however, Bambaeichi et al. (2004) found that the diurnal fluctuations in muscle strength did not change after partial sleep loss. Moreover, the time awake (associated with fatigue) and sleepiness has been shown to influence the time-of-day-specific performance (Araujo et al., 2011; Carrier and Monk., 2000; Edwards et al., 2007), as performance efficiency on a specific task may decrease over the day because of the amount of hours since awakening (Carrier and Monk, 2000).

Also Edwards et al. (2013) have suggested that several factors may account for circadian variations in muscle strength, including effects of motivation, subjective arousal and sleepiness, hormones (thyroid hormones, T/C ratio) and ionic changes. In the present study, the analysis of serum hormone concentrations revealed that diurnal variation in serum testosterone and cortisol was similar to those found in previous studies (Hayes et al., 2012), with the highest values observed in the morning for both hormones. However, our results failed to show any significant relationships between the daily changes in peak force and concentrations of serum testosterone, cortisol or T/C ratio and, thereby, reconfirmed what has been reported by other studies (Hayes et al., 2013; Teo et al., 2011). Our high morning performance group and high evening performance group showed significantly different morning to evening change in the T/C ratio. The high evening performance types showed significantly larger morning to evening fluctuation in the T/C ratio compared to the high morning performance types. This indicates that the high evening performance types may have been in a more desirable physiological condition for strength performance in the evening compared to the morning (Hayes et al., 2013). However, as the strength performance and hormonal data was collected on different days, we have to be careful with the conclusions.

This is the first study observing the inter-individual differences in maximal voluntary force production in the condition were extreme chronotypes were excluded from the sample. So far the efforts to determine the circadian phenotype and thereby the time-of-day preferences, are based on the scores obtained from the chronotype questionnaires, rather than in actual timing (phase of entrainment). These questionnaires assess the self-reported preferences to perform certain activities and should provide the data about when physical function, hormone levels, body temperature, cognitive faculties and eating and sleeping patterns are active (Levandovski et al 2013). In the present study, the high morning and high evening performance types showed in addition to significant differences in force production, also distinct rhythms in myoelectric activity and serum T/C. This means that persons not belonging to the extreme chronotypes may still demonstrate either high morning or high evening strength performance. Therefore, the questionnaires designed to determine the chronotype may not always be sensitive enough to determine the morningness or eveningness in maximal neuromuscular performance. Our results indicate that the ability to produce maximal voluntary force is not related to the morningness-eveningness determined by the chronotype questionnaire.

In summary, diurnal variations in strength performance are likely to be multi-factorial and the mechanisms behind the diurnal rhythms seem to vary between the individuals as suggested also by the previous studies (Chtourou & Souissi, 2012; Giacomoni et al., 2005). The present results from the total group of participants are in accordance with previous studies, showing that isometric knee extension force is higher in the evening when compared to the morning (Guette et al., 2005a; Nicolas et al., 2005; Sedliak et al., 2008). However, we were able to identify the high morning performance types who showed significantly higher voluntary maximal isometric force levels in the morning and the high evening performance types who showed large morning to evening fluctuations in knee extensor force with higher values observed in the evening. As to the mechanisms, central factors seem not to be exclusively responsible for these daily variations but possibly peripheral mechanisms were also affecting the diurnal fluctuation in maximal strength performance. Nevertheless, in the high evening performance group the diurnal fluctuations in maximal force were associated with accompanying diurnal variations in the central activation. The mechanisms behind high morning performance in the high morning performance types, however, need further investigation. This means that persons not belonging to the extreme chronotypes may still have the ability to demonstrate either high morning or high evening strength performance and chronotype questionnaires may not be sensitive enough to predict the best time for physical performance. In the future also training-induced adaptations should be studied for the persons showing high morning or evening strength performance.

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