Menstrual cycle associated modulations in neuromuscular function and fatigability of the knee extensors in eumenorrheic females.

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ABSTRACT

Sex hormone concentrations of eumenorrheic females typically fluctuate across the menstrual cycle and can affect neural function such that oestrogen has neuro-excitatory effects, and progesterone induces inhibition. However, the effects of these changes on corticospinal and intracortical circuitry, and the motor performance of the knee-extensors, are unknown. The present two-part investigation aimed to i) determine the measurement error of an exercise task, transcranial magnetic stimulation (TMS) and motor nerve stimulation (MNS) derived responses in females ingesting a monophasic oral contraceptive pill (hormonally-constant), and ii) investigate whether these measures were modulated by menstrual cycle phase (MCP), by examining them before and after an intermittent isometric fatiguing task (60% of maximal voluntary contraction, MVC) with the knee-extensors until task failure in eumenorrheic females on days 2, 14, and 21 of the menstrual cycle. The repeatability of neuromuscular measures at baseline and fatigability ranged between moderate-excellent in females taking the oral contraceptive pill. Maximal voluntary contraction was not affected by MCP (P=0.790). Voluntary activation (MNS and TMS) peaked on day 14 (P=0.007 and 0.008, respectively). Whilst corticospinal excitability was unchanged, short-interval intracortical inhibition was greatest on day 21 compared to days 14 and 2 (P=0.001). Additionally, time to task failure was longer on day 21 compared to both days 14 and 2 (24 and 36%, respectively; P=0.030). The observed changes were larger than the associated measurement errors. These data demonstrate that neuromuscular function and fatigability of the knee-extensors varies across the menstrual cycle, and may influence exercise performance involving locomotor muscles.

Keywords: corticospinal excitability, fatigue, intracortical inhibition, menstrual cycle, voluntary activation

NEW AND NOTEWORTHY

The present two-part study first demonstrated the repeatability of transcranial magnetic stimulation and electrical motor nerve stimulation evoked variables in a hormonally-constant female population. Subsequently, it was demonstrated that the eumenorrheic menstrual cycle affects neuromuscular function. Changing concentrations of neuroactive hormones corresponded with greater voluntary activation on day 14, greater intracortical inhibition on day 21, and lowest fatigability on day 21. These alterations of knee extensor neuromuscular function have implications for locomotor activities.

INTRODUCTION

The cyclical changes in concentrations of multiple sex hormones, including oestrogen and progesterone (55), across the eumenorrheic menstrual cycle can affect central nervous system (CNS) function due to their ability to cross the blood-brain barrier (62). *In vitro* models have shown direct evidence for the effect of sex hormones on neuronal function. For instance, oestradiol (an oestrogenic steroid hormone) binds to oestrogen receptor α (ER-α) sites on *γ*-aminobutyric acid (GABA) mediated neurons, causing an attenuation in GABA synthesis and release (54, 70). Additionally, oestrogen potentiates the effects of excitatory glutamatergic (both NMDA and non-NMDA) receptors (61), resulting in a net excitatory effect. Additionally, oestrogen has been shown to decrease firing thresholds and increase discharge frequency of cerebral neurons (58, 72). On the contrary, progesterone has a net inhibitory effect on the nervous system, as the activity and effects of GABA are potentiated, leading to decreased neuronal discharge rate (60) and increased inhibition of pyramidal neurons in rats (37). Some evidence also suggests that the presence of progesterone directly antagonises estrogenic actions by lowering the available ER-α and -β receptor numbers on various sites of neuronal cells (47). Indeed, as Smith and Woolley (61) outlined, the neurosteroidal actions of hormones act upon the neurotransmitter receptors. Thus, given GABAergic and glutamatergic synapses are located within the motor cortex (43), a hormonal effect would be expected

The differential effect of oestrogen and progesterone concentrations on indices of nervous system excitability have also been established in humans. Transcranial magnetic stimulation (TMS) studies show increased intracortical excitability and reduced intracortical inhibition in the late-follicular phase, when oestradiol concentration is high and progesterone low (56, 57), substantiating the alterations seen in the aforementioned

in vitro studies. While these *in vivo* studies show clear changes in human CNS function, they were conducted in the resting upper limbs, specifically in hand muscles associated with fine motor control. However, properties of intracortical and corticospinal circuits vary between upper and lower limb projections (7, 15). Thus, the menstrual cycle associated modulations in neural function of a small, upper limb muscle group cannot be extrapolated to larger, lower limb muscle groups. Understanding how the menstrual cycle affects the neural control of large locomotor muscle groups has significant implications for everyday locomotive tasks, injury rehabilitation, and athletic performance. For instance, neuroplasticity following stroke (17), and strength training (71) are influenced by GABAergic inhibition.

To date, there is minimal research investigating menstrual cycle induced changes in nervous system and motor function of the knee extensors (KE). Previous studies investigating motor function, such as the ability to produce maximum voluntary contraction force (MVC), are equivocal, with studies showing 8-23% greater maximal force with the KE mid-cycle (4, 53, 64). Multiple studies however, report no difference in maximal strength (20, 24, 40, 46). The studies that have shown changes in maximal strength have suggested that mechanisms such as motor unit firing rates (65) and intracortical excitability (56, 57) could be contributing factors. However, the proposed mechanistic factors, and the neuromuscular response (e.g. MVC), have not been concurrently studied. Voluntary activation (VA) of the quadriceps has been assessed using motor nerve stimulation twice with no menstrual cycle effect shown (40, 46). However, as it is thought that the assessment of VA using TMS (VA_{TMS}) reflects the ability of the motor cortex to activate the motor units within the target muscle group (68), if supraspinal properties are modulated by the menstrual cycle (i.e. 56, 57), VA_{TMS} could

provide a more appropriate measure to discern whether the ability to voluntarily activate the KE is affected.

Other aspects of motor performance, such as performance fatigability (38), have also been studied throughout the menstrual cycle with inconclusive results. Sarwar et al. (53) showed that the KE of eumenorrheic females were less fatigable in the luteal phase during an electrically stimulated isometric fatiguing protocol. However, this finding has not been corroborated with dynamic, voluntary contractions performed with the KE (21, 40). Additionally, none of the aforementioned studies were open-ended, with a fatigue index calculated after a set amount of time/contractions. Thus, due to the causes of fatigability being task specific (66), discrepancies in the aforementioned investigations could be due to the differences in fatiguing protocols used and their respective limiting factors.

The effect of hormonal fluctuations on neuromuscular function and fatigability of the KE remains unclear. Conflicting literature exists for the majority of neuromuscular variables, despite a rationale for change based on neuroendocrine and upper-limb studies. The inclusion of statistical measures of error are recommended for investigations utilising methods of neurostimulation to inform the contribution of random variation to any modifications in neuromuscular function (29). Therefore, the present investigation recruited a population of monophasic oral contraceptive (mOCP) users to discern test-retest repeatability of neuromuscular function measures without the influence of endogenous hormones (*Study A*). The consistent dosage of exogenous oestrogen and progesterone in the mOCP precludes ovulation (28), creating a physiological environment in which the effects of endogenous hormones are negated. Thereafter, *Study B* aimed to investigate KE neuromuscular function and fatigability across the menstrual

cycle. It was hypothesised that when oestrogen levels increased (and progesterone remained low) from day 2 to 14, maximum force production would concomitantly rise, alongside a reduction in intracortical inhibition and an increase in VA. Secondly, it was hypothesized that the rise in progesterone from day 14 to 21 would occur alongside a reversal in these changes, and improved time to task failure (TTF).

METHODS

Ethical Approval

The study received institutional ethical approval from the Northumbria University Health and Life Sciences Research Ethics Committee (HLSPA301116) and was conducted according to all aspects of the Declaration of Helsinki, apart from registration in a database. Participants provided written, informed consent to volunteer for the study

Participants

A total of 30 participants volunteered to participate in the study. Fifteen mOCP users (age: 23 ± 2 years; stature: 170 ± 6 cm; mass: 70.6 ± 8.5 kg) and fifteen eumenorrheic females (age: 25 ± 4 years; stature: 169 ± 6 cm; mass: 68.3 ± 7.8 kg; mean cycle duration: 29 ± 3 days, range: 24-34 days). The mOCPs reported taking an mOCP for at least 6 months as prescribed (i.e. a seven-day break after every 21-day pill consumption period), whereas eumenorrheic females reported having regular cycles without using any form of hormonal contraceptives for at least six months. A full list of the mOCPs taken by participants is presented in Table 1. Participants arrived at the laboratory rested and hydrated, with strenuous physical activity avoided for 48 hours, and caffeine and alcohol prohibited for 24 hours.

TABLE 1 HERE

Experimental Design

Study A

Oral contraceptive users visited the laboratory three times, completing a familiarisation and two experimental visits. Experimental visits were completed during the final 14 days

of the pill cycle, with a minimum of 48 h between visits to allow recovery (13). The visits were identical to those described below for *Study B*, however, blood sampling was not performed as it is established that endogenous hormone concentrations do not fluctuate throughout the consumption phase of the mOCP cycle (10).

Study B

Eumenorrheic females visited the laboratory four times, completing a familiarisation session prior to three experimental visits. Participants completed experimental visits on days 2 (D2, Early-follicular), 14 (D14, Late-follicular), and 21 (D21, Mid-luteal) of the menstrual cycle. Testing days were counted from the onset of menstruation and, to verify menstrual cycle phase, fasted venous blood samples were taken between the hours of 0600 – 0900 on testing days to analyse serum oestradiol and progesterone concentrations. The order of visits was pseudorandomized and counterbalanced to minimize order effects, with five participants beginning on each testing day (D2, D14 or D21). All testing visits occurred within the same menstrual cycle (order: D2, D14, D21), or two consecutive cycles (order: D14, D21, D2 or D21, D2, D14). Experimental visits consisted of a baseline neuromuscular assessment, intermittent, isometric contractions at 60% MVC until task failure, followed immediately by a post-task neuromuscular assessment. The intensity for the fatiguing task was likely far greater than the critical torque (~30% MVC [12]), and therefore an unsustainable intensity, with task failure attributable to decrements in neuromuscular function (1, 11).

Experimental Procedures

For *Study B*, upon arriving between the hours of 0600 – 0900, fasted venous blood samples were taken following 10 minutes of seated rest. Participants were then

instructed to consume a typical breakfast and return to the laboratory at their designated testing time. The breakfast and time of testing were replicated (± 1 hour) for each experimental visit to control for diurnal variations in corticospinal excitability and maximal force production (63). Time of testing was also controlled for in *Study A*, with participants consuming the mOCP a constant time before trials in order to standardise circulating exogenous hormone concentrations between visits. In both studies, experimental sessions began with participants completing a standardised voluntary isometric contraction warm up (2 × contractions at 25, 50, and 75% perceived maximal effort) followed by a baseline neuromuscular assessment (described below). The fatiguing task involved sets of intermittent isometric contractions (3 s contraction, 2 s rest at 60% MVC) to task failure. Contractions were paced with an audible metronome to ensure the duty cycle was maintained. One set was defined as 11 submaximal contractions followed by a 3 s MVC with motor nerve stimulation (MNS) delivered at the peak force and 2 s post, lasting one minute. Task failure was defined as an inability to reach the target force three times at any stage of the protocol. Rating of perceived exertion (RPE; 6) was recorded using a 6-20 scale following each MVC throughout the fatiguing task. Real-time visual force feedback using target forces set as percentages of maximum force was provided to participants on a computer screen to aid a constant force level. The post-task neuromuscular assessment began immediately following task failure.

Neuromuscular Assessments

Measures of neuromuscular function were assessed pre- and post- exercise with MNS of the femoral nerve, and TMS of the contralateral motor cortex at rest and during voluntary contractions of the right knee-extensors. Pre-exercise neuromuscular assessments began with two practice MVCs to ensure potentiation of subsequent evoked measures, followed

by three ~3 s MVCs, all separated by 30 s. During these 3 MVCs, MNS was delivered when peak force plateaued, and then ~2 s after the MVC to measure voluntary activation (VA_{MNS}) and potentiated twitch amplitude ($Q_{tw.pot}$) of the knee-extensors. Single-pulse TMS was subsequently delivered during two sets of five 3-5 s contractions at 100, 87.5, 75, 62.5 and 50% MVC, with 5 s rest between contractions and 10 s rest between sets, to determine VA_{TMS} (19). The TMS silent period (SP) was determined during the 50% MVC contraction of each set. Participants were instructed to maintain a constant force on the guideline and "push through the stimulation" (52). Finally, ten single- and ten paired-pulse TMS stimulations were delivered during a 10% MVC contraction in an alternate order to determine corticospinal excitability and short-interval cortical inhibition (SICI), respectively. The neuromuscular assessment was repeated immediately post-exercise. Measures of neuromuscular function (MVC, $Q_{tw.pot}$, VA_{MNS}) were measured within 30 s of task failure, and VA_{TMS} measured within 2-2.5 minutes, in an attempt to minimise the dissipation of fatigue, however it is possible that the sensitivity of these measures was compromised due to the rapid recovery of central fatigue post-exercise (32).

Force and Electromyographical Recordings

During assessments of neuromuscular function and fatiguing tasks, participants sat on a custom-built chair with knee and hip angles kept constant (both 90° flexion). A calibrated load cell (MuscleLab force sensor 300, Ergotest technology, Norway) was attached via a non-compliant cuff positioned 2 cm superior to the ankle malleoli on the participants' right leg, to measure knee extensor force (N). Surface Ag/AgCl electrodes (Kendall H87PG/F; Covidien, Mansfield, MA) were placed over the *rectus femoris* (RF), and *biceps femoris* (BF) with a 2 cm inter-electrode distance, to record the compound muscle action potential (M-wave) elicited by the electrical stimulation of the femoral nerve, the MEP

elicited by TMS, and the root-mean-square amplitude during isometric contractions (rmsEMG). Electrode placement was consistent with SENIAM guidelines (35), and a reference electrode was placed over the patella. Prior to placement, the skin-electrode contact area was cleaned using a 70% IPA alcohol wipe (FastAid, Robinson Healthcare, Worksop, UK). Signals were amplified: gain ×1000 for EMG and ×300 for force (CED 1902; Cambridge Electronic Design, Cambridge, UK), bandpass filtered (EMG only: 20–2000 Hz), digitized (5 kHz; CED 1401, Cambridge Electronic Design), and analysed offline (Spike2 v8, Cambridge Electronic Design).

Motor Nerve Stimulation

Single electrical stimuli (200 μs duration) were delivered to the right femoral nerve using a constant current stimulator (DS7AH Digitimer Ltd, Welwyn Garden City, UK) via adhesive surface electrodes (CF3200; Nidd Valley Medical Ltd., Harrogate, UK). The cathode was placed over the nerve, high in the femoral triangle, in the position that elicited the greatest twitch amplitude (Q_{tw}) and M-wave in the RF at rest. The anode was placed halfway between the greater trochanter and iliac crest. Optimum stimulus intensity was determined as the minimum current that elicited maximum values of Q_{tw} and M-wave (M_{max}) at rest. To ensure a supramaximal stimulus, the optimum stimulus intensity was increased by 30% and was not different between trials in either study (*A*: 230 ± 61 vs. 241 ± 65 mA, $P = 0.271$; *B:* 233 ± 72, 244 ± 68, and 262 ± 67 mA, $P = 0.125$).

Transcranial Magnetic Stimulation

Single and paired pulse stimuli (1 ms duration) were delivered to the contralateral (left) motor cortex via a concave double cone coil (110 mm diameter, maximum output 1.4 T) powered by two linked monopulse stimulators (Magstim Bistim and Magstim^{200}, The

Magstim Company, Whitland, UK). Optimal coil placement was determined as the position that elicited the greatest RF motor evoked potential (MEP) with concomitant smallest antagonist (BF) MEP during a 10% MVC at 50-70% stimulator output. This position was marked on the scalp with indelible marker to ensure consistent placement during trials. Stimulator intensity for VA_{TMS} was determined as the intensity that elicited the greatest superimposed twitch (SIT) during a contraction at 50% MVC. Stimulator intensity was increased in 5% intervals from 50% stimulator output and two stimuli were delivered during a ~5 s contraction, with the mean of two SITs recorded (9, 18). Mean stimulator intensity was not different between trials in either study (*A*: 67 ± 10 vs. 66 ± 10%, $P = 0.737$; *B*: 63 ± 10, 63 ± 11, and 63 ± 12%, $P = 0.984$). The stimulator output activated a large proportion of the KE motoneuron pool at baseline in each experimental visit with no difference between trials in *Study A* (69 ± 35 vs. 68 ± 36% M_{max} amplitude, $P = 0.916$) or *Study B* (61 ± 17; 57 ± 17; 55 ± 14% M_{max} amplitude; $P = 0.788$). Small co-activation of the antagonist muscle (BF) was observed in response to TMS and did not differ between trials in *Study A* (0.60 ± 0.37 vs. 0.72 ± 0.53 mV, $P = 0.106$) or *Study B* (0.53 ± 0.39, 0.71 ± 0.42 and 0.60 ± 0.34 mV, $P = 0.211$).

Active motor threshold (aMT) was determined as the stimulator intensity that elicited a MEP of > 200 μV in three out of five stimulations during a 10% MVC contraction. Stimulator intensity was increased in 5% steps from 35% of stimulator output until a consistent MEP amplitude >200 μV was found. Thereafter, stimulus intensity was reduced in 1% steps until the lowest intensity to elicit a MEP of >200 μV was found. aMT was not different on any testing visit in *Study A* (40 ± 6 vs. 40 ± 7%, $P = 0.746$) or *Study B* (43 ± 9, 42 ± 8, and 43 ± 9%, $P = 0.874$). SICI was assessed with ten paired and ten single pulse stimulations delivered. Paired-pulse TMS consisted of a conditioning pulse at 70%

of aMT, and a test pulse at 120% aMT, with an inter-stimulus interval of 2 ms. All stimuli were delivered during a 10% contraction. This paradigm has previously been demonstrated as the optimal configuration for eliciting SICI in the KE (8), and has been used previously in our laboratory in male populations (31). Two sets of 10 stimuli were used, with a 10 s rest between contractions.

Blood Sampling and Hormone Analysis (*Study B only*)

Venous blood sampling was performed on the morning of each testing session. A 10 mL blood sample was drawn from an antecubital vein into a silica coated tube by a trained phlebotomist, then left upright for 15 minutes to coagulate before centrifuging. Samples were centrifuged at 2,500 rpm for 10 minutes at room temperature (Allegra-X22R, Beckman Coulter, USA). Using a 500-1000 μl pipette, the supernatant serum was separated into three aliquots (~1000 μl each) and stored at −80°C until oestradiol and progesterone analysis were performed. Total concentrations of 17-β oestradiol and progesterone were measured in duplicate using hormone-specific enzyme-linked immunoassay kits (Cayman Chemical, Ann Arbor, MI). All samples were analysed using the ELISA technique with absorbance detection (wavelength 405 nm). The minimal oestradiol and progesterone detection was 15 pg/ml and 7.5 pg/mL, respectively. To calculate 17-β oestradiol and progesterone levels, a standard curve was plotted using eight standards against their absorbance. Using the mean absorbance from the duplicate of each sample, the concentration of the sample was interpolated directly from the standard curve. The coefficients of variation (CV) for the ELISA kits, as provided by the manufacturer, were 8-12% for 17-β oestradiol, and 5-8% for progesterone. In one instance, the CV of a duplicate sample exceeded the manufacturer's CV due to an excessively high (non-physiological) reading in one well. Therefore, the lower of the two was used for data analysis.

Participants' hormonal profiles were deemed 'acceptable' when a peak in progesterone concentration was observed during the luteal phase (D21) and an increase in 17-β oestradiol was observed from D2 to D14. If neither peak was observed, then participants were deemed anovulatory and excluded from further analyses.

Data Analysis

Voluntary activation using motor nerve stimulation was determined using the ITT (48) by comparing the amplitude of the superimposed twitch (SIT) with the amplitude of the potentiated resting twitch ($Q_{tw.pot}$) using the following formula: VA_{MNS} (%) = (1 – [SIT ÷ $Q_{tw,pot}$]) × 100]. Voluntary activation using TMS was assessed during two sets of contractions at 100, 87.5, 75, 62.5 and 50% MVC (19). Single pulse TMS was delivered during each contraction, and the linear regression between SIT amplitude and contraction intensity was extrapolated to the *y* intercept to obtain an estimated resting twitch (ERT; 68) . In order to achieve significant linearity ($P < 0.05$), a total of 4 out of 300 SITs across all trials in *Study A* were excluded (1.3%), which led to four regressions containing 9 data points rather than 10 (1 pre-exercise, 3 post-exercise). In *Study B*, six out of 870 SITs were excluded from all linear regressions (0.7%), meaning that there were 86 ten point regressions, three nine point regressions, and one eight point regression used to estimate resting twitches. Mean r^2 values for ERTs in *Study A* were 0.94 ± 0.04 pre exercise vs. 0.94 ± 0.04 post exercise, and in *Study B* were 0.92 ± 0.05 pre exercise, and 0.89 ± 0.07 post-exercise. The SIT during 100% MVC was compared with the ERT using the following formula: VA_{TMS} (%) = (1 – [SIT ÷ ERT]) × 100. SICI was quantified as the percentage ratio between the amplitude of conditioned MEPs to the amplitude of unconditioned MEPs. Corticospinal excitability was determined by expressing the mean MEP amplitude during the 10% MVC as a percentage of M_{max}. The

root-mean-square of EMG activity (rmsEMG) was recorded during the middle 500 ms epoch of each 3 s contraction during the fatiguing task. rmsEMG was then expressed as a percentage of M_{max}. For the data presented as %TTF, the MVC, VA_{MNS}, and $Q_{tw.pot}$ for the nearest minute during the fatiguing protocols were taken, and the average rmsEMG for the nearest full set of contractions to the target percentage (i.e. 25, 50, or 75% TTF) was taken, for 0 and 100% TTF, the first and last complete set was used. All data analysis was performed offline. In *Study* B, despite a rigorous familiarisation and verbal encouragement, one participant failed to maintain the intermittent contractions for the required 3 s during the fatiguing task, thus invalidating the TTF duration. Therefore, it was deemed appropriate to remove the participant's TTF duration and post-trial neuromuscular assessment from further analysis (n = 14), however, baseline data was included for statistical analysis (n = 15).

Statistical Analysis

Data are presented as mean ± SD within the text and figures. Normal Gaussian distribution of data was confirmed using the Kolmogorov–Smirnov test. If a violation was detected, the data was logarithmically transformed. The alpha for all statistical tests was set at $P \leq 0.05$.

For Study A, between session and pre-post exercise differences were explored using two-way (2 × 2) repeated measures ANOVAs, if assumptions of sphericity were violated, then the Greenhouse-Geisser correction was applied. If significant main or interaction effects were detected, Bonferroni-corrected post hoc tests were performed. For between session test-retest reliability multiple indices were calculated (paired samples t-tests, typical error, intraclass correlation coefficient, 2, 36) between the two time points. Within-

subjects variation was calculated as the standard deviation of the mean differences divided by the square root of 2, and termed typical error (TE) throughout the manuscript. Typical error was expressed as absolute raw values and as a percentage of the mean (coefficient of variation, CV). Intraclass correlation coefficients ($ICC_{3,1}$) were calculated according to Bland and Altman (5). ICC values were defined as follows: <0.5 = poor, 0.5-0.75 = moderate, 0.75-0.9 = good, >0.9 = excellent (45). Due to the ceiling effect (i.e. all values grouped close to 100%) associated with VA_{MNS} and VA_{TMS}, the ICCs were not calculated (16, 67).

For study B, one-way repeated measures ANOVAs were run for all pre-exercise dependent variables to assess MCP changes in neuromuscular function and hormone concentrations. Sphericity was assessed using Mauchly's test and if necessary, controlled using the Greenhouse-Geisser correction. Two-way repeated measures ANOVAs were run using pre and post exercise variables to obtain both fatigue, and MCP × fatigue interaction effects. To explore potential differences in the fatigue profiles of neuromuscular and perceptual variables, two-way repeated measures ANOVAs were run including data points from baseline, 25%, 50%, 75%, and 100% of TTF. Significant main and interaction effects were explored using Bonferroni-corrected tests.

RESULTS

Study A

Exercise performance and pre-post exercise changes

The TTF was not different between experimental visits (560 ± 275 s vs. 603 ± 357 s respectively, P = 0.314). When assessing exercise-induced changes in neuromuscular function, the two-way ANOVAs detected no between-trial differences in change scores

411 (trial × time interactions: $P \geq 0.331$), therefore to assess the pre-post change, data from
412 both visits were pooled. The MVC decreased pre-post exercise (time effect: 507 ± 95 vs.
413 379 ± 85 N; $F_{1,14} = 136.66$, $P < 0.001$, $\eta^2 = 0.91$). Similarly, indices of contractile function
414 ($Q_{tw.pot}$ and ERT) decreased pre-post trial ($Q_{tw.pot}$: 169 ± 24 vs. 109 ± 21 N; $F_{1,14} = 92.61$, P
415 < 0.001, $\eta^2 = 0.87$; ERT: 120 ± 36 vs. 93 ± 28 N; $F_{1,14} = 19.07$, $P = 0.001$, $\eta^2 = 0.56$). Indices
416 of VA also decreased pre-post trial: VA_{MNS} (93.6 ± 3.2 vs. 85.1 ± 6.8%; $F_{1,14} = 36.60$, $P <$
417 0.001, $\eta^2 = 0.72$) and VA_{TMS} (94.6 ± 3.1 vs. 83.1 ± 10.6%; $F_{1,14} = 20.82$, $P < 0.001$, $\eta^2 = 0.60$).
418 Corticospinal excitability (MEP/M_{max}) was not different pre-post exercise ($P = 0.057$).
419 There were no changes in SICI pre-post exercise (80.8 ± 14.2 vs. 79.8 ± 13.9%, $P = 0.667$),
420 whereas SP duration lengthened (189 ± 46 vs. 202 ± 50 ms, $F_{1,14} = 5.49$, $P = 0.034$, $\eta^2 =$
421 0.28). Lastly, M_{max} was not different pre-post exercise (3.02 ± 1.19 vs. 2.81 ± 1.01 mV, $P =$
422 0.362).
423
424 TABLE 2 HERE
425
426 *Reliability of neuromuscular measures*
427 Pre-exercise data from mechanical variables (Table 2) showed good (ERT, and TTF) and
428 excellent (MVC, and $Q_{tw,pot}$) reliability. The TE and CV were also low for the majority of
429 variables (CV ≤ 12.5%), except TTF (CV = 20.0%). Post-exercise reliability (Table 2) was
430 weaker, but still interpreted as predominantly good ($Q_{tw,pot}$, ERT) or excellent (MVC).
431 These values were all poorer post-exercise; however, remained relatively low (CV ≤
432 14.9%). The relative reliability (ICCs) of the pre-post change was either moderate (MVC,
433 ERT, VA_{MNS}) or good ($Q_{tw,pot}$), however there was a high degree of random error (CV
434 range: 19.7 – 62.7%).
435

436 TABLE 3 HERE

437

438 Surface EMG variables (Table 3) showed moderate (MEP/M_{max}, SP) or good (SICI, M_{max})
439 reliability pre-exercise, but with larger test-retest CVs than mechanical variables (range:
440 9.2 – 30.1%). Post-exercise reliability was similar to pre- for most variables, with ICCs
441 either moderate (MEP/M_{max}, SP) or good (M_{max}), and comparable CVs (range 13.0 –
442 31.0%). Despite this, the post-exercise reliability of SICI was poor (ICC = 0.42), which was
443 further supported by a significant bias between visits 1 and 2 (−9.1%, P = 0.031). When
444 the pre-post change was significant for a variable, i.e. SP, the relative reliability of change
445 value was deemed poor (ICC = 0.44), with a high degree of random error (CV: 155.1%).
446

Study B

Hormonal Profiles

TABLE 4 HERE

Thirteen out of 15 participants presented a regular hormonal profile (see Table 4). Two participants had no increase in progesterone on D21; given the hypothesis that changing hormone concentrations would modulate neuromuscular function, and these participants did not exhibit any change in hormone concentrations, they were excluded from further statistical analyses. The repeated measures ANOVAs showed an effect of MCP on 17-β oestradiol ($F_{1.4,19.5}$ = 3.55, P = 0.040, η_p^2 =0.18) and progesterone concentration ($F_{=1.0,14.1}$ = 8.35, P = 0.012, η_p^2 =0.37). Post hoc tests revealed that 17-β oestradiol concentrations were greater on D14 compared to D2 (P = 0.033), and greater on D21 than D2 (P = 0.029). Progesterone was greater on D21 than D2 and D14 (P = 0.011, and 0.012, respectively).

Baseline Neuromuscular Function

FIGURE 1 HERE

MVC force was unaffected by MCP (Figure 1A, $F_{1.4,16.8}$= 0.15, P = 0.790, η_p^2 = 0.01). Potentiated twitch force was also unchanged (Figure 1B, $F_{2,24}$ = 0.25, P = 0.782, η_p^2 = 0.02); however, the SIT elicited by MNS was affected by MCP ($F_{2,28}$ = 3.69, P = 0.040, η_p^2 = 0.24), with greater SITs on D14 compared to D2 (mean difference: 2 N, P = 0.031). The reduced SIT on D14 meant that VA_{MNS} was affected by MCP (Figure 1C, $F_{2,28}$ = 9.23, P = 0.001, η_p^2

= 0.44) with post hoc tests showing greater VA_{MNS} on D14 compared to D2 (mean difference: 1.9%, P = 0.007), however, there was no difference between D14 and D21 (mean difference: 1.0%, P = 0.059). VA_{TMS} was also affected by MCP (Figure 1D, $F_{2,28}$ = 5.89, P = 0.008, η_p^2 = 0.33) with greater values on D14 compared to D21 (mean difference: 3.0%, P = 0.016), however, D14 and D2 were not different (mean difference: 2.5%, P = 0.080). Despite the change in VA_{TMS}, neither of its constituent parts were altered by MCP: ERT ($F_{1.3,15.3}$ = 0.25, P = 0.784, η_p^2 = 0.02) and SIT elicited by TMS ($F_{1.3,15.3}$ = 2.17, P = 0.136, η_p^2 = 0.15).

FIGURE 2 HERE

As shown in Table 3, M_{max} was unaffected by MCP ($F_{2,28}$ = 0.24, P = 0.786, η_p^2 = 0.02), nor was normalized MEP amplitude (Figure 2A, $F_{2,28}$ = 2.24, P = 0.129, η_p^2 = 0.16). However, SICI was affected (Table 5 and Figure 2B, $F_{1.4, 16.8}$ = 13.52, P < 0.001, η_p^2 = 0.53) with post hoc tests showing greater inhibition on D21 compared to D2 (mean difference: −10%, P = 0.048) and D14 (mean difference: −14%, P = 0.001). The pre-stimulus normalised rmsEMG activity was not different between MCPs (D2: 1.16 ± 0.43, D14: 1.07 ± 0.53, D21: 1.20 ± 0.64% M_{max}, $F_{2,28}$ = 0.31, P = 0.736, η_p^2 = 0.025) and neither was the SP (Figure 2C, Table 5, $F_{2,28}$ = 0.53, P = 0.594, η_p^2 = 0.04).

FIGURE 3 HERE

Fatigability

Time to task failure during the intermittent, isometric, fatiguing task was significantly affected by MCP (see Figure 3, $F_{1.4, 14.8} = 6.89$, $P = 0.030$, $\eta_p^2 = 0.32$), with post hoc tests showing greater TTF on D21 compared to D2 (mean difference: 187 s, $P = 0.025$). However, there was no difference between D21 and D14 (mean difference: 135 s, $P = 0.103$), or D2 and D14 ($P = 0.594$). The two-way ANOVA (MCP × time) time effect showed that MVC decreased pre-post exercise ($F_{1,11} = 80.056$, $P < 0.001$, $\eta_p^2 = 0.88$), as did $Q_{tw.pot}$ ($F_{1,11} = 123.53$, $P < 0.001$, $\eta_p^2 = 0.92$), VA_{MNS} ($F_{1,11} = 15.219$, $P = 0.002$, $\eta_p^2 = 0.58$), and VA_{TMS} ($F_{1,11} = 13.99$, $P = 0.003$, $\eta_p^2 = 0.56$). SP also increased pre-post exercise ($F_{1,11} = 9.68$, $P = 0.010$, $\eta_p^2 = 0.468$). The MCP × time interaction effects for the aforementioned variables that changed pre-post exercise indicated no difference between MCPs (all $P \geq 0.128$). The only exception to this was VA_{TMS} ($F_{2,22} = 3.48$, $P = 0.049$, $\eta_p^2 = 0.24$), however, post hoc tests revealed that the differences were only apparent pre-exercise (as indicated above), and not post-exercise ($P \geq 0.670$). Despite no time effect ($P = 0.578$), SICI displayed a MCP × time interaction effect ($F_{1.4, 15.0} = 5.26$, $P = 0.028$, $\eta_p^2 = 0.32$), however, the only differences were evident pre-exercise (as indicated above), with no post-exercise difference ($P \geq 0.247$).

TABLE 5 HERE

All variables measured during the fatiguing tasks (see Figure 4) demonstrated time effects ($P \leq 0.024$), however, only some (MVC, $Q_{tw.pot}$, VA_{MNS}) demonstrated an absence of MCP × time interaction effects ($P \geq 0.205$). MVC (Figure 4A) decreased progressively from baseline to 75% TTF (all intervals $P \leq 0.001$), however, between 75% and 100% TTF no further decrease was observed ($P = 0.776$). A similar pattern was observed with $Q_{tw.pot}$

(Figure 4B), with decreases exhibited until 50% TTF (both intervals $P \leq 0.009$), however, between 50-100% TTF $Q_{tw.pot}$ did not further decrease ($P \geq 0.593$). VA_{MNS} (Figure 4C) demonstrated the inverse time course, with no change from 0 to 50% TTF ($P \geq 0.345$), then a progressive decrease from 50 to 100% TTF ($P \leq 0.034$). rmsEMG (Figure 4D) and RPE (Figure 4E) exhibited phase × time interaction effects ($P \leq 0.032$). RPE increased progressively throughout all trials ($P \leq 0.008$), however, at 25% TTF RPE was greater on D21 compared to D14 (+2, $P = 0.006$), at 50% TTF D21 was greater than D2 (+2, $P < 0.001$), and at 75% TTF D21 was greater than D2 (+1, $P = 0.005$). The only significant increase in rmsEMG was between 25 and 50% TTF ($P = 0.003$), and despite the phase × time interaction effect, no post hoc differences between phases were apparent ($P \geq 0.205$).

FIGURE 4 HERE

DISCUSSION

The present investigation aimed to assess the influence of modulations in female sex hormones across the eumenorrheic menstrual cycle on neuromuscular function and fatigability. The data from *Study A* established repeatability of the measures in a hormonally-constant female population (mOCP users). Subsequently, *Study B* showed that in eumenorrheic females, the hormone-induced changes in neuromuscular function and fatigability across the menstrual cycle were greater than the associated error from hormonally-constant females in *Study A*. Whilst one index of neuromuscular function (MVC) did not change, modulations in CNS control of muscle contraction were observed. Specifically, VA was greatest on D14 which was concurrent with an increase in the concentration of oestrogen. Additionally, parallel to an increase in progesterone, SICI was

greatest on D21. Time to task failure during the open-ended intermittent, isometric protocol was greatest on D21 of the cycle. Collectively, the present data suggest that neuromuscular function and fatigability are modulated by the eumenorrheic menstrual cycle.

Maximum strength and voluntary activation across the menstrual cycle

There was no effect of MCP on MVC force. As mentioned, previous data regarding maximum voluntary strength across the menstrual cycle is equivocal. In agreement with the present study, multiple studies have shown no effect (24, 40, 46), however, several studies have shown that strength peaks mid-cycle (49, 53, 64). Previously, discrepancies such as the time of day (4), or variability in menstrual cycle duration, as well as the chosen days of the menstrual cycle for testing (27) have been used as explanatory reasons for this discrepancy. The present study controlled these factors within Study B, by testing at the same time of day and confirming participants were in the correct phase by serum hormone analysis, yet no effect of MCP was observed.

Interestingly, Study B demonstrated changes in VA (assessed by both MNS and TMS) despite no change in MVC. VA_{MNS} peaked on D14 and VA_{TMS} was greater on D14 compared to D21 (see Figure 1). As $Q_{tw.pot}$ and ERT were not affected by MCP, these changes in VA were mediated by a decreased SIT amplitude on D14 in response to both motor nerve and motor cortical stimulation. This could indicate that there was a decrease in the capacity of the CNS to elicit extra force in response to stimulation. The TMS and MNS evoked SITs represent the extra force from motor units that the CNS is not able to voluntarily recruit or discharge at a sufficient rate (68). As acknowledged by Todd *et al*

571 (68), a change in SIT force could be caused by changes in the CNS altering activation of
572 the motoneuron, therefore changes within the motor cortex could provide an explanation
573 for the change in VA. An alternative explanation could be the magnitude of the respective
574 measurement errors of these variables. In *Study* A, when hormones were controlled, the
575 CVs of VA_{MNS} (1.7%) and VA_{TMS} (3.0%) are lower than the typical error for MVC (5.0%).
576 Whilst changes seen in the present data set (i.e. the 1.8% increase in VA_{MNS} between D2
577 – D14, or the 3.1% decrease in VA_{TMS} between D14 – D21) were similar to typical error,
578 it could be the case that the increase in VA was not large enough to elicit a detectable
579 increase in MVC due to its larger typical error. Previous studies that have shown VA_{MNS}
580 not to change have used the CAR equation (40, 46), which is less sensitive to change than
581 the ITT (50). It is likely therefore, that the magnitude of menstrual cycle effect on VA_{MNS}
582 and VA_{TMS} is marginally greater than the random error associated with the techniques
583 used to assess it, thus based on current evidence, the true effect is unclear. Also of note is
584 the MCP × time interaction effect for VA_{TMS}, which would indicate that the magnitude of
585 change from pre-post exercise was different between MCPs. However, this appears to be
586 driven by the increased VA_{TMS} pre-exercise on D14, as there were no differences in post-
587 exercise values. Therefore, it is unlikely that participants experienced a greater degree of
588 CNS adjustment following exercise during the late-follicular phase (D14).
589

590 *Corticospinal and intracortical function across the menstrual cycle*

591 As mentioned, the increase in both measurements of VA on D14 (VA_{MNS} and VA_{TMS}) could
592 represent changes in supraspinal properties altering synaptic drive to the motoneuron
593 pool across the menstrual cycle (51). To investigate the state of the corticospinal tract
594 and motor cortex, the present study employed single- and paired-pulse TMS. No

menstrual cycle effect was observed on corticospinal excitability, however, intracortical inhibition was increased on D21. Single pulse MEPs in the resting FDI muscle have previously been shown not to be affected by oestrogen concentrations (day 1 vs. day 14 of the menstrual cycle, 39), and the present study extends this conclusion to the active knee extensors, whilst demonstrating that the increase of progesterone concentrations on D21 is not concurrent with changes in corticospinal excitability. When considering paired-pulse responses, however, the increase in progesterone concentrations were concomitant with a ~14% increase in SICI, which when considered with previous evidence (37, 59), was likely through potentiation of $GABA_A$ inhibition. Indeed, GABA agonist pharmacological interventions (e.g. baclofen and gabapentin) have shown similar changes (74). The difference between days 14 and 21 demonstrated in *Study B* (14%) was double the measurement error of SICI observed in *Study A* (7%), however, there was no difference between D2 and D14 (difference = 4%). Interestingly, SICI followed a similar pattern to the E:P ratio (see Table 4), with the only significant change demonstrated on D21 concurrent to a decrease in the E:P ratio. Furthermore, the MCP × time interaction effect for SICI in *Study B* would suggest that intracortical inhibition is differentially modulated by exercise throughout the menstrual cycle. Whilst this is a concept that has been postulated before (23), and the present data appears to show this phenomenon, the interaction should be treated with caution as the post-exercise reliability of SICI in *Study A* was poor. A significant bias was observed ($P = 0.031$), with a poor ICC value (0.42), thus, a conclusion regarding MCP specific changes in intracortical inhibition following exercise cannot be confidently made using the present data.

The TMS SP, thought to partly reflect $GABA_B$ inhibitory mechanisms (14) was not affected by MCP, supporting previous data recorded in the FDI muscle (33). However, the

conclusion that the menstrual cycle affects only $GABA_A$ neurotransmission cannot be made with the current data, as Yacyshyn et al. (73) showed that the SP has a large spinal contribution. Additionally, glutamatergic intracortical facilitation (ICF) was not measured in the present study, but has previously been shown to be affected by the menstrual cycle, with augmented ICF demonstrated mid-cycle (56, 57). Whilst the causal link between intracortical function and voluntary activation is under researched, it is possible that the adjustments of intracortical circuitry altered the capacity of TMS and MNS to evoke a SIT. For instance, if intracortical excitability was greatest on D14, there may have been a 'ceiling effect', meaning the stimulations were not able to induce additional excitation in the motor cortex, thus, innervating fewer additive motor units during MVCs, and evoking a smaller SIT, and the contrary occurring on D21, when inhibition was greatest. The modulation of neurotransmitters has previously shown to affect VA, with pharmacological increases in noradrenaline (44) and serotonin (42) resulting in a ~1-2% increase in VA. Indeed the effects of serotonin have been shown to be augmented by oestrogen (3), and inhibited by progesterone (34). Therefore, it is possible that the modulation of inhibitory and facilitatory intracortical circuitry across the menstrual cycle might collectively contribute to the changes in VA_{MNS} and VA_{TMS}.

Fatigability across the menstrual cycle

Fatigability, as measured by the TTF of the open-ended fatiguing protocol, was lowest on D21 (i.e. greatest TTF), thus supporting the findings of Sarwar et al. (53), who showed that fatigue index was lowest in the luteal phase during a three-minute intermittent involuntary contraction protocol. The present data, however, contradicts Janse de Jonge et al. (40), who showed no effect of MCP during voluntary or electrically evoked fatiguing protocols performed with the knee extensors. The differences between tasks could

explain these discrepancies. The voluntary task used by Janse de Jonge et al. (44) involved both dynamic knee extension and flexion, rather than a single muscle group. This anisometric, multi-muscle group exercise likely elicits a different pattern of sensory afferent feedback (30), and was not open-ended like the present study, which could explain the discrepancies in fatigability. The same reasons might also apply to why the findings of DiBrezzo et al. (21) are inconsistent with the present study, who similarly demonstrated no menstrual cycle effect on fatigue during a set amount of dynamic contractions. Thus, the task employed in the present study likely permitted a greater degree of fatigue to develop, allowing the aforementioned MCP differences to be discerned.

As widely acknowledged, fatigability has both physiological and perceptual components that interact to determine exercise tolerance (26, 66). The fatiguing task in the present study involved high intensity (60% MVC) intermittent, isometric contractions, which were assumed to be far greater than the critical torque (~30% MVC [12]), and limited by decrements in neuromuscular adjustments (1, 11). With no MCP x time interaction effects displayed for neuromuscular variables (MVC, Qtw.pot, and VA), the degree of pre-post exercise adjustment was not different between menstrual cycle phases. Accordingly, one hypothesis for why TTF was longer on D21 could be the influence of neurotransmitter systems on perceptions of fatigue. The present study measured GABAergic inhibition and demonstrated a large increase in SICI on D21 (Figure 2B), and it has previously been shown that GABA can have anti-nociceptive properties (25) acting as an analgesic (41). Indeed, it has recently been postulated that "luteal analgesia" occurs in eumenorrheic females when progesterone is elevated, where the affective response to nociceptive pain is reduced due to alterations in functional connectivity in the emotional regulation

network (69). Thus, it could be possible that the analgesic effects of enhanced GABAergic neurotransmission permitted participants to continue exercising for a longer period due to a lower perception of pain. However, more evidence is needed to explore the effects of GABAergic inhibition on exercise-induced fatigue.

Further Considerations

In *Study* B it would appear that there was substantial between-subject variation in neuromuscular function and the changes across the menstrual cycle (Figures 1-3). Potential explanations for this could be the large standard deviations in hormone concentrations at each time point (Table 4), which has been reported in previous investigations (55). Additionally, inter-individual differences in hormone receptor numbers and sensitivity could contribute to the variation in changes across the menstrual cycle. In muscle tissue (22), expression of sex hormone receptors is altered by changing hormonal environments, which could conceivably occur in neuronal tissues such as the motor cortex, however the present data cannot answer this research question.

Whilst serum hormones were quantified for the eumenorrheic females in Study B, the mOCP users' serum hormone concentrations were not quantified in Study A. These data would have provided useful information about the measurement error of the sample, however individual 'meaningful' changes might differ between participants in order to achieve ovulation.

Conclusion

The present investigation demonstrated that when neuro-active hormones are constant, females demonstrate stable neuromuscular function (*Study A*). In contrast, when

695 eumenorrheic females were tested at three distinct phases of the menstrual cycle (Study
696 B), the changing hormonal environment coincided with large changes in CNS function,
697 which affected aspects of motor performance. Specifically, oestrogen had neuro-
698 excitatory effects that were associated with an increase in VA on D14, whereas
699 progesterone's neuro-inhibitory effects was concurrent with an increased intracortical
700 inhibition and decreased VA. Additionally, fatigability was modulated by MCP, with the
701 greatest TTF seen on D21, concurrent with an increase in progesterone. Thus, the
702 menstrual cycle elicits changes in neuromuscular function and fatigability in locomotor
703 muscle of eumenorrheic females.

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Competing Interests

The authors have no competing interests of any kind to declare.

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Author Contributions

PA, KT, GH, SKH and SG devised the study protocol. PA, CB, JS, KMH and DCMS collected the data. PA analysed the data. PA, KMH, KT, GH, and SG interpreted the results. PA drafted the manuscript. All authors revised and approved the final manuscript.

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Tables

Table 1: Monophasic combined oral contraceptive pills (mOCPs) taken by participants in Study A.

OCP brand	No. Participants	Synthetic Estrogen	Dosage (μg)	Synthetic Progestin	Dosage (μg)
Rigevidon®	6	Ethinylestradiol	30	Levonorgestrel	150
Cilest®	3	Ethinylestradiol	35	Norgestimate	250
Yasmin®	2	Ethinylestradiol	30	Drospirenone	300
Gedarel®	1	Ethinylestradiol	20	Desogestrel	150
Gedarel®	1	Ethinylestradiol	30	Desogestrel	150
Microgynon®	1	Ethinylestradiol	30	Levonorgestrel	150
Levest®	1	Ethinylestradiol	30	Levonorgestrel	150

Table 2: Reliability data for mechanical variables pre- and post-exercise in Study A. Pre-post change (Δ) is presented when a significant ($P < 0.05$) change was observed.

Measure		**Visit 1**	**Visit 2**	***P***	**Bias**	**TE**	**CV (%)**	**ICC (95% CI)**
MVC	Pre	502 ± 90	511 ± 103	0.314	-9	23	4.5	0.96 (0.89 - 0.98)
(N)	Post	375 ± 93	383 ± 81	0.356	-8	24	6.2	0.94 (0.82 - 0.98)
	Δ	-128 ± 43	-128 ± 51	0.972	0	30	23.9	0.62 (0.18 - 0.85)
$Q_{tw.pot}$	Pre	168 ± 27	170 ± 22	0.589	-2	9	5.0	0.90 (0.72 - 0.96)
(N)	Post	110 ± 22	110 ± 21	0.942	0	11	10.4	0.75 (0.40 - 0.91)
	Δ	-58 ± 29	-61 ± 21	0.643	-2	12	19.7	0.81 (0.53 - 0.93)
ERT	Pre	121 ± 38	118 ± 34	0.689	3	15	12.5	0.85 (0.62 - 0.95)
(N)	Post	94 ± 30	91 ± 27	0.605	3	14	14.9	0.79 (0.48 - 0.92)
	Δ	-27 ± 25	-27 ± 28	0.943	0	17	62.7	0.63 (0.20 - 0.86)
VA_{TMS}	Pre	94.3 ± 3.3	94.8 ± 2.9	0.679	-0.5	2.8	3.0	-
(%)	Post	82.3 ± 11.1	83.9 ± 10.4	0.406	-1.6	5.1	6.1	-
	Δ	-12.0 ± 11.2	-10.9 ± 9.6	0.573	1.1	5.2	45.4	0.78 (0.46 - 0.92)
VA_{MNS}	Pre	93.6 ± 3.0	93.7 ± 3.2	0.834	-0.1	1.6	1.7	-
(%)	Post	84.5 ± 7.2	85.8 ± 6.7	0.942	-1.3	3.6	4.2	-
	Δ	-9.1 ± 6.0	-7.9 ± 6.1	0.390	1.2	3.7	43.2	0.66 (0.24 - 0.87)
TTF (s)		560 ± 275	603 ± 357	0.338	-43	117	20.0	0.88 (0.69 - 0.96)

CV: Coefficient of variation; ICC: Intraclass correlation coefficient; MVC: Maximum voluntary contraction; $Q_{tw.pot}$: Potentiated quadriceps twitch; ERT: Estimated resting twitch; VA_{TMS}: Voluntary activation assessed with TMS; VA_{MNS}: Voluntary activation assessed with MNS; TE: Typical error; TTF: Time to task failure

Table 3: Reliability values for electromyographical data pre- and post-exercise. Pre-post change (Δ) is presented when a significant (P < 0.05) change was observed.

Measure		Visit 1	Visit 2	*P*	Bias	TE	CV (%)	ICC (95% CI)
MEP/M_{max}	Pre	22.4 ± 12.0	19.8 ± 10.5	0.291	2.6	6.40	30.1	0.71 (0.34 - 0.89)
(%)	Post	17.8 ± 9.0	16.9 ± 10.1	0.677	0.9	5.4	31.0	0.72 (0.34 - 0.90)
	Δ	-	-	-	-	-	-	-
SICI	Pre	78.7 ± 15.0	82.9 ± 13.5	0.148	-4.2	7.4	9.2	0.75 (0.42 - 0.91)
(%)	Post	75.3 ± 13.3	84.3 ± 13.3	**0.031**	-9.1	10.4	13.0	0.42 (0.00 – 0.75)
	Δ	-	-	-	-	-	-	-
M_{max}	Pre	2.96 ± 1.13	3.08 ± 1.28	0.507	-0.12	0.48	15.9	0.86 (0.64 – 0.92)
(mV)	Post	2.74 ± 1.01	2.88 ± 1.03	0.466	-0.14	0.50	17.8	0.79 (0.47 - 0.92)
	Δ	-	-	-	-	-	-	-
SP	Pre	187 ± 45	190 ± 50	0.791	-3	31	16.4	0.60 (0.24 - 0.82)
(ms)	Post	201 ± 56	202 ± 46	0.947	-8	37	19.2	0.63 (0.19 - 0.86)
	Δ	14 ± 29	12 ± 23	0.815	2	19.8	155.1	0.44 (0.00 - 0.77)

CV: Coefficient of variation; ICC: Intraclass correlation coefficient; MEP: Motor evoked potential, MVC: maximum voluntary contraction, SICI: Short interval cortical inhibition, M_{max}: maximum compound action potential; TE: Typical error

Table 4: Group average concentrations for 17-β oestradiol and progesterone across the three tested phases of the menstrual cycle. * = greater than d2, # = greater than d14, † = greater than d21 (all P < 0.05).

	Day 2	Day 14	Day 21
17-β Oestradiol ($pg.ml^{-1}$)	248 ± 129	328 ± 160*	341 ± 186*
Progesterone ($ng.ml^{-1}$)	1.27 ± 0.50	1.38 ± 0.69	4.41 ± 4.60*#
E:P ratio	0.20 ± 0.13	0.28 ± 0.18	0.12 ± 0.10*#

Table 5: Variables assessed throughout the pre- and post-exercise testing battery across the menstrual cycle. P values from the baseline ANOVA (1 × 3 repeated measures), and the pre-post exercise ANOVA (2 × 3 repeated measures) are reported. When a significant effect of exercise was found, the Δ in a variable from pre-post exercise was reported. * =greater than day 2, # = greater than day 14, † = greater than day 21.

	Day 2			Day 14			Day 21			MCP effect	Pre-post exercise	MCP × Exercise
	Pre	**Post**	**Δ**	**Pre**	**Post**	**Δ**	**Pre**	**Post**	**Δ**	**1×3 ANOVA**	**2×3 ANOVA**	**2×3 ANOVA**
MVC (N)	457 ± 79	344 ± 59	−25%	454 ± 78	337 ± 67	−26%	460 ± 87	355 ± 93	−24%	0.790	**<0.001**	0.236
SIT_{MNS} (N)	9 ± 5#	11 ± 7	-	7 ± 4	10 ± 8	-	8 ± 4	11 ± 6	-	**0.040**	0.069	0.452
$Q_{tw.pot}$ (N)	148 ± 20	95 ± 24	−36%	150 ± 20	91 ± 22	−39%	151 ± 23	94 ± 26	−38%	0.782	**<0.001**	0.634
VA_{MNS} (%)	93.4 ± 2.8	88.4 ± 8.8	−6%	95.3 ± 1.9*	87.9 ± 6.9	−8%	94.3 ± 2.2	88.0 ± 5.6	−7%	**0.001**	**0.002**	0.303
SIT_{TMS} (N)	6 ± 2	10 ± 8	67%	5 ± 3	9 ± 7	80%	7 ± 6	10 ± 9	43%	0.136	**0.028**	0.292
ERT (N)	94 ± 43	74 ± 37	−35%	96 ± 39	64 ± 60	−40%	93 ± 43	70 ± 36	−35%	0.784	**0.001**	0.128
VA_{TMS} (%)	93.2 ± 2.8	87.5 ± 8.5	−6%	95.7 ± 2.4†	84.3 ± 9.4	−12%	92.6 ± 3.2	86.7 ± 9.7	−7%	**0.008**	**0.003**	**0.049**
MEP/M_{max} (%)	17 ± 5	17 ± 9	-	22 ± 7	18 ± 9	-	18 ± 10	15 ± 8	-	0.129	0.278	0.485
SICI (%)	77 ± 11*	84 ± 14	-	82 ± 10*	74 ± 22	-	67 ± 12	75 ± 19	-	**0.001**	0.578	**0.028**
SP (ms)	160 ± 42	176 ± 49	12%	173 ± 70	176 ± 54	2%	174 ± 48	176 ± 49	3%	0.594	**0.010**	0.360
M_{max} (mV)	4.05 ± 2.19	3.93 ± 2.27	-	4.39 ± 1.88	4.06 ± 2.12	-	4.30 ± 2.50	3.80 ± 2.28	-	0.786	0.087	0.436
TTF (s)	519 ± 164			571 ± 179			706 ± 262*			**0.030**	-	-

MVC: maximum voluntary contraction, SIT_{MNS}: superimposed twitch elicited by motor nerve stimulation; $Q_{tw.pot}$: potentiated quadriceps twitch; VA_{MNS}: voluntary activation assessed with motor nerve stimulation; SIT_{TMS}: superimposed twitch elicited by transcranial magnetic stimulation; ERT: estimated resting twitch; VA_{TMS}: voluntary activation assessed with TMS; MEP/M_{max}: corticospinal excitability; SICI: short-interval cortical inhibition; SP: TMS evoked silent period; M_{max}: maximum compound muscle action potential; TTF: time to task failure.

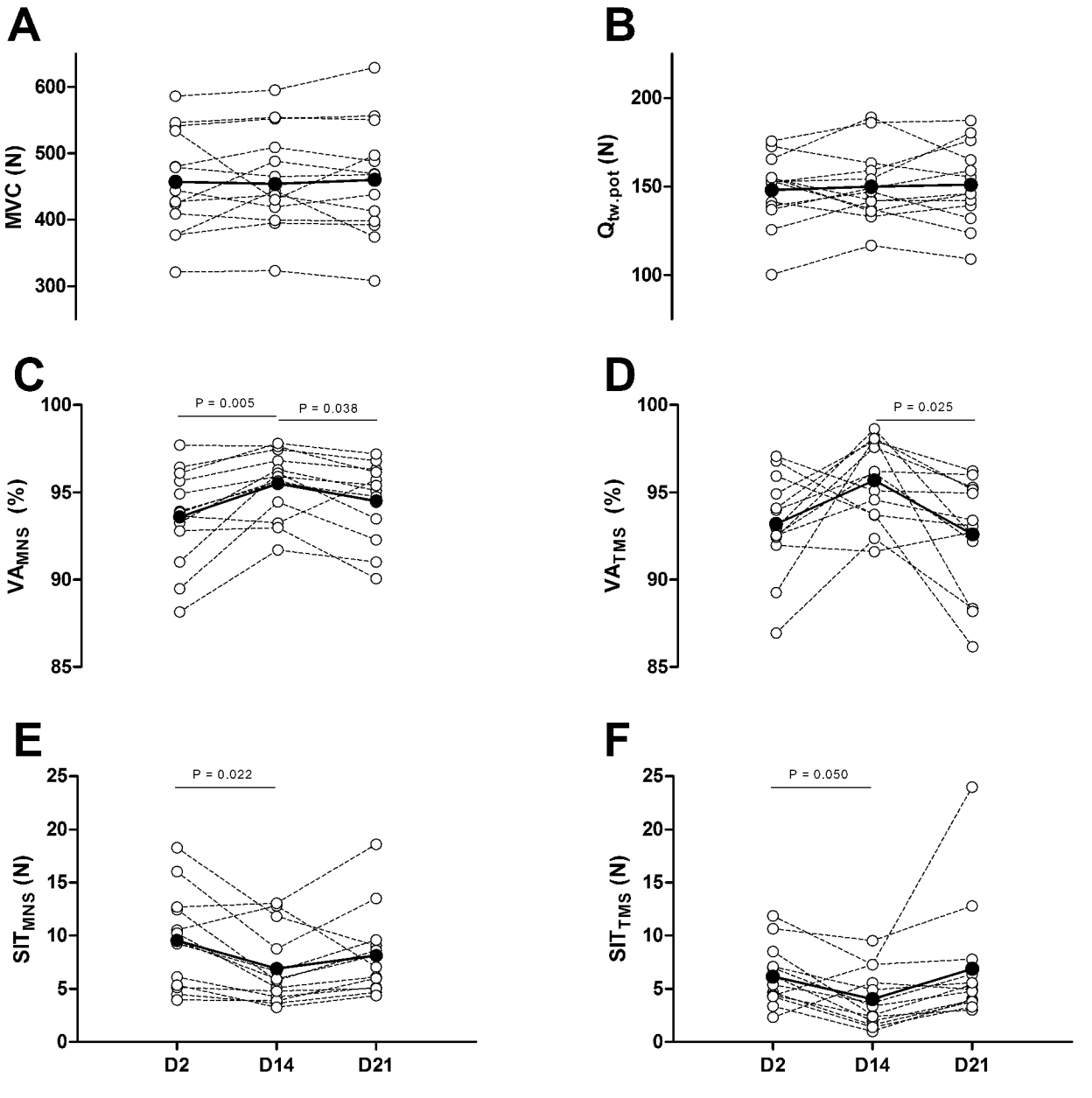
Figure Legends

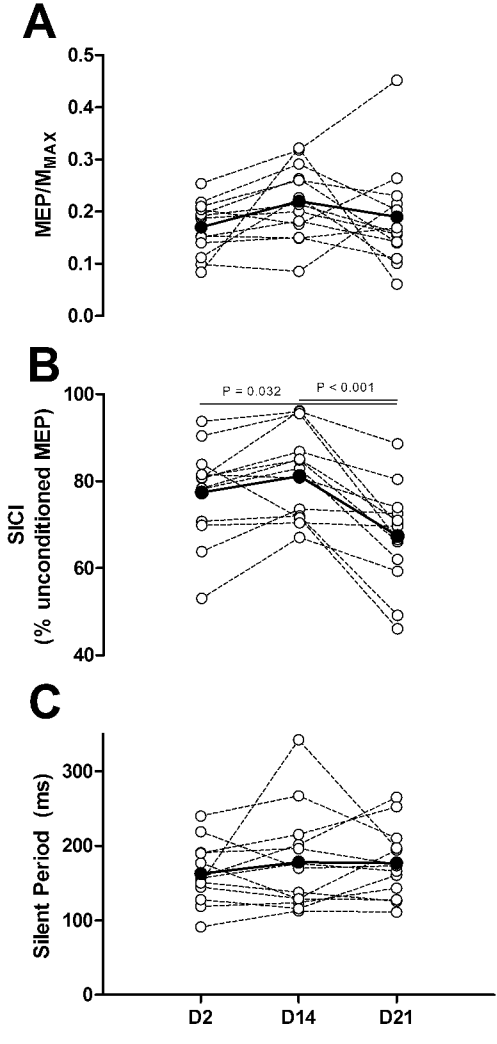
Figure 1: Baseline neuromuscular measures across the three timepoints. Panel A: maximum voluntary contraction; Panel B: potentiated twitch force; Panel C: voluntary activation assessed with motor nerve stimulation; Panel D: voluntary activation assessed with transcranial magnetic stimulation; Panel E: superimposed motor nerve stimulation evoked twitch during a maximum voluntary contraction; Panel F: superimposed transcranial magnetic stimulation evoked twitch during a maximum voluntary contraction. Individual data are shown with mean data overlaid as the filled symbols and connecting line.

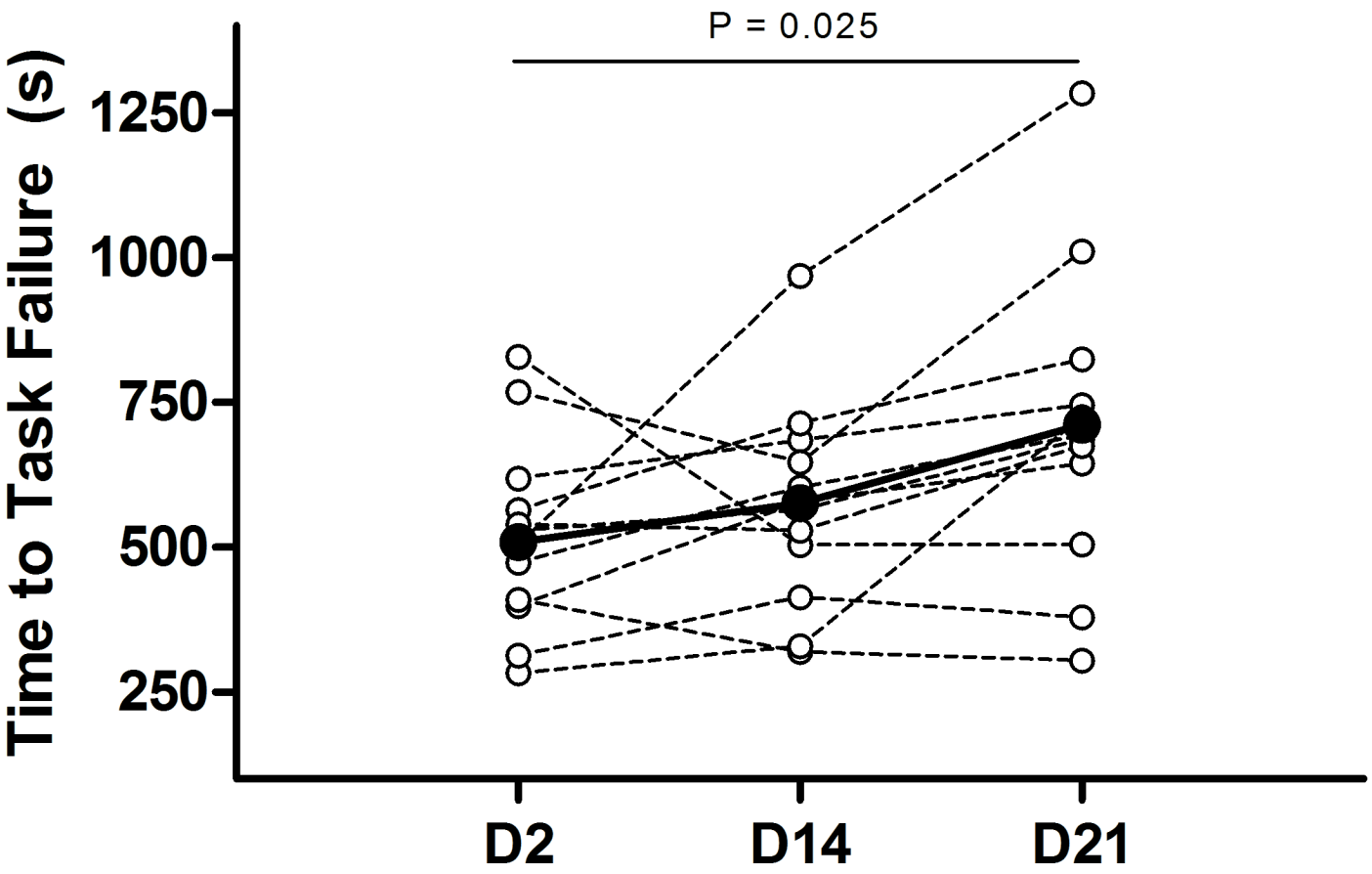
Figure 2: Transcranial magnetic stimulation evoked responses across the three testing time points. Panel A: corticospinal excitability; Panel B: short interval cortical inhibition; Panel C: TMS evoked silent period. Individual data are shown with mean data overlaid as the filled symbols and connecting line.

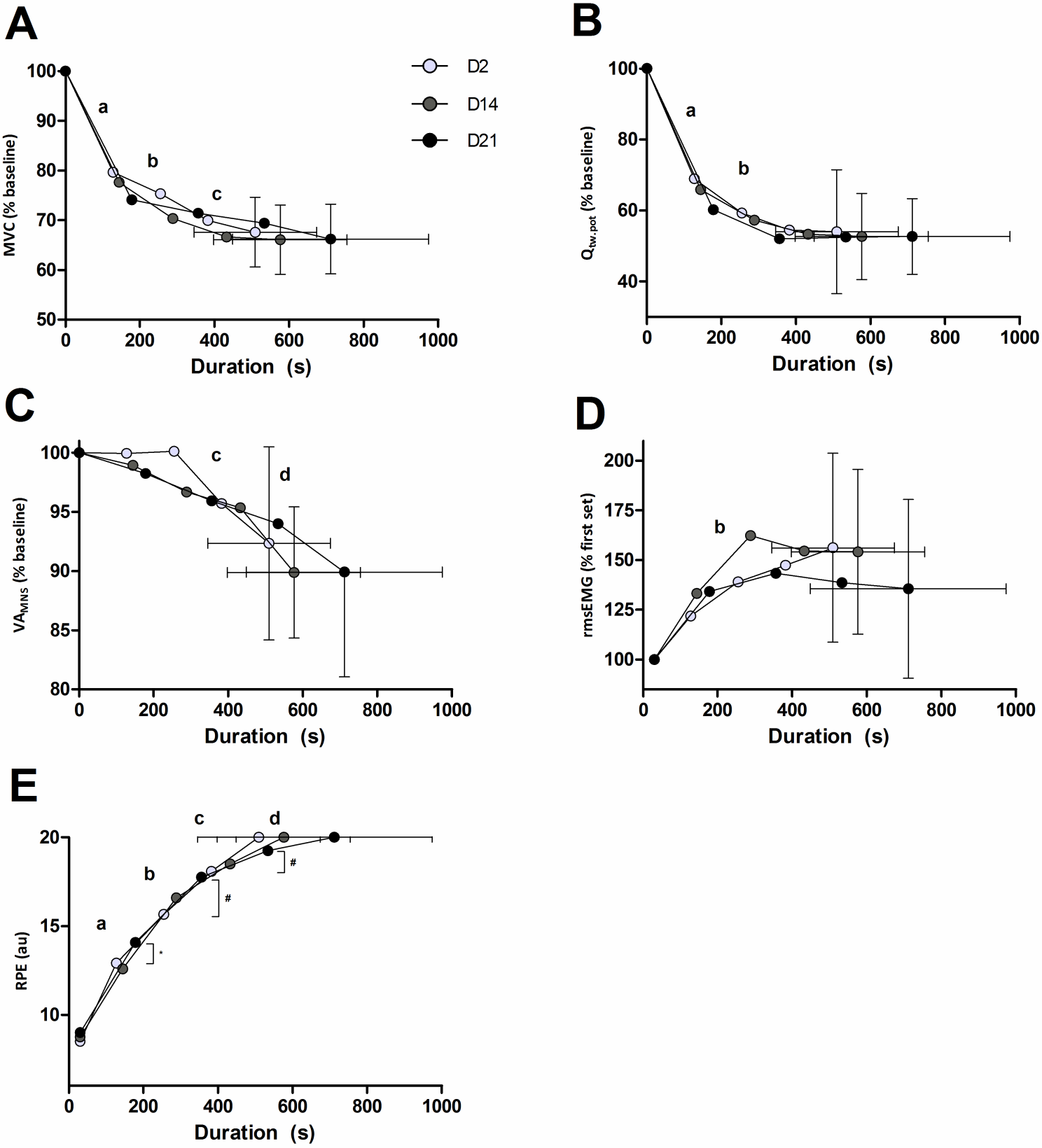
Figure 3: Time to task failure during the submaximal intermittent isometric fatiguing task at the three testing time points. Individual data are shown with mean data overlaid as the filled symbols and connecting line.

Figure 4: Neuromuscular variables assessed at 25, 50, 75 and 100% TTF throughout the fatiguing tasks in each MCP. Panel A: maximum voluntary contraction; B: potentiated quadriceps twitch; C: voluntary activation assessed with motor nerve stimulation; D: root-mean-squared EMG; E: rating of perceived exertion. Data are means with the standard deviation shown in panels A-D for the final point. Data are displayed as % baseline, although statistical analyses were performed on absolute data. Statistical differences ($P < 0.05$) are depicted by a: significantly difference between baseline-25% TTF; b: significant difference between 25-50% TTF; c: significant difference between 50-75% TTF; d: significant difference between 75-100% TTF; *: significant difference between D21-D14; #: significant difference between D21-D2.


A
MVC (N)
600
500
400
300
B
$Q_{tw.pot}$ (N)
200
150
100
C
VA_{MNS} (%)
100
95
90
85
P = 0.005
P = 0.038
D
VA_{TMS} (%)
100
95
90
85
P = 0.025
E
SIT_{MNS} (N)
25
20
15
10
5
0
P = 0.022
D2
D14
D21
F
SIT_{TMS} (N)
25
20
15
10
5
0
P = 0.050
D2
D14
D21


A
0.5
0.4
0.3
0.2
0.1
0.0
MEP/M_MAX
B
100
80
60
40
SICI
(% unconditioned MEP)
P = 0.032
P < 0.001
C
300
200
100
0
Silent Period (ms)
D2
D14
D21


P = 0.025
Time to Task Failure (s)
1250
1000
750
500
250
D2
D14
D21


A
D2
D14
D21
MVC (% baseline)
Duration (s)
a
b
c
B
$Q_{tw.pot}$ (% baseline)
Duration (s)
C
VA_{MNS} (% baseline)
Duration (s)
d
D
rmsEMG (% first set)
Duration (s)
E
RPE (au)
Duration (s)
*
#