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1 **Menstrual cycle associated modulations in neuromuscular function and fatigability**
2 **of the knee extensors in eumenorrheic females.**
3

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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.

2

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

81 The cyclical changes in concentrations of multiple sex hormones, including oestrogen and

82 progesterone (55), across the eumenorrheic menstrual cycle can affect central nervous

83 system (CNS) function due to their ability to cross the blood-brain barrier (62). *In vitro*

84 models have shown direct evidence for the effect of sex hormones on neuronal function.

85 For instance, oestradiol (an oestrogenic steroid hormone) binds to oestrogen receptor α

86 (ER-α) sites on γ-aminobutyric acid (GABA) mediated neurons, causing an attenuation in

87 GABA synthesis and release (54, 70). Additionally, oestrogen potentiates the effects of

88 excitatory glutamatergic (both NMDA and non-NMDA) receptors (61), resulting in a net

89 excitatory effect. Additionally, oestrogen has been shown to decrease firing thresholds

90 and increase discharge frequency of cerebral neurons (58, 72). On the contrary,

91 progesterone has a net inhibitory effect on the nervous system, as the activity and effects

92 of GABA are potentiated, leading to decreased neuronal discharge rate (60) and increased

93 inhibition of pyramidal neurons in rats (37). Some evidence also suggests that the

94 presence of progesterone directly antagonises estrogenic actions by lowering the

95 available ER-α and -β receptor numbers on various sites of neuronal cells (47). Indeed,

96 as Smith and Woolley (61) outlined, the neurosteroidal actions of hormones act upon the

97 neurotransmitter receptors. Thus, given GABAergic and glutamatergic synapses are

98 located within the motor cortex (43), a hormonal effect would be expected

99

100 The differential effect of oestrogen and progesterone concentrations on indices of

101 nervous system excitability have also been established in humans. Transcranial magnetic

102 stimulation (TMS) studies show increased intracortical excitability and reduced

103 intracortical inhibition in the late-follicular phase, when oestradiol concentration is high

104 and progesterone low (56, 57), substantiating the alterations seen in the aforementioned

105 *in vitro* studies. While these *in vivo* studies show clear changes in human CNS function,

106 they were conducted in the resting upper limbs, specifically in hand muscles associated

107 with fine motor control. However, properties of intracortical and corticospinal circuits

108 vary between upper and lower limb projections (7, 15). Thus, the menstrual cycle

109 associated modulations in neural function of a small, upper limb muscle group cannot be

110 extrapolated to larger, lower limb muscle groups. Understanding how the menstrual

111 cycle affects the neural control of large locomotor muscle groups has significant

112 implications for everyday locomotive tasks, injury rehabilitation, and athletic

113 performance. For instance, neuroplasticity following stroke (17), and strength training

114 (71) are influenced by GABAergic inhibition.

115

116 To date, there is minimal research investigating menstrual cycle induced changes in

117 nervous system and motor function of the knee extensors (KE). Previous studies

118 investigating motor function, such as the ability to produce maximum voluntary

119 contraction force (MVC), are equivocal, with studies showing 8-23% greater maximal

120 force with the KE mid-cycle (4, 53, 64). Multiple studies however, report no difference in

121 maximal strength (20, 24, 40, 46). The studies that have shown changes in maximal

122 strength have suggested that mechanisms such as motor unit firing rates (65) and

123 intracortical excitability (56, 57) could be contributing factors. However, the proposed

124 mechanistic factors, and the neuromuscular response (e.g. MVC), have not been

125 concurrently studied. Voluntary activation (VA) of the quadriceps has been assessed

126 using motor nerve stimulation twice with no menstrual cycle effect shown (40, 46).

127 However, as it is thought that the assessment of VA using TMS (VA_{TMS}) reflects the ability

128 of the motor cortex to activate the motor units within the target muscle group (68), if

129 supraspinal properties are modulated by the menstrual cycle (i.e. 56, 57), VA_{TMS} could

130   provide a more appropriate measure to discern whether the ability to voluntarily activate

131   the KE is affected.

132

133   Other aspects of motor performance, such as performance fatigability (38), have also

134   been studied throughout the menstrual cycle with inconclusive results. Sarwar et al. (53)

135   showed that the KE of eumenorrheic females were less fatigable in the luteal phase during

136   an electrically stimulated isometric fatiguing protocol. However, this finding has not been

137   corroborated with dynamic, voluntary contractions performed with the KE (21, 40).

138   Additionally, none of the aforementioned studies were open-ended, with a fatigue index

139   calculated after a set amount of time/contractions. Thus, due to the causes of fatigability

140   being task specific (66), discrepancies in the aforementioned investigations could be due

141   to the differences in fatiguing protocols used and their respective limiting factors.

142

143   The effect of hormonal fluctuations on neuromuscular function and fatigability of the KE

144   remains unclear. Conflicting literature exists for the majority of neuromuscular variables,

145   despite a rationale for change based on neuroendocrine and upper-limb studies. The

146   inclusion of statistical measures of error are recommended for investigations utilising

147   methods of neurostimulation to inform the contribution of random variation to any

148   modifications in neuromuscular function (29). Therefore, the present investigation

149   recruited a population of monophasic oral contraceptive (mOCP) users to discern test-

150   retest repeatability of neuromuscular function measures without the influence of

151   endogenous hormones (*Study A*). The consistent dosage of exogenous oestrogen and

152   progesterone in the mOCP precludes ovulation (28), creating a physiological

153   environment in which the effects of endogenous hormones are negated. Thereafter, *Study*

154   *B* aimed to investigate KE neuromuscular function and fatigability across the menstrual

6

155   cycle. It was hypothesised that when oestrogen levels increased (and progesterone

156   remained low) from day 2 to 14, maximum force production would concomitantly rise,

157   alongside a reduction in intracortical inhibition and an increase in VA. Secondly, it was

158   hypothesized that the rise in progesterone from day 14 to 21 would occur alongside a

159   reversal in these changes, and improved time to task failure (TTF).

160

METHODS

162 **Ethical Approval**

163 The study received institutional ethical approval from the Northumbria University Health

164 and Life Sciences Research Ethics Committee (HLSPA301116) and was conducted

165 according to all aspects of the Declaration of Helsinki, apart from registration in a

166 database. Participants provided written, informed consent to volunteer for the study

167

168 **Participants**

169 A total of 30 participants volunteered to participate in the study. Fifteen mOCP users (age:

170 23 ± 2 years; stature: 170 ± 6 cm; mass: 70.6 ± 8.5 kg) and fifteen eumenorrheic females

171 (age: 25 ± 4 years; stature: 169 ± 6 cm; mass: 68.3 ± 7.8 kg; mean cycle duration: 29 ± 3

172 days, range: 24-34 days). The mOCPs reported taking an mOCP for at least 6 months as

173 prescribed (i.e. a seven-day break after every 21-day pill consumption period), whereas

174 eumenorrheic females reported having regular cycles without using any form of

175 hormonal contraceptives for at least six months. A full list of the mOCPs taken by

176 participants is presented in Table 1. Participants arrived at the laboratory rested and

177 hydrated, with strenuous physical activity avoided for 48 hours, and caffeine and alcohol

178 prohibited for 24 hours.

179

180 TABLE 1 HERE

181

182 **Experimental Design**

183 *Study A*

184 Oral contraceptive users visited the laboratory three times, completing a familiarisation

185 and two experimental visits. Experimental visits were completed during the final 14 days

8

186   of the pill cycle, with a minimum of 48 h between visits to allow recovery (13). The visits

187   were identical to those described below for *Study B*, however, blood sampling was not

188   performed as it is established that endogenous hormone concentrations do not fluctuate

189   throughout the consumption phase of the mOCP cycle (10).

190

191   *Study B*

192   Eumenorrheic females visited the laboratory four times, completing a familiarisation

193   session prior to three experimental visits. Participants completed experimental visits on

194   days 2 (D2, Early-follicular), 14 (D14, Late-follicular), and 21 (D21, Mid-luteal) of the

195   menstrual cycle. Testing days were counted from the onset of menstruation and, to verify

196   menstrual cycle phase, fasted venous blood samples were taken between the hours of

197   0600 – 0900 on testing days to analyse serum oestradiol and progesterone

198   concentrations. The order of visits was pseudorandomized and counterbalanced to

199   minimize order effects, with five participants beginning on each testing day (D2, D14 or

200   D21). All testing visits occurred within the same menstrual cycle (order: D2, D14, D21),

201   or two consecutive cycles (order: D14, D21, D2 or D21, D2, D14). Experimental visits

202   consisted of a baseline neuromuscular assessment, intermittent, isometric contractions

203   at 60% MVC until task failure, followed immediately by a post-task neuromuscular

204   assessment. The intensity for the fatiguing task was likely far greater than the critical

205   torque (~30% MVC [12]), and therefore an unsustainable intensity, with task failure

206   attributable to decrements in neuromuscular function (1, 11).

207

208   **Experimental Procedures**

209   For *Study B*, upon arriving between the hours of 0600 – 0900, fasted venous blood

210   samples were taken following 10 minutes of seated rest. Participants were then

211 instructed to consume a typical breakfast and return to the laboratory at their designated

212 testing time. The breakfast and time of testing were replicated (± 1 hour) for each

213 experimental visit to control for diurnal variations in corticospinal excitability and

214 maximal force production (63). Time of testing was also controlled for in *Study A*, with

215 participants consuming the mOCP a constant time before trials in order to standardise

216 circulating exogenous hormone concentrations between visits. In both studies,

217 experimental sessions began with participants completing a standardised voluntary

218 isometric contraction warm up (2 × contractions at 25, 50, and 75% perceived maximal

219 effort) followed by a baseline neuromuscular assessment (described below). The

220 fatiguing task involved sets of intermittent isometric contractions (3 s contraction, 2 s

221 rest at 60% MVC) to task failure. Contractions were paced with an audible metronome to

222 ensure the duty cycle was maintained. One set was defined as 11 submaximal

223 contractions followed by a 3 s MVC with motor nerve stimulation (MNS) delivered at the

224 peak force and 2 s post, lasting one minute. Task failure was defined as an inability to

225 reach the target force three times at any stage of the protocol. Rating of perceived

226 exertion (RPE; 6) was recorded using a 6-20 scale following each MVC throughout the

227 fatiguing task. Real-time visual force feedback using target forces set as percentages of

228 maximum force was provided to participants on a computer screen to aid a constant force

229 level. The post-task neuromuscular assessment began immediately following task failure.

230

231 *Neuromuscular Assessments*

232 Measures of neuromuscular function were assessed pre- and post- exercise with MNS of

233 the femoral nerve, and TMS of the contralateral motor cortex at rest and during voluntary

234 contractions of the right knee-extensors. Pre-exercise neuromuscular assessments began

235 with two practice MVCs to ensure potentiation of subsequent evoked measures, followed

236   by three ~3 s MVCs, all separated by 30 s. During these 3 MVCs, MNS was delivered when

237   peak force plateaued, and then ~2 s after the MVC to measure voluntary activation

238   (VA_{MNS}) and potentiated twitch amplitude ($Q_{tw.pot}$) of the knee-extensors. Single-pulse

239   TMS was subsequently delivered during two sets of five 3-5 s contractions at 100, 87.5,

240   75, 62.5 and 50% MVC, with 5 s rest between contractions and 10 s rest between sets, to

241   determine VA_{TMS} (19). The TMS silent period (SP) was determined during the 50% MVC

242   contraction of each set. Participants were instructed to maintain a constant force on the

243   guideline and "push through the stimulation" (52). Finally, ten single- and ten paired-

244   pulse TMS stimulations were delivered during a 10% MVC contraction in an alternate

245   order to determine corticospinal excitability and short-interval cortical inhibition (SICI),

246   respectively. The neuromuscular assessment was repeated immediately post-exercise.

247   Measures of neuromuscular function (MVC, $Q_{tw.pot}$, VA_{MNS}) were measured within 30 s of

248   task failure, and VA_{TMS} measured within 2-2.5 minutes, in an attempt to minimise the

249   dissipation of fatigue, however it is possible that the sensitivity of these measures was

250   compromised due to the rapid recovery of central fatigue post-exercise (32).

251

252   *Force and Electromyographical Recordings*

253   During assessments of neuromuscular function and fatiguing tasks, participants sat on a

254   custom-built chair with knee and hip angles kept constant (both 90° flexion). A calibrated

255   load cell (MuscleLab force sensor 300, Ergotest technology, Norway) was attached via a

256   non-compliant cuff positioned 2 cm superior to the ankle malleoli on the participants'

257   right leg, to measure knee extensor force (N). Surface Ag/AgCl electrodes (Kendall

258   H87PG/F; Covidien, Mansfield, MA) were placed over the *rectus femoris* (RF), and *biceps*

259   *femoris* (BF) with a 2 cm inter-electrode distance, to record the compound muscle action

260   potential (M-wave) elicited by the electrical stimulation of the femoral nerve, the MEP

11

261   elicited by TMS, and the root-mean-square amplitude during isometric contractions

262   (rmsEMG). Electrode placement was consistent with SENIAM guidelines (35), and a

263   reference electrode was placed over the patella. Prior to placement, the skin-electrode

264   contact area was cleaned using a 70% IPA alcohol wipe (FastAid, Robinson Healthcare,

265   Worksop, UK). Signals were amplified: gain ×1000 for EMG and ×300 for force (CED 1902;

266   Cambridge Electronic Design, Cambridge, UK), bandpass filtered (EMG only: 20–2000

267   Hz), digitized (5 kHz; CED 1401, Cambridge Electronic Design), and analysed offline

268   (Spike2 v8, Cambridge Electronic Design).

269

270   *Motor Nerve Stimulation*

271   Single electrical stimuli (200 μs duration) were delivered to the right femoral nerve using

272   a constant current stimulator (DS7AH Digitimer Ltd, Welwyn Garden City, UK) via

273   adhesive surface electrodes (CF3200; Nidd Valley Medical Ltd., Harrogate, UK). The

274   cathode was placed over the nerve, high in the femoral triangle, in the position that

275   elicited the greatest twitch amplitude (Q_{tw}) and M-wave in the RF at rest. The anode was

276   placed halfway between the greater trochanter and iliac crest. Optimum stimulus

277   intensity was determined as the minimum current that elicited maximum values of Q_{tw}

278   and M-wave (M_{max}) at rest. To ensure a supramaximal stimulus, the optimum stimulus

279   intensity was increased by 30% and was not different between trials in either study (*A*:

280   230 ± 61 vs. 241 ± 65 mA, *P* = 0.271; *B:* 233 ± 72, 244 ± 68, and 262 ± 67 mA, *P* = 0.125).

281

282   *Transcranial Magnetic Stimulation*

283   Single and paired pulse stimuli (1 ms duration) were delivered to the contralateral (left)

284   motor cortex via a concave double cone coil (110 mm diameter, maximum output 1.4 T)

285   powered by two linked monopulse stimulators (Magstim Bistim and Magstim[200], The

286   Magstim Company, Whitland, UK). Optimal coil placement was determined as the

287   position that elicited the greatest RF motor evoked potential (MEP) with concomitant

288   smallest antagonist (BF) MEP during a 10% MVC at 50-70% stimulator output. This

289   position was marked on the scalp with indelible marker to ensure consistent placement

290   during trials. Stimulator intensity for VA_{TMS} was determined as the intensity that elicited

291   the greatest superimposed twitch (SIT) during a contraction at 50% MVC. Stimulator

292   intensity was increased in 5% intervals from 50% stimulator output and two stimuli

293   were delivered during a ~5 s contraction, with the mean of two SITs recorded (9, 18).

294   Mean stimulator intensity was not different between trials in either study (*A*: 67 ± 10 vs.

295   66 ± 10%, *P* = 0.737; *B*: 63 ± 10, 63 ± 11, and 63 ± 12%, *P* = 0.984). The stimulator output

296   activated a large proportion of the KE motoneuron pool at baseline in each experimental

297   visit with no difference between trials in *Study A* (69 ± 35 vs. 68 ± 36% M_{max} amplitude,

298   *P* = 0.916) or *Study B* (61 ± 17; 57 ± 17; 55 ± 14% M_{max} amplitude; *P* = 0.788). Small co-

299   activation of the antagonist muscle (BF) was observed in response to TMS and did not

300   differ between trials in *Study A* (0.60 ± 0.37 vs. 0.72 ± 0.53 mV, *P* = 0.106) or *Study B* (0.53

301   ± 0.39, 0.71 ± 0.42 and 0.60 ± 0.34 mV, *P* = 0.211).

302

303   Active motor threshold (aMT) was determined as the stimulator intensity that elicited a

304   MEP of > 200 μV in three out of five stimulations during a 10% MVC contraction.

305   Stimulator intensity was increased in 5% steps from 35% of stimulator output until a

306   consistent MEP amplitude >200 μV was found. Thereafter, stimulus intensity was

307   reduced in 1% steps until the lowest intensity to elicit a MEP of >200 μV was found. aMT

308   was not different on any testing visit in *Study A* (40 ± 6 vs. 40 ± 7%, *P* = 0.746) or *Study B*

309   (43 ± 9, 42 ± 8, and 43 ± 9%, *P* = 0.874). SICI was assessed with ten paired and ten single

310   pulse stimulations delivered. Paired-pulse TMS consisted of a conditioning pulse at 70%

311   of aMT, and a test pulse at 120% aMT, with an inter-stimulus interval of 2 ms. All stimuli

312   were delivered during a 10% contraction. This paradigm has previously been

313   demonstrated as the optimal configuration for eliciting SICI in the KE (8), and has been

314   used previously in our laboratory in male populations (31). Two sets of 10 stimuli were

315   used, with a 10 s rest between contractions.

316

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

318   Venous blood sampling was performed on the morning of each testing session. A 10 mL

319   blood sample was drawn from an antecubital vein into a silica coated tube by a trained

320   phlebotomist, then left upright for 15 minutes to coagulate before centrifuging. Samples

321   were centrifuged at 2,500 rpm for 10 minutes at room temperature (Allegra-X22R,

322   Beckman Coulter, USA). Using a 500-1000 μl pipette, the supernatant serum was separated

323   into three aliquots ($\sim$1000 μl each) and stored at $-80°$C until oestradiol and progesterone

324   analysis were performed. Total concentrations of 17-β oestradiol and progesterone were

325   measured in duplicate using hormone-specific enzyme-linked immunoassay kits

326   (Cayman Chemical, Ann Arbor, MI). All samples were analysed using the ELISA technique

327   with absorbance detection (wavelength 405 nm). The minimal oestradiol and

328   progesterone detection was 15 pg/ml and 7.5 pg/mL, respectively. To calculate 17-β

329   oestradiol and progesterone levels, a standard curve was plotted using eight standards

330   against their absorbance. Using the mean absorbance from the duplicate of each sample,

331   the concentration of the sample was interpolated directly from the standard curve. The

332   coefficients of variation (CV) for the ELISA kits, as provided by the manufacturer, were 8-

333   12% for 17-β oestradiol, and 5-8% for progesterone. In one instance, the CV of a duplicate

334   sample exceeded the manufacturer's CV due to an excessively high (non-physiological)

335   reading in one well. Therefore, the lower of the two was used for data analysis.

14

336 Participants' hormonal profiles were deemed 'acceptable' when a peak in progesterone

337 concentration was observed during the luteal phase (D21) and an increase in 17-β

338 oestradiol was observed from D2 to D14. If neither peak was observed, then participants

339 were deemed anovulatory and excluded from further analyses.

340

341 **Data Analysis**

342 Voluntary activation using motor nerve stimulation was determined using the ITT (48)

343 by comparing the amplitude of the superimposed twitch (SIT) with the amplitude of the

344 potentiated resting twitch ($Q_{tw.pot}$) using the following formula: VA_{MNS} (%) = (1 – [SIT ÷

345 $Q_{tw,pot}$]) × 100]. Voluntary activation using TMS was assessed during two sets of

346 contractions at 100, 87.5, 75, 62.5 and 50% MVC (19). Single pulse TMS was delivered

347 during each contraction, and the linear regression between SIT amplitude and

348 contraction intensity was extrapolated to the y intercept to obtain an estimated resting

349 twitch (ERT; 68) . In order to achieve significant linearity ($P < 0.05$), a total of 4 out of 300

350 SITs across all trials in *Study A* were excluded (1.3%), which led to four regressions

351 containing 9 data points rather than 10 (1 pre-exercise, 3 post-exercise). In *Study B*, six

352 out of 870 SITs were excluded from all linear regressions (0.7%), meaning that there

353 were 86 ten point regressions, three nine point regressions, and one eight point

354 regression used to estimate resting twitches. Mean r^2 values for ERTs in *Study A* were

355 0.94 ± 0.04 pre exercise vs. 0.94 ± 0.04 post exercise, and in *Study B* were 0.92 ± 0.05 pre

356 exercise, and 0.89 ± 0.07 post-exercise. The SIT during 100% MVC was compared with

357 the ERT using the following formula: VA_{TMS} (%) = (1 – [SIT ÷ ERT]) × 100. SICI was

358 quantified as the percentage ratio between the amplitude of conditioned MEPs to the

359 amplitude of unconditioned MEPs. Corticospinal excitability was determined by

360 expressing the mean MEP amplitude during the 10% MVC as a percentage of M_{max}. The

361 root-mean-square of EMG activity (rmsEMG) was recorded during the middle 500 ms

362 epoch of each 3 s contraction during the fatiguing task. rmsEMG was then expressed as a

363 percentage of M_{max}. For the data presented as %TTF, the MVC, VA_{MNS}, and $Q_{tw.pot}$ for the

364 nearest minute during the fatiguing protocols were taken, and the average rmsEMG for

365 the nearest full set of contractions to the target percentage (i.e. 25, 50, or 75% TTF) was

366 taken, for 0 and 100% TTF, the first and last complete set was used. All data analysis was

367 performed offline. In *Study* B, despite a rigorous familiarisation and verbal

368 encouragement, one participant failed to maintain the intermittent contractions for the

369 required 3 s during the fatiguing task, thus invalidating the TTF duration. Therefore, it

370 was deemed appropriate to remove the participant's TTF duration and post-trial

371 neuromuscular assessment from further analysis (n = 14), however, baseline data was

372 included for statistical analysis (n = 15).

373

374 **Statistical Analysis**

375 Data are presented as mean ± SD within the text and figures. Normal Gaussian

376 distribution of data was confirmed using the Kolmogorov–Smirnov test. If a violation was

377 detected, the data was logarithmically transformed. The alpha for all statistical tests was

378 set at $P ≤ 0.05$.

379

380 For Study A, between session and pre-post exercise differences were explored using two-

381 way (2 × 2) repeated measures ANOVAs, if assumptions of sphericity were violated, then

382 the Greenhouse-Geisser correction was applied. If significant main or interaction effects

383 were detected, Bonferroni-corrected post hoc tests were performed. For between session

384 test-retest reliability multiple indices were calculated (paired samples t-tests, typical

385 error, intraclass correlation coefficient, 2, 36) between the two time points. Within-

16

386 subjects variation was calculated as the standard deviation of the mean differences

387 divided by the square root of 2, and termed typical error (TE) throughout the manuscript.

388 Typical error was expressed as absolute raw values and as a percentage of the mean

389 (coefficient of variation, CV). Intraclass correlation coefficients ($ICC_{3,1}$) were calculated

390 according to Bland and Altman (5). ICC values were defined as follows: <0.5 = poor, 0.5-

391 0.75 = moderate, 0.75-0.9 = good, >0.9 = excellent (45). Due to the ceiling effect (i.e. all

392 values grouped close to 100%) associated with VA_{MNS} and VA_{TMS}, the ICCs were not

393 calculated (16, 67).

394

395 For study B, one-way repeated measures ANOVAs were run for all pre-exercise

396 dependent variables to assess MCP changes in neuromuscular function and hormone

397 concentrations. Sphericity was assessed using Mauchly's test and if necessary, controlled

398 using the Greenhouse-Geisser correction. Two-way repeated measures ANOVAs were

399 run using pre and post exercise variables to obtain both fatigue, and MCP × fatigue

400 interaction effects. To explore potential differences in the fatigue profiles of

401 neuromuscular and perceptual variables, two-way repeated measures ANOVAs were run

402 including data points from baseline, 25%, 50%, 75%, and 100% of TTF. Significant main

403 and interaction effects were explored using Bonferroni-corrected tests.

404

405   **RESULTS**

406 ***Study A***

407 *Exercise performance and pre-post exercise changes*

408 The TTF was not different between experimental visits (560 ± 275 s vs. 603 ± 357 s

409 respectively, *P* = 0.314). When assessing exercise-induced changes in neuromuscular

410 function, the two-way ANOVAs detected no between-trial differences in change scores

17

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

447   ***Study B***

448   *Hormonal Profiles*

449

450   TABLE 4 HERE

451

452   Thirteen out of 15 participants presented a regular hormonal profile (see Table 4). Two

453   participants had no increase in progesterone on D21; given the hypothesis that changing

454   hormone concentrations would modulate neuromuscular function, and these

455   participants did not exhibit any change in hormone concentrations, they were excluded

456   from further statistical analyses. The repeated measures ANOVAs showed an effect of

457   MCP on 17-β oestradiol ($F_{1.4,19.5}$ = 3.55, *P* = 0.040, η_p^2 =0.18) and progesterone

458   concentration ($F_{=1.0,14.1}$ = 8.35, *P* = 0.012, η_p^2 =0.37). Post hoc tests revealed that 17-β

459   oestradiol concentrations were greater on D14 compared to D2 (*P* = 0.033), and greater

460   on D21 than D2 (P = 0.029). Progesterone was greater on D21 than D2 and D14 (*P* = 0.011,

461   and 0.012, respectively).

462

463   *Baseline Neuromuscular Function*

464

465   FIGURE 1 HERE

466

467   MVC force was unaffected by MCP (Figure 1A, $F_{1.4,16.8}$= 0.15, *P* = 0.790, η_p^2 = 0.01).

468   Potentiated twitch force was also unchanged (Figure 1B, $F_{2,24}$ = 0.25, P = 0.782, η_p^2 = 0.02);

469   however, the SIT elicited by MNS was affected by MCP ($F_{2,28}$ = 3.69, *P* = 0.040, η_p^2 = 0.24),

470   with greater SITs on D14 compared to D2 (mean difference: 2 N, *P* = 0.031). The reduced

471   SIT on D14 meant that VA$_{MNS}$ was affected by MCP (Figure 1C, $F_{2,28}$ = 9.23, *P* = 0.001, η_p^2

20

472 = 0.44) with post hoc tests showing greater VA_{MNS} on D14 compared to D2 (mean

473 difference: 1.9%, P = 0.007), however, there was no difference between D14 and D21

474 (mean difference: 1.0%, P = 0.059). VA_{TMS} was also affected by MCP (Figure 1D, $F_{2,28}$ =

475 5.89, P = 0.008, η_p^2 = 0.33) with greater values on D14 compared to D21 (mean difference:

476 3.0%, P = 0.016), however, D14 and D2 were not different (mean difference: 2.5%, P =

477 0.080). Despite the change in VA_{TMS}, neither of its constituent parts were altered by MCP:

478 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,

479 η_p^2 = 0.15).

480

481 FIGURE 2 HERE

482

483 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

484 was normalized MEP amplitude (Figure 2A, $F_{2,28}$ = 2.24, P = 0.129, η_p^2 = 0.16). However,

485 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

486 hoc tests showing greater inhibition on D21 compared to D2 (mean difference: −10%, P

487 = 0.048) and D14 (mean difference: −14%, P = 0.001). The pre-stimulus normalised

488 rmsEMG activity was not different between MCPs (D2: 1.16 ± 0.43, D14: 1.07 ± 0.53, D21:

489 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,

490 Table 5, $F_{2,28}$ = 0.53, P = 0.594, η_p^2 = 0.04).

491

492 FIGURE 3 HERE

493

494

495

496

Fatigability

498   Time to task failure during the intermittent, isometric, fatiguing task was significantly

499   affected by MCP (see Figure 3, $F_{1.4, 14.8}$ = 6.89, P = 0.030, η_p^2 = 0.32), with post hoc tests

500   showing greater TTF on D21 compared to D2 (mean difference: 187 s, P = 0.025).

501   However, there was no difference between D21 and D14 (mean difference: 135 s, P =

502   0.103), or D2 and D14 (P = 0.594). The two-way ANOVA (MCP × time) time effect showed

503   that MVC decreased pre-post exercise ($F_{1,11}$ = 80.056, P < 0.001, η_p^2 = 0.88), as did $Q_{tw.pot}$

504   ($F_{1,11}$ = 123.53, P < 0.001, η_p^2 = 0.92), VA_{MNS} ($F_{1,11}$ = 15.219, P = 0.002, η_p^2 = 0.58), and

505   VA_{TMS} ($F_{1,11}$ = 13.99, P = 0.003, η_p^2 = 0.56). SP also increased pre-post exercise ($F_{1,11}$ =

506   9.68, P = 0.010, η_p^2 = 0.468). The MCP × time interaction effects for the aforementioned

507   variables that changed pre-post exercise indicated no difference between MCPs (all P ≥

508   0.128). The only exception to this was VA_{TMS} ($F_{2,22}$ = 3.48, P = 0.049, η_p^2 = 0.24), however,

509   post hoc tests revealed that the differences were only apparent pre-exercise (as indicated

510   above), and not post-exercise (P ≥ 0.670). Despite no time effect (P = 0.578), SICI

511   displayed a MCP × time interaction effect ($F_{1.4, 15.0}$ = 5.26, P = 0.028, η_p^2 = 0.32), however,

512   the only differences were evident pre-exercise (as indicated above), with no post-

513   exercise difference (P ≥ 0.247).

514

515   TABLE 5 HERE

516

517   All variables measured during the fatiguing tasks (see Figure 4) demonstrated time

518   effects (P ≤ 0.024), however, only some (MVC, $Q_{tw.pot}$, VA_{MNS}) demonstrated an absence of

519   MCP × time interaction effects (P ≥ 0.205). MVC (Figure 4A) decreased progressively from

520   baseline to 75% TTF (all intervals P ≤ 0.001), however, between 75% and 100% TTF no

521   further decrease was observed (P = 0.776). A similar pattern was observed with $Q_{tw.pot}$

522   (Figure 4B), with decreases exhibited until 50% TTF (both intervals $P \leq 0.009$), however,

523   between 50-100% TTF $Q_{tw.pot}$ did not further decrease ($P \geq 0.593$). VA_{MNS} (Figure 4C)

524   demonstrated the inverse time course, with no change from 0 to 50% TTF ($P \geq 0.345$),

525   then a progressive decrease from 50 to 100% TTF ($P \leq 0.034$). rmsEMG (Figure 4D) and

526   RPE (Figure 4E) exhibited phase × time interaction effects ($P \leq 0.032$). RPE increased

527   progressively throughout all trials ($P \leq 0.008$), however, at 25% TTF RPE was greater on

528   D21 compared to D14 (+2, $P = 0.006$), at 50% TTF D21 was greater than D2 (+2, $P <$

529   0.001), and at 75% TTF D21 was greater than D2 (+1, $P = 0.005$). The only significant

530   increase in rmsEMG was between 25 and 50% TTF ($P = 0.003$), and despite the phase ×

531   time interaction effect, no post hoc differences between phases were apparent ($P \geq$

532   0.205).

533

534   FIGURE 4 HERE

535

536   **DISCUSSION**

537   The present investigation aimed to assess the influence of modulations in female sex

538   hormones across the eumenorrheic menstrual cycle on neuromuscular function and

539   fatigability. The data from *Study A* established repeatability of the measures in a

540   hormonally-constant female population (mOCP users). Subsequently, *Study B* showed

541   that in eumenorrheic females, the hormone-induced changes in neuromuscular function

542   and fatigability across the menstrual cycle were greater than the associated error from

543   hormonally-constant females in *Study A*. Whilst one index of neuromuscular function

544   (MVC) did not change, modulations in CNS control of muscle contraction were observed.

545   Specifically, VA was greatest on D14 which was concurrent with an increase in the

546   concentration of oestrogen. Additionally, parallel to an increase in progesterone, SICI was

547 greatest on D21. Time to task failure during the open-ended intermittent, isometric

548 protocol was greatest on D21 of the cycle. Collectively, the present data suggest that

549 neuromuscular function and fatigability are modulated by the eumenorrheic menstrual

550 cycle.

551

552 *Maximum strength and voluntary activation across the menstrual cycle*

553 There was no effect of MCP on MVC force. As mentioned, previous data regarding

554 maximum voluntary strength across the menstrual cycle is equivocal. In agreement with

555 the present study, multiple studies have shown no effect (24, 40, 46), however, several

556 studies have shown that strength peaks mid-cycle (49, 53, 64). Previously, discrepancies

557 such as the time of day (4), or variability in menstrual cycle duration, as well as the chosen

558 days of the menstrual cycle for testing (27) have been used as explanatory reasons for

559 this discrepancy. The present study controlled these factors within Study B, by testing at

560 the same time of day and confirming participants were in the correct phase by serum

561 hormone analysis, yet no effect of MCP was observed.

562

563 Interestingly, Study B demonstrated changes in VA (assessed by both MNS and TMS)

564 despite no change in MVC. VA_{MNS} peaked on D14 and VA_{TMS} was greater on D14 compared

565 to D21 (see Figure 1). As $Q_{tw.pot}$ and ERT were not affected by MCP, these changes in VA

566 were mediated by a decreased SIT amplitude on D14 in response to both motor nerve

567 and motor cortical stimulation. This could indicate that there was a decrease in the

568 capacity of the CNS to elicit extra force in response to stimulation. The TMS and MNS

569 evoked SITs represent the extra force from motor units that the CNS is not able to

570 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

620 conclusion that the menstrual cycle affects only GABA$_A$ neurotransmission cannot be

621 made with the current data, as Yacyshyn et al. (73) showed that the SP has a large spinal

622 contribution. Additionally, glutamatergic intracortical facilitation (ICF) was not

623 measured in the present study, but has previously been shown to be affected by the

624 menstrual cycle, with augmented ICF demonstrated mid-cycle (56, 57). Whilst the causal

625 link between intracortical function and voluntary activation is under researched, it is

626 possible that the adjustments of intracortical circuitry altered the capacity of TMS and

627 MNS to evoke a SIT. For instance, if intracortical excitability was greatest on D14, there

628 may have been a 'ceiling effect', meaning the stimulations were not able to induce

629 additional excitation in the motor cortex, thus, innervating fewer additive motor units

630 during MVCs, and evoking a smaller SIT, and the contrary occurring on D21, when

631 inhibition was greatest. The modulation of neurotransmitters has previously shown to

632 affect VA, with pharmacological increases in noradrenaline (44) and serotonin (42)

633 resulting in a ~1-2% increase in VA. Indeed the effects of serotonin have been shown to

634 be augmented by oestrogen (3), and inhibited by progesterone (34). Therefore, it is

635 possible that the modulation of inhibitory and facilitatory intracortical circuitry across

636 the menstrual cycle might collectively contribute to the changes in VA$_{MNS}$ and VA$_{TMS}$.

637

638 *Fatigability across the menstrual cycle*

639 Fatigability, as measured by the TTF of the open-ended fatiguing protocol, was lowest on

640 D21 (i.e. greatest TTF), thus supporting the findings of Sarwar et al. (53), who showed

641 that fatigue index was lowest in the luteal phase during a three-minute intermittent

642 involuntary contraction protocol. The present data, however, contradicts Janse de Jonge

643 et al. (40), who showed no effect of MCP during voluntary or electrically evoked fatiguing

644 protocols performed with the knee extensors. The differences between tasks could

645   explain these discrepancies. The voluntary task used by Janse de Jonge et al. (44) involved

646   both dynamic knee extension and flexion, rather than a single muscle group. This

647   anisometric, multi-muscle group exercise likely elicits a different pattern of sensory

648   afferent feedback (30), and was not open-ended like the present study, which could

649   explain the discrepancies in fatigability. The same reasons might also apply to why the

650   findings of DiBrezzo et al. (21) are inconsistent with the present study, who similarly

651   demonstrated no menstrual cycle effect on fatigue during a set amount of dynamic

652   contractions. Thus, the task employed in the present study likely permitted a greater

653   degree of fatigue to develop, allowing the aforementioned MCP differences to be

654   discerned.

655

656   As widely acknowledged, fatigability has both physiological and perceptual components

657   that interact to determine exercise tolerance (26, 66). The fatiguing task in the present

658   study involved high intensity (60% MVC) intermittent, isometric contractions, which

659   were assumed to be far greater than the critical torque (~30% MVC [12]), and limited by

660   decrements in neuromuscular adjustments (1, 11). With no MCP x time interaction effects

661   displayed for neuromuscular variables (MVC, Qtw.pot, and VA), the degree of pre-post

662   exercise adjustment was not different between menstrual cycle phases. Accordingly, one

663   hypothesis for why TTF was longer on D21 could be the influence of neurotransmitter

664   systems on perceptions of fatigue. The present study measured GABAergic inhibition and

665   demonstrated a large increase in SICI on D21 (Figure 2B), and it has previously been

666   shown that GABA can have anti-nociceptive properties (25) acting as an analgesic (41).

667   Indeed, it has recently been postulated that "luteal analgesia" occurs in eumenorrheic

668   females when progesterone is elevated, where the affective response to nociceptive pain

669   is reduced due to alterations in functional connectivity in the emotional regulation

28

670 network (69). Thus, it could be possible that the analgesic effects of enhanced GABAergic

671 neurotransmission permitted participants to continue exercising for a longer period due

672 to a lower perception of pain. However, more evidence is needed to explore the effects of

673 GABAergic inhibition on exercise-induced fatigue.

674

675 *Further Considerations*

676 In *Study* B it would appear that there was substantial between-subject variation in

677 neuromuscular function and the changes across the menstrual cycle (Figures 1-3).

678 Potential explanations for this could be the large standard deviations in hormone

679 concentrations at each time point (Table 4), which has been reported in previous

680 investigations (55). Additionally, inter-individual differences in hormone receptor

681 numbers and sensitivity could contribute to the variation in changes across the menstrual

682 cycle. In muscle tissue (22), expression of sex hormone receptors is altered by changing

683 hormonal environments, which could conceivably occur in neuronal tissues such as the

684 motor cortex, however the present data cannot answer this research question.

685

686 Whilst serum hormones were quantified for the eumenorrheic females in Study B, the

687 mOCP users' serum hormone concentrations were not quantified in Study A. These data

688 would have provided useful information about the measurement error of the sample,

689 however individual 'meaningful' changes might differ between participants in order to

690 achieve ovulation.

691

692 *Conclusion*

693 The present investigation demonstrated that when neuro-active hormones are constant,

694 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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Tables

908 Table 1: Monophasic combined oral contraceptive pills (mOCPs) taken by participants in Study
909 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

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914 Table 2: Reliability data for mechanical variables pre- and post-exercise in Study A. Pre-post
915 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

916 Table 3: Reliability values for electromyographical data pre- and post-exercise. Pre-post change
917 (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

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

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

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931 Table 5: Variables assessed throughout the pre- and post-exercise testing battery across the menstrual cycle. P values from the baseline ANOVA (1 ×
932 3 repeated measures), and the pre-post exercise ANOVA (2 × 3 repeated measures) are reported. When a significant effect of exercise was found, the
933 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 1×3 ANOVA	Pre-post exercise 2×3 ANOVA	MCP × Exercise 2×3 ANOVA
	Pre	Post	Δ	Pre	Post	Δ	Pre	Post	Δ			
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.

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Figure Legends

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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; #: sign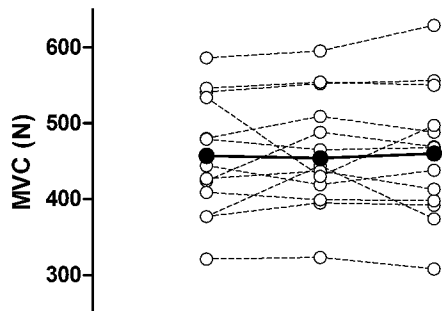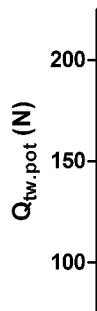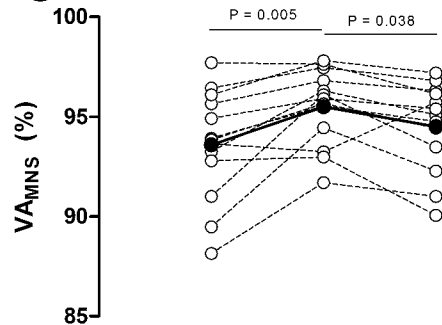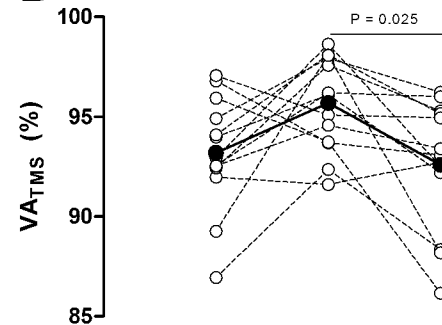ificant difference between D21-D2.