Health & Medicine | Lancaster University

Effects of time of day and menstrual cycle phase (low and high progesterone) on female's cognitive and strength performance.

NTC number:

Approved: 05/01/2026

Protocol

This study aims to investigate the effects of time-of-day and menstrual cycle phase on tier 2 female's cognitive and strength performance through several measures. The independent variables for this study are (i) time-of-day (three levels: 07:00, 12:00, and 18:00 h) and (ii) menstrual cycles phase in eumenorrheic females (two levels: low levels of progesterone (phase 1) and high levels of progesterone (phase 4) (>16nmol L^{-1}; Verhagen *et al.*, 2025). The dependent variables of this study include muscle strength, measured across three domains (maximal handgrip strength, flight time and height performing CMJ and SJ, and maximal lower limb strength), and executive function using a modified version of Eriksen's original Flanker task, (Koch and Krenn, 2021), and electroencephalography (EEG) alpha wave activity, measured with an Enobio 8 EEG system (Neuroelectrics/Cambridge/MA). Each measure will have at least one cross validation measure, to ensure greater validity in our findings.

An a-priori analysis was conducted using G*Power version 3.1.9.6 for this study (Faul et al. 2007); chosen due to the intention to analyse the data using a MANOVA repeated measures within factors statistical test. The sample size for this study was determined based on a medium effect size (f=0.25), using a statistical power of 80% and an alpha value of 0.05. This study involves one group (tier 2 females partaking in sports) with two independent variables of time of day (07:00, 12:00, and 18:00 h) and menstrual cycle phase (highest and lowest levels of progesterone). G*Power indicated a minimum sample size of 24, however, to account for any potential data loss or dropouts, this study will aim to collect data from 30 participants.

Participants will be recruited via opportunity sampling with the use of posters placed around Lancaster University campus, online advertisements on social media platforms and through word of mouth. For individuals to participate, they must meet the extensive eligibility criteria to ensure that this study tests the effects of time-of-day and menstrual cycle phases on tier 2 female cognitive and strength performance. Participants must fit the following criteria:

<u>Inclusion criteria (must have all the following):</u>

- Female (assigned at birth)
- Aged 18-30
- Tier 2 athlete which requires the participants to identify with a specific sport, train with a purpose to compete, regularly train ~3 times per week and compete at a local level (for example being a member of a BUCS sport and competing regularly with that sport)
- Regular menstrual cycle – considered between 21–35-day cycles with at least 9 periods a year.

<u>Exclusion criteria (cannot have any of the following):</u>

- The current/previous (in the last 3 months) use of any contraceptive method that alter the menstrual cycle
- The use of the emergency contraceptive pill either 3 months prior to the study or during the study
- Diagnosis of menstrual disorders including (but not exclusively) endometriosis and PCOS
- A formal diagnosis of sleep disorders such as insomnia
- Consume >400mg/day of caffeine
- A musculoskeletal injury in the past 3 months
- A formal diagnosis of neurological or psychiatric disorders such as ADHD, ASD, Dyslexia or schizophrenia
- Taking prescribed medication that may affect sleep for example stimulants such as Methylphenidate

- No regular night shift work
- No trans-meridian travel within 10 days of their expected participation dates (travel that will cause jetlag)
- Have given blood in the last 12 weeks – this is not strictly an exclusion; participants will just need to wait until after 12 weeks before taking part in the study

Following confirmation from the participant that they meet all inclusion criteria, they would be invited for a pre-screening and familiarisation visit to the Human Performance Laboratory (HPL). Participants must refrain from alcohol, drugs and caffeine 24 h before the screening and must attend the screening in active wear.

Commented [CG1]: Re screening - at the screeing visit I'd suggest you ask Ash to have a look at the participant's veins.

Some young females can be very tricky to get blood from. It doesn't mean we need to exlcude but might be worth having extra help in the lab - we need to be confidence we're getting blood from anyone who particiaptes...

Participants will be provided with an information sheet on arrival, outlining the study, followed by a consent form that must be signed before continuing. A questionnaire pack entitled "Screening questionnaire pack" must then be completed by each participant. The screening questionnaire pack includes 4 sections: 'Section 1 – The ACSM', 'Section 2 – The menstrual cycle questionnaire' and 'Section 3 – The Composite Morningness questionnaire' (Smith *et al.*, 1989) and 'Section 4 – daily caffeine consumption questionnaire'.

Section 1 assesses the risk factors of each participant, asking for family history, health conditions, and measuring resting blood pressure, anthropometrics, blood analysis and checking for atrial fibrillation. Each participant must pass this assessment prior to continuing. If at this stage, the participants answer's require medical clearance, they will be asked to provide this before continuing with the study. If a participant does not receive medical clearance, they will no longer be able to take part in the study and be provided with a debrief sheet. Throughout the study, if a participant presents as unable to continue in the study or wishes to withdraw from the study, they too will be provided with a debrief sheet.

Section 2 is further divided into part A and B. Part A will ensure the participants are eligible for the study by asking them about the regularity of their menstrual cycle, as well as asking when their next cycle starts (the first day of bleeding) which will also inform the possible dates the participant can carry out the testing days. Part B contains the menstrual distress questionnaire (MEDI-Q), an evaluation tool of a participant's menstruation-related distress, investigating participants experience of pain, discomfort, psychic and cognitive changes, gastrointestinal symptoms and changes in physiological function during their periods over the last 12 months (Vannuccini *et al.*, 2021).

Section 3 assesses the participants morningness chronotype by asking questions surrounding their ideal wake-sleep routine, how they would feel if they had to rise in the early morning and how well they function after waking. From this, their chronotype can be determined using a scoring system of 1-5 on each option of the questionnaire; morning type (44 and above), intermediate type (22-43) and evening type (22 and less; Shahid *et al.*, 2011).

Section 4 assesses how much caffeine the participant typically consumes day-to-day; asking what form of caffeine they consume (drinks such as tea or tablets) and the quantity.

Familiarisation of the executive function task and muscle strength tests will follow, to allow participants to practice the tests. The familiarisation visit must be conducted 1-2 weeks before the first testing visits to minimise learning effects.

Commented [CG2]: I would insert a table here or where approrpaite that says (i) what we're going to measure and (ii) in what biofluid and (iii) why

e.g. Oestrogen (E2) in blood at morning visit of visit 1 and 2 to distinguish follicular vs luteal phase

Progesterone - blood

LH - urine?

FSH?

Participants will be provided with five Luteinising hormone (LH) ovulation strips (Easy@Home, Easy Healthcare Corporation, IL, USA) to take home and use three to four days prior to expected ovulation date (known by self-tracking of their menstrual cycle). These strips require participants to urinate onto the strip and wait 2-3 minutes (min) for the indicator to appear. For a positive test, the indicator line must show up darker than the control line. LH is always present in the body however there is a rise in

LH levels 12-36 h prior to ovulation and therefore the positive test on the strip is an indicator of when ovulation will occur for the participant. If a participant does not get a positive test, they will be sent the debrief sheet and will no longer be able to partake in the study as some females do not ovulate (anovulation/ oligo-ovulation) and therefore are not eumenorrheic (Mihm *et al.*, 2011). Participants in this study will follow a similar structure to the best practice highlighted by Verhagen *et al.*, (2025) in which one cycle of the following is required: self-reporting the onset of menses to schedule phase 1 testing, a day 1,2,3 or 4 blood sample for the determination of β-oestradiol and progesterone to confirm phase 1, daily LH urine sticks from 5 days before estimated day of ovulation until the LH surge to schedule phase 2 testing, and a blood sample for the determination of progesterone (≥16 nmol.L^{-1}) +7 days (±1) days after confirmation of the LH surge to confirm phase 4 testing.

24 h before both testing visits, participants will be asked to refrain from alcohol and drug consumption and retire before 23:00 h. On the day of both tests, participants will be asked to wake at 06:00 h and arrive at HPL fasted and having consumed no caffeine. Participants will visit the laboratory 3 times; 07:00, 12:00 and 18:00 h and carry out the same battery of tests at each time. On the first visit, participants will be provided with a food diary, asking them to record exactly what they ate and when, so that this can be replicated on the second test day to reduce variability in individual data. Participants would be asked to arrive at 12:00 and 18:00 h having fasted for 3 h prior; they would be told to eat immediately after each testing session at 08:00, 13:00 and 19:00 h to limit the thermic effect of food (TEF) on core body temperature (Reed and Hill, 1996).

Commented [CG3]: as discussed yesterday it might be better to specify when they should eat rather than when not - just a thought

Test day protocol:

Figure 1 shows a schematic of the research design and the experimental procedure. The participants would perform the full process described above at 07:00 and 18:00 h, whilst at 12:00 h participants would carry out all steps except the strength measures to reduce the fatiguing effect on these measures.

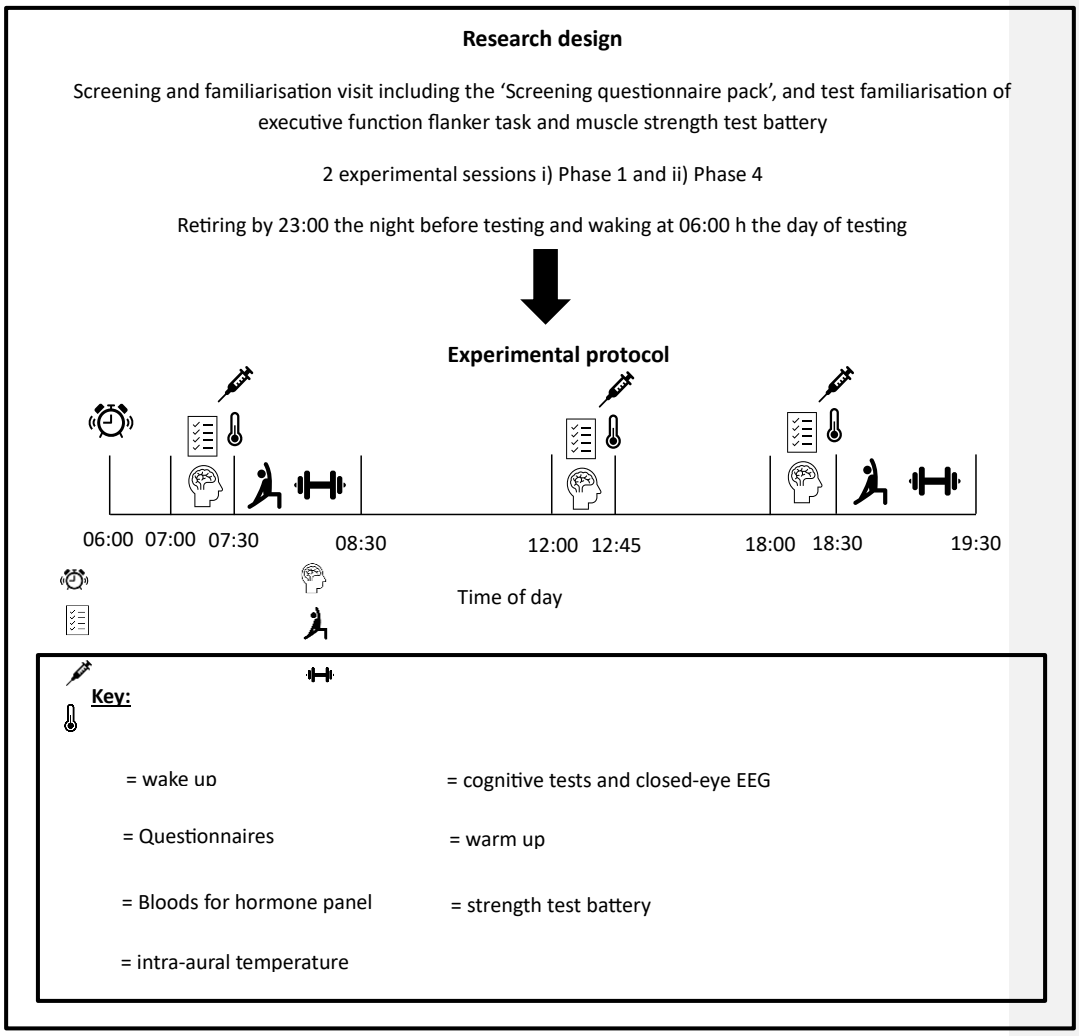

Figure 1: Schematic of research design and experimental protocol

On arrival at 07:00 h participants will have several measures taken before being seated for an acclimatisation period. Participants height and weight will be taken using bioelectrical impedance scales (DC-430P/Tanita/Tokyo, Japan). Participants will then be seated at a desk surrounded by a screen which is required to help reduce external stimuli which could later affect the cognitive element of the study. A heart rate monitor (H10/Polar/Kempele, Finland) will be fitted around the participants chest to measure heart rate (HR) and heart rate variability (HRV) throughout the acclimatisation period. Participants will then remain seated for a 30 min acclimatisation period to reduce any transient influences of the pre-laboratory environment which could affect core body temperature reading.

During this 30-min acclimatisation period, participants would complete the '**test day questionnaire'** containing four sections; 'Section A: menstrual symptoms', 'Section B: sleep quality' 'Section C: Profile of Mood states' (Terry *et al.*, 1999) and 'Section D: caffeine withdrawal questionnaire'.

Section A will ask participants to categorise several menstrual symptoms with a number from 0-4 with 0 being not present and 4 being extremely present.

Section B will assess the participants quality of sleep over the past month and of the night before test day compared to their 'normal'.

Section C contains 32 feelings that people experience frequently and asks the participant to circle a number 0-4 for each feeling, with 0 being 'not at all' and 4 being 'extremely'.

Section D asks participants asks participants to circle a number 0-4 for each feeling listed which related to caffeine withdrawal, having not consumed caffeine for 24 h.

Section E will ask participants to draw a line along a number scale to indicate how tired/alert they feel. These questionnaires should take 5-10 minutes for the participant to complete.

After the completion of questionnaires, participants will have the **EEG** system fitted. Using the Enobio 8 EEG system (Neuroelectrics/Cambridge/MA), neural activity at resting-state will be measured. The sliver-silver chloride (Ag|AgCl) electrodes are prepositioned (Cz, F3, F4, Pz, P7, P8, O1 and O2) according to the international 10-20 montage system (figure 2) in a head cap that is placed on the participants head prior to recording, with adjustments made after correct placement of Cz electrode. The reference electrode will be placed on the right earlobe (A2) with a small volume of gel as recommended by the manufacturer. Participants will relax for 2 min and ear plugs will be given, and they will be asked to close their eyes to reduce the level of external stimuli. After 2 min relaxation, participants will be told that the EEG recording is about to happen. Firstly, for a period of 5 min, participants will sit with their eyes closed whilst EEG is recorded (resting eyes closed [REC]). Then participants will be asked to open their eyes and focus on a black cross displayed on a computer screen in front of them, this will happen for another 5 min (resting eyes open [REO]). Participants will then be asked several temporal questions which focus on their perception of time, the questions will ask about the amount of time the participants spent with their eyes closed/open and if these times vary between TOD and phase.

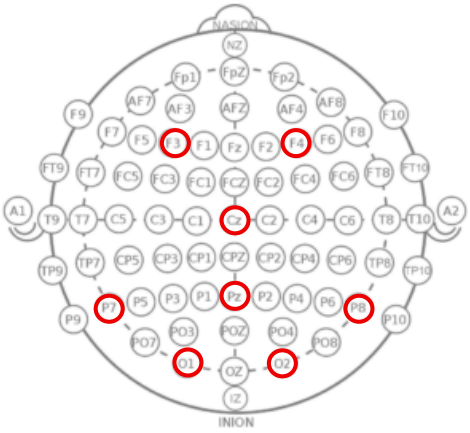

Figure 2: EEG electrode placement

Following this, participants will be instructed to carry out an **executive function test battery**. The test carried out will be a modified version of Eriksen's original Flanker task (Eriksen and Eriksen, 1974; Koch and Krenn, 2021). The Flanker task is used as a measure of inhibition and cognitive flexibility; asking participants to respond to a visual stimulus depending on what is displayed on the screen and the instructions given. This modified flanker task uses arrows displayed on a screen and asking participants to focus on the central arrow that is flanked by arrows to its left and right, these flanking arrows are either congruent or incongruent to the central arrow. This test battery consists of two sections:

Section 1 requires the participants to react to the middle arrow by pressing C on an electronic keyboard if the arrow was facing to the left and press M if it was facing to the right – measuring simple reaction time.

Section 2 measures cognitive flexibility – this section requires participants to switch their reaction response depending on the colour of the arrows. If the middle arrow appears red, they must press C if it is pointing right and M if it is pointing to the left. If the middle arrow is green, they must treat them as in the previous section, pressing C for left facing and M for right facing. (Koch and Krenn, 2021).

Reaction time and number of correct answers for congruent and incongruent sequences (arrows in the same or different directions) would be determined and analysed as an indicator of cognitive flexibility and inhibition (two measures of executive function).

After the 30-min acclimatisation period, participants intra-aural **temperature** will be measured; two measures will be taken 2 minutes apart, if the difference between the values is >0.2°C then a third measure would be taken. Intra-aural temperature will be measured using a tympanic thermometer (TH889J/Radiant Innovation/Hsinchu, Taiwan). The average temperature would be calculated as the mean of the closest pair of measurements (Reilly *et al.*, 2007, Edwards *et al.*, 2005). The rectal probe is the gold standard measure of core body temperature however after discussions with a group of female athletes, the decision to measure core body temperature using intra-aural was made, as it was concluded that the rectal probe would reduce participant willingness to volunteer for the study.

Commented [CG4]: need blood pressure too

Blood pressure (BP) will be measured as a cross-validation method of circadian rhythm using an automatic blood pressure monitor (M3 Comfort/Omron/Kyoto, Japan). Participants will remain seated with their feet flat to the floor and arm relaxed on the desk in front of them for the measure of BP.

Venipuncture of the arm would then be performed to draw blood from the participants. The blood samples would be collected in several vacutainers (gold topped for Oestrogen, progesterone and hsCRP, and purple top for inflammatory markers) with a small volume extracted and placed on ProWipes pads where the participant's blood can be drawn up in a capillary tube for **glucose and lactate** analysis using a blood analyser (Biosen C-Line GP+/EKF/Barleben, Germany). This measure will only be performed at the morning visit to consider blood glucose and lactate levels after the overnight fast. The remaining **blood samples** collected in vacutainers would be stored at -80°C before carrying out various assays, according to manufacturer's guidelines, to measure progesterone (P), oestradiol (E_2) and Interleukin (IL-6), Tumour necrosis factor (TNFα) and high sensitivity C reactive protein (hsCRP). Blood analysis of progesterone and oestrogen would be carried out in duplicate using commercially available serum and plasma enzyme linked immunosorbent assays (**ELISA**). The blood analysis would act to confirm correct phase identification during testing, with the exclusion of participants occurring after testing due to E_2 or P levels not matching those defined for Phase 1 and 4 above.

Table 1: summary table of proteins measured, their biofluid and the purpose of the measurement

	Biofluid	**Assay**	**Purpose of measure**
Progesterone	Serum	ELISA	As a confirmation of phase of participant's cycle.
Oestradiol	Serum	ELISA	As a confirmation of phase of participant's cycle.
Luteinising hormone	Urine	Easy@Home, Easy Healthcare Corporation, IL, USA	Carried out 5 days prior to expected the LH surge which occurs 24-48 hours prior to ovulation to determine when ovulation will fall.
IL-6	Serum and Plasma	LEGENDplex Human inflammation panel 1	To measure key inflammatory markers to determine a possible relationship between inflammation and the menstrual cycle/time-of-day, resulting in possible discrepancies in strength and cognitive measures.
TNF-α	Serum and plasma	LEGENDplex Human inflammation panel 1	To measure key inflammatory markers to determine a possible relationship between inflammation and the menstrual cycle/time-of-day, resulting in possible discrepancies in strength and cognitive measures.
hsCRP	Serum and plasma	ELISA	To measure key inflammatory markers to determine a possible relationship between inflammation and the menstrual cycle/time-of-day, resulting in possible discrepancies in strength and cognitive measures.

Following a 5-min warm up on a static bike at 80-120 watts, the participants would complete the **strength test battery** section of the study. The handgrip dynamometer measures voluntary isometric grip force (N) of the hand flexors, which is known as handgrip strength (HGS) (McGrath *et al.*, 2022). Participants would be seated on a chair with a straight back with their shoulder adducted and neutrally rotated, elbow flexed to 90° with a neutral forearm and wrist, in accordance with the American Society of Hand Therapists'. Participants would grip the dynamometer with their whole hand at the

predetermined handle position, set at the familiarisation visit, they will be asked to maximally squeeze the dynamometer for 3-seconds. 3 repeats will be carried out with the participants dominant hand, with 1-min of rest between each, recording the maximal values to determine both peak and mean values, along with height as these have shown to strongly corelate with HGS (Fiebert *et al*., 1998).

Following this, participants would perform both countermovement (CMJ) and squat jumps (SJ), measuring flight time (s) and vertical jump height (cm). The Jump height meter (Optojump/Microgate/Bolzano, Italy) consists of two parallel bars (one receiver and one transmitter bar) that are positioned approximately 1m apart on the floor, with the athlete stood with their feet running parallel between the two bars. For SJ, participants start in an upright position with their hands on their hips, lowering into a squat position with their knees at approximately 90°, and holding this position until the researcher counts them down. On the count of 3 the participant was instructed to jump as high as they could from the predetermined squat position, keeping their hands on their hips. For CJM, participants also begin with their hands on their hips, in the upright position. On the count of 3, they are encouraged to assume a similar squat position (knees at approximately 90°) as in the SJ as quickly as possible, jumping as high as possible immediately after. For both SJ and CMJ, participants are asked to take-off with extended knees and ankles, and land in a similar way, bending their knees as they land. Each jump type will be repeated 3 times with a rest interval of 30-seconds between each jump repetition and a 2-minute rest interval between jump types.

Participants will then carry out lower limb strength testing using the **Biodex system** (S3/Biodex/New York, U.S.A.) on their dominant leg. Seating position would be predetermined in the familiarisation visit and participants would be strapped to the chair as seen in. Participant's exact range of motion and limb weight will be determined for each visit before carrying out maximal isometric Voluntary contraction of knee extensors and flexors measured over 4s at 60° flexion. Between away and towards contractions, participants will have 30-sec and between each set, participants will have 3-min passive recovery.

Commented [CG5]: Flexibility would be good to measure here too

IKD dynamometry (torque-angle) with EMG confirmation?

Goniometry for something cheap and quick but above more physiological

Commented [AS6R5]: I'm a little confused by this comment - what do you mean? Have the changes to detail answered this question? What do you mean by flexibility?