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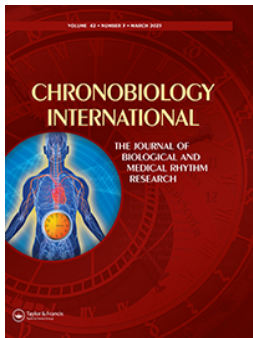
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To cite this article: Ben J. Edwards, Kevin P. Gibbins, Chris J. Morgan, Magali Giacomoni, Colin M. Robertson, David A. Low & Samuel A. Pullinger (28 Apr 2025): Investigating effects of moderate hyperthermia at two phases of the circadian cycle for core temperature (heat gain and peak), on quadriceps maximal voluntary contraction force, Chronobiology International, DOI: [10.1080/07420528.2025.2494631](https://doi.org/10.1080/07420528.2025.2494631)

To link to this article: <https://doi.org/10.1080/07420528.2025.2494631>



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Published online: 28 Apr 2025.



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Investigating effects of moderate hyperthermia at two phases of the circadian cycle for core temperature (heat gain and peak), on quadriceps maximal voluntary contraction force

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ABSTRACT

Athletes often perceive a performance disadvantage in the morning, in part, because of a recognised deficit in functional muscle force capacity. This diurnal variation in muscle force production has been attributed to higher rectal (T_{rec}) and muscle (T_{m}) temperatures in the evening as well as motivational, peripheral, and central factors. A warm-up is an essential component of sporting performance, however moderate hyperthermia reduces sporting gross muscular performance although possibly to a lesser degree in the morning (raising phase) than the peak of the core temperature rhythm (~17:00 h). We investigated whether i) increasing morning T_{rec} temperatures to evening resting values by an active warm-up leads to quadriceps muscle force production becoming equal to evening values. Or ii) raising T_{rec} passively in the morning or evening to 38.5°C results in greater quadriceps muscle force production reductions in the evening. Eight active males (mean±SD: age, 25.5 ± 1.9 yrs; body mass, 71.0 ± 6.7 kg; height, 1.79 ± 0.06 m) volunteered and randomly completed five sessions (separated by > 48 h): control morning (M, 07:30 h) and evening (E, 17:30 h) sessions (both with an active 5-min warm-up) and three further trials – an active warm-up 07:30 h trial (M_{E} , until resting evening temperatures were reached), a morning ($M_{38.5}$) and an evening ($E_{38.5}$) passive warm-up trial which continued until T_{rec} values reached 38.5°C (immersed in a water-bath @ ~40°C, 45–50% Relative humidity). During each trial, 5-measures of maximal voluntary contraction (MVC) of the quadriceps on an isometric dynamometer (utilizing the twitch-interpolation technique) were performed with force (peak and mean of the 5-trials) and percentage activation recorded. T_{rec} , ratings of perceived exertion (RPE) and thermal comfort (TC) were measured. Measurements were made after the participants had reclined for 30-min at the start of the protocol and after the warm-ups/passive heating and prior to the measures for isometric dynamometry. T_{rec} and T_{m} (at 3, 2 and 1 cm depths) temperatures were taken at rest, after the passive warm-up, and immediately before the isometric MVC measurements. Data were analysed by general linear models with repeated measures. Isometric force for knee extension showed higher values in the evening than morning (peak $\Delta 83.2$ N, mean $\Delta 67.8$ N; $p < 0.05$). T_{rec} and T_{m} (at 3 cm depth) values were higher at rest in the evening than the morning (by 0.47 and 0.85°C respectively; $p < 0.05$) increasing from rest by 0.54 and 2.2°C, 1.78 and 2.2°C, and 1.31 and 1.8°C, in the M_{E} , $M_{38.5}$ and $E_{38.5}$ conditions, respectively; ratings of thermal comfort reflecting this ($p < 0.05$). There was no significant effect of active M_{E} warm-up and moderate hyperthermia $M_{38.5}$ compared to morning control peak (peak or mean) torque (M). $E_{38.5}$ reduced “mean” but not “peak” torque in the evening ($\Delta 61.9$ N, $p = 0.009$; $p = 0.051$). In summary, active warm-up did not improve isometric MVC in the morning and moderate hyperthermia reduced isometric MVC “mean” force only during the peak of the core temperature rhythm (~17:00 h).

ARTICLE HISTORY

Received 13 September 2024
Revised 27 February 2025
Accepted 13 April 2025



KEYWORDS

Rectal and muscle temperature; MVC force; percutaneous stimulation; diurnal variation

Introduction

Muscle force production and power output consistently peak in the mid-afternoon or early evening, this evidence is predominantly in male participants in a temperate environment (around 17–20°C, Robertson et al. 2024). This seems to be regardless of the muscle group measured (hand, elbow, leg or back, for example)

or speed of contraction (see reviews by Drust et al. 2005; Reilly and Waterhouse 2009). Recently, in strength conditioned individuals, when comparing the circadian characteristics outputs for Biodex Isokinetic and Isometric MVC methods as well as the Muscle Lab force-velocity linear encoder, measures of “peak torque” and “peak velocity” were consistently higher in the

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evening (Acrophase $\Phi \sim 17:00$ h). “Time-to-peak” torque/velocity was repeatedly slower during the morning ($\Phi \sim 06:00$ – $08:00$ h, Robertson et al. 2024) whatever the testing device, the angular velocity or the muscle group tested. We have discussed this before (Edwards et al. 2013; Robertson et al. 2024) but, in brief, the proposed mechanisms to explain daily rhythms in force production include the following: 1) Peripheral or muscle-related variables (contractibility, metabolism and morphology of muscle fibres and local muscle temperature), which can be influenced by hormonal and ionic muscle process variations (Araujo et al. 2011; Bambaiechi et al. 2005; Reilly and Waterhouse 2009). And/or 2) Neurological factors (central nervous system command, perceived alertness, motivation and mood: Castaingts et al. 2004; Giacomoni et al. 2005; Racinais et al. 2005; Racinais 2010) which are complimentary to explain the force production. Whereas 3) Input from the body clock and proteins and peripheral clocks – that is, an endogenous component to the daily variation in muscle force production has been suggested to be important (Zhang et al. 2009). And/or 4) A causal link between “resting” core and muscle temperatures and performance both will affect the variations in peripheral or neurological factors. It is suggested that the higher evening resting core temperature ($T_c \sim 0.8^\circ\text{C}$ in rectal and gut sites: Edwards et al. 2002) and local muscle temperature ($T_m \sim 0.35^\circ\text{C}$ in vastus lateralis at depths of 3 cm: Edwards et al. 2013; Robinson et al. 2013) produces an increase in force-generating capacity of the muscle (Bernard et al. 1998; Giacomoni et al. 2005) and neural function (reduced twitch time course or increase in speed of contraction: Martin et al. 1999).

A warm-up is an essential component of sporting performance (Bishop 2003) and the link between T_c and physical performance has been studied in chronobiology and sporting studies. Predominantly using either passive (raising T_c values by external means) or active “warm-up” exercises, to increase T_c values in the morning to rectal temperature values found in the evening. Then investigating if this change in T_c resulting in increases morning performance to evening values, where either time to complete a cycling or swimming time-trial or muscle force production has been investigated (see Edwards et al. 2013 for further details). However, using an active or passive warm-up to increase T_c has shown no concurrent increases in morning performance indices (flexibility, muscle force output, times for swimming or cycling time-trials, sprints, power output from Wingate test or in a loaded counter-movement jump) to evening values (Arnett 2002;

Atkinson et al. 2005; Martin et al. 1999; Racinais et al. 2004a, 2004b, 2005a, 2005b; Souissi et al. 2010; Taylor et al. 2011). Interpretation of some of these results is compromised by the complexity of the protocols employed, with many measures being taken. This inevitably leads to a long protocol (~ 2.5 h), during which core and muscle temperatures continue to cool, defeating the purpose of the control of T_c warm up. It has been suggested therefore that only a single key performance measurement should be focused upon (Edwards et al. 2013; Robinson et al. 2013).

Considering only passive heating of the body rather than active warm-up, hence no depletion of energy substrates and where no prior exercise fatiguing effect is apparent. Moderate (T_c values of 38.5°C) and severe hyperthermia (T_c values $\sim 39.5^\circ\text{C}$) have been shown to decrease MVC performance (Todd et al. 2003; Periard et al. 2014). This is due to a decrease in the central nervous system function associated primarily with this high T_c rather than peripheral temperature changes (Rodrigues et al. 2023). Corresponding decreases in voluntary nerve activation levels elicited by peripheral nerve stimulation (induced by superimposed twitch) and cortical activation (Schaefer and Bittmann 2019) are also evident. However, the ability to generate force during brief (3–5 s) MVC of the knee and elbow extensor muscle groups, albeit in a small sample size ($n = 3$), was unaltered after exhaustive exercise under heat stress (Oesophageal temperature = $39.8 \pm 0.13^\circ\text{C}$; Nielsen et al. 1993). With passive increases in T_m improving evoked muscle contractile function (such as time-to-peak torque Rodrigues et al. 2023; and peak relaxation rate of muscle; Todd et al. 2005). In previous research, effects of time-of-day have not been considered. However, “resting” core temperature measured from the rectal site (T_{rec}) shows a daily variation where values are lowest in the early morning at about 05:00 h increasing to peak (acrophase) in the evening at about 17:00 h to fall thereafter to 05:00 h (Waterhouse et al. 2005). Based on these observations it has been proposed the human is in a “heat-gain” mode during the rising phase and a “heat-loss mode” on the falling phase, with the peaks and troughs representing the transition phase between heat loss and gain (Aldemir et al. 2000; Otani et al. 2020; Waterhouse et al. 2004, 2007, Figure 2). Pullinger et al. (2018) have previously investigated a possible time-of-day effect to moderate hyperthermia (passive heating to T_{rec} values of 38.5°C) on repeated sprint performance (RS) lasting ~ 5 mins (10×3 s sprints with 30 s recovery). They reported increasing T_{rec} values by a passive warm-up to 38.5°C in the morning (07:30 h; a rise of 1.77°C in the heat gain phase) and evening

(17:30 h; a rise of 1.23°C, at or around the peak in core temperature) resulted in no change in key variables for RS in the morning compared to normal diurnal values (or the control evening data). However, hyperthermia at the peak in the core temperature rhythm resulted in a reduction in evening RS values (distance covered, average power and average velocity, $p < 0.05$) compared to evening control with all variables being like those of the morning control ($p > 0.05$). Whether MVC where time-on-task is 4 s rather than a repeated sprint protocol (5-min) shows differences in response to moderate hyperthermia dependent on the phase of the circadian rhythm and time-of-day is unknown.

Therefore, the present study is designed to investigate whether increasing morning and evening resting T_{re} by passive warm-ups to 38.5°C (in the “heat-gain” and “peak-phase” of core temperature rhythm) will result in similar decrements in muscle force output and activation, using isometric dynamometry with percutaneous stimulation. A second research aim investigates whether our previous finding – that an active morning warm-up which raises rectal temperature to previously measured resting evening values (which increases core and muscle temperature as well as factors independent of temperature) offsets diurnal variation in MVC force output – can be confirmed. We hypothesized the following:

- (1) There will be a diurnal variation in T_{rec} and mean and peak MVC force output.
- (2) Increasing morning and evening resting T_{rec} by passive warm-ups to 38.5°C (in the “heat-gain” and “peak-phase” of core temperature rhythm) will result in decrements in muscle force output (mean and peak) and activation in the evening but not in the morning.
- (3) Using an active morning warm-up on a cycle ergometer to increase T_{rec} to previously measured resting evening values offsets diurnal variation in MVC (means and peak) force output.

Methods

Participants and Ethical Approval

Eight non-heat acclimatized male participants as identified by sex and gender with age (mean \pm SD) 25.5 \pm 1.9 y, height 1.79 \pm 0.06 m, body mass 71.0 \pm 6.7 kg and habitual retiring and waking times at 23:15 \pm 1:12 h:min and 07:30 \pm 0:31 h:min, respectively, were recruited for the study. Using statistical power software (Minitab v16.2.2, State College, PA, USA), the sample size required for this repeated-measures study was estimated to be seven. For

our primary aim, this estimation was based on detecting a practically important difference of > 93 N between morning and afternoon isometric MVC force, a statistical power of 0.8, an alpha level of 0.05, and a within-subject standard deviation of 66 N (Giacomoni et al. 2005). Inclusion criteria were healthy males (18–30 years), with previous weight/strength training experience (≥ 2 years). Participants habitually retired between 22:00–23:30 h and rose at 06:00–07:30 h and agreed to retire to bed at 22:30 and rise at 06:30 h; which is not too dissimilar to their natural sleep patterns. None of the participants were receiving any pharmacological treatment (including non-steroidal anti-inflammatory drugs, NSAIDs) throughout the study period. Habitual caffeine consumption was assessed using the caffeine consumption questionnaire (CCQ) and those with > 150 mg per day were excluded (Landrum 1992). Further, all participants expressed no preference to training regarding time-of-day by a weekly self-reported 2-week training diary. Recruiting participants with this specific type of exercise history meant that the known neuromuscular facilitative responses, which are typically associated with acute increases in muscular strength amongst untrained individuals due to neural adaptations and responses, were reduced (Häkkinen 1989). Exclusion criteria included depressed mood (from the Beck depression inventory), poor sleep quality (a Pittsburgh sleep quality index global score > 5), recent shift-work or travel across multiple time-zones (within the last month) and “extreme” chronotype (assessed via the Composite Morningness Questionnaire, Smith et al. 1989; Mean chronotype score was 34.1 \pm 3.1, all intermediate types) and risk factors and/or symptoms of cardiovascular disease. Through interviews, it was established that the volunteers had minimal knowledge of the effects of time of day or time-since-sleep on human performance. Verbal explanation of the experimental procedure was provided to everyone; this included the aims of the study, the possible risks associated with participation and the experimental procedures to be utilized. Any questions were answered. Individuals then provided written, informed consent before participating in the study. The experimental procedures were approved by the Human Ethics Committee at Liverpool John Moores University (ETHICS CODE: 08/SPS/027). The study was conducted in accordance with the ethical standards of the journal and complied with the principles of the Declaration of Helsinki.

Research Design

All sessions took place under standard laboratory conditions (lighting, room temperature and humidity were 200–250 lux, 20.7 \pm 0.4°C and 55.0 \pm 1.0%, respectively,

unless stated otherwise). Before taking part in the main experiment, each participant first completed three to seven familiarisation sessions (4.6 ± 1.2 sessions) starting at 12:00 h and each attendance was separated by at least 3 days. These sessions ensured that participants were fully familiarised with the all measures of the protocol such as MVC of the quadriceps on an isometric dynamometer (utilizing the twitch-interpolation technique), T_{rec} , T_{m} , T_{sk} and all subjective measures (see Figure 2). Following the familiarisation process, each participant completed five experimental sessions which took place 3 days apart. Participants were randomly allocated into 2 groups who completed the control conditions in a counterbalanced in order so either i) morning condition then ii) evening trial (07:30 and 17:30 h; M and E) after a standardised 5-min warm-up at 150 W, on a stationary cycle ergometer (Daum Electric GmbH, Flugspatzstr, Fürth, Germany) or vice versa. Having established evening resting temperatures, the participants were allocated into three groups and the sessions were administered in a counter-balanced design to minimise any learning or order effects (Edwards et al. 2024). Sessions consisted iii) an active warm-up morning trial (M_{E} , @ 150 W, on a Daum cycle ergometer until resting evening rectal temperatures from condition E were reached), a iv) morning ($M_{38.5}$) and an v) evening ($E_{38.5}$) passive warm-up trial which continued until rectal temperature reached 38.5°C (immersed in a water-bath up to the nipple line @ $39.94 \pm 0.14^{\circ}\text{C}$, 45–50% Relative room humidity [RH]; see Figure 1).

Protocol and Measurements

Participants were free to live a “normal life” between sessions, sleeping at home at night and attending lectures and doing light office work in the day. They were told to refrain from drinking alcoholic or caffeinated beverages and from other training or heavy exertion for the 48-h before the experiments or during them. Volunteers recorded the type, amount and timing of the food they ate for the period of 24-h before and during the first day of the experimental session and were asked to replicate this diet for the days before the other experimental conditions. For the morning sessions, participants arrived in an overnight fasted state and were asked not to eat in the 4-h prior to the evening session. The protocol is displayed in Figure 1. Participants arrived 1-h before the start of the test and inserted a soft flexible rectal probe (Mini-thermistor, Grant Instruments Ltd, Shepreth, UK) ~10 cm beyond the external anal sphincter and lay down and relaxed in the laboratory. To assess skin temperature (T_{sk}) during the 30 min resting period, skin thermistors (Grant Instruments, Squirrel 2010 series, UK) were attached using surgical tape (Micropore, 3 m UK) at four different locations on the left side of the body (chest[ch], forearm[f], thigh[th] and calf[ca]). The location was highlighted in permanent marker pen and the participant topped up the pen mark between sessions, this location was assessed using measures from anatomical points for reproducibility before testing. Mean T_{sk} was calculated using the equation proposed by Ramanathan

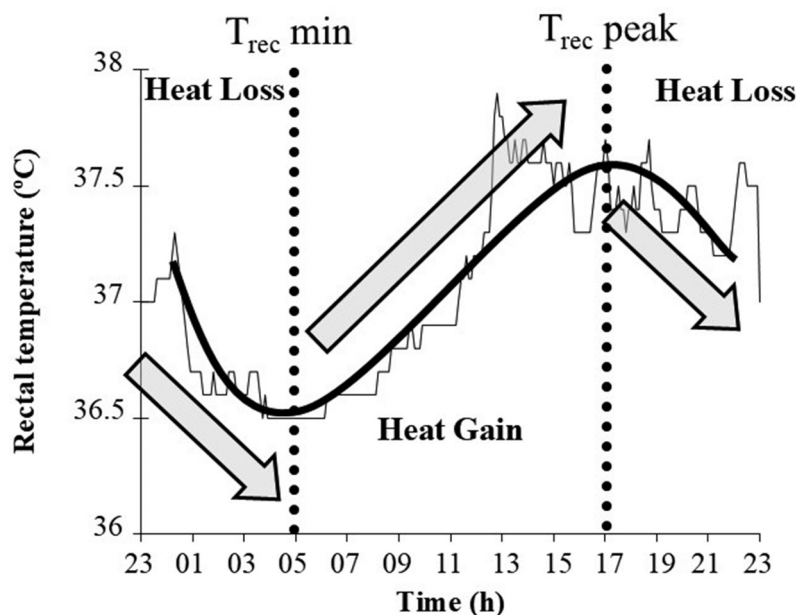


Figure 1. Circadian rhythm of rectal temperature with “peak,” “minimum,” “heat loss” and “heat gain” phases on the curve. Adapted from Edwards et al. (2009).

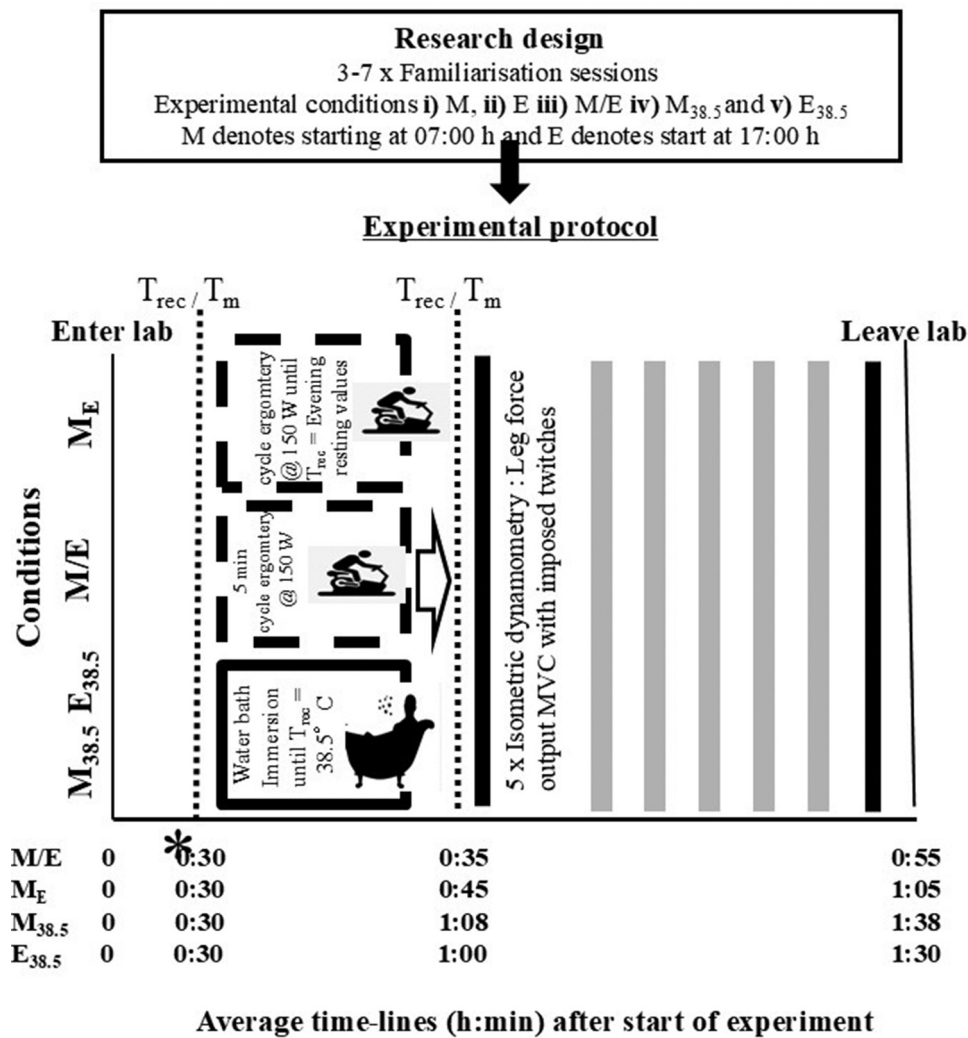


Figure 2. Schematic of the protocol for either passive or active warm-up performed in the morning (07:30 h) or evening (17:30 h). Rectal (T_{rec}) and muscle (T_m) temperatures, thermal comfort (TC) and profile of mood states (POMS) were measured after the subjects had reclined for 30 min at the start of the protocol and again after the warm-ups and prior to the measures for isometric dynamometry. * denotes start of 5-min continuous T_{rec} baseline measurement. The average value of these 5-min of recording was defined as resting T_{rec} and used in the subsequent analysis. Rating of effort (0–10 cm VAS), TC and ratings of perceived exertion (RPE) were taken after each strength measure. **Black dotted bars** indicate when T_{rec} and T_m temperature (at 3 depths) were taken. Grey bars indicate 5-min recovery periods between tests of muscle performance.

(1964): $T_{sk} = (0.34 \times T_{ch}) + (0.33 \times T_{th}) + (0.18 \times T_{ca}) + (0.15 \times T_f)$. Mean body temperature (T_{MBT}) was calculated as $[(0.64 \times T_{rec}) + (0.36 \times T_{sk})]$, (Burton 1935), and Thermal gradient by $T_{rec} - T_{sk}$ (T_G). Rectal temperature (T_{rec}) and T_{sk} was then recorded continuously over the next 30 min by means of a Squirrel 1000 data logger (Grant Instruments Ltd, Shepreth, UK) while participants remained semi-supine but awake. The average value of the last 5-min of recording was defined as resting T_{rec} and T_{sk} and used in the subsequent analysis. The standard body position (lying down) was used since T_{rec} is subject to other influences than that produced by the endogenous 24-h period oscillator, such as muscle activity, feeding and sleep (Edwards et al. 2002). At this

time “resting” muscle temperature (T_m) was assessed using a needle thermistor inserted into the right vastus lateralis (VL) muscle (13050, ELLAB, Hilleroed, Denmark). The area was marked with a permanent marker to minimise site variation between testing sessions. Thigh skinfold thickness was measured using Harpenden skinfold callipers (HSK BI, Baty International, West Sussex, UK) and divided by two to determine the thickness of the thigh subcutaneous fat layer over the participant’s VL (Enwemeka et al. 2002). The needle thermistor was then inserted at a depth of 3 cm plus one-half the skinfold measurement for determination of deep T_m . For determination of T_m at 2 and 1 cm below the subcutaneous fat layer, the thermistor

was then withdrawn by 1 cm increments (in compliance with procedures set out by Gregson et al. 2011; Saltin et al. 1968; Sargeant 1987). Muscle temperature was recorded using an ELLAB electronic measuring system (CTF 9004, ELLAB, Denmark).

Following these temperature measurements, participants completed a Profile of Mood States (POMS) questionnaire (Terry et al. 2003). Thermal comfort (Bakkevig and Nielsen 1993), ratings of perceived exertion (on a 6–20 scale, Birk and Birk 1987) and ratings of effort (on a 0 to 10 scale; “0” meaning no effort and “10,” maximal) were measured after the warm-ups and after each measure of muscle performance.

Passive Warming Strategy [$M_{38.5}$ and $E_{38.5}$]

Participants lowered themselves into a purpose-built water tank (3 m × 2 m × 1.5 m) until semi-reclined and immersed up to their nipple line, wearing just sports swimming jammers/trunks. They were provided with 150 ml of water to consume within every 15-min whilst immersed. Additionally, body mass measures were obtained pre- and post-immersion (after participants had towelled themselves dry). Previously advised compensatory water intake (Shirreffs and Maughan 1998) was provided immediately after the post-immersion body mass measurement. Water temperature was monitored and maintained at ~40°C throughout the warming period using mains supplied water at ~41°C and a heater in the waterbath that circulated the water and helped maintain temperature. Participants T_{rec} and T_{sk} were measured throughout the warming process and they were allowed to leave the water tank once their T_{rec} had increased to 38.5°C - measured continuously by rectal thermometry. Volunteers then dried themselves and T_m at the three depths was again measured. The participants then commenced the rest of the experimental protocol. The dynamometer was set up for the participant and positioned ~2 m from the water tank and the time between leaving the water bath and performing the strength tests was recorded at $2:45 \pm 0:32$ min:s.

Maximal Isometric Quadriceps Force and Percutaneous Stimulation

For every session, the position of the participant in the customised isometric chair (Lido Active, Loredan, Davis, CA, USA) was standardized in accordance with the guidelines set by the manufacturers and considering any adjustment required by the individual (established during familiarisation sessions). In brief, these guidelines were as follows: The backrest was positioned to ensure 80° of hip flexion and the knee joint was set at

90° flexion. A belt and straps were tightly secured across the shoulders, hips and chest to stabilize the upper body. Quadriceps muscle force was measured from the ankle where the attachment was connected to a strain gauge (Tadea, Tension/Compression cell, Huntleigh, Sweden) by a metal force transducer (previously calibrated with known weights). Participants performed isometric maximum voluntary contractions (MVCs) of the quadriceps muscles (4-s duration), both with and without percutaneous electrical stimulation, i.e. the twitch interpolation technique (ITT). During the initial session, participants practised performing MVCs without ITT, to become accustomed to achieving and maintaining maximum force voluntarily for the time required. This session was also used to establish the supra-maximal current amplitude for superimposition during an MVC (Digimeter, DS7, Hertfordshire, UK). Whilst remaining at rest, the amperage of a 240 V square-wave pulse (100 μ s, 1 Hz) was progressively increased until the point was reached beyond which further increases in intensity caused no further increase in resting twitch force (Morton et al. 2005; Newman et al. 2003).

The quadriceps muscles were electrically stimulated using two moistened surface electrodes (Dura-stick II, Chattanooga Group, Hixson, TN, USA, 7 × 12.7 cm) positioned on the anterolateral side of the thigh, on the belly of the rectus femoris and vastus lateralis muscles. The skin was prepared prior to the placement of each electrode by shaving and light abrasion of the skin followed by cleansing with an isopropyl alcohol swab. A permanent marker pen was used to identify the position of each electrode to minimise electrode placement variability between sessions (Giacomoni et al. 2005; Keogh et al. 1999). Eight single square-wave electrical impulses (100 μ s) were delivered during the 8-s sampling period. Each impulse was computer driven and was delivered at 240 V AC (RMS) at 50 Hz. Two impulses were delivered before and after the contractions; the other four impulses were administered during the contraction period and tested the peak value of the MVC. The peak forces of the pre- and post-contraction twitches were then averaged, allowing comparison of resting twitch amplitudes in both un-potentiated and potentiated condition (Oskouei et al. 2003). The amplitude of supra-maximal superimposed current was identified for each participant in familiarisation sessions and corresponded to 10% above the level required to evoke a resting muscle twitch of maximal amplitude (Todd et al. 2004).

Data were acquired for 8 s and analysed with a commercially designed software programme (AcqKnowledge III software for Windows, Biopac Systems Inc, Aero Camino Goleta, CA, USA). The

calculation of voluntary activation was conducted according to the interpolated twitch ratio (as recommended by Merton 1954), whereby the size of the interpolated twitch is expressed as a ratio of the amplitude elicited by the same stimulus delivered to a relaxed and potentiated muscle. The average of the force recorded during the 100-ms period before the application of each stimulus during the contraction and, subsequently, the maximal force recorded during the 100-ms period after each stimulus was used. The highest pre-stimulus force (taken as MVC force) and the resulting maximal post-stimulus force were then used to calculate the size of the interpolated twitch. Interpolated twitch size was calculated by subtraction of the mean pre-stimulus force from the maximal post-stimulus force.

Voluntary Activation = $[1 - (\text{Size of interpolated twitch} / \text{Size of resting twitch})] \times 100$ Equation 1

During familiarisation sessions, participants alternated between performing MVCs with and without twitch interpolation, so that approximately three trials of each were performed within each session. This approach was suggested by Morton et al. (2005), who reported that many participants performed weaker contractions when they were expecting stimulation (attributed to apprehension due to the prospect of receiving noxious stimuli) compared to when they were not expecting stimulation. Familiarisation sessions were conducted until the participant's MVC force and voluntary activation demonstrated a plateau effect and overall percentage muscle activation (see Equation 1) was repeatedly above 85% (again, compliant with observations made by Morton et al. 2005). This level of initial consistency was typically achieved within three to seven sessions, after which participants were then considered eligible to participate in the main experiment. Standardised strong verbal encouragement was given during each familiarisation session/trial and real-time visual feedback of their performance was provided to the participants *via* a computer display projected onto a large screen placed in front of them. The participants performed 5 repetitions, where peak force outputs for extension of the right knee were recorded. A 3-min rest was allowed between each effort and the highest and mean value for the 5 efforts was utilized for subsequent analysis.

Statistics

The data were analysed by means of the Statistical Package for Social Sciences (SPSS) for Windows, IBM version 29, using a General linear model (GLM) with repeated measures (condition [5 levels, M, E, M_E, M_{38.5} and E_{38.5}]) for “resting” T_{rec}, “Pre-strength

measure” T_{rec}, “resting” T_m (for 3, 2 and 1 cm depths independently), “Pre-strength measure” T_m (for 3, 2 and 1 cm depths independently), change in pre-post (T_{rec}, T_{sk}, T_{MBT}, T_G), post T_m (3–1 cm depths), “peak” and “mean” force production, percentage activation, RPE, rated “Effort” and all variables of the POMS questionnaire. A GLM with repeated measures was used for “resting” T_m, and “Pre-strength” measure T_m (condition [5 levels] x depth [3 levels, 3, 2 and 1 cm]). To correct violations of sphericity, the degrees of freedom were corrected in a normal way, using Huynh-Feldt ($\epsilon > 0.75$) or Greenhouse-Geisser ($\epsilon < 0.75$) values for ϵ , as appropriate (Field 2000). Graphical comparisons between means and Bonferroni pairwise comparisons were made where main effects were present. For correlations, to investigate the relationship between temperature and MVC, effort etc. The method of Bland and Altman (1995) was used, which considers multiple values being obtained from each participant. Following convention, the alpha level of significance was set at 5%. Effect sizes were calculated from Cohen's d for mean and peak force production only where the magnitude of the ES was classified as trivial (≤ 0.2), small (> 0.2 – 0.6), moderate (> 0.6 – 1.2), large (> 1.2 – 2.0) and very large (> 2.0) based on guidelines from Batterham and Hopkins (2006). The results are presented as the mean \pm the standard deviation throughout the text unless otherwise stated. Ninety-five percent confidence intervals are presented where appropriate as well as the mean difference between pairwise comparisons.

Results

Tables 1 and 2 show the group means (\pm SD) for all variables recorded in each condition, together with the statistical analyses.

Rectal and Muscle Temperatures at Rest

Rectal Temperature

There was a significant diurnal variation present for T_{rec}, with higher resting values in the E vs. the M condition (mean difference = $+0.47^\circ\text{C}$; see Table 1 and Figure 3). The CI for this comparison was 0.02 to 0.91°C ($p = 0.038$). This variation was consistent and in the expected direction for resting values for the other conditions where morning values at rest were lower than evening ones (E_{38.5} vs. M = $+0.50^\circ\text{C}$; $p = 0.042$; and a trend that did not reach significance for E_{38.5} vs. M_{38.5} = $+0.47^\circ\text{C}$; $p = 0.075$ and E vs. M_{38.5} = $+0.44^\circ\text{C}$; $p = 0.079$). There was no statistical difference between resting T_{rec} levels in the morning (07:30 h) for M, M_E or

Table 1. Mean (\pm SD) values for resting and pre-isometric temperature (T_{rec} and T_m at 3, 2 and 1 cm depths), force production, % activation, rating of perceived exertion (RPE), thermal comfort (TC), effort and resting POMS variables measured under the five experimental conditions.

Variable	Morning (M)	Evening (E)	M_E	$M_{38.5}$	$E_{38.5}$	P-value
Resting temp ($^{\circ}$C)						
T_{rec}	36.7 \pm 0.2	37.2 \pm 0.2 a d	36.6 \pm 0.2	36.7 \pm 0.2	37.2 \pm 0.2 a d	<0.0005
T_m (3 cm depth)	35.7 \pm 0.4	36.6 \pm 0.4 a c d	35.6 \pm 0.4	35.6 \pm 0.7	36.4 \pm 0.4 a d	<0.0005
T_m (2 cm depth)	34.9 \pm 0.9	35.7 \pm 0.5 c	34.7 \pm 0.7	35.1 \pm 0.8	35.1 \pm 0.3	= 0.011
T_m (1 cm depth)	33.4 \pm 1.4	34.0 \pm 1.1	33.0 \pm 1.4	33.3 \pm 1.5	33.4 \pm 0.7	= 0.593
Pre-Isometric temp ($^{\circ}$C)						
T_{rec}	36.8 \pm 0.2	37.1 \pm 0.2 <i>a</i>	37.2 \pm 0.2 <i>a</i>	38.5 \pm 0.0 a b c	38.5 \pm 0.0 a b c	<0.0005
T_m (3 cm depth)	37.0 \pm 0.7	37.7 \pm 0.3	38.0 \pm 0.4 a	37.9 \pm 0.5	38.4 \pm 0.3 a b	= 0.003
T_m (2 cm depth)	36.8 \pm 0.8	37.2 \pm 0.6	37.6 \pm 0.6 a	37.9 \pm 0.5	38.2 \pm 0.3 a	= 0.003
T_m (1 cm depth)	35.6 \pm 1.2	35.9 \pm 1.2	35.9 \pm 1.3	36.3 \pm 1.5	37.1 \pm 0.8 a b c	= 0.046
Isometric						
Peak MVC (N)	835 \pm 97	918 \pm 105 a d c e	839 \pm 96	820 \pm 120	857 \pm 127 d	= 0.014
Activation (%)	86 \pm 10	83 \pm 7	85 \pm 10	83 \pm 11	83 \pm 10	= 0.699
Mean MVC (N)	806 \pm 90	874 \pm 81 a d c e	788 \pm 79	770 \pm 106	812 \pm 111 d	= 0.007
Subjective measures						
Effort (0–10 cm VAS)	9.6 \pm 0.7	9.9 \pm 0.4	9.8 \pm 0.5	9.5 \pm 0.8	9.8 \pm 0.5	= 0.385
RPE (6–20 scale)	14.4 \pm 2.6	14.9 \pm 2.7	15.4 \pm 2.6	16.4 \pm 2.8	16.0 \pm 2.8	= 0.188
Thermal comfort (1–9)	6.1 \pm 1.1	6.4 \pm 1.1	8.0 \pm 1.1 a b	8.4 \pm 0.5 a b	8.9 \pm 0.4 a b	<0.0005
Resting POMS						
Vigour	5.1 \pm 2.3	9.9 \pm 2.1 a d	5.1 \pm 1.8 <i>b</i>	5.0 \pm 2.4	10.0 \pm 2.1 a c d	<0.0005
Fatigue	6.5 \pm 4.9	2.4 \pm 3.3	6.0 \pm 2.8	6.0 \pm 4.2	2.6 \pm 2.7	= 0.052
Happy	6.5 \pm 2.3	8.5 \pm 2.3 <i>a</i>	6.5 \pm 2.4	7.8 \pm 2.7	9.1 \pm 2.7	= 0.037
Depression	1.1 \pm 1.7	0.4 \pm 0.7	0.5 \pm 0.9	0.4 \pm 0.5	0.3 \pm 0.7	= 0.179
Calm	9.5 \pm 2.0	10.3 \pm 3.2	8.4 \pm 2.7	8.4 \pm 2.4	10.6 \pm 2.9	= 0.101
Anger	0.9 \pm 2.1	1.4 \pm 1.7	1.1 \pm 1.7	0.0 \pm 0.0	0.0 \pm 0.0	= 0.222
Confusion	0.6 \pm 1.2	1.3 \pm 1.8	0.9 \pm 1.2	0.6 \pm 0.7	0.8 \pm 1.2	= 0.582
Tension	0.6 \pm 0.7	1.9 \pm 2.4	0.4 \pm 0.7	0.4 \pm 0.7	0.0 \pm 0.0	= 0.115

Statistical significance ($p < 0.05$) is indicated in **bold**, and a trend (where $0.10 > P > 0.05$) is indicated in *italics*. a – different from M condition; b – different from E condition; c – different from M_E condition; d – different from $M_{38.5}$ condition and e – different from $E_{38.5}$ condition.

Table 2. Mean (\pm SD) values for change in temperature pre-post ($^{\circ}$ C) for T_{rec} , T_{Sk} , T_{MBT} and T_G and well as change in T_m temperature gradient post warm-up for 3–1 cm depths measured under the five experimental conditions.

Variable	Morning (M)	Evening (E)	M_E	$M_{38.5}$	$E_{38.5}$	P-value
Change in temp pre-post ($^{\circ}$C)						
T_{rec}	0.14 \pm 0.29 c d e	−0.04 \pm 0.07 c d e	0.54 \pm 0.27 d e	1.78 \pm 0.27 <i>e</i>	1.31 \pm 0.22	<0.001
T_{Sk}	0.90 \pm 0.37 c d e	0.85 \pm 0.45 c d e	−0.27 \pm 0.23 d e	−8.91 \pm 0.35	−8.71 \pm 0.31	<0.001
T_{MBT}	0.23 \pm 0.17 c d e	0.33 \pm 0.16 c d e	−0.45 \pm 0.18 d e	−4.11 \pm 0.21	−3.74 \pm 0.23	<0.001
T_G	−1.04 \pm 0.17 c d e	−0.80 \pm 0.47 c d e	−0.27 \pm 0.37 d e	7.06 \pm 0.44	7.37 \pm 0.19	<0.001
Change in temp post warmup (3–1 cm depth, $^{\circ}$C)						
T_m	1.49 \pm 0.87	1.65 \pm 1.15	2.06 \pm 1.17 e	1.31 \pm 1.26	1.03 \pm 0.88	= 0.026

Statistical significance ($p < 0.05$) is indicated in **bold**, and a trend (where $0.10 > P > 0.05$) is indicated in *italics*. a – different from M condition; b – different from E condition; c – different from M_E condition; d – different from $M_{38.5}$ condition and e – different from $E_{38.5}$ condition.

$M_{38.5}$ conditions; and T_{rec} levels in the evening (17:30 h) for E or $E_{38.5}$ conditions ($p > 0.05$).

Muscle Temperatures Compared at 1, 2 and 3 cm Depths

There was a main effect for condition ($F_{4, 28} = 2.844$; $p = 0.043$) where pairwise comparisons showed T_m values (at all depths) were lower in the morning (M, M_E or $M_{38.5}$) conditions than the E condition (−0.72 $^{\circ}$ C, −0.98 $^{\circ}$ C and −0.73 $^{\circ}$ C, respectively; all $p < 0.05$; see Figure 3). Pairwise comparisons showed a trend for $E_{38.5}$ values to be higher than the M condition (+0.56 $^{\circ}$ C; $p = 0.09$), there were no significant differences between any other conditions ($p > 0.05$). Resting T_m values increased with depth (1, 2 or 3 cm; $F_{1,1, 7,7} =$

211.848; $p < 0.0005$). Pairwise comparisons showed 3 cm was greater than 1 cm (+2.54 $^{\circ}$ C; CI = 2.01 to 3.07 $^{\circ}$ C; $p < 0.0005$) and 2 cm (+0.91 $^{\circ}$ C; CI = 0.71 to 1.11 $^{\circ}$ C; $p < 0.0005$), and 2 cm was greater than 1 cm depth (+1.63 $^{\circ}$ C; CI = 1.26 to 2.01 $^{\circ}$ C; $p < 0.0005$). There was no interaction between depth x condition, T_m values falling from depths of 3 cm to 1 cm irrespective of condition ($F_{2,8, 19,8} = 1.034$; $p = 0.396$; see Figure 2).

Muscle Temperatures Assessed Separately at the Three Depths

At 3 cm depth: There was a main effect for condition ($F_{4, 28} = 17.365$; $p < 0.0005$; see Table 1) where pairwise comparisons showed T_m values at 3 cm were lower in the morning (M, M_E or $M_{38.5}$)

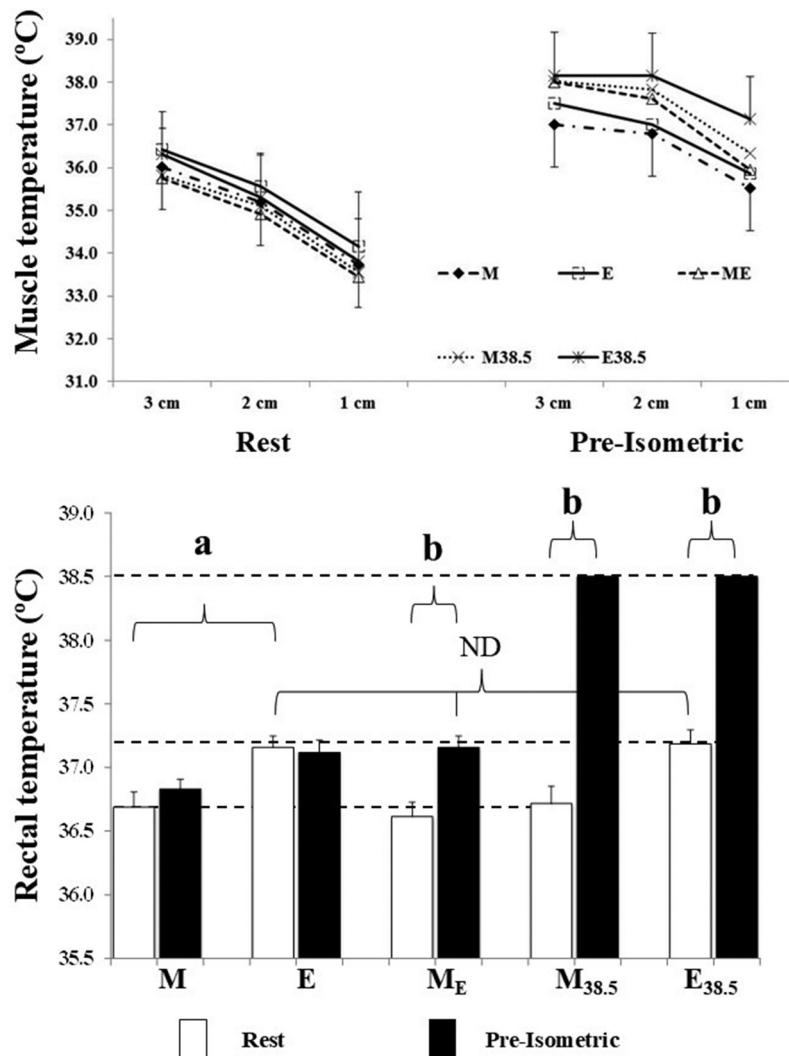


Figure 3. Mean and 95% confidence intervals (corrected for between-subject variability) for rectal temperature and muscle temperature (at 3 depths) at rest (\square) and pre-isometric effort (\blacksquare). A - significant diurnal variation in T_{rec} , where the morning value (07:30 h) is lower than the evening value (17:30 h; $p < 0.05$). b - T_{rec} significantly lower at rest than after the warm-up ($p < 0.05$). ND - no difference.

conditions than the E condition (-0.85°C , -1.98°C and -0.73°C , respectively; $p < 0.05$). Pairwise comparisons showed $E_{38.5}$ to be higher than $M_{38.5}$ ($+0.81^{\circ}\text{C}$; CI = 0.15 to 1.47°C ; $p = 0.017$) and a trend for $E_{38.5}$ T_m to be higher than M condition values ($+0.67^{\circ}\text{C}$; $p = 0.072$). There were no significant differences between any other conditions ($p > 0.05$).

At 2 cm depth: There was a main effect for condition ($F_{4, 28} = 3.966$; $p = 0.011$; see Table 1) where pairwise comparisons showed a trend for T_m values at 2 cm depth to be lower in the M_E condition than the E condition (-1.01°C ; $p = 0.067$). There were no differences between any other conditions ($p > 0.05$).

At 1 cm depth: There was no main effect for condition ($F_{4, 28} = 0.708$; $p = 0.593$; see Table 1, Figure 3)

Rectal Temperature and Muscle Temperatures at Three Depths After Warm-Up and Before the Muscle Force Measures

Rectal Temperature

T_{rec} values post warm-up and pre-MVC for $M_{38.5}$ and $E_{38.5}$ condition were higher than M ($+1.67^{\circ}\text{C}$), M_E ($+1.38^{\circ}\text{C}$), and E ($+1.38^{\circ}\text{C}$) conditions ($F_{1.8, 12.6} = 360.920$; $p < 0.0005$). There was a trend for T_{rec} values in the M condition to be lower after the warm-up than in the E and M_E conditions ($p = 0.09$ and $p = 0.06$, respectively). M_E values were the same as resting E condition values. That is, the protocol successfully produced the changes in temperature (to 38.5°C or to resting values previously observed in the evening) that were required to test the basic research question.

Muscle Temperatures at Different Depths

There was a main effect for condition ($F_{1.8, 12.3} = 7.408$; $p = 0.009$), with T_m values before MVC strength measures lower in the M than M_E condition (-0.78°C ; $CI = -0.06$ to -1.50°C ; $p = 0.033$) and a trend for $E_{38.5}$ values to be higher than the M condition ($+1.48^\circ\text{C}$; $p = 0.052$). There was no difference between any other conditions ($p > 0.05$). The post warm-up pre-MVC T_m values increased with depth of insertion of the needle ($F_{1.1, 7.1} = 21.415$; $p = 0.002$). Pairwise comparisons showed: 3 cm vs. 1 cm ($+1.61^\circ\text{C}$; $CI = 0.50$ to 2.72°C ; $p = 0.008$); 2 cm vs. 1 cm ($+1.34^\circ\text{C}$; $CI = 0.49$ to 2.20°C ; $p = 0.005$); and 3 cm vs. 2 cm ($+0.27^\circ\text{C}$; $p = 0.053$). There was no interaction such that the profiles for T_m dropped in parallel from 3 cm to 1 cm irrespective of condition ($F_{2.8, 18.8} = 1.034$; $p = 0.396$; see Figure 3).

Muscle Temperature Assessed Separately at Different Depths

At 3 cm depth: There was a main effect for condition ($F_{2.1, 14.4} = 8.855$; $p = 0.003$; see Table 1), where pairwise comparisons showed T_m values at 3 cm depths pre-MVC efforts were lower in the M condition than the M_E or $E_{38.5}$ conditions (-0.98°C , -1.35°C , respectively; $p < 0.05$). Pairwise comparisons showed a trend for $E_{38.5}$ values to be higher than in the E condition ($+0.67^\circ\text{C}$; $p = 0.088$), but there were no significant differences between values for any other conditions ($p > 0.05$).

At 2 cm depth: There was a main effect for condition ($F_{2.0, 14.0} = 8.863$; $p = 0.003$; see Table 1), where pairwise comparisons showed T_m values to be lower in the M condition than the M_E or $E_{38.5}$ conditions (-0.81°C , -1.36°C , respectively; $p < 0.05$). There was no difference between any other conditions ($p > 0.05$).

At 1 cm depth: There was a main effect for condition ($F_{1.6, 11.3} = 4.425$; $p = 0.046$; see Table 1), where pairwise comparisons showed T_m values to be higher in the $E_{38.5}$ condition than the M, E and M_E conditions (-1.49°C , -1.28°C and -1.20°C , respectively; $p < 0.05$). There were no significant differences between any other conditions ($p > 0.05$).

Change in Temperature (From Rest to Post Warm-Up)

There was a main effect for condition ($p < 0.001$, see Table 2), where pairwise comparisons showed ΔT_{rec} , ΔT_{sk} , ΔT_{MBT} , ΔT_G values to be different (higher or lower) in $M_{38.5}$ and $E_{38.5}$ conditions than the M, E and M_E conditions. M_E values were different than M or E values for ΔT_{rec} , ΔT_{sk} , ΔT_{MBT} , ΔT_G . There was a trend for ΔT_{rec} values to be higher in $M_{38.5}$ vs $E_{38.5}$ conditions ($+0.47^\circ\text{C}$, $p = 0.075$). Lastly, post warm-up 3 to 1 cm values

hence temperature gradient from deep to 1 cm for T_m showed higher values in M_E vs $E_{38.5}$ conditions (1.03°C , $p = 0.012$).

Isometric Measures

There were significant changes in peak force production ($p < 0.05$; See Table 1 and Figure 3), but not activation levels, across the 5 conditions ($p > 0.05$, see Table 1). In answer to the first hypothesis – pairwise analysis showed “peak” and “mean” force production to be higher in the E than M condition (83.2 N, $p = 0.001$, $d = 0.754$ and 67.8 N, $p = 0.007$, $d = 0.781$), with a CI of 26.0 to 140.5 N and 24.8 to 110.7 N respectively. Further, peak and mean force production in the E condition was significantly higher than in the M_E and $M_{38.5}$ conditions (78.7 N, $d = 0.082$ and 97.9 N, $d = 0.139$; 85.9 N, $d = 0.016$ and 104.0 N, $d = 0.06$; all $p < 0.05$). Mean force production was higher in the E condition than the $E_{38.5}$ condition (61.9 N, $p = 0.019$, $d = 0.626$), with a trend for peak force to also be lower in the $E_{38.5}$ condition ($p = 0.051$, $d = 0.525$ see Table 1). There was no difference in peak and mean force production between the M, M_E or $M_{38.5}$ conditions.

Ratings of Perceived Exertion (RPE), Thermal Comfort (TC) and Effort Levels

Mean (\pm SD) values for subjective rating of thermal comfort (TC) were significantly different between conditions ($F_{2.0, 14.0} = 18.822$; $p < 0.0005$, see Table 1), where pairwise analysis showed values for the M and E conditions (rated as “slightly warm”) to be lower than those of the M_E , $M_{38.5}$ and $E_{38.5}$ conditions (rated as “hot to very hot”), but with no significant differences between the M vs. E conditions or the M_E , $M_{38.5}$ and $E_{38.5}$ conditions. RPE (subjectively rated as “hard to very hard”) and effort (rated as “maximal”) were not significantly different in the 5 conditions ($p > 0.05$).

Resting POMS Questionnaire Variables

Resting subjective measures of “vigour” and “happy” values differed significantly with Condition, where levels of “vigour” were statistically lower at rest in the morning (M, M_E , $M_{38.5}$) than the evening sessions (E, $E_{38.5}$). And “happiness” values were lower in M than E condition only ($p < 0.05$, see Table 1). Resting subjective values for “anger,” “fatigue,” “depression,” “calmness,” “confusion” and “tension” were not significantly different between the conditions ($p > 0.05$).

Table 3. Correlations between temperatures (T_{rec} and T_{m} at 3, 2 and 1 cm depths), performance for isometric MVC dynamometry, RPE, effort and TC.

Dependent/covariate	Peak force	T_{rec}	T_{m} 3 cm	T_{m} 2 cm	T_{m} 1 cm	Activation	RPE	EFFORT	TC
Peak force (N)		+	+	+	-	+	- (0.35)	+	-
T_{rec} (°C)			+ (0.54)	+ (0.66)	+ (0.52)	-	+ (0.44)	+	+ (0.73)
T_{m} 3 cm (°C)				+ (0.90)	+ (0.76)	-	+	+	+ (0.68)
T_{m} 2 cm (°C)					+ (0.83)	-	+	-	+ (0.72)
T_{m} 1 cm (°C)						- (0.32)	+	-	+ (0.62)
Activation (%)							-	-	-
RPE (6–20 cm VAS)								+	+ (0.32)
EFFORT (0–10 cm VAS)									+

Method of Bland and Altman (1995) used. Correlations either negative or positive are denoted by the sign + or -. Where **emboldened**, $p < 0.05$, and correlation coefficient given; *italicized* values represent a statistical trend ($0.10 > p > 0.05$) and correlation coefficient given. If $p > 0.05$, values not emboldened, and no correlation coefficient given.

Correlations Between Measured Variables

Correlations between the measured variables are shown in Table 3. Important findings were: there were no significant correlations between peak force and T_{rec} or T_{m} (at either 1, 2 or 3 cm depths); there was a significant negative correlation between RPE and peak force; there were significant positive correlations between T_{rec} and T_{m} (at 3, 2 and 1 cm), RPE and TC; muscle temperatures at the three depths were positively correlated with each other and with TC; and there was a positive trend between RPE and TC with higher values for perceived exertion at subjectively higher rating of the thermal comfort scale.

Discussion

We found in the current investigation that increasing T_{rec} values to 38.5°C in highly motivated individuals [effort scores of ~96%] by passive immersion (in a water bath at ~40°C) in the morning ($\Delta T_{\text{rec}} \sim 1.78^\circ\text{C}$) and evening ($\Delta T_{\text{rec}} \sim 1.31^\circ\text{C}$) did not lead to similar variations in muscle force output (peak and mean) and activation, using isometric dynamometry with percutaneous stimulation (see Figure 4 and Table 1). Rather, there was no difference in peak and mean force production between the M and $M_{38.5}$ conditions – so mild hyperthermia did not significantly reduce peak and mean force production (Δ 1.8 and 4.5%, $d = 0.139$ and $d = 0.374$ respectively). However, mean force production was reduced in the $E_{38.5}$ condition compared to the evening control (-7.1% , $p = 0.019$, $d = 0.626$), with a trend for reductions in peak force (-6.7% , $p = 0.051$, $d = 0.525$). This reduction in peak and mean force produced values no different to those in the control morning condition (M). Others have shown either no effect of moderate hyperthermia on MVC, in agreement with the current investigations morning condition results (Lloyd et al. 2015; Nielsen et al. 1993). Or a reduction in MVC, similar but smaller

than our evening condition results (~2% Brazaitis et al. 2012; Racinais 2013; Morrison et al. 2004; Todd et al. 2003; Periard et al. 2014). However, the time-of-day of the experiments of these previous studies was not reported and some recruited both male and female participants without establishing menstrual cycle stage or maintained the thermal stress during the performance measures which makes comparisons with our results challenging.

To our knowledge, this is the second study to explore a potential time-of-day effect on the influence of moderate hyperthermia on physical performance and our findings are like that of Pullinger et al. (2018) who investigated in the morning and evening, the effects of passive heating (to T_{rec} values of 38.5°C) on repeated sprint performance (RS) lasting ~5 mins. They reported that increases in T_{rec} values of 1.77 and 1.23°C in the morning and evening respectively (07:30 h, a rise of in the heat gain phase and 17:30 h, at or around the peak in core temperature) resulted in no change in RS morning performance. However, hyperthermia at the peak of the core temperature circadian rhythm, resulted in a reduction in evening RS values (distance covered, average power and average velocity, $p < 0.05$) compared to evening control with all variables being like those of the morning control trial ($p > 0.05$). Differences in response to moderate hyperthermia appear dependent on the phase of the circadian rhythm of core temperature and time-of-day in both force output (where time-on-task is 4 s) and repeated sprints (5-min). Where moderate hyperthermia effects performance more at the peak in the rhythm of core temperature than the rising phase, whereas the level of activation or central activation capacity remain the same regardless of time-of-day and hyperthermia. Voluntary activation refers to the level at which the motor neuron pool has been voluntarily excited to produce tension in the muscle of interest. In the current investigation no difference in motivation such as subjective rating of effort (~95–99%), rating of perceived exertion ($p > 0.05$) and activation in all conditions suggests

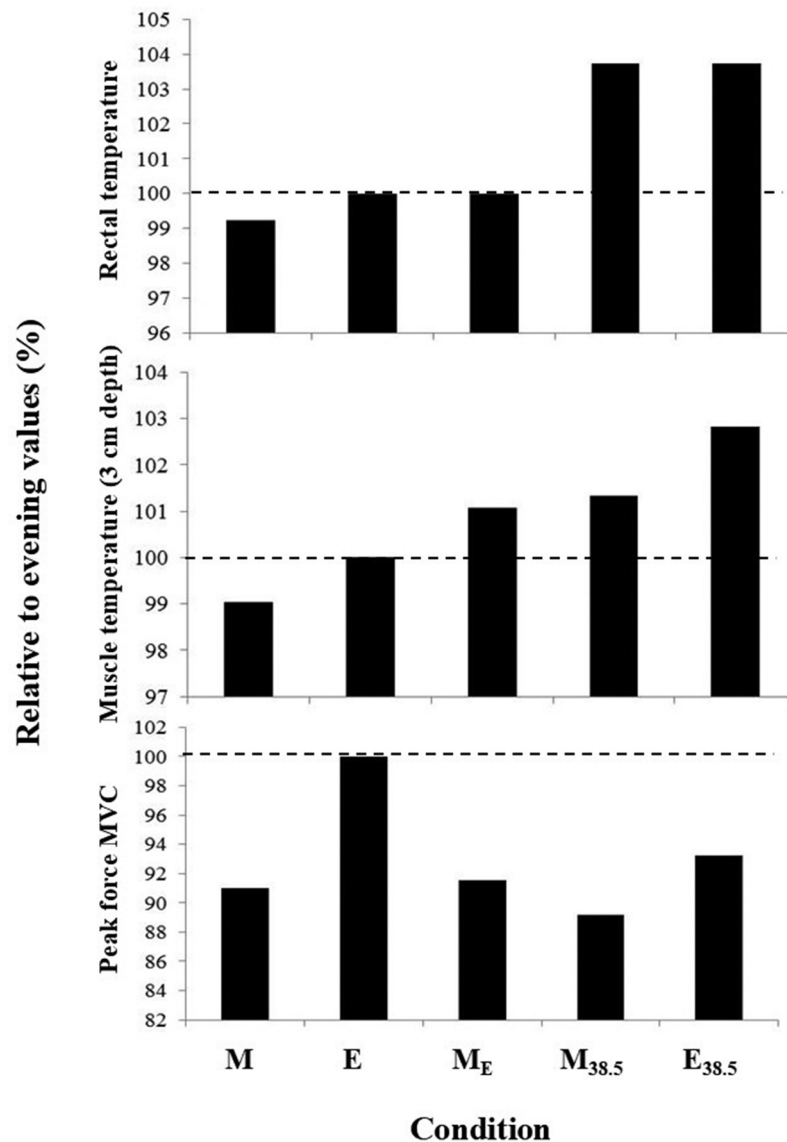


Figure 4. Percentage changes for temperatures before the MVCs (rectal and muscle at 3 cm depth) and isometric MVC peak force expressed relative to evening values (hence, evening values always 100%) for the five conditions.

that torque or force appears not to be centrally modulated by time-of-day, raising morning to evening levels of T_{rec} or moderate hyperthermia. Others that have observed a decrease in short (~ 4 s) MVC performance (Todd et al. 2003; Periard et al. 2014) due to a decrease in the central nervous system function, with voluntary activation increasing with the duration of the contraction (Todd et al. 2005). This reduction in voluntary activation is associated primarily with this high core temperature, rather than peripheral temperature changes (Thomas et al. 2006; Rodrigues et al. 2022). Values in our study were around 84%, lower than those found by others using small muscle groups (98%) though like values of 91% found using the same technique by others (Morton et al. 2005). However, it has been suggested that voluntary activation estimation is subject to error when tendon

properties (force elongation relationships) are not considered (Pearson and Onambele 2006). Peripheral fatigue is largely caused by processes in the muscle such as disturbances in excitation-contraction coupling and accumulation of metabolites. Although the loss of force production capacity is thought to originate from both central and peripheral fatigue factors, with the combination of heat stress and prior contractile activity (i.e. exercise) exacerbating the rate of decline (Racinais et al. 2017). Currently the extent of central and peripheral mechanisms that influence neuromuscular force during passively induced hyperthermia (whether this is affected by time-of-day) is unclear (Rodrigues et al. 2022).

Lastly, unlike others we removed the participants from the thermal stress when their core temperatures reached 38.5°C and all T_{sk} sites values were

representative of the water temperature (40°C). At this time (the heat stimulus) the participants were in a heat-gain mode as the thermal gradient was 1.5°C from skin to core and this was representative in T_{MBT} values, for ethical and medical reasons we were not allowed to measure T_{m} whilst the participants were in the hot water but similar values to T_{rec} or T_{sk} site were expected. Therefore, although we did not measure T_{rec} just before isometric measures we did measure T_{m} values after the participant had towelled down and mounted the ergometer (~3 mins), at this time deep T_{m} values (3 cm) were less than T_{rec} in the hot bath for $M_{38.5}$ and $E_{38.5}$ conditions respectively ($38.00 \pm 0.37^\circ\text{C}$ vs $38.03 \pm 0.36^\circ\text{C}$). It is thought that during the delay in transition from the water bath to the isometric ergometer, to the actual highest peak torque for MVC of the 5 trials (~3–9 mins) T_{c} and T_{m} values could have responded differently between the morning and evening sessions – at ~4 min the thermal gradient from 3 cm to 1 cm depths were no different with a small effect size ($d = 0.26$, $1.31 \pm 1.26^\circ\text{C}$ and $1.03 \pm 0.88^\circ\text{C}$). Waterhouse et al. (2007) stated that evidence for an effect of the body clock on thermoregulatory responses dependent on the time-of-day come from several sources i) there are circadian rhythms in core temperature at which increasing sweating and cutaneous vasodilation are initiated, ii) the sensitivity of thermoregulatory reflexes exhibit a circadian rhythm, where sensitivity is defined as the relationship between their output (increase in vasodilation or sweating) and input (rise in core temperature above a threshold), iii) core temperature increases are greater for a given amount of spontaneous activity performed around the trough (e.g. morning) rather than the peak of the resting circadian rhythm of core temperature. And, iv) T_{rec} shows a clear resting circadian rhythm with $\Phi \sim 17:00$ h and troughs $\sim 05:00$ h, with recent evidence of T_{m} also showing a clear rhythm with $\Phi \sim 17:03$ h (Robertson et al. 2024). The daily fluctuations in distal (hands and feet) skin temperature are inverted to that of T_{rec} with maximum values occurring $\sim 04:00$ h and proximal temperature (infraclavicular region and sternum) appearing to vary in phase to that of T_{rec} (Cuesta et al. 2017). The mechanisms and interactions that explain the current investigation findings regarding thermoregulatory responses to time-of-day and moderate hyperthermia (T_{c} values of 38.5°C) on MVC performance through the central nervous system, peripheral temperature changes and voluntary nerve activation levels and cortical activation is presently unknown.

A second main finding was that increasing morning rectal temperatures to exactly those found in the

evening (M_{E} , producing a rise of 0.5°C in T_{rec} and 2.2°C in T_{m}) by an extended active “warm-up” did not result in the muscle force output becoming equal to evening values (see Figure 4 and Table 1). These findings agree with previous ones by us (Edwards et al. 2013), but as the previous protocol was long (~2 h) and the MVC was conducted at the end of this protocol. The lack of effectiveness of the warm-up could simply be due to the human cooling down. The different thermal response (core vs. muscle) to the passive vs. active warm-up allows us to differentiate between effects of type of warm-up on core and/or muscle temperature increases, such as high core and muscle vs low core and high muscle to see if this leads to beneficial modifications in muscle performance, as suggested by others (Racinais et al. 2009). However, a performance change offsetting diurnal variation of muscle force production was not associated with either warm-up (Edwards et al. 2013). This lack of a beneficial increase in force with passive or active warming in the morning indicates that the daily variation of muscular function is not wholly explained by muscle (or core) temperatures, and this agrees with the view that some other factor(s) contribute to the observed rhythms in muscle performance (Edwards and Waterhouse, 2013). Direct comparisons between previously published results and our present ones are difficult for several reasons: a) We did not use a passive warm-up in a hot and humid environment, which challenges the thermoregulatory heat gain/loss mechanisms differently from a hot but non-humid condition (Gagge and Gonzalez 1996); b) The end-criteria for both active and passive warm-ups in our study were previously measured resting evening rectal temperatures or 38.5°C , after which the participants was removed from the heat source and the rest of the experiment was conducted in a thermoneutral laboratory (hence heat challenge removed). Using a warm-up protocol (either active or passive) of standard duration (as used by others, for example: Atkinson et al. 2005) would produce a large individual variation in resultant core temperature, either over- or under-shooting the required value; and c) A pre-requisite for the exploration of a warm-up effect is evidence of a diurnal variation in both core temperature and the performance variable (s) being examined, in the population of participants recruited for the study. This has not always been the case (see Table 1 of Edwards et al. 2013 for further information).

In the present study, effects of time-of-day were seen in temperature (rectal and muscle at a 3 cm depth) and isometric peak force, in agreement with past research (Edwards et al. 2013; Pullinger et al. 2018; Robertson et al. 2024; Robinson et al. 2013). The lack of

a significant correlation between isometric peak force and increases in T_{rec} in the range of temperature we chose (moderate hypothermia) and/or T_{m} (in our case at depths of 1–3 cm, see Table 1) is in agreement with the results of others, who either found no, or only a minor, effect (Asmussen et al. 1977; Bergh and Ekblom 1979; Binkhorst et al. 1979; Falls 1972) – even though they had small sample sizes and/or did not impose percutaneous stimulations. Indeed, the relationship between muscle and/or rectal temperature and force production in the ranges we measured may not be linear (Racinais et al. 2017). Some implications of our findings – namely, that raising core and muscle temperature in the morning and evening to levels of moderate hyperthermia (38.5°C) on observed diurnal variation in isometric performance – are considered below.

In addition to effects of muscle and core temperatures upon muscle performance, several other factors have been suggested to account for circadian variations in muscle strength. One of these is the neural activity that is associated with muscle activity, which comprises several components: (1) an initial output from the motor centres of the brain; (2) transmission of nerve impulses along motor fibres and across the neuromuscular junction to activate the muscles, the rate of nerve conduction being proportional to core temperature; and (3) activities of sensory receptors in muscles (muscle spindles) which control muscle contraction via spinal (stretch and crossed extensor) reflexes and pathways involving the cerebellum. Further factors include motivation, subjective arousal and sleepiness, hormones (including thyroid hormones and testosterone – cortisol ratio) and ionic changes. Motivation is a central mechanism that could be involved in the daily rhythm in muscle performance (Drust et al. 2005). In the current study, subjective ratings of perceived exertion showed no diurnal variation. As is to be expected in this highly motivated group, ratings of muscular effort were near always close to “maximum” irrespective of the experimental condition. The results of this study support the view that morning-evening differences in muscle force generated through isometric contraction, when diurnal variation in core and deep muscle temperatures (3 cm) are evident, involve peripheral mechanisms that are not only dependent on muscle temperature (see Guette et al. 2005; Martin et al. 1999) but also possibly due to other factors determined by the environment and outputs from the body clock (endogenous factors). We have considered this previously (Robinson et al. 2013), but direct evidence that there is a large endogenous component to the daily variation in muscle force production (from the body clock and peripheral clocks) is

presently unsubstantiated (Sargent et al. 2010). As highlighted previously (Kline et al. 2007; Reilly and Waterhouse 2009), to investigate this internal component would entail time-consuming and challenging (for both researchers and participants) protocols. Chronobiological protocols which attempt to standardise or reduce the exogenous component of the rhythm using constant routines, forced desynchronisation or ultra-short sleep-wake-cycle protocols would be required but, currently any results from them have not been published. Such approaches may further extend our knowledge of the hypothesised “causal link” between T_{rec} and T_{m} and isometrically or isokinetically assessed MVC. Further, “very little is known about circadian aspects of skeletal muscle function/metabolism but some progress has been made on understanding the molecular clock in skeletal muscle” (Lefta et al. 2011). More than 200 skeletal muscle genes exhibit circadian rhythmicity (McCarthy et al. 2007) and have known roles in muscle development, contraction, metabolism and atrophy, and these genes could influence power output and responsiveness to mechanical stimuli. We hypothesise diurnal changes in the muscle proteins may explain differences in muscle performance, and proteomic techniques (Burniston and Hoffman 2011) may offer a practical means to investigate these effects.

Limitations

We chose 07:30 and 17:30 h for our time points (rising and peak) to coincide with the morning and evening time points most used in research investigation diurnal variations in muscle strength (Robertson et al. 2024). However, we and others have also looked at temperature heat-gain/loss mechanisms in the mid phase in rising (~11:00 h) and mid phase in falling (23:00 h; Aldemir et al. 2000; Otani et al. 2020; Waterhouse et al. 2004, 2007). At this moment it is uncertain how moderate hyperthermia timed at the lowest (~05:00 h), and mid-falling phase of the circadian rhythm (23:00 h) effects isometric force output. Secondly, we created the conditions in T_{rec} to test the hypotheses to examine with precise modelling of T_{rec} values by removing the individual from the stimulus when T_{rec} reached values required such as 38.5°C. However, from the participants leaving the water bath to getting onto the isokinetic dynamometer to the last MVC was ~20 min. Although all participants obtained their peak MVC in their first or second effort this represented ~6–9 mins after leaving the heat and others have shown a 0.1°C/min loss in T_{m} after a 20-min active warmup. Hence the participants could have been at T_{m} values from ~36.5 to 37.6°C (Kapnia et al. 2023) by the time of their first

or second MVC with a corresponding decrease in T_{rec} – this could explain the lack of effectiveness of M_E condition in force production as the benefits of the warm-up could have been lost (Kapnia et al. 2023; Mallette et al. 2019). In the current investigation we did not measure distal (hands and feet – the main sites for sensible heat loss) as well as proximal skin sites, rather used a weighted formula. As distal skin temperature does not follow the same circadian rhythm of T_{rec} or proximal and represents the health loss mechanism it is important measure (Vellei et al. 2023). Lastly, in our study we recruited intermediate chronotypes, future work could investigate the sensitivity hyperthermia to find a daily variation for muscle performance using outright morning or evening types (Tamm et al. 2009).

Summary

In this population, diurnal variation in T_{rec} , T_m and mean and peak isometric force output values were found. However, raising morning rectal temperature to evening values, or raising morning and evening rectal temperature to 38.5°C, by two different types of heating did not increase isometric muscle force. Rather, differences in response to moderate hyperthermia appear dependent on the phase of the circadian rhythm of core temperature and time-of-day in both MVC force output. Where moderate hyperthermia reduces performance more at the peak in the rhythm of core temperature than the rising phase and activation levels remain the same regardless of time-of-day and hyperthermia. Lastly, an active morning warm-up on a cycle ergometer to increase T_{rec} to previously measured resting evening values has no effect on morning isometric (mean and peak) force output or activation levels.

Practical Considerations

Implications of the current study's data suggest that raising morning T_{rec} to evening levels (1.38°C and corresponding increase in T_m of 0.98°C) did not improve isometric peak force so other methods to improve muscle force output are needed. Moderate hyperthermia is more likely to negatively affect isometric muscle force in the evening than the morning, this has implications to athletes warming up in the heat before a short power-based event. The current findings may influence the choice of time of day on heat adaption strategies, to get the most effective stress-strain adaptation effect (Gibson et al. 2019).

Future Considerations

Considering the applied and chronobiological issues that remain to be addressed, further research questions

generated from the present study include: 1) Can the athlete in a thermoneutral environment overcome the poorer muscle force production normally found in the morning, perhaps by using the phase-response curves for light, melatonin and/or exercise? 2) What role does the endogenous component of muscle force output play? 3) Is the diurnal variation in muscle strength still robust when considering whole body muscular efforts (such as squats, clean-and-jerks, etc.) and using reliable measurements of these variables? 4) Effects of hyperthermia on muscle strength mid-phase for rise and fall of circadian rhythm of core temperature.

Acknowledgments

We would like to thank the participants who participated in the study. Further, thanks go to Dr Robert Erskine for providing helpful comments and editorial advice on this manuscript.

Disclosure Statement

No potential conflict of interest was reported by the author(s).

Funding

This research received funding through internal grants in the Research Institute for Sport and Exercise Sciences at Liverpool John Moores University.

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Data Availability Statement

The data presented in this study are not available due to ethical restrictions.

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