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Diurnal variation in repeated sprint performance cannot be offset when rectal and muscle

temperatures are at optimal levels (38.5°C).

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Abstract

The present study investigated whether increasing morning rectal temperatures (Trec) to evening levels, or increasing morning and evening Trec to an "optimal" level (38.5°C), resulting in increased muscle temperatures (Tm), would offset diurnal variation in repeated sprint (RS) performance in a causal manner. Twelve trained males underwent five sessions [age (mean \pm SD) 21.0 \pm 2.3 yr, peak oxygen uptake ($\dot{V}O_2$ peak) 60.0 \pm 4.4 mL.kg-1min-1, height 1.79 \pm 0.06 m, body mass 78.2±11.8 kg,]. These included control morning (M, 07:30 h) and evening (E, 17:30 h) sessions (5-min warm-up), and three further sessions consisting of a warm-up morning trial (ME, in 39-40°C water) until Trec reached evening levels; two "optimal" trials in the morning and evening (M38.5 and E38.5, in 39-40°C water) respectively, until Trec reached 38.5°C. All sessions included 3x3-s task-specific warm-up sprints, thereafter 10x3-s repeated sprints with 30-s recoveries were performed a non-motorised treadmill. Trec and Tm measurements were taken at the start of the protocol and following the warm-up periods. Values for Trec and Tm at rest were higher in the evening compared to morning values (0.48°C and 0.69°C, P<0.05). RS performance was lower in the M for distance covered (DC), average power (AP) and average velocity (AV) (7.8-8.3%, P<0.05). Increasing Trec in the morning to evening values or optimal values (38.5°C) did not increase RS performance to evening levels (P>0.05). However, increasing Trec in the evening to "optimal" level through a passive warm-up significantly reduced RS performance variables to values found in the M condition (6.0-6.9%, P<0.05). Diurnal variation in Trec and Tm is not wholly accountable for time-of-day oscillations in RS performance on a non-motorised treadmill; the exact mechanism(s) for a causal link between central temperature and human performance are still unclear and require more research.

Introduction

In male participants in a temperate environment (around 17-20°C), many human performance variables display diurnal variation (Drust et al., 2005). Repeated sprint (RS) performance which can be defined as the ability to produce the best possible short-term performance over a series of sprints (<6 s) with minimal recovery intervals (<60 s) between bouts (Bishop, 2003), is an important component of team-sports performance (Bangsbo et al., 1991; Bishop, 2003; Spencer et al., 2005). Time-of-day variations in RS performance have previously been investigated (Giacomoni et al., 2006; Racinais et al., 2005c; Zarrouk 2012). As far as we are aware, only one study has investigated the effects of time-of-day on RS performance on a non-motorised treadmill - a more externally valid mode of measurement for RS performance in most team sports rather than a cycle ergometer. Pullinger et al. [5] reported in a population of 20 trained male participants, who were familiarised with the protocol about 5 times - distance covered, peak and average power, peak and average speed - all showed significantly higher values in the evening compared to the morning (a range of 3.3-8.3%).

The exact mechanisms for this observed diurnal variation in human performance are still unknown but have been attributed to a number of factors (See Edwards et al., 2013 and Pullinger et al., 2013; 2014). One factor which has been proposed is the causal link of the temperature rhythm, which might be implicated directly or indirectly, where the higher evening resting core body temperature (~0.8°C in rectal and gut sites, Edwards et al., 2002) and local muscle temperature (≥ ~0.35°C in vastus lateralis at depths of 3 cm, Edwards et al., 2013; Robinson et al., 2013; Pullinger et al., 2013; 2017) produce an increase in force-generating capacity of the muscle (Bernard et al., 1998; Coldwells et al., 1994; Giacomoni et al., 2005; Melhim, 1993) and neural function (reduced twitch time course or increase in speed of contraction, Martin et al., 1999). The link between core

temperature and performance has been studied predominantly using either active (by means of exercise) or passive (by means of a chamber or water bath) "warm-ups" to increase rectal body temperature in the morning, to approximately, or precisely, the temperature found in the evening; and then examining whether this increases morning performance to evening levels. In brief, using an active warm-up shows conflicting results. Numerous studies have shown improvements in muscular performance (O'Brien et al., 1997; Sargeant & Dolan, 1987; Souissi et al., 2010; Stewart & Sleivert, 1998) to take place following an active warm-up while others have reported no significant effects or a decrease in performance (McCutcheon et al., 1999; Mitchell & Huston, 1993; Pyke et al., 1968; Sargeant & Dolan, 1987; Stewart & Sleivert, 1998). Further, when raising morning rectal temperature by active warm-up to values precisely found in the evening, RS performance did not increase to evening values (Pullinger et al., 2017). However, passive exposure to hot environments (either a 30-min exposure to a hot bath at 38°C, with legs from feet to pelvis immersed or 1 h at 29.5°C, 74 ±10% relative humidity ambient air) blunts muscle force diurnal variation in performance (a squat jump [SJ], a counter-movement jump [CMJ], and a brief maximal sprint on cycle ergometer; Racinais et al., 2004; 2009). Interpretation of some of these results are compromised by the complexity of the protocols), with core and muscle temperature cooling evident as the protocol progressed – with focus on only one key measure suggested (Edwards et al., 2013; Robinson et al., 2013). Interestingly, when combining both internal (i.e., afternoon central temperature) and external passive warm-up in the afternoon, evidence points towards a 'ceiling' effect, whereby muscular force cannot be increased further (Racinais et al. 2005a, 2005b, 2009).

Considering that according to Åstrand & Rodahl (1986) and Stickler et al. (1990), physical performance is 'optimal' at a rectal temperature ~38.3-38.5°C with corresponding muscle

temperature of $\geq 39.0^{\circ}$ C, and that previously mentioned investigations that have measured both T_{rec} and T_m have elicited central temperatures $\leq 37.6^{\circ}$ C with corresponding muscle temperature of $\leq 38.0^{\circ}$ C (Edwards *et al.* 2013; Robinson *et al.* 2013), more in depth research would be beneficial. As far as we are aware, only one investigation has looked at increasing rectal temperature to 'optimal' levels. In this study it was established that raising morning and evening rectal temperature values to 38.5° C by passive 'warm-up' did not increase isometric muscle torque values. Therefore, the diurnal variation in muscle torque observed to peak in the evening cannot be attributed wholly to body temperature. In order to further reaffirm this 'ceiling' hypothesis, and also examine the central temperature diurnal variation causal factor theory, passive warm-ups eliciting central temperatures considered 'optimal' are required.

Therefore this study aimed to control rectal temperature to see whether diurnal variation in Trec and Tm is accountable for time-of-day oscillations in RS performance on a non-motorised treadmill. This was investigated through increasing morning rectal temperature (by a passive warm-up) to evening resting values or increasing morning and evening rectal temperatures (by a passive warm-up) to optimal (38.5°C) values.

Methods

Subjects

Twelve well-trained male athletes were recruited for this study (mean \pm SD: age 21.0 \pm 2.3 y, maximal oxygen uptake ($\dot{V}O_2$ peak) 60.0 \pm 4.4 mL.kg⁻¹min⁻¹, height 1.79 \pm 0.06 m, body mass 78.2 \pm 11.8 kg). The 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 and retire at 23:30 h and waking at 06:30 h to be in the laboratory for 07:00 h. Participants recorded the type, amount and timing of the food they ate for the period of 24 h before and taken during the day of the experimental session and replicated this for experimental conditions. All participants gave their written informed consent. The study was approved by the Human Ethics Committee of Liverpool John Moores University.

Experimental overview

All sessions took place under standard laboratory conditions (lighting, room temperature and humidity, and barometric pressure were 200-250 lux, 21.1 ± 1.1 °C, 34.6 ± 5.1 %, and 755.3 ± 6.6 mmHg, respectively). Prior to the main experiment, each player completed three familiarisation sessions each separated by at least three days until the RS performance demonstrated a plateau effect, revealing no additional learning effects. The overall coefficient of variation and 95% ratio limits of agreement were lower than 5 % and 10 %, respectively. Thereafter each participant completed five experimental sessions three days apart: a morning and an evening trial (07:30 and 17:30 h; M and E) after a standardized 5-min warm-up at 10 km.h⁻¹ on a motorized treadmill (Pulsar, H/P cosmos, Germany) which were counterbalanced in order of administration; a passive

warm-up morning trial (M_E), and a morning and evening "optimal" passive warm-up trial (M_{38.5} and E_{38.5}) in a 39-40°C water immersion bath where subjects semi-reclined immersed up to their neck (Hitema ECA.002 water chiller, TO1B2620, Italy). These were administered in a counterbalanced design to minimize any learning or order effects (Edwards *et al.*, 2013). In the M_E session, starting rectal temperature (Trec) was modelled to become equal to that found in the participant in the previous E session at rest. In the M_{38.5} and E_{38.5} starting Trec temperatures were modelled to become equal to optimal values of 38.5°C. Participants' T_{rec} and T_m were again measured when they mounted the treadmill (after towel drying) and they then commenced the rest of the experimental protocol.

Participants arrived 1h before the start of the test, inserted a soft flexible rectal probe (Minithermistor; Grant instruments Ltd, Shepreth, UK) \sim 10-cm beyond the external anal sphincter, strapped on a heart rate monitor (Polar FT1; Polar Electro Oy, Kempele, Finland) and then lay down and relaxed in the laboratory. Rectal temperature (T_{rec}) was then recorded continuously over 30-min by means of a Squirrel 1000 data logger (Grant Instruments Ltd, Shepreth, UK) while subjects remained semi-supine but awake. The average value of the last 5-min recording was defined as resting T_{rec} and used for subsequent analysis. At this time 'resting' muscle temperature (T_m) was assessed using a needle thermistor inserted into the right leg vastus lateralis (13050, ELLAB, Hilleroed, Denmark). The area was marked with a permanent marker to minimize site variation between testing sessions. Thigh skinfold thickness was measured using Harpenden skinfold callipers (HSK BI, West Sussex, UK) and halved to determine the thickness of the thigh subcutaneous fat layer of the participant's vastus lateralis (Emwemeka et al., 2002). The needle thermistor was then inserted a depth of 3 cm plus one-half the skinfold measurement for

determination of deep T_m (in compliance with procedures set out by 13, 14). Muscle temperature was recorded using an ELLAB electronic measuring system (CTF 9004, ELLAB, Denmark). The subjects then undertook a standardized 5-min warm-up run at 10 km.h-1 on a motorised treadmill, the extended active warm-up (M_E) or one of the cool down procedures (E_{Mrec} and E_{Mmuscle}). A second Tm measure was assessed following the warm-up. Thermal comfort (Bakkevig & Nielsen, 1994), ratings of perceived exertion (Birk & Birk, 1987), ratings of effort (on a 0 to 10 cm scale; '0' meaning no effort and '10', maximal) and heart rate were measured after the warm-ups and after each repeated sprint.

RS performance

Repeated sprint (RS) performance were performed on a non-motorized treadmill (Woodway Force 3.0; WI, USA). The exact protocol has been described previously, where subjects sprint for 3-s x 10 times with 30-s recovery between sprints (Pullinger et al., 2013). During the test, treadmill speed, power output and distance covered were sampled at a rate of 200 Hz, leading to 600 samples per variable over the 3-s sprint. Sprint data for peak power (PP), average power (AP), peak velocity (PV), average velocity (AV) and distance covered (DC) were recorded with a commercially-designed software program (Pacer Treadmill Software; Innervations, Australia) and used in the subsequent analysis. Fatigue was calculated using the % decrement method as advised by Glaister et al. (2008):

During all sessions, participants undertook a task-specific warm-up procedure, consisting of 3 x 3-s sprints at 50, 70 and 80 % according to self-perceived maximum effort, respectively. The task-specific bursts of activity were brief enough not to cause significant fatigue. Standardized strong verbal encouragement was given during all sessions.

****Insert Figure 1 here****

Statistical analysis

All data were analysed using statistical software (SPSS, Chicago, IL, USA). Differences between conditions were evaluated using a general linear model with repeated measures. 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. Graphical comparisons between means and Bonferroni pairwise comparisons were made where main effects were present. Effect sizes (ES) were calculated from the ratio of the mean difference to the pooled standard deviation. 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. The alpha level of significance was set at 5 %.

Resting

A main effect for condition was observed for T_{rec} with higher resting values in the E (mean difference = $0.48 \pm 0.05^{\circ}$ C, P < 0.0005, 95% CI: $0.32 - 0.64^{\circ}$ C; ES=3.48) compared to the M condition (Table 1 and Fig2). This variation was consistent and in the expected direction with resting values of Trec also higher in the evening (E, E_{38.5}) conditions (P < 0.0005). There was no statistical difference between resting T_{rec} levels in the morning (07:30 h) for M, M_E or $M_{38.5}$ condition; and T_{rec} levels in the evening (17:30 h) for E or $E_{38.5}$ conditions (P > 0.05).

A main effect for condition was observed for Tm (Table 1). Values were lower in the morning (M, M_E or $M_{38.5}$) conditions compared to the evening condition (-0.71°C, -0.65°C and -0.62°C; P < 0.05) and $E_{38.5}$ condition (-0.82°C, -0.76°C and -0.72°C; P < 0.05). There was no difference between any values for any other conditions (P > 0.05; Table 1).

****Insert Figure 2 and Table 1 here****

Post warm-up

A main effect for condition was observed for Trec with higher values in the E (mean difference = $0.45 \pm 0.06^{\circ}$ C, P < 0.0005, 95% CI: 0.26- 0.65° C; ES=2.81) compared to the M condition post warm-up. Pairwise comparisons showed M_{38.5} and E_{38.5} values to be higher than all conditions (P < 0.0005). Further, M_E values were exactly the same as resting E. In summary, the protocol produced the changes in Trec (to resting values previously observed in the morning and 38.5°C) that were required to test the basic research questions. There was no difference between any values for any other conditions (P>0.05).

A main effect was observed for T_m values. Values were lower in the M condition than the $M_{38.5}$ and $E_{38.5}$ condition (-0.92°C and -1.10°C; P<0.05, see Table 1). Pairwise comparisons showed $M_{38.5}$ and $E_{38.5}$ values to be higher than the evening condition (+0.58°C and +0.76°C; P<0.05) and M_E condition (+1.13°C and

+1.31°C; P<0.05). Further, E values showed a trend to be higher than M_E values. There was no difference between any values for any other conditions (P>0.05).

RS performance

A main effect for condition was observed for distance covered (DC), average velocity (AV), average power (AP), peak velocity (PV) and peak power (PP) (Table 1 and Fig 3). There were time-of-day effects in RS performance with lower values of 8.2 % for DC (mean difference = 1.31 ± 0.23 m, P=0.002, 95% CI: 0.49-2.13m; ES=0.89), 8.3% for AV (mean difference = 1.56 ± 0.28 km.h⁻¹, P=0.002, 95% CI: 0.59-2.53km.h⁻¹; ES=0.88), and 7.8% for AP (mean difference = 209.00 ± 54.99 W, P=0.029, 95% CI: 16.75-401.26W; ES=0.79) in the M condition compared to the E condition. The passive warm-up strategy (M_E) in the morning did not significantly increase any RS performance values (P>0.05) to E levels. Increasing core temperature in the morning (M_{38.5}) to optimal levels still showed time-of-day effects in RS performance with lower values compared to the E condition (P>0.05). However, Increasing core in the evening to optimal levels (E_{38.5}) resulted in a decrease in RS performance for DC, AV and AP (6.0-6.7%) when compared to the E condition (P < 0.05) and no different to the M condition (P < 0.05). There was no difference between any values for any other conditions (P > 0.05).

Insert Figure 3*

Heart rate, rating of perceived exertion (RPE), thermal comfort (TC) and effort levels.

A main effect for condition was observed in heart rate and TC levels (Table 1). Values for heart rate were lower in the ME to be lower compared to the E and E38.5 conditions (P < 0.05). Values for TC were higher in the M38.5 compared to the E and ME conditions (P < 0.05) during RS performance. RPE was no different between conditions (P > 0.05). There was a significant "sprint" effect for TC, heart rate and RPE (P < 0.0005), where values increased from the first to the last

sprint. This rise was not present for self-rated effort, which was rated as 10 or maximal for each sprint for all conditions and showed the motivation of the subjects to perform to the best of their ability in each sprint (P > 0.05).

Discussion

Therefore this study aimed to control rectal temperature to see whether diurnal variation in Trec and Tm is accountable for time-of-day oscillations in RS performance on a non-motorised treadmill. This was investigated through increasing morning rectal temperature (by a passive warm-up) to evening resting values or increasing morning and evening rectal temperatures (by a passive warm-up) to optimal (38.5°C) values. The main findings of the study were: 1) increasing morning Trec by a passive warm-up to exactly that found in the evening, a rise of 0.48°C, did not result in RS performance becoming equal to evening values (Fig 3); 2) Increasing morning Trec by a passive warm-up to "optimal levels", a rise of 1.77 °C, did not result in RS performance becoming equal to evening values (Fig 3); 3) Increasing evening Tree by a passive warm-up to "optimal" levels, a rise of 1.23°C, resulted in a decrease in RS performance that was not statistically significantly different from morning values (a drop of 6.0-6.9%; see Fig 3). To the best of our knowledge, this is the first study to demonstrate these results, with precise modelling of pre-exercise temperature (by removing individuals from the warming when the required Trec was reached). Using a warm-up (active or passive) of standard duration, as used by others (Sim et al., 2009; Taylor et al., 2013; Yaicharoen et al., 2012), produces large variation in core and/or muscle temperatures within individuals, either resulting in over- or under-shooting the required value. It was observed that variation in the time taken to reach the required temperatures differed between participants and conditions (18:12±07:39 min:s for M_E, 46:26±17:26 min:s for M_{38.5}, 22:28 \pm 02:32 min:s for E_{38.5}, respectively).

It has been well recognised that a passive warm-up (by air, a water bath, water-perfused suits or by showering) increases Trec and Tm, which potentially benefits force development [Asmussen & Boje, 1945; Racinais et al., 2005b]. It has previously been established that increasing core

temperature to levels of 39.0±0.2°C by passive air (46-50°C) resulted in a decrease in MVC torque and percentage of voluntary activation [26]. Further, RS performance in a warm environment, preceded by a period of water immersion (24:00±04:00 min:s), which resulted in elevations of core temperature to 39.6±0.1°C and Tm to 40.0±0.2°C was found to impair performance (Drust et al., 2005). Both aforementioned studies found that such elevations in core temperature following a passive warm-up induced hyperthermia and therefore negated the anticipated potential beneficial effect of the elevated core temperature and Tm during performance. In agreement with Waterhouse et al., (2005) it is our view that, if an "optimum" core and/or muscle temperature is sought in order to hypothetically overcome diurnal variation in RS performance, individuals should be warmed up in the in the morning or evening to exactly those values deemed "optimal" for short term gross muscular performance. In the literature, physical performance is deemed "optimal" at Trec levels of ~38.3-38.5°C, with a corresponding Tm of \geq 39.0°C (Åstrand & Rodahl, 1986; Stickler et al., 1990). Therefore, following the issues of hyperthermia present in both aforementioned studies, to test our primary hypothesis Trec was increased to levels of 38.5°C both in the M and E. Values of 38.5°C would not result in RS performance to be negatively affected by hyperthermia and were therefore cautious not to increase Trec beyond this value. An "optimal" passive warm-up by water immersion resulted in increases in Tm at 3 cm depth to 38.2°C and 38.4°C, rises of 2.4°C and 2.0°C from resting state, in the M and E, respectively. These Tm values were slightly lower than those suggested to be optimal (Åstrand & Rodahl, 1986; Stickler et al., 1990). In spite of these changes, there was no plateau effect at Trec values of 38.5°C for RS performance values, and no differences were present between $M_{38.5}$ and $E_{38.5}$ (P > 0.05). Therefore, the suggested values of 38.5°C which have been suggested to be optimal in the literature (Åstrand & Rodahl, 1986; Stickler et al., 1990) are not. A plateau effect occurred, but not as originally anticipated, such that values

between M and E following passive warm-up to 38.5°C were no different and significantly lower to E values. Further, these values were no different to M values. This lack of a 'plateau' indicates that the link between Trec and RS performance is not as simple as suggested and we believe that a value of 38.5°C in rectal temperature causes mild-hyperthermia. Such elevations in core temperature have previously been shown to alter and have a detrimental effect on cardiovascular (Gonzalez-Alonso et al., 2005), metabolic (Brooks et al., 1971; Nybo et al., 2002) and physiological (Febbraio et al., 1994; Hancock et al., 2003) responses to exercise. However, these do not seem to fully explain the decreases present in performance and it is believed possible reductions in the central nervous system's neural drive to the active musculature acting as a protective mechanism trying to prohibit from actual hyperthermia might be the reason (Drust et al., 2005)

Taken together the results of this study support the view that morning-evening differences in RS performance, where diurnal variations in core and deep muscle temperatures (3 cm) are evident, involve peripheral mechanisms that are not only dependent on muscle temperature (in accord with the views of others: 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). Direct evidence that there is a large endogenous component to the daily variation in muscle force production (from the body clock and peripheral clocks: Zhang et al., 2009), although suggested in the literature by many authors, is presently unsubstantiated (Sargent et al., 2010).

Summary

In this highly motivated population, raising morning rectal temperature to evening values, or raising morning and evening Trec to 38.5°C passively did not increase RS performance nor offset

diurnal variation. Therefore, Trec (and Tm) cannot fully explain time-of-day oscillations in RS performance on a non-motorised treadmill. Although central temperature may provide some endogenous rhythm to RS performance, the exact mechanism(s) for a causal link between central temperature and human performance are still unclear, and involve multiple components and mechanisms which require further research. It may be beneficial to pursue the contribution of the body clock to the diurnal variation in performance and look to use either chronobiotics such as bright light or exercise, to directly shift the body clock or dawn simulators to improve mood (Thompson et al., 2014) – to ultimately increase morning gross muscular performance and aid athletes have to compete or undergo training in the morning.

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Practical implications

Muscle force production and RS performance is higher in the evening than the morning. Our results showed no evidence that increasing morning rectal temperature (by a passive warm-up) to evening resting values or optimal values leads to a positive change in RS performance on a non-motorised treadmill. However, increasing evening rectal and/or muscle temperatures (by immersion in a hot bath) to optimal values leads to a negative effect. These findings help us to un-pick the contribution of core and muscle temperature to the diurnal variation in RS performance.

Declaration of interest statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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