

# Chronobiology International

## The Journal of Biological and Medical Rhythm Research

ISSN: 0742-0528 (Print) 1525-6073 (Online) Journal homepage: [www.tandfonline.com/journals/icbi20](http://www.tandfonline.com/journals/icbi20)

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**To cite this article:** Ben J. Edwards, Wayne Edwards, Chloe Gallagher, Magali Giacomoni, Adrian Markov, Samuel A. Pullinger, Theresa Toussaint & Benoit Mauvieux (23 Jan 2026): Is there a diurnal variation in flexibility in extreme morning and evening-types where a standardised approach has been employed: Effect of an extended warm-up in the morning?, Chronobiology International, DOI: [10.1080/07420528.2025.2611854](https://doi.org/10.1080/07420528.2025.2611854)

**To link to this article:** <https://doi.org/10.1080/07420528.2025.2611854>



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Published online: 23 Jan 2026.



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REVIEW ARTICLE



## Is there a diurnal variation in flexibility in extreme morning and evening-types where a standardised approach has been employed: Effect of an extended warm-up in the morning?

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### ABSTRACT

Evidence of a diurnal variation on flexibility is equivocal in the literature. This is in part due to familiarisation of the participant to the test, inter-individual variation in chronotype (“morningness” and “eveningness”), bias due to methodological issues and outcome, as well as level of warm-up before the measure. Therefore, the main objective of this study was to establish the effects of time-of-day on flexibility in eight outright “morning-type” [M] and eight “evening-type” [E] where a “standardised protocol” has been employed to reduce bias. A second objective was to determine the effect of a 30-min warm-up on the early morning measures of flexibility. Sixteen recreationally active adults, eight M-types (three males and five females) and eight E-types (three males and five females) were recruited. The participants completed (i) a  $\dot{V}O_2$  peak test on a cycle ergometer, (ii) three familiarisations where intra-aural ( $T_{IA}$ ) temperature was measured at rest and after a 5-min warm-up on a cycle ergometer. Thereafter, participants undertook grip strength (right and left hand), subjective arousal (0–10 cm Likert scale), and a battery of five static flexibility tests. Measuring whole-body range of movement (ROM, cm), spinal ROM during hyper-extension ( $^\circ$ ), lateral ROM of the spine ( $^\circ$ ), and ROM during ankle plantar-flexion and dorsi-flexion ( $^\circ$ ). Thereafter, (iii) five experimental sessions (using the same protocol) at 08:00, 12:00, 16:00, 20:00 and a further 08:00 h session (administered in a counterbalanced order), where a 30-min warm-up at 70% of  $\dot{V}O_2$  peak was performed on a cycle ergometer. Each session was separated by 48-h. Data were analysed using general linear models with repeated measures. M-type showed greater ankle dorsi-flexion than E-types (8.0°ROM). Diurnal variations (08:00–20:00 h) in temperature ( $T_{IA}$ ,  $\Delta 0.64^\circ\text{C}$ ), whole-body flexibility ( $\Delta 3.0$  cm), lateral-movement of the spine ( $\Delta 4.4^\circ\text{ROM}$ ), ankle dorsi-flexion (1.9°ROM), right grip strength ( $\Delta 4.0\text{N}$ ), and arousal ( $\Delta 2.4$  cm) were observed ( $p < 0.05$ ); the highest values for all variables were recorded at 16:00 h, apart from arousal which peaked at 12:00 h. Resting  $T_{IA}$  showed a significant interaction between chronotype and time-of-day where the peak in the M-type was 16 h and E-type later at 20 h ( $p = 0.002$ ); although not significant there was a trend for the M-type profiles for whole-body flexibility to decrease from 16 to 20 h and E-types to plateau following the temperature profile ( $p = 0.093$ ). The 30-min warm-up in the morning showed an increase in  $T_{IA}$  temperature of  $0.58 \pm 0.41^\circ\text{C}$  and whole-body flexibility ( $2.4 \pm 2.5$  cm) which is measured with specially designed apparatus compared to the morning session with 5-min warm-up. The other flexibility measures which involved goniometry showed no detectable effect under current measurement precision for both time-of-day and a 30-min warm-up. M-types showed a greater increase in ankle dorsi-flexion flexibility than E-types after the extended warm-up ( $\Delta 2.3^\circ\text{ROM}$ ).

### ARTICLE HISTORY

Received 7 July 2025  
Revised 1 December 2025  
Accepted 29 December 2025

### KEYWORDS

Time-of-day; range-of-motion; stiffness and core-temperature; Intra-aural temperature; Random variation; systematic bias

## Introduction

A large body of research has demonstrated that in a temperate environment (around 17–22°C) physiological variables associated with sporting performance exhibit daily variations aligned with the body’s temperature rhythm. Typically, showing lower values in the morning

and peaks in the afternoon (Drust et al. 2005; Edwards et al. 2023). Flexibility has for a long time been a very under-rated component of physical fitness (Corbin and Noble 1980), with only specialised sports attaching any significance to it (specifically Gymnastics and Martial arts). Recent evidence suggests that in addition to

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flexibility, long-term static stretching training induces positive effects on muscle strength, muscle power, and muscle hypertrophy irrespective of age and sex (Bougezzzi et al. 2023). Research investigating effects of diurnal variation in flexibility measures in healthy adult populations has been sparse. However, existing studies do report a time-of-day variation in some, but not all flexibility measures, with least flexibility in the morning and greater flexibility in the evening (Adams et al. 1987; Gifford 1987; Atkinson et al. 1993; Fathallah et al. 1995; Fernández-Ruiz et al. 2025). One explanation for this lack of clarity in laboratory-based chronobiological research has been attributed to methodological issues, such as a lack of rigor and standardisation in method employed. These limitations reduce the likelihood of detecting time-of-day effects. In addition, there has often been inadequate control for factors specifically important to chronobiological investigations (Bommasamudram et al. 2022; Ravindrakumar et al. 2022; Edwards et al. 2024).

Although there is no consensus on what exactly a standardised approach should entail, an attempt to consider some of these concerns and standardise this approach has recently been presented (Edwards et al. 2024). This involves a checklist of considerations for chronobiological studies on humans and sporting performance, covering participant selection, environmental control, methodological rigor, and equipment. This check list has already been employed in studies investigating diurnal variation in time-trial performance (Edwards et al. 2024), circadian rhythms in muscle strength (Robertson et al. 2024), broad jump performance in males and females after sleep deprivation (Edwards 2025), and the effect of moderate hyperthermia at two phases of the circadian cycle on core temperature (Edwards et al. 2025).

Two chronotype extremes have been identified in the population called “Larks” and “Owls” also referred to as morning and evening types (M-types and E-types). These groups exhibit distinct characteristics in body temperature rhythm and shape, with M-types tending to perform better in the morning and E-types in the evening (Burgoon et al. 1992; Vitale and Weydahl 2017; Maheshwari et al. 2022). Arguably as physical exercise performance is closely linked to core temperature, there are implications for time-of-day effects on performance in different chronotypes. However, studies into this field are few and fewer still of these studies looked at performances in a sporting context, with the majority being interested in the effect on shiftwork or aging (Atkinson 1994; Vitale and Weydahl 2017; Maheshwari et al. 2022). Similarly, although circadian rhythms in many components of physical performance have been investigated in men, there is limited information on females

(Drust et al. 2005). The exclusion of women as participants in exercise physiology research has been attributed mainly to (i) the potential confounding effects of female hormonal fluctuations, (ii) the increased cost and time required to account for menstrual cycle phases, and (iii) the need for additional resources (e.g. blood or salivary samples to assess ovarian steroid levels) to produce high-quality data. However, recent work suggests that in females who are on a monophasic oral contraceptive pill, exercise performance is consistent across the oral contraceptive pill cycle allowing for greater flexibility in study design (Elliott-Sale et al. 2021).

Finally, the effect of a “warm-up” on body temperature and its corresponding benefits to physical and mental performance is well documented (Bishop 2003). Studies have shown that increases in intramuscular temperature result in a decrease in intramuscular resistance. This translates to an increased range of motion at the joints (Sepega et al. 1981). Therefore, it could be suggested that an aerobic warm-up of sufficient intensity and duration, similarly external heat applications (ultrasound, shortwave diathermy, and hot packs) to increase deep tissue temperature without causing marked fatigue, will lead to a corresponding increase in flexibility (Stewart and Sleivert 1998; Nakano et al. 2011). This “effect” of warm-up on flexibility is of particular interest in the early morning, when body temperature in both morning and evening-types is at its lowest values. One of the most employed methods to reduce diurnal variation in performance incorporates various forms of warm-up, with mixed results (Kusumoto et al. 2021). In recreational athlete populations, warming the body either passively (e.g. water baths) or actively (e.g. exercise) in the morning to match previously recorded evening temperatures has shown no improvement in repeated sprint performance (Pullinger et al. 2019), time-trial cycling (Atkinson et al. 2005), or muscle strength measures (Edwards et al. 2024). However, others have shown that an extended warm-up in the morning raising core temperatures to the level observed at 12:00 h can eliminate diurnal variations in jump height (Taylor et al. 2011). Similarly exercising in a warm environment has been shown to increase morning power in jump tests to approximately that of afternoon (Racinais et al. 2004a) and eliminate diurnal variation in vertical jump and Wingate cycle ergometry performance (Racinais et al. 2004b).

Therefore, the aims of this study were to investigate (i) diurnal variations in flexibility in individuals (male or female), we hypothesise (1) greater flexibility in the evening (16:00–20:00 h) and least in the morning (08:00 h); (ii) whether this overall effect is dependent on the participants circadian type, we hypothesise (2)

that M-types profiles expected to show greater flexibility earlier in the day than E-types; (iii) whether an extended 30-min warm-up at 08:00 h increases flexibility compared to a control incremental 5-min warm-up at the same time, we hypothesise (3) that 30-min will result in greater  $T_{IA}$  and corresponding higher flexibility than the 5-min warm-up condition.

## Methods

### Participants

Recruitment followed a three-part process: (i) Initially, 500 Composite Morningness Questionnaires (Barton et al. 1990) were administered to a cohort of first, second, and third-year sports science degree students at the university. These questionnaires on a 13–52 scale were given at the start of a lecture, during which the teacher gave specific time for the students to fill in their responses, which were given back to the experimenters after the lecture. This administration was done at four different times-of-day to maximise the chance of catching the whole cohort of students. Of the 500 students, 35 “morning” (20 males and 15 females) and 22 outright “evening” types (12 males and 10 females) were identified, based on scores of greater than 44 (M-types) or less than 22 (E-types).

For this cohort, additional *inclusion criteria* for selection were applied: (ii) injury-free with no diagnosed sleep disorders and no history of shiftwork or travel outside the local time-zone in the past month. Participants had to be healthy males and females (18–30 y) and agree to retire to bed at 23:00 and rise at 07:00 h, which reflects an intermediate time between M-type and E-type weekday sleep patterns. *Additional inclusion criteria specifically for the females* were (iii) self-reported menstrual function and use of an oral monophasic contraceptive pill (for at least 3 months) containing an estrogen combined with a progestin, with participants being allocated sessions within 21-days of pill ingestion with ~48 h recovery like the men (Giacomoni and Falgairette 2000).

*Exclusion criteria:* 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). Of the identified chronotypes, eight “morning” (three males and five females) and eight outright “evening” types (three males and five females) were selected based on scores and meeting the

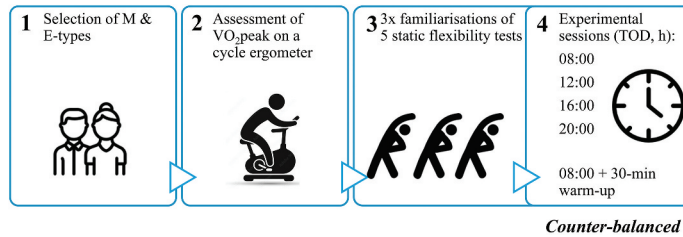
inclusion criteria. The last step involved (iii) a sleep diary being administered, in which participants kept daily records of their bedtimes and times of final awakening for a period of 2 consecutive weeks. The M-types generally went to bed ~21:00–22:30 h and rose from 05:00 to 05:55 h, and E-types retired from 24:00 to 01:00 h and rose from 09:00 to 10:00 h. Habitual total sleep time was as follows: M-types:  $8.4 \pm 0.2$  decimal h vs E-types:  $8.7 \pm 0.9$  decimal h. The identification of participants to chronotype was managed by a disinterested party. Hence, the author (BE) did not know the participants’ chronotype until after the data was collected.

From the initial process, 6 males and 10 females ( $n = 16$ ), who identified gender and biological sex the same, were classified as “recreationally active” by the “Participant Classification Framework” (McKay et al. 2021), met the criteria, and volunteered for the study (Figure 1A). Mean  $\pm$  SD for M-types vs E-types: age =  $21.8 \pm 1.8$  vs  $22.8 \pm 1.8$  yrs, body mass =  $70.3 \pm 10.8$  vs  $67.4 \pm 10.3$  kg, height =  $168.1 \pm 9.2$  vs  $170.3 \pm 10.3$  cm and aerobic activity and strength/week of  $7:30 \pm 0:47$  vs  $3:11 \pm 0:07$  h:min and  $2:15 \pm 0:13$  and  $0:45 \pm 0:003$  h:min. A 14-day recall leisure-time physical activity questionnaire (Lamb and Brodie 1991) was administered. Participants were required to arrive fasted and abstain from alcohol, caffeine, and exercise 24 h preceding a testing session. All participants gave their written informed consent. The study was conducted in accordance with the Local Ethics committee at JMU, the ethical standards of the journal, and complied with the Declaration of Helsinki.

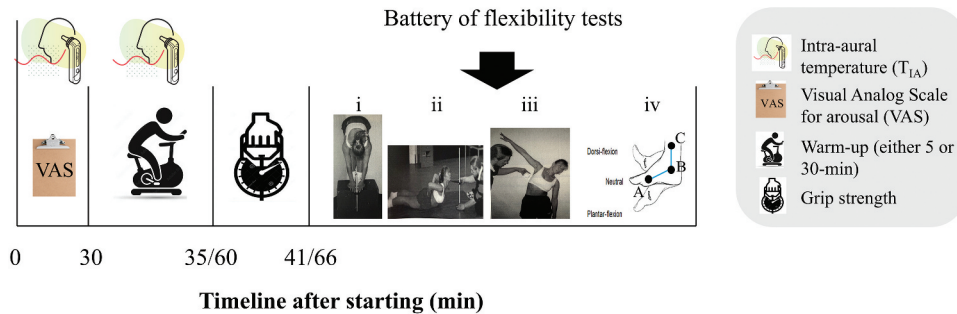
### Experimental Design

The participants attended the environmentally controlled chamber (Edge, Nottingham, UK) on seven occasions, and the chamber was held at a dry temperature, humidity, barometric pressure, and ambient light of  $20.3 \pm 0.7^\circ\text{C}$ ,  $54.4 \pm 5.2\%$ , 750–760 mmHg, and 750 lux, respectively, for all sessions. First, assessment of  $\dot{V}O_2$  peak/max was conducted on a cycle ergometer by each participant during a session taking place from 12:00 to 14:00 h. Participants were then familiarised with the battery of tests at 12:00 h for three sessions (see Familiarization section). Five experimental sessions were allocated in a randomised counterbalanced method (08:00, 12:00, 16:00, 20:00 h, and an extended 08:00 h warm-up session) where each session was separated by 48 h (Figure 1A). The participant entered the laboratory at 30-min prior to the hour, with the end of the warmup being either 5 or 30 mins after this (see Figure 1). All experiments were completed between the months of October and December (Autumn to Winter in the UK) to ensure the

## A. Study design



## B. Experimental protocol



**Figure 1.** Schematic of the experimental study design (A) and experimental protocol (B) for conditions undertaken. The two timelines reflect both the 5 and 30-min warm-up.

individual's exposure to sunlight in the mornings when entering the laboratories was <80 lux (Light-watch, Neurotechnologies, Cambridge, UK), with sunrise and sunset range from start to the end of the experiment being 07:31 to 08:03 h and 18:04 to 19:54 h, respectively.

### Protocol and Measurements

Participants completed a  $\dot{V}O_2$  peak/max test using the Metalyzer 3B (Cortex GmbH, Leipzig, Germany). Tests were conducted on cycling ergometers with reusable masks (7450 Series Silicone V2, Hans-Rudolph Inc, KS, USA) following the CASES guidelines (British Association of Sport and Exercise Sciences 1997). Five mins of resting data were collected before participants completed a 5-min warm-up (WU) at 100 or 150 W for females and males, respectively, which also served as the first testing stage. Resistance was then increased by 30 W every 3 mins until volitional exhaustion. The test will be referred to a “peak” rather than “max” if the participants did not reach the criterial of a plateau in the  $\dot{V}O_2$  data between the last and penultimate stage.

The participants were then brought into the laboratory and became accustomed to the tests and equipment being used by three-familiarisation sessions. This was done to reduce possible learning effects, as shown by the results of

the test re-test on some of the flexibility variables. This also decreased the overall time taken to do the tests in the experiment. The height and body mass of the volunteers were established, as well as seat position on the cycle ergometer (Excalibur Sport, Lode, Groningen, Netherlands) and grip position for the grip strength dynamometer (Takeikiki Kogoyo, Japan; Edwards et al. 2000).

### Familiarisation

The random error between the three initial reliability trials was heteroscedastic and the data were therefore expressed as 95% ratio limits of agreement. For simplicity, only the 95% ratio limits of agreement for the second and third battery of tests were investigated for the 16 participants; these were  $\ast/\div$  1.202 to 1.649 (about 20.2–64.9%) and coefficient of variance of 11.6–33.1%, indicating little to large measurement error (Atkinson and Nevill 1998). Paired *t*-test showed no difference ( $p > .005$ ) with whole-body flexibility (cm) and spinal hyperextension (cm) showing the least random bias (Table 1).

### Protocol and Measurements for the Experimental Sessions

The main experiment consisted of five visits: at 08:00, 12:00, 16:00, 20:00 and a further 08:00 h session



**Table 1.** Systematic and random bias values ( $n = 16$ ) for familiarisation 3 and 2 for a series of five static flexibility tests measuring whole-body flexibility, spinal hyper-extension, lateral-movement of the spine, ankle plantar-flexion, and dorsi-flexion.

Flexibility tests	Familiarisation 2	Familiarisation 3	CV%, P-value and SEM
Whole-body flexibility (cm)	6.2 $\pm$ 11.5	6.6 $\pm$ 12.1	11.6%; $p = 0.167$ ; 0.27
Spinal hyper-extension (cm)	26.3 $\pm$ 5.6	27.1 $\pm$ 17.9	10.3%, $p = 0.381$ , 0.26
Lateral-movement of the spine right side ( $^{\circ}$ ROM)	44.5 $\pm$ 23.0	40.7 $\pm$ 22.1	13.0%, $p = 0.118$ , 0.14
Ankle plantar-flexion (left foot, $^{\circ}$ ROM)	16.6 $\pm$ 11.2	14.9 $\pm$ 9.2	17.1%, $p = 0.143$ ; 0.31
Ankle dorsi-flexion (left foot, $^{\circ}$ ROM)	70.3 $\pm$ 20.2	71.3 $\pm$ 22.5	33.1%, $p = 0.864$ ; 0.15

Mean  $\pm$  SD values and CV%,  $p$ -value from a paired  $t$ -test, and standard error of the mean (SEM) are given.

(administered in a counterbalanced order), where a 30-min warm-up at 70% of  $\dot{V}O_2$  peak was performed on a cycle ergometer in the environmental chamber. In all conditions, they were asked not to consume food 4 h before each session (Edwards et al. 2024). In all sessions, the participants were instructed to retire at 23:00 h and rise at 0700 h, verified by their response to a scheduled automated text message at 23:00 h and upon waking. Compliance was high, with all confirming they had adhered to the schedule as assessed by the automated text response and verbal check in the morning of the experimental sessions. This information was recorded and the delay was never more than  $\pm 3$  mins of the text. The volunteers were free to live a “normal life,” between sessions sleeping at home at night, attending lectures and doing light office work in the day. As the participants were students and the day of testing in the week was the same for all conditions, although we did not measure activity the participants had the same series of time-tabled lectures which dictated their movement the day of and before the scheduled testing. They were asked to refrain from any other training or heavy exertion for the 48 h before the experiments or during them. Participants recorded the type, amount, and timing of the food they ate for the period of 24 h before the day of the first session and were asked to replicate this diet for the days before each experimental condition. Water and non-caffeinated/non-alcoholic, calorie-free beverages were allowed ad libitum up to a total volume of 3 L per day. The clothing of the participants was standardised to lose fitting tracksuit and T-shirt for the warm-up with shorts underneath for the men, the same with the addition of a sports bra for the females.

### Temperature, Arousal, and Grip Strength

The volunteers arrived in the laboratory 30 mins before the experiments and were asked to relax in a supine position. After 25-min period intra-aural (ear), temperatures ( $T_{IA}$ ; Genius first-temp infrared, Nottingham, UK) were taken every min for 5 mins and the average value was recorded as resting temperature (Edwards et al. 2002). The participants were then asked to mount an assigned cycle-ergometer,

previously set up for their height (identified *via* the familiarisation session) and calibrated (according to the instruction manual). The participants indicated their level of arousal on a 10 cm visual analogue scale, where Zero represented ‘almost asleep’ and 10 corresponded to ‘fully alert as possible’ (VAS, to the nearest whole number for facilitation of interpretation; Edwards et al. 2024). They completed a general 5-min warm-up at 100 W for the females and 150 W for the males. At the end of the warm-up, the average of two measures for  $T_{IA}$  was taken to represent warm-up temperature, for the extended warmup condition the participant exercised for 30 mins at the workload corresponding to that of 70% of  $\dot{V}O_{2\text{ peak}}$ . Grip strength was measured in both arms *via* a grip strength dynamometer (with a digital visual display); the participant had three attempts with 2-min rest between each go, with the highest value kept for subsequent analysis (Edwards et al. 2024).

### Battery of Five Static Flexibility Tests

The tests chosen were a battery of five flexibility tests recommended by Watson (1990). The procedure and test-environmental conditions were kept constant. Methods for testing for static flexibility were modified according to the results from the pilot study. The participants took off their shoes and socks and completed a series of five static flexibility tests measuring whole-body flexibility, spinal hyper-extension, lateral-movement of the spine, ankle plantar-flexion, and ankle dorsi-flexion (see Figure 1B). No verbal encouragement was given, the tests were conducted three times, and the peak value was recorded. The movement was executed slowly, and due to the nature of the stretch being “static,” the participants held the final position for 5-s. The experimenter indicated that when the “5 s” period was up, the volunteer was then instructed to relax.

### Whole-Body Flexibility

The participant stands with feet together, toes touching the bar on the forward flexometer (Takei Kiki Kogyo,

Tokyo, Japan) mounted on a stable box 40 cm off the ground (Figure 1.B.i). The participants bend forward with their arms outstretched and hands together, pushing the movable cursor as far as possible down the scale whilst keeping their knees fully extended and legs straight. This distance was recorded in cm.

### **Spinal Hyper-Extension**

The participants lie prone on the floor and bend themselves backward with their hands clasped behind their waist with their big toes 45 cm apart at 90°, and the chin rest is slid up until it nearly meets the chin of the participant (Takei Kiki Kogyo, Tokyo, Japan). The projecting end of the chin rest is adjusted to encounter the chin properly and the chin rest bends in the centre, while the cursor remains in the same position to indicate the height for reading (Figure 1.B.ii). This distance was recorded in cm.

### **Lateral Movement of the Spine**

The participant stands upright with feet hip width apart, and the heels, buttocks, shoulders, and head should be in contact with a smooth wall and must remain so throughout the test. The opposite arm is raised horizontal with the shoulder to that of the direction of rotation. The Bubble Motion clinical inclinometer (MIE, Leeds, UK) is placed (2 × length of the inclinometer) from the auxiliary (pit) of the arm against the corresponding rib and this point is marked. A further point is marked for the other end of the inclinometer (to standardise the effects of rotation of the inclinometer in repeated tests). The inclinometer is now calibrated for measuring the lateral range of motion of the spine and the volunteer leans as far as possible to the left (Figure 1.B.iii).

### **Left Ankle Plantar and Dorsi-Flexion Assessment**

The standard body position in relation to both the ankle plantar and dorsi-flexion tests was the same. The volunteers sit with their back upright and at 90 degrees to a backrest, on a conventional National Health Service (NHS) hospital bed. Buttocks, shoulders, and head should be in contact with the smooth backrest and must remain so throughout the test. The legs should be relaxed and straight out in front, with feet hip width apart, the heels on the mat provided. Participants ankle-foot shown in the neutral position with markers for measurement of both plantar and dorsi-flexion. The points “A,” “B,” and “C” are marked on the skin of the left leg and left foot, respectively, and are used as reference points. The individual plantar flexes and Point “B”

is marked on the skin corresponding to the ankle joint (lateral malleolus) to eliminate error associated with movement from the skin away from the bone during the motion. Point “C” is marked 13–16 cm away from point “B” along the fibula. And point “A” corresponds to a point 14–16 cm from point “B” where the foot can be seen to ball, depending on the individual. The participant assumes the standard position and is instructed to either plantar flex or dorsi flex and the stationary arm is lined up with points “C” (shaft of fibula) and “B” (the axis of rotation) and the moveable arm point “A” using a clinical goniometer (MEDPRO™ Protractor 12 Inch Goniometer, Singapore) is lined up with the three points and a measurement taken (Figure 1.B.iv).

### **Statistical Analysis**

Data were analysed using the Statistical Package for Social Sciences version 30.0 (IBM SPSS Statistics, IBM Corp. 2024). All data were checked for normality using the Shapiro–Wilk test. Independent t-tests were used to compare demographic characteristics of each group. General linear models with repeated measures (time-of-day or 08:00 with 5-min warm-up vs 08:00 h with extended warm-up) and group factor (M-type vs E-types) were used for all measurements collected. To correct sphericity violations, the degrees of freedom were corrected using Greenhouse–Geisser ( $\epsilon < 0.75$ ) or Huynh–Feldt ( $\epsilon > 0.75$ ). Post-hoc Bonferroni pairwise and graphical comparisons were made where main effects were found. Significance was set at  $p < 0.05$ . Effect sizes are referred to as partial eta squared values ( $\eta^2_p$ ) with values of 0.01, 0.06, and 0.14 corresponding to a small, medium, and large effect, respectively (Cohen 1988). The results are presented as the mean ± 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**

Mean ± SD values and the results from the ANOVA statistical analyses are displayed in Tables 2 and 3. Statistical significances of the results can be seen in Figures 2 and 3.

Table 3. Mean ± SD values at 08:00 h for variation in intra-aural temperature ( $\Delta T_{IA}$ ) between the two warm-up modalities (5-min vs 30-min), and values of battery of flexibility tests and grip strength recorded after the extended warm-up in Morning (M) and Evening (E)-

**Table 2.** Mean (SD) values for M-types and E-types for resting intra-aural ( $T_{IA}$ ) temperature, a battery of 5 flexibility tests, visual arousal scale, and right and left grip strength.

	M-types	E-types	Main effect group (GP)	Time-of-day	Interaction
$T_{IA}$ temperature ( $^{\circ}\text{C}$ )	36.75 $\pm$ 0.38	36.83 $\pm$ 0.40	$p = 0.491$ , $\eta^2_p = 0.035$ , OP = 10.1%	$P < 0.001$ , $\eta^2_p = 0.657$ , OP = <b>100%</b>	$P = 0.002$ , $\eta^2_p = 0.297$ , OP = <b>93.7%</b>
Whole-body flexibility (cm)	11.0 $\pm$ 6.8	8.1 $\pm$ 3.3	$p = 0.312$ , $\eta^2_p = 0.073$ , OP = 16.5%	$P < 0.001$ , $\eta^2_p = 0.373$ , OP = <b>95.1%</b>	$p = 0.093$ , $\eta^2_p = 0.140$ , OP = 53.7%
Spinal hyper-extension (cm)	37.9 $\pm$ 10.6	29.6 $\pm$ 8.3	$p = 0.178$ , $\eta^2_p = 0.125$ , OP = 26.2%	$p = 0.174$ , $\eta^2_p = 0.111$ , OP = 41.6%	$p = 0.086$ , $\eta^2_p = 0.144$ , OP = 54.9%
Lateral-movement of the spine left side rotation ( $^{\circ}\text{ROM}$ )	53.4 $\pm$ 8.3	45.4 $\pm$ 9.4	$p = 0.070$ , $\eta^2_p = 0.216$ , OP = 44.8%	$P < 0.001$ , $\eta^2_p = 0.335$ , OP = <b>96.6%</b>	$p = 0.199$ , $\eta^2_p = 0.104$ , OP = 38.8%
Left ankle dorsi-flexion ( $^{\circ}\text{ROM}$ )	95.1 $\pm$ 5.8*	88.0 $\pm$ 8.2	$p = 0.038$ , $\eta^2_p = 0.272$ , OP = <b>56.7%</b>	$P = 0.031$ , $\eta^2_p = 0.189$ , OP = <b>70.5%</b>	$p = 0.861$ , $\eta^2_p = 0.018$ , OP = 9.3%
Left ankle plantar-flexion ( $^{\circ}\text{ROM}$ )	33.0 $\pm$ 7.2	30.8 $\pm$ 4.0	$p = 0.450$ , $\eta^2_p = 0.041$ , OP = 11.2%	$p = 0.089$ , $\eta^2_p = 0.142$ , OP = 54.3%	$p = 0.322$ , $\eta^2_p = 0.079$ , OP = 29.8%
Right grip strength (N)	36.8 $\pm$ 6.7	30.7 $\pm$ 9.2	$p = 0.205$ , $\eta^2_p = 0.112$ , OP = 23.6%	$P < 0.001$ , $\eta^2_p = 0.396$ , OP = <b>99.3%</b>	$p = 0.246$ , $\eta^2_p = 0.093$ , OP = 35.2%
Left grip strength (N)	32.8 $\pm$ 5.9	27.1 $\pm$ 8.6	$p = 0.202$ , $\eta^2_p = 0.114$ , 23.9%	$p = 0.066$ , $\eta^2_p = 0.155$ , OP = 59.3%	$p = 0.386$ , $\eta^2_p = 0.069$ , OP = 26.1%
Visual arousal (0–10 cm VAS)	5.8 $\pm$ 2.0	5.3 $\pm$ 2.5	$p = 0.451$ , $\eta^2_p = 0.041$ , OP = 11.2%	$P = 0.005$ , $\eta^2_p = 0.262$ , OP = <b>88.0%</b>	$p = 0.920$ , $\eta^2_p = 0.011$ , OP = 6.6%

Significance ( $p$ -value),  $\eta^2_p$ , and observed power (%) for main effects for group (GP), time-of-day, and the interaction are given. Statistical significance ( $p < 0.05$ ) is indicated in **bold**. \* indicates a significant GP effect. ROM = range of motion.

**Table 3.** Mean  $\pm$  SD values at 08:00 h for variation in intra-aural temperature ( $\Delta T_{IA}$ ) between the two warm-up modalities (5-min vs 30-min), and values of battery of flexibility tests and grip strength recorded after the extended warm-up in Morning (M) and Evening (E)-types.

	M-types	E-types	Main effect group (GP)	5 vs 30-min warm-up	Interaction
$\Delta T_{IA}$ temperature ( $^{\circ}\text{C}$ )	0.60 $\pm$ 0.43	0.53 $\pm$ 0.37	$p = 0.443$ , $\eta^2_p = 0.043$ , OP = 11.4%	$p < 0.001$ , $\eta^2_p = 0.684$ , OP = <b>99.9%</b>	$p = 0.685$ , $\eta^2_p = 0.012$ , OP = 6.7%
Whole-body flexibility (cm)	11.9 $\pm$ 6.7	8.0 $\pm$ 2.6	$p = 0.138$ , 0.150, OP = 31.0%	$p = 0.003$ , $\eta^2_p = 0.490$ , OP = <b>95.6%</b>	$p = 0.566$ , $\eta^2_p = 0.024$ , OP = 8.5%
Spinal-hyper extension (cm)	39.3 $\pm$ 10.7	28.7 $\pm$ 9.5	$p = 0.051$ , $\eta^2_p = 0.246$ , OP = 51.3%	$p = 0.744$ , $\eta^2_p = 0.008$ , OP = 6.1%	$p = 0.206$ , $\eta^2_p = 0.112$ , OP = 23.5%
Lateral-movement of the spine left side rotation ( $^{\circ}\text{ROM}$ )	54.6 $\pm$ 9.7	45.3 $\pm$ 9.3	$p = 0.065$ , $\eta^2_p = 0.223$ , OP = 46.3%	$p = 0.613$ , $\eta^2_p = 0.019$ , OP = 7.7%	$p = 0.463$ , $\eta^2_p = 0.039$ , OP = 10.9%
Left ankle dorsi-flexion ( $^{\circ}\text{ROM}$ )	94.9 $\pm$ 4.6*	85.8 $\pm$ 9.2	$p = 0.027$ , $\eta^2_p = 0.302$ , OP = <b>62.9%</b>	$p = 0.209$ , $\eta^2_p = 0.110$ , OP = 23.2%	$p = 0.035$ , $\eta^2_p = 0.279$ , OP = <b>58.2%</b>
Left ankle plantar-flexion ( $^{\circ}\text{ROM}$ )	32.4 $\pm$ 7.9	31.6 $\pm$ 10.2	$p = 0.743$ , $\eta^2_p = 0.008$ , OP = 6.1%	$p = 0.285$ , $\eta^2_p = 0.081$ , OP = 17.9%	$p = 0.941$ , $\eta^2_p = 0.000$ , OP = 5.1%
Right grip strength (N)	34.6 $\pm$ 7.8	28.3 $\pm$ 10.2	$p = 0.187$ , $\eta^2_p = 0.121$ , OP = 25.3%	$p = 0.988$ , $\eta^2_p = 0.000$ , OP = 5.0%	$p = 0.762$ , $\eta^2_p = 0.007$ , OP = 6.0%
Left grip strength (N)	31.9 $\pm$ 7.1	26.2 $\pm$ 10.0	$p = 0.213$ , $\eta^2_p = 0.108$ , OP = 22.9%	$p = 0.536$ , $\eta^2_p = 0.028$ , OP = 9.1%	$p = 0.487$ , $\eta^2_p = 0.035$ , OP = 10.2%
Visual arousal (0–10 cm VAS)	4.5 $\pm$ 1.5	3.4 $\pm$ 1.9	$p = 0.139$ , $\eta^2_p = 0.149$ , OP = 30.9%	$p = 0.677$ , $\eta^2_p = 0.013$ , OP = 6.8%	$p = 0.337$ , $\eta^2_p = 0.066$ , OP = 15.3%

Significance ( $p$ -value),  $\eta^2_p$ , and Observed power (%) for main effects for group (GP), warm-up duration and the interaction are given. Statistical significance ( $p < 0.05$ ) is indicated in **bold**. \* Indicates a significant group (GP) effect. ROM = range of motion.

types. Significance ( $P$ -value),  $\eta^2_p$ , and observed power (%) for main effects for group (GP), warm-up duration, and the interaction are given. Statistical significance ( $p < 0.05$ ) is indicated in **bold**. \*Indicates a significant group (GP) effect. ROM = range of motion.

### Comparison of Demographic Characteristics of Two Groups

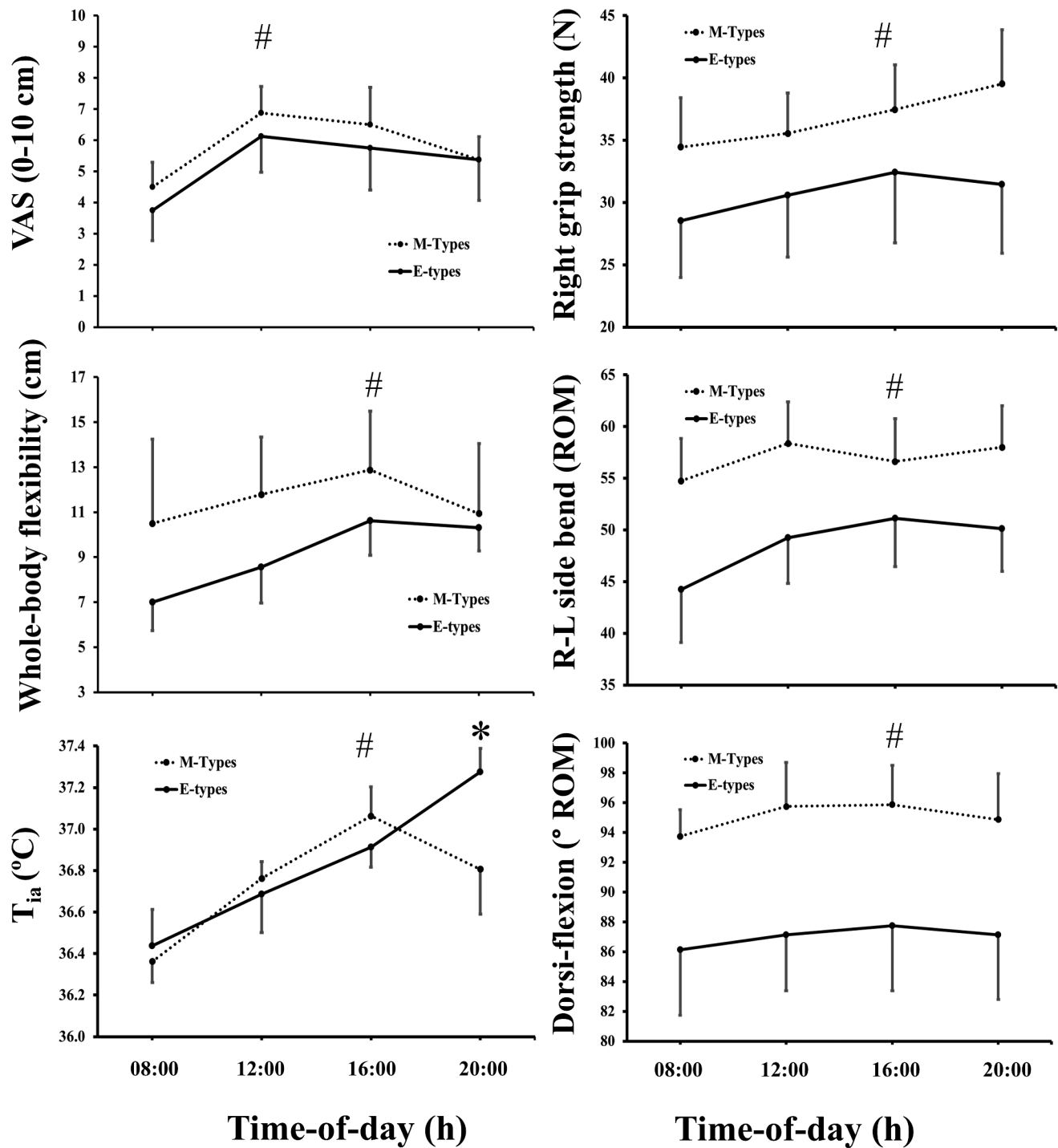
There were no differences for age (mean difference: 0.28 yrs,  $p = 0.283$ ), height (2.2 cm,  $p = 0.619$ ), mass (2.86 kg,

$p = 0.596$ ), or strength training activity (0.5 decimal h,  $p = 0.579$ ). However, the morning types spent significantly greater aerobic training activity than the evening types (3.81 decimal h, CI = 1.29–6.35 h;  $\eta^2_p = 0.43$ ,  $p = 0.006$ ).

### Effect of Time-of-day on Resting $T_{IA}$ Body Temperature

There was no main effect for group such that mean  $T_{IA}$  values for M-types and E-types were similar ( $p > 0.05$ , Table 2). A diurnal variation was found where

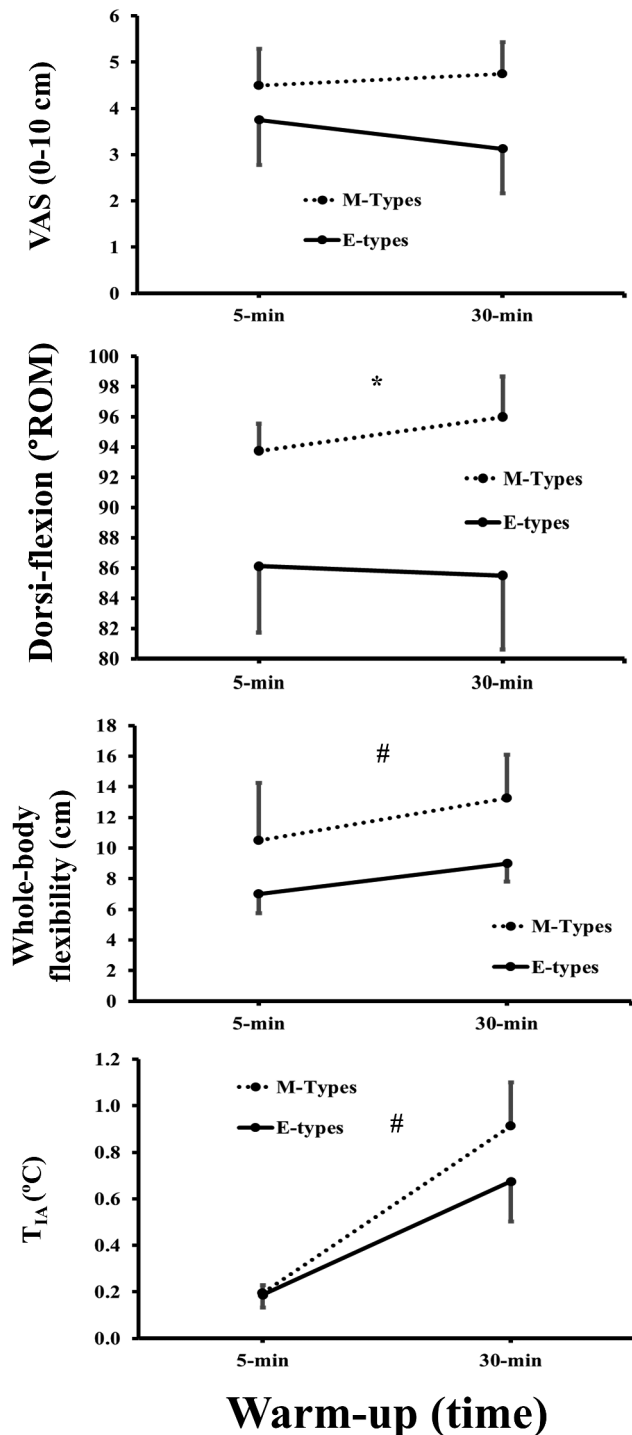




**Figure 2.** Mean (CI) time-of-day effects for visual analogue scale for arousal (VAS), right grip strength, whole-body flexibility, R-L side bend (lateral-movement of the spine right side), ankle dorsi-flexion, and resting  $T_{IA}$  (intra-aural temperature) for M-types and E-types. GLM with repeated measures: #, main effect for time-of-day; \*, interaction (time-of-day  $\times$  chronotype). In summary, flexibility peaked at 16:00–20:00 h with  $T_{IA}$  peaking earlier in M-types than E-types.

$T_{IA}$  values increased from 08:00 to 20:00 h ( $0.64^{\circ}\text{C}$ ,  $\text{CI} = 0.38\text{--}0.91^{\circ}\text{C}$ ,  $\eta^2_p = 0.66$ ,  $p < 0.001$ ; **Figure 2**). A significant interaction between chronotype and

time-of-day was found where the peak in the M-type was 16:00 h and E-type later at 20:00 h ( $\eta^2_p = 0.30$ ,  $p = 0.002$ ; **Figure 2**).



**Figure 3.** Mean (CI) values for pre-post warmup for 5 and 30-min warm-ups for visual arousal (VAS), dorsi-flexion, whole-body flexibility, and pre-post  $\Delta T_{IA}$  temperature for M-types and E-types. GLM with repeated measures: #, main effect for warm-up, \*, interaction effect (warm-up  $\times$  chronotype). In summary, extended warm-up increased  $T_{IA}$  and whole-body flexibility and reduced morning deficits in dorsi-flexion in M-types only.

#### Effect of Time-of-Day on Performance and Subjective Visual Arousal

M-types showed greater flexibility in ankle dorsi-flexion than E-types ( $8.0^{\circ}\text{ROM}$ ,  $\text{CI} = 0.50\text{--}15.60^{\circ}$

$\text{ROM}$ ,  $\eta^2_p = 0.27$ ,  $p = 0.038$ ; Table 2), with a trend for lateral movement of the spine left side rotation ( $p = 0.070$ ; Table 2). Diurnal variations were found in whole-body flexibility ( $3.0\text{ cm}$ ,  $\text{CI} = 0.72\text{--}5.3\text{ cm}$ ,  $\eta^2_p = 0.37$ ;  $p < 0.001$ ), lateral-movement of the spine types ( $4.4^{\circ}\text{ROM}$ ,  $\text{CI} = 0.86\text{--}7.9^{\circ}\text{ROM}$ ,  $\eta^2_p = 0.34$ ;  $p < 0.001$ ), ankle dorsi-flexion ( $1.9^{\circ}\text{ROM}$ ,  $\text{CI} = 0.36\text{--}3.4^{\circ}\text{ROM}$ ,  $\eta^2_p = 0.19$ ;  $p = 0.031$ ), right grip strength ( $4.0\text{ N}$ ,  $\text{CI} = 1.7\text{--}6.4\text{ N}$ ,  $\eta^2_p = 0.40$ ;  $p < 0.001$ ), and visual arousal ( $2.4\text{ cm}$ ,  $\text{CI} = 0.96\text{--}3.79\text{ cm}$ ,  $\eta^2_p = 0.26$ ;  $p = 0.005$ ). Flexibility and grip strength values peaked at  $\sim 16:00\text{--}20:00\text{ h}$  and VAS at  $12:00\text{--}16:00\text{ h}$  (Figure 2). A trend for both left grip strength and ankle plantar-flexion was observed with a peak  $\sim 16:00\text{--}20:00\text{ h}$  ( $p = 0.066$ ;  $p = 0.089$ ). There were no significant interactions between chronotype and time-of-day, for any of the variables studied. Exploring the M-type profiles for whole-body flexibility to decrease from  $16:00$  to  $20:00\text{ h}$  and E-types to plateau ( $p = 0.093$ , Figure 2, Table 2); with the opposite for spinal hyper-extension ( $p = 0.086$ , Table 2).

#### Effect of a 30-Min Extended Warm-Up at 08:00 h on $T_{IA}$ Temperature

There was no significant main effect for chronotype for  $T_{IA}$  temperature change from a 5-min to 30-min warm-up ( $p > 0.05$ , Table 3). A significant condition effect was found, where the extended warm-up increased morning temperature values more than the 5-min warm-up ( $0.58^{\circ}\text{C}$ ,  $\text{CI} = 0.36\text{--}0.81^{\circ}\text{C}$ ,  $\eta^2_p = 0.66$ ;  $p < 0.001$ ) and no interaction ( $p > 0.05$ , Table 3; Figure 3).

#### Effect of a 30-Min Extended Warm-Up at 08:00 h on Performance

There was a significant main effect for chronotype for left ankle dorsiflex-flexion ( $+9.1^{\circ}\text{ROM}$ ,  $\eta^2_p = 0.30$ ,  $\text{CI} = 1.2\text{--}17.0^{\circ}\text{ROM}$ ;  $p = 0.027$ ) and a trend for spinal hyper-extension ( $p = 0.051$ ) and lateral movement of the spine left side rotation ( $p = 0.065$ ) where values were greater in the M-types than the E-types (Table 3). A significant condition effect for condition was found where the extended 30-min warm-up resulted in increased morning whole-body flexibility than the 5-min warm-up ( $+2.4\text{ cm}$ ,  $p = 0.003$ ,  $\text{CI} = 1.0\text{--}3.8\text{ cm}$ ,  $\eta^2_p = 0.49$ ; Figure 3). A significant interaction for chronotype and warm-up condition was found for left ankle dorsiflex-flexion, where pairwise comparisons showed M-types had a greater increase in flexibility than E-types after the extended

warm-up (+2.3°ROM, CI = 0.4–4.1°ROM,  $\eta^2_p = 0.28$ ,  $p = 0.035$ , Table 3; Figure 3).

## Discussion

A significant diurnal variation in the static flexibility test for whole-body flexibility and right grip strength peaking 16–20 h was found, which was seen to follow that of  $T_{IA}$  temperature profile. A daily variation of 0.64°C in  $T_{IA}$  temperature and of 4.0 N in right grip strength agrees with reported findings from other studies (Horne and Ostberg 1976a; 1976b; Refinetti and Menaker 1992) and previous data from our laboratory (Edwards et al. 2000; Drust et al. 2005; Edwards et al. 2024). The observed diurnal variation in whole-body flexibility of  $\Delta 3.0$  cm ( $\eta^2_p = 0.37$ , large effect) confirms our first hypothesis and agrees with the  $\Delta 2$ –3 cm observed by Atkinson et al. (1993) using the same flexometer as the current study. Larger diurnal variations of  $\Delta 14.4$  cm were reported by Gifford (1987) who assessed whole-body flexibility from a “stand-and-reach test.” The technique used in Gifford study was slightly different as the participant had to stand on a wooden platform and push a velcro strip down to the floor, the distance reached being measured in cm. Forward flexibility, assessed from the sit-and-reach test, also showed diurnal variations with changes from 3 to 4 cm (Russell et al. 1992; Reilly et al. 2007; Guariglia et al. 2011) to 5.1 cm (Rahnama et al. 2009). These diurnal changes in whole-body flexibility have been mostly attributed to two factors: (i) daily changes in spinal mechanics and stature  $\sim 1.9$  cm which would explain 63% of our findings on whole-body flexibility (Wright et al. 1969; Adams et al. 1987, 1990), such as intervertebral disc height in the lumbar region ( $\sim 1.5$  mm in the height of each lumbar disc; Zander et al. 2009). And (ii) the diurnal variation in body temperature, as any increase in core temperature would increase flexibility through changes in viscoelastic property of musculotendinous unit (increase compliance and reduce stiffness) with increases in muscle temperature. Literature exploring the link and quantification of core or muscle temperature change and corresponding effect on flexibility in humans is sparse. Further, in the current study, the data was collected after specific training by an expert and observed in day-to-day practice and technique corrected for 6 months before the study commenced. Level of expertise and training is rarely reported in other research. We also found significant daily variations, with large effect sizes (where  $\eta^2_p > 0.14$ ) in lateral movement of the spine (4.4°ROM,  $\eta^2_p = 0.34$ ) and

ankle dorsi-flexion (1.9°ROM,  $\eta^2_p = 0.34$ ). The presence of a variation in static measures of flexibility either using a backward flexometer, inclinometers, or goniometer is unclear. With some authors measuring straight leg raise test, ankle dorsi-flexion or ROM (using joint angles) of knee and hip reporting no variation (Fathallah et al. 1995; Lericollais et al. 2011; Gaos et al. 2024; Fernández-Ruiz et al. 2025). Whereas others show a significant daily variation in sit-and-reach, lumbar flexion, straight leg raise test, hamstring flexibility, and ankle dorsi-flexion (Adams et al. 1987; Gifford 1987; Reilly et al. 2007; Manire et al. 2010; Lericollais et al. 2011; Fernández-Ruiz et al. 2025).

Comparison between our study and others is further complicated, as we recruited and included both males and females, where only a few studies have done this (Gifford 1987; Manire et al. 2010). Differences in flexibility in biological sex exist, where females are generally more flexible than males at the tests used such as hamstring flexibility and lumbar flexion (Manire et al. 2010). However, as the females in the current study were on the monophasic pill, the session occurred in the first 21 d of the pill ingestion phase. Hence, maintaining constant the hormonal status due to the monophasic pill and therefore standardising the menstrual cycle potential effect. The current study unlike others sought to use a standardised approach to reduce the “bias in measurement of the outcome” and “bias in selection of the reported result” (Edwards et al. 2024). One of the strengths of this study was the use of three familiarisation sessions, unlike other studies. The random bias was lowest in the forward and backward flexometer (CV = 10–11%) and larger in the inclinometer and goniometer assessments (Table 1), which could partially explain the lack of evidence of a rhythm in the current study. The techniques may be less accurate and were like that of other re-test results for whole-body flexibility but with smaller sample sizes ( $n = 1$  CV = 8.5%, Gifford 1987). With greater reliability in the sit and reach test (CV = 3.5%; Bozic et al. 2010; 3.8%; Sporis et al. 2011), further comparison is limited, and the random error for the tests ranged from 10.3% for spinal hyper-extension to 17.1% for left foot dorsi-flexion (Table 1). In other studies, investigating the test–retest reliability of the ankle, both dorsi and plantar flexion (knee extended), the correlation coefficients were low, but the authors reported a lack of reliability (Hamill et al. 1989; Baumhauer et al. 1995). Clinicians can be 95% confident that an observed change between two measures larger than 47° and 50°ROM for the flexibility measures obtained from the ankle dorsi-flexion with knee extended and flexed (Cejudo et al. 2015). Finally, it

should be noted that equipment and technique differ between studies for the same measure, which may affect bias of measure (Burton 1991).

We hypothesized that M-types profiles would show earlier peaks in  $T_{IA}$  temperature profiles and corresponding greatest flexibility earlier in the day than E-types. A significant interaction between chronotype and time-of-day for  $T_{IA}$  temperature profiles was found, where the peak in the M-type was at 16:00 h and E-type later at 20:00 h (Figure 2). In agreement, Horne and Ostberg (1976a) and Kerkhof and Lancel (1991) reported that M-types tended to have an earlier circadian peak time of oral temperature ( $\sim 2\text{--}2.5$  h) and higher day time temperature than E-types, the difference between M-types and E-types being not only in the time of the peak of the curve, but in the shape of the curve as well. M-types showed an elevated temperature early in the morning and only a slight increase thereafter until a peak at 19:30 h, whereas E-types temperature rises steadily throughout the day and peaks about  $\sim 21:30$  h. However, due to the limited time-of-day points for measurement taken in our study (with only 4 hours between each point), further interpretation of the profiles for core temperature is limited. Hill et al. (1988) reported no difference in morning and evening core temperatures between morning and evening types; they proposed that this was because these participants were measured at so early in the morning that the morning types did not have time to demonstrate this rapid increase reported by Horne and Ostberg (1976a).

Our second hypothesis was not confirmed where no interaction of chronotype and time-of-day for flexibility was found. Although not significant, there was a trend for the M-type profiles for whole-body flexibility to decrease from 16:00 to 20:00 h and E-types to plateau following the temperature profile from 16:00 to 20:00 h ( $p = 0.093$ , Figure 2, Table 2). No other interactions were observed for other variables. Unfortunately, the observation of only 7% outright M-types and 44% E-types in the university recruitment pool meant that when the 35 “morning” (20 males and 15 females) and 22 outright “evening” types (12 males and 10 females) were put through the inclusion criteria, they resulted in two groups of eight participants. These strict selection criteria led to a low sample size in each group ( $n = 8$ ) which reduced the power of the statistics. However, as there is sparse research on chronotype and sporting performance not widening the pool to “almost M or E-types” was not considered. To explore sex-related differences, we ran an ANCOVA test with “sex” as a covariate for the flexibility tests that showed a time-of-day effect (whole-body flexibility, lateral-movement of the spine, and ankle dorsi-flexion), and we found no evidence that

“sex” masked or mimic chronotype effects on flexibility. A physical activity questionnaire (PAQ) was administered to further investigate factors such as an activity/exercise: Several investigations report that active people tend to be more flexible than inactive people (Beaulieu 1980). Results from 14-d recall leisure-time physical activity questionnaire (Lamb and Brodie 1991) revealed activity and exercise undertaken by an individual participant in an average week. The exercise periods were broken down into two aspects “Aerobic” and “Strength” training, respectively. A potential masking effect of the level of physical activity cannot be excluded in the present study, as M-types individuals’ mean aerobic activity/exercise was significantly greater than that of E-types ( $p = 0.05$ ), although there was no statistical significance between the outright chronotypes in terms of “Strength” training ( $p = 0.160$ ). The training aerobic history as well as the mode of exercise (swimming) may explain the greater able dorsi-flexion for the morning vs evening-types. Such training can increase ROM independent of time-of-day. However, we ran an ANCOVA test with “aerobic activity” as a covariate for the flexibility tests that showed a time-of-day effect (whole-body flexibility, lateral-movement of the spine, and ankle dorsi-flexion), and no significant differences in main effects for time-of-day, chronotype, or interactions were found ( $p > 0.05$ ). Therefore, we found no evidence that having greater aerobic activity levels in the M-type population did not mask or mimic chronotype effects on flexibility.

The last aim of the study was to investigate if an extended 30-min warm-up at 08:00 h will increase flexibility performance due to the association of increase in core temperature and performance measures in comparison to that of a control incremental 5-min warm-up at 08:00 h. Further, the greater use of the cardiovascular system in the extended warm-up has been shown to increase flexibility by various direct and indirect means. These include improved and increased blood flow in the muscles (Watson 1990) and offset a circadian variation in joint stiffness usually associated with a 24-h cycle in the temperature of tissues within and around these joints (Wright et al. 1969). As well as increases in intramuscular temperature result in a decrease in intramuscular resistance which may result in an increase in range of motion in association with joints (Sepega et al. 1981).

Both active (exercise) warm-ups increased  $T_{IA}$  ( $0.28 \pm 0.13$  vs  $0.86 \pm 0.35^\circ\text{C}$ , 5-min and 30-min respectively), however as predicted the 30-min warm-up increased morning temperature (irrespective of chronotype) to a higher level ( $\Delta 0.58^\circ\text{C}$ ,  $\eta^2_p = 0.72$ ; Figure 2) than the 5-min standard warm-up suggested by CASES

for this population of recreational athletes (100–150 W for 5-min; British Association of Sport and Exercise Sciences (1997). Therefore, our third hypothesis was correct where the extended warm-up resulted in an increased morning whole-body flexibility values (a more sensitive measure than the goniometer tests) compared to the 5-min warm-up. This increase was that of 80% of the observed diurnal variation, with a large effect size ( $\Delta 2.4$  cm,  $\eta^2_p = 0.49$ ). In similar tier 2 populations (McKay et al. 2021) whether warming the human both passively (water baths), or as in our case actively (exercise) in the morning to previously measured evening temperatures, has shown mixed results (Kusumoto et al. 2021). Either there is no improvement in repeated sprint performance (Pullinger et al. 2019), time-trial cycling (Atkinson et al. 2005), as well as muscle strength measures (Edwards et al. 2024) or an improvement in morning countermovement jump (Taylor et al. 2011), vertical jump, cycling tests (Racinais et al. 2004b), and Wingate 30-s all out cycle ergometry (Gauthier et al. 1996; Chaari et al. 2015). Exercising at 60, 70 or 80% of  $\dot{V}O_2$  peak but for 15 min have previously shown little effect on ROM, since ankle dorsiflexion and hip extension significantly increased in all warm-up conditions, hip flexion significantly increased only after the 80% of  $\dot{V}O_2$  peak warm-up, and knee flexion did not change after any warm-up (Stewart and Sleivert 1998).

There was a significant main effect for chronotype for left ankle dorsi-flexion ( $\Delta 9.1^\circ$  ROM,  $p = 0.027$ ) and a trend for spinal hyper-extension ( $p = 0.051$ ) and lateral movement of the spine left side rotation ( $p = 0.065$ ) where values were greater in the M-types than the E-types (Table 3). A significant interaction for chronotype and warm-up condition effect was found for left ankle dorsi-flexion, where M-types showed a greater increase in flexibility than E-types after the extended warm-up ( $\Delta 2.3^\circ$  ROM,  $p = 0.035$ ; Table 3, Figure 3). This may partially be explained by the anecdotal (as we did not measure this) general greater physical activity of the M-type cohort, being mainly recruited from the university swim team. However, both groups were classified as “recreationally active” by the “Participant Classification Framework” (McKay et al. 2021).

## Limitations

Due to the limited population of outright chronotypes and inclusion criteria, sample size for the present study was small and this will reduce the power of the study. To standardize the time-of-day of familiarisation of the five static flexibility tests and reduce time-of-day learning

variability, we chose a time roughly in the middle of the testing periods for all participants. We acknowledge that learning effects can still be phase-dependent but chose not to balance familiarization across times of testing. Further, an optimal warm-up normally incorporates dynamic movements due to the “specific” nature of flexibility (specific to various joints throughout the body), as well as a warm-up component that does not induce fatigue. Hence, our choice of 70%  $\dot{V}O_2$  peak for 30 mins may not be optimal (although elicited HR of 63% Max HR considered to be a warm-up intensity) and this may explain the lack of a significant increase in flexibility in some measures, as well as the practical applicability of the findings (Williford et al. 1986). Participants were 20–24 y old and classified as “recreationally active” by the “Participant Classification Framework” (McKay et al. 2021); hence, the findings of this study cannot be generalized to elite athletes or older adults without caveat. Intra-aural temperature site was used, even though we standardised the method to collect these measures in different conditions. We acknowledge that this method as a measure of core temperature is subject to environmental influences (Edwards et al. 2000). We did not note the brand/estrogen-progestin dose range, and there may still have been endogenous temperature fluctuations associated with the menstrual cycle that may have confounded the results. Finally, we used a 14-d recall leisure-time physical activity questionnaire (Lamb and Brodie 1991), and we acknowledge that more sophisticated measures of aerobic and strength weekly activities are available.

## Conclusion

The present study demonstrates clear diurnal rhythms in  $T_{IA}$  temperature, whole-body flexibility, and grip strength, with peaks at 16:00 h, apart from arousal which peaked at 12:00 h. Chronotype modulated these patterns: M-types peaked at 16:00 h, whereas E-types showed delayed maxima around 20:00 h, and M-type flexibility tended to decline thereafter, while E-types plateaued. Extending the morning warm-up to 30 mins elevated  $T_{IA}$  by  $0.58 \pm 0.41^\circ\text{C}$  and increased flexibility by  $2.4 \pm 2.5$  cm versus a 5-min routine, suggesting that prolonged warm-ups can partly offset suboptimal training times. These findings support chronotype-tailored exercise scheduling and highlight warm-up duration as a practical intervention when training outside one’s optimal circadian window. Flexibility measures which involved goniometry were unaffected by both time-of-day and a 30-min warm-up, and this reflects the low sensitivity of these measures on the population which



the familiarisation sessions in the current study provided.

## Future Research

We suggest that future research should explore diurnal variation in flexibility in an elite population of athletes, using dynamic flexibility rather than static measures. The training and exposure to measuring flexibility of the researcher should be acknowledged in the method section. Diet and sleep should be measured more objectively (e.g. actigraphy and controlled meals) to reduce variability. Core temperature should be measured using a site that reflects more brain temperature (i.e. rectal, gut, or oesophageal).








## Disclosure Statement

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

## Funding

The author(s) reported that there is no funding associated with the work featured in this article.

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