

Time-of-Day Variation in Knee Extensor Strength and Fatigue Is Associated With Circadian Phenotype in Healthy Young Adults

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Abstract

Time-of-day variation in neuromuscular performance is well established, but the contribution of circadian phenotype remains unclear. This study examined whether maximal knee-extensor strength and fatigue resistance differ between morning and evening and whether chronotype and actigraphy-derived sleep-timing are associated with diurnal performance variation. Forty-four healthy young adults (21 males, 23 females) completed a randomised-crossover protocol consisting of neuromuscular testing in the morning (0700–0900) and evening (1700–1900). Participants completed 7 days of actigraphy monitoring and the Composite Morningness Questionnaire to characterise behavioural circadian phenotype. Sleep timing was assessed using habitual sleep midpoint (between sleep-onset and wake-time), with night-to-night variability in sleep midpoint used as an index of sleep-timing variability. Maximal voluntary isometric contraction (MVIC) torque, fatigue index, contractions completed, and time-to-exhaustion were assessed during each trial. Baseline MVIC torque was greater in the evening compared with the morning (2.96 ± 0.80 vs 2.85 ± 0.77 N m kg⁻¹; $P < .0001$), with post-fatigue torque also remaining higher in the evening (2.00 ± 0.60 vs 1.84 ± 0.54 N m kg⁻¹; $P < .0001$). Participants demonstrated a lower fatigue-index (32.57 ± 6.99 vs $35.58 \pm 6.26\%$; $P < .0001$), completed more contractions (37.95 ± 8.29 vs 35.09 ± 7.58 ; $P < .0001$), and exhibited longer time-to-exhaustion (6.36 ± 1.51 vs 5.85 ± 1.26 minutes; $P < .0001$) during evening testing. Chronotype was strongly associated with the PM-AM difference in MVIC torque ($r = -.684$, $P < .0001$), explaining 47% of the variance in diurnal performance responses. Neither habitual sleep midpoint ($r = -.029$, $P = .848$) nor night-to-night variability in sleep midpoint ($r = -.055$, $P = .721$) was associated with performance differences. No significant sex-related differences were observed. Maximal knee extensor strength and fatigue resistance were enhanced during evening compared with morning testing. The magnitude of this response was strongly associated with behavioural circadian phenotype, but not actigraphy-derived sleep-timing. Findings suggest that chronotype influences time-of-day differences in neuromuscular performance and should be considered when evaluating exercise performance.

Keywords

circadian rhythm, chronotype, neuromuscular performance, fatigue resistance, time-of-day, strength

Introduction

The circadian timing system orchestrates daily rhythms in physiology and behaviour, enabling organisms to anticipate predictable environmental cycles and optimise biological function across the 24-hour day.^{1–3} In humans, circadian regulation influences numerous physiological systems relevant to physical performance, including endocrine function, substrate metabolism, cardiovascular physiology, neural activation, thermoregulation, and skeletal muscle function.^{4–7} Consequently, many aspects of exercise performance exhibit robust diurnal rhythmicity, with endurance capacity,

anaerobic power, repeated-sprint performance, and maximal strength frequently reported to be superior during the late afternoon or early evening compared with the morning.^{7–15} Although these group-level patterns are well established, substantial inter-individual variability exists in both the

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magnitude and timing of diurnal performance rhythms, and the biological factors responsible for this variability remain poorly understood.

Growing evidence suggests that this variability may reflect differences in circadian phenotype rather than clock time alone. Chronotype describes an individual's preferred timing of sleep, wakefulness and daily activity, reflecting behavioural manifestations of the underlying circadian system.¹⁶⁻¹⁸ Individuals exist along a continuum from morning to evening preference and differ substantially in the timing of peak cognitive, physiological and physical performance behaviour.¹⁹⁻²¹ These observations have prompted increasing interest in whether biological timing should be considered when interpreting exercise performance and prescribing exercise interventions. Rather than representing a uniform physiological response to clock time, diurnal variation in performance may instead reflect the interaction between experimental testing time and an individual's circadian phenotype.

This concept is supported by accumulating evidence demonstrating that skeletal muscle is itself a circadian tissue. Autonomous molecular clocks within skeletal muscle regulate transcriptional programmes controlling mitochondrial function, substrate utilisation, contractile biology, protein turnover and adaptive responses to exercise.²²⁻²⁴ Experimental disruption of molecular clock function abolishes normal daily rhythms in exercise capacity in rodents, demonstrating that an intact circadian system is required for normal rhythmicity in physical performance.^{25,26} Likewise, exercise performed at different biological times elicits distinct metabolic and molecular responses in both rodents and humans,²⁵ while recent work from our laboratory demonstrated that endurance training performed during the early active phase induces superior physiological adaptation compared with training performed later in the active period despite lower absolute workloads.²⁷ Collectively, these findings support the concept that biological timing is a fundamental regulator of skeletal muscle physiology and exercise adaptation rather than simply a modifier of performance.

Despite these advances, relatively little is known regarding whether behavioural circadian phenotype explains inter-individual differences in human neuromuscular performance across the day. Most previous investigations have focused on endurance exercise or whole-body performance tasks, while relatively few studies have combined objective behavioural circadian phenotyping with controlled laboratory assessments of isolated neuromuscular function.¹⁹⁻²¹ Furthermore, females remain substantially underrepresented in both circadian and neuromuscular performance research despite recognised sex-related differences in endocrine physiology, sleep-wake regulation and exercise responses that may influence circadian regulation of skeletal muscle function performance.²⁸⁻³⁰ Consequently, it remains unclear whether the well-established diurnal variation in maximal strength and fatigue resistance reflects intrinsic differences in circadian

phenotype or is simply a consequence of testing at different times of day.

Therefore, the aim of this study was to determine whether behavioural circadian phenotype is associated with inter-individual variation in the diurnal rhythm of neuromuscular performance in healthy young adults using a controlled randomised crossover design. Specifically, we compared maximal voluntary isometric contraction (MVIC) torque, fatigue resistance and indices of neuromuscular performance between morning and evening testing while characterising behavioural circadian phenotype using validated chronotype questionnaires together with 7 days of actigraphy-derived sleep timing and sleep regularity. Sex was included as a planned analytical factor to examine potential sex-related differences in diurnal neuromuscular responses. We hypothesised that (1) maximal force production and fatigue resistance would be enhanced during evening compared with morning testing, (2) behavioural circadian phenotype would explain a significant proportion of the inter-individual variability in diurnal neuromuscular performance, and (3) these responses may differ between males and females.

Methods

Participants

Forty-four healthy young adults aged 18-35 years (21 males: age 26.7 ± 4.3 years, body mass 75.05 ± 9.02 kg, height 180.24 ± 7.95 cm; and 23 females: age 25.9 ± 4.7 years, body mass 64.21 ± 7.05 kg, height 168.37 ± 6.02 cm) were recruited to participate in this study. Participants were recreationally active, engaging in moderate physical activity 2-5 times per week, but were not involved in structured resistance training programmes. All participants were free from known cardiovascular, neurological, metabolic, musculoskeletal, or diagnosed sleep disorders. Habitual caffeine intake was assessed using a validated caffeine consumption questionnaire, and participants reporting habitual caffeine consumption >150 mg·day⁻¹ were excluded. Additional exclusion criteria included shift work within the previous 3 months, transmeridian travel within 4 weeks of testing, current smoking, use of medications known to affect sleep, neuromuscular function, or cardiovascular responses (e.g., sedative/hypnotic medications, stimulants, muscle relaxants, cardiovascular medications such as β -blockers), and lower-limb injury within the preceding 6 months. No participants were excluded on the basis of medication use.

To minimise variability associated with hormonal fluctuations, all female participants were tested between days 10 and 15 following the onset of menstruation, based on self-report of the first day of their most recent menstrual cycle and typical cycle length, which were confirmed verbally at each laboratory visit. Participants using hormonal contraceptives were excluded. All participants provided written informed consent before participation. The study was

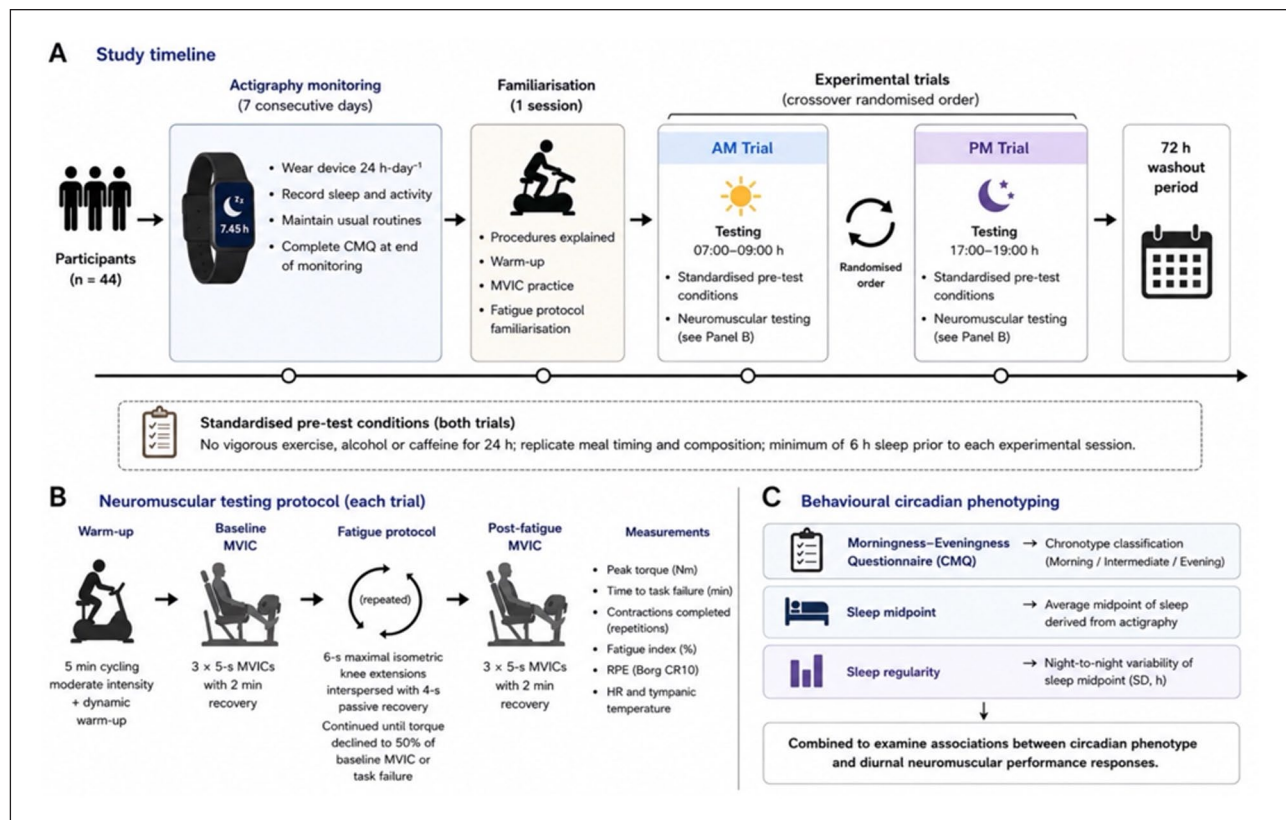


Figure 1. Experimental design and behavioural circadian phenotyping procedures. (A) Overview of the study timeline and experimental design. Participants completed 7 consecutive days of actigraphy monitoring before experimental testing, followed by a familiarisation session and 2 neuromuscular testing trials performed in randomised crossover order during the morning (AM; 0700–0900 hours) and evening (PM; 1700–1900 hours), separated by a minimum of 72 hours washout period. Standardised pre-test conditions were maintained before each trial. (B) Neuromuscular testing protocol performed during each experimental session, including warm-up procedures, baseline maximal voluntary isometric contraction (MVIC) assessment, repeated maximal isometric knee extension fatigue protocol, post-fatigue MVIC assessment, and physiological/perceptual outcome measurements. (C) Behavioural circadian phenotyping measures derived from actigraphy and questionnaire assessments, including Composite Morningness Questionnaire (CMQ) score, sleep midpoint, and sleep regularity metrics used to examine associations with diurnal neuromuscular performance responses.

conducted in accordance with the Declaration of Helsinki and received approval from the Ethics Committee of the School of Medicine and Dentistry, University of Lancashire.

Sample Size Estimation and Justification

Sample size estimation was informed by previous studies investigating diurnal variation in neuromuscular performance using repeated-measures designs,^{31–33} which have typically reported moderate effect sizes for differences in maximal force production between morning and evening conditions. A total sample size of 44 participants was selected to provide >80% statistical power to detect moderate within-subject effects (Cohen's $d \approx 0.5$ – 0.7) for the primary outcome measure, maximal voluntary isometric contraction (MVIC) torque. Previous studies examining sex-related differences in diurnal neuromuscular performance were also considered when planning recruitment^{34,35} to examine sex as a planned secondary factor in the analysis. Associations

between circadian phenotype variables and AM-PM differences in neuromuscular performance were examined across the whole cohort, with sex-stratified association analyses treated as exploratory.

Study Design

A randomised crossover design was employed (Figure 1A). Each participant attended the laboratory on 3 occasions: 1 familiarisation session, followed by 2 experimental trials performed in the morning (AM; 0700–0900) and evening (PM; 1700–1800). Trial order was randomised and counterbalanced using a computer-generated randomisation sequence. Experimental trials were separated by a minimum of 72 hours to minimise residual fatigue effects.

Before the first experimental trial, participants completed 7 consecutive days of wrist-worn actigraphy monitoring (Fitbit Inspire 3, Fitbit Inc.) and a validated chronotype questionnaire (Composite Morningness Questionnaire; CMQ) to

characterise habitual sleep-wake behaviour and circadian preference (Figure 1A). Actigraphy monitoring was maintained throughout the experimental period. In addition to fixed clock-time testing, habitual wake time and time elapsed since waking were recorded before each experimental session to provide additional context regarding circadian timing relative to individual sleep-wake behaviour.

Pre-Trial Standardisation

Participants were instructed to maintain habitual sleep-wake patterns throughout the study period. During the 24 hours preceding each experimental session, participants refrained from strenuous physical activity and alcohol consumption and avoided caffeine intake for at least 12 hours before testing. Participants were also instructed to replicate dietary intake, meal timing, and pre-exercise feeding patterns between conditions as closely as possible. Participants were instructed to obtain a minimum of 6 hours sleep before each experimental session. Wake time before each laboratory visit was obtained from actigraphy, and time elapsed since waking was calculated as the interval between the actigraphy derived wake time and the start of the corresponding experimental trial. Adherence was assessed using actigraphy-derived sleep duration for the night preceding each experimental trial.

Actigraphy and Chronotype Assessment

Habitual sleep-wake behaviour was assessed using wrist-worn actigraphy (Fitbit Inspire 3, Fitbit Inc.) over 7 consecutive days before the first experimental trial and throughout the experimental period. Actigraphy-derived variables included sleep duration, sleep onset and wake time, sleep midpoint, sleep efficiency, and night-to-night variability in sleep midpoint.³⁶⁻³⁸ Actigraphy-derived wake time on each experimental day was also used to determine time elapsed since waking before each trial. Sleep midpoint was calculated as the midpoint between sleep onset and wake time and averaged across the monitoring period as a behavioural proxy of circadian timing.³⁹⁻⁴¹ Sleep midpoint was additionally calculated separately for weekdays and weekends, and the weekend-weekday differences in sleep midpoint were determined to characterise intraweek variation in sleep timing. Variability in sleep midpoint across monitoring days was used as an index of sleep-wake regularity. Participants also completed the Composite Morningness Questionnaire (CMQ)⁴² to assess circadian preference (Figure 1C). Chronotype score, overall, weekday and weekend sleep midpoint, weekend-weekday variation in sleep midpoint, and sleep-wake regularity variables were included in exploratory analyses examining associations with AM-PM differences in neuromuscular performance and fatigue outcomes.

Familiarisation Session

Participants attended a familiarisation session at least 72 hours before the first experimental trial. Anthropometric measurements were recorded, including height (Seca 213 stadiometer, Seca GmbH & Co. KG., Germany) and body mass (Seca GmbH & Co. KG., Hamburg, Germany). Dominant leg was identified using a validated question-based method.⁴³ Participants then completed a standardised warm-up consisting of 5 minutes cycling on a stationary ergometer (Keiser M3i, Keiser Corporation) at 80 ± 10 W and 90 r/min, followed by familiarisation with all neuromuscular testing procedures on the isokinetic dynamometer. Participants performed both submaximal and maximal practice contractions to minimise learning effects and establish reliable performance measures before experimental testing.

Neuromuscular Testing

Neuromuscular performance was assessed using an isokinetic dynamometer (HUMAC2015, Version 15.000.0236; CSMi, Stoughton, MA), calibrated before each testing session. Participants were seated with the dominant leg secured to the dynamometer lever arm, with the lateral femoral epicondyle aligned to the axis of rotation. The lower leg was secured above the malleoli using a padded Velcro strap, and the arms were crossed over the chest to minimise extraneous movement. Participant positioning was standardised and replicated across all visits. Following completion of the standardised warm-up, tympanic temperature (Braun ThermoScan IRT 4520, Braun GmbH, Germany) and resting heart rate were measured immediately before neuromuscular testing under standardised conditions. Participants first completed maximal voluntary isometric contractions (MVICs) of the knee extensors at 90° knee flexion. Each contraction consisted of a 5-s maximal effort separated by 2-minute recovery. Three maximal contractions were performed, with the highest peak torque value retained for analysis. Standardised verbal encouragement and visual feedback were provided throughout testing (Figure 1B).

Following baseline MVIC assessment, participants completed a fatiguing protocol adapted from Pethick et al, consisting of repeated 6-second maximal isometric contractions interspersed with 4-second passive recovery periods. The protocol continued until torque output declined to 50% of baseline MVIC or task failure occurred. Immediately following completion of the fatigue protocol, post-fatigue MVICs were performed to assess reductions in maximal force-generating capacity (Figure 1B).

Experimental Outcomes

The primary outcome measure was pre-fatigue MVIC peak torque. Secondary outcomes included post-fatigue MVIC

Table 1. Participant Characteristics and Habitual Sleep Behaviours.

Variable	Total cohort	Male	Female
<i>n</i>	44	21	23
Age (y)	26.25 ± 4.49	26.67 ± 4.32	25.87 ± 4.70
Height (cm)	174.04 ± 9.16	180.24 ± 7.95	168.37 ± 6.02
Body mass (kg)	69.38 ± 9.66	75.05 ± 9.02	64.21 ± 7.05
CMQ score	32.07 ± 11.79	31.67 ± 11.57	32.43 ± 12.23
Sleep midpoint (h)	3.50 ± 0.70	3.00 ± 0.50	3.95 ± 0.53
Habitual sleep duration (h · night ⁻¹)	6.85 ± 0.23	6.82 ± 0.23	6.88 ± 0.22

Data are presented as mean ± SD unless otherwise stated. CMQ, Composite Morningness Questionnaire. Higher CMQ scores indicate greater morning preference. Participants were classified as evening-type (<22), intermediate-type (23–43), or morning-type (>44) based on established CMQ thresholds. Sleep midpoint was calculated as the midpoint between habitual sleep onset and wake time derived from 7-day actigraphy monitoring.

torque, fatigue index, number of contractions completed, time to exhaustion, rating of perceived exertion (RPE),⁴⁴ resting heart rate, and tympanic temperature. Peak torque values were normalised to body mass to facilitate comparison between participants. Fatigue index was calculated as the percentage decline in torque relative to baseline MVIC values.

Statistical Analysis

All statistical analyses were performed using GraphPad Prism (GraphPad Prism version 11.0.1, San Diego, CA). Data were assessed for normality using the Shapiro-Wilk test before analysis. Descriptive data are presented as mean ± standard deviation (SD). Statistical significance was accepted at $P < .05$. Baseline and post-fatigue maximal voluntary isometric contraction (MVIC) torque values were analysed using a 2-way repeated-measures ANOVA with time of day (AM vs. PM) and fatigue condition (Pre vs. Post) as within-subject factors. Where significant main effects or interactions were identified, Fisher's least significant difference (LSD) post hoc comparisons were performed. Within-participant differences in fatigue-induced torque loss (Δ torque), fatigue index, time-to-exhaustion, resting heart rate, pre-exercise tympanic temperature, rating of perceived exertion (RPE), and contractions completed between AM and PM trials were analysed using paired 2-tailed t tests. Associations between circadian phenotype variables, including Composite Morningness Questionnaire (CMQ) score, overall, weekday and weekend sleep midpoint, weekend-weekday difference in sleep midpoint, and sleep midpoint variability, and within-participant PM-AM differences in neuromuscular performance outcomes were explored using simple linear regression analyses. Associations between CMQ score and overall, weekday and weekend sleep midpoint, and the weekend-weekday difference in sleep midpoint were similarly examined using Pearson correlation and simple linear regression. Pearson correlation coefficients (r), coefficients of determination (R^2), regression equations, and 95% confidence intervals were calculated. Differences in sleep midpoint between

chronotype categories (morning, intermediate, and evening) were analysed using 1-way ANOVA with Tukey's post hoc multiple-comparisons testing where appropriate. To explore potential sex-related influences on diurnal performance responses, between-sex differences in PM-AM changes in torque (Δ torque), fatigue index, time-to-exhaustion, and CMQ score were analysed using unpaired Welch-corrected 2-tailed t tests. The association between CMQ score and the PM-AM difference in normalised MVIC torque was additionally examined separately in males and females using Pearson correlation and simple linear regression analyses, with regression slopes formally compared between sexes. The association between the between-trial difference in time elapsed since awakening and the PM-AM difference in normalised MVIC torque was examined using Pearson correlation and simple linear regression. To determine whether the association between CMQ score and diurnal variation in MVIC torque was independent of time elapsed since awakening, multiple linear regression was performed with PM-AM difference in normalised MVIC torque as the dependent variable and CMQ score and between-trial difference in time elapsed since awakening (PM-AM) as predictors.

Results

Participant characteristics and habitual sleep behaviours are summarised in Table 1. The cohort consisted of recreationally active young adults with a broad distribution of chronotype scores and habitual sleep timing patterns, enabling examination of associations between circadian phenotype and diurnal variation in neuromuscular performance. All participants maintained regular sleep-wake schedules throughout the monitoring period.

Chronotype classification identified representation across morning ($n=9$), intermediate ($n=22$), and evening ($n=13$) phenotypes, with intermediate chronotypes comprising the largest proportion of the cohort. Habitual sleep timing characteristics demonstrated broad overlap across chronotype categories, supporting subsequent analyses of circadian phenotype and neuromuscular performance outcomes. For each

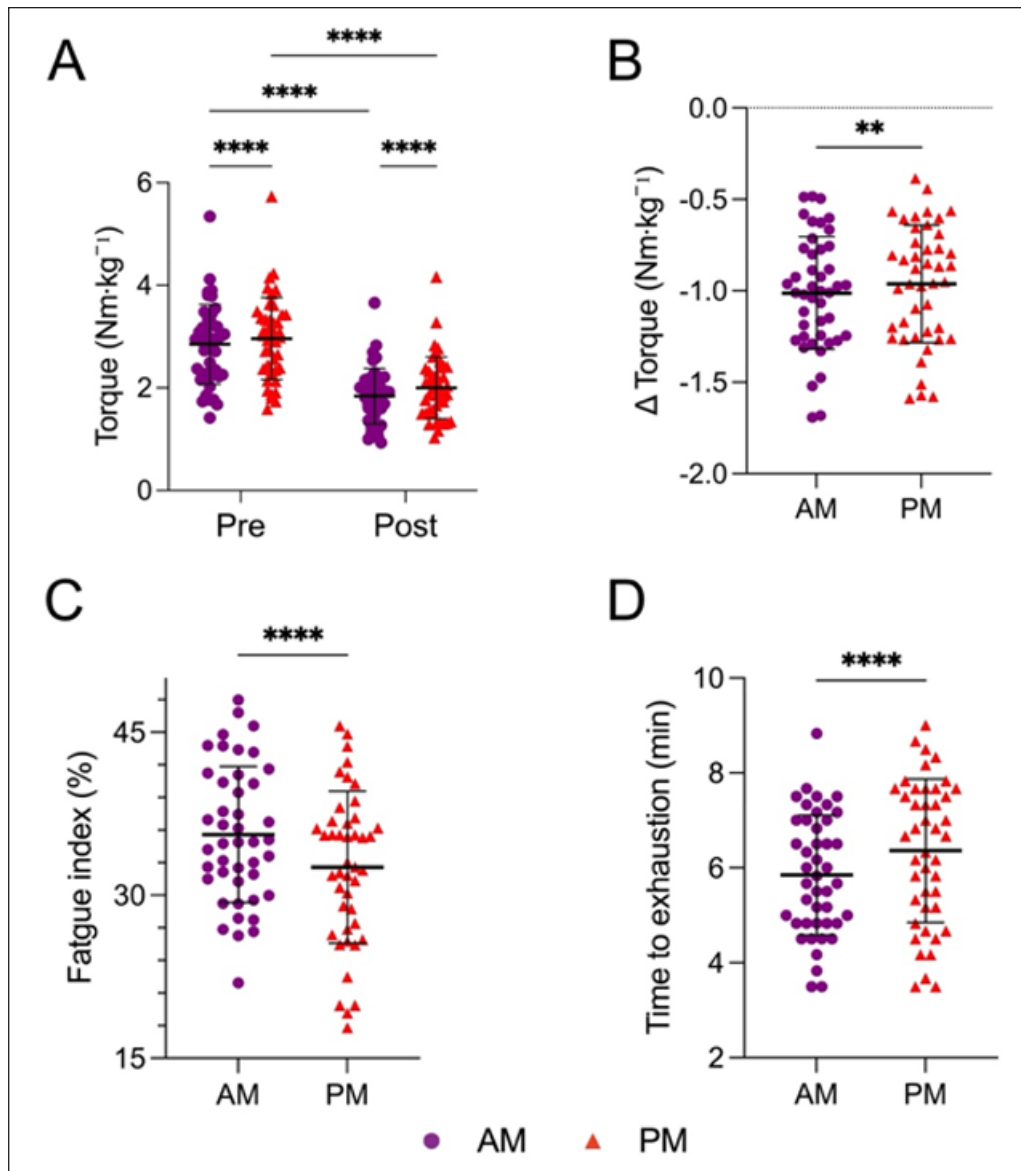


Figure 2. Diurnal variation in neuromuscular performance and fatigue responses. (A) Maximal voluntary isometric contraction (MVIC) torque measured before (Pre) and after (Post) the fatigue protocol during AM and PM trials. (B) Within-participant reduction in MVIC torque (Δ torque) following the fatigue protocol during AM and PM conditions. (C) Fatigue index during AM and PM trials. (D) Time-to-exhaustion during AM and PM trials. Data are presented as individual participant values with mean $\pm$ SD; AM condition represented as purple circles and PM condition represented by red triangles. Statistical comparisons were performed using 2-way repeated-measures ANOVA with Fisher's LSD post hoc testing (A) and paired 2-tailed *t* tests (B-D). ***P* < .01, *****P* < .0001.

experimental trial, prior night sleep duration was 7.20 ± 0.70 hours before the AM trial and 7.30 ± 0.77 hours before the PM trial (*P* = .493), with 100% (44/44) of participants meeting the ≥ 6 hours criterion.

Neuromuscular Performance and Fatigue Responses

Baseline MVIC torque was significantly greater during the PM trial compared with the AM trial (PM: 2.96 ± 0.80

Nm·kg⁻¹ vs. AM: 2.85 ± 0.77 Nm·kg⁻¹, *P* < .0001; Figure 2A). Following the fatigue protocol, MVIC torque significantly decreased in both conditions (main effect of fatigue, *P* < .0001), with post-fatigue torque remaining significantly greater during the PM trial compared with the AM trial (PM: 2.00 ± 0.60 Nm·kg⁻¹ vs. AM: 1.84 ± 0.54 Nm·kg⁻¹, *P* < .0001). No significant time-of-day $\times$ fatigue interaction was observed (*P* = .1103), indicating that the relative magnitude of fatigue-induced torque loss was broadly comparable between AM and PM trials.

Table 2. Diurnal Differences in Neuromuscular Performance and Fatigue-Related Outcomes.

Variable	AM	PM	PM-AM Δ	% Diff	95% CI	Effect size (η^2)	P value
Pre-fatigue MVIC (Nm)	197.53 $\pm$ 57.90	205.07 $\pm$ 59.07	7.54 $\pm$ 7.69	3.82	4.67 to 10.41	0.314	<.0001
Post-fatigue MVIC (Nm)	127.64 $\pm$ 40.00	138.51 $\pm$ 43.16	10.87 $\pm$ 11.14	8.52	7.99 to 13.74	0.314	<.0001
Pre-fatigue MVIC (Nm kg ⁻¹)	2.85 $\pm$ 0.77	2.96 $\pm$ 0.80	0.11 $\pm$ 0.12	3.86	0.07 to 0.15	0.342	<.0001
Post-fatigue MVIC (Nm kg ⁻¹)	1.84 $\pm$ 0.54	2.00 $\pm$ 0.60	0.16 $\pm$ 0.17	8.70	0.12 to 0.20	0.342	<.0001
Fatigue index (%)	35.58 $\pm$ 6.26	32.57 $\pm$ 6.99	-3.01 $\pm$ 4.18	-8.46	-4.28 to -1.74	0.348	<.0001
Time-to-exhaustion (min)	5.85 $\pm$ 1.26	6.36 $\pm$ 1.51	0.51 $\pm$ 0.64	8.72	0.32 to 0.71	0.395	<.0001
Resting heart rate (bpm)	65.05 $\pm$ 4.47	61.93 $\pm$ 3.35	-3.11 $\pm$ 1.26	-4.78	-3.50 to -2.73	0.862	<.0001
Pre-exercise tympanic temperature (°C)	36.37 $\pm$ 0.27	36.60 $\pm$ 0.21	0.23 $\pm$ 0.05	0.63	0.13 to 0.33	0.343	<.0001
Perceived exertion (CR10)	7.41 $\pm$ 0.95	7.07 $\pm$ 1.04	-0.34 $\pm$ 0.22	-4.60	-0.79 to 0.11	0.052	.133
Contractions completed (n)	35.09 $\pm$ 7.58	37.95 $\pm$ 8.29	2.86 $\pm$ 2.36	8.15	2.15 to 3.58	0.601	<.0001

Data are presented as mean $\pm$ SD. PM-AM Δ represents the within-participant difference between PM and AM conditions. Effect sizes are reported as partial eta squared (η^2). % Diff, percent difference. Tympanic temperature values represent measurements obtained following the standardised warm-up and immediately before baseline neuromuscular testing. Fatigue index (%) was calculated as ((pre-fatigue MVIC - post-fatigue MVIC) / pre-fatigue MVIC) $\times$ 100. MVIC, maximal voluntary isometric contraction. Statistical comparisons were performed using paired 2-tailed *t* tests. Statistical significance was accepted at *P* < .05.

Analysis of within-participant AM-PM differences nevertheless demonstrated a significantly smaller reduction in MVIC torque during the PM condition compared with the AM condition (Δ torque: -0.96 ± 0.32 vs. -1.01 ± 0.31 Nm kg⁻¹, *P* = .0051; Figure 2B). Fatigue index was significantly lower during the PM trial compared with the AM trial (PM: $32.57 \pm 6.99\%$ vs. AM: $35.58 \pm 6.26\%$, *P* < .0001; Figure 2C), indicating attenuated performance fatigability later in the day. Similarly, time-to-exhaustion was significantly greater during the PM trial compared with the AM trial (PM: 6.36 ± 1.51 minutes vs. AM: 5.85 ± 1.26 minutes, *P* < .0001; Figure 2D). Collectively, these findings demonstrate enhanced neuromuscular performance and fatigue resistance during the evening condition relative to the morning condition.

No significant AM-PM differences were observed for rating of perceived exertion (RPE) (*P* = .133). In contrast, participants completed significantly more contractions during the PM trial compared with the AM trial (*P* < .0001). Pre-exercise tympanic temperature was significantly higher, whereas resting heart rate was significantly lower, during the PM trial compared with the AM trial (both *P* < .0001; Table 2).

Circadian Phenotype and Interindividual Variability in Diurnal Torque Responses

A significant negative association was observed between CMQ score and the PM-AM difference in pre-fatigue normalised MVIC torque ($r = -.684$, $R^2 = .468$, *P* < .0001; Figure 3A). Individuals with lower CMQ scores (greater evening preference) demonstrated larger improvements in torque production during the PM trial relative to the AM trial, whereas participants with higher CMQ scores (greater morning preference) exhibited smaller diurnal differences.

In contrast, no significant association was observed between sleep midpoint and the PM-AM difference in normalised MVIC torque ($r = -.029$, $R^2 = .0009$, *P* = .848; Figure 3B). Sleep midpoint also did not significantly differ between chronotype categories (*P* = .6407; Figure 3C). Mean sleep midpoint values were 3.40 ± 0.66 hours for morning types, 3.60 ± 0.65 hours for intermediate types, and 3.39 ± 0.83 hours for evening types. Tukey's post hoc analyses confirmed no significant pairwise differences between groups (all *P* > .05). Similarly, sleep midpoint variability was not associated with the PM-AM difference in normalised MVIC torque ($r = -.055$, $R^2 = .003$, *P* = .721), suggesting that habitual night-to-night variability in sleep timing was not related to diurnal variation in neuromuscular performance.

Separating actigraphy-derived sleep midpoint according to weekday and weekend did not alter these findings. Neither weekday ($r = .093$, *P* = .550) nor weekend sleep midpoint ($r = .046$, *P* = .766) was associated with CMQ score, and neither weekday ($r = -.045$, *P* = .771) nor weekend sleep midpoint ($r = .013$, *P* = .936) was associated with the PM-AM differences in normalised MVIC torque. Weekend-weekday differences in sleep midpoint were similarly unrelated to CMQ score or diurnal MVIC variation (Supplementary Table 2).

Multiple linear regression demonstrated that CMQ score remained independently associated with the PM-AM difference in normalised MVIC torque after accounting for between-trial differences in time awake ($B = -0.00680 \pm 0.00104$, *P* = .0001). Difference in time awake was also independently associated with the response ($B = -0.255 \pm 0.091$, *P* = .0179). The overall model explained 53.7% of the variance in diurnal MVIC responses, $F(2, 41) = 23.75$, *P* < .0001, adjusted $R^2 = .514$ (Supplementary Table 3).

Substantial variability was observed in the magnitude of diurnal torque responses across participants (Figure 3D).

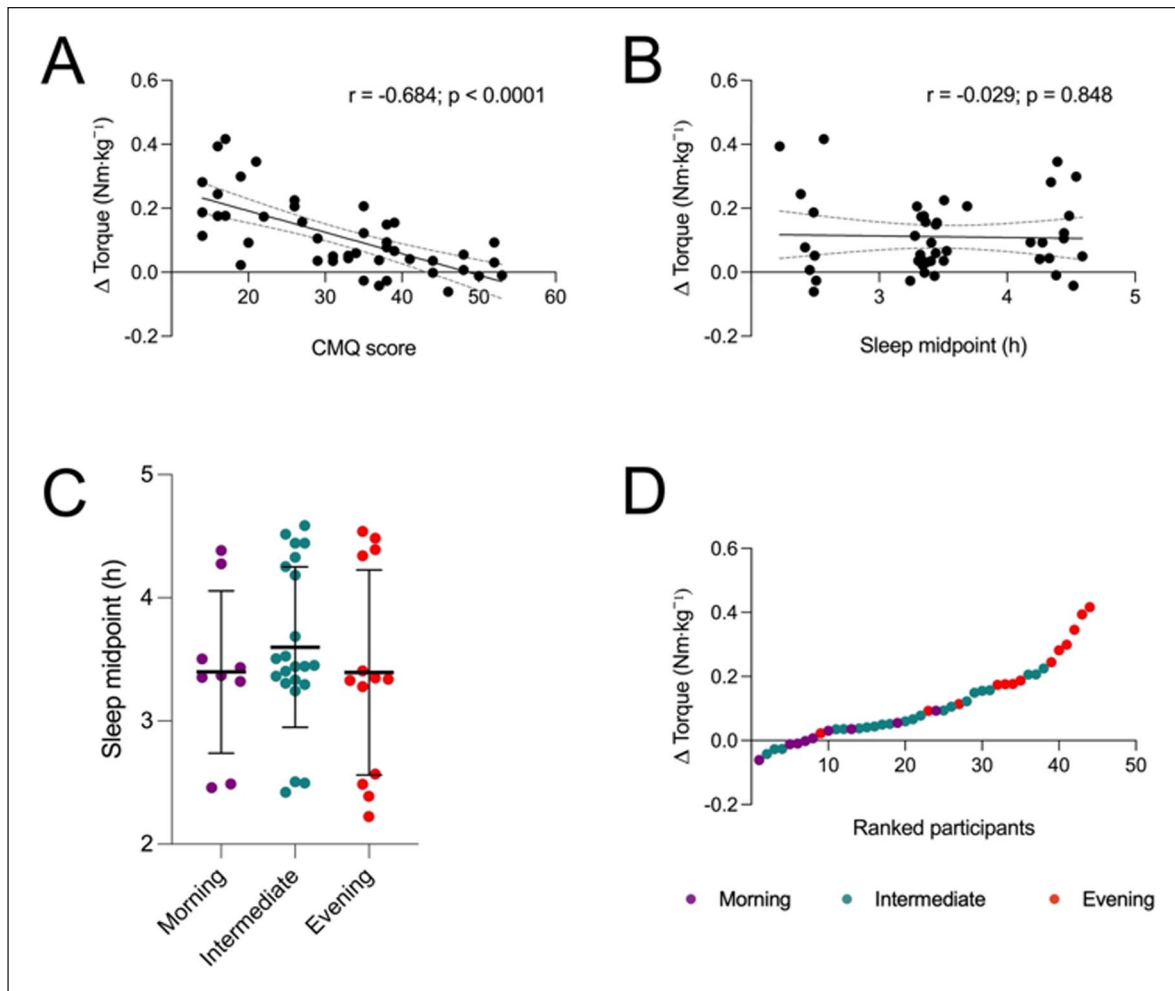


Figure 3. Associations between circadian phenotype and diurnal variation in neuromuscular performance. (A) Association between Composite Morningness Questionnaire (CMQ) score and the PM-AM difference in normalised maximal voluntary isometric contraction (MVIC) torque (Δ torque). Lower CMQ scores were associated with larger PM-associated increases in torque production. (B) Association between sleep midpoint and the PM-AM difference in normalised MVIC torque (Δ torque). (C) Sleep midpoint across chronotype categories (morning, intermediate, and evening). (D) Ranked individual PM-AM differences in normalised MVIC torque demonstrating substantial variability in time-of-day responsiveness across participants. Solid lines in panels A and B represent simple linear regression fits with dashed lines indicating 95% confidence intervals. Data in panel C are presented as individual values with mean $\pm$ SD.

Ranked PM-AM torque differences ranged from approximately -0.06 to $+0.42 \text{ Nm}\cdot\text{kg}^{-1}$, demonstrating marked heterogeneity in time-of-day responsiveness despite the overall group-level PM advantage. Collectively, these findings suggest that self-reported chronotype, as assessed by CMQ score, is more strongly associated with diurnal variation in neuromuscular performance independent of differences in time elapsed since waking and was not reproduced by actigraphy-derived measures of habitual sleep timing or sleep timing variability.

Table 2 summarises the primary neuromuscular performance and fatigue-related outcomes obtained during the AM and PM exercise trials. Participants demonstrated significantly greater baseline and post-fatigue torque production, lower fatigue index values, and longer time-to-exhaustion

during the PM condition compared with the AM condition, consistent with enhanced neuromuscular performance and fatigue resistance later in the day.

Sex-Related Influences on Diurnal Neuromuscular Responses

To explore potential sex-related influences on diurnal performance responses, AM-PM differences in neuromuscular outcomes were compared between male and female participants (Figure 4). No significant between-sex difference was observed for normalised torque responses (Δ torque; males: $0.12 \pm 0.13 \text{ Nm}\cdot\text{kg}^{-1}$ vs. females: $0.10 \pm 0.11 \text{ Nm}\cdot\text{kg}^{-1}$; $P = .4670$; Figure 4A). Similarly, PM-AM changes in fatigue index did not differ between sexes (males: $-3.08\% \pm 4.24\%$

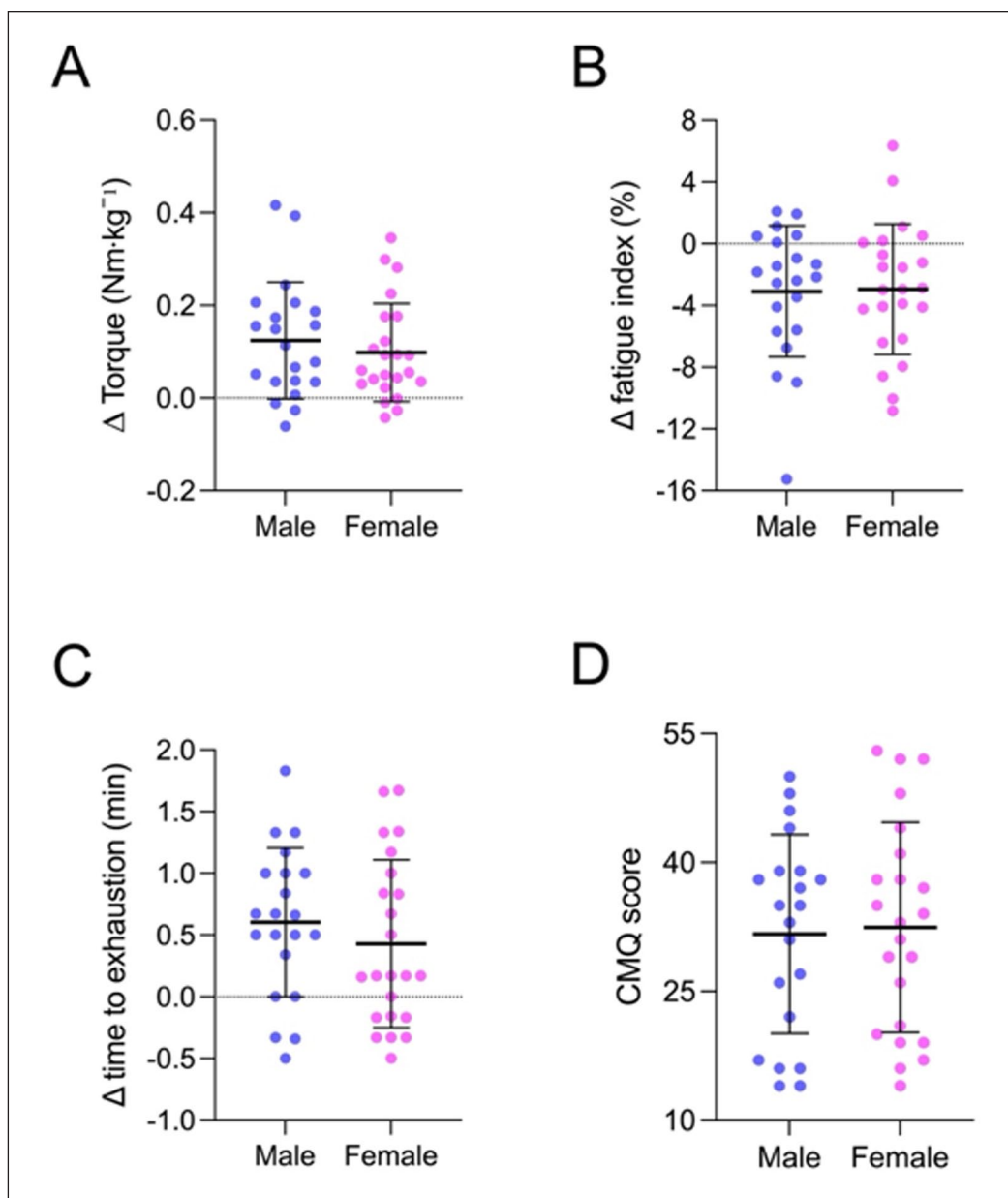


Figure 4. Sex-related influences on diurnal neuromuscular performance responses and chronotype characteristics. (A) Between-sex comparison of within-participant PM-AM differences in normalised maximal voluntary isometric contraction (MVIC) torque (Δ torque). (B) Between-sex comparison of PM-AM differences in fatigue index. (C) Between-sex comparison of PM-AM differences in time-to-exhaustion. (D) Composite Morningness Questionnaire (CMQ) scores in male and female participants. Positive values in panels A-C reflect greater PM-associated responses relative to the AM trial. Data are presented as individual participant values with mean $\pm$ SD. Statistical comparisons were performed using unpaired Welch-corrected 2-tailed *t* tests. No significant between-sex differences were observed for any variable (all *P* > .05).

vs. females: $-2.95\% \pm 4.22\%$; *P* = .9185; Figure 4B). No significant between-sex difference was identified for PM-AM changes in time-to-exhaustion (males: 0.60 ± 0.60 minutes vs. females: 0.43 ± 0.68 minutes; *P* = .3710; Figure 4C). Composite Morningness Questionnaire (CMQ) scores were also comparable between males and females (males: 31.67 ± 11.57 vs. females: 32.43 ± 12.23 ; *P* = .8316;

Figure 4D). Collectively, these findings suggest that sex did not meaningfully influence the magnitude of diurnal variation in neuromuscular performance or fatigue-related responses within the present cohort.

Sex-stratified analyses demonstrated that this relationship was evident in both males ($r = -.740$, 95% CI -0.888 to -0.454 , *P* = .0001) and females ($r = -.638$, 95% CI -0.831 to

-0.306 , $P=.0011$). The slopes of the regression relationships did not differ significantly between sexes, $F(1, 40)=1.309$, $P=.259$, providing no evidence that sex modified the association between CMQ score and diurnal variation in normalised MVIC torque (Supplementary Figure 1).

Discussion

This study demonstrates that maximal knee extensor strength and fatigue resistance exhibit clear time-of-day variation in healthy young adults, with neuromuscular performance consistently enhanced during evening compared with morning testing. Specifically, participants demonstrated greater baseline and post-fatigue MVIC torque, lower fatigue index values, longer time-to-exhaustion, and a greater number of contractions completed during the PM condition relative to the AM condition (Figure 2A-D; Table 2). These findings are consistent with previous reports demonstrating superior late-afternoon and evening exercise performance across a range of strength, power, and endurance tasks.^{10,30,45,46} Importantly, we extend this literature by demonstrating that behavioural circadian phenotype, assessed using the Composite Morningness Questionnaire, was strongly associated with the magnitude of the PM-associated increase in torque production (Figure 3A), whereas objectively derived sleep timing measures, including sleep midpoint and sleep midpoint variability, were not associated with diurnal performance responses (Figure 3B and C).

The observation that maximal force production and fatigue resistance were enhanced during the PM condition is consistent with previous studies demonstrating superior neuromuscular performance during the late afternoon and early evening.^{7,15} Racinais et al,⁴⁶ reported a $\sim 3\%$ to 6% increase in maximal muscle force and contractile performance later in the day, while Sedliak et al,³² demonstrated greater maximal strength capacity during evening compared with morning testing in resistance-trained men. Similarly, this study identified a $\sim 4\%$ increase in baseline MVIC torque during the PM condition, accompanied by lower fatigue index values and an $\sim 9\%$ longer time-to-exhaustion (Table 2), indicating that the evening advantage extended beyond maximal force production to include performance fatigability and the capacity to sustain repeated maximal contractions.

One frequently proposed explanation for superior late-afternoon and evening exercise performance is the circadian rise in body temperature.^{4,5} Under controlled laboratory conditions, core temperature typically increases by approximately 0.5 – 1.0 °C from the early morning to the late afternoon, coinciding with peak performance.^{5,9} This increase has been suggested to enhance neuromuscular performance through improvements in nerve conduction velocity, muscle compliance, cross-bridge cycling kinetics, and excitation-contraction coupling.^{5,12,47} Consistent with this hypothesis, pre-exercise tympanic temperature was significantly higher

during the PM trial in this study (36.60 ± 0.21 °C vs. 36.37 ± 0.27 °C; Table 2). However, the magnitude of this difference (~ 0.23 °C) was substantially smaller than the amplitude typically reported for circadian variation in core temperature,^{5,47} suggesting that thermal factors alone are unlikely to fully explain diurnal variation in neuromuscular performance. Importantly, tympanic temperature was measured following the same standardised warm-up in both trials, which may have attenuated thermal differences between conditions. Previous work has similarly demonstrated that experimentally raising morning body temperature to evening levels does not abolish the diurnal difference in muscle force production,⁴⁸ further suggesting that temperature alone cannot explain the observed time-of-day effect.

The persistence of time-of-day differences in performance despite relatively modest thermal changes has led to increasing interest in alternative physiological mechanisms. Previous studies have proposed contributions from central neural drive, motor unit recruitment, muscle metabolic status, hormonal milieu, and intrinsic variation in skeletal muscle contractile function across the day.^{12,25,26,49–51} While this study was not designed to directly evaluate these mechanisms, several observations suggest that the evening advantage was not simply a consequence of heightened physiological activation. Resting heart rate was lower during the PM condition despite greater subsequent force production and fatigue resistance (61.93 ± 3.35 vs 65.05 ± 4.47 bpm; Table 2), arguing against a simple explanation based on increased arousal. Experimental studies in both humans and animal models have demonstrated that exercise capacity, skeletal muscle metabolism, and exercise-responsive signalling pathways exhibit marked time-of-day dependence.^{25–27} Although the present findings cannot identify the specific physiological mechanisms responsible, they are consistent with the view that daily variation in neuromuscular performance arises from the interaction of multiple thermal, neural, metabolic, and circadian processes rather than a single explanatory factor.

A key finding of this study was the strong association between behavioural circadian phenotype and the magnitude of the diurnal difference in neuromuscular performance. Lower CMQ scores, indicative of greater evening preference, were associated with larger PM-associated increases in torque production, with chronotype score explaining approximately 47% of the variance in PM-AM torque responses (Figure 3A). This observation is consistent with previous work by Facer-Childs and Brandstaetter,²⁰ who reported that athletes with later chronotypes exhibited greater evening exercise capacity and delayed peak performance times relative to earlier chronotypes. Similarly, Facer-Childs et al,¹⁹ demonstrated that both physical and cognitive performance are influenced by circadian phenotype and time since awakening. However, these previous studies primarily examined whole-body exercise performance, whereas this study

demonstrates a comparable relationship using an isolated neuromuscular model. This suggests that associations between circadian phenotype and time-of-day performance may extend beyond complex behavioural or sport-specific tasks and be evident during isolated neuromuscular performance testing. Collectively, these findings support the concept that optimal neuromuscular performance may occur when testing is performed at a time more closely aligned with an individual's preferred timing of activity and wakefulness.

In contrast to the chronotype findings, neither habitual sleep midpoint nor sleep midpoint variability was associated with the magnitude of the PM-AM difference in torque production (Figure 3B and C). Importantly, this absence of association persisted when sleep midpoint was examined separately for weekdays and weekends and when the weekend-weekday difference was considered (Supplementary Table 2). This observation is noteworthy given that sleep midpoint is frequently used as a behavioural proxy of circadian timing and has been widely adopted within chronobiology research as an indicator of phase preference. The absence of an association may appear inconsistent with the relationship observed between CMQ score and neuromuscular performance. However, chronotype questionnaires and sleep timing metrics capture related, but not identical, aspects of circadian behaviour. Whereas sleep midpoint reflects when an individual typically sleeps, chronotype questionnaires incorporate broader dimensions of behavioural preference, including preferred timing of activity, alertness, and performance across the day. Consequently, it is plausible that behavioural preference may be more closely related to time-of-day variation in neuromuscular performance than sleep timing behaviour alone.

An alternative, although more speculative, interpretation is that behavioural sleep timing may incompletely reflect the biological and behavioural factors that influence exercise performance across the day. Individuals with similar habitual sleep schedules may nevertheless differ in their preferred timing of activity, exposure to social or occupational constraints, time since awakening, and the degree to which daily behaviours are aligned with endogenous circadian physiology. However, the association between CMQ score and PM-AM torque remained significant after accounting for time since awakening (Supplementary Table 3). This is consistent with previous work demonstrating that chronotype and sleep timing are related but distinct constructs and may not always provide equivalent information regarding circadian organisation.^{18-20,51-54} Importantly, the present findings should not be interpreted as evidence that sleep timing is unimportant for exercise performance. Rather, they suggest that commonly used behavioural measures may differ in their ability to capture factors relevant to diurnal variation in neuromuscular function. Future studies incorporating physiological markers of circadian phase alongside behavioural assessments will be required to determine whether chronotype, sleep timing, or

other indices of circadian alignment provide the most informative predictors of time-of-day differences in exercise performance.

The present findings also have implications for the design and interpretation of exercise physiology research. Time of day is increasingly recognised as an important source of biological variation,^{51,52} yet many studies continue to assess neuromuscular performance at a single clock time without considering individual circadian characteristics. The observed differences in strength and fatigue-related outcomes between AM and PM testing conditions highlight the importance of standardising testing time and reporting relevant circadian variables when evaluating physical performance. Furthermore, the association between chronotype and diurnal variation in torque production suggests that behavioural circadian phenotype may represent an additional factor worthy of consideration when comparing individuals or interpreting responses to exercise interventions.

No significant between-sex differences were observed in the magnitude of diurnal variation in torque production, fatigue index, time-to-exhaustion, or chronotype score within the present cohort (Figure 4). Furthermore, the association between CMQ score and the PM-AM difference in normalised MVIC torque was evident in both males and females, with no significant difference between regression slopes, providing no evidence that sex modified this relationship (Supplementary Figure 1). These findings suggest that the relative enhancement in evening neuromuscular performance was broadly comparable between males and females under the controlled conditions employed in this study. Although sex-related differences in skeletal muscle physiology, endocrine regulation, and sleep-wake behaviour have been reported previously,^{28,55} evidence regarding sex-specific time-of-day effects on exercise performance remains limited and inconsistent. Importantly, females continue to be under-represented in both circadian and exercise physiology research, particularly in studies incorporating chronotype assessment alongside neuromuscular performance measures. While the present findings provide preliminary evidence that diurnal variation in neuromuscular performance may be broadly similar between sexes, the study was not specifically powered to detect sex-by-time-of-day interactions. Consequently, larger studies incorporating hormonal verification and physiological measures of circadian phase are required to determine whether sex influences the magnitude or timing of optimal neuromuscular performance.

Several limitations should be acknowledged. First, only 2 testing time points were examined, limiting assessment of the full temporal profile of neuromuscular performance across the day. Second, although chronotype questionnaires and actigraphy-derived sleep measures provide valuable behavioural information, physiological markers of circadian phase, such as dim-light melatonin onset or continuous core body temperature assessment, were not measured. Consequently, the present findings should be interpreted in

the context of behavioural circadian phenotype rather than endogenous circadian phase. Third, sleep characteristics were derived from commercially available wearable technology and associated proprietary algorithms, which may provide lower precision than research-grade actigraphy or polysomnography. Fourth, menstrual cycle phase was determined by self-report rather than hormonal verification. Finally, the cohort consisted of healthy young adults, and therefore the findings may not generalise to older individuals, elite athletes, clinical populations, or shift workers in whom circadian disruption and time-of-day effects may differ substantially.

The present findings also highlight important avenues for future research. While time-of-day differences in exercise performance are now well established,⁷ the physiological factors underpinning these responses remain incompletely understood. In particular, it remains unclear whether diurnal variation in performance is best explained by clock time, behavioural chronotype, endogenous circadian phase, circadian alignment, or interactions between these factors. The contrasting associations observed for chronotype and sleep timing suggest that behavioural markers may differ in their ability to capture biologically relevant circadian influences on exercise performance. Future studies should therefore integrate behavioural assessments with physiological measures of circadian timing, including dim-light melatonin onset and continuous temperature monitoring, to better characterise these relationships and provide a stronger evidence base for the emerging concept of chrono-exercise medicine.⁵⁶

In conclusion, maximal knee extensor strength and fatigue resistance were enhanced during evening compared with morning testing in healthy young adults. Furthermore, the magnitude of the PM-associated increase in torque production was strongly associated with behavioural circadian phenotype, whereas objectively derived sleep timing measures were not. These findings suggest that chronotype may be more closely associated with time-of-day differences in neuromuscular performance than sleep timing behaviour alone. Collectively, this study provides further evidence that circadian factors influence human neuromuscular performance and highlights behavioural circadian phenotype as a potentially important consideration when investigating time-of-day responses to exercise.

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Ethical Considerations

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of the School of Medicine and Dentistry, University of Lancashire.

Author Contributions

SJH conceived and designed the study. KDJ, MSL, RP, SSL, IJT, and SJH collected the data. SJH analysed the data and drafted the manuscript. KDJ, MSL, and SSL contributed to manuscript preparation and revision. All authors read and approved the final manuscript.

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Data Availability Statement

The data supporting the findings of this study are available in the Figshare repository, <https://doi.org/10.6084/m9.figshare.32630727>.

Supplemental Material

Supplementary material is available for this article online.

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