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Correlating diurnal variations in peak athleticism with buccal gene expression in youth football players

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ABSTRACT

Hourly variations in athletic performance are a well-documented physiological phenomenon in individual and team sports including football. Measuring the timing of maximal performance could potentially improve performance. Evidence suggests that buccal gene expression correlates with athletic performance, yet this has not been studied in sport-specific participants. Therefore, the study aimed to (A) examine the expression of *Per2* and *Bmal1* genes in 45 youth football players and correlate with countermovement jump (CMJ) performance; (B) investigate the fluctuations in *Per2* and *Bmal1* expression levels and CMJ performance at various timepoints during a regular training day. CMJ metrics (Reactive Strength Index-Modified, Force at Peak Power, Eccentric Duration, and Peak Power) and gene expression levels were measured in 45 youth football players at 08:30 and 17:30. Exercise timing made a significant difference in each measured performance metric at the individual and squad level. Thirty-four participants displayed increases in RSI-mod ($p < 0.001$) over the morning measurements. This contributed to an 8% increase in overall squad performance ($p = 0.0009$), which significantly correlated to buccal gene expression ($R^2 = 0.94$). Profiling player buccal gene expression could inform the timing of training and rehabilitation protocols to coincide with maximal athletic performance in football, other team sports, and individual sports.

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KEYWORDS
Football; gene expression; athletic performance; exercise timing; counter-movement jump

1. Introduction

Research across sports has shown that variations in athletic performance occur throughout the day (Ayala et al., 2021; Pradhan et al., 2024). In both male and female football players, for example, performances in sprint drills, a cardiovascular

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endurance test (BLEEP), and skills/accuracy tests fluctuated by almost 15% when performed at different times of the day (E. Facer-Childs & Brandstaetter, 2015b). Another study showed even greater effects (E. R. Facer-Childs et al., 2018), but for alternative performance measures. Therefore, the timing of peak athletic performance is an important consideration in performance maximisation. From a practical perspective, athletes and coaches should be aware of the potentially significant effects of the time-of-day variation in performance (Kunorozva et al., 2014; Roy & Forest, 2018).

The timing of peak performance of an individual athlete is multifaceted (Souissi et al., 2022), possibly governed by the circadian rhythm and chronotype biology (Thun et al., 2015). The circadian rhythm dictates many aspects of human physiology including sleep-wake cycles, which manifest as an individual's chronotype; whether a morning or evening person (Ayyar & Sukumaran, 2021; Nobari et al., 2023). Several valid questionnaires are available to assess Chronotype, such as the Morning-Eveningness Questionnaire (MEQ) (Horne & Östberg, 1977). While useful, the questionnaires are subjective, relying on recall and the individual maintaining accurate sleep pattern records. Thus, a more objective measure is needed.

Gene expression levels have recently been shown to correlate with resting muscle tone and importantly, the timing of peak performance (Basti et al., 2021). The study measured *Per2* and *Bmal1* expression, which reportedly fluctuated throughout the day for all 10 participants. The level of *Per2* correlated with athletic performance as measured by the hand-grip strength (HST)-test (Basti et al., 2021). Despite being a robust study, the small sample size limits its generalisability, especially with sport-relevant subjects. Furthermore, the authors also measured countermovement jump (CMJ) as a performance metric, although no correlation with CMJ was reported. To replicate and extend this previous study, the present study used CMJ metrics, which have been significantly correlated with explosive actions such as jumping and sprinting, thus making it a vital measure for football players' overall athletic performance (Meylan & Malatesta, 2009).

The aims of the present study were to: (A) examine the expression of *Per2* and *Bmal1* in 45 youth football players alongside a CMJ without arm-swing; (2) investigate the fluctuations in *Per2* and *Bmal1* expression levels and CMJ performance at different times of a regular training day. The study hypothesis was that each player would show differences in athletic performance at different times of the day and the levels of *Per2* and *Bmal1* would correlate with these temporal changes in athletic performance.

2. Materials and methods

2.1. Study design

This cross-sectional study was conducted in the off-season, on day two of an international age-group camp. There was no training load or games before testing. To achieve significance ($p \leq 0.05$) with sufficient power (80%) to detect at least a correlation of 0.5, the minimum required sample size for this study was 29 (Bujang & Baharum, 2016).

2.2. Participants

Forty-five youth football players from a National Association Programme were involved in the study (average age; 14.8 ± 0.6 years, weight; 62.2 ± 8.7 kg and height; 1.73 ± 0.09 m), recruited during camp induction on 20th of August 2023. The study was independently approved by the Institutional Review Board at Cardiff Metropolitan University and the Chief Football Officer at the Football Association of Wales (approval number: Sta-9930). The study was conducted under the ethical standards of the Helsinki Declaration. The participant's parents/legal guardians signed an informed consent form before participation (Murtagh et al., 2020). Participants were free to withdraw without reason during the study.

Participants must be actively competing in age-level football, minimum of one year of consistent training, free from illness or injuries that affect performance, be able to complete standard fitness tests particularly counter movement jumps, available for all testing time points, willingness to provide informed consent and comply with the study protocol, Participants were excluded on the basis of participation only in recreational sports, presence of illness, musculoskeletal injuries, or other conditions that could impair performance or safety, use of medications or supplements that may affect athletic performance, significant irregularity in sleep patterns, current use of performance-enhancing drugs or excessive alcohol consumption, inability or unwillingness to comply with all study requirements, including testing sessions and data collection.

The participants were enrolled in age-grade academies of professional English Premier League and Championship teams and one layer was based in a La Liga team and participated in routine training at their respective clubs in the days leading up to testing. All players were rested the day before testing (camp induction). Participants were free from previous significant time-out injuries.

All data were anonymised before analysis. No dietary interventions were undertaken. The players had a predetermined schedule of daily activities that included mealtimes, training, and educational activities. All players followed the same schedule, which also included standardised bedtimes and wake times.

2.3. Counter movement jump

Data on CMJ without arm swing were collected for all participants within 30 minutes of 08:30 and 17:30. All participants were familiar with the jumping protocols, having completed jumps as part of regular National Team programme assessments and participating in several practice testing sessions. All jump tests were conducted at an indoor facility to avoid any variations in surface that might affect results. Commercially available force plates (Vald, Brisbane, Australia) were used for data collection, which has been validated for field-based testing (Lake et al., 2018). To standardise jump tests, participants were instructed to perform all attempts following previously validated protocols (Cormack et al., 2008). Following a standardised 2-minute warm-up routine, consisting of a variety of running patterns (jogging, high knees, skipping) and dynamic stretching (Chtourou et al., 2013), the participants then performed three practice jumps prior to measurement. Participants were

informed to self-select the jumping depth and to jump as high as possible. Three trials were performed with 20 seconds of rest between each. The best result was utilised for analysis. The players jumped after sample collection.

The chosen CMJ metrics were: Reactive Strength Index-modified (RSI-mod (metres/second, m/s)); a robust measure of jump performance (Vieira & Tufano, 2021) and explosive power, regarded as the "first sightline in all-purpose monitoring" (Suchomel et al., 2015); Force at Peak Power (FPP (Newtons, N)); Eccentric Duration (ED (milliseconds, ms)); a measure of eccentric performance and a highly sensitive indicator of readiness, strength, and power expression (Mike et al., 2017); and Peak Power (PP (Watts, W)), a highly reliable indicator of power during the contact phase (Warr et al., 2020), to correlate with absolute eccentric and concentric peak force and overall jump performance to indicate lower body strength (Bridgeman et al., 2018).

2.4. Biological samples

Participants were instructed to collect buccal samples at 08:30 and again at 17:30 using a sterile Isohelix swab (Cell Projects, United Kingdom (UK)), previously validated (Archer et al., 2016) for the non-invasive collection of nucleic acids by novice users for gene expression applications. The swab was rubbed on the inside of each cheek for 30 seconds, then placed in a 15 ml falcon tube. Participants were observed by trained practitioners to ensure the risk of contamination was eliminated. Samples were stored in ALLprotect® reagent (New England Biolabs, UK) to preserve sample quality at ambient temperature for seven days (Mohammad Najafi, 2014). All samples were processed within this time.

2.5. Molecular analysis

Total ribonucleic acid (RNA) was extracted using the Monarch® RNA Miniprep Kit (New England Biolabs, UK) for high-yield, quality RNA (Sharma et al., 2023). Samples were quantified by spectrophotometry using the Nanodrop (ThermoFisher Scientific, UK), a previously validated approach (García-Alegría et al., 2020). The expression levels of *Per2*, *Bmal1* and *GAPDH* were quantified using TaqMan probes (Rn01427704_m1, Hs00154147_m1 and Hs02786624_g1 all from ThermoFisher, UK) that have been validated for the specific determination of gene expression levels in human samples (Basti et al., 2021; Jiang et al., 2019). All TaqMan assays were run using the CFX Opus Real-time PCR system (BioRad, UK).

The PCR cycle threshold (Ct) values were determined using CFX Maestro Software 2.3 (Bio-Rad Laboratories, UK) widely used in gene expression studies (Robarts et al., 2022). The raw Ct values were used in downstream analysis. Gene expression levels were normalised as previously described (Basti et al., 2021; Livak & Schmittgen, 2001). Specifically, expression levels were initially normalised to *GAPDH* (ΔCT), followed by normalisation to the target gene's expression at the earliest time point (ΔΔCT). Relative quantification was calculated using the 2^−ΔΔCT method. One technical replicate was performed. The efficiency of real-time PCR was measured in a separate serial dilution experiment for each probe (data not shown). PCR efficiency was shown to be 90–100%, although this was not factored into the calculations.

2.6. Statistical data analysis

For each player, both gene expression and CMJ performance variables were transformed into binary values to indicate the direction of change between the morning (08:30) and evening (17:30) sessions. A value of + 1 represented an increase, while −1 denoted a decrease. These binary indicators were then used as inputs in machine learning models to evaluate how consistently directional shifts in performance and gene expression align. This approach emphasises pattern discovery and classification accuracy, rather than the prediction of raw values, and facilitates exploratory insights into potential synchrony between physiological and molecular rhythms.

The dataset was approached through two experimental scenarios. Firstly, a Gaussian Mixture Model (GMM) was employed for clustering to identify player sub-groups exhibiting similar performance characteristics over the morning measurements. The dataset was constructed, assigning a value of 1.0 for increases and −1.0 for decreases for each player. The resulting 45x4-sized dataset was then employed in a GMM clustering algorithm (Saranya et al., 2020). Given the presence of four athletic measures and two value options (1.0 or −1.0), a maximum of 16 potential clusters existed. To determine the optimal number of clusters, the silhouette score, a metric indicating how effectively samples are grouped with others that share similarities was utilised (Shahapure & Nicholas, 2020). The second experimental case involved regression analysis.

Three machine-learning models XGBoost (XGB), Multilayer Perceptron (MLP) and Random Forests (RF) were trained using the genetic measurements to reflect observed athletic performance changes. The machine learning models (XGBoost, multilayer perceptron (MLP), and Random Forest) were selected to represent diverse algorithmic strategies. XGBoost (boosted trees) was chosen for its efficiency and ability to handle non-linear relationships, MLP (neural network) for its representational flexibility, and Random Forest (ensemble learning) for its robustness through ensemble learning. This allowed us to assess which types of patterns were consistently detected across methods, rather than aiming to select a single "best" model. These results may also inform future applications in sports science research, where larger datasets could support exploration of more advanced ML or AI techniques to further enhance predictive power.

The training process utilised the leave-one-out cross-validation (LOOCV) technique (Cheng et al., 2017). The average training coefficient of determination (R^2) value was recorded to establish a correlation between the molecular analysis modelled against each performance metric.

2.7. Main effect analysis

To assess the main effect of time on each of the measured metrics, squad averages were determined for each performance metric, at each timepoint or separated into directions of morning-to-evening performance change (increase/decrease), and statistical significance determined by a two-tailed or one-tailed paired T-test, respectively using SPSS version 28 (IBM Corp. Released 2021. IBM SPSS Statistics for Windows, Version 28.0. Armonk, NY: IBM Corp). Where $p \leq 0.05$, the difference was deemed significant.

3. Results

3.1. Temporal changes in measured performance metrics

3.1.1. RSI-mod performance

Thirty-four players displayed increases in RSI-mod ($p = 0.7 \times 10^{-6}$), 10 players showed decreases ($p = 0.001$) from the morning measurements, and one player's measurement remained unchanged at 0.52 m/s (Figure 1(a)). One player showed a 41.9% increase in RSI-mod from 0.31 m/s to 0.44 m/s, conversely, another player showed a 16.7% decrease from 0.60 m/s to 0.50 m/s over the morning measurements.

3.1.2. FPP performance

Decreases in FPP were seen in seven players ($p = 0.009$). The largest decrease was 11.1% (from 1383 N to 1229 N) in morning to evening FPP measurements (Figure 1(b)). Conversely, 37 players showed increased FPP in the evening ($p < 0.001$). The largest increase was 18.3% from 1342 N to 1587 N in the evening. One player showed no difference from the morning measurements, remaining at 1219 N.

3.1.3. ED-performance

ED decreased for 27 players in the evening ($p = 0.002$) (Figure 1(c)). The largest decrease was 26.7%, from 832 to 610 ms. In contrast, 18 players had increased ED over the morning performances ($p = 0.0001$). The largest morning-to-evening increase was 38.7%; from 460 ms to 638 ms.

3.1.4. PP-performance

Twelve players showed a decrease in PP in the evening ($p = 0.02$) (Figure 1(d)). The largest morning-to-evening decrease was 39.2% from 2961 W to 1800 W. Conversely, 33/45 players showed increased PP ($p < 0.001$). The largest morning-to-evening increase in PP was 29.9% (from 3797 W to 4931 W).

3.2. Temporal changes in player performance

3.2.1. RSI-mod

Twenty players showed at least a 10% change in RSI-mod over the morning measurements (S1A Fig). Morning to evening changes in RSI-mod showed the third-highest range compared to the other performance metrics. Twenty-two players showed 1.0–13.5% increases over the morning timepoint with two players showing even greater change (S2 Fig).

3.2.2. ED

Nineteen players showed a change of at least 10% over morning ED performances (S1B Fig). Changes in morning to evening ED performances showed the second largest range of 65.4% of the metrics measured. Twenty-two players displayed a change between −8.4% and 5.8% over the morning ED measurements (S2 Fig).

3.2.3. FPP

Seven players showed at least a 10% absolute change in FPP (S1C Fig). FPP was the least variable affected by time of day of the four metrics measured amongst the 45 players with

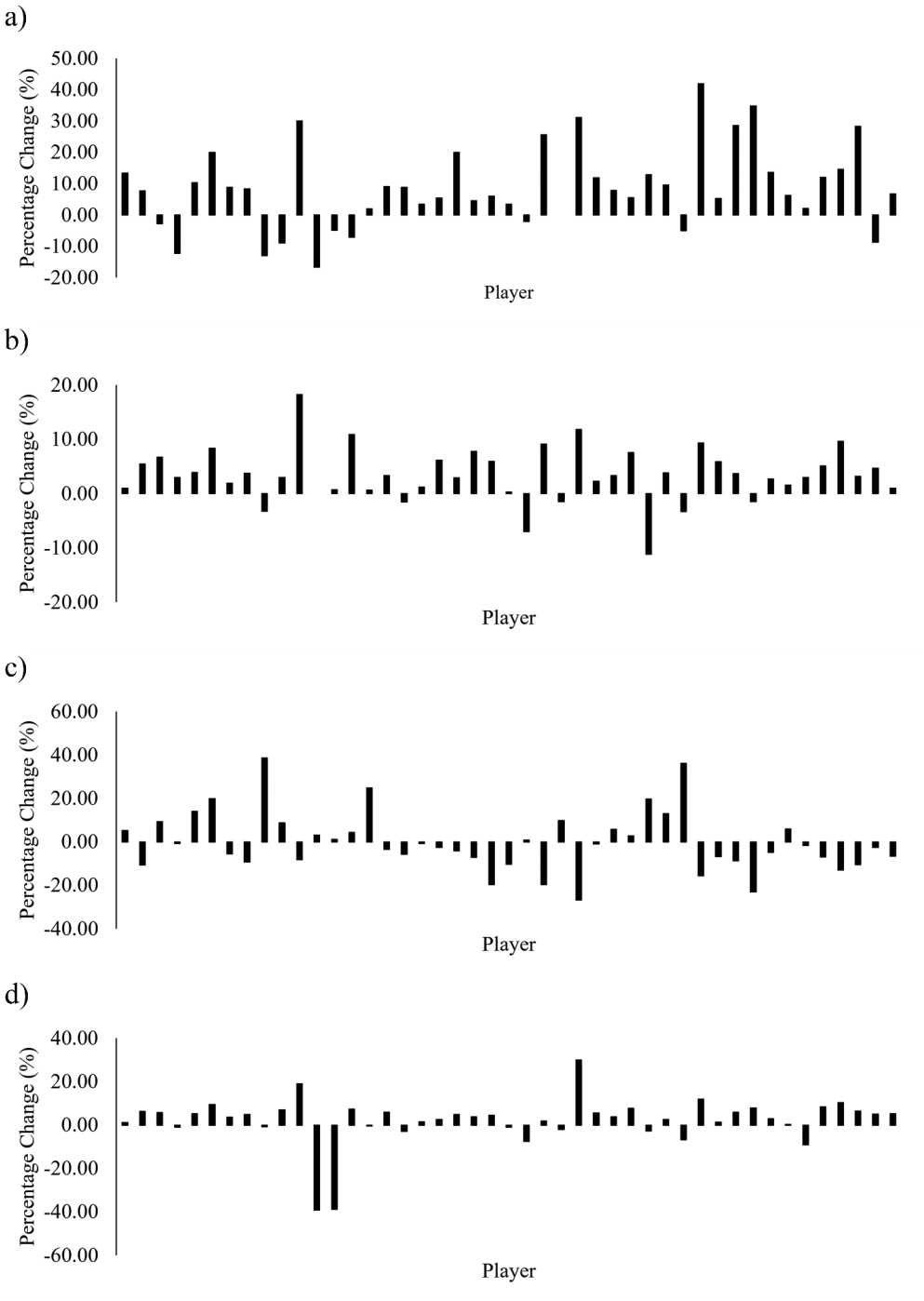

Figure 1. Variation in performance metrics (%) from morning to evening. a) reactive strength index-modified. b) force at peak power. c) eccentric Duration. d) peak power $n = 45$.

a difference of 29.4% from the largest decrease to the largest increase over the morning measurements. Twenty-two players lay between 0.84% and 6% increase in FPP over the morning timepoints (S2 Fig).

3.2.4. PP

At least a 10% absolute change in PP was observed for eight players (S1D Fig). Twenty-two players fell between the range of −0.6% and 6.3% with two positive and one negative outlier (38.8% decrease) (S2 fig).

3.3. Temporal changes in squad performance

3.3.1. RSI-mod

The squad average increased from 0.48 to 0.52 m/s ($p = 0.0009$) in the evening (Figure 2(a)).

3.3.2. FPP

The squad average increased from 1307 to 1349 N ($p = 0.0002$) from morning to evening (Figure 2(b)).

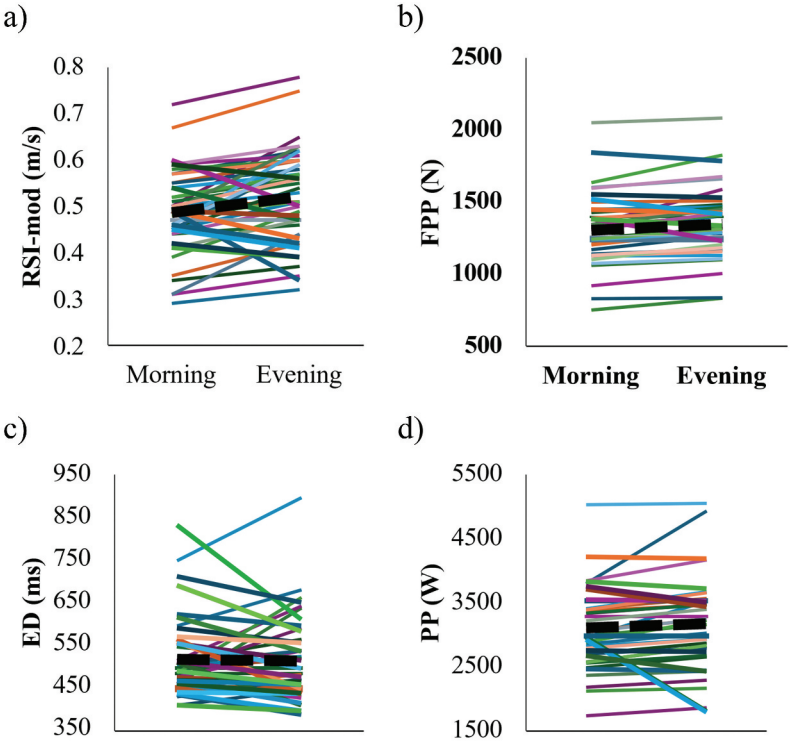

Figure 2. Metric-specific changes across the entire squad. Reactive strength index-modified (RSI-mod). Eccentric Duration (ED). Force at peak power (FPP). Peak power (PP).

3.3.3. ED

The squad average decreased from 518 to 513 ms ($p = 0.069$) from morning to evening (Figure 2(c)).

3.3.4. PP

The squad average increased from 3106 to 3173 W ($p = 0.19$) from morning to evening (Figure 2(d)).

3.4. Performance metrics; cluster analysis

Morning to evening increases in RSI-mod, FPP and PP was accompanied by a decrease in ED, the most common performance change profile observed in 21 of 45 players (Figure 3(a) and S3A Fig). Seven players exhibited increases in all metrics from morning to evening (Figure 3(b) and S3B Fig), five players only increased ED (Figure 3(c) and S3C Fig), and three players showed a decrease in only RSI-mod (Figure 3(d) and S3D Fig). Two players displayed a decrease in ED and PP and increases in RSI-mod and FPP (Figure 3(e) and S3E Fig).

Seven unique profiles of performance changes were observed amongst the players (S3F-L Fig). The players can be most optimally divided into 12 clusters based on the changes in performance profiles, given the highest silhouette score of 0.82 (Table 1), the features of each cluster are described (Table 2).

Fold change in gene expression is varied across the 45 players (Figure 4). Nineteen players exhibited increases in *Per2* and decreases in *Bmal1* over the morning levels. Incidentally, only one player exhibited the opposite. Ten players exhibited decreases, and five players exhibited increases in both genes over the morning measurements. A maximum of four clusters can be obtained, but the numbers of players within each cluster were similar to the top four performance metrics clusters.

3.5. Modelling gene expression against each performance metric

RSI-mod. XGB gave the highest R^2 value for RSI-mod of 0.94 (Table 3). Owing to this, R^2 values for RSI-mod were the second highest for correlation with gene-normalised Ct values across the 45 players for all three methods of modelling.

3.5.1. FPP

XGB and MLP both gave an R^2 value of 0.99 for FPP (Table 3). All three methods of R^2 analysis revealed that FPP was the highest correlating performance metric.

3.5.2. ED

XGB and MLP both gave an R^2 value of 0.93 for ED (Table 3). All three methods of R^2 analysis revealed that ED was the third highest correlating performance metric, as for RSI-mod.

3.5.3. PP

XGB gave an R^2 value of 0.85 for PP (Table 3). All three models scored PP as the lowest-ranked correlation performance metric.

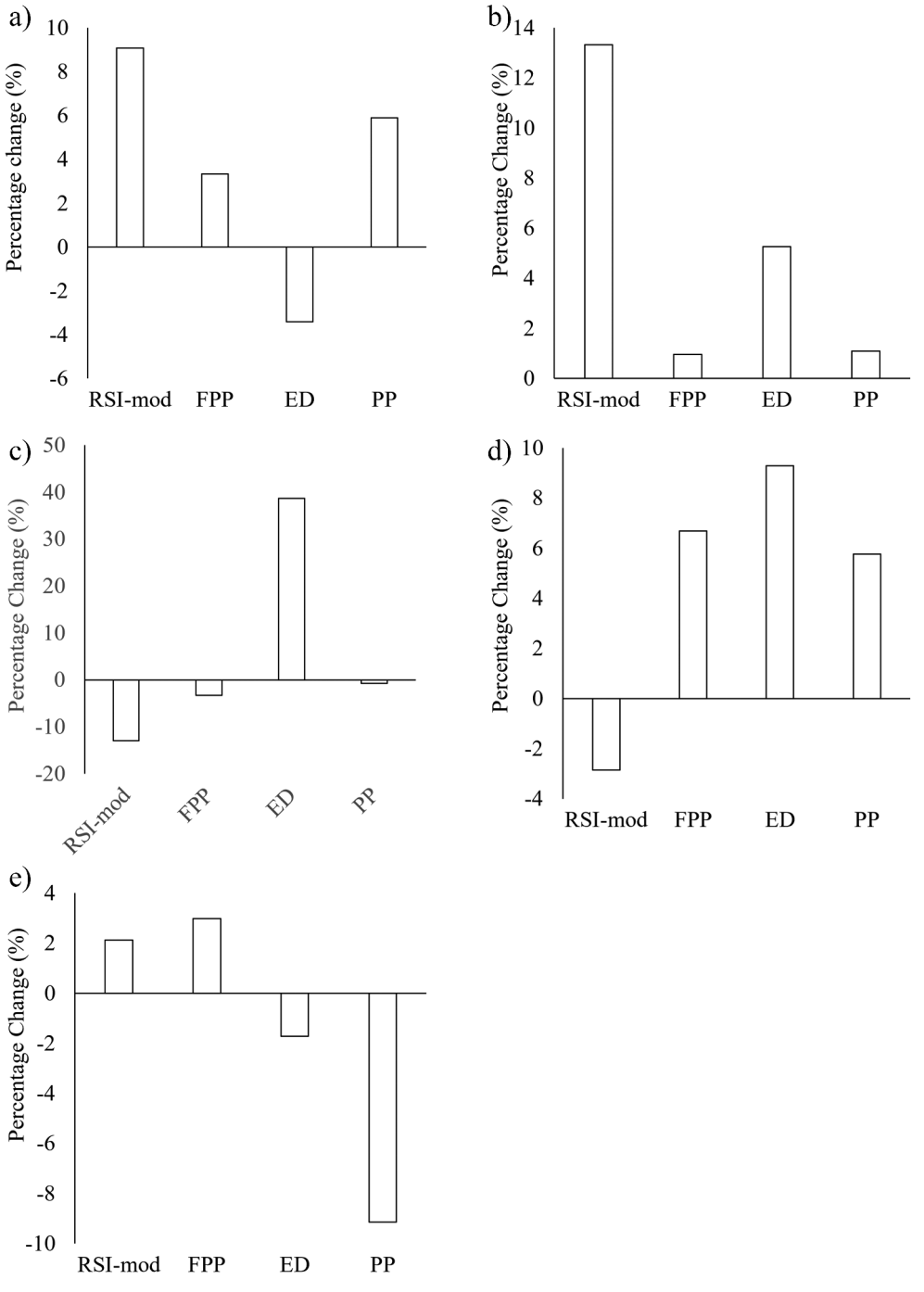

Figure 3. Representative profiles illustrating the predominant patterns of morning-to-evening performance changes. Eccentric Duration (ED). Reactive strength index-modified (RSI-mod). Peak power (PP). Force at peak power (FPP).

Table 1. Silhouette score for each of the different clusters.Twelve clusters were identified as the optimal number, achieving a silhouette score of 0.82 (in bold).

Number of Clusters	Silhouette Score
2	0.520
3	0.593
4	0.631
5	0.678
6	0.734
7	0.748
8	0.726
9	0.773
10	0.774
11	0.781
12	**0.822**
13	0.811
14	0.800
15	0.800
16	0.800

Table 2. Cluster characteristics derived using a Gaussian mixture model (GMM) in each performance metric when measured in the evening relative to the morning baseline. The directionality of change is indicated as ↑ (increase), ↓ (decrease), or 0 (no change).

Cluster number	Number of players	RSI-mod	FPP	ED	PP	Study Figure
1	21	↑	↑	↓	↑	Fig. 3(a) and Suppl. Fig. S2A
2	7	↑	↑	↑	↑	Fig. 3(b) and Suppl. Fig. S2B
3	5	↓ **or (0)**	↓ **or (0)**	↑	↓	Fig. 3(c) and Suppl. Fig. S2C
4	3	↓	↑	↑	↑	Fig. 3(d) and Suppl. Fig. S2D
5	2	↑	↑	↓	↓	Fig. 3(e) and Suppl. Fig. S2E
6	1	↑	↑	↑	↓	Suppl. Fig. S2F
7	1	↓	↑	↑	↓	Suppl. Fig. S2G
8	1	↑	↓	↓	↑	Suppl. Fig. S2H
9	1	↑	↓	↓	↓	Suppl. Fig. S2I
10	1	↓	↑	↓	↑	Suppl. Fig. S2J
11	1	↓	↑	↓	↓	Suppl. Fig. S2K
12	1	↑	↓	↑	↓	Suppl. Fig. S2L

3.6. Relative importance of Per2 and Bmal1 in modelling against each performance metric

Relative importance obtained by an XGB model showed that *Per2* was higher than that of *Bmal1* for all performance metrics (Figure 5). Gain values for *Per2* and *Bmal1* were 84 and 65 (RSI-mod), 128 and 110 (FPP), 121 and 99 (ED) and 120 and 110 (PP). In modelling capability, *Per2* expression levels had a higher influence on athletic performance.

4. Discussion

The study aimed to (A) examine the expression of *Per2* and *Bmal1* in 45 youth football players alongside a CMJ without arm-swing; (B) investigate the fluctuations in *Per2* and *Bmal1* expression levels and CMJ performance at different times of a regular training day.

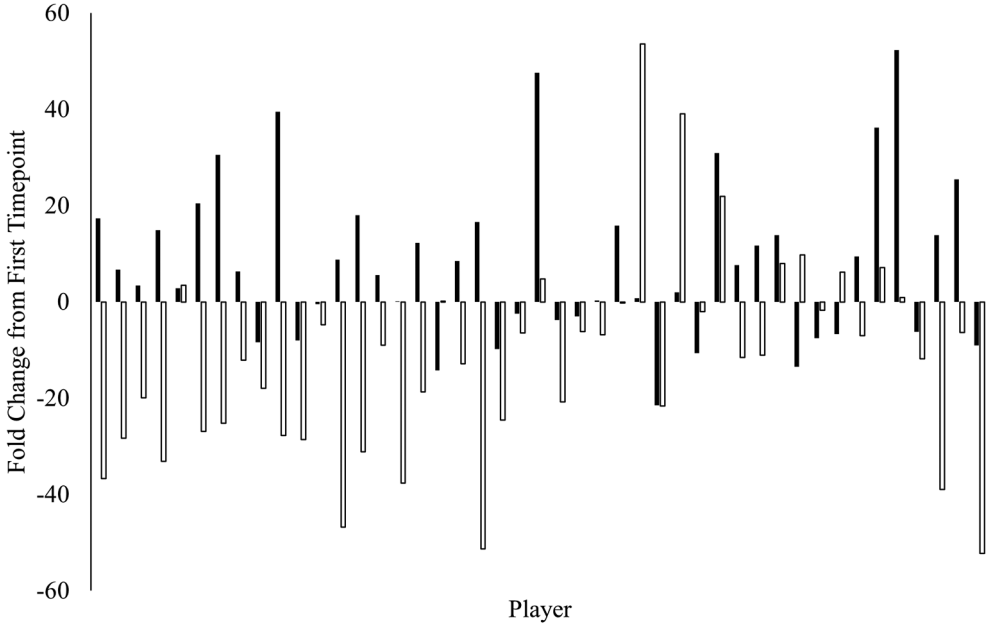

Figure 4. Evening expression levels of *Per2* (black) and *Bmal1* (white) shown as fold changes relative to morning baseline values. $n = 45$.

Table 3. The coefficient of determination (R^2) for each of the measured performance metrics as modelled against our molecular analysis. (XGBoost (XBG). Multilayer perceptron (MLP). Random Forest (RF). $n = 45$.

	R^2			
Performance metric	XGB	MLP	RF	RANK
RSI-modified [m/s]	0.94	0.93	0.79	2
Force at Peak Power [N]	0.99	0.99	0.81	1
Eccentric Duration [ms]	0.93	0.93	0.79	3
Peak Power [W]	0.86	0.85	0.71	4

The main findings were that RSI-mod, FPP, ED, and PP showed diurnal variations at the individual and squad levels. This was accompanied by changes in the expression of *Per2* and *Bmal1*, respectively, which were shown to correlate with performance.

4.1. All athletic performance measurements displayed temporal differences

Performance changes were not equal amongst the participants. Several participants showed substantial differences, which contributed to the overall changes observed throughout the squad. It has been suggested that diurnal changes in CMJ performance may be negated by dynamic stretches (Chtourou et al., 2013). The authors observed improvements in jump height over morning measurements of 20 football players. The study reports that the difference was removed if each player stretched before the morning squat- and CMJs. This is in contrast to another study reporting that afternoon improvements in cyclists' time trial improvements over

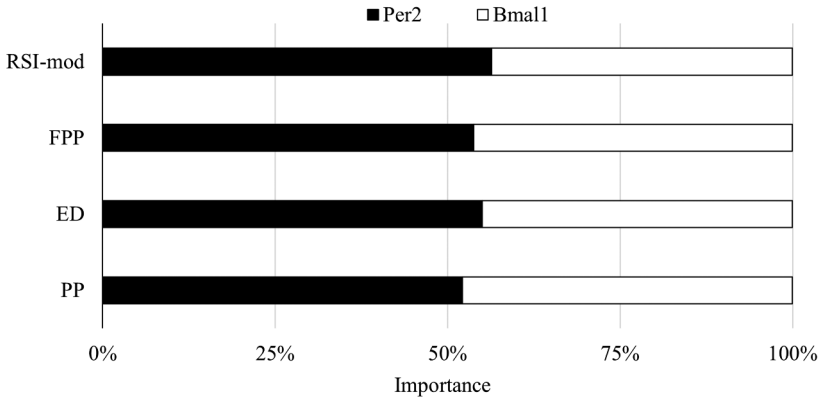

Figure 5. Feature importance from XGBoost analysis. *Per2* (black) was a more influential genetic parameter than *Bmal1* (white) in predicting athletic performance across all measured performance metrics. Eccentric Duration (ED). Reactive strength index-modified (RSI-mod). Peak power (PP). Force at peak power (FPP).

16.1 km were not abolished by a warm-up routine, actually suggesting that timing is a more important factor (Atkinson et al., 2005). In the present study, some players jumped higher in the morning than in the evening (jump height data not shown) and the data presented here suggest that CMJ performance varies throughout the day. Exercise timing may also affect individuals differently and contributes to a substantial difference in athletic performance for some individuals.

The awareness of when to train in football is becoming more apparent. A very recent comparison of time-affected training performance was carried out over 28 weeks when training the day before a match in an elite football team, varied between the morning and afternoon. For each measured metric, Total Distance (TD), High-Speed Running (HSR), Dynamic Stress Load (DSL), Accelerations (ACC), Decelerations (DEC), Explosive Distance (ED), the average values were higher in the morning than in the afternoon training sessions (Owen et al., 2023). In contrast, another study showed that football-specific performance peaked between 16:00 and 20:00 (Reilly et al., 2007). While firm conclusions cannot be drawn with limited study participants and differences in performance parameters, both studies provide evidence of temporal differences in athletic ability pertinent to football. Even kicking accuracy is time-of-day affected (Palucci Vieira et al., 2022). The performance results of the studies were reported as squad averages and not as individual players. Therefore, differences in findings may reflect differences in terms of chronotypes and gene expression levels of individual players, which could be an important factor in performance optimisation (Rae et al., 2015). For example, a football squad composed of morning player types would perform better in the morning than a squad composed of evening-type individuals. Although, Basti et al. (2021) found that chronotype alone was not correlated with performance. Indeed, morning-type cyclists performed better in the morning (Atkinson et al., 2005).

4.2. Changes in CMJ metrics correlate with gene expression

The expression levels of *Per2* and *Bmal1* were quantified using real-time PCR (Basti et al., 2021) for all participants in this study. *Per2* and *Bmal1* gene expression levels were normalised against the levels of *GAPDH*, a reliable and validated housekeeper gene, to ensure the technical validity of the data (Basti et al., 2021; Livak & Schmittgen, 2001). The focus was on whether the patterns identified in performance were reflected in the gene expression signatures to support growing evidence that gene expression may correlate to the timing of maximal athletic performance. There was a high degree of variability in gene expression data across the participants where the same pattern of gene expression (higher *Per2* and lower *Bmal1* in the evening), and the degree of change between the examined cohort were significantly different.

Therefore, changes in expression levels, much like the degree of change in athletic performance, as measured by CMJ metrics in the present study, are individualised. Although the player's age range in the present study was small, there may be other influential covariates, such as chronotype, maturity status and hormone levels, which may reflect inter-individual differences in performance and gene expression. For this reason, the focus of this study was to correlate performance on an individual basis. The lack of this data could be considered a limitation of this study. Future studies should incorporate such data.

The R^2 value was used to evaluate the effectiveness of predictive models and understand the degree of association between molecular analyses and athletic performance measurements. It can be inferred that FPP demonstrates the highest level of interpretability based on the genetic measurements, surpassing both RSI-mod and ED, which exhibit comparable, but lower interpretability. Notably, PP yields the lowest R^2 values among the four-performance metrics, despite still offering 86% fit within the XBG model. Overall, all four metrics and three methods of calculation showed high levels of correlation with the molecular analysis. Compared to RF, XGB and MLP were consistently higher and had similar inter-variations. RF returned the lowest R^2 values of the three methods (0.71–0.81). Despite this, all three methods revealed a consistent pattern between each metric and the modelled molecular analysis. PP was the lowest across all three methods, FPP consistently showed the highest R^2 value.

Although the R^2 values reported are high, they were obtained using leave-one-out cross-validation (LOOCV), a method chosen for its rigour and appropriateness in small-sample studies. LOOCV allows each data point to serve as a test case once, reducing the risk of overfitting relative to simpler resampling approaches. Still, we interpret these results as exploratory in nature; intended to highlight potential associations rather than provide conclusive predictive models

The correlations between clock gene expression levels and peak athletic ability shown here are similar to those previously reported (Basti et al., 2021). The authors also measured *Per2* and *Bmal1* gene expression in parallel from hair and blood samples over two days to show the applicability and stability of peripheral gene expression analysis over a longer period. In this previous, independent study, R^2 values were not determined, although the authors did show a correlation for nine out of ten participants between *Per2* and HST performance and the correlation was significantly less for *Bmal1*.

Similar results are reported and furthered in the present study by examining the relative importance of each gene.

The relative importance values generated by XGB, helped to quantify the influence of each gene in modelling that significantly impacts the target variable (performance metric). High relative importance values (gain) suggest that a particular feature plays a crucial role in the model's decision-making process, while lower values indicate comparatively lesser impact. This information aids in prioritising and understanding the relevance of each gene guiding model selection and enhancing model interpretability. Effective interpretation of relative importance facilitates a more nuanced understanding of the underlying relationships within the dataset, thereby contributing to informed decision-making in various domains. The results demonstrate that *Per2* is more important than *Bmal1* in this context, which supports a previous study (Basti et al., 2021).

The relationship between buccal gene expression levels and chronotype, often used to define temporal preference for exercise, is not fully understood. Thus, it is difficult to comment on whether higher gene expression levels in the morning equate to a morning chronotype or vice versa. Indeed, it is not surprising that changes in gene expression levels are unique, depending on many inherited and environmental factors (De Jong et al., 2019). While sampling mucosal tissue is a well-validated method for obtaining clock gene expression data (Bjarnason et al., 2001), it is important to recognise that buccal-derived gene expression data may be subject to biological variability introduced by several external factors. These may include recent food or beverage intake, oral hygiene practices (e.g. brushing, use of mouthwash or toothpaste), hydration status, and the composition of the oral microbiota. For example, components in toothpaste and dietary intake have been shown to modulate microbial gene expression and may influence host epithelial responses (Hu et al., 2024). Although the direct impact of these variables on human buccal clock gene expression is not yet fully characterised, their influence on gene transcription in oral tissues is biologically plausible and warrants consideration. Indeed, saliva may provide a more stable alternative in this regard (Nelson et al., 2025). The biological meaning of the gene expression changes measured in the present study is beyond the scope of this research. Indeed, it is important to state that this study did not aim to define chronotype but to use molecular markers as an objective surrogate for daily variations in peak jump performance from CMJs. Suffice it to say that the weight of evidence highlights the applicability of molecular profiling a squad of athletes to ascertain optimal peak training times.

Another important factor in exercise timing may be "time since awakening" (E. Facer-Childs & Brandstaetter, 2015a, 2015b). In the present study, all participants had the same sleep schedule, and all had the same wake time. Despite this, heterogeneity in the timing of peak athletic performance was seen, so this predictor would also be individualised and dependent on individual biology where evening types would wake later and would therefore peak later in the day (Taillard et al., 2021). It remains to be seen if the time since waking could be intertwined with the change in *Per2* and *Bmal1* expression levels, which would be reflected in the molecular analysis to give a complete, objective view of internal biological time.

Furthermore, assessing the peak time of athletic performance and adapting sessions accordingly has potential implications to enhance recovery. In a study of weightlifters, markers of muscle damage and oxidative stress (including reactive oxygen species)

were measured post-exercise in the morning, afternoon, and evening (Ammar et al., 2016). The authors found higher levels of oxidative stress, muscle damage, and inflammation following the morning sessions. These findings were supported elsewhere (Aloui et al., 2017), and in a study of 12 football players, muscle fatigue was more pronounced post-evening exercise (Hammouda et al., 2011). It is conceivable that the participants involved were more suited to evening exercise (weightlifting) and morning exercise (football players), although this was not assessed. Therefore, the potential misalignment of training time with biological time, possibly increased adverse outcomes. It would be interesting to examine if tailoring training according to individual biological time allows the body to cope better with the demands of physical performance.

4.3. Limitations and future directions

Due to logistical constraints, the study was limited to two performance measurements. Therefore, while the study is valid for individual comparative performance improvements and shows promise in analysis, actual peak athletic performance may likely exist between the morning and evening measurements. Nevertheless, the data presented here evidentially supports the rationale adding to the growing body of evidence that expression levels of key genes in buccal cells can serve as markers of exercise timing. Furthermore, the methodology of sample collection performed here was non-invasive and easy to perform, allowing the profiling of exercise timing of individual and team sports. Future studies should be replicated with different categories of football players, including female athletes across other sports. Additionally, other load variables (e.g. high-speed running, sprinting, accelerations, and decelerations) and physical attributes (e.g. agility, change of direction, cardiorespiratory, or other strength tests) may be utilised to improve the clarity of results.

5. Practical applications

Overall, these findings highlight the importance of squad profiling and considering both the type and timing of training to maximise physical and tactical performance in different age groups of national teams. Specifically, the present results suggest that athletic performance is time-of-day dependent and individually variable. These findings may have practical implications for coaches in offering a method to profile players and squads providing information to tailor training session times to optimise physical performance and tactical understanding considering time-of-day scheduling. This may help practitioners design more effective training programmes based on the timing of optimal player performance (Morgans et al., 2024).

6. Conclusions

The examined youth football players in the present study showed significant time-of-day differences in CMJ performance. This adds to the evidence that athletic performance is time-of-day dependent and individually different. Measuring the expression levels of *Per2* and *Bmal1* serves as an accessible, objective marker of peak exercise timing for the

individualisation of certain training aspects. By extension, peak performance may be accompanied by reduced muscle fatigue and adversity post-exercise to enhance recovery.

Disclosure statement

In accordance with Taylor & Francis policy and my ethical obligation as a researcher, I am reporting that I have business interests in a company that may be affected by the research reported in the enclosed paper. I have disclosed those interests fully to Taylor & Francis, and I have in place an approved plan for managing any potential conflicts that may arise.

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Study Contributions

Authors [1], [10] and [8] was involved in study conceptualisation and project administration. [6] and [7] collected the CMJ data, who are experienced sport scientists and active football practitioners at EPL clubs. [1], [2], [3] and [4] performed the molecular methodology and hold degrees; Masters or higher (PhD) in genetics or biomedical science. [5] performed formal analysis. [1], [10], [9] and [5] contributed to the manuscript writing. All authors reviewed and edited the final manuscript.

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