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Original Research

The Simple, Conventional Markers of Fatigue: Variations in Neuromuscular Performance, Creatine Kinase and Hydration Status in Elite Soccer Players Over a season.

Catherine E. Beattie, Ph.D.[1], Samuel A. Pullinger, Ph.D.[2], Cristian Savoia, Ph.D[3], Ben J. Edwards, Ph.D.[3], Colin M. Robertson, Ph.D.[4]

[1] Sport Science & Medical Department Bolton Wanderers Football Club, School of Sport and Biomedical Sciences Department, University of Bolton, Bolton, UK., [2] Sports Science Department., Aspire Academy, Doha, Qatar, [3] Research Institute for Sport and Exercise Sciences., Liverpool John Moores University, Liverpool, UK., [4] School of Sport and Biomedical Sciences Department, The University of Bolton, Bolton, UK.

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Background and Purpose

Fixture congestion, game-intensities and limited recovery negatively influence physical/ physiological responses during a competitive soccer season. Therefore, the aim of the investigation was to examine weekly alterations in neuromuscular performance markers, creatine kinase and hydration in elite soccer players throughout a season.

Study Design

Longitudinal Observational Study.

Methods

Sixteen male professional soccer players competing in the English Football League Championship were assessed over the course of a season. All players provided a urine sample, a blood sample to assess creatine-kinase concentration and performed a countermovement jump test at the start of the season, in-season, pre-and post-match over 38 weeks.

Results

Jump height was the most common marker of performance to be significantly reduced in-season compared to baseline (-5.4 to -11.3%, P<0.05) with 45.2% of the time-points affected. Measures of FT:CT (-7.5 to -12.4%) and AP (-9.4 to -11.5%), also showed significant deteriorations throughout the season compared to baseline (P<0.05) at several time-points. Max force (MF) significantly increased in-season (+5.1 to 7.0%) in 20% of the observed time-points compared to baseline. CK concentration significantly increased during 19% of the time-points (P<0.05; 62 to 159%). Urine osmolality demonstrated significant differences in-season compared to baseline, but none to levels of dehydration.

Conclusion

Monitoring elite soccer players over the course of a competitive season shows alterations in neuromuscular performance and hydration status. These data suggest that assessing counter-movement jump performance may be a useful marker for monitoring responses to training/competition, while creatine-kinase and hydration status may be limited.

Introduction

In elite soccer, fixture congestion and limited recovery time during a competitive season leads to accumulated levels of physiological stress.[1] This imbalance in homeostasis predisposes players with the inability to cope with the physiological demands of training/competition and as a result increases their susceptibility to potential injury.[1,2] In an attempt to monitor a player's physiological and physical response to match and/or training load, several tests and biomarkers are currently being utilised in elite sport.[3] The most common include creatine kinase (CK), cortisol, testosterone, hydration levels, jump tests and wellness questionnaires.[4–8] These tests and markers help provide a clearer understanding of the physiological impact of training and competition and ultimately aid in the design and development of more effective strategies to accelerate recovery prior to the next fixture.[9]

The neuromuscular system is largely taxed by high-intensity manoeuvres such as accelerations, decelerations, repeated sprints, tackles and duel plays during soccer match-play.[10,11] Neuromuscular fatigue (NMF), caused by such actions, has the potential to arise at several points during the pathway between the central nervous system and the contracting muscle.[2] A common method used to assess NMF in team sports is the countermovement jump (CMJ) test.[12] Jump height is a commonly collected marker in sport,[2] yet recent recommendations have suggested a more comprehensive analysis should be utilised to allow observations that provide more information into movement strategy.[13] Alternative CMJ outputs such as the ratio between flight time and contraction time (FT:CT) and average power (AP) have been favoured due to their increased sensitivity to external load completed during games and cumulative load over a season.[14,15] Nonetheless, there is limited understanding on the long-term alterations in NMF across a soccer season and its potential effect on gross physical performance output. The investigation of long-term hormonal and biochemical responses in elite soccer however has been researched more extensively.[4–7] Several muscle metabolism parameters, such as CK and myoglobin have shown to increase after exercise.[16] These increases represent muscular stress and tissue damage following muscle-damaging actions with a high eccentric component, such as accelerations/decelerations.[17] Investigations in elite soccer have supported associations between post-match CK concentrations and match-related load,[7,8] however to the authors knowledge no investigations have detailed weekly responses across a competitive season. The weekly observation of such markers may help prevent situations of accumulated stress and avoid over-training syndrome thus reducing potential increased injury risk. Nevertheless, the validity/reliability of such markers have been questioned due to high inter-individual variability and further research is required to conclude on sensitivity and application of CK testing in elite soccer.[14,18] In addition, individual differences in CK may limit the diagnostic accuracy of group-based reference ranges, with a need to derive reference ranges from repeated measures for each player in order to provide accurate and individualised baseline and reference ranges.[14]

Therefore, monitoring physical and biochemical changes are of paramount importance to determine the recovery-fatigue status of athletes. Additional markers such hydration status have become key observational markers due to the known adverse effects of dehydration on aerobic, endurance exercise and cognitive performance.[19] Re-hydration post physical activity is of upmost importance, with research reporting that intracellular fluid volume loss can impair glycogen and protein resynthesis rate - an essential part of the recovery process.[20] As such, maintaining a state of euhydration is an imperative factor in optimising an athlete's performance. Although not gold standard, a common technique utilised in the field is urine osmolality (Uosm), measured via a handheld refractometer, that has previously been shown as a reliable measure of acute body water loss.[21] Considering the limited literature available on the alterations and reference ranges in this marker over a season in elite soccer players, additional findings need to be established.

Whilst a comprehensive body of research has investigated acute and residual fatigue responses post-match,[4,7,8,14] limited research is available on season-long responses in elite soccer players. A clear understanding of the weekly physiological and physical impact of training and competition will help in the design and development of more effective strategies to accelerate recovery prior to the next fixture.[9] Therefore, the purpose of this research was to: (1) examine weekly alterations in neuromuscular parameters, creatine kinase and hydration status in a cohort of elite senior soccer players across a competitive season compared to baseline measures and (2) establish appropriate reference ranges in elite soccer players to aid the decision-making process for practitioners and coaches.

Materials and Methods

Experimental Approach to the Problem

To assess whether responses to neuromuscular parameters, creatine kinase and hydration status can be observed weekly throughout the competitive season in professional soccer players. The study included measurement points pre-and post-match on a weekly basis. These markers were compared to baseline measures throughout the season.

Subjects

Sixteen first-team professional male soccer players competing in the English Football League (EFL) Championship during the 2015-16 season were utilised for this study [age (mean ± SD) 27.6 ± 4.8 yrs; body mass 80.1 ± 8.7 kg; stature 1.8 ± 0.9 m; body fat 9.6 ± 1.8%]. Inclusion criteria required players to achieve 20 or more competitive league appearances over the course of the season to observe effects of those who were playing regularly and available for selection. Exclusion criteria included any players that sustained an injury during the data collection windows. Goalkeepers were excluded from the sample due to their specialised role within the team. They were given a verbal explanation of the study protocols and provided written informed consent. All players had at least ten years of playing experience in national and international competitions. The study was approved by the Ethics Committee of the School of Sport and Biological Sciences, University of Bolton and conformed to the recommendations of the Declaration of Helsinki.

This investigation was completed as part of routine testing and monitoring from pre-season until the end of season (July 2015 to May 2016). Because of this approach, a traditional scientific experimental design was not employed. The generic training and competition plan followed by the team was developed by the technical staff. The typical weekly schedule is displayed in Table 1. Baseline data were collected in a rested state 36-h before the first match of the season (BAS) and subsequent data collections on 38 occasions throughout the 40-week season. This measure was taken to represent the players training baseline rather than an absolute resting baseline. Data were collected every week three days apart: on a Friday 24-h before match-day (MD -1, 24-h pre-match) and on a Monday 48-h post-match day (MD +2, 48-h post-match), either side of a scheduled Saturday fixture. All measures were carried out at the same time-of-day (09:30 to 11:00-h) to reduce the potential influence of circadian rhythmicity.[22] Due to time constraints, injury or illness, the collected data were averaged per week and re-

Table 1. A typical weekly micro-cycle based on a one or two match-play week.

	MONDAY 1 (MD +2)	TUESDAY 2	WEDNESDAY 3	THURSDAY 4	FRIDAY 5 (MD -1)	SATURDAY 6 (MD)	SUNDAY 7 (MD+1)
AM	Data Collection & Training	Training if no Match	Off / recovery	Training	Data Collection & Training	Off	Off / recovery
PM	Off	Match or Weights/ Training	Off	Weights	Off	Match	Off

'Off' denotes athletes had no prescribed session or fixture and did not need to attend to the clubs training facilities.
MD denotes 'Match Day'.

sulted in a varied number of data sets across the study period, mean (± SD) of 10.7 ± 2.4 participants per data collection point. In a fasted state, where no food or water was consumed prior to arriving at the training ground, all players provided a urine sample, followed by a blood sample and then performed a CMJ test.

Procedures

Urine Osmolality (Hydration Testing)

Players reported to the training facilities and provided a urine sample, the first urine void of the day, to assess hydration status using a handheld urinary refractometer (Osmocheck pocket pal OSMO, Vitech Scientific Ltd, Japan). If this was not possible, players were asked to ingest 300 mL of water and the second void was used. They were asked to provide a sample of midstream morning urine directly into a 30 mL, clear, sterile, plastic container, analysed within 30-min of collection to provide urine osmolality (Uosm). Prior to analysis, the device was calibrated according to the manufacturer's guidelines, by pipetting a drop of distilled water directly on the face of the prism to calibrate the refractometer to point zero. Between samples the prism was rinsed with distilled water and dried. The reproducibility for Uosm measured via the Osmocheck has previously been reported (CV% 34; ICC 0.98; TE 0.15).[23]

Creatine Kinase Testing

Upon completion of the hydration test, the players provided a blood sample in a rested state to test plasma CK concentrations. Capillary blood (32 µL) samples were collected via a finger prick using a safety lancet (Sarstedt DS1588, Numbrecht, Germany) into a heparinized capillary tube (Reflotron®). From this, the blood was directly pipetted on a CK test strip (Reflotron®) and subsequently analysed through a Boehringer Mannheim Reflotron Analyzer (Reflotron®). The measurement range for CK using this method was 24.4-1400 µL. The reproducibility for CK measured via the Reflotron® has previously been reported (CV% 18 - 20; ICC 0.90; TE 94).[24,25]

Counter Movement Jump (Physical Performance Test)

The CMJ (a vertical jump test) was then performed using a portable force platform (HUR Labs Force Platform 3.8.0.2, Kokkola, Finland). After familiarisation the CMJ test was performed according to previously described methods.[14] All players performed a standardised warm-up consisting of a 5-min cycle at 80-W power load (Keiser M3, Fresno, CA, USA), dynamic stretching and three submaximal CMJ efforts. The test was repeated three times with a rest period of 30-s provided in between each jump with the highest value used for further analysis. To minimise the influence of external factors, all testing sessions were conducted at the same indoor gym facility to provided consistent stable flooring. CMJ data was recorded for jump height calculated by impulse-momentum (height - cm); contraction time (CT, representing the time from the initiation of the countermovement until the participant leaves the force plate - ms); flight time (FT, representing the moment the participant leaves the force plate until landing - ms); flight time:contraction time ratio (FT:CT); peak power (PP - Watts); max force (MF - Newtons); take off velocity (TV – m/s); average power (AP - Watts) and average force (AF - Newtons).

Statistical Analyses

All data were analysed by means of the Statistical Package for Social Sciences for Windows (SPSS, Chicago, IL), IBM version 28. All data were log transformed to improve the normality of distribution. After log transformation the outliers more than three interquartile ranges below the first quartile or above the third quartile were removed from the analysis. To overcome the varied number of data sets across the study period a linear mixed-models procedure was used. Following convention, the alpha level of significance was set at 5% ($P < 0.05$). Geometric means were calculated by taking the exponential and 95% confidence intervals (CI) reported alongside the P-value. Descriptive statistics for the season were calculated from the raw data including the mean (±SD), median, and interquartile range. After log-transformation a 95% reference range was derived from the data based on the normal distribution.

Table 2. Descriptive values for all variables over the course of the season including the mean ±SD, the median (and interquartile range) and the 95% reference interval.

Measure	Mean ± SD	Median (IQR)	95 % Reference Interval
Uosm (mOsmols)	338 ± 155	300 (240 to 395)	137 to 675
CK (u/l)	476 ± 338	366 (244 to 593)	98 to 1417
CMJ H (cm)	35 ± 4	35 (32 to 38)	28 to 43
CT (ms)	837 ± 107	837 (774 to 891)	646 to 1075
FT (ms)	551 ± 32	549 (526 to 575)	491 to 615
FT:CT	0.67 ± 0.10	0.66 (0.61 to 0.72)	0.50 to 0.88
MF (N)	1881 ± 169	1859 (1751 to 1977	1576 to 2210
PP (W)	4104 ± 442	3988 (3791 to 4320)	3342 to 4873
AF (N)	1055 ± 90	1048 (988 to 1087)	839 to 1232
AP (W)	1072 ± 179	1061 (939 to 1197)	768 to 1462
TV (m/s)	2.62 ± 0.15	2.61 (2.51 to 2.72)	2.34 to 2.92

Results

Table 2 shows the mean ± SD, the median (and interquartile range) and the 95% reference intervals for hydration, CK concentrations and CMJ performance measures collected over the entire season. Hydration levels (Uosm) were significantly different in-season compared to baseline values ($P = 0.001$) during 30% of the collected time-points (Figure 1A). Only weeks 25, 26, 27 and 32 found Uosm values to exceed 400 mOsmols with an increase between 56 to 131% higher compared to the observed baseline value ($P = 0.001$; 95% CI: 0.18 – 1.10).

Creatine kinase concentration established significant differences in-season compared to baseline values ($P = 0.001$) during 19% of the collected time-points (Figure 1B). Values for CK ranged from 236 to 670 u/l in-season, rising as much as 117% compared to baseline values observed. Significant differences were observed for weeks 7, 14, 15, 21 and 23 ($P = 0.05$; 95% CI: 0.02 – 1.42).

Several measures related to CMJ performance displayed significant differences in season-compared to baseline values observed ($P < 0.005$). In-season, jump height was significantly reduced during 45% of the time-points compared to baseline (-5.4 to -11.3%, $P < 0.05$), with the greatest impairment occurring during week 19 (Figure 1C), which saw CMJ FT, FT:CT, AP and TV to be significantly affected (-4.8 to -11.5%, $P < 0.05$). Measures of TV observed significant impairments in 43% of time-points compared to baseline (Figure 1K), with FT in 20% (Figure 1E), FT:CT in 17% (Figure 1F) and AP in 15% (Figure 1J) of time-points ($P < 0.05$). Values for TV decreased by 3 to 6%, FT by 2.9 to 4.8%, FT:CT by 7.5 to 12.4%, and AP by 9.4 to 11.4%, respectively. Only measures of MF demonstrated significant increases to baseline in 20% of time points over the season (Figure 1G; $P < 0.05$) ranging from 5.1 to 7.0%. A total of 45% of in-season time-points demonstrated at least two variables to be significantly different to baseline values (Figure 1C-1K). CMJ PP and AF however showed no significant differences for any of the time-points in-season when compared to baseline values ($P > 0.05$).

Discussion

The overall analyses of data highlights significant alterations in most of the physiological and neuromuscular performance measures assessed within this study in a cohort of professional players in-season when compared to their training baseline values. The current investigation demonstrated significant increases in both CK concentrations and Uosm compared to baseline, over a limited number of time-points. Further, increases were observed in MF during the CMJ, while other measures showed significant decreases in-season compared to baseline. These changes across the season in CMJ outputs, closely related to jump strategy, are potentially indicative of low-level NMF observed in this cohort of players. To the authors knowledge, this is the first study that investigates weekly variations in several recovery-fatigue measures over a competitive season in elite senior soccer players.

A clear pattern of depression in CMJ jump height and FT:CT alongside increases in plasma CK concentration have previously been demonstrated following 90-min of elite competitive soccer.[14] Therefore, it is reasonable to assume that substantial alterations observed in this study are indicative of low-level NMF and increased muscular stress. The monitoring of plasma CK has been widely adopted in elite sport, but due to large inter-individual variability observed in this biomarker it has been questioned for use as a valid marker of recovery.[8,18] The expression of CK is influenced by factors such as ethnicity, age and fibre type composition.[18] Cultural diversity among the soccer population is commonplace and was present within the current cohort of players, with 31% (5 out of 16) of the sub-group coming from black ethnicities who have previously shown higher than average CK concentrations.[25] This inter-individual variability will have influenced the group median and the associated large variance observed in Figure 1B. Therefore, changes in CK concentrations should be compared to individual baseline measures and only comparisons between groups should be used when of similar ethnicity and age. Despite the high inter-individual variability in this marker, there were significant increases in CK from

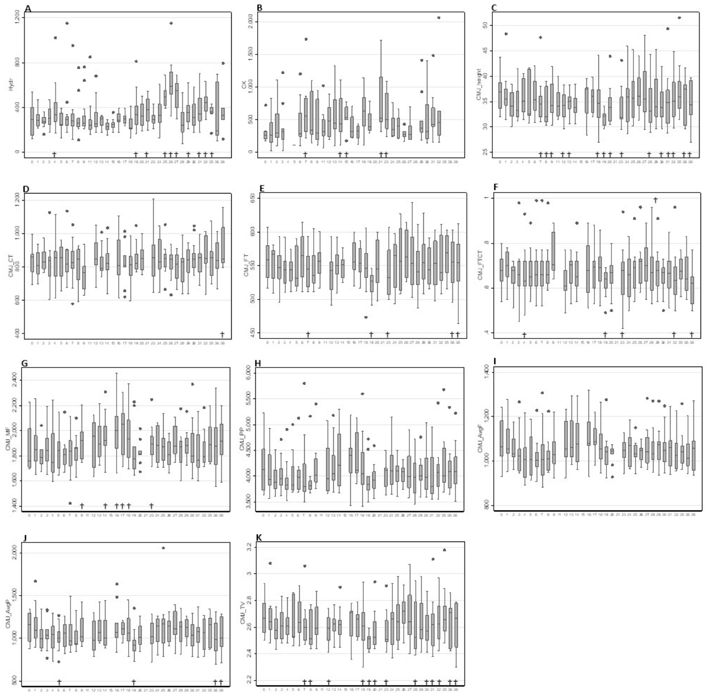

Figure 1.

a Boxplots of recovery-fatigue markers over the course of a competitive season (weeks 1 to 38) for A) Uosm (mOsmols); B); Creatine kinase (CK) concentration (u/l); C) CMJ height (height – cm); D) CMJ contraction time (CT - ms); E) CMJ flight time (FT -ms); F) flight time:contraction time ratio (FT:CT); G) max force (MF - Newtons); H) peak power (PP – Watts); I) average force (AF – Newtons); J) average power (AP – Watts) ; K) take off velocity (TV – m/s).
•Denotes an outlier †Denotes a significant difference to baseline values (P < 0.05)

baseline measures at five time points recorded in the first half of the competitive season. This trend is consistent with observations established by Lazarim et al.[26] who found higher concentrations of CK at the beginning of the competitive season and reductions in CK activity as the season progressed in Brazilian national championship soccer players. These results are indicative of a positive muscular adaptive response, perhaps due to better player preparation and management leading to higher load tolerance and lower fatigue responses, and potentially due to higher training loads usually observed within pre-season. Due to the irregularity of testing time points in previous investigations however it is difficult to draw accurate comparisons to the present study findings. Nowakowska et al.[7] found higher CK concentrations in the 'spring round' in comparison to the 'autumn round' in midfielders and a weak-positive correlation between CK and cumulative match-time in midfielders/defenders across a season. The increased trend in CK levels in this sub-group is to be expected due their increased physical output,[27] but it does not support the notion of muscular adaptation in elite soccer players as previously suggested in the literature. The inconsistent findings on the validity of CK testing highlights the importance of utilising more than one biomarker when attempting to detect over-training and/or fatigue.[16] The measure of CK as a marker of muscle damage and the recovery process is of little diagnostic value when used alone. Additional biomarkers such as nitrogen urea, lactate dehydrogenase (LDH), myoglobin, and others could be used in combination with CK to provide more detailed information on the recovery process and adaptations to training.[16] Some biomarkers however are difficult to measure other than in venous blood samples. In the context of high-level athletes and the need for frequent sampling points, the use of non-invasive markers ought to be considered.[28] Therefore, surrogate markers e.g., related to oxidative stress [29] or visual assessment tests should be considered in combination to CK.

Interestingly, this investigation observed several alterations in CMJ outputs derived from the 'push-off' phase and movement strategy variables from the 'countermovement' phase across the season. Jump height displayed the greatest variation to baseline measures with 14 out 31 (45%) of time points significantly lower. The magnitude of change during these time points ranged from 5.4 to 11.3%, which is outside the CV % reported in previous research (3.8 to 5%).[12] Although CMJ height is a commonly collected and reported variable due to its simplicity, it has been criticised for its use in the detection of NMF due to its lack of insight into movement mechanics.[12] In elite soccer, Meister et al.[30] previously observed a tendency towards higher CMJ heights during a three-week period of high match exposure, indicating an improvement in jump performance despite a congested schedule. Furthermore, Thorpe et al.[31] observed a positive, non-significant, trivial to small relationship between changes in CMJ height and high intensity running distance accumulated over two to four days. These findings add controversy to the original rationale of using CMJ height to detect signs of NMF and alternative measures that provide greater sensitivity to alterations in load should and need to be considered.[14] Therefore, CMJ output FT:CT has been suggested as a more effective marker for detecting NMF due to its reflection of outputs concerning concentric push-off (FT) and altered movement strategy (CT). In the present study, FT:CT demonstrated significant reductions to baseline at only 5 out of 30 (17%) time points. The only comparison to the extent of data collection similar to the present study is from Australian Rules soccer (ARF) where CMJ FT:CT as well as two hormone markers were analysed across the season.[10] Cormack et al. found that FT:CT was substantially lower than baseline at 12 out of 20 (60%) of time points, with one time point in particular showing a substantial decrease of -17.1 ± 21.8%. In the present study, the most substantial decrease in FT:CT was noted at week 38, towards the end of the competitive season (-12.4%). This time point also demonstrated reductions in height, FT, AP, TV and increases in CT, although no significant change in MF or PP. These alterations may reflect fatigue manifested in an altered movement strategy whereby countermovement range and speed are changed to achieve/maintain peak outputs. Nonetheless, the alterations in movement strategy may have a stronger association to that of increased injury risk.[32] Research has suggested that increased levels of NMF may lead to increased electromechanical delay in muscular contraction and reduced rate of force development, that in-turn can predispose to noncontact injuries.[33] Max force and power outputs might be recovered to baseline/preinjury levels, but these gross outcome measures can mask latent deficiencies in movement strategy. Therefore, alterations in movement strategy may provide greater insight in the profiling of elite athletes.[34]

The longest period of observed NMF was during three consecutive weeks (18-20 and 30-32). It can be suggested that extended periods of reductions in physical performance indicate inappropriate periodisation resulting in non-functional overreaching and/or overtraining syndrome. If consecutive weeks of attenuated physical output from CMJ testing are observed in addition to spikes/changes in CK concentrations appropriate interventions need to be implemented by coaching staff and practitioners to reduce injury risk and the well-being of players. The body has the capacity to adapt to stressors if periods of accumulated fatigue from match and training can be reduced to restore levels of homeostasis.[35] In support of this, significant increases in MF were observed over the competitive season with 20% of time points displaying significantly higher values compared to baseline. Most of these increases were observed during the middle of the competitive season. These observations in MF would indicate that the players were adapting to training and match loads as the season progressed. The repeated and routine testing of the CMJ over the course of the season should allow these athletes to execute this movement with well-rehearsed motor-patterns that may contribute to maintenance of peak performance outputs. Although if players are taking longer to achieve peak performance outputs this could be the marginal difference in outmanoeuvring an opposing player to allow a goal scoring opportunity that may yield a competitive advantage.

Lastly, hydration testing revealed that Uosm levels were substantially higher than baseline at 30% time points. The Uosm levels were however below the accepted euhydration cut-off of <700 mOsmols [36] suggesting that the players were adequately hydrated despite the significant increase to baseline measures. In support of these findings, the observed 95% reference range for Uosm (137 to 675 mOsmols) were below the accepted euhydration cut-off. Players involved in this investigation were aware of the importance of maintaining a euhydrated state, allowing for optimal recovery and the restoration of physiological homeostasis post training and competition. Therefore, the observed alterations in Uosm are not of clinical significance to affect physical or cognitive performance.[19,20]

A limitation to the current investigation was the exclusion of training/match external load across the season to observe if these alterations aligned to changes in preceding and/or cumulative load. Recent investigations in elite soccer have shown inconsistent findings in relation to the sensitivity of the CMJ and CK to match and training demands.[5,6,8,14] Rowell et al.[6] demonstrated a large number of unclear or trivial effects on CMJ FT:CT as a result of changes in internal training load (rate of perceived exertion) over a period of 3 to 28 days. Hecksteden & Meyer[8] observed a highly significant association between total running distance and high-speed running distance with CK concentrations from players competing in the FIFA World Championship 2014 and UEFA European Championship 2016. Other investigations however have struggled to establish any significant associations.[5,30] Further longitudinal research is required to determine the sensitivity of these measures to accumulated load. Lastly, due to the nature of collecting data in the field there were many uncontrollable variables, such as player absences or limited testing time that led to missing data points and limited the ability to analyse the data on an individual level or by playing position. Further, the use of therapeutic interventions, nutritional strategies and sleep should be taken into consideration when interpreting the data. These are major factors which can affect recovery,[37] although are largely out of practitioners control when athletes are away from the elite sport setting. Future research assessing weekly alterations in neuromuscular performance markers needs a clear focus on eliminating intraindividual and interindividual variations by deriving reference ranges from individual players to improve diagnostic accuracy. In addition, assessing additional markers related to CMJ, such as the eccentric phase could provide useful information related to fatigue in elite soccer players.

Conclusions

Elite soccer players were able to increase and/or maintain peak force and power outputs despite changes in outputs related to jump strategy indicative of low-level NMF. It is reasonable to suggest that although players demonstrated physiological and neuromuscular alterations across a season, they have the capacity to adapt their movement strategy to maintain gross performance outputs. Further, increases in CK were present during the first half of the competitive season, while players were able to maintain a state of euhydration across the season, although these measures may be of limited value. Nevertheless, regular and routine observation of several CMJ output variables are more useful markers for monitoring responses to training and competition in elite players when normative reference ranges are established. The observation of such markers may help prevent situations of accumulated stress through better training provision and reducing potential injury risk helping coaches and practitioners.

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