INTERNATIONAL JOURNAL OF
MEDICAL BIOCHEMISTRY

DOI: 10.14744/ijmb.2023.48278
Int J Med Biochem 2024;7(1):26–32

Research Article

Biomarkers of a Football Match-play - Internal load analysis using Technical Soccer Specific Aerobic Field Test (TSAFT^{90})

Janani Gopalakrishnan[1], Kaveen Dharanirajan[2], Zanetta Brewart Petra[2], Santhi Silambanan[3], Thiagarajan Alwar[1], Arumugam Sivaraman[1]

[1]Department of Arthroscopy and Sports Medicine, Sri Ramachandra Centre for Sports Science, Sri Ramachandra Institute of Higher Education and Research, Chennai, India

[2]Department of Sports Science, Sri Ramachandra Centre for Sports Science, Sri Ramachandra Institute of Higher Education and Research, Chennai, India

[3]Department of Biochemistry and Clinical Nutrition, Sri Ramachandra Institute of Higher Education and Research, Chennai, India

Abstract

Objectives: The study measured the magnitude of physiological, immune, endocrine, and muscle damage markers of exercise-induced fatigue using the Technical Soccer Specific Aerobic Field Test (TSAFT^{90}). We examined the effect of fatigue on performance and recovery at 24 hours post-exercise using biochemical indices.

Methods: Professional football players (n=30) with a mean age of 19.20 years participated in the study during their preseason. To induce fatigue, participants underwent a 90-minute fatigue simulation program, TSAFT^{90}. Venous blood samples were collected at baseline, 0-hour, and 24 hours post-fatigue. Analyzed markers included fatigue metabolites (lactate and uric acid), endocrine response marker (cortisol), muscle damage marker (creatine kinase), immunological markers (leukocytes, lymphocytes, neutrophils, and monocytes), inflammatory marker (CRP), hydration indicator (serum osmolality), and recovery marker (magnesium). Ball velocity and a 7-point Likert scale for muscle soreness were recorded to assess performance and perception of fatigue, respectively.

Results: All biomarkers studied were significantly elevated ($p<0.05$) at 0 hours post-fatigue. Uric acid, creatine kinase, leukocytes, monocytes, CRP, serum osmolality, and magnesium remained altered at 24 hours. Ball velocity significantly reduced post-fatigue ($p=0.04$) from 94.67 km/hr to 90.47 km/hr, whereas there was no change in the soreness scale.

Conclusion: The failure of the biomarkers to return to baseline levels within 24 hours indicates disrupted homeostasis. Monitoring the internal load with biomarkers aids in formulating strategies that can delay or mitigate fatigue and help achieve optimal performance and recovery, thus reducing the likelihood of injury.

Keywords: Athletic performance, biomarkers, fatigue, football, recovery, sports injury

How to cite this article: Gopalakrishnan J, Dharanirajan K, Brewart Petra Z, Silambanan S, Alwar T, Sivaraman A. Biomarkers of a Football Match-play - Internal load analysis using Technical Soccer Specific Aerobic Field Test (TSAFT^{90}). Int J Med Biochem 2024;7(1):26–32.

Playing a 90-minute football match leads to a decrease in physical performance with associated disturbances in psychophysiological and biochemical parameters. Neuromuscular fatigue is defined as an exercise-induced reduction in the maximal voluntary force that a player can exert. Fatigue causes a breakdown in internal homeostasis due to an increase in energy production demanded by an external stimulus [1]. This incomplete force restoration can significantly influence performance. Most football injuries occur in the final 15 minutes of each half, with a higher injury risk in the second half than in the first [2]. Fatigue monitoring and management are crucial for controlling athletes' training adaptations and reducing

Address for correspondence: Janani Gopalakrishnan, MD. Department of Arthroscopy and Sports Medicine, Sri Ramachandra Centre for Sports Science, Sri Ramachandra Institute of Higher Education and Research, Chennai, India
Phone: +919444861493 **E-mail:** shubajanani@gmail.com **ORCID:** 0000-0002-3808-6079
Submitted: July 21, 2023 **Revised:** September 08, 2023 **Accepted:** September 13, 2023 **Available Online:** January 10, 2024

their susceptibility to injury as they face higher physical demands coupled with fixture congestion [3]. Among the tools available to monitor fatigue, the serum biochemical profile reflects the ideal internal load. Biomarker fluctuations could affect performance, and recovery is thought to be influenced by these changes [4]. Immunosuppression tends to occur following a rapid increase in training load, leading to a higher risk of illness and injury for athletes who do not return to baseline levels within the latency period [5].

Existing literature categorizes the peripheral biomarkers of exercise-induced fatigue into several groups: Fatigue metabolites (lactate and uric acid) [6, 7], endocrine response markers (cortisol and testosterone) [7, 8], muscle damage markers (creatine kinase and myoglobin) [7–10], immunological markers (lymphocytes, leukocytes, neutrophils, and monocytes)[4, 8, 11], oxidative stress and inflammatory markers (reactive oxygen species, TAS, CRP, and interleukin 6) [6, 7, 11], hydration indices (hematocrit, haemoglobin, and serum osmolality) [12, 13], and recovery markers (magnesium) [12, 14]. Measuring these biomarkers helps understand the effects of training load, nutrition, hydration, and recovery methods on an athlete's homeostasis.

Although various football-specific simulation programs for fatigue are mentioned in the literature, such as free running, motorized, and non-motorized treadmill programs [15], those involving technical actions like passing, dribbling, shooting, and jumping are essential for a simulation protocol's success. The Technical Soccer Specific Aerobic Field Test (T-SAFT90) is one such program with proven external validity [16]. Data related to fatigue in football in the Asian sub-continent is scarce. This study is the first in the region using TSAFT^{90}, a novel football-specific simulation program, to analyze fatigue biomarkers and their influence on kicking velocity and recovery in Indian professional football players. The primary objective was to measure the magnitude of physiological, immune, endocrine, and muscle damage markers of exercise-induced fatigue at baseline, 0 hours, and 24 hours post-TSAFT^{90}. The secondary objective was to assess the effect of fatigue on performance using kicking velocity and recovery from muscle soreness, thereby drawing recommendations to monitor and manage match-induced fatigue in footballers.

Materials and Methods

Study participants

Professional male football players (n=30) representing the first division league level with a mean age of 19.20 (1.56) years and training experience of 7.90 (2.6) years consented to participate in this quasi-experimental study. Ethical clearance was obtained from the Institutional Ethical Committee board before the start of the study (IEC-NI/21/APR/78/82). A medical clearance and injury screening were conducted by a sports physician prior to participation. The study was conducted during the pre-season. Questions on the number of weekly training sessions, strength and conditioning sessions, and matches played were recorded to maintain homogeneity. Additionally, players' recovery strategies post-match/training, average sleep hours, and a 24-hour dietary recall were documented. All players were familiarized with the TSAFT^{90} well before the test day. A training-free period of 48 hours was observed before the fatigue testing. All tests were conducted at the same time of day for participants in batches. Participants were asked to fill in a 7-day food recall diary. Food and water intake on the test day and the following day were standardized for all participants.

TSAFT 90 setup

The players underwent a 90-minute audio-guided football-specific fatigue simulation program, TSAFT^{90} [16]. A schematic representation of the fatigue program is depicted in Figure 1. The protocol involves a 20-meter shuttle circuit, with poles, barriers, and a goalpost positioned strategically for players to navigate through utility movements. The movements were directed by verbal signals from an audio file at both the 0-meter ("outbound") and 20-meter cones ("return activities"). Each 15-minute protocol segment represents the rhythm and various types of activities demanded (13 in total; "stand still", "walk," "jog," "run," "sprint," "jump," "passes," "shots on target", "ball dribbling", and other combinations) [16]. Six cycles of the program were completed to achieve 90 minutes of simulation (45 minutes [3 cycles] - 15-minute rest - 45 minutes [3 cycles]), inducing fatigue.

Blood sampling and processing

Venous blood samples were collected at baseline, 0-hour post-fatigue, and 24 hours post-fatigue. The baseline sample was collected one week before the test day, following a non-training period of 48 hours. The laboratory analyzed blood samples for the following serum markers: lactate, uric acid, cortisol, Creatine Kinase, magnesium, osmolality, leukocytes, neutrophils, monocytes, lymphocytes, and CRP. Hematological markers, namely leukocytes, neutrophils, monocytes, and lymphocytes, were analyzed using fluorescence flow cytometry with Sysmex SP-50 (Sysmex Co., Kobe, Japan). The AU 5800 and AU 680 (Beckman Coulter, Brea, California, USA) measured serum uric acid, magnesium (serum xylidyl blue), creatine kinase (IFCC, NAC activated), and lactate (enzymatic method). Serum osmolality was calculated using the Smithline and Gardner formula (Serum osmolality = 2(Na) + glucose/18 + BUN/2.8). Cortisol was measured using an electrochemiluminescent immunoassay (ECLIA) on a Cobas 8000 module e602 (Roche Diagnostics, Mannheim, Germany). CRP levels were assayed by rate turbidimetry on an Immage 800 Immunochemistry System CRPH (Beckman Coulter, Brea, CA, USA).

Performance indicators

Post-strike ball velocity is an established performance variable [17]. Each player kicked a ball from a distance of 11 meters

Figure 1. TSAFT[90] (Technical Soccer Specific Aerobic Field Test) set up in the indoor training area.

from the goal post. Kicking velocity was measured before and after TSAFT[90] using a handheld radar speed gun (Pocket Radar Ball Coach, Pro-Level Speed Training Tool, USA) from 1 meter behind the goal post. The perception of lower limb muscle soreness was noted at 0 and 24 hours post-fatigue using a lower limb-specific 7-point Likert scale, adapted from Impellizzeri 2007 [18]. Heart rate and rate of perceived exertion were monitored throughout the protocol.

Statistics

Data were analyzed using R statistical software version 4.0.2. An unpaired t-test was used for performance indicators and one-way ANOVA followed by Tukey HSD (Honestly Significant Difference) post hoc test to compare the mean difference across groups for fatigue biomarkers. Statistical significance was set at a p-value of <0.05.

Results

Among the participants, 31% were forwards, 36% were midfielders, and 33% were defenders. Fatigue metabolites, namely lactate and uric acid, the endocrine response marker cortisol, the muscle damage marker creatine kinase, immunological markers leukocytes, lymphocytes, neutrophils, and monocytes, the inflammatory marker CRP, all remained significantly elevated at the end of 0 hours post-fatigue compared to baseline ($p<0.05$). The hydration status indicator, serum osmolality, and the recovery marker, serum magnesium, were also deranged and did not return to normal levels post-fatigue (Table 1).

Uric acid, creatine kinase, leukocytes, monocytes, CRP, serum osmolality, and magnesium remained altered at 24 hours. Graphical representations of the biomarkers are depicted in Figure 2a-d. The kicking velocity significantly decreased post-fatigue ($p=0.04$) from 94.67 km/hr to 90.47 km/hr, and the Likert scale showed no significant difference after 24 hours ($p=0.07$). Average heart rate and RPE during all six blocks of TSAFT[90] are shown in Figure 2e, f.

Discussion

A 90-minute football match induces acute physiological changes that stress aerobic metabolism. The utility of biochemical indices to study fatigue in football and its practical implications for managing recovery and preventing injury is still under investigation. This study aimed to measure the magnitude of various subtypes of fatigue biomarkers using a football-specific simulation program and assess the effect of fatigue on performance and recovery over 24 hours. Despite numerous studies on biochemical profiles during fatigue, no data involving Asian football athletes have been found to date. This study utilized a simulation program encompassing

Table 1. Fatigue analysis of various biomarkers at Baseline, 0 hour and 24 hour post fatigue

Biomarkers/ (mean [SD])	Baseline	0 Hour	24 Hour	Baseline vs 0 hr	0 hr vs 24 hr	Baseline vs 24 hr
Fatigue metabolites						
Lactate (mg/dL)	24.63 (7.51)	45.89 (13.04)	24.80 (8.13)	p=0.001 ↑	p=0.001 ↑	p=0.33
Uric acid (mg/dL)	5.58 (1.10)	6.01 (1.20)	5.82 (1.08)	p=0.05 ↑	p=0.002 ↑	p=0.05 ↑
Endocrine response						
Cortisol (µg/dL)	9.34 (2.68)	13.50 (5.68)	9.59 (3.29)	p=0.001 ↑	p=0.001 ↑	p=0.70
Muscle damage						
CK (U/L)	233.17 (122.83)	525.93 (396.45)	658.47 (563.80)	p=0.001 ↑	p=0.004 ↑	p=0.001 ↑
Hydration and muscle contraction						
Serum mg (mg/dL)	2.06 (0.13)	1.92 (0.14)	2.02 (0.13)	p=0.02 ↓	p=0.05 ↓	p=0.02 ↓
Serum osmolality (mOsm/kg)	285.00 (3.01)	292.20 (3.52)	287.77 (3.53)	p=0.02 ↑	p=0.04 ↑	p=0.01 ↑
Immunological state						
Leukocytes (cu/mm)	6.458.00 (1091.13)	11.543.00 (3572.52)	6.920.00 (1584.27)	p=0.001 ↑	p=0.002 ↑	p=0.05 ↑
Lymphocytes	37.22 (9.33)	25.60 (9.89)	37.68 (9.37)	p=0.002 ↑	p=0.001 ↑	p=0.22
Neutrophils	53.74 (10.03)	68.07 (10.73)	53.01 (10.63)	p=0.001 ↑	p=0.01 ↑	p=0.77
Monocytes	4.33 (1.04)	3.63 (0.84)	4.44 (1.32)	p=0.42	p=0.01 ↑	p=0.04 ↑
Eosinophils	3.95 (3.01)	1.79 (2.05)	4.13 (3.06)	p=0.001 ↑	p=0.001 ↑	p=0.07
Inflammatory markers						
C Reactive Protein	0.10 (0.10)	0.19 (0.12)	0.30 (0.25)	p=0.02 ↑	p=0.001 ↑	p=0.01 ↑

↑ Statistical significance set to p <0.05. One-way ANOVA followed by Tukey HSD (Honestly Significant Difference) post hoc test was done. SD: Standard deviation; CK: Creatine Kinase.

all technical activities in an actual football match, showing that the players' internal systems exhibit inadequate physiological recovery adaptation mechanisms to fatigue. At 0 hours post-fatigue, all biomarkers were elevated. Specifically, lactic acidosis impairs contractile function by reducing sarcoplasmic release and reuptake of calcium, myofibrillar sensitivity, the activity of ATPase, and key glycolysis enzymes such as phosphofructokinase and phosphorylase [6]. Blood lactate measurement estimates anaerobic metabolism contribution and interprets an athlete's resistance to fatigue, acting both as a fatigue agent and signaling molecule. Our study indicates a return to baseline for serum lactate at 24 hours. Another fatigue metabolite, uric acid, the end product of purine metabolism, was significantly elevated until 24 hours [5.82 (1.08) mg/dl]. Ascensao et al. [11] 2008 found that uric acid levels in 16 male soccer players were significantly elevated up to 72 hours post-match. Uric acid is associated with total antioxidant status as it scavenges hydroxyl ions and other free radicals produced in human plasma and skeletal muscle after acute strenuous exercise [19], attenuating the rise of plasma oxidative damage due to exercise.

White blood cell (WBC) activity reflects not only the status of the immune system but also the extent of muscle damage caused by exercise. Knight et al. [20] suggested that monitoring the redox status of WBCs is an emerging approach for the long-term management of elite athletes to better prevent overtraining and treat early infections. Our study showed significantly elevated WBC indices at 0-hour vs. 24-hour measurements. After 24 hours, leukocytes [6,920.00 (1584.27) cu/mm] and monocytes [4.44 (1.32) cu/mm] remained elevated, failing to return to baseline, illustrating the effect of fatigue on the immune system. Becatti et al. [21] and Carrera et al. [22] have identified established links between the redox status of leukocyte subpopulations with performance, injury, and training adaptations, demonstrating that training status and antioxidant supplementation impact reactive oxygen species production by WBCs.

Evidence of tissue damage is indicated by the increase in creatine kinase even 24 hours after the fatigue program. The increase in CK is 2-fold (658.47 U/L) in Indian players compared to Europeans (301.4 U/L) [10], which explains the dissimilarity between the study populations regarding nutrition, training load, recovery, and resistance levels to fatiguing conditions. This is consistent with the 24-hour CK values obtained by da Silva CD[16] in Brazilian football players (812±383 U/L). Stajer et al. [9] have elucidated a complex metabolic relationship between creatine and cortisol response to exhaustive exercise training, suggesting a link between cortisol and exercise-induced impaired bioenergetics, specifically the GAA-creatine axis. The biomarkers of creatine metabolism are positively associated with a cortisol stress response to exercise. The study shows a significantly increased stress response with serum cortisol values from 9.34 (2.68) µg/dL at baseline to 13.50 (5.68) µg/dL immediately post-TSAFT[90], but the values seem to be nearing baseline levels 24 hours after fatigue [9.59 (3.29) µg/dL]. The trendline of serum cortisol was found to be similar to that reported in previous studies [16, 23].

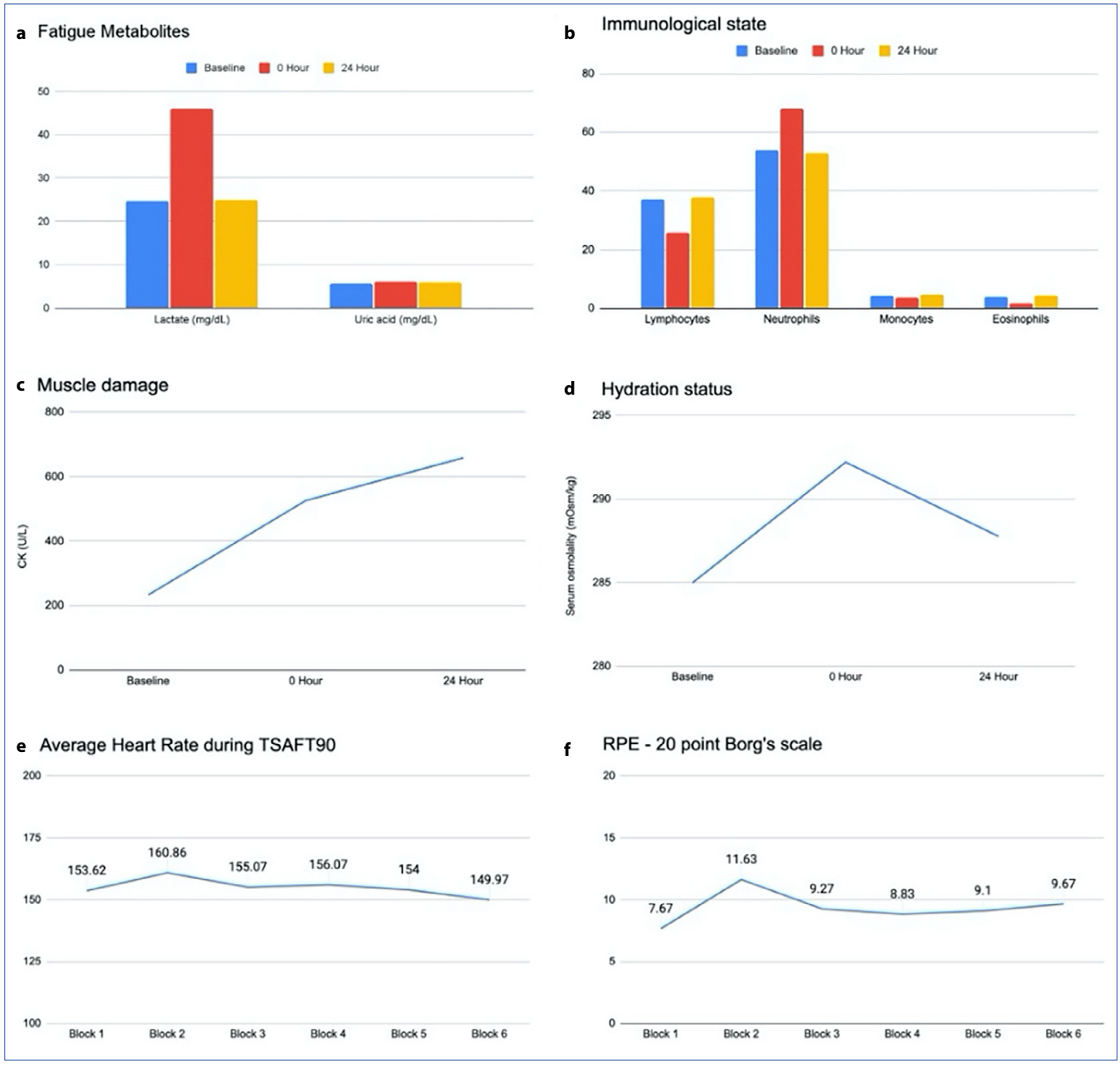

Figure 2. Graphical representation of Biomarkers over three timelines (Baseline, 0 hour, and 24-hour post-fatigue). (a) Fatigue metabolites, (b) immunological markers, (c) muscle damage markers, (d) hydration indicator, (e) average heart rate throughout the fatigue program, (f) RPE using 20-point Borg's scale during six blocks of the fatigue program

RPE: Rate of Perceived Exertion.

Exercise-induced muscle damage leads to an inflammatory response, indicated by increased levels of biomarkers such as CRP, IL-6, IL-1β, and TNF-α [24]. C-reactive protein and interleukins are linked with the physical stress of match-play and an increased susceptibility to injury risk, overreaching, and overtraining [5]. Jatene P et al. [25] have stated that CRP is a reliable biochemical marker for internal load monitoring after training or match-play with high external loads. In this study, CRP levels were significantly elevated at both 0 Hr ($p=0.02$) and 24 Hr ($p=0.01$) post-fatigue. Recent reviews [24] also state that football matches affect the circulating levels of CRP, which tend to peak 24 hours postgame and return to baseline within 72 hours of recovery time. Therefore, a congested match schedule can adversely affect an athlete's physiological system's inflammatory response.

Sweat is hypotonic, and exercise-induced dehydration primarily results in a decrease in extracellular fluid volume due to plasma water loss, a condition known as hypertonic hy-

povolemia. Biomarkers of hemoconcentration have been extensively used as indicators of dehydration. Serum osmolality is the gold standard for assessing acute and dynamic changes in hydration status, particularly in sports [12, 26]. Various studies have identified a dehydration threshold for blood osmolality at 295 mOsm/kg of plasma water [27, 28]. In the current study, football players were within threshold limits at 0 hours and 24 hours, yet there was a significant statistical derangement in hydration status. Magnesium, a crucial micronutrient for recovery [29], plays a vital role in energy production and storage, blood glucose level maintenance, and normal muscle function [14]. Our footballers showed a significant downward trend in magnesium levels post-fatigue. Plasma volume and blood micronutrient concentration, especially magnesium, are interrelated, as demonstrated in this study.

The secondary objective was to evaluate the impact of fatigue on performance through kicking velocity and recovery from muscle soreness. There was a significant decrease in ball velocity post-fatigue ($p=0.04$) from 94.67 km/hr to 90.47 km/hr, reflecting reduced performance. The perceptual measure for grading muscle soreness, Likert's 7-point scale for lower limb soreness, showed no significant change after 24 hours ($p=0.07$). This finding contrasts with the magnitude of disrupted homeostasis in relation to the biochemical internal load of the players. One hypothesis is that when players feel less fatigued, they may train or play at higher intensities, potentially increasing the risk of injuries [30].

Limitations

The levels of biochemical parameters may fluctuate based on an individual's lifestyle, fitness status, training history, nutrition, psychological state, genetics, previous injuries, chronic diseases, and immune status. Additionally, exhausting players for 90 minutes in an indoor environment may differ from an actual outdoor football match. Future studies should aim to bridge the gap between biochemical adaptations to fatigue and their practical applications in preventing injuries and overtraining in football.

Practical implications

- The internal homeostasis of football players remains severely deranged 24 hours after the TSAFT 90 fatigue program. This information can assist in scheduling training sessions and matches to allow for adequate recovery.
- Elevated CK, CRP, UA, and leukocytes may indicate muscle damage and ongoing inflammation. This finding underscores the need for sports scientists to adapt appropriate recovery strategies and monitor the training load to improve the fatigue threshold. These parameters can serve as performance and recovery indicators.
- An increase in serum osmolality and a decrease in magnesium disrupt hydration levels, contributing to fatigue and causing burnout that affects performance. Thus, magnesium supplementation can aid in achieving euhydration and ultimately promote recovery. Tailored planning of pre, during, and post-match nutritional supplementation by sports nutritionists can significantly manage fatigue.
- From an injury prevention and management perspective, these biochemical indices can assist in examining neuromuscular fatigue related to injury risk parameters and in evaluating the late-stage rehabilitation status of players returning from injury. Monitoring the athletes with periodic checks using biomarkers can contribute to longitudinal management to reduce injury and illness risk.
- There was no significant increase in soreness scale scores at 24 hours, which allows players to engage in intensive training or matches, thus increasing the risk of acute and overuse injuries. The mismatch between perceived and physiological differences in fatigue demonstrates that biomarkers may be more reliable than subjective soreness assessments.

Conclusion

All fatigue biomarkers analyzed in the study were significantly elevated immediately post-TSAFT 90 simulation program. The failure of these biomarkers (UA, CK, leukocytes, CRP, serum osmolality, and magnesium) to return to baseline levels within 24 hours suggests disrupted homeostasis. Poor internal recovery at this time can affect performance and increase the likelihood of injury.

Acknowledgements: The authors would like to thank all participants for their commitment and effort to the study. The authors would also like to thank Ric Lovell [Associate Professor (Sport & Exercise Science), School of Science and Health, Western Sydney University, Australia] and Cristiano Diniz da Silva [Assistant Professor, Juiz de Fora Federal University, Brazil]for sharing the TSAFT[90] program details and helping in effectively replicating the program for our players.

Conflict of Interest: The authors declare that there is no conflict of interest.

Ethics Committee Approval: The study was approved by The Sri Ramachandra Institute of Higher Education and Research Ethics Committee (No: IEC-NI/21/APR/78/82, Date: 07/07/2021).

Financial Disclosure: The authors declared that this study has received financial support from ICMR (Indian Council for Medical Research) through their ICMR TSS fellowship scheme (Reg No. U04M180052).

Peer-review: Externally peer-reviewed.

Authorship Contributions: Concept – J.G., T.A., A.S.; Design – J.G., S.S.; Supervision – S.S., T.A.; Materials – J.G., K.D., Z.B.P.; Data collection &/or processing – K.D., Z.B.P.; Analysis and/or interpretation – S.S.; Literature search – J.G., S.S.; Writing – J.G., S.S.; Critical review – S.S., A.S.

References

1. Abd-Elfattah HM, Abdelazeim FH, Elshennawy S. Physical and cognitive consequences of fatigue: A review. J Adv Res 2015;6(3):351–8. [CrossRef]
2. Rahnama N. Prevention of football injuries. Int J Prev Med 2011;2(1):38–40.
3. Zouhal H, LeMoal E, Wong DP, BenOunis O, Castagna C, Duluc C, et al. Physiological responses of general vs. specific aerobic endurance exercises in soccer. Asian J Sports Med 2013;4(3):213–20. [CrossRef]
4. Magalhães J, Rebelo A, Oliveira E, Silva JR, Marques F, Ascensão A. Impact of Loughborough Intermittent Shuttle Test versus soccer match on physiological, biochemical and neuromuscular parameters. Eur J Appl Physiol 2010;108(1):39–48. [CrossRef]
5. Jones CM, Griffiths PC, Mellalieu SD. Training Load and Fatigue Marker Associations with Injury and Illness: A Systematic Review of Longitudinal Studies. Sports Med Auckl NZ. 2017;47(5):943–74. [CrossRef]
6. Theofilidis G, Bogdanis GC, Koutedakis Y, Karatzaferi C. Monitoring exercise-induced muscle fatigue and adaptations: Making sense of popular or emerging indices and biomarkers. Sports 2018;6(4):153. [CrossRef]
7. Silva JR, Ascensão A, Marques F, Seabra A, Rebelo A, Magalhães J. Neuromuscular function, hormonal and redox status and muscle damage of professional soccer players after a high-level competitive match. Eur J Appl Physiol 2013;113(9):2193–201. [CrossRef]
8. Greenham G, Buckley JD, Garrett J, Eston R, Norton K. Biomarkers of physiological responses to periods of intensified, non-resistance-based exercise training in well-trained male athletes: A systematic review and meta-analysis. Sports Med 2018;48(11):2517–48. [CrossRef]
9. Stajer V, Vranes M, Ostojic SM. Correlation between biomarkers of creatine metabolism and serum indicators of peripheral muscle fatigue during exhaustive exercise in active men. Res Sports Med 2020;28(1):147–54. [CrossRef]
10. Twist C, Waldron M, Highton J, Burt D, Daniels M. Neuromuscular, biochemical and perceptual post-match fatigue in professional rugby league forwards and backs. J Sports Sci 2012;30(4):359–67. [CrossRef]
11. Ascensão A, Rebelo A, Oliveira E, Marques F, Pereira L, Magalhães J. Biochemical impact of a soccer match - analysis of oxidative stress and muscle damage markers throughout recovery. Clin Biochem 2008;41(10–11):841–51. [CrossRef]
12. Lee EC, Fragala MS, Kavouras SA, Queen RM, Pryor JL, Casa DJ. Biomarkers in sports and exercise: Tracking health, performance, and recovery in athletes. J Strength Cond Res 2017;31(10):2920. [CrossRef]
13. Lau WY, Kato H, Nosaka K. Effect of oral rehydration solution versus spring water intake during exercise in the heat on muscle cramp susceptibility of young men. J Int Soc Sports Nutr 2021;18(1):22. [CrossRef]
14. Volpe SL. Magnesium and the athlete. Curr Sports Med Rep 2015;14(4):279–83. [CrossRef]
15. Field AC, Harper LD, W F Aldous J, Page RM. A commentary on soccer match-play simulations for applied research and practice. Sci Med Footb 2023;7(2):93–105. [CrossRef]
16. da Silva CD, Lovell R. External validity of the T-SAFT90: A soccer simulation including technical and jumping activities. Int J Sports Physiol Perform 2020;15(8):1074–80. [CrossRef]
17. Rađa A, Kuvačić G, Giorgio AD, Sellami M, Ardigò LP, Bragazzi NL, et al. The ball kicking speed: A new, efficient performance indicator in youth soccer. PLoS ONE. 2019;14(5):e0217101. [CrossRef]
18. Impellizzeri FM, Maffiuletti NA. Convergent evidence for construct validity of a 7-point likert scale of lower limb muscle soreness. Clin J Sport Med Off J Can Acad Sport Med 2007;17(6):494–6. [CrossRef]
19. Tauler P, Aguiló A, Gimeno I, Fuentespina E, Tur JA, Pons A. Influence of vitamin C diet supplementation on endogenous antioxidant defences during exhaustive exercise. Pflugers Arch 2003;446(6):658–64. [CrossRef]
20. Knight JSM, Webster S, Purdue J. Managing fatigue and early infection in football: A novel approach. Proc Footb Med Strateg Return Play Lond, 9–10 Apr, London UK, 2016, s. 227–229.
21. Becatti M, Mannucci A, Barygina V, Mascherini G, Emmi G, Silvestri E, et al. Redox status alterations during the competitive season in elite soccer players: Focus on peripheral leukocyte-derived ROS. Intern Emerg Med 2017;12(6):777–88. [CrossRef]
22. Carrera-Quintanar L, Funes L, Vicente-Salar N, Blasco-Lafarga C, Pons A, Micol V, et al. Effect of polyphenol supplements on redox status of blood cells: A randomized controlled exercise training trial. Eur J Nutr 2015;54(7):1081–93. [CrossRef]
23. Perroni F, Migliaccio S, Borrione P, Vetrano M, Amatori S, Sisti D, et al. Can haematological and hormonal biomarkers predict fitness parameters in youth soccer players? A pilot study. Int J Environ Res Public Health 2020;17(17):6294. [CrossRef]
24. Pérez-Castillo ÍM, Rueda R, Bouzamondo H, López-Chicharro J, Mihic N. Biomarkers of post-match recovery in semi-professional and professional football (soccer). Front Physiol 2023;14:1167449. [CrossRef]
25. Jatene P, Santos GS dos, Portella DL. C-Reactive protein serum levels as an internal load indicator of sprints in competitive football matches. Int J Sports Med 2019;40(12):762–7. [CrossRef]
26. Armstrong LE, Maughan RJ, Senay LC, Shirreffs SM. Limitations to the use of plasma osmolality as a hydration biomarker. Am J Clin Nutr 2013;98(2):503–4. [CrossRef]
27. Cheuvront SN, Kenefick RW, Charkoudian N, Sawka MN. Physiologic basis for understanding quantitative dehydration assessment. Am J Clin Nutr 2013;97(3):455–62. [CrossRef]
28. Sawka MN, Burke LM, Eichner ER, Maughan RJ, Montain SJ, et al; American College of Sports Medicine. American College of Sports Medicine position stand. Exercise and fluid replacement. Med Sci Sports Exerc 2007;39(2):377–90.
29. Zhang Y, Xun P, Wang R, Mao L, He K. Can magnesium enhance exercise performance? Nutrients 2017;9(9):946. [CrossRef]
30. Killen NM, Gabbett TJ, Jenkins DG. Training loads and incidence of injury during the preseason in professional rugby league players. J Strength Cond Res 2010;24(8):2079–84. [CrossRef]