Effect of 12-week endurance training on biochemical parameters in elite football players: A comprehensive analysis

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ABSTRACT
Endurance training plays a pivotal role in football performance, shaping players’ cardiovascular fitness and metabolic adjustments. The purpose of this study is to investigate the different changes in biochemical parameters after exposure to 12 weeks of circuit training based on endurance in football. A total of fifteen elite football players, with an average age of 16.87 ± 1.13 years, an average weight of 61.87 ± 5.94 kg, and an average height of 172.82 ± 5.18 cm, participated in the study. On average, they had been training for 5.0 ± 1.0 years and had intermediate experience in national-level competitions for 4.0 ± 1.0 years. Following the initial data collection, the participants engaged in a twelve-week endurance training programme, involving one hour of daily training for four days a week. Pre-experimental designs were used in the data collection process. Further, for the analysis and extraction of data, descriptive statistics and t-tests were employed. The significance level was set at P < 0.05. Significant changes were found from baseline to post-treatment in each player’s biochemical parameters. A significant increase in HDL, haemoglobin, creatine, and RBC and a significant decrease in LDL and VLDL were observed. The findings of this study underscore the potential benefits of endurance training for elite football players, as reflected in positive alterations in various biochemical parameters. However, for more comprehensive conclusions and a deeper understanding of the broader implications, future research is recommended with larger sample sizes, control groups, and more detailed performance outcome measurements. This research contributes to our knowledge of the physiological responses of elite football players to endurance training and the potential advantages, it offers in optimising their performance and overall health.

Keywords: HDL; LDL; VLDL; creatine; hemoglobin

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INTRODUCTION
Circuit training, a popular type of exercise, integrates strength training and cardiovascular exercise in a series of consecutive stations (Paoli et al., 2013; Mohammed et al., 2018). This dynamic approach to fitness
offers a range of benefits, including improved cardiovascular fitness, muscular strength and endurance (Fragala et al., 2019). However, while circuit training provides these benefits, its direct effects on certain blood components, such as red blood cells (RBCs), high-density lipoprotein (HDL), low-density lipoprotein (LDL), creatine, very low-density lipoprotein (VLDL), and haemoglobin, remain relatively limited (Kostrzewa-Nowak et al., 2020). Nevertheless, understanding these different effects may provide valuable insights into the holistic impact of circuit training on overall health and well-being.

Circuit training, a holistic exercise approach that combines strength and cardiovascular elements (Martin-Niedecken et al., 2020), may indirectly affect haemoglobin levels through improving cardiovascular fitness and oxygen transport capacity (Zivkovic et al., 2013). This is due to regular exercise can stimulate the production of more red blood cells and increase the efficiency of oxygen transport throughout the body, ultimately contributing to changes in performance patterns among athletes (Apostolidis et al., 2014). These interconnected physiological responses highlight the intricate relationship between circuit training, haemoglobin levels and athletic performance.

In the context of football, a sport that demands excellent fitness and endurance due to its dynamic nature, players undergo various forms of training to reach peak performance (Silva et al., 2023; Mujika et al., 2018). The rigorous demands go beyond daily training sessions, encompassing additional responsibilities such as national cups and other competitive events, creating a situation where football players experience significant levels of stress (McKay et al., 2022; Archiza et al., 2018). This co-occurring stress can put pressure on various physiological systems, including the musculoskeletal, neurological, immunological and metabolic systems, often resulting in observable changes in biochemical and haematological indices (Huggett, 2018). Therefore, the rigorous nature of the sport of football underscores the importance of understanding the interactions between training regimens, physiological responses and athletic performance.

Numerous epidemiological studies have consistently shown beneficial changes in blood lipid and lipoprotein levels resulting from regular aerobic exercise among both sexes (Sotiropoulos et al., 2009; Ruiz-Ramie et al., 2019; Martin-Niedecken et al., 2020). However, the lack of research dedicated to elite football athletes, particularly regarding their biochemical status, is noteworthy. Elite athletes engage in specialised training routines that may give rise to unique metabolic adaptations, potentially affecting their blood lipid profiles (Kostrzewa-Nowak et al., 2020). Thus, understanding specific lipid profile changes in elite players may provide insights to optimise training, nutrition and recovery strategies, which may ultimately improve cardiovascular health and longevity in the context of an intensive athletic career.

Furthermore, competitive stressors in sport can trigger physiological responses that affect the musculoskeletal, neurological, immunological and metabolic systems (Kostrzewa-Nowak et al., 2020). This can manifest as changes in stress-related biomarkers such as cortisol, serotonin, dopamine, creatine kinase (CK), lactate dehydrogenase (LDH), and myoglobin (Oglodek, 2022). Despite the recognition and admiration for football, limited research has explored the biochemistry and haematology of football players, especially regarding the physiological stressors imposed by the nature of the sport (Azmi & Kusnanik, 2018). Therefore, delving into this aspect has become very urgent to not only improve our knowledge of athletes’ health, but also to develop better strategies in managing competitive stress that may significantly affect their performance.

Based on these considerations, this study investigated the impact of circuit training on biochemical parameters. Circuit training, which resembles high-intensity interval training, combines resistance and endurance training and involves a series of exercises performed at different stations (Silva et al., 2023). We conducted this study over half a competitive season on elite U-19 football players, aiming to deepen our understanding of their biochemical responses. The insights gained may prove valuable to coaches, physiotherapists and exercise experts, informing strategies to optimise training and performance among elite football players.

**METHOD**

The current study was carried out at Poloi Academy of Manipur, India, and involved fifteen elite football players who were part of the U-19 national team. These players had an average age of 16.87 ± 1.13 years, an
average weight of 61.87 ± 5.94 kg, and an average height of 172.82 ± 5.18 cm. They had a consistent track record of participating in competitions for the past 3-4 years and had also taken part in National Level Competitions. Before the training program commenced, the players were provided with detailed information about the study procedures, and informed consent was obtained from both the individuals and their parents through the academy. The study protocol was adhered to for each participant. Following the baseline data collection, all selected players underwent a 12-weeks endurance training program (Luo et al., 2023). The research took place between June and October 2022. The Institutional Human Ethical Committee approved the study protocol (Reference: TU/IHEC/3/1/22), ensuring compliance with the principles outlined in the declaration of Helsinki concerning human experimentation. The study focused on analyzing several biochemical variables, including red blood cells (RBC), creatine, hemoglobin, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL). The circuit training schedule program is represented in Fig 1.

### Table 1. 12 Weeks Circuit Training Program on Elite Football Players

<table>
<thead>
<tr>
<th>S. No</th>
<th>Exercise</th>
<th>Repetitions</th>
<th>Sets</th>
<th>Total duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Push up</td>
<td>25-30sec</td>
<td>40-45sec</td>
<td>3</td>
</tr>
<tr>
<td>2.</td>
<td>High knee</td>
<td>25-30sec</td>
<td>40-45sec</td>
<td>3</td>
</tr>
<tr>
<td>3.</td>
<td>Plank</td>
<td>25-30sec</td>
<td>40-45sec</td>
<td>3</td>
</tr>
<tr>
<td>4.</td>
<td>Burpee</td>
<td>25-30sec</td>
<td>40-45sec</td>
<td>3</td>
</tr>
<tr>
<td>5.</td>
<td>Butt kick</td>
<td>25-30sec</td>
<td>40-45sec</td>
<td>3</td>
</tr>
<tr>
<td>6.</td>
<td>Mountain climbing</td>
<td>25-30sec</td>
<td>40-45sec</td>
<td>3</td>
</tr>
<tr>
<td>7.</td>
<td>Skipping</td>
<td>25-30sec</td>
<td>40-45sec</td>
<td>3</td>
</tr>
<tr>
<td>8.</td>
<td>Sit up</td>
<td>25-30sec</td>
<td>40-45sec</td>
<td>3</td>
</tr>
</tbody>
</table>

**Figure 1. Schematic Diagram of Circuit Training Schedule**

**Procedure of Data Collection**

For the blood collection, players were asked to place their forearm in a comfortable position. Before and after the training, a total of five milliliters of blood were drawn from the antecubital vein of each player...
using two test tubes for the biochemical study, following the method described (Guo et al., 2022). Subsequently, the collected blood samples were used for the biochemical analysis.

**Data Analysis**

The data obtained from the study was analyzed using statistical software called SPSS version 27. Descriptive analysis, including calculations of mean, standard deviation (SD), and standard error mean (SEM) for each test, was conducted. Additionally, inferential statistics, specifically the pair sample t-test, were employed to determine the significance of differences between the Pre and Post tests.

**Ethics**

The study adhered to ethical guidelines outlined in the Declaration of Helsinki and received approval from the Institutional Human Ethical Committee (Reference: TU/IHEC/3/1/22). Prior to their involvement in the study, all participants or their legal guardians provided informed consent. Detailed information about the study protocol was provided to the participants, and they willingly signed the consent statement before participating in the study.

**RESULTS AND DISCUSSION**

The purpose of this study was to investigate how a 12-week circuit training program would influence both Biochemical Parameters and Skill Proficiency in U-19 elite football players. The researchers achieved this by comparing the baseline data with the data collected after the 12-week training period. The data collected from a group of 15 carefully selected subjects underwent comprehensive statistical analysis, and the findings of this study are elaborated upon below.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>N</th>
<th>Test</th>
<th>Mean</th>
<th>SD</th>
<th>SEM</th>
<th>t-Value</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC</td>
<td>15</td>
<td>Pre test</td>
<td>5.57</td>
<td>.37</td>
<td>.09</td>
<td>3.51</td>
<td>.003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post test</td>
<td>5.65</td>
<td>.36</td>
<td>.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatine</td>
<td>15</td>
<td>Pre test</td>
<td>.91</td>
<td>.11</td>
<td>.02</td>
<td>3.59</td>
<td>.003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post test</td>
<td>.99</td>
<td>.10</td>
<td>.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>15</td>
<td>Pre test</td>
<td>14.95</td>
<td>1.15</td>
<td>.29</td>
<td>3.09</td>
<td>.008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post test</td>
<td>15.27</td>
<td>1.19</td>
<td>.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL</td>
<td>15</td>
<td>Pre test</td>
<td>54.60</td>
<td>8.50</td>
<td>2.19</td>
<td>9.93</td>
<td>.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post test</td>
<td>60.53</td>
<td>10.22</td>
<td>2.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL</td>
<td>15</td>
<td>Pre test</td>
<td>65.53</td>
<td>21.97</td>
<td>5.67</td>
<td>3.19</td>
<td>.006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post test</td>
<td>57.80</td>
<td>17.73</td>
<td>4.57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLDL</td>
<td>15</td>
<td>Pre test</td>
<td>16.20</td>
<td>4.57</td>
<td>4.57</td>
<td>6.34</td>
<td>.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post test</td>
<td>13.73</td>
<td>4.16</td>
<td>4.16</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The effect of circuit training on RBC evoked a significant increase from pre to post data (5.57 ± .37) to (5.65 ± .36) which shown a t value of 3.51 having p-Value of .003 which is significant at .003. When we compare with this pre value with biological reference frame by the WHO (4.5-5.5) reference value, the effect in the changes has surpass the borderline of RBC which need a concern on this specific variable for further studies. The same changes have also observed on creatine variables after 12 weeks circuit training.

The mean and SD observed in the pre were (.91 ± .11) which further increase to (.99 ± .10) after long 12 weeks circuit training. In addition, significant changes were observed when we compared the pre data to post data. The observed t value in the comparison was 3.59 which is significant at (p = .003). The hemoglobin changes depicted from the training of circuit training of 12 weeks was (14.95 ± 1.15) pre and (15.27 ± 1.19) post. And the significant difference found between pre and post were p = .008 having a t value of 3.09 which is significant at 0.05 significant level.

Evidence shown by the table 4.2 on HDL shown a significant increase from pre to post (54.60 ± 8.50) and (60.53 ± 10.22). Further shown a significant t value of 9.93 in compare to pre and post which is significant at 0.05 level. The study shown a significant reduction in the mean value from pre to post in case of LDL which
shown positive effect on the changes of biochemical variables. The mean value of LDL from pre to post were (65.53 ± 21.97) and (57.80 ± 17.73) respectively. The result has been compared to find the significant difference and the t value of 3.19 was observed with significant value of p = .006. The mean value and Sd observed for pre and post of VLDL were (16.20 ± 4.57) and (13.73 ± 4.16) respectively. In addition, significant difference was also observed at 0.05 level of significance.

The findings of this study suggest that the circuit training programme induced significant changes in selected variables, including various physiological alterations that led to improved individual performance. Red blood cells (RBCs) play a crucial role in microcirculation by facilitating the exchange of oxygen and carbon dioxide with surrounding tissues (Beunders et al., 2020; Gates et al., 2013). To navigate microscopic capillaries, RBCs must deform, and their primary function is to transport respiratory gases. During exercise, the circulatory system ensures the supply of substrates to working muscles. The primary role of RBCs during exercise is to transport oxygen from the lungs to tissues in response to the body’s demands and to transport metabolically produced carbon dioxide to the lungs for elimination (Mairbäurl, 2013; Kostrzewa-Nowak et al., 2015). They also contribute to energy production and repair. This study observed significant changes in red blood cell counts in both training methods. Untrained healthy adults, as per Calahorro (2011), can benefit from a regular moderate running training programme due to the regeneration of a younger RBC population with improved characteristics, aiding oxygen supply in the microcirculation.

Creatinine levels in the human body vary among individuals. Individuals engaged in moderate or intense physical exercise typically exhibit higher serum creatinine levels than sedentary individuals (Zivkovic et al., 2013). Factors such as age, gender, ethnicity, dietary protein intake, lean mass, and intensity of physical activity can influence creatinine concentrations, either decreasing or increasing them (Arwandi & Firdaus, 2021; Beunders et al., 2020). The current study noted a substantial increase in creatinine levels in football players after a 12-week circuit training program. This increase can be attributed to the various stress-induced muscle changes that result in muscle tear and injury, with creatinine being expelled as a waste product through the kidneys (Bompa & Buzzichelli, 2019). Muscle requires a certain amount of creatine for energy synthesis to adapt to training-induced stress and increased loads.

The study’s alignment with Ruiz-Ramie et al. (2019) findings underscores the consistency of the impact of light to moderate exercise on net protein catabolism and creatinine excretion. Additionally, this perspective on creatine concentration, as introduced in the presented study, sheds light on the significance of monitoring creatinine expulsion as an indicator of metabolic adaptations in response to exercise (Asadi, 2016). These insights contribute to our understanding of how exercise-induced stress influences metabolic processes and further emphasise the relevance of creatinine as a biomarker for muscular stress and adaptation (Guo et al., 2022).

Exercise has the potential to elevate haemoglobin levels and improve the overall quality of life. Although this study focused on women, it addressed a specific community of male U-19 high-level players. The observation of RBCs suggests a likely favourable effect on haemoglobin concentration due to their direct association. As (Törpel et al., 2020) suggests, an increase in RBC volume leads to a higher haemoglobin concentration in the blood, enhancing oxygenated blood distribution in the body and supporting various bodily functions. The current study indicates a trend of rising RBC levels after 12 weeks of circuit training for football players, suggesting a concurrent increase in haemoglobin levels.

Athletes serve as models of HDL regulation due to their elevated HDL levels (Manna et al., 2010). Low HDL concentrations have been identified as a risk factor for cardiac diseases, with a 1 mg/dl increase in HDL lowering heart disease risk by 4% (Gordon et al., 2014). Notably, high-profile players may experience unexpected attacks during matches for unknown reasons. According to Osei et al. (2020), short-term, low-intensity exercise can influence HDL concentration, but unaltered evidence also exists. It has been reported that prolonged activity exceeding 1.5 hours is required for HDL changes to occur during, immediately after, and the day following exercise. In contrast, the present study's statistical data indicate a significant increase in HDL levels in football players after a 12-week circuit training programme, highlighting a pronounced shift in the pattern of HDL levels among these athletes (Sepriadi et al., 2020).
CONCLUSION

The 12-week endurance training programme has shown promise as an avenue for enhancing the performance potential of elite football players. The observed favourable changes in various biochemical markers suggest that focused endurance training can significantly contribute to improving the overall athletic capacity of these players. However, this study acknowledges certain limitations, including the relatively small sample size and the absence of a control group, which may affect the generalizability and causality of the findings. Moreover, individual variations in lifestyle and dietary habits among participants introduce confounding factors that warrant further investigation. Therefore, it is recommended that future research endeavors involve larger-scale studies with diverse participant pools, controlled trials to establish causality, and longitudinal studies to assess the sustainability of the observed changes. This research contributes to our understanding of the potential benefits of endurance-based circuit training for elite football players and underscores the need for further clinical research to precisely elucidate its role in enhancing individual performance and skill development within the professional sports context.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES


