

Modulatory Effects of Physical Fitness Test on Haematological Parameters and Some Enzymes in Some Football Referees in Abeokuta, Ogun State, Nigeria

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Publication Date: 2026/10/06

Abstract: The study comprises of fifty male active referees within the age bracket 30 to 40 years as test subjects and thirty (30) male non-sports men age 30 to 40 years as control subjects. Ethical approval was obtained from the ethical committee of college of Biosciences at the Federal University of Agriculture, Abeokuta, Nigeria. Haematological tests and determination of the activities of glucose kinase, glucose 6-phosphate dehydrogenase, glycogen phosphorylase and glucose 6-phosphatase were carried out on the pre- and post-fitness exercise blood samples of the sports men (Soccer Referees) while same analysis were performed on the control blood samples. The data obtained from the Pre-fitness (PRE-T) and Post-fitness exercise (POST-T) were compared with the control samples. The data (PRE-T, POST-T and Control) were subjected to one way analysis of variance (one-way anova), using statistical package for social sciences (SPSS) V16.0 and the results were presented as mean $\pm$ standard deviation. Post hoc test and test of significance was carried out using LSD at $p < 0.05$. The results of this study on haematocrits reveals that, physical exercise caused significant increase ($p < 0.05$) in the percentage packed cell volume (%PCV), Haemoglobin concentration (Hb), and red blood cells count in the blood of the football referees after subjected to physical fitness exercises compared to their values before the fitness exercise while there were significant difference in the %PCV, Hb and Rbc values of the football referees compared to the values obtained in the control (sedentary people). Fluid was lost as a result of heavy sweating and there was movement of plasma water into the extracellular matrix as a results of accumulation of osmotically active metabolites and filtration due to increased capillary hydrostatic pressure. The study also revealed that the physical fitness test/exercise of the soccer referees have no significant effects on haematological parameters such as MCV, MCH and MCHC when compared with the control. This implies that the exercise does not lead to noticeable changes in the blood corpuscles and haemoglobin of the Soccer referees. This may be attributed to the facts that, the fitness exercise of the soccer referees is regimented in which they have 40 seconds to walk briskly through a distance of 50m at intervals and another 30seconds to run and cover 150m. These altogether is embarked upon 24 times simultaneously covering a distance of 4,800 metres in 28 minutes. The 40seconds walking along the track allows for muscle relaxation and thus reducing rushing of blood to the muscles for oxygen distribution. Significant reduction ($p < 0.05$) in the total white blood cell counts and percentage Neutrophils, Lymphocytes, Eosinophils, Monocytes and Basophils in the blood of the Soccer referees immediately after the fitness test compared with the control white blood cells counts were recorded. Enzymatic activities of glucokinase, glucose 6-phosphate dehydrogenase, glycogen phosphorylase and glucose 6-phosphatase were observed to increase significantly ($p < 0.05$) in the blood of the soccer referees due to fitness exercise compared to the activities of these enzymes in the blood of the control (non sports men) while there were no observed significant difference in the activities of these four enzymes in the blood of the Referees prior to the fitness exercise compare with the control. These suggests that during exercise, rapid metabolic activities such as glycogenolysis, gluconeogenesis, and glycolysis takes place as evident in the activities of the four enzymes under study.

How to Cite: Olurinde, Oluremi Joseph; Olurinde, Olukorede Daniel; Rahman, Samson Adisa; Sulaiman, Lateef Adegboyega (2026) Modulatory Effects of Physical Fitness Test on Haematological Parameters and Some Enzymes in Some Football Referees in Abeokuta, Ogun State, Nigeria. *International Journal of Innovative Science and Research Technology*, 11(9), 3052-3059. <https://doi.org/10.38124/ijisrt/26sep1058>

I. INTRODUCTION

➤ Physical Exercises

Physical exercise involves movement of parts of the body for the purpose of exercising the body parts or the entire body. Such activities could be field and track events, indoor games, gymnastics, swimming and so on. These activities enhance or maintain physical fitness and overall health and wellness of individual (Kylasov, *et al.*, 2011). Frequent and regular physical exercise boosts the immune system and helps prevent certain "diseases of affluence" such as coronary heart disease, (Stampfer *et al.*, 2000) type 2 diabetes and obesity (Hu *et al.*, 2001).

Getting involved in physical exercise could be for the purpose of recreation or professional training as in the case of football referees

➤ The Football Referees

A football referee is an official that is directly involves in the officiating of a football match. They are tasked with the assignment of enforcing the laws of the game and ensure that the game is not put in a state of disrepute.

Football refereeing is a professional hobby in Nigeria registered under the auspices of Nigeria (football) Referees Association (N.R.A) and an affiliate of Nigeria Football Federation (NFF). Members caught across all the 36 states in the country including the Federal Capital Territory, Abuja. Referees are subjected to constant physical fitness test to determine individual referee's level of fitness to officiate in the football leagues in the country based on this fact, various Referees Councils organizes weekly keep-fit exercises for their members to maintain some degree of fitness in referees. There is no doubt that this group of sport men and women are unique set of people that need to maintain good health status in order to remain physically fit to be able to discharge their duties.

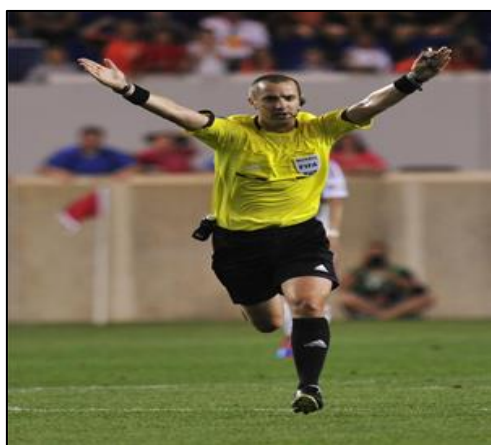


Fig 1 Picture of a Referee on Duty. Typical photograph of a football referee on duty.
<https://www.fifa.com>

➤ Haematology

Haematology is the branch of medicine concerned with the study of the cause, diagnosis, treatment and prevention of diseases related to blood such as iron deficiency, leukaemia, etc. It also involves treating diseases that affect the production of blood and its components, such as blood cells, haemoglobin, blood proteins, bone marrow, platelets, blood vessels, spleen, and the mechanism of coagulation (Ramnik, 2003). Haematological indices are those test parameters used to detect or diagnose specific diseases in blood such as: Packed cell volume (PCV), haemoglobin (Hb), red blood cell counts (Rbc), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and white blood cell counts (WBC).

➤ The Blood

Blood is the transport medium in the body system. It helps to transport metabolites to different parts of the body. It is a constantly circulating fluid that provides the body with oxygen and help in waste removal. Blood comprises of: red blood cells, white blood cells and platelets.

➤ Enzymes

Enzymes are macromolecular biological catalysts they convert substrates into different molecules known as products. They are protein molecules in the cells and works as catalyst. They speed up chemical reactions in the body, but do not get used up. Enzymes possess many characteristics such as : colloidal nature, catalytic nature, specificity, heat sensitivity, reversibility of reaction and P^H sensitivity.

The union between the substrate and the enzyme takes place at the active site more or less in a manner in which key fits a lock and results in the formation of an enzyme substrate complex. The enzyme-substrate complex is highly unstable and almost immediately decomposes to produce the end products of the reaction and regenerate the free enzyme (Jain, *et al.*, 2013).

➤ Chemicals and Reagents

Haemoglobin colorimetric assay kits (Cayman chemical), Normal saline, Hydrochloric acid, EDTA, diagnostic kits and reagents for: glucose 6-phosphate dehydrogenase (Randox), glucokinase, glycogen phosphorylase and glucose-6-phosphatase (Sigma-Aldrich).

➤ Equipment and Apparatus

Weighing balance, Needles and syringes, Plain bottles, Latex gloves, EDTA bottles, Glass slides, Plain capillary tubes, Cotton wool, Methylated Spirit, Haematocrit Centrifuge, Haematocrit reader, plate reader, ELISA machine and UV/Visible Spectrophotometer.

➤ Sample Collection

Thirty (30) male football referees, age between 30-40 years were used for this research work. They were advised against strenuous activities 24 hours before the collection of their venous blood sample, that is, a day prior to the physical fitness test. The blood samples were collected at Moshood Kashimawo Olawale International Stadium, Kuto Abeokuta, Ogun State, training venue of the Football Referees in the state. Venous blood sample of the sport men (Referees) were collected into EDTA bottles before they commenced training for the day. After embarking on the physical fitness exercise, their blood samples were also collected into EDTA bottles. Control samples were also collected from ten (10) volunteers that were not in any form involves in sporting activities. These controls were administered a questionnaire and after analyzing the questionnaires, ten were picked and their blood samples collected. The blood samples (controls and tests) were used for haematological and biochemical tests.

➤ Procedures for Washing of Red Blood Cells:

Washing of the red blood cells was carried out following the method of Handin *et al.*, 2003. Phosphate Buffered Saline (pH 7.0 -7.2) was used; however, 0.9% saline may also be used for the washing.

➤ Determination of Percentage Packed Cell Volume (%PCV).

Microhematocrit method as described by Brian *et al.*, 2000 was used to determine the percentage packed cell volume (%PCV). The determination of the packed cell volume (PCV) using a small quantity of whole blood, a capillary tube, and a high-speed centrifuge. Packed cell volume, PCV. The measure of the ratio of the volume occupied by the red blood cells to the volume of whole blood, expressed as a fraction.

$$\%PCV = \frac{\text{VOLUME OF PACKED RBC} \times 100}{\text{TOTAL VOLUME}}$$

➤ Determination of Haemoglobin (Hb) Concentration (Mg/Dl).

Haemoglobin concentration was calculated from percentage PCV as follows:

- hemoglobin (g/dL) = hematocrit (% PCV) x 0.34
- haemoglobin (g/dL) = hematocrit (decimal fraction) x 34

To convert a hemoglobin result from g/dL to mmol/L, multiply the displayed result by 0.621.

➤ Determination of MCV, MCH And MCHC

Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration were calculated using the formulas:

$$MCV = \frac{PCV \times 10}{Rbc} \quad (fL)$$

$$MCH = \frac{Hb \times 10}{Rbc} \quad (pg).$$

$$MCHC = \frac{Hb \times 100}{PCV} \quad (g/dl).$$

➤ Enzymatic Assay of Glucokinase Activity

Glucokinase specific activity was assayed using Continuous Spectrophotometric Rate Determination according to the method of Womack, *et al.*, 1973 following the procedures described in Sigma Aldrich protocol for the enzymatic assay of Glucokinase (EC 2.7.1.2).

➤ Enzymatic Assay of Glycogen Phosphorylase Activity

Glycogen phosphorylase specific activity was assayed using Continuous Spectrophotometric Rate Determination according to the method of Cori and Green, 1943 following the procedures described in Sigma Aldrich protocol for the enzymatic assay of Glycogen phosphorylase (EC 2.4.1.1).

➤ Enzymatic Assay of Glucose-6-Phosphate Dehydrogenase Activity

Glucose-6-phosphate dehydrogenase specific activity was assayed according to method described by Olive, *et al.*, 1971 following the procedures in Randox Manual for glucose-6-phosphate dehydrogenase.

➤ Enzymatic Determination of Glucose-6-Phosphatase Activity

Specific activity of glucose-6-phosphatase was carried out using Spectrophotometric Stop Rate Determination according to method described by Nordlie and Arion, 1966 following the procedures in Sigma-Aldrich protocol manual.

➤ Statistical Analysis of Data

The data obtained were subjected to one way analysis of variance (one-way anova), using statistical package for social sciences (SPSS) V16.0 and the results were presented as mean ± standard deviation. Post hoc test and test of significance was carried out using LSD at p < 0.05.

II. RESULTS

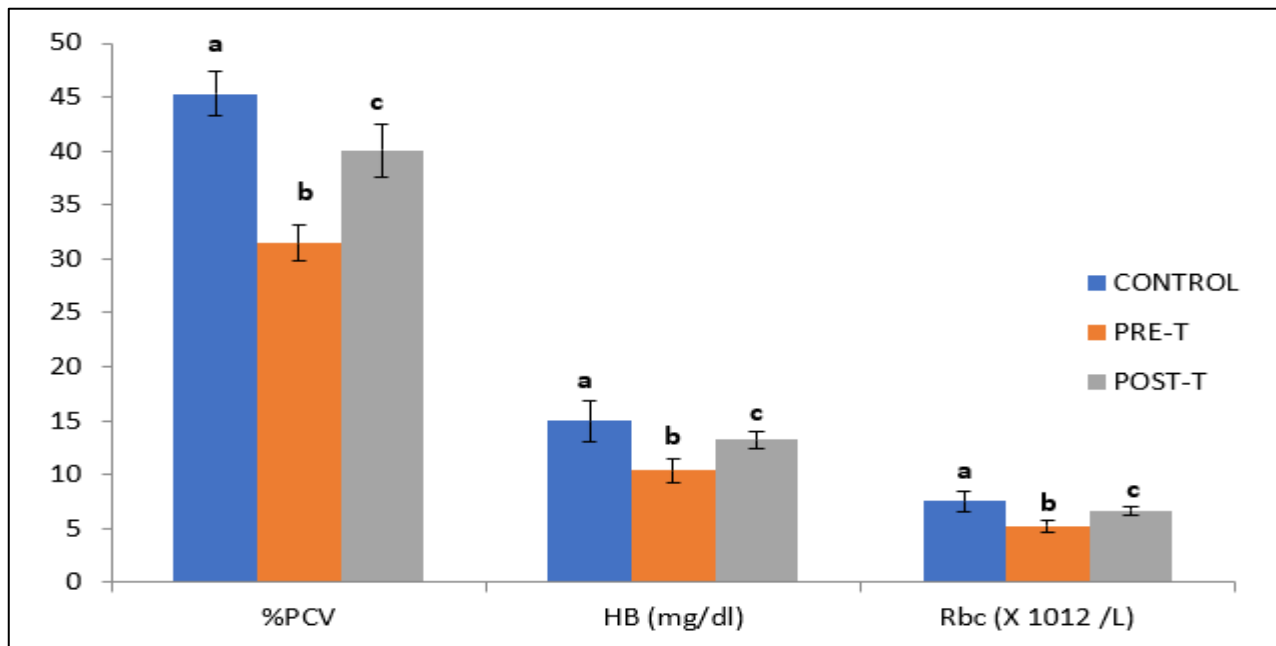


Fig 2 Graph of %PCV, Hb and Rbc

Effect of physical fitness exercise on percentage packed cell volume, haemoglobin concentration and red blood cell counts. Values are expressed as mean $\pm$ standard deviation (Test, n=50, Control n=30). Bars bearing different alphabets are significantly different at $p < 0.05$

Figure 2 shows that there were significance increase in the %PCV, HB and Rbc of the soccer referees after the fitness exercise compared to the values before they embarked on fitness exercise. %PCV, HB and Rbc at both PRE- and POST- fitness exercise were found to be significantly lower compared to control samples.

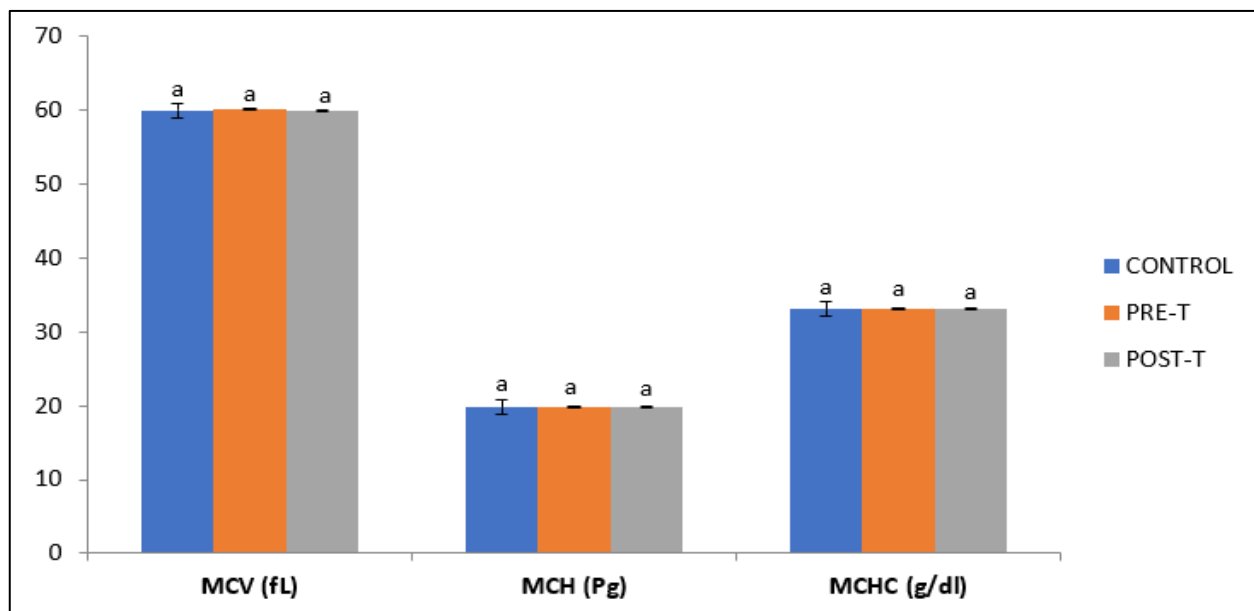


Fig 3 Graph of MCV, MCH and MCHC

Effects of physical fitness exercise on mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration. Values are expressed as mean $\pm$ standard deviation (Test, n=50, Control n=30). Bars bearing the same alphabet are insignificantly different at $p > 0.05$.

Figure 3 reveals that there were no significance difference in the values of MCV, MCH and MCHC at both PRE- and POST-fitness levels compared with the control.

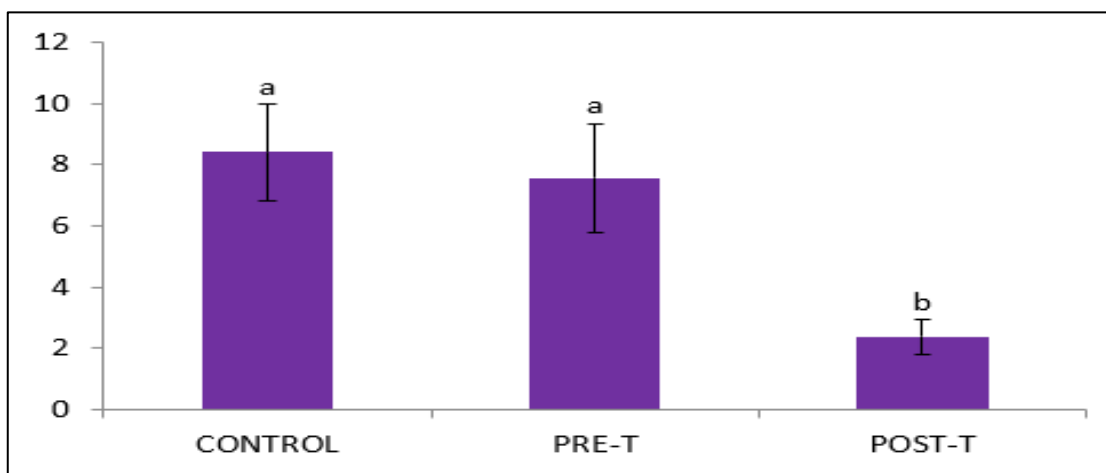


Fig 4 Graph of White Blood Cell Counts (WBC X 10⁹).

Effects of physical fitness exercise on white blood cell counts.

Values are expressed as mean $\pm$ standard deviation (Test, n=50, Control n=30). Bars bearing different alphabets are significantly different while those with the same alphabet are insignificantly different at $p < 0.05$

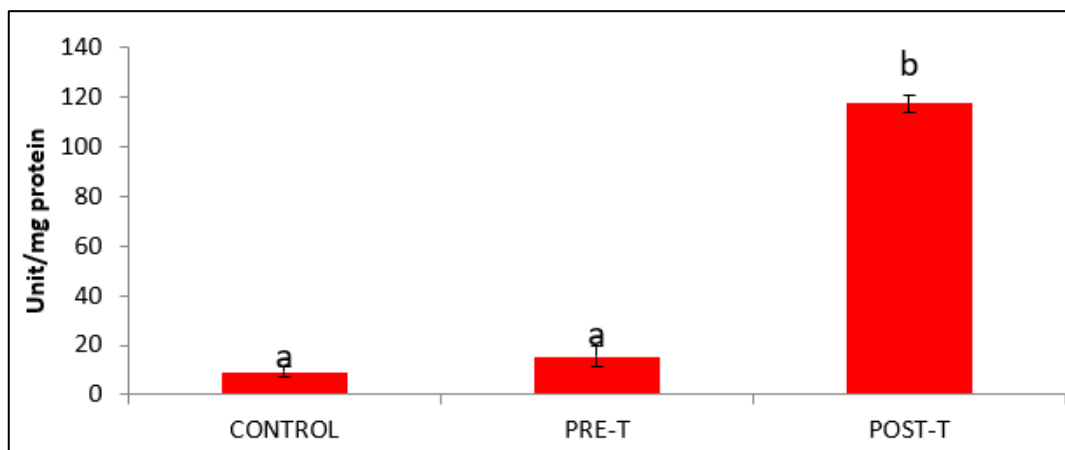


Fig 5 Graph of Glucokinase Specific Activity (Unit/Mg Protein Min⁻¹).

Effects of physical fitness exercise on glucokinase activity.

Values are expressed as mean $\pm$ standard deviation (Test, n=50, Control n=30). Bars bearing different alphabets are significantly different while those with the same alphabet are insignificantly different at $p < 0.05$.

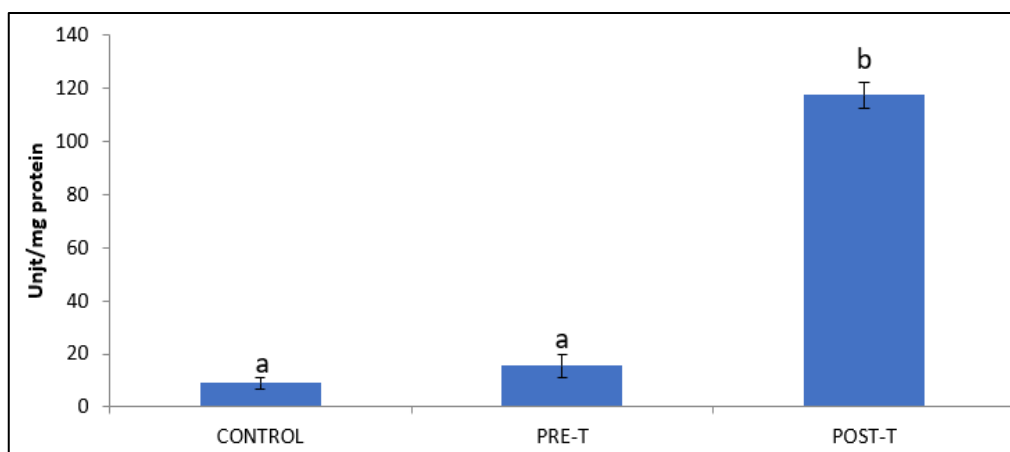


Fig 6 Graph of Glucose-6-Phosphate Dehydrogenase Specific Activity (Unit/Mg Protein Min⁻¹)

Effects of physical fitness exercise on glucose 6-phosphate dehydrogenase activity. Values are expressed as mean $\pm$ standard deviation (Test, n=50, Control n=30). Bars

bearing different alphabets are significantly different while those with the same alphabet are insignificantly different at $p < 0.05$.

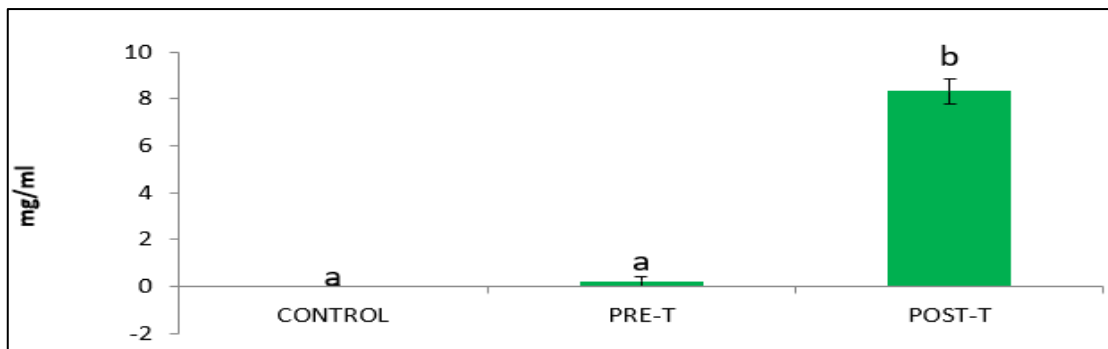


Fig 7 Graph of Glycogen Phosphorylase Specific Activity (Unit/Mg Protein Min⁻¹)

Effects of physical fitness exercise on glycogen phosphorylase activity.

Values are expressed as mean $\pm$ standard deviation (Test, n=50, Control n=30). Bars bearing different alphabets are significantly different while those with the same alphabet are insignificantly different at $p < 0.05$.

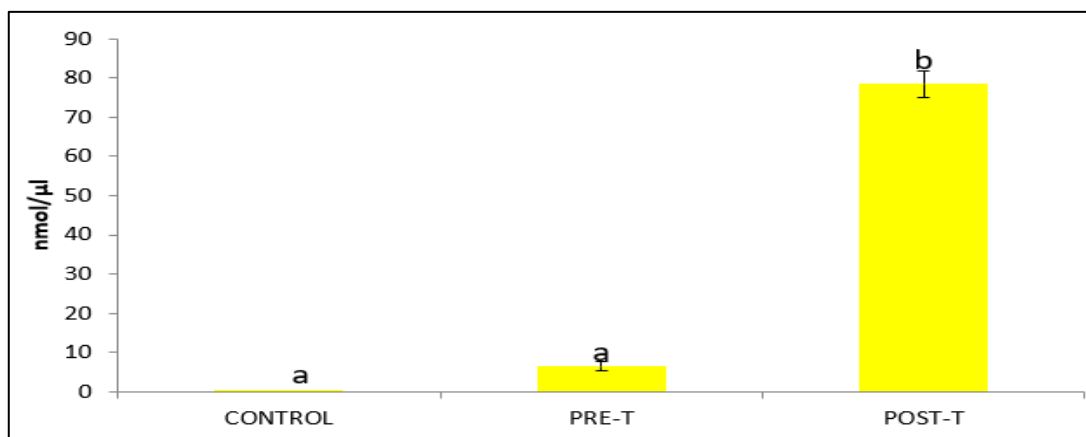


Fig 8 Graph of Glucose6-Phosphatase Specific Activity (Unit/Mg Protein Min⁻¹)

Effects of physical fitness exercise on glucose 6-phosphatase activity.

Values are expressed as mean $\pm$ standard deviation (Test, n=50, Control n=30). Bars bearing different alphabets are significantly different while those with the same alphabet are insignificantly different at $p < 0.05$.

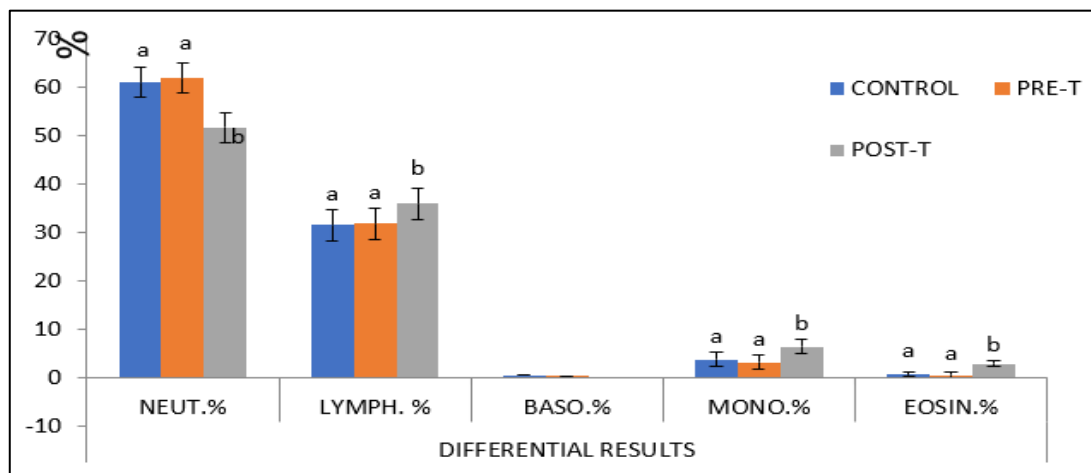


Fig 9 Graph of Differential Results

Effects of physical fitness exercise on Neutrophils (%NEUT.), Lymphocytes (%LYMPH.), Basophils (%BASO.), Monocytes (%MONO.) and Eosinophils (%EOSIN.)

Values are expressed as mean $\pm$ standard deviation (Test, n=50, Control n=30). Bars bearing different alphabets are significantly different while those with the same alphabet are insignificantly different at $p < 0.05$.

➤ **Keys:**

- CONTROL = Blood samples from non sports men
- PRE-T = Blood samples collected prior to fitness exercise.
- POST-T = Blood samples collected immediately after the fitness exercise.

III. DISCUSSION

Physical exercises are carried out for different purposes. It may be to: increase growth and development, prevent aging, strengthening muscles and cardiovascular system, increase athletic skills, weight maintenance, and also enjoyment. Frequent and regular physical exercise aids the immune system and prevent certain diseases such as coronary heart diseases, (Stampfer *et al.*, 2000), type-II diabetes and obesity (Hu. *et al.*, 2001). Exercising the body helps to prevent stress and depression, increase quality of sleep and serves as a non-pharmaceutical sleep aid to treat diseases such as insomnia, help promote or maintain positive self-esteem and improve mental health (Wilmore and Knuttgen 2003).

This study monitored the effects of physical exercise on the haematological indices, white blood cells and activity of some enzymes of football Referees. When exercising, the cardiovascular system has to harness substrate supply to working muscles (Heimo, 2013). The substrates has to be immediately and continually acted upon by enzymes such as glycogen phosphorylase, glucose-6-phosphatase, glucokinase and glucose-6-phosphatedehydrogenase. Un-disturbed blood circulation is also of paramount important during exercise, red blood cells primarily transport oxygen from the lungs to the tissues and deliver CO₂ produced metabolically while exercising to the lungs for expiration (Crawford *et al.*, 2006). Increased blood flow and improved oxygen off-loading from haemoglobin assists the body in meeting up with the increasing O₂ demand during exercise. (Laughlin *et al.*, 2012; Mairbaur, 1994).

The integrity of the red blood cells must be maintained when exercising to be able to perform all its functions thus the necessity for the improved activity of glucose-6-phosphate dehydrogenase to generate NADPH which is much needed in reducing oxidized glutathione to its reduced form.

The results of this study on haematocrits reveals that, physical exercise caused significant increase in the percentage packed cell volume, Haemoglobin concentration (Hb), and red blood cells count of the football referees after subjected to physical fitness exercises compared to their

values before the fitness exercise while there were significant decrease in the %PCV, Hb and Rbc values of the football referees compared to the values obtained in the control (sedentary people). Decreased plasma volume caused haematocrit to increase during exercise because fluid replacement during exercise is limited (Costill *et al.*, 1974). Fluid is lost as a result of high sweating rate, movement of plasma water into the extracellular space as a result of the concentration of osmotically active metabolites and filtration due to increased capillary hydrostatic pressure.

The study also revealed that the physical fitness test/exercise of the soccer referees have no significant effects on haematological parameters such as MCV, MCH and MCHC when compared with the control. This implies that the exercise does not lead to noticeable destruction of the blood corpuscles and haemoglobin in the Soccer referees. This may be attributed to the facts that, the fitness exercise of the soccer referees is regimented in which they have 40 seconds to walk briskly through a distance of 50m at intervals and another 30seconds to run and cover 150m. These altogether is embarked upon 24 times simultaneously covering a distance of 4,800 metres in 28 minutes. The 40s walking along the track allows for muscle relaxation and thus reducing rushing of blood to the muscles for oxygen distribution. The level at which haematocrit fluctuates depends on the intensity of the exercise and the type of the exercise (Hu and Lin, 2012). Despite stimulated erythropoiesis, the red blood mass could decrease when exercising as a results of intravascular haemolysis of senescent red blood cells. During exercise, red blood cells passes through capillaries in contracting muscles, resulting in the compression of red cells thus leading to mechanical rupture e.g., in foot soles during running and in the palms of weightlifters (Heimo, 2013). The insignificance difference in the value of MCH, MCV and MCHC of the soccer referees as compared with the Control (sedentary people), suggest that the regimented fitness tests of the referees requires endurance and does not have negative effect on the haematological indices.

The study further revealed significant reduction in the total white blood cell counts of the Soccer referees immediately after the fitness test compared with the control white blood cells counts as it is evident in the differential counts in percentage (Neutrophils, Lymphocytes, Eosinophils, Monocytes and Basophils) of the football referees compared with the control. The reduction in the white blood cells may be attributed to stress and high temperature encountered by the white blood cells during period of running on the track.

Consequently, enzymatic activities of glucokinase, glucose-6-phosphate dehydrogenase, glycogen phosphorylase and glucose-6-phosphatase were observed to increase significantly in the blood of the soccer referees during exercising compared to the activities of these enzymes in the blood of the control (sedentary people) while there were no observed significant difference in the activities of these four enzymes in the blood of the Referees prior to the fitness exercise compare with the control. These suggests that during exercise, rapid metabolic activities such as

glycogenolysis, gluconeogenesis, and glycolysis takes place as evident in the activities of the four enzymes under study.

IV. CONCLUSION

The results of this study revealed that physical fitness exercise improves the activities of glucose 6-phosphate dehydrogenase, glucokinase, glycogen phosphorylase and glucose 6-phosphatase studied in this work. The improved activities of G6PDH concurrently may lead to generation of more NADPH in the body of the soccer referees thereby resulting in generation of more reduced glutathione which apart from its antioxidant properties plays a vital role in the maintenance of red cells integrity thereby leading to improved haematology in the body of sports men while continuous glycolysis as evident in the activities of glucokinase and other metabolic activities furnishes more ATP to the system hence resulting in improved muscular activities in athletes.

Conclusively, this study shows that physical fitness exercise does not have negative effects on the haematological indices in the body of the football referees, rather it resulted into improved blood circulations and generation of NADPH a reducing equivalent that is highly essential for the maintenance of red cells integrity and reduction of oxidized glutathione (antioxidant) to its reduced form, through improved enzymatic activities. However, it is also worthy of note that observed decrease in plasma volume was recorded. This may largely be attributed to dehydration.

RECOMMENDATIONS

- Sports men and women are encouraged to take in more fluids during and after training to compensate for the lost fluids in the body during and after exercise and maintain adequate balance diet.
- Further studies on the effect of sporting activities on sports men and women involving more rigorous activities and longer time (e.g Marathon race) is suggested.

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