

# EVALUATION OF PLASMA LEVELS OF INTERLEUKIN 6 AND IRON STATUS OF FOOTBALL PLAYERS IN A NIGERIAN UNIVERSITY

## ABSTRACT

To determine the levels of interleukin 6 (IL-6) and iron status of football players in Madonna University, Elele, Rivers State, Nigeria. A total number of 100 subjects were recruited for the study, comprising of 50 of football players before playing football (25 males and 25 females) and 50 of football players after playing football (25 males, 25 females) from Madonna University Nigeria, Elele Campus, Rivers State, Nigeria. The data obtained from the study were presented as Mean  $\pm$  SD in tables and analysed using student t-test for parametric data using SPSS version 20. The level of significance was set at  $p < 0.05$ . The results showed significant increase ( $p = 0.004$ ) in interleukin 6 (IL-6) of football players after playing compared to it before playing and no significant change ( $p = 0.505$ ) in the iron level of football players after playing compared to before playing respectively. The results also showed no significant change in interleukin 6 compared among football players based on gender and age groups and no significant change in the iron level of all the subjects. The study showed increase in interleukin 6 (IL-6) of the football players after playing compared to the level before playing which shows that the physical activity increases the level of interleukin 6 and but has no effect on the iron level after football game.

**Keywords:** interleukin 6, iron, football, exercise, students, University

## INTRODUCTION

It has been reported that in Physical exercise like football that there was a lower load to muscle to perform contraction (Moreira *et al.*, 2014). Muscle adapted to physical load by secreting interleukin-6 into blood stream. Interleukin-6 is an important myokine for muscle adaptation during sports especially football (Chowdhury *et al.*, 2020). It is responsible for inflammatory regulation, protein synthesis, lipid deposition, metabolism and muscle development. Interleukin-

6 was also related to iron deposition involving ferritin, hepcidin and haemoglobin (Nakagawa *et al.*, 2014).

Interleukin-6 is a pro-inflammatory cytokine that can increase following physical exercise (White *et al.*, 2020). Higher level of interleukin-6 is linked to high response of inflammation due to physical exercise like in football which involves the entire body (Cabral-Santos *et al.* 2015). Interleukin-6 stimulates synthesis of hepcidin so that its level raised in the blood during inflammation (Nemeth *et al.*, 2004; D'Angelo, 2013). It is reported by Cullen *et al.* (2016) that the effect of exercise intensity and volume on the interleukin-6 response increases in the high intensity group compared to the low intensity group.

Interleukin 6 (IL-6) is a cytokine that plays a role in the specific antigen immune response and acute inflammatory response (Wolf *et al.*, 2014, Obeagu *et al.*, 2019; Ifeanyi *et al.*, 2020). It is produced in several types of cells and can act in a large number of tissues (Hirano *et al.*, 1990). IL-6 plays a crucial role in the defense response and has a pleiotropic characteristic that can determine more than one phenotypic characteristic (Hirano *et al.*, 1990; Kang *et al.*, 2020). When moderate to extreme intensity exercise (>85–90% of maximal heart rate) is performed, the IL-6 level in the blood circulation increases. Skeletal muscle contraction is the stimulus for its release; thus, it is considered a myokine as it is produced, expressed, and released by muscle and has paracrine and endocrine effects (Pedersen *et al.*, 2004; Reihmane and Dela, 2014). A reduction in the availability of carbohydrates for exercise stimulates the release of IL-6 as it can assist in the maintenance of serum glucose levels during exercise (Reihmane and Dela, 2014). IL-6 is an important marker since an increase in its concentration is associated with an increase in the levels of acute-phase inflammatory proteins, such as C-reactive protein (Estrela *et al.*, 2017), the risk of cardiovascular events, and the process of rupture (Zhao *et al.*, 2017).

Hepcidin plays a key role of ferroportin opening and iron transport via membrane regulation (Coates, 2014). Hepcidin inhibits ferroportin opening so iron fail to export across membrane of erythrocyte and macrophage (Ganz and Nemeth, 2012).

The role of haeme and nonhaeme iron in biological function and sports has been clarified via human and animal studies, and several classic reviews have been published (Finch and Huebers, 1982; Dallman, 1982) and updated (Azevedo *et al.*, 1989). Not surprisingly, haemoglobin iron, when lacking, can greatly affect sports through a reduction in oxygen transport to exercising

muscle. Endurance performance at reduced exercise intensities, however, is more closely related to tissue iron concentrations because of the strong association between the ability to maintain prolonged submaximal exercise and the activity of iron-dependent oxidative enzymes.

The study was done to determine the levels of interleukin 6 (IL-6) and iron status of football players in Madonna University, Elele, Rivers State, Nigeria

## **MATERIALS AND METHODS**

### **Study Design**

The project is a cross-sectional study involving subjects recruited from football players of Madonna University Nigeria, Elele Campus. The subjects encompass males and females football players and apparently healthy individuals age and sex-matched as the controls. The study is a quantitative research to assess the levels of interleukin 6 and iron status of the football players among the students of the University.

### **Study area**

The research was carried out on football players in Madonna University Nigeria, Elele Campus, Rivers State, Nigeria. It is located in the South South part of Nigeria.

### **Study population**

A total number of 100 subjects were recruited for the study, comprising of 50 subjects before playing football (25 males and 25 females) and 50 subjects after playing football (25 males, 25 females) for the normal 90 minutes of football playing in Madonna University Nigeria, Elele Campus, Rivers State, Nigeria. They all gave consent to participate in this study.

### **Inclusion criteria**

Students of Madonna University Nigeria, Elele Campus that are footballers without any sign of disease and apparently healthy individuals were selected for the study.

### **Exclusion criteria**

Any Student of Madonna University Nigeria, Elele Campus that is sick or showed s any sign of disease, pregnant, smoker, alcoholics or aged were excluded for the study.

### **Procurement of iron**

A commercially prepared serum iron test kit product of BioSystems reagents and instruments company limited were used to assay the iron level.

### **Ethical consideration**

The approval for the study was obtained from the Department of Medical Laboratory Science, Madonna University Nigeria, Elele Campus, Rivers State.

### **Laboratory Investigations**

#### **Interleukin 6 (IL-6) determination using Elabscience (Catalog No: E-EL-H0102)**

#### **Procedure**

1. 100 $\mu$ L standard or sample (**plasma**).was added to the wells and incubated for 90 min at 37°C
2. The liquid was discarded, immediately added 100 $\mu$ L Biotinylated Detection Ab working solution to each well and incubated for 60 min at 37°C.
3. The plate was aspirated and washed for 3 times
4. 100 $\mu$ L HRP conjugate working solution was added, incubated for 30 min at 37°C and aspirate d and washed the plate for 5 times
5. 90 $\mu$ L Substrate Reagent was added and incubated for 15 min at 37°C
6. 50 $\mu$ L Stop Solution was added
7. The plate was read at 450nm immediately and the results calculated.

#### **Statistical analysis**

The data obtained from the study were presented as Mean  $\pm$  SD in tables and analysed using student t-test for parametric data using SPSS version 20. The level of significance was set at  $p < 0.05$ .

## RESULTS

**Table 1: Mean  $\pm$  SD values of interleukin 6 (IL-6) and Iron status of the subjects before and after playing football**

Parameters	Before Playing	After Playing	t-value	P-value
IL-6 (pg/ml)	11.55 $\pm$ 2.28	19.81 $\pm$ 3.60	4.291	0.004*
Iron ( $\mu$ g/dl)	82.70 $\pm$ 18.92	93.23 $\pm$ 26.02	0.703	0.505

Table 1 showed significant increase ( $p=0.004$ ) in interleukin 6 (IL-6) **football players** after playing (19.81 $\pm$ 3.60 pg/ml) compared to before playing (11.55 $\pm$ 2.28 pg/ml) and no significant change ( $p=0.505$ ) in **the iron level of football players** after playing (93.23 $\pm$ 26.02  $\mu$ g/dl) compared to before playing (82.70 $\pm$ 18.92  $\mu$ g/dl) respectively.

**Table 2: Mean  $\pm$  SD values of interleukin 6 (IL-6) and Iron status of the Football players based on sex**

Parameters	Male	Female	t-value	P-value
IL-6 (pg/ml)	17.99 $\pm$ 2.43	18.86 $\pm$ 6.53	-0.177	0.876

Iron ( $\mu\text{g/dl}$ )	95.20 $\pm$ 36.49	81.95 $\pm$ 10.40	0.494	0.670
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Table 2 showed no significant change ( $p=0.876$ ) in interleukin 6 (IL-6) of male football players (17.99 $\pm$ 2.43 pg/ml) compared to female football players (18.86 $\pm$ 6.53 pg/ml) and no significant change ( $p=0.670$ ) in the iron level of male football players (95.20 $\pm$ 36.49  $\mu\text{g/dl}$ ) compared to female football players (81.95 $\pm$ 10.40 $\mu\text{g/dl}$ ) respectively.

**Table 3: Mean  $\pm$  SD values of interleukin 6 (IL-6) and Iron status of the Football players based on age bracket**

Parameters	Below 20 years	21-30 Years	t-value	P-value
IL-6 (pg/ml)	13.19 $\pm$ 4.36	18.86 $\pm$ 6.53	-1.021	0.414
Iron ( $\mu\text{g/dl}$ )	90.05 $\pm$ 43.77	81.95 $\pm$ 10.40	0.255	0.823

Table 3 showed no significant change ( $p=0.414$ ) in interleukin 6 (IL-6) of football players aged below 20 years (13.19 $\pm$ 4.36 pg/ml) compared to football players aged above 21- 30 years (18.86 $\pm$ 6.53 pg/ml) and no significant change ( $p=0.823$ ) in the iron level of football players aged below 20 years (90.05 $\pm$ 43.77  $\mu\text{g/dl}$ ) compared to football players aged 21- 30 years (81.95 $\pm$ 10.40  $\mu\text{g/dl}$ ) respectively.

## DISCUSSION

The study showed increase in interleukin 6 (IL-6) of football players after playing that was statistically significant. It is also known that muscular exercise enhances plasma levels of some cytokines (Ronsen *et al.*, 2002). Several studies

demonstrated that strenuous exercise is accompanied by an increase in circulating pro-inflammatory responsive cytokines along with other bioactive stress molecules having some similarities with the response to sepsis and trauma (Hoffman-Goetz and Pedersen, 1994; Pedersen *et al.*, 1997). It has been shown that physical activity such as exercises to the muscles increase the level of secretion and release of interleukin 6 from the muscles as well as from the lymphocytes. Despite the difficulties inherent in measuring plasma cytokines concentrations (Ruiz-Argüelles, 1995), studies of subjects exercising intensively reported conflicting results. Some authors reporting increase (Ostrowski *et al.*, 1998) and others no changes (Rivier *et al.*, 1994) in IL-6 production after strenuous exercise. The stress and oxidation may increase the inflammatory process that will raise the levels of interleukin 6 and regulate iron production through hepcidin regulation. This study also shows a significant increase in IL-6 concentrations for footballers after playing. Thus, it has been demonstrated that plasma concentrations of IL-6 increases up to more than 100-fold during prolonged muscular exercise (Pedersen *et al.*, 2001). The augmented IL-6 plasma concentrations following football was associated with muscle damage in an earlier study (Pedersen *et al.*, 1998), but today it is very clear that exercise without any muscle damage also induces marked production of IL-6 and that IL-6 is produced as a direct consequence of contraction per se (Pedersen *et al.*, 2001).

When interleukin 6 was compared among the males and females, there was no significant change. This shows that gender has no effect on the levels of interleukin 6 as well as the iron status of footballers. Also, when interleukin 6 (IL-6) and iron were compared based on age groups such as below 20 years and 21-30 years of the footballers, there were no significant changes among the age groups.

## **Conclusion**

The study showed increase in interleukin 6 (IL-6) of the **football players** after playing compared to the level before playing which shows that the physical activity increases the level of interleukin 6 and but has no effect on the iron level after football game.

#### COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

#### REFERENCES

- Azevedo, J.L.Jr, Willis, W.T., Turcotte, L.P., Rovner, A.S., Dallman, P.R. and Brooks, G.A. (1989). Reciprocal changes of muscle oxidases and liver enzymes with recovery from iron deficiency. *American Journal of Physiology*. **256**:E401–E405.
- Cabral-Santos, C., Gerosa-Neto, J. and Inoue, D.S. (2015). Similar anti-

- inflammatory acute responses from moderate-intensity continuous and high-intensity intermittent exercise. *Journal of Sports Science and Medicine* **14**, 849-856.
- Chowdhury, S., Schulz, L. and Palmisano, B. (2020). Muscle-derived interleukin 6 increases exercise capacity by signaling in osteoblasts. *Journal of Clinical Investigation* **130**, 2888-2902.
- Coates, T.D. (2014). Physiology and pathophysiology of iron in hemoglobin-associated diseases. *Free Radical Biology and Medicine* **72**: 23-40.
- Cullen, T., Thomas, A.W. and Webb, R. (2016). Interleukin-6 and associated cytokine responses to an acute bout of high-intensity interval exercise: The effect of exercise intensity and volume. *Applied Physiology, Nutrition and Metabolism* **41**, 803-808.
- Dallman, P.R. (1982). Manifestations of iron deficiency. *Seminars in Hematology*. **19**:19–30.
- D'Angelo, G. (2013). Role of hepcidin in the pathophysiology and diagnosis of anemia. *Blood Research*. **48**:10-15.
- Estrela, A.L., Zaparte, A., Da Silva, J.D., Moreira, J.C., Turner, J.E., Bauer, M.E. (2017). High volume exercise training in older athletes influences inflammatory and redox responses to acute exercise. *Journal of Aging and Physical Activity*. **25**(4):559–569.
- Finch, C.A. and Huebers, M.D. (1982). Perspectives in iron metabolism. *New England Journal of Medicine*. **25**:1520–1525.
- Ganz, T. and Nemeth, E. (2012). Hepcidin and iron homeostasis. *Biochimica et Biophysica Acta - Molecular Cell Research* **1823**, 1434-43.
- Hoffman-Goetz, L. and Pedersen, B.K. (1994). Exercise and the immune system: a model of the stress response? *Immunology Today* **15**:382-387.

Hirano, T., Akira, S., Taga, T. and Kishimoto, T. (1990). Biological and clinical aspects of interleukin 6. *Immunology Today*. **11**:443–449.

Ifeanyi, O. E., Uzoma, O. G., Amaeze, A. A., Ijeko, A. E., Felix, C. E., Ngozi, A.

F., Nchekwubedi, C. S., & Chinenye, K. S. (2020). Maternal Expressions (Serum Levels) of Alpha Tumour Necrosis Factor, Interleukin 10, Interleukin 6 and Interleukin 4 in Malaria Infected Pregnant Women Based on Parity in a Tertiary Hospital in Southeast, Nigeria. *Journal of Pharmaceutical Research International*, **32**(23), 35-41.

Kang, S., Narazaki, M., Metwally, H. and Kishimoto, T. (2020). Historical overview of the interleukin-6 family cytokine. *Journal of Experimental Medicine*. **217**(5):4.

Moreira, L.D.F., de Oliveira, M.L. and Lirani-Galvão (2014). Physical exercise and osteoporosis: effects of different types of exercises on bone and physical function of postmenopausal women. *Arquivos Brasileiros de Endocrinologia and Metabologia* **58**:1-9.

Nakagawa, H., Tamura, T. and Mitsuda, Y.(2014). Inverse correlation between serum interleukin-6 and iron levels among Japanese adults: A cross-sectional study. *BMC Hematology* **14**:1-6.

Nemeth, E., Rivera, S. and Gabayan, V. (2004). IL-6 mediates hypoferrremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. *Journal of Clinical Investigation*. 113, 1271-6.

Obeagu, E.I., Vincent, C.C.N. and Chinedu-Madu, J.U. (2019). Studies on some cytokines of apparently healthy Nigerian women aged 10-40 years. *International Journal of Current Research in Medical Sciences*. **5**(12): 24-30

Ostrowski, K., Rohde, T., Zacho, M., Asp, S. and Pedersen, B.K. (1998). Evidence

that IL-6 is produced in skeletal muscle during intense long-term muscle activity. *Journal of Physiology*. **508**:949-953.

Pedersen, B.K., Ostrowski, K., Rohde, T. and Bruunsgaard, H. (1998). The cytokine response to strenuous exercise. *Canadian Journal of Physiology and Pharmacology*. **76**:505-511.

Pedersen, B.K., and Hoffman-Goetz, L. (2000). Exercise and the immune system: regulation, integration and adaptation. *Physiological Reviews* **80**:1055-1081.

Pedersen, B.K., Steensberg, A., Fischer, C. (2004). The metabolic role of IL-6 produced during exercise: is IL-6 an exercise factor? *Proceedings of the Nutrition Society*. **63**(2):263–267.

Pedersen, B.K., Bruunsgaard, Klokke, H.M., Kappel, D.A., Maclean, H.B. and Nielsen, T. (1997). Exercise induced immunomodulation-possible roles of neuroendocrine and metabolic factors. *International Journal of Sports Medicine*. **18**:S2-S7.

Pedersen, B.K., Steensberg, A., Fischer, C., Keller, C., Ostrowski, K. and Schjerling, P. (2001). Exercise and cytokines with particular focus on muscle derived IL-6. *Exercise Immunology Review*. **7**:18-31.

Pedersen, B.K., Steensberg, A. and Schjerling, P. (2001) Exercise and interleukine-6. *Current Opinion in Hematology*. **8**:137-141.

Reihmane, D. and Dela, F. (2014). Interleukin-6: possible biological roles during exercise. *European Journal of Sport Science*. **14**(3):242–250.

- Rivier, A., Pene, J., and Chanez, P. (1994). Release of cytokines by blood monocytes during strenuous exercise. *International Journal of Sports Medicine*. **15**:192-198.
- Ronsen, O., Tor, L., Roald, B., Pedersen, B.K. (2002). Enhanced plasma IL-6 and IL-1ra responses to repeated vs. single bouts of prolonged cycling in elite athletes. *Journal of Applied Physiology*. **92**:2547-2553.
- Rossander-Hulthén, L. and Hallberg L. (1996). Prevalence of iron deficiency in
- Ruiz-Argüelles, G.J. (1995). Laboratory measurement of human cytokines. *Journal of the International Federation of Clinical Chemistry* **7**:12-15.
- White, G.E., West, S.L. and Caterini, J.E. (2020). Massage therapy modulates inflammatory mediators following sprint exercise in healthy male athletes. *Journal of Functional Morphology and Kinesiology* **5**:1-11.
- Wolf, J., Rose-John, S. and Garbers, C. (2014). Interleukin-6 and its receptors: a highly regulated and dynamic system. *Cytokine*. **70**(1):11–20.
- Zhao, L., Wang, X. and Yang, Y. (2017). Association between interleukin-6 and the risk of cardiac events measured by coronary computed tomography angiography. *International Journal of Cardiovascular Imaging*. **33**(8):1237–1244.