

EVALUATION OF PLASMA LEVELS OF INTERLEUKIN 6 AND IRON OF VOLLEYBALL PLAYERS BASED ON HEIGHTS AND WEIGHT OF A NIGERIAN UNIVERSITY STUDENTS

Abstract

Sports is known to increase inflammation which may in turn affect the levels of interleukin 6 and iron in the players such as in volleyball. The study was done to determine the levels of interleukin 6 (IL-6) and iron *levels* of volleyball players based on heights and weights of a Nigerian University *students*. A total number of 80 subjects were recruited for the study, comprising of 40 subjects before playing volleyball (20 males and 20 females) and 40 subjects after playing volleyball (20 males, 20 females) from Madonna University Nigeria, Elele Campus, Rivers State, Nigeria. The level of significance was set at $p < 0.05$. The results showed that there was significant increase ($p = 0.032$) in interleukin 6 (IL-6) of volleyball players of 5.1-6.5M compared to volleyball players of 4.0-5.5M and no significant change ($p = 0.663$) in iron of volleyball players 4.0-5.5M compared to volleyball players 5.1-6.5M respectively. The study showed that there was no significant increase ($p = 0.978$) in interleukin 6 (IL-6) of volleyball players of 50-65Kg compared to volleyball players of 66-85Kg and no significant change ($p = 0.294$) in iron of volleyball players 50-65Kg compared to volleyball players 66-85Kg respectively. During exercise, interleukin 6 (IL-6) but not the iron level increase significantly based upon the height of volleyball players. Both of these parameters do not change significantly based upon their weight

Keywords: interleukin 6, iron, inflammation, iron, volleyball, sports, exercise, muscle

INTRODUCTION

It has been reported that Physical exercise like volleyball 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 volleyball (Chowdhury *et al.*, 2020). It is responsible for inflammatory regulation, protein synthesis, lipid deposition, metabolism and muscle development. Interleukin-6 is also related to iron deposition involving ferritin, hepcidin and haemoglobin (Nakagawa *et al.*, 2014; Obeagu *et al.*, 2021).

It is shown that 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 sports like in volleyball which involves the entire body (Cabral-Santos *et al.* 2015). Interleukin-6 stimulates synthesis of hepcidin so that its level increases 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 of muscles.

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 sports (>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 that 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 stress on the muscles and lymphocytes together with monocytes may change the levels of interleukin 6 after volleyball game and becomes necessary to carry out this research to ascertain what happens in the players the variables will affect the quality of life and wellbeing of the volleyball players. The study was done to **evaluate** the levels of interleukin 6 (IL-6) and iron **in** volleyball players based on **their** heights and weights in Madonna University, Elele, Rivers State, Nigeria

MATERIALS AND METHODS

Study Design

The project is a cross-sectional study involving subjects recruited from volleyball players of Madonna University Nigeria, Elele Campus. **The subjects encompass males and females volleyball players before playing as the control and after playing as the test and were age and sex-matched as the controls.**

Study area

The research was carried out on volleyball 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 80 subjects were recruited for the study, comprising of 40 subjects (**control**) before playing volleyball (20 males and 20 females) and 40 subjects (**test**) after playing

volleyball (20 males, 20 females) from 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 volleyball players without any sign of disease and apparently healthy were selected for the study.

Exclusion criteria

Any student of the volleyball team of Madonna University Nigeria, Elele Campus who was sick or showed any sign of disease, pregnant, smoker, alcoholics or aged were excluded for the study.

Procurement of iron of test kit

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)

Assay procedure

100 μ L standard or sample was added to the wells and incubated for 90 min at 37°C. The liquid was discarded, immediately added 100 μ L Biotinylated Detection Ab working solution to each well and incubated for 60 min at 37°C. The plate was aspirated and washed for 3 times. 100 μ L HRP conjugate working solution was added, incubated for 30 min at 37°C and aspirate d and washed the plate for 5 times. 90 μ L Substrate Reagent was added and incubated for 15 min at 37°C. 50 μ L Stop Solution was added. 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 volleyball players based on heights

Parameters	Heights		t-value	P-value
	4.0-5.5M	5.1-6.5M		
IL-6 (pg/ml)	16.24 \pm 2.42	25.21 \pm 3.43	-3.229	0.032*
Iron (ug/dl)	81.75 \pm 26.52	90.28 \pm 18.78	-0.469	0.663

Table 1 showed that there was significant increase ($p=0.032$) in interleukin 6 (IL-6) of volleyball players of 5.1-6.5M (25.21 \pm 3.43 ug/dl) compared to volleyball players of 4.0-5.5M (16.24 \pm 2.42 pg/ml) and no significant change in iron ($p=0.663$) of volleyball players 4.0-5.5M (81.75 \pm 26.52 ug/dl) compared to volleyball players 5.1-6.5M (90.28 \pm 18.78 ug/dl) respectively.

Table 2: Mean \pm SD values of interleukin 6 (IL-6) and Iron status of volleyball players based on weights

Parameters	Weights		t-value	P-value
	50-65Kg	66-85Kg		
IL-6 (pg/ml)	23.82 \pm 6.12	23.68 \pm 1.58	0.031	0.978
Iron (ug/dl)	85.03 \pm 17.98	103.25 \pm 9.83	-1.268	0.294

Table 2 showed that there was no significant increase ($p=0.978$) in interleukin 6 (IL-6) of volleyball players of 50-65Kg (23.82 ± 6.12 ug/dl) compared to volleyball players of 66-85Kg (23.68 ± 1.58 pg/ml) and no significant change in iron ($p=0.294$) of volleyball players 50-65Kg (85.03 ± 17.98 ug/dl) compared to volleyball players 66-85Kg (103.25 ± 9.83 ug/dl) respectively.

DISCUSSION

The study showed that there was significant increase in interleukin 6 (IL-6) of volleyball players of 5.1-6.5M height compared to volleyball players of 4.0-5.5M and no significant change in iron of volleyball players 4.0-5.5M height compared to volleyball players 5.1-6.5M height respectively. It is also known that muscular exercise enhances plasma levels of some cytokines (Ronsen *et al.*, 2002). Several studies demonstrated that tedious sports 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.

The results also showed that there was no significant increase in interleukin 6 (IL-6) of volleyball players of 50-65Kg weight compared to volleyball players of 66-85Kg weight and no significant change in iron of volleyball players 50-65Kg weight compared to volleyball players 66-85Kg weight respectively. This study also shows a significant increase in IL-6 concentrations for volleyball players 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; Obeagu *et al.*, 2022).

Conclusion

During exercise, interleukin 6 (IL-6) but not the iron level increase significantly based upon the height of volleyball players. Both of these parameters do not change significantly based upon their weight

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, Tand 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., Ijego, 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

Obeagu, E.I., Obeagu, G.U., Emeonye, O.P. and Jakheng, S.P.E.(2022). An update on interleukin 6 and iron status of volleyball players. *Madonna University Journal of Medicine and Health Sciences*. **2**(2): 41-74.

Obeagu, E. I., Amedu, G. O., Okoroiwu, I. L., Okafor, C. J., Okun, O., Ochiabuto, O. M. T. B., & Ukeekwe, C. O. (2021). Evaluation of Plasma Levels of Interleukin 6 and Iron Status of Football

Players in a Nigerian University. *Journal of Pharmaceutical Research International*, 33(59B), 383-388. <https://doi.org/10.9734/jpri/2021/v33i59B34393>

Ostrowski, K., Hermann, C., Bangash, A., Schjerling, P., Nielsen, J.N., Pedersen, B.K. (1998). A trauma-like elevation in plasma cytokines in humans in response to treadmill running. *Journal of Physiology*. (Lond) **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., 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 interleukin-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..

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.