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

Comment [CC1]: This is redundant, needs deletion

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 status of volleyball players based on heights and weights of a Nigerian University 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 50-65Kgcompared to volleyball players 66-85Kgrespectively. The study showed difference in interleukin 6 (IL-6) of the volleyball players based on height and not change in iron based on height and weight together with interleukin 6.

Comment [CC2]: Something missing here, please complete the sentence

Comment [CC3]: This sentence is confusing, needs clarification. Replace it with the following sentence:
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. Interluekin-6 was 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 increased 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 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).

Comment [CC4]: Replace with is

Comment [CC5]: Replace with increases

Comment [CC6]: Add here of muscles like?

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Comment [CC8]: What is the effect of this phenomenon on muscles

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 and ~~iron levels~~after volleyball game.~~and becomes necessary to carry out~~.This is the rationale of presentresearch to ascertain what happens in the players? The variables will affect their quality of life and wellbeing ~~of the volleyball players~~.

The study was done to ~~determine~~ the levels of interleukin 6 (IL-6) and iron ~~status of~~in volleyball players based on theirheights and weights ~~of a students~~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 football players 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 volleyball players in Madonna University Nigeria, Elele Campus, Rivers State, Nigeria. It is located in the South-South part of Nigeria.

Study population

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Comment [CC20]: This is confusing sentence. Kindly clarify who were the subjects and who were the controls

Comment [CC21]: Mere repetition, needs deletion

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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. They all gave consent to participate in this study.

Comment [CC23]: Oral or written?

Comment [CC24]: Where is the control group?

Inclusion criteria

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

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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.

Comment [CC26]: This is confusing
It should be like:
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 test kit

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

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Comment [CC28]: Put inverted commas

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)

Comment [CC29]: You have mentioned the test material and procedure for Ferritin only.
You have not mentioned how the iron was tested

Assay procedure

Comment [CC30]: What is the name of this procedure, Is it ELISA?

100µL standard or sample was added to the wells and incubated for 90 min at 37°C**.** The liquid was discarded, immediately added 100µL 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**.** 100µL HRP conjugate working solution was added, incubated for 30 min at 37°C and aspirate d and washed the plate for 5 times**.** 90µL Substrate Reagent was added and incubated for 15 min at

37°C**.** 50µL Stop Solution was added**.** The plate was read at 450nm immediately and the results calculated.

Statisticalanalysis

The data obtained from the study were presented as Mean ± 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 ± 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±2.42	25.21±3.43	-3.229	0.032*
Iron (**ug/dl**)	81.75±26.52	90.28±18.78	-0.469	0.663

Comment [CC31]: Reformate the Table

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±3.43ug/dl) compared to volleyball players of 4.0-5.5M (16.24±2.42 pg/ml)and no significant change in iron (p=0.663) of volleyball players 4.0-5.5M (81.75±26.52 ug/dl) compared to volleyball players 5.1-6.5M (90.28±18.78ug/dl) respectively.

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

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

Comment [CC32]: Reformate the Table o the analogy of tale 1

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±6.12ug/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±9.83ug/dl) respectively.

DISCUSSION

The study showed that there was significant increase 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 in iron of volleyball players 4.0-5.5M compared to volleyball players 5.1-6.5M 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 form 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 (Riviere*t 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 compared to volleyball players of 66-85Kgand no significant change in iron of volleyball players 50-65Kgcompared to volleyball players 66-85Kg 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

Comment [CC33]:
Discussion is deficient
Generate discussion by comparing your results with those of the other researchers and discuss the possible causes of differences or similarities with references. Include the key studies in discussion

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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

The study showed difference in interleukin 6 (IL-6) of the volleyball players based on height and not change in iron based on height and weight together with interleukin 6.

Comment [CC36]: The conclusion is confusing. Needs rewriting to make it crisp and in line with the main objective of the study as:
During exercise, interleukin 6 (IL-6) but not the iron level increases significantly based upon the height of volleyball players. Both of these parameters do not change significantly based upon their weight.

Comment [CC37]: Full stop

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