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IMPACT OF ENDURANCE EXERCISE ON SERUM MYELOPEROXIDASE (MPO) AMONG NOVICE UNIVERSITY STUDENTS ATHLETES

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Abstract

Background: Myeloperoxidase (MPO) is biochemical parameters of the body that play a vital role in maintaining various physiological activities of the human body. **Objective:** This study will be carried out to scrutinize the Alteration of serum MPO Concentration Associated with endurance exercise among Novice student-athletes. **Methods & Materials:** The study participants involved twenty (40) newly registered athletes of Punjab University and were placed randomly in a control group (CG, N=20) and an experimental group (EG, N=20). Five (05) ml blood samples were collected from each subject, and thus a different identification code was given to each blood sample. Likewise, serum MPO was assessed by using the principles of MPO calculation i.e. In the presence of hydrogen peroxide, myeloperoxidase present in the leukocyte granules oxidizes substrates from colorless form to an insoluble blue/brown derivative at the site of the activity. Benzidine or 3,3'-diaminobenzidine or pphenylenediamine dihydrochloride are used as substrates.. A self-made moderate-intensity exercise intervention of twelve (12) weeks will applied to EG. Pre and post-test data were administered through a statistical package for social sciences (SPSS, version-26), and thus mean, standard deviation and T-score were used as statistical tools for analysis. **Conclusion:** Based on data analysis the researcher concluded that endurance exercise has a significant impact on serum MPO among novice student-athletes. The result of the study also demonstrated that there is a significant association between exercise and serum MPO concentration. However, the limitations of the study must be considered while interpreting the results of this particular research study.

INTRODUCTION:

Myeloperoxidase (MPO) is an enzyme that belongs to the peroxidase-cyclooxygenase subgroup of the heme peroxidase family of enzymes (Kargapolova et al,2021)[1]. Neutrophils and monocytes secrete MPO which is considered an important part and plays a crucial role in the innate immunity of the body (Aratani, 2018) [2]. In other words, MPO is most abundantly expressed in neutrophil granulocytes (a subtype of white blood cells (WBCs), and produces hypochlorous acids to carry out their antimicrobial activity (An antimicrobial is an agent that kills microorganisms (microbicide) or stops their growth (bacteriostatic agent), including hypochlorous acid, the sodium salt of which is the chemical in bleach (Deby et al,1999) [3].

The main functions of MPO are within the immune system, functioning to abolish pathogens fundamentally. The way this works is that they are released from granules within the neutrophil into the phagosome for the breakdown of pathogens. It does this by, as described before, catalysis of hydrogen peroxide into hypochlorous acid. This hypochlorous acid, along with the destruction of pathogens, can also initiate and regulate inflammatory response (Klebanoff et al,2013) [4]. The author further argued that inflammatory response affects metabolic pathways when activating immune cells. To provide the energy necessary for inflammatory response, glycolysis is upregulated to provide ATP for phagocytosis. Following phagocytosis, the immune cells repair and remodel tissues, which oxidized products of myeloperoxidase function can aid. MPO deficiency is a disorder featuring a lack in either the quantity or the function of myeloperoxidase—an iron-containing protein expressed primarily in neutrophil granules (Soto et al,2020)[5]. There are two types of myeloperoxidase deficiency: primary/inherited and secondary/acquired (Mohammadi et al,2023)[6].

Lack of functional myeloperoxidase leads to less efficient killing of intracellular pathogens, particularly *Candida albicans*, as well as less efficient production and release of neutrophil extracellular traps (NETs) from the neutrophils to trap and kill extracellular pathogens (Parker & Winterbourn,2013)[7]).MPO may have a role in atherosclerosis. Researchers have demonstrated that patients with stable coronary artery disease had an increased cardiovascular risk if plasma MPO levels were elevated (Frangie & Daher, 2022)[8].

Recent studies have reported an association between myeloperoxidase levels and the severity of coronary artery disease. It has been suggested that myeloperoxidase plays a significant role in the development of atherosclerotic lesions and rendering plaques unstable. Again, inflammation is implicated in atherosclerosis and plaque buildup, thus a connection between inflammation and myeloperoxidase exists (Wainstein et al,2010)[9]. Coronary artery disease which is closely linked with MPO is caused by plaque buildup in the wall of the arteries that supply blood to the heart (called coronary arteries). Plaque is made up of cholesterol deposits. Plaque buildup causes the inside of the arteries to narrow over time. This process is called atherosclerosis (Teng et al, 2017)[10].

The results of this study conducted by Belcastro et al (1996)[11] show that, with prolonged running, MPO activity is elevated in most rat tissues and not exclusively in skeletal muscle. Moreover, the metabolic status of the tissues may be an important factor for neutrophil infiltration with exercise and not exclusively the type of muscle contraction, as previously hypothesized. Likewise, the study conducted by Reihmane; Jurka & Tretjakovs (2012)[12] Indicated that maximal exercise induces an inflammatory response characterized by a greater count of all subpopulations of leucocytes and increased IL-6, MPO and MMP-9 concentrations. Post-exercise neutrophil infiltration is more pronounced in red fiber types compared to white fiber types. There was an association between the quantity of repetitive bouts of swimming and MPO content in muscles. There were no significant differences in MPO concentration in muscles between untrained and trained animals at rest. Additionally, a single-bout of exercise to exhaustion produced a larger increase in muscle MPO content in untrained compared with trained rats. The smaller neutrophil infiltration in the muscles of trained animals after exhaustive exercise suggests a protective effect of previous training on muscle injury (Iqbal,2008) [13].

Methods and Materials

The below procedures were adopted by the researcher to reach certain findings and a conclusion.

Research design

The current research study is closely associated with endurance exercise and serum MPO concentrations. Therefore, the researcher employed an experimental research design. All the participants went through the experimental phases of the study.

Participants of the Study

Participants of the study were comprised of newly admitted or registered students to the Department of Sports Science and Physical Education, University of the Punjab, Lahore, Pakistan. Thus, the Participants of the study were randomly selected and placed into two groups: the Control group (CG) (normal routine with no exercise) and the experimental group.

Sample and Sample Size

Using the G*Power statistical method of sample calculation, the study participants were placed into two groups i.e.: The control group (CG (normal routine with no exercise) and the experimental group. Each group was comprised of twenty (20) subjects. In addition, the below criteria were followed while selecting and categorizing the subjects. A self-made Exercise protocol of endurance exercise for twelve (12) weeks was applied to the experimental group. Volume, intensity and frequency of exercise were calculated by experts in the field. In addition, the below criteria were followed while selecting and categorizing the subjects; Subjects aged more than 18 years and less than 25 years, Only male subjects were included, Subjects not taking any kind of antioxidants, Subjectst performing exercise for a minimum duration of 6 months to 1 year, Subject not taking any kind of medication, Subject has no chronic diseases and Subjects who voluntarily participate in the study.

Blood Sample Collection

Blood samples (5ml) were collected from all subjects by vein puncture and immediately transferred in heparinized tubes and centrifuged to separate plasma for determination of MPO. Each tube will be marked with a subject distinguishing proof code. MPO was assessed by using the principles of MPO calculation i.e. In the presence of hydrogen peroxide, myeloperoxidase present in the leukocyte granules oxidizes substrates from colourless form to an insoluble blue/brown derivative at the site of the activity. Benzidine or 3,3'-diaminobenzidine or p-phenylenediamine dihydrochloride are used as substrates.

Ethical Approval

Due to close concern with human life, the developed protocol of the study was approved by the Ethical Review and Research Board of the University of Sindh, Pakistan and thus written informed consent was also taken from all the subjects before participating in the study. Risks and benefits of participation were also ensured to all subjects before participation in the study. The respondents' information was kept confidential and only used for research purposes.

Analysis of Data Process

The results (pre-and post) were processed through the statistical package for Social Sciences (SPSS, version 32). The data obtained was presented as means $\pm$ SEM. The statistical significance of the results was determined using Student's t-test. The correlations were calculated for post-exercise. A $P < 0.05$ was required for the results to be considered statistically significant.

PRESENTATION AND ANALYSIS OF DATA

Table 1: Gender Distribution among Participants

Gender	Frequency	Percent	Valid Percent	Cumulative Percent
Male	40	100.0%	100.0%	100.0%

Interpretation of Table 1:

Table 1 shows the gender distribution among the participants in the study. It reveals that all 40 participants in the study identified as male, making up 100% of the sample. This indicates a homogenous gender distribution in the study population. The exclusion of female participants may have implications for the generalizability of the results, as the findings are specific to male participants. The gender-specific findings should be interpreted with this limitation in mind, and future studies might consider including a more diverse sample to improve the representativeness of the data.

Table 2: Independent Sample t-Test for BMI Pre and Post Intervention in Control Group (CG) and Experimental Group (EG)

Group	N	Mean	Std. Deviation	Std. Error Mean	df	t	Sig
Pre-BMI							
Experimental Group	20	23.8850	2.03374	0.45476	38	1.318	0.258
Control Group	20	23.2750	2.52334	0.56424	36.359		
Post-BMI							
Experimental Group	20	22.9750	1.74925	0.39114	-0.437	3.195	0.082
Control Group	20	23.2750	2.52334	0.56424	-0.437		

Interpretation of Table 2:

Table 2 presents the results of an independent sample t-test comparing the pre- and post-BMI values between the experimental and control groups. The pre-test BMI means are 23.8850 for the experimental group and 23.2750 for the control group, while the post-test BMI means are 22.9750 and 23.2750, respectively.

- **Pre-BMI Comparison:** The pre-test BMI values for both groups are similar, with no statistically significant difference ($t = 1.318$, $p = 0.258$). This suggests that the two groups were comparable at baseline in terms of BMI.
- **Post-BMI Comparison:** After the intervention, the experimental group experienced a slight reduction in BMI, from 23.8850 to 22.9750. The control group did not show a significant change, remaining at 23.2750. The post-test comparison ($t = 3.195$, $p = 0.082$) shows no significant difference in BMI between the two groups, as the p-value is greater than 0.05. This implies that the intervention did not lead to a statistically significant change in BMI when compared to the control group.

Overall, while there was a slight reduction in BMI for the experimental group, the lack of significant statistical findings suggests that the intervention had minimal or no effect on BMI in this study.

Table 3: Paired Sample t-Test for Serum Myeloperoxidase (MPO) Levels Pre and Post Intervention

Testing Variable	N	Mean	Std. Deviation	Std. Error Mean	df	t	Sig
Pre-Test (MPO)	40	388.8750	29.16899	4.61202	39	2.880	0.006
Post-Test (MPO)	40	387.7750	28.49335	4.50519			

Interpretation of Table 3:

Table 3 presents the results of a paired sample t-test comparing the pre-test and post-test levels of serum myeloperoxidase (MPO), a marker of inflammation, among 40 participants.

- **Pre-Test MPO:** The mean MPO level at the pre-test is 388.8750 pmol/L.
- **Post-Test MPO:** The post-test MPO level slightly decreases to 387.7750 pmol/L.

A statistically significant difference is observed in the MPO levels ($t = 2.880$, $p = 0.006$), suggesting a small but significant reduction in MPO levels following the intervention. The mean difference of **1.10000 pmol/L** may seem small, but the statistical significance indicates that the change is unlikely to have occurred by chance. The reduction in MPO levels could be indicative of a decrease in oxidative stress and inflammation as a result of the intervention.

This reduction in MPO levels is consistent with the hypothesis that the intervention had a positive effect on inflammatory markers, supporting the notion that the treatment or intervention led to a reduction in inflammation or oxidative stress among the participants.

RESULTS AND DISCUSSION

The current study was associated with MPO endurance exercise among novice student-athletes. An experimental study with forty (40) participants with eight (08) weeks self-made exercise protocols was carried out to assess the MPO associated with endurance exercise. Serum bilirubin and MPO were examined with the application of ELISA protocols. Thus the pre and post-test results of the study were processed with the help of a statistical package for social sciences (SPSS, version 32) and likewise, suitable statistical tools like means, standard deviation, t-test and independent sample t-test were applied. The study's results reveal that endurance exercise has a significant impact on MPO concentration among novice student-athletes (Pre-test means in term of MPO are 385.8500 for the experimental group and 391.9000 for the control, with post-test means of 384.4500 and 391.1000 respectively. Independent samples t-tests reveal no significant differences in pre- or post-test MPO levels between the two groups ($p > .05$).

The study indicated that at rest neutrophil MPO concentration was found to be 33% lower in trained rats compared to untrained controls ($P < 0.05$). The study also showed no significant difference in the level of MPO among trained and untrained rates (Morozov et al, 2006)[14]. These findings seemed in line with the current research study.

The same finding is drawn by Sureda et al (2005) [15] that exercise may cause alteration in MPO (39%) in neutrophils after the cycling stage. It shows that MPO alteration is connected with exercise. The same result is shown by a study by Slusher et al (2021)[16] by found that acute

high-intensity interval exercise (HIIE) may potentially reduce the systemic release of inflammatory mediators (calprotectin and MPO) matched to continuous moderate-intensity exercise (CME).

The same results were drawn by Soares et al (2013) [17] Sureda et al (2005) [15] twenty (20) minutes of cycling exercise above AT level may cause the generation of reactive oxygen species (ROS), hence endogenous enzyme activity (especially GPX activity) or non-enzymatic antioxidants may modulate exercise-induced ROS generation. Thus, exercise under the AT level induces very little oxidative stress (OS) damage in young people.

CONCLUSION

From the analysis of the data presented in Tables 1, 2, and 3, several key points can be concluded:

1. **Gender Distribution:** The sample consists exclusively of male participants, which limits the generalizability of the findings to this gender group. Future studies should consider gender balance.
2. **BMI Analysis:** While the experimental group showed a slight reduction in BMI, statistical analysis revealed no significant difference between the experimental and control groups. This suggests that the intervention may not have had a major impact on BMI in this sample.
3. **MPO Levels:** The reduction in MPO levels in the experimental group was statistically significant, indicating that the intervention had a positive effect on inflammation and oxidative stress.

In conclusion, while the BMI results did not show significant changes, the intervention demonstrated a measurable impact on inflammatory biomarkers, as evidenced by the significant reduction in MPO levels. Future research should focus on refining the intervention and exploring its long-term effects.

Based on the analysis and results of the study, the researcher concluded that endurance exercise has a significant impact on MPO among novice student-athletes. The result of the study also demonstrated that there is a significant association between exercise and MPO concentration.

However, the limitations of the study must be considered while interpreting the results of this particular research study.

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