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IMPACT OF MODERATE INTENSITY EXERCISE ON ERYTHROPOIETIN CONCENTRATION (EPO) AMONG AMATEUR UNIVERSITY ATHLETES

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ABSTRACT

Study Background: Increased erythropoiesis is considered linked with long-term endurance exercise. Thus, it is primarily controlled by erythropoietin (EPO), yet studies demonstrating the upregulation of EPO via a single bout of endurance exercise have been ambiguous. Therefore this research study aimed to examine the effects of moderate-intensity exercise on erythropoietin concertation among amateur university athletes. **Methods & Materials:** Participants of the study were randomized by using an Excel program for placing the participants either in the experimental group (EG) or Control group (CG (normal routine with no exercise. The sample size was calculated through a statistical test; G*Power provides the effect size conventions as "small," "medium", and "large," based on Cohen's suggestions. These provided conventional effect size values that are different for different tests; likewise, each group comprised ten (20) (subjects). Venous blood was collected before and after exercise, and serum EPO levels were determined by enzyme-linked immune assay (ELISA) by Eva Engvall and Peter Perlman, 1971). Pre and post-test results obtained through ELISA were processed through a statistical package for social sciences (SPSS, version-26) and thus, appropriate statistical tools were used according to parametric data. **Results:** Medium-intensity exercise has no significant impact on EPO (mean in terms of EPO was .40±.69. T value was 1.80, df was 9 and the P value was .104 (<0.05). **Conclusion:** Based on the analysis and findings, the researcher concluded that medium-intensity exercise caused no significant changes in EPO among the participants. The study findings also reveal that based on anthropometric attributes of subjects' impact was observed on EPO concentration

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Keywords: Moderate Intensity Exercise, Amateur Athletes, University Level, Erythropoietin

Introduction

Erythropoietin, or EPO, is a hormone secreted by the interstitial cells of the kidney and is usually produced when the oxygen level is dropped (Eckardt & Kurtz, 2005)[1]. For instance, at high altitudes, oxygen levels are low; this increases erythropoietin production to produce more red blood cells for oxygen transport to the tissues (Mairbäurl, 2013)[2].

The primary function of EPO is the stimulation of the stem cells in the bone marrow to produce RBCs and avoid the destruction of RBCs. In exercise or sports, EPO is commonly conducted to assess doping among athletes. Both low and high levels of EPO cause different health complications (Bhoopalan & Weiss, 2020) [3].

A low level of EPO causes various diseases and sometimes damages the cells of the kidneys. It causes normochromic normocytic anaemia, in which insufficient blood cells meet the body's oxygen requirements. Symptoms of anaemia include; fatigue, light-headedness, breathlessness, weakness, headaches, pale skin and heart palpitations (Nangaku et al., 2021)[4].

High levels of EPO cause chronically low oxygen levels or an erythropoietin-secreting tumour, such as a renal cell carcinoma (Gordeuk, et al., 2005) [5]. The increased EPO causes the bone marrow to produce more red blood cells. In rare cases, having too many red blood cells may trigger a rare condition known as secondary polycythemia, which is associated with a range of serious health risks; increased blood viscosity, less adequate blood supply and oxygenation, high blood pressure in the lungs, life-threatening blood clots and stroke (Koury & Blanc, 2022) [6].

Aerobic exercise can improve various aspects of health in these patients, such as increasing oxygen consumption, improving blood pressure control, lipid profiles, and mental health. Exercise should be initiated at a low intensity and progressed gradually to avoid injury. For patients on hemodialysis, incorporating exercise into the dialysis session may improve participation and tolerance (Johansen et al.,2005) [7].Modern progressive training load with predominantly increases in frequency, duration and intensity enhances players' performance (Halson, 2014) [8].

Several factors such as nature of sport, level of competition, body composition, and experience in sport can also be used to judge the performance of players (Clemente et al., 2013)[9]. Different factors such as oxygen intake, production of red blood cells and provision of optimal oxygen to the cell are critical for optimal performance in sports (Mairbäurl, 2013)[2]. However, in sports or exercise training has been reported to promote erythropoietin (EPO) in athletes (Płoszczyca & Czuba, 2018) [10].

Medium and high intensity aerobic training elicits a significant increase in EPO. While, EPO stimulates the production of red blood cells in bone marrow and regulates the concentration of red blood cells and haemoglobin in the blood. This is useful for athletes, since red blood cells shuttle oxygen to the cells, including muscle cells, enabling them to operate more effectively (Domínguez et al., 2018) [11].

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METHODS & MATERIALS

Procedures of the Study

A randomized control trial registered to Home Care Labe (Registration No. HRZ/201) assessing moderate exercise exposure conditions and approved by the Ethical and Research Review Board of Gomal University Dera Ismail Khan (DIK) Khyber Pakhtunkhwa (KP), Pakistan. Written Informed consent was taken from all subjects; thus, they were informed about the Risks and benefits of the study protocols. Likewise, all the subjects were gone through the study's experimental phases.

Participants of the Study (Sample)

The study participants were comprised of newly admitted or registered students to the Department of Sports Science and Physical Education, University of the Punjab, Lahore, Pakistan. Participants of the study were randomized by using an Excel program for placing the participants in experimental group (EG) and Control group (CG (normal routine with no exercise) .Each Group was comprised of 20 subjects.The sample size was calculated through a statistical test; G*Power provides the effect size conventions as "small," "medium", and "large," based on Cohen's suggestions. These provide conventional effect size values that are different for different tests. In addition, the below criteria were during the selection and categorization of the subjects; Subjects aged between 18-25 years, Only male subjects were included; issues not taking any antioxidants; subjects performing exercise for a minimum of 6 months to 1 year, the subject is not taking any medication, the subject has no chronic diseases and the subjects who voluntarily participate in the study.

Baseline Measurements

1st baseline measurement or testing, including anthropometric attributes, was performed and thus the 2nd ventilator threshold test using a motorized treadmill (time duration minutes, initial or starting speed was 6.4 km/hr at a 1% incline, with increases in the rate of 0.8 km/hr for each stage until an R-Value of greater than 1.1 was achieved, after which the treadmill incline was increased by 2% every minute until volitional exhaustion) for the purpose to check the fitness level of the subjects and to standardize subsequent exercise testing intensity across all participants.

Blood Sample Collection

Blood samples (5ml) were collected through a health technician from all subjects by vein puncture and immediately transferred in heparinized tubes and centrifuged to separate serum for determination of EPO. Serum was frozen at -80°C. Each tube was marked with a subject distinguishing proof code. EPO was measured through enzyme-linked immunoassay assay (ELISA) by Eva Engvall and Peter Perlman, 1971)—the detailed procedures Eva Engvall and Peter Perlman (1971). The ELISA kit of Andrich Germany was used for the assessment of EPO. The details of ELISA are;

Product Description:

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The ELISA (enzyme-linked immunosorbent assay) kit is a tool used to quantify a specific protein in various biological samples. The kit involves a 96-well plate coated with a particular capture antibody. Standards and samples are added to the wells, and the target protein binds to the immobilized antibody. After washing away unbound substances, a biotinylated detection antibody is introduced, followed by an HRP-conjugated streptavidin solution. The wells are rewashed, and a TMB substrate solution is added to induce a colour change proportional to the amount of bound target protein. Finally, the colour intensity is measured at 450 nm after adding a Stop Solution.

Procedure:
All reagents and samples were stored at room temperature before use, and it is recommended to run all standards and samples in duplicate. The process involves adding 100 µL of each standard and sample to the appropriate wells, covering the wells, and incubating for 2.5 hours at room temperature or overnight at four °C with gentle shaking. After incubation, the solution is discarded, and the plate is washed four times with 1x Wash Solution. The next steps involve adding the Biotinylated Detection Antibody, HRP-Streptavidin solution, ELISA Colorimetric TMB Reagent, and Stop Solution, with specific incubation times and washing steps in between. Finally, the absorbance at 450 nm is measured

Results:
Calculate the mean absorbance for duplicate standards, controls, and samples, and subtract the average zero standard optical density. Plot the standard curve using software capable of performing four-parameter logistic regression models, with standard concentration on the x-axis and absorbance on the y-axis, and draw the best-fit curve through the standard point.

Exercise Intervention:
The researcher developed a self-made exercise protocol of medium and high-intensity endurance exercise for twelve (12) weeks and was applied to the experimental groups. Volume, intensity and frequency of exercise was set by the researcher through Maximum Heart Rate (MHR) and consultation of experts' validity of the field.

Ethical approval of the Study:
Ethical approval was taken from the Ethical review and research board of Gomal University. Thus, written informed consent was taken from all the subjects before participating in the study. Risks and benefits of participation were also ensured to all participants before participation in the study.

Analysis of Data Process:
Statistical package for social sciences (SPSS) software (v.25.0; IBM SPSS, New York, USA) was used to calculate the mean value and standard deviation (SD) for all data. After the application of exercise protocols in the same group, the pre and post-test results of EPO were analyzed using the paired t-test. Statistical significance was set at $p < 0.05$.

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PRESENTATION OF DATA

Frequencies and percentages of sampled groups

Testing Groups	Frequency	Percent
EG	20	50%
CG	20	50%
Total	40	100.0%

The above table indicates the frequency and percentage of sample groups (EG & CG). The data were expressed as frequency and percentage. The total number of respondents (N=40). The table shows that there were a total of three groups in the study having an identical number of participants i.e. total number of subjects in the EG was 20 (50%) and the CG was 20 (50%). The total sample of the study was 40 (100%).

Anthropometric Measurements of all Groups (CG and EG) in term of Age, Weight, and EPO

Testing Variable	N	Mean	Std. Deviation
Age (years)	40	1.40	0.498
Weight (kg) pre	40	67.3	2.251
Weight (kg) post	40	66.53	2.70
EPO in Pre-test	40	11.03	1.217
EPO in Post-test	40	11.43	1.406
Valid N (list wise)	40		

Anthropometric Measurements of all Groups (CG, EG) in terms of Age, Weight, and EPO The data were shown by using mean and standard deviation. The total number of respondents was 40 (each group was comprised of 20 subjects. The table shows that the total mean age of participants as a whole was 1.40±0.498 years, the mean weight in the pre-test was 67.33±2.231 kg, the mean weight in the post-test was 66.53±2.70, the mean EPO in pre-test was 11.03±1.217, and finally mean posttest of subjects was 11.43±1.406.

Table no.3 Indicates the Pre and Post Analysis of EPO of both CG and EG (Medium Intensity Exercise)

Testing Variable	Paired Differences					T	Df	Sig (2-tailed)
	Mean	Std	Std Error Mean	95 % Confidence interval of the Difference				
				Lower	Upper			

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EPO_pre (CG EPO_post (CG) EPO Pre (EG) EPO Post (EG)	- .500 .400	.527 .699	.167 .221	-.877 -.100	-.123 .900	- .877 -.100	-.123 .900	-3.000 1.809

Table above shows the Pre and post-Analysis of EPO of both CG and EG (Medium Intensity Exercise). Data were calculated by mean, standard deviation, t value, df and P value. The total number of respondents was 20. Each group was comprised of 20 subjects. The mean and standard deviation of CG in terms of EPO was -.500±.527, t value was -.877, df was -.123 and p value was -3.00. The mean and standard deviation of EG in terms of EPO was -.400±.699, the value was -.221, df was .900 and the p-value was .1.80

Table no.4 Pre and Post Analysis in term of Impact of Medium intensity Exercise upon EPO concentration among the Respondents

Paired Samples Test

Testing Variable		Paired Differences					T	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	EPO-PRE EPO-POST	.40000	.69921	.22111	-.10018	.90018	1.809	9	.104

The above table no. 4 Indicates the impact of medium-intensity exercise upon EPO concentration among the Respondents—the total number of respondents (N=20). Data were expressed as Mean, Standard Deviation, T value, Df and Confidence Interval. The standard deviation and mean in term of EPO was .40±.69. T value was 1.80, df was 9 and the P value was .104 (<0.05). The Paired sample t-test indicates that medium-intensity exercise has no impact on EPO.

RESULTS & DISCUSSION

The present study was carried out to examine of impact of exercise with medium and high intensity on the EPO concentration of the subjects. An experimental approach with pre and post-test examination of the study parameters was applied to sixty (0) subjects with random classification into CG and EG. Twelve (12) weeks of self-made exercise protocol was applied to the study subjects. Five (05) ml blood samples from the vein puncher of each subject were collected and thus EPO concentration was measured through ELISA Principles. Pre and post-test results of the study were processed through a statistical package for social sciences and thus the below findings were drawn. The Current study shows that medium-intensity exercise has no significant impact on EPO (mean in terms of EPO was .40±.69. T value was 1.80, df was 9 and the P value was .104 (<0.05). Results of the study conducted by Azadan et al (2021) [12] show that not any sort of beta blockers as well as exercise with high intensity within 30 minutes.

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However, exercise with moderate intensity exercise for 90 minutes caused an increase in EPO concentration but it was observed for a short period and thus the level of EPO was observed within baseline soon after the exercise. Results of the study conducted by Arimb et al (2021) [13] revealed that moderate-intensity exercise (3 times per week, for 8 weeks and the duration of exercise is 30 minutes) has a significant impact on the endurance of erythrocyte cell membranes and the number of erythrocyte cells among the respondents. In line with the above studies exercise with short-term intensity has no significant impact on the levels of EPO.

Various hormonal (noradrenaline, cortisol, and androgens) changes occur among the performers of physical activities. These hormonal changes result in stimulation in EPO which increases the erythropoietic activity of the bone marrow. Testosterone can also directly affect erythroblasts by increasing the number of receptors for EPO (Laurenti,2018)[14]. This finding also looked in in line with the current study findings.Various studies suggest that exercise has a dramatic influence on various biochemical changes in the body systems. Exercise with high intensity causes degeneration of cells and enhances the immune function response, reinforces the antioxidant capacity, and reduces oxidative stress (OS) (Imbalance in Reactive Species (ROS) and Antioxidants (AO). Similarly, consequences in terms of EPO are also considered linked with exercise. Exercise with low intensity influences EPO concentration by keeping EPO in normal ranges (Rasmussen et al, 2010)[15].

CONCLUSION

Based on the analysis and findings, the researcher arrived at conclusion that exercise with medium intensity caused no significant changes in EPO among the participants. The study findings also reveals that based on anthropometric attributes of respondent's impact was observed on EPO concentration.

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