



Original Article

Impact of High-Intensity Exercise on Antioxidant System and Liver Enzymes

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ABSTRACT

Liver is a vital organ of human body performing a variety of functions. In addition, maintenance of antioxidant system is also considered important. **Objective:** To observe the impact of high volume and high intensity exercise on blood redox state and enzymatic function of the liver. It was a comparative cross-sectional study conducted from January 2017 to Jan 2018 at Gomal University, Dera Ismail Khan Pakistan **Methods:** An observational study was conducted among twenty (40) voluntarily selected subjects. Measurement of both enzymatic functions of liver and blood redox state of the body were performed through liver functions tests (LFTS) and ferric reducing assay protocol (FRAP assay) by using blood samples collected from the subjects. **Results:** The collected data were analyzed through statistical package for social sciences (SPSS) version 25 by using different statistical tools such as Mean, Standard Deviation and T Score. **Conclusions:** Data analysis disclosed that high intensity exercise significantly affected the levels of ALT ($t_{38} = -4.369, p < 0.05$), ALP ($t_{38} = -.757, p > .05$), AST ($t_{38} = -2.246, p < .05$) and FRAP ($t_{38} = 4.308, p < 0.05$).

INTRODUCTION

Free radicals and reactive oxygen and nitrogen species (RONS) are produced during the metabolic process of cell. The enzymatic and non-enzymatic system helps in reducing the level of free radicals [1]. For smooth functioning of body, the balanced production of both antioxidants and reactive oxygen species (ROS) is important. Imbalance production of both antioxidants and ROS may cause oxidative stress which negatively influences the redox state of blood [2]. As a result of oxidative stress, a person may lead to many health complications such as lungs problems, kidney failure, liver problems and so on. Exercises with low intensity strengthen the functions of body systems. Similarly, high

intensity exercise may cause disruption of blood redox state [3]. Pro- and anti-inflammatory cytokine formation is also affected by exercise. Wadley, Van Zanten, & Aldred, (2013) stated that protein is fundamental unit of cell which may be affected by high intensity exercise. The affected state of protein may be due to oxidative stress [4]. According to Martinović, et al., (2009), free radicals are basically molecules or parts of molecules having one or more odd number of electrons in the outer cloud layer. It has a very little life span but having high level of reactivity in the body. For getting electronic stability it attacks on nearer stable molecule taking away an electron and creating a new free radical thus the effected molecule

become unbalanced and enter or attack on another nearer molecule which cause the disruption of different cell components [5]. According to Hollander et al., (2004) antioxidants play its role in regulating cellular homeostasis and curbing the destiny of reactive oxygen. The two utilities of antioxidants are to reduce or control ROS through giving of an electron to RONS. He was of the opinion that an antioxidant is a factor that withdraws a free radical without gaining instability itself. The brightly colored fruits and vegetables also called phytonutrients possess them. Exercise is a great source of antioxidant and ROS production [6]. There are two groups of antioxidants i.e., location wise group of antioxidants e.g., plasma antioxidants (uric acid, ascorbic acid, bilirubin, transferrin, caeruloplasmin). Cell membrane antioxidants like α -tocopherol have also been regarded as membranous chain breaking antioxidant. Catalase, glutathione reductase, superoxide dismutase (SOD) and glutathione peroxidase (GSH) all are intracellular antioxidants. Various antioxidant markers are used to specify plasma for evaluating the antioxidant response during exercise. Total antioxidant status (TAS) or total antioxidant capacity (TAC) is the commonly used methodologies for measuring the antioxidants activity in plasma. The very significant elements which affect the antioxidant's activities are exercising, its intensity, duration and fitness levels. Furthermore, antioxidant also helps in fighting against age related molecular degeneration and supports the body immune system [7]. Cooper et al., (2002) stated that high intensity exercise causes oxidative stress in both human as well as in animals. [8]. Oxidative stress has remained to be one of the basic reasons of different health complication always found among the energetic exercise performers. According to Leeuwenburgh & Heinecke (2001), it is marked that mitochondria of cell have remained an important source of reactive materials such as superoxide, hydrogen peroxide, and probably hydroxyl radical. [9]. Anaerobic physical activity and oxidative stress do have an affinity as long-term anaerobic activity causes harm to the muscles cells by upsetting the protein, lipids, and nucleic acids [9, 10]. Low intensity exercise such as normal walk and jogging is deliberately helpful in strengthening the antioxidants system of the body [11]. It has also been suggested that there are complicated cross-talks among pathological factors, inflammation, free radicals and immune responses [12]. Vitality of liver function is important area of consideration on part of health issues. Ignorance of liver function can cause failure of whole functions of the body systems. It is deemed that high intensity exercise will adversely affect the liver functions and blood redox state. There are a few studies reported in the literature on the effects of high intensity exercise on liver functions and

redox system in humans. So, there is a dire need of conducting research to investigate effects of high intensity exercise in order have a comprehensive and generalized view of the research problem. Keeping in view the importance the present study was initiated to examine the effect of high intensity exercise on liver functions as well as the on blood redox state.

METHODS

The following methodologies were implemented for obtaining the results for confirmation of hypothesis. A case control study was carried out from January 2017 to Jan 2018 at Gomal University, Dera Ismail Khan. The risks and the benefits of participation the research study was deliberately communicated to all the participants. Likewise all the participants were informed about information's confidentiality and will be utilized for the purposes of research. The low intensity exercise is defined as the exercise which can produce about 40–50% of maximum heart rate (MHR) Dayan et al. (2005) and Clark et al. (2005) and include walking routine jogging. The high intensity activities are those which can produce approximately 70% of MHR like exhaustive aerobic exercise in gymnasium. High intensity exercise performers were selected as subjects of the study using this rationale and by utilizing the international physical activity questionnaire (IPAQ) [13, 14]. Two different groups each group comprising of twenty participants were recruited. The high intensity exercise (EG) was enrolled from Gomal University Fitness Center whereas the second group consisting of twenty subjects that were not doing any type of exercise (CG) was enrolled from various departments of the Gomal University, KPK Pakistan. Alike only those participants were enrolled in the study those represented the exercise-trained cohort, and age- and sex-matched sedentary control. The following type subjects were not enrolled for the study: with complete sedation, using any type of medication for lengthy term, suffering from chronic diseases, refused to sign written informed consent and those aged more than 30 years. Subjects that were not using any type of antioxidants, doing exercise for 6 months to 1 year, subjects not using any type of medicine as well as those subjects free from chronic diseases were enrolled in the study. Blood sample (5 mL) was collected from each participant by vein puncture and instantly transmitted in heparinized tubes. The plasma was separated by centrifuging of all blood samples at 15000 rpm for 20 minutes which was used for the determination of Alanine transaminase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST) and Ferric reducing antioxidant power FRAP. The blood samples were assigned a unique subject identification code. ALT and AST were determined using method of Schumann et al., (2002) [15]. Four hundred μ l R1

(reagent1) and 100µl R2 (reagent2) were taken from the kit by the help of pipette into a test tube and then 50µl serum were added. Mixture was observed at 500nm on a UV-Visible Spectrophotometer (Microlab 300 Japan). Alkaline phosphatase was measured by Schumann et al., (2011) methodology [16]. Four hundred µl of R1 (diethanolamine and magnesium chloride) and 100µl of R2 (P-nitrophenyl phosphate) were transferred to a test tube after that 10µl of serum was added to the reaction solution and was analyzed at 405nm on UV-Visible spectrophotometer (Microlab- 300 Japan). For the estimation of the oxidative stress in participants FRAP assay protocol was used. The procedure involved the formation of blue colored ferrous-tripyridyltriazine (Fe²⁺-TPTZ) complex by reduction of ferric (Fe³⁺) to ferrous (Fe²⁺) ion at low pH. The absorbance was measured at 593nm [17]. Calculation of FRAP value was done by the following equation:

$$\text{FRAP } \mu\text{Mole Value} = \frac{0 - \text{to } 4 \text{ min } \Delta_{593} \text{ nm test sample}}{0 - \text{to } 4 \text{ min } \Delta_{593} \text{ nm test standard}} \times \text{FRAP}_{\text{STD}} \mu\text{M}$$

For data analysis different statistical tools such as Mean, Standard Deviation and T Score were employed according to parametric data by SPSS version 25.0. The p-value <0.05 was considered significant.

RESULTS

Testing Variable	N	Range	Mini:	Maxi:	Mean	SD	Variance
BMI	20	8.00	18.00	26.00	21.3500	2.27746	5.187
ALT (IU/L)	20	47.00	27.00	74.00	47.8500	14.53589	211.292
ALP (IU/L)	20	160.00	132.00	292.00	249.2500	51.59955	2662.513
AST (IU/L)	20	15.00	20.00	35.00	28.5000	4.01969	16.158
FRAP (µmole/L)	20	39.80	98.20	138.00	113.7440	13.74323	188.876

Table 1: Descriptive Analysis of High Intensity Exercise Group (EG) in Term of BMI, ALT, ALP, AST and FRAP

Descriptive analysis of data of EG, N=20 in term of various parameters i.e. BMI, ALT, ALP, AST & FRAP are expressed as Mean, and Standard Deviation is given in Table 1. The data about; BMI indicated; range of the data was 8.00, minimum record was 18.00, maximum was 26.00, mean and standard deviation was 21.35 ± 2.27 , variance was 5.187. ALT indicated; range of the data was 47.00, minimum record was 27.00, maximum was 74.00, mean and standard deviation was 47.85 ± 14.53 , variance was 221.29. ALP indicated; range of the data was 160.00, minimum record was 132.00, maximum was 292.00, mean and standard deviation was 249.25 ± 4.01 , variance was 16.15. AST indicated; range of the data was 15.00, minimum record was 20.00, maximum was 35.00, mean and standard deviation was 28.50 ± 5.33 , variance was 28.46. FRAP indicated; range of the data was 39.80, minimum record was 98.20, maximum was 138.00, mean and standard deviation was 113.74 ± 13.74 , variance was 188.87.

Testing Variable	Category of the Respondents	N	Mean	SD	T-score	Sig.
BMI	CG	20	20.9500	1.79106	-.617	.541
	EG	20	21.3500	2.27746		
ALT (IU/L)	CG	20	33.5000	2.11511	4.389	.000
	EG	20	47.8500	14.53589		
ALP (IU/L)	CG	20	236.9000	51.54548	-.757	.454
	EG	20	249.2500	51.59955		
AST (IU/L)	CG	20	25.3500	4.81527	-2.246	.031
	EG	20	28.5000	4.01969		
FRAP (µmole/L)	CG	20	113.7440	21.83079	4.308	.000
	EG	20	138.5925	13.74323		

Table 2: Mean difference in term of BMI, ALT, ALP, AST and FRAP of CG and EG

Comparison of both groups i.e. CG and EG in EG, N in term of various parameters i.e. BMI, ALT, ALP, AST & FRAP are expressed as Mean, and Standard Deviation is given in Table 2. Mean, standard deviation, T-Score and P-Value were used for expressing the data. The data of both groups CG and EG in term of BMI showed that Mean of CG was 20.95 ± 1.79 , Mean of EG was 21.35 ± 2.27 , T Value of both CG and EG was -.617, P-Value was .541 ($t_{38} = -2.709$, $p > .05$). The data indicates no significant difference in term of BMI of both CG and EG. Data about ALT indicates that mean of CG was 33.50 ± 2.11 , Mean of EG was 47.85 ± 14.53 , T Value of both CG and EG was -4.389, P-Value was .000. ($t_{38} = -4.369$, $p < .05$) it means that there is significant difference between CG and EG in term of ALT. ALP showed that mean of CG was 236.90 ± 51.59 , Mean of EG was 249.25 ± 51.59 , T Value of both CG and EG was -1.44, P-Value was .454. ($t_{38} = -1.44$, $p > .05$). Therefore, no significance difference was found in ALP of both CG and EG. The ALP CG was less than the ALP value of EG. AST showed that mean of CG was 25.35 ± 4.81 , mean of EG was 28.50 ± 4.01 , T Value of both CG-I and EG was -2.246, P-Value was 0.031. ($t_{38} = -2.246$, $p < .05$). Therefore, significance difference was found in AST of both CG and EG. The AST of CG-I was less than the AST of EG. FRAP showed that Mean of CG was 113.74 ± 13.74 , Mean of EG was 138.59 ± 13.74 , T Value of both CG and EG was 4.308, and P-Value was .000. ($t_{38} = 4.308$, $p < .05$) Therefore significance difference is found in FRAP of both CG and EG. The FRAP value of CG was less than the FRAP value of EG (Table 2).

DISCUSSION

The results of present study exhibited that enzymatic functions of liver (ALT ($t_{38} = -1.74$, $p < 0.05$) and AST ($t_{38} = -2.246$, $p < 0.05$) were significantly affected by high intensity exercise. A significant difference in liver biomarkers among the performers of high intensity exercise was reported by Pettersson et al. (2007) [18]. The very heavy manual labor and strength training produced alteration in liver enzymes as mentioned by Sjogren et al., (2007) [19]. Similarly, marathon runners were also found elevated ALT and potentially they may to develop rhabdomyolysis in extreme circumstances. It has been reported by Bakowski

et al. (2008) that liver enzymes such as ALT, ALP and AST were not affected by exercise [20]. Even though, it has been mentioned by Fallon and Colleagues (1999) that a significant increase in the level of liver enzymes have been observed after exercise [21]. Sharma & Kansal (2010) witnessed that level of AST ($p < 0.001$) and ALT ($p < 0.01$) were deliberately increased after exercise and there was no enhancement in glutathione (GSH) levels afterwards exercise $p < 0.05$ [22]. No significant difference was observed in ALP of both CG and EG as shown by results i.e. Mean of EG was 236.90 ± 50.96 , mean of CG was 236.90 ± 51.59 , T Value of both CG and EG was 1.44, P- Value was .454. ($t_{38} = -0.757, p > 0.05$). The study of Statland & Bokelund (1973) also confirmed these findings that ALP was nearly unaffected during the one-week exercise period [23]. There was imbalance between ROS and antioxidants (Mean of CG was 113.74 ± 21.83 , Mean of EG was 138.59 ± 13.74 , T Value of both EG and CG was 4.308 and P- Value was 0.000 ($t_{38} = -4.308, p = 0.000$) as shown by results of recent study. It had been reported by Berzosa et al. (2011) that both moderate and low intensity exercise in young male athletes produced oxidative stress. Low volume and intensity resistance exercise caused significant proliferation in malondialdehyde (MDA) during pre and post treatment in un-trained athletes ($P < 0.05$) as reported by Liu et al., (2009) [24]. Low intensity exercise assists in circumventing the complications of oxidation amongst the athletes ($P < 0.05$) as mentioned by Gholamnezhad et al., [25]. Bioenergetics and xenobiotic detoxification are actively carried out by liver. Furthermore, the liver plays the vital character in the mitochondrial system in metabolism, ion homeostasis and redox signaling regulation and cellular energy creation, also cellular remodeling and revision. The progress of liver diseases results from Liver mitochondrial dysfunction. Certainly, liver mitochondrial deterioration has been connected to hepatocyte death and inflammation, consequently producing degenerative processes. Moreover, mitochondria are said to be substantial originators and goals of ROS associated to liver ailments. The malfunction of mitochondria to harmonize subcellular metabolic procedures in the liver causes the diminished regulation of hepatic lipid metabolism and subsequent lipid buildup in hepatocytes [26]. Because mitochondria are involved in cell metabolism therefore, the effects of exercise on mitochondria have been broadly studied. Exercise-related alterations are assumed to principally upset muscle mitochondria; yet liver mitochondria are also a target throughout exercise. Liver mitochondria are dire since the liver's involvement in glucose and lipid metabolism demands the creation of energy for these energy-intensive processes. Consequently, exercise excites systemic pathways that secondarily normalize liver

mitochondria bioenergetics, function and structure as a stimulus for liver mitochondria to make ATP to maintain homeostasis of those metabolic activities [27]. Factually, the skeletal muscle fibers are capable of generating many of muscle-derived chemicals known as myokines that are secreted into the bloodstream and attack non-contractile tissues such as liver, brain and adipose tissue during exercise-related contractions. Besides that, numerous other possible pathways, the communication of myokines with tissue-specific receptors on those tissues controls chemicals that may ultimately initiate PGC-1 α , causing in improved mitochondrial biogenesis regulation. Its valuable observing that, based on the level of the negative repercussions, physical action may too activate autophagy in several tissues, comprising the liver [28]. If the study would have conducted with a large sample size and including a third group with nutritional supplementation would have produced the excellent outcome.

CONCLUSIONS

High intensity exercise may cause disruption of blood redox state which consequently lead to abnormality in normal function of the body. The high intensity exercise (EG) was enrolled from Gomal University Fitness Center whereas the second group consisting of twenty subjects that were not doing any type of exercise (CG) was enrolled from various departments of the Gomal University, KPK Pakistan. Alike only those participants were enrolled in the study those represented the exercise-trained cohort, and age- and sex-matched sedentary control.

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