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Oxidative Stress in Sports Persons after a bout of Intense Exercise: A Cross Sectional Study.

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Abstract

The Aim of this Study was to see the effect of single bout of Intense Exercise on production of Pro- Oxidant molecules or Oxidative Stress promoters by measuring the Serum Malondialdehyde (MDA) in the Sports Persons. Malondialdehyde (MDA) is produced after the action of Reactive Oxygen Species (ROS) on Lipids in the membranes of the cells specially contracting Muscle cells. This Study was done on 60 Sports Persons who were playing outdoor sports like Hockey,Football,Medium distance running for last 1 year or more. Informed Written Consent was taken from the Subjects and the Institutional Ethical Committee clearance was obtained. Blood samples were taken before and after the bout of intense exercise and Serum MDA levels were estimated. Paired't' test was applied for statistical evaluation of the data generated using SPSS(Statistical package for Social Studies) Version 17.0 software.The study showed statistically significant increase in the levels of Serum MDA after the bout of Intense Exercise (p value <0.05 with Confidence Interval of 95%).The Study shows increased Oxidative Stress during the Exercise in Sports Persons which there by decreases the performance of the Sports Persons by decreasing the contraction of the muscles and producing fatigue. Further Studies in future needs to be done after giving various Antioxidants like Vitamin E &C and food supplements having high Antioxidant levels to these Sports Persons and see the effect of dietary supplementation of these on the performance of the Sports Persons so that if beneficial effect is seen the performance of Sport persons could be increased.

Keywords: Oxidative Stress, Sports Persons, single bout of Intense Exercise, MDA

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Introduction

Increased lipid peroxidation has been reported by various authors[1,2,3] following Aerobic Exercise protocols in untrained Subjects, This Study was done to see the effect of single bout of Intense Exercise on production of Pro- Oxidant molecules or Oxidative Stress promoters by measuring the Serum Malondialdehyde (MDA) in the Sports Persons who were trained athletes (training for minimum of one year, 4 times a week, which consisted of aerobic workout or activity of 1 hour per day),so that the performance of these Sports persons could be improved in future by dietary supplementation or other interventions if role of oxidative stress in trained Sports Persons in found in this study.

Oxidative stress is a condition in which the delicate balance existing between Pro-Oxidant (free radicals) production and their subsequent amelioration via the Antioxidant defence system becomes in favour of Free Radical expression [4]. The production or formation of Free Radicals in vivo is primarily initiated by the consumption of molecular oxygen, which, due to its structure is in fact a Free Radical it-self. A Free Radical is any Species which is capable of existence and it contains one or more unpaired Electrons [5]. Many types of Free Radicals exist

[Hydrogen atoms, transition metal ions, Carbon centred radicals (e.g., Trichloromethyl), Sulphur centred Radicals (e.g., Thiyl)] [5] but those derived from either Oxygen and/or Nitrogen are the most important Free Radicals generated in living systems [6,7]. Both the Free Radicals themselves as well as the Non-Radical Species created via interaction with Free Radicals are collectively referred to as Reactive Oxygen/Nitrogen Species (RONS)[8]. The body's Antioxidant defence system serves to protect the cells from excess RONS production which may be Endogenous (Bilirubin, Uric Acid, Superoxide dismutase, Catalase, Glutathione Peroxidase, etc) or Exogenous (Carotenoids, Tocopherols, Ascorbate, Bioflavonoids, etc) compounds [9]. The Exogenous compounds are consumed in the diet and come mostly from ingestion of Fruits and Vegetables [10].

Indirect assessment of Oxidative Stress involves the measurement of the more stable molecular products formed via the reaction of RONS with certain Biomolecules. Common products include stable metabolites (e.g., Nitrate/Nitrite), and/or concentrations of Oxidation target products, including Lipid peroxidation end products [Isoprostanes, Malondialdehyde (MDA), Thiobarbituric Acid Reactive Substances (TBARS), Lipid hydroperoxides (LOOH), Conjugated Dienes (CD), oxidized Low Density Lipoprotein (oxLDL)], oxidized Proteins [Protein Carbonyls (PC), individual oxidized Amino Acids, Nitro tyrosine (NT)], and Nucleic Acids [8-hydroxy-2-deoxyguanosine (8-OHdG), oxidized DNA bases] [11] etc.

Material and Methods

Subjects

Inclusion Criteria

- Healthy Sports persons(playing Hockey, Football or were Medium distance runners),
- training for last one year or more, minimum 4 times a week in form of doing aerobic training or activity 1 hour per day ,
- Those who gave consent for the Study.

Exclusion Criteria:

- Sports persons on any Antioxidant Supplements like oral vitamin E or C Tablets were excluded,
- Sports persons on Antioxidant rich diet, or other food supplements were excluded,
- Sports Persons who Smokers were excluded ,
- Sports Persons residing in area with high environmental pollution were excluded,
- Sport persons having any known Chronic Illnesses like Diabetes, Hypertension were excluded.

Informed written Consent was taken from all the Subjects (Sports Persons). Also, the Institutional Ethical Committee clearance was taken.

Sample collection and processing

All the Sport Persons were subjected to rigorous 1 hour and 30 minutes of Aerobic Exercise(Subjects were asked to run at an average speed of about 10 Kilometres / hour for about 1 hour and 30 minutes). Under aseptic conditions and with prior Consent of the Subjects, 5 ml of Blood Sample was drawn from the peripheral vein of the Subjects. The Blood was drawn from the Subjects just before the bout of intense Aerobic Exercise and 6 hours after the Exercise. Blood was centrifuged at 3000 RPM for fifteen minutes and Serum was separated.

Analytical method

Free radicals were estimated by the method, adopted by Philpot J. for Serum Malondialdehyde (MDA) levels, which is a Lipid Peroxidation end product[12].

MDA Estimation

Principle. One molecule of MDA reacts with two molecules of Thiobarbituric acid (TBA) at the pH 3.5.The pink colour Chromogen can be measured Spectrophotometrically at 532 nm.

Procedure. For Assay1 ml. of Serum was mixed with 2.5 ml. of 20% Trichloroacetic acid(TCA) and 1 ml of 0.67% of aqueous solution of TBA.The mixture was heated for 30 minutes in boiling water bath,the pink pigment was extracted with 2 ml. of n-Butonol and its absorbance was read at 532 nm against n-Butonol as blank.

MDA= (OD of sample/OD of standard) X concentration of standard MDA in Nano grams/ml of serum.

Statistical Analysis. Paired 't' test was applied for Statistical evaluation of the data generated using SPSS (Statistical package for Social Studies) Version 17.0 Software. The Statistical significance level was put at 'p' value <0.05 with Confidence Interval (CI) of 95%.

Results

As shown in the above Table 2 it was found that the Mean Serum MDA levels in Sports person was raised 6 hours after the Intense Exercise as compared to Pre-Exercise levels, which was a Statistically Significant change[with p value of<0.001 at Confidence Interval(CI) of 95%] .This Shows that after a bout of Intense Exercise(Short duration <2 hours ,Intense Aerobic Exercise) the Sport persons are under Oxidative Stress.

Table 1. *Anthropometric Profile of the Sports Persons*

n=60	Value
Age(Years)	22.50 ± 4.55
Height(Meters)	1.72 ± 0.09
Weight(Kilograms)	68.35 ± 7.65
BMI(Kilograms/meter square)	23.12 ± 0.75

Table 2. *Serum MDA level in Sport Persons before and after Exercise*

n(Number of subjects)=60	Mean value Serum MDA (Nanograms/millilitre)	Standard Deviation(S.D.) (Nanograms/millilitre)
Pre Exercise	5.73	1.39
Post Exercise	36.40	4.07

**p value was found to be <0.001 that is Statistically Significant change,*
*** With CI (Confidence Interval) of 95%.*

Discussions

Overproduction of RONS can result from a variety of Stressors, such as exposure to Environmental Pollutants [5] excessive nutrient intake [13] or Physical Exercise [14]. Infact any situation in which the consumption of Oxygen is increased, as during Physical Exercise, could result in an Acute state of Oxidative Stress.

Increased lipid peroxidation, measured via TBARS[1] , F_2-isoprostanes[2] and LOOH[3] has been reported by other authors following Aerobic Exercise protocols in untrained Subjects while in our study we found that there is increased Lipid Peroxidation in trained subjects too .Also we found this elevation by a different method of estimation that is MDA. This study would provide further evidence for the increased migration of Phagocytic cells following intense Aerobic Exercise, resulting in increased RONS production and subsequent Oxidative damage among trained Sport persons.

Primary RONS generation in response to Acute Exercise can occur via several pathways. These include Mitochondrial respiration (electron leakage from electron transport chain and subsequent production of the superoxide radical), Prostanoid Metabolism, the Auto-oxidation of Catechol-amines, and Oxidase enzymatic activity (NAD(P)H Oxidase, Xanthine Oxidase)[15]. The initial increase in RONS during Exercise, as well as following cessation of the work bout can lead to additional secondary generation of Pro-oxidants via phagocytic respiratory burst, a loss of Calcium Homeostasis and/or the destruction of Iron-containing Proteins [15].

Although the role of oxidative stress in exercise-induced adaptations, as well as in human Physiology remains to be completely elucidated, it appears based on the extensive body of literature that a currently undefined optimal level of RONS production may be vital in order for optimal adaptive potential and physiological function to be achieved that is muscle force generation [16,17]and in production of fatigue which decreases the performance of the Athletes [18,19].

Conclusion

The Study shows increased Oxidative Stress during the Exercise in Sports Persons which there by decreases the performance of the Sports Persons by decreasing the contraction of the muscles and producing fatigue. Further Studies in future needs to be done after giving various Antioxidants like Vitamin E &C and food supplements having high Antioxidant levels to these Sports Persons and see the effect of dietary supplementation of these on the performance of the Sports Persons so that if beneficial effect is seen the performance of Sport persons could be increased in future.

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