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Lipid Peroxidation ---- An Exercise Induced Oxidative Stress Marker And Impact Of Multivitamin Supplementation In Exercising Females.

Authors:

1. **Chandana Bera**, Assistant Professor, Department of Physiology, NC Medical College, Panipat, Haryana, India. Email:berachandana24@gmail.com
2. **Dr. Manila Jain**, Professor & HOD, Department of Physiology, Index Medical College, Hospital and Research Center, Indore, MP, India.Email:gbubai90@gmail.com
3. **Subarna Ghosh**, Assistant Professor, Department of Physiology, NC Medical College, Panipat, Haryana, India. Email: gbubai@rocketmail.com
4. **Bijay Kumar Mahaseth**, Assistant Professor, Department of Biochemistry, NC Medical College, Panipat, Haryana, India. Email: gbubai82@gmail.com

Corresponding Author:

Subarna Ghosh, Assistant Professor, Department of Physiology, NC Medical College, Panipat, Haryana, India. Email: gbubai@rocketmail.com

Phone : 8930971149

Abstract

Background: Physical exercise is associated with a 10-20 fold increase in whole body oxygen uptake. Oxygen flux in the active peripheral skeletal muscle fibers may increase by as such 100 to 200 fold during exercise. Studies during the past 2 decades suggest that during strenuous exercise, generation of reactive oxygen species (ROS) is elevated to a level that overwhelms tissue antioxidant defense systems. The result is oxidative stress.The present study aims to investigate the effect of multivitamin supplementation on the oxidative stress marker—lipid peroxidation before and after exercise of hundred healthy sedentary females of 18- 21 years age and comparable height and weight. **Materials & Methods:** Endurance capacity (min) of each subject was determined through exercise on a Magnetic Break Bicycle Ergometer at a fixed workload of 600KgM/min till exhaustion. Lipid peroxidation (LP) levels were analyzed at pre and post exercise levels. The subjects (N=100) were divided into two groups – control (n=50) receiving placebo supplementation for 15 days and experimental group (n=50) (V_M) receiving for 15 days. The same procedure was repeated after supplementation.**Results :** Results indicates endurance exercise was responsible for increase significantly ($p<0.001$). But after supplementation of multivitamin for 15 days post exercise lipid peroxidation was significantly reduced ($p<0.001$) when it was compared with post exercise pre supplementation level. Placebo supplemented control group showed no significant change ($p>0.05$) when it was compared with post-exercise pre- supplementation level. **Conclusion :** Multivitamin has significant effect in enhancing the antioxidant defense and reducing oxidative stress marker—lipid peroxidation.

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Key Words : Exercise, Oxidative stress, Multivitamin, Lipid Peroxidation.

Introduction

The existence of free radicals in the living cells was first reported in 1954 and the important finding helped launch the field of free radical biology. However, the discovery that muscular exercise is associated with biomarkers of oxidative stress did not occur until 1978. Following the initial report that exercise promotes oxidative stress in humans, many studies have confirmed that prolonged or short- duration high intensity exercise results in increased radical production in active skeletal muscles resulting in the formation of oxidized lipids and proteins in the working muscles. Since these early descriptive studies, the investigation of radicals and redox biology related to exercise and skeletal muscle has grown as a discipline and the importance of this research in the biomedical sciences is widely recognized.[1] Physical exercise is characterized by an increase in oxygen consumption by the whole body. Two to five percent of oxygen used in mitochondria forms free radicals. As oxidative phosphorylation increases in response to exercise, there will be concomitant increase in free radicals. Catecholamines that are released during exercise can lead to free radical production. Other sources of free radical increase with exercise include prostanoid metabolism, xanthine oxidase, NAD(P)H oxidase, and several secondary sources, such as the release of radicals by macrophages recruited to repair damaged tissue.[2] This leads to a decrease in antioxidant levels that could promote both an increase in the markers of lipoprotein peroxidation and damage to the erythrocyte membrane with consequent modification of membrane fluidity.[3]

Antioxidant supplements are marked to use by athletes as a means to counteract oxidative stress of exercise. If the increase in free radicals is greater than the ability to neutralize them, the radicals will attack cellular components, especially lipids. The attack on lipids initiates a chain reaction called lipid peroxidation, which leads to generation of more radicals and ROS that can harm other cellular components. The body appears able to withstand a limited increase in free radicals and in fact, data suggest that an increase in ROS is necessary for muscle adaptation to occur. These free radicals are neutralized by an elaborate antioxidant defense system consisting of enzymes such as catalase, superoxide dismutase, glutathione peroxidase, and numerous non-enzymatic antioxidants, including vitamin A, E and C, glutathione, ubiquinone and flavonoids.[2]

To examineacute oxidative stress in response to exercise, most researches have assessed various stress markers in blood and urine. Most commonly measured are by products of lipid peroxidation. It is an indirect measure of free radical activity.

Thus, the present study aims to elucidate the correlation between the immediate effect of physical exercise on lipid peroxidation (LP) and the first-hand effect of multivitamin supplementation in combating exercise induced oxidative stress marker- lipid peroxidation.

Materials and Methods

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Ethical Clearance: This study was conducted in Index Medical College, Hospital and Research Center, Department of Physiology. Ethical clearances were obtained from the Institutional Ethical Committee before carrying out the study.

The entire study procedures were explained and written consent was taken from each volunteer, individually for doing the study. Consent was also taken for publishing this article.

Hundred (N=100), physically fit (no cardiorespiratory disorder, no menstrual disorder) , females of Index Medical College, Hospital and Research Center students of comparable height and weight and aged 18 to 21 years were selected for the study. The subjects were subdivided into two groups – the first group (n=50) served as the control with placebo supplementation for 15 days and the second group was experimental group (V_M) (n=50) was given Multivitamin (New A to Z Gold; By Alkem Laboratories Ltd) supplementation in capsular form for the same period of time. The subjects had no history of any major disease, were not undergoing physical conditioning training. The composition of Multivitamin capsule (New A to Z Gold; By Alkem Laboratories Ltd) was as follows:

Docosahexaenoic acid (Dha) (60.0Flu) + Eicosapentaenoic Acid (Epa) (90.0mg) + Alpha Lipoic Acid (30.0mg) + Vitamin C/ Ascorbic Acid (25.0mg) + Vitamin E/ Tocopherol (8.0mg) + Beta-Carotene Dispersion (2.4mg) + Vitamin B6/ Pyridoxine (1.5 mg) + Vitamin B1/ Thiamine (0.8mg) + Vitamin B9/ Folic Acid/ Folate (60.0mcg) + Vitamin B12/ Mecobalamin / Cyanocobalamin/ Methylcobalamin (0.6mcg) + Zinc sulphate Monohydrate (0.9 mg) + Sodium Selenate (30.0mcg) + Chromium chloride (30.0mcg).

Before the actual experiment, the details of the experimental procedure were explained to the subjects to allay apprehension. They were asked to refrain from eating at least for an hour prior to the test and allowed to take complete rest for half an hour before the actual experiment, so that the heart rate could settle to a constant value.

Before the actual exercise, height(Cm), weight(Kg), oral temperature (in°F by clinical thermometer) and blood pressure(mmHg) by sphygmomanometer (Auscultatory method) and pre- exercise heart rate by feeling carotid artery pulsation in beats/min were recorded. Endurance capacity (min) of each subject was determined through exercise on a Magnetic Break-Bicycle Ergometer with a fixed workload of 600KgM/min till exhaustion.

At the end of exercise, endurance capacity, peak heart rate, recovery heart rate upto 30 minutes of recovery heart rate upto 30 minutes of recovery period were recorded. The entire procedure was repeated on each subject before and after beta carotene, minerals and antioxidants supplementation.

Blood (5ml) was collected from the ante cubital vein before and after exercise for the determination of serum MDA (Malondialdehyde) for estimation of lipid peroxidation.[4] Blood Hb[5] was determined only before exercise.

The room temperature varied between 20°C- 24°C and the relative humidity was about 77%.

Statistical Analysis:

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A two-tail 't' test by difference method was asked for testing the significance of difference between the sample means.

Results:

The physical parameters of the female subjects are shown in Table 1.

Endurance capacity (min) of the exercising female showed a significant increase ($P<0.001$) after Multivitamin supplementation, as shown in Table 2.

1^{st}, 2^{nd}, 3^{rd},4^{th} and 5^{th} min recovery heart rates showed significant ($P<0.001$) decrease after Multivitamin, as shown in Table 3. Recovery of heart rate after exercise is a good indicator of physical fitness and faster the recovery is, the better fitness is reflected.

There were insignificant effects of Multivitamin supplementation on resting hemoglobin concentration, as shown in Table 4.

In Table 5. it was found that the exercise caused a significant ($P<0.001$) increase in serum Lipid Peroxidation ($P<0.001$) and significant increase in GP_X level ($P< 0.05$)before Multivitamin supplementation. Lipid Peroxidation level ($P<0.001$) was significantly reduced after exercise after Multivitamin supplementation for 15 days when it was compared with post- exercise pre supplementation level(Table 5.).

Discussion

The study reflects that multivitamin is highly effective in combating exercise – induced oxidative stress as the study shows that multivitamin is highly effective in reducing the post- exercise lipid peroxidation level.

The study shows that lipid peroxidation level significantly ($p<0.001$) increases after exercise. On the other hand multivitamin supplementation which contains Vitamin E, Vitamin C, Vitamin E, Vitamin B complex and all the antioxidant minerals for 15 days post –exercise lipid peroxidation level was significantly ($p<0.001$) reduced when compare to post-exercise pre supplementation level. So multivitamin seems to be combating against ROS and lipid peroxidation level was reduced after multivitamin supplementation.

Previous experiment cohesively suggested that the moderate multivitamin-mineral supplementation prevented the transient lipid peroxidation level during exercise competition.[6] Vitamin E, vitamin C, Zinc and Selenium supplementation improves maximal voluntary contraction and endurance limit time of the dominant and non-dominant quadriceps by enhancing the antioxidant defense and reducing oxidative stress.[7]

It is also reported that vitamin C and vitamin E have been used together to determine the effects of antioxidant supplementation on exercise. This combination is thought to be more effective than either vitamin alone since vitamin C can regenerate vitamin E.[8]

Study also explained that deficiency of antioxidant nutrients appear to hamper antioxidant systems and augment exercise-induced oxidative stress and tissue damage.[9]

By the previous research and present study we can be able to conclude that multivitamin enriched with beta carotene, vitamin C, vitamin E and vitamin B complex and antioxidant minerals acts in the synergistically and reduce the burden of ROS by the endogenous antioxidant

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enzyme and ultimately help to improve exercise performance and decreasing lipid peroxidation level which is an oxidative stress marker.

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Tables:

Table 1. Physical Characteristics of Female Participants (Mean±SD)

Groups	Age (Years)	Height (cm)	Weight (kg)
Placebo (n=50)	21.4±0.63	154.78±3.06	60.69±5.85
V_M (n=50)	21.67±0.82	153.73±6.70	54.29±7.67

Table 2. Endurance Capacity of Female Participants before and after Multivitamin supplementation (Mean±SD)

Groups	Endurance Capacity	
	Before Supplementation	**After Supplementation**
Placebo (n=50)	7.56±0.72	7.71±0.73 NS
V_M (n=50)	6.76±0.50	14.80±0.54**

NS = Not Significant

**$p<0.001$ = Significant

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Table 3. Recovery Heart Rates (Beats/min) of Female Participants before and after Multivitamin supplementation (Mean±SD)

Group		1st min	2nd min	3rd min	4th min	5th min
Placebo (n=50)	**Before Supplementation**	160.0±3.78	148.13±2.77	124.00±2.00	109.33±2.47	99.2±2.37
	After Supplementation	160.27±2.60 **NS**	149.20±2.70 **NS**	124.53±2.45 **NS**	109.47±2.56 **NS**	99.47±2.88 **NS**
V_M (n=50)	**Before Supplementation**	160.00±3.55	149.73±2.71	123.87±2.07	109.07±2.60	98.80±1.97
	After Supplementation	150.6±1.99 **	139.87±1.85 **	109.00±1.96 **	93.1±2.28 **	88.00±2.39 **

NS = Not Significant
**p<0.001 = Significant

Table 4. Hemoglobin Concentration (g%) of Exercising Female Participants before and after Multivitamin supplementation (Mean±SD)

Groups	Hemoglobin Concentration (g%)		Level of Significance
	Before Supplementation	After Supplementation	
Placebo (n=50)	12.33±0.11	12.40±0.11	NS
V_M (n=50)	11.97±0.18	11.83±0.13	NS

NS = Not Significant

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Table 5. Exercise induced changes in Oxidative stress Markers serum lipid peroxidation(K x 10^{-1} nmole/ml of serum) of Exercising Female Participants before and after Multivitamin supplementation (Mean±SD)

Enzyme Parameters	Groups								Level of Significance
	Placebo (n=50)				V_M(n=50)				
	Before Supplementation		After Supplementation		Before Supplementation		After Supplementation		
	Pre-Exercise	Post-Exercise	Pre-Exercise	Post-Exercise	Pre-Exercise	Post-Exercise	Pre-Exercise	Post-Exercise	
Serum lipid peroxidation (K x 10^{-1} nmole/ml of serum)	36.78±0.51	42.53±0.30	36.91±0.53	42.67±0.48	36.88±1.28	42.86±1.07	31.50±1.18	34.80±1.18	1 & 2 = p<0.001 3 & 4 = p<0.001 5 & 6 = p<0.001 2 & 4 = NS 6 & 8 = p<0.001
	1	2	3	4	5	6	7	8	

NS = Not Significant, p<0.001 highly significant.

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Authors' Contribution:

TypeOf Contribution	Description	Contributors
Conception	Constructinganideaorhypothesisforresearch and/ormanuscript	Chandana Bera Dr. Manila Jain
Design	Planningmethodologytoreachtheconclusion	Chandana Bera
Supervision	Organizingandsupervisingthecourseoftheproject or the article and taking the responsibility	Dr. Manila Jain
Fundings	Providingpersonnel,environmentalandfinancialsupport andtoolsandinstrumentsthatarevitalfortheproject	NONE
Materials	Biologicalmaterials,reagentsandreferredsubjects	Chandana Bera Subarna ghosh
Data collection and/orprocessing	Takingresponsibilityinexecutionoftheexperiments, patient follow-up, data management and reporting	Chandana Bera Subarna Ghosh Bijay Kumar Mahaseth
Analysis and/or interpretation	Takingresponsibilityinlogicalinterpretationand presentation of the results	Chandana Bera Subarna Ghosh
Literaturereview	Taking responsibility in this necessary function	Chandana Bera
Writer	Takingresponsibilityintheconstructionofthewhole or body of the manuscript	Chandana Bera Bijay Kumar Mahaseth
Criticalreview	Reviewingthearticlebeforesubmissionnotonlyfor spelling and grammar but also for its intellectual content.	Chandana Bera Dr. Manila Jain

Conflict of Interest:

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Author: Subarna Ghosh	Signature:	Date: 02/03/2022
Author: Bijay Kumar Mahaseth	Signature:	Date: 02/03/2022
Author:	Signature:	Date:
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TYPE OF CONTRIBUTION	DESCRIPTION	CONTRIBUTORS
CONCEPTION	Constructing an idea or hypothesis for research and/or manuscript	Chandana Bera Dr. Manila Jain
DESIGN	Planning methodology to reach the conclusion	Chandana Bera
SUPERVISION	Organising and supervising the course of the project or the article and taking the responsibility	Dr. Manila Jain
FUNDINGS	Providing personnel, environmental and financial support and tools and instruments that are vital for the project	— None —
MATERIALS	Biological materials, reagents and referred patients	Chandana Bera Subarna Ghosh
DATA COLLECTION AND/OR PROCESSING	Taking responsibility in execution of the experiments, patient follow-up, data management and reporting	Chandana Bera Subarna Ghosh Bijay Kumar Maheseth
ANALYSIS AND/OR INTERPRETATION	Taking responsibility in logical interpretation and presentation of the results	Chandana Bera Subarna Ghosh
LITERATURE REVIEW	Taking responsibility in this necessary function	Chandana Bera
WRITER	Taking responsibility in the construction of the whole or body of the manuscript	Chandana Bera Bijay Kumar Maheseth
CRITICAL REVIEW	Reviewing the article before submission not only for spelling and grammar but also for its intellectual content	Chandana Bera Dr. Manila Jain
OTHER		

CORRESPONDING AUTHOR : Subarna Ghosh.

SIGNATURE :

DATE : 02/03/2022

This form should be signed by corresponding author and submitted during the initial submission with the rest of the manuscript files.

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