Research Work and Publications

(1) Presented Poster entitled “Role of Vitamin E in Ameliorating Oxidative Damages and Some Aspects of Lipid Profiles in Response to Smoking and Exercise” in Interactive Workshop in Biological Sciences-2000 at Calcutta.

(2) Presented paper entitled “Antioxidant and G-6-PD Level: A key combination in oxidative stress management in trained females” in Third Congress of “Federation of Indian Physiological Societies (FIPS)” in November 2000 at Calcutta.


ANTIOXIDANT AND G-6-PD LEVEL : A KEY COMBINATION IN OXIDATIVE STRESS MANAGEMENT IN TRAINED FEMALES

ANANDI BAGCHI, SUBRATA GHOSH, SATIPATI CHATTERJEE and PRATIMA CHATTERJEE

Sports and Exercise Physiology Laboratory
Department of Physiology, University College of Science and Technology
Calcutta University
92, Acharya Prafulla Chandra Road, Kolkata 700009

ABSTRACT

Antioxidants like vitamin E, vitamin C and β-carotene are known to act synergistically in an antioxidant chain reaction. An attempt has been made to observe the effect of antioxidant vitamin mixture supplementation on endurance capacity and other allied physiological parameters and also to establish serum glucose-6-phosphate dehydrogenase (G-6-PD) level as a crucial marker of post-exercise oxidative stress management in trained females.

Three experimental groups A, B and placebo, each consisting of ten trained females of 18-21 years age range were chosen. Group A was given 400 mg of vitamin E supplementation daily while group B was given a mixture of vitamins E, C and β-carotene daily in capsular form for a period of 21 days.

Endurance capacity determined by bicycle ergometric method was increased more significantly (P<0.001) in mixture of antioxidant vitamin supplemented group B than vitamin E supplemented group A (P<0.01), and first minute recovery heart rate decreased significantly (P<0.05) in both groups. G-6-PD level was shown to increase more significantly (P<0.01) with antioxidant vitamin mixture supplementation than with vitamin E supplementation singly (P<0.05) in trained females.

INTRODUCTION

Oxidative stress is often induced by strenuous physical exercise (Jenkins, Sen et al.), in that state production of reactive oxygen species (ROS) within the body transcends the antioxidant defense from within. Earlier reports proved that even submaximal exercise may elevate the stress indices like plasma lipid peroxides and higher blood glutathione oxidation (Sen et al.). ROS are known to have a wide variety of pathophysiological characteristics (Kehrer). Moreover, ROS may also contribute to fatigue in oxidative skeletal muscle (Barclay and Hansel). Recently, a number of studies have been directed towards exercise-induced oxidative stress management programme. Antioxidant supplementation studies have revealed a beneficial trend (Jenkins, Sen et al.).

In this particular study, female subjects were only chosen, where estrogen plays a
very crucial role as an antioxidant hormone (Halliwell and Gutteridge) Moreover, the mixture of vitamin E, vitamin C and β-carotene are being supplemented here as the major non-enzymatic antioxidants available, at clinical dose, for the purpose of stress management. Again, in this particular study, a new oxidative stress index is being attempted to be developed, i.e., glucose-6-phosphate dehydrogenase (G-6-PD) level, which is probably involved with strenuous physical exercise. Therefore, the study aims in revealing the effects of antioxidant vitamin supplementation on exhaustive exercise-induced oxidative stress in females.

The aims of the study are to reveal

1) the fate of oral administration of antioxidant mixture in the form of vitamin E, vitamin C and β-carotene at clinical dose and its effectiveness in controlling exercise induced oxidative stress in trained females;

2) whether any improvement in endurance, at all, occurs after supplementation;

3) to evaluate the exercise-mediated changes in G-6-PD level considered as a probable marker of oxidative stress management programme.

However, in this humble study, moderate exercise (720 kgm min⁻¹) in Magnetic Brake Bicycle Ergometer was carried out.

MATERIALS AND METHODS

Thirty young, physically fit, trained females (state level athletes collected from Sports Section of Eastern Railway) coming from comparable socioeconomic background, with age ranging from 18-21 years volunteered for this study. They were debarred from taking extra vitamin supplementation three months before and during the study. None of these subjects had any history of chronic cardiac and respiratory diseases. Before starting the experiment, they were familiarized with the Bicycle Ergometer in the laboratory.

A double-blind study was carried out whereby the subjects were divided into three groups. Two groups each of ten subjects were randomly assigned for Experimental group A and Experimental group B, who were given 400 mg of pure vitamin E only and a mixture of vitamin E, vitamin C and β-carotene respectively for a period of 21 days. Experimental group C consisting of 10 female subjects of the placebo group, has also been studied.

Each of the subjects was allowed to take rest for a minimum of half an hour, at the end of which pre-exercise heart rate and physical parameters (height, weight, etc) were measured.

Endurance capacity (min) of each subject was determined by exercising them on a Magnetic Brake Bicycle Ergometer with a workload of 720 kgm min⁻¹, till exhaustion

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

Blood was taken before and after endurance exercise for the determination of haemoglobin (gm%) by cyanomethemoglobin method (Harold et al) Assessment of glucose-6-phosphate dehydrogenase was also carried out with the blood drawn after exercise by UK-Kinetic Method (Kachmar and Moss).

The room temperature varied between 20°C-24°C and the relative humidity was about 78%. Statistical analysis was done using a two-tail ‘t’ test by difference method.
RESULTS

Studies on thirty trained females had shown that after 400 mg of vitamin E supplementation (Group A), the mean endurance capacity (mm) rose from 13.05 ± 4.37 to 20.01 ± 4.60, while after supplementation of a mixture of vitamin E, vitamin C and β-carotene (Group B), the value increased from 13.86 ± 3.30 to 23.27 ± 1.91. It is thus seen that the antioxidant mixture produces a much more significant (P < 0.001) enhancement in endurance capacity as compared to vitamin E supplementation singly (P < 0.05). In group C (Placebo group) there is, however, no change.

Recovery heart rate recorded at the first minute of recovery period showed significant (P < 0.05) decrease in both experimental groups A and B. Recovery heart rate after exercise series as an effective indicator of physical fitness; faster the recovery rate higher the physical fitness. Antioxidant vitamin supplementation produces a faster recovery immediately after exercise. Thus antioxidant vitamin supplementation increases the physical fitness of the subjects.

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (years)</th>
<th>Height (cm)</th>
<th>Weight (Kg)</th>
<th>Body Surface Area (square metres)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>19.05 ± 0.48</td>
<td>152.13 ± 7.32</td>
<td>47.16 ± 8.20</td>
<td>1.34 ± 0.10</td>
</tr>
<tr>
<td>Group B</td>
<td>19.61 ± 0.31</td>
<td>156.28 ± 6.67</td>
<td>48.23 ± 6.41</td>
<td>1.48 ± 0.36</td>
</tr>
<tr>
<td>Placebo</td>
<td>19.98 ±0.22</td>
<td>160 ± 5.38</td>
<td>45.65 ± 7.20</td>
<td>1.41 ± 0.63</td>
</tr>
</tbody>
</table>

Values are mean ± S.D.

### Table 2A

Endurance Capacity (mins) of Trained Females Before and After Antioxidant Vitamin Supplementation

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Endurance Capacity (mins) Before Supplementation</th>
<th>Endurance Capacity (mins) After Supplementation</th>
<th>Level of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>13.05 ± 3.37</td>
<td>20.01 ± 4.60</td>
<td>Significant (P &lt; 0.01)</td>
</tr>
<tr>
<td>Group B</td>
<td>13.86 ± 3.30</td>
<td>23.27 ± 1.91</td>
<td>Highly significant (P &lt; 0.001)</td>
</tr>
<tr>
<td>Placebo</td>
<td>13.02 ± 1.03</td>
<td>13.04 ± 0.91</td>
<td>Not significant</td>
</tr>
</tbody>
</table>

Values are mean ± S.D.
Table 2B
First Minute Recovery Heart Rate (beats min\(^{-1}\)) of Trained Females Before and After Antioxidant Vitamin Supplementation

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>First Minute Recovery Heart Rate (beats mm(^{-1}))</th>
<th>Level of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before Supplementation</td>
<td>After Supplementation</td>
</tr>
<tr>
<td>Group A</td>
<td>157±2</td>
<td>152±3</td>
</tr>
<tr>
<td>Group B</td>
<td>153±3</td>
<td>148±7</td>
</tr>
<tr>
<td>Placebo</td>
<td>155±1</td>
<td>155±1</td>
</tr>
</tbody>
</table>

Values are mean ± S.D

Table 3A
Haemoglobin Concentration (gm\%) of Trained Females Before and After Antioxidant Vitamin Supplementation

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Haemoglobin Concentration (gm%)</th>
<th>Level of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before Supplementation</td>
<td>After Supplementation</td>
</tr>
<tr>
<td>Group A</td>
<td>11.6±0.6</td>
<td>11.9±0.8</td>
</tr>
<tr>
<td>Group B</td>
<td>12.1±0.7</td>
<td>12.7±0.4</td>
</tr>
<tr>
<td>Placebo</td>
<td>12.0±0.3</td>
<td>11.9±0.6</td>
</tr>
</tbody>
</table>

Values are mean ± S.D

Table 3B
Glucose-6-phosphate dehydrogenase (G-6-PD) (U/gm Hb) of Trained Females Before and After Antioxidant Vitamin Supplementation

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>G-6-PD Level (U/gm Hb)</th>
<th>Level of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before Supplementation</td>
<td>After Supplementation</td>
</tr>
<tr>
<td>Group A</td>
<td>9.36±0.74</td>
<td>10.36±0.82</td>
</tr>
<tr>
<td>Group B</td>
<td>9.63±0.78</td>
<td>10.64±0.54</td>
</tr>
<tr>
<td>Placebo</td>
<td>9.30±0.60</td>
<td>9.21±0.54</td>
</tr>
</tbody>
</table>

Values are mean ± S.D
G-6-PD level showed a more significant \((P \leq 0.01)\) increase after antioxidant vitamin mixture supplementation as compared to supplementation of vitamin E alone \((P \leq 0.05)\). G-6-PD level increased from 9.36 ± 0.74 to 10.36 ± 0.82 in group A while the value increased from 9.63 ± 0.78 to 10.64 ± 0.54 in experimental group B. In placebo group (C), however, there is no change.

Antioxidant vitamin supplementation showed insignificant effects on resting heart rate, peak heart rate and haemoglobin concentration.

**DISCUSSION**

Antioxidants like vitamin E, vitamin C and glutathione (GSH) are known to act synergistically \((\text{Constantinescu et al} 2, \text{Sen et al} 10)\) in the form of antioxidant chain reaction \((\text{Sen et al} 11)\). Lipophilic vitamin E is a major lipid peroxidation chain breaking antioxidant. The water-soluble antioxidants like ascorbate and GSH may be involved in regenerating \(\alpha\)-tocopherol from its radical products like tocopherol 0, etc. \((\text{Constantinescu et al} 2)\). In plasma, ascorbate \((\text{vitamin C})\) is a potent antioxidant, the first to be depleted on exposure to peroxyl radicals and other types of oxidative stress \((\text{Free et al} 3)\). The study is a unique one for supplementing \(\beta\)-carotene, along with vitamin E and vitamin C - where \(\beta\)-carotene actively scavenge and deactivates free radicals both in vitro and in vivo. Additionally it acts as a single oxygen quencher.

G-6-PD is the rate-limiting enzyme in the first step of Pentose Phosphate pathway, which is essential for the production of NADPH in the RBC for the recycling of endogenous antioxidant GSH \((\text{Jacobasch and Rapoport} 6)\). Some of those NADPH importantly neutralizes hydrogen peroxide, glutathione and other organic peroxides produced in the oxidative stress \((\text{Stryer} 12)\). These information made the G-6-PD level an even more important marker for post-exercise oxidative stress management system, particularly for females, where the natural antioxidant hormone, estrogen, is acting. This is the first time that such a model has been used for the study of exercise-induced oxidative stress.

Our hypothesis that endogenous whole blood G-6-PD status is crucially important in protecting against exercise-induced oxidative stress, at least in females and thus in influencing endurance to exhaustive exercise, was tested using the comparative data between the pre-supplementation and post-supplementation of vitamin mixture.

To conclude, one might say:

1) antioxidant vitamins help to act as an effective protocol in "exercise-induced oxidative stress management" on exercising females, specifically by increasing G-6-PD level and thus play a permissive role in the enhancement of endurance of trained females;

2) antioxidant vitamin mixture \((\text{vitamin E, vitamin C and } \beta\text{-carotene})\) appears to be more effective over vitamin E supplementation singly in increasing G-6-PD level.

**REFERENCES**


10 Sen, C K, L Packer and O Hanninen (Editors), Exercise and oxygen toxicity Amsterdam Elsevier Science (In Press)


No. 30/2002-2003

Dear Sir/Madam,

I am glad to inform you that your paper has been accepted for presentation under ISCA Young Scientists Award Programme in the Section of Physics (Condensed Matter Physics) at the 90th Indian Science Congress to be held at Bangalore University, Bangalore from January 3 to 7, 2003.

You are requested to kindly present your full paper, membership card, certificate of age (original) and a copy of your biodata at the Indian Science Congress Association Camp Office, at Bangalore on January 4, 2003.

F.A./D.A. will be paid by ISCA at the venue by cheque (maximum of first class/A.C.III Tier, which is available, train fare by the convenient shortest route to and from residence/institute to venue and back and D.A. as per ISCA rules not more than 7 days). You will be required to submit xerox copy of the ticket for reimbursement of your T.A.

Thanking you,

Yours faithfully,

(A. B. Banerjee)
General Secretary (Hqrs.)