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EFFECT OF VITAMINS E AND C ON EXERCISE-INDUCED OXIDATIVE STRESS

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ABSTRACT

The effects of ingesting antioxidant vitamins E and C for 4 weeks on serum malondialdehyde (MDA) levels at rest and after exercise was studied in humans. Twenty-four young healthy males aged 15 – 30 years participated in the study. They were randomly assigned to either an antioxidant vitamin supplemented group (daily doses of 1000IU of α– tocopherol and 1000mg of ascorbic acid) or a control group. Exercise consisted of 40 min of treadmill running at 60% of maximal O_2 consumption (VO_2 max) followed by 10min of running at 90% of VO_2 max. Blood samples were collected at rest and immediately after two exercise bouts. The mean values of serum MDA concentrations (marker of lipid peroxidation) in antioxidant vitamins E and C supplemented group increased from 4.0 ± 0.04 nmol MDA /ml at rest to 4.95 ± 0.06 nmol MDA /ml at moderate exercise and then to 5.88 ± 0.4 nmol MDA /ml at high intensity exercise. It was concluded that taking 1000mg vitamin C and 1000IU of vitamin E daily lowered the makers of lipid peroxidation at rest and after exercise but does not prevent the exercise induced increase in oxidative stress.

KEYWORDS: Vitamin E, Vitamin C, Oxidative stress, exercise.

INTRODUCTION

It is now well established that free radicals and other reactive oxygen species are continuously produced in physiological systems. These free radicals and other reactive oxygen species serve useful physiological functions but they can be toxic when generated in excess (Aruoma *et al*, 1991). Excess generation of reactive oxygen species within tissues can cause damage to DNA, lipids, proteins and carbohydrates. Which of these is the most important target of damage depends upon the cell type subjected to the oxidative stress and upon how it is imposed (Halliwell *et al*, 1992).

There is growing evidence that free radical production and subsequent lipid peroxidation are normal sequelae to the rise in oxygen consumption concomitant with exercise and are positively correlated with increase in selected muscle damage (Kenter *et al*, 1985).

The potential of dietary antioxidants to detoxify the peroxides produced during exercise has received increasing attention in recent year (Langseth, 1992). The nutrients that have shown promise as protective antioxidants are lipid - soluble antioxidants such as vitamins E and A in humans because of their association with membrane lipids (Hortons and Fairhurst, 1987). Vitamin C serves directly as an antioxidant by scavenging aqueous peroxyl radicals and indirectly by regenerating reduced vitamin E (Frei, 1991).

The potential of these dietary antioxidants to enhance the glutathione status of human blood during exercise has received increasing attention (Kenter *at al*, 1985). However, there is paucity of information regarding the ability of these vitamins, whether ingested alone or in combination, to protect against exercise – induced oxidative stress. It is therefore, the purpose of this work to examine the role of vitamins C and E mixture on exercise – induced oxidative stress.

MATERIALS AND METHODS

Subjects: Volunteers were recruited from within the Imo State University environment. The study protocol was carefully explained to them before they gave written consent to participate in this study as required by WHO (TDR, 2001 and TDR, 2002). Candidates were in good health by history and physical examination.

Diet Levels and Serum Vitamins Levels: A diet history and 3 – day diet record was obtained from each subject before commencement of the study. Subjects taking vitamin supplements were excluded from participating in this study. Maintenance of established dietary patterns was encouraged throughout the study. Fasting blood samples were obtained for measurement of serum Vitamins E and C levels.

Vo_2 Max and Body Weight Measurement: VO_2 max, was measured during a graded treadmill exercise test to exhaustion, as described by Coyle *et al* (1984). Body weight was also taken and recorded.

Study Design: Twenty-four young non-smoking males between the age range of 15 – 30 years participated in the study. The subjects were randomly assigned to either an antioxidant supplemented (AS) or a control group. The VS group consumed one capsule containing 1000IU of α-tocopherol and 2 tablets containing 500mg of ascorbic acid. The placebo was composed of saturated triacylglycerols spray-dried onto a dextrin matrix and coloured gelatin beadlets (product of Pfizer Nigeria Ltd). Subjects consumed one capsule of Vitamin E and one tablet of Vitamin C before breakfast and one tablet of Vitamin C in the evening before dinner.

The subjects were instructed to refrain from strenuous physical exercise on the day preceding the exercise test. On the day of the test, subjects reported to the laboratory after a 10 hr fast. After 15 min of seated rest, blood samples were obtained for measurement of serum malondialdehyde (MDA).

The subjects performed 40 min of treadmill running at an intensity corresponding to 60% of $V0_2$ max. At the conclusion of the exercise bout, subjects were seated for 15 min and then blood samples for measurement of MDA obtained. The subjects then ran on the treadmill for an additional 10 min, during which the speed and elevation were generally increased so that they were exercising at 90% of their VO_2 max. Blood samples for serum MDA measurement were obtained at the conclusion of 10 min bout exercise.

After four weeks of antioxidant vitamins or placebo ingestion subjects returned to the laboratory to repeat exercise.

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ANALYTICAL PROCEDURES

Serum malondialdehyde (MDA) was measured by a thiobarbituric acid assay procedure (Albro *et al*, 1986), which was calibrated using 1, 1, 3, 3, – tetraethoxypropane (Sigma chemical, St louis, MO) as a standard. Results were expressed as nanomoles of MDA per millimetre of serum.

Serum Vitamin C (ascorbic acid) was assayed by the 2 – 4 –nitrophenyl – hydrazine methods of Tietz (1986). Vitamin E (∝- tocopherol) was determined by reduction of ferric to ferrous ion by vitamin E, which then forms a red complex with ∝ - ∝– dipyridyl (Quaife *et al*, 1949).

STATISTICS

All values were expressed as mean ± S.D. The statistical analysis was carried out using Duncan Multiple range test to detect differences in the concentrations of lipid peroxide product (MDA), Vitamin E and C. Test with P< 0.05 were considered significantly different. All results were analysed by the Statistical Analysis System (SAS) program (SAS institute, Inc. Cary, N. C)

RESULTS

Table I shows the mean values of body weight changes before and after 4 weeks of vitamin mixture or placebo ingestion. The results show that there was no significant change in all. It also shows the mean values of serum vitamins C and E concentrations before and after 4 weeks of vitamin mixture or placebo ingestion. This shows that there were significant changes in the mean values of serum vitamins C and E concentrations after 4 weeks of vitamin mixture ingestion ($p<0.05$).

Table II shows the comparism between mean values of serum MDA levels at rest, moderate and high intensity exercises in both vitamin supplemented group and placebo group. The mean serum MDA concentrations in the placebo group significantly increased from 4.99 ± 0.6 nmol MDA /ml at rest to 6.11 ± 1.0 nmol MDA /ml at moderate exercise and then to 7.24 ± 0.9 nmol MDA /ml at high intensity exercise ($p < 0.05$).

Also the mean values of serum MDA concentrations in vitamin supplemented group increased from 4.0 ± 0.04 nmol MDA /ml at rest to 4.95 ± 0.06 nmol MDA /ml at moderate exercise and then to 5.88 ± 0.4 nmol MDA /ml at high intensity exercise.

While Table 111 shows the comparism between mean values of serum malondialdehyde level in the vitamin supplement group before and after four weeks of Vitamin C and E supplementation. The mean serum MDA concentrations 4.0 ± 0.04, 4.95 ± 0.06 and 5.88 ± 0.4 obtained at rest, moderate and high intensity exercise respectively were significantly reduced ($p < 0.05$) in subject after vitamin supplementation when compared to 4.99 ± 0.6, 6.11 ± 1.0 and 7.24 ± 0.9 obtained at rest, moderate and high intensity exercise respectively in placebo group ($P > 0.05$).

Table 1: Serum Body weight and Vitamins C and E Concentrations before and after 4 Weeks of Vitamin Mixture or Placebo Ingestion.

	VS		P	
	Before	After	Before	After
Body weight, kg	72.3 ±6.8	71.8 ± 5.4	71.6 ± 8.2	71.2 ± 7.5
∝ tecopherol (mg/dl)	0.76 ± 0.21[a]	1.98 ± 0.64[b]	0.78 ± 0.23[a]	0.86 ± 0.1[a]
Vit C (mg/dl)	1.42 ± 0.35[a]	2.01 ± 0.31[b]	1.39 ± 0.38[a]	1.43 ± 0.38[a]

Means with the same superfix are not significant at $p > 0.05$. Results are shown as Means ± SD.

TABLE II: The comparism between mean values of serum MDA levels at rest, moderate and high intensity exercises in both vitamin supplemented group and placebo group (nmol/ml).

	At rest	After Moderate Exercise	After High Intensity Exercise
P	4.99 ± 0.6	6.11 ± 1.0*	7.24 ± 0.9**
VS	4.0 ± 0.4	4.95 ± 0.6*	5.88 ± 0.4**

Values are in mean ± S.D.

* Significantly different from group at rest $p < 0.05$

** Significantly different from group at rest and at moderate exercise $p < 0.05$

Table III: Comparism between mean values of serum MDA level in groups before and after four weeks of placebo and Vitamin C and E supplementation (nmol/ml).

	P	VS	Level of significance
Before exercise	4.99 ± 0.6	4.0 ± 0.4	$p < 0.05$
After moderate exercise	6.11 ± 1.0	4.95 ± 0.6	$p < 0.05$
After high intensity exercise	7.24 ± 0.9	5.88 ± 0.4	$p < 0.05$

Values are in mean ± S.D.

DISCUSSION

The rise in oxygen consumption associated with physical exercise has been increasingly implicated in the production of damaging free radicals of oxygen resulting in the formation of lipid peroxides (Davies et al, 1982). The observed significant high levels of serum MDA concentrations (end products of lipid peroxidation) after moderate and high intensities of exercise are in conformation with previous reports that showed lipid peroxidation product levels to be either not affected or increased at various intensities and duration of exercise. Dillard *et al*, (1978), reported two to three fold increases in expired pentane production in subject exercising at 50% of VO_2 max. Allessio *et al* (1988) reported a relationship between exercise intensity and serum MDA values. Sumikawa *et al* (1993) reported elevated markers of lipid peroxidation as a result of physical training and acute exercise.

Considering the rise in peroxidative bye – products as a result of exercise, the issue of protection from free radical damage becomes an important one. Various researchers have reported significant increases in the activity of the antioxidant enzymes catalase, superoxide dismutase and/or glutathione peroxidase in both animal (Kanter *et al*, 1985) and in human (Jenkins *et al*, 1984) models after exercise training. However as Higuchi *et al*, (1985) suggested, increases in respiratory chain constituents after training are greater than the changes in antioxidant capacity. Therefore the potential of exogenous antioxidants to detoxify free radicals produced during exercise is of considerable interest. The results of this study show that an antioxidant vitamin supplement brings about significant reductions in serum MDA concentration. After exercise, however it is not readily apparent that the vitamin E and C mixture provides additional protection. Post exercise values were significantly greater than resting as well as post exercise MDA levels. Therefore although vitamin E and C mixture did not produce additional lipid peroxide lowering effects once exercise began, it is apparent that the antioxidant effect manifested at rest did carry over to exercise as well.

Various studies have indicated that deficiencies of individual antioxidant vitamins can potentiate oxidant stress and that supplementation with individual vitamins particularly vitamin E, may alternate lipid peroxidation (Frei, 1991). The results of the present study provide evidence that supplementation with two antioxidant vitamins simultaneously can decrease the absolute levels of lipid peroxide marker produced during exercise. Whether this apparently beneficial effect is attributable to an individual vitamin or is the cumulative effect of the vitamin mixture remains to be elucidated. Though it has been shown that ascorbic acid and α- tocopherol can act synergistically in the inhibition of oxidation. Packer *et al* (1979) have shown in the pulse radiolysis study that α - tocopherol which has higher reaction rate (Kinh) than ascorbic acid scavenges the peroxyl radical quickly than ascorbic acid but the α - tocopheroxyl radical derived from α - tocopherol is reduced back to regenerate α - tocopherol by ascorbic acid.

SUMMARY AND CONCLUSION

We conclude that daily ingestion of 1000mg of ascorbic acid and 1000IU of α - tocopherol equivalents for 4 weeks results in a significant reduction in the absolute levels of serum MDA, both at rest and after moderate and heavy exercise. Such a beneficial combination may therefore be recommended to persons involved in strenuous exercise or other activities that endanger oxidative stress.

REFERENCES

Albro, P. W., Corbelt, J. T. and Schroeder, J. L., 1986. Application of the thiobarbiturate assay to the measurement of lipid products in microsomes. *Chem. Biol. Interact* 86: 185 – 194

Allesio, H. M., 1993. Exercise induced oxidative stress: *Med. Sci. Sports – Exercise* 25 (2): 218 – 224

Aruoma, O.; Kaur, H. and Halliwel, B. G., 1991. Oxygen free radicals and human diseases. *J. Roy.*Soc. Health. 9: 172 – 177

Coyle, E. F., Martin, W. H., Sinacore, D. R., Joyner, M. J., Hagberg, J. M. and Holloszy, J. O., 1984. Time course of loss of adaptations after stopping intense endurance training. *J. Appl. Physiol. 57*:1859 – 1864.

Davies, K. J. A.; Quintanilha, A. T.; Brooks, G. A. and Packer, L., 1982. Free radicals and tissue damage produced by exercise. *Biochem. Biophys. Res. Commun.* 107: 1198 – 1205.

Dillard, C. J., Litov, R. E., Sowin, W. M., Dumelin, E. and Tappel, A. L., 1978. Effects of exercise, vitamin E, and ozone on pulmonary function and lipid peroxidation. *J. Appl. Physiol.* 45: 927 – 932.

Frei, B., 1991. Ascorbic acid protects lipids in human and low, density lipoprotein against oxidative damage. *Am. J. Clin. Nutr.* 54: 135 – 185.

Halliwell, B. Gutteridge, J. M. C. and Cross, C. E., 1992. Free radicals antioxidants and human diseases: where are we now? *J. Lab. Cli. Med.* 119(16): 598 – 620

Higuchi M., Carter, J., Chen, M and Hollozy. 1985. Superoxide dismuatse and catalase in skeletal muscle: adaptive response to exercise. *J. Gerontol.* 40: 281 – 296.

Horton, A. A. and Fairhurst, S., 1987. Lipid peroxidation and mechanisms of toxicity CRC. *Crit. Rev. Toxicol.* 18: 27 – 29

Jenkins, R. R.; Friedland, R. and Howald, H., 1984. The relationship of oxygen uptake to superoxide dismutase and catalase activity in human skeletal muscle. *Int. J. Sports Med. 5*: 11 – 14.

Kanter, M. M., Hamlin, R. L., Unverferth, D. V., Davis, H. W. and Merola, A. J., 1985. Effect of exercise training on antioxidant enzymes and cardiotoxicity of doxorubicin. *J. Appl. Physiol. 59:* 1298 – 1303.

Langseth, L., 1992. Antioxidant vitamins. *News Letter. Bull* 2: 10 – 25.

Packer, J. E.; Slater, T. F. and Oibon, R. L., 1979. Direct observation of a free radical intersection between vitamin E and C. *Nature Lord 278:* 737 – 738.

Sumikawa, K., Mu, Z., Inons, T., Okochi, T., Yoshide, T. and Adachi, K.., 1993. Changes in erythrocyte membrane phospholipid composition induced by physical training and physical exercise. *Gur. Appl. Physical.* 67(2): 132 – 137.

Tiezt, W., 1986. Ascorbic acid. In: Fundamentals of Clinical Chemistry. W.B. Saunders Company, Britain. Pp 422-519.

Quaife, M. L., Scrimshan, N. S. and Lowry, O. H., 1949. A micromethod for assay of total tocopherols in blood serum. *J Biol. Chem. 180:* 1229-1236.