# THE EFFECT OF COMBINED VITAMIN C, E, b-CAROTENE AND SELENIUM SUPPLEMENTATION ON PHYSICAL PERFORMANCE AND ANTIOXIDANT STATUS IN YOUNG MEN

**by**

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The aim of the research was to evaluate the effect of antioxidant vitamin supplementation on physical performance in young males. The research material included 24 male students, divided into two groups: C – control, S – supplemented with vitamin C, E, β-carotene and selenium. Both groups performed a identical one-hour ergocycle effort with varied intensity before and after 3 and 6 weeks of the experiment. Maximal oxygen uptake ($VO_2$max) and total work output (TWO) were evaluated for all subjects. Blood concentration of lactate (LA), α-tocopherol, γ-tocopherol, selenium, retinol, malondialdehyde (MDA), creatine kinase (CK) activity and activities of antioxidant enzymes – SOD, GSH-Px, CAT was evaluated. The students supplemented with antioxidant vitamins increased their $VO_2$max by 11% and TWO by 9%, yet these differences were statistically insignificant. After 3 weeks of supplementation the concentration of α-tocopherol increased significantly, while that of vitamin C did not. The level of γ-tocopherol and retinol did not change after 6 weeks of supplementation, the level of the metabolites returned to initial values, except that of γ-tocopherol which decreased significantly ($p<0,05$). The improvement of physical work capacity was much higher for the group receiving antioxidant vitamins, thus the authors suggest supplementation with such vitamins over the period of 3 weeks for endurance athletes. Supplementation with antioxidant enzymes did not changes activities of SOD, GSH-Px and CAT. Since all of the vitamins and selenium present in the Zellschutz products have antioxidant properties, it is difficult to establish which one has the greatest effect on physical work capacity. It seems necessary to conduct further research in this area with particular vitamins at different doses.

**Key words**: supplementation, $VO_2$max, physical work capacity, antioxidants

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The effect of supplementation with antioxidant vitamins on physical performance is controversial. Earlier studies indicated a improvement of physical work capacity following supplementation, yet current, more advanced research do not confirm these results (Takanami et al. 2000). Currently it is thought that antioxidants protect the muscles against the free radicals, generated during endurance exercise (Evans 2000, Kanter et al. 1993). Antioxidants may also influence the production of energy by increasing the level of substrates or coenzymes necessary for particular biochemical reactions. It is also supposed that antioxidants help in the removal of $H^+$ ions which inhibit many of those reactions. At the moment it is difficult to establish which of the antioxidants have the greatest influence on these properties, what doses are optimal and how long the supplementation process should take.

The established functions of antioxidant vitamins predispose them for improving physical work capacity. It is a known fact that the concentration of these vitamins increases after supplements yet further migration to tissues is hindered do to the structure and the accompanying biochemical properties, for example: water-soluble (vitamin C) and lipid-soluble (vitamin E) (Schröder et al. 2001, Thompson et al. 2001). A crucial role in maintaing the proper concentration of antioxidant vitamins in the body is played by the system responsible for reconstructing these substances from radical forms (Gerster 1989).

Bioavailabity is a very important property of antioxidant vitamins. The dose of these vitamins should cause a significant increase of their blood concentration. Previous and current research has used a varied scope of dosages, from physiologically justified to megadoses (Beaton et al. 2000, Davson et al. 2002, Peters et al. 2001). Takanami et al. (2000) suggested supplementing endurance athletes with vitamin E at doses of 100- to 200 mg/day. Supplementation of athletes with vitamin C usually occurs at doses ranging from 500 to 1500 mg/day over period of few days or several weeks (Nieman et al. 2002, Tauler 2002, Thompson et al. 2001). In this research the subjects were supplemented with a highly bioavailable antioxidant complex (vitamin C, E, β-carotene and selenium) over a period of 6 weeks.

The main objective of this work was to evaluate the effect of oral supplementation with combined vitamins: E, C, β-carotene and selenium on maximal oxygen uptake ($VO_2max$) and work volume (WV) as well as antioxidant status in men (enzymatic and non enzymatic antioxidants)

## *Material and Methods*

The research included 24 male students of physical education randomly divided into 2 groups: (S, n=12) and (C, n=12). The basic characteristics of the subjects are presented in Table 1. All student were informed of the purpose and the nature of the study before giving their written consent to participate in the experiment, which had been approved by the Ethics Committee at the Medical University of Silesia in Katowice. None of the men had taken medicines and vitamin for at last 30 days before and during the experiment.

Table 1. Physical characteristics of subjects

| *Parameter* | Supplemented group (S) | | Control group (C) | |
|---|---|---|---|---|
| | | SD | $\bar{x}$ | SD |
| Age (years) | 20,7 | 0,6 | 20,7 | 1,1 |
| Body mass (kg) | 75,0 | 6,9 | 70,8 | 5,7 |
| Body height (cm) | 178,0 | 6,9 | 175,9 | 7,5 |
| $VO_2max$ ($ml \cdot kg^{-1} \cdot min^{-1}$) | 51,6 | 8,9 | 50,4 | 8,9 |

Participants from S group was supplemented daily with a Zellschutz supplement (40 g) (FitLine – Germany) consisting of vitamins C (150 mg), E (24 mg), β-carotene (14,4 mg) and selenium (80 μg) for an overall period of 6 weeks. The control group (C) – did not receive any supplement. During the entire period none of the participants performed any kind of specific training. The students only performed obligatory physical exercise (3 h/day) which were included in the education program. One week before the first experimental trial maximal workload (Wmax) was determined using a graded exercise test to determine the 60% workload used in the experimental protocol. Shortly before the first and after the last exercise test, $VO_2max$ was evaluated on a cycloergometer (Monark 829E) during a submaximal test (according to

Astrand). The subjects from both groups were subjected to a identical one-hour ergocycle effort (Monark 814 E), with varied intensity, before and after 3 and 6 weeks of the experiment. The test consisted of continuous cycling (45 min) at 60% W max at a constant speed (60 rpm), followed by 15 min of work with progressive intensity to perform as much work as possible (Jekendrup et al. 1996). Oxygen uptake (Oxycon Alpha– Jaeger -Germany) was recorded during the test. Relative work was monitored during the last 15 min of the exercise protocol (PC MCE v-2.3 program-JBA; Poland). The total amount of work performed during the last 15 min (power output over 15 min) and $VO_2$max were taken as a measure of physical work capacity. The average energetic values of the diets and the amount of vitamins A, C, E we calculated with the use of a computer program „Dietus BUI InFit" 1995 (Poland).

Blood samples for biochemical analysis were drawn from the anticubital vein at rest, immediately after the exercise protocol and after 1 h of restitution. The concentration of α-tocopherol and γ-tocopherol as well as retinol were evaluated in plasma with the use of liquid chromatography (HPLC) (Sobczak et al. 1999), lactate (LA) concentration was evaluated in plasma with commercial kits of Biomerieux. Plasma creatine kinase activity (CK, EC 2.7.3.2) were determined with Analco (Poland) kit and malondialdehyde level (MDA) with Buege-Aust'a method (Buege and Aust (1978). Whole blood vitamin C concentration was evaluated with the Omaye method (Omaye et al. 1979) and selenium level with spectrofluorymeter by Danch at al. (1996) method. Glutathione peroxidase (GSH-Px, EC 1.11.1.9) activity was determined in whole blood by the method of Flohe and Gunzler (1984) with tert-butyl-hydroperoxide as substrate, superoxide dysmutase (SOD, EC 1.15.1.1) in erythrocytes using commercially available RANSOD (UK) kit, catalase (CAT, EC 1.11.1.6) by the method of Aebi (1974).

For statistical purposes the „Statistica" (Software 1995) was used, as mean values (X) and standard deviations (SD) were calculated. The Student „t" test for depended and independent variables was applied to determine the significance of changes in- and between both student groups. All data were tested for homogeneity of variances using the Levene's test and then analyzed using 2-way analysis of variance (ANOVA) in order to determine the effects of independent factors: 1- supplementation (group S and C), 2-time of

supplementation (0, 3, 6 weeks). Significant main effects and interactions were further analyzed using a Tukey *post hoc* test. The CK data was not normally distributed, so it was logarithmically transformed and the log-transformed data was then subsequently analyzed using the nonparametric Friedman test. The level of significance for all analyses was accepted at $p<0,05$.

## *Results*

The analysis of the tested subjects diet is presented in table 2. The data indicates no significant difference in the caloric value of diets of both groups (51,4kcal for group S, and 52,8 kcal for control group). The amount of vitamin A,C,E and selenium were consistent with RDA and after supplementation were much higher in the group supplemented with Zellschutz product. The research indicated a body mass loss of 0,63 kg before supplementation, 0,63 kg after 3 weeks and 0,58 kg after 6 weeks, during the 1 h exercise protocol. The loss of body mass amounted close to 1%. It was assumed that this did not effect performance.

Table 2. The average daily caloric value of the diets and the amount of vitamins A, E and C in them, in supplemented (S) and control groups (C)

| Group | | Energetical value (kcal) | Vitamin A[1] (μg) | **Vitamin C** (mg) | **Vitamin E** (mg) |
|---|---|---|---|---|---|
| S | $\bar{x}$ | 3856,7 | 844,6 (+2500)[2] | 59,74 (+150) | 8,36 (+24) |
| | SD | 409,7 | 239,2 | 30,6 | 3,7 |
| C | $\bar{x}$ | 3746,0 | 710,9 | 86,5 | 8,5 |
| | SD | 1136,3 | 280,7 | 54,9 | 4,8 |

[1]equivalents of retinol
[2]in parenthesis the additional amount of vitamins given through supplementation

During the test, oxygen uptake was varied. At rest oxygen uptake equaled 387±41,3 $ml \cdot min^{-1}$. During the first 10 min it rose to 2414,3±198,7 $ml \cdot min^{-1}$, and maintained at this level for 45 min. During the last 15 min of progressive intensity oxygen uptake rose to 3521,0±50,2 $ml \cdot min^{-1}$ .

The supplementation with antioxidant vitamins caused a statistically insignificant rise in oxygen uptake. In the supplemented group the initial $VO_2$max equaled 51,6±8,9 ml·kg$^{-1}$·min$^{-1}$ while this value rose to 57,4±9,1 ml·kg$^{-1}$·min$^{-1}$ after 6 weeks. The values of $VO_2$max for the control group were respectively 49,2±10,1 and 51,4±8,3 ml·kg$^{-1}$·min$^{-1}$. The increase in the supplemented group was 11% (440 ml $O_2$/min).

During the 3rd week of supplementation the experimental group showed a insignificant increase in relative work (9%), yet was significantly ($p<0,05$) greater than for the control group, at 6 weeks no further improvement was observed and the differences between groups were insignificant (tab. 3). The analysis of variance (2-way ANOVA) indicated a statistically significant effect of supplementation on the amount of relative work performed at last 15 min. of endurance exercise ($F=8,5$ and $p<0,01$).

Table 3. Relative work (during 15 min. of 1 h exercise protocol in the supplemented (S) and control groups (C)

| **Group** | Evaluation I (J/kg b.m.) | | *Evaluation II (J/kg b.m.)* | | Evaluation III (J/kg b.m.) | |
|---|---|---|---|---|---|---|
| | $\bar{x}$ | SD | $\bar{x}$ | SD | $\bar{x}$ | SD |
| S | 2128,8 | 335,9 | 2326,2* | 348,1 | 2353,4 | 346,8 |
| C | 2001,7 | 269,6 | 2052,0 | 261,1 | 2125,3 | 248,8 |

*Significantly different from the control group value at: $p<0,05$

The concentration of lactate after 45 min of exercise was close to 3 mmol/l, over 8 mmol/l after the protocol and 1,8 mmol/ after 1 h of restitution. The ANOVA also indicated a statistically significant influence of antioxidant vitamin supplementation on the concentration of α-tocopherol ($F=13,2$, and $p<0,001$). The interaction between independent factor was also significant ($F=6,0$ and $p<0,01$). The *post hoc* Tukey tests showed a insignificant increase in the concentration of α-tocopherol after 3 weeks of supplementation ($p=0,06$) yet this increase was significantly greater ($p<0,001$) in comparison to the control group. After 6 weeks such tendencies, were not observed (tab. 4). The same analysis indicated a significant influence of time on the

Table 4. α-tocopherol, γ-tocopherol, ascorbic acid (vit. C), selenium, retinol, malondialdehyd (MDA), concentrations and creatine kinase (CK) activity in supplemented (S) and control (C) groups

| **Variables** | *Group* | **Trial I** | | **Trial II** | | **Trial III** | |
|---|---|---|---|---|---|---|---|
| | | $\bar{x}$ | SD | $\bar{x}$ | SD | $\bar{x}$ | SD |
| α-tocopherol (μg/ml) | S | 14,6 | 2,3 | 17,9# | 3,1 | 13,7 | 2,3 |
| | C | 13,6 | 4,6 | 12,2 | 2,4 | 13,1 | 1,4 |
| γ-tocopherol (μg/ml) | **S** | 0,94 | 0,21 | 0,98 | 0,39 | 0,69* | 0,15 |
| | **C** | 0,82 | 0,34 | 0,87 | 0,27 | 0,61 | 0,16 |
| Vitamin C (μg/ml) | **S** | 27,3 | 6,8 | 29,9 | 8,7 | 27,9 | 6,6 |
| | **C** | 24,3 | 4,9 | 25,4 | 3,8 | 24,2 | 5,0 |
| Selenium (ng/ml) | **S** | 75,3 | 12,1 | 80,2 | 15,7 | 69,5 | 12,4 |
| | **C** | 73,6 | 10,4 | 58,1* | 7,5 | 68,2 | 14,8 |
| *Retinol (mg/ml)* | **S** | 0,47 | 0,06 | 0,46 | 0,09 | 0,48 | 0,10 |
| *CK (log U/l)* | **S** | 2,17 | 0,28 | 2,17 | 0,28 | 2,17 | 0,22 |
| | **C** | 1,98 | 0,11 | 1,94 | 0,11 | 1,98 | 0,08 |
| *MDA (nmol/l)* | **S** | 4,5 | 0,6 | 4,2 | 0,7 | 4,6 | 0,6 |
| | **C** | 4,3 | 0,7 | 4,5 | 0,9 | 4,5 | 0,7 |

* Significantly different from the pre-research value at: $p<0,05$
#Significantly different from the control group at: $p<0,001$

γ-tocopherol ($F=7,1$ and $p<0,01$) and a insignificant effect of supplementation. The interaction between independent factors was insignificant**.** The level of ascorbic acid also increased after 3 weeks of supplementation, yet this difference was not statistically significant (tab. 4). The ANOVA indicated a statistically significant influence of antioxidant supplementation on the concentration of ascorbic acid in blood ($F=6,7$ and $p<0,01$). The interaction between independent factors was insignificant. The level of retinol did not change (tab. 4). After 6 weeks of supplementation the concentration of α-tocopherol and ascorbic acid returned to initial values, that of retinol, did not

change, while the level of γ-tocopherol decreased significantly ($p<0,05$). The analysis of variance also indicated a significant effect of supplementation on the concentration of selenium in the blood ($F=8,1$ and $p<0,01$). Statistically significant was also the interaction of independent factors which included the time of the experiment and supplementation ($F=5,5$ and $p<0,01$). The post hoc Tukey's test results showed, a statistically significant decrease ($p<0,05$) in the concentration of selenium in the control group after 3 weeks of the research (tab. 4). The concentration of selenium was significantly different between the control and supplemented groups ($p<0,001$). There was no significant effect of supplementation with antioxidant vitamins on the plasma concentration of malondialdehyde(MDA) (tab. 4). The application of the nonparametric Friedman's test, did not confirm the effect of 3 and 6 week supplementation on the activity of plasma CK (tab. 4). The results of antioxidant enzyme activities are presented in table 5. Supplementation with combined vitamins E, C, β-carotene and selenium had no significant effect (2-way ANOVA) on resting activities of SOD, CAT, GSH-Px.

Table 5. Glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT) activities in supplemented (S) and control (C) groups, in trials I, II and III

| **Variables** | *Group* | **Trial I** | | **Trial II** | | **Trial III** | |
|---|---|---|---|---|---|---|---|
| | | $\bar{x}$ | SD | $\bar{x}$ | SD | $\bar{x}$ | SD |
| GSH-Px (U/gHb) | S | 14,9 | 2,9 | 15,4 | 2,1 | 15,1 | 3,1 |
| | C | 15,1 | 2,6 | 14,9 | 2,7 | 14,9 | 2,2 |
| SOD (U/gHb) | **S** | 855,6 | 106,8 | 912,8 | 102,4 | 919,6 | 97,6 |
| | **C** | 862,1 | 71,6 | 914,1 | 51,8 | 879,4 | 85,9 |
| CAT (k/gHb) | **S** | 169,6 | 25,1 | 178,3 | 23,3 | 185,1 | 24,3 |
| | **C** | 173,2 | 23,8 | 179,9 | 17,8 | 173,3 | 31,6 |

## *Discussion*

Aerobic work capacity depends to a large extent on the effectiveness of the cardiovascular system and may be improved by physical training and supplementation (Haymes 1991). Work capacity is also dependent on the synthesis of structural and enzymatic proteins. The last ones act as catalysts of

chemical reactions, allowing the athlete to reach the steady state. This helps to supply the working muscles in ATP (Packer 1997). It is know fact that vitamin E activates the reactions of acetyl-Co A with oxaloacetate initiating Krebs cycle. Vitamin E activates and stimulates the of electrons flow in respirator chain. It also diminishes the consequences of hypoxia, controls the metabolism of creatine and improves circulation, by increasing stroke volume and the diffusion of oxygen to active tissues (Shephard 1983) . Supplementation of athletes with vitamin E of different doses has not proven to be ergogenic.

Vitamin E is the major antioxidant vitamin of lipid environments, while vitamin C is the major antioxidant vitamin in aquaeus environments. Recent evidence indicates that the two are tightly interlinked both with each other and with other antioxidant systems. Vitamin E is the major chain-breaking antioxidant in membranes and lipoproteins by breaking the chain of lipid peroxidation.

Vitamin C is an essential cofactor in number of hydroxylases such as prolyl hydroxylase and lysyl hydroxylase. Since hydroxylation adds stability to the collagen triple helix, many of the symptoms of vitamin C deficiency, such as blood vessel fragility, can be traced to lack of proper collagen strength but in addition to its role in hydroxylation, vitamin C probably functions as an antioxidant. Vitamin C acts as a free radical scavenger, neutralizing such reactive oxygen species as superoxide hydrogen peroxide and hypochlorous acid in the process being converted to dehydroascorbic acid. Dehydroascorbic acid may be recycled to ascorbic acid by various mechanism (e.g. glutathione) (Packer 1997).

In the present research a low weight vitamin complex was used in which the metabolic effects of vitamin E and C was supported by β-carotene and selenium. A insignificant rise in $VO_2$max occurred in 60% of the tested subjects. It seems that a increase of 440 ml/min in maximal oxygen do to supplementation may by significant to competitive athletes despite the lack of statistical significance in this research project. Some authors assume that higher doses of antioxidant vitamins may cause of greater changes in aerobic capacity (Antosiewicz 1998). A review of scientific literature indicates the lack of influence of antioxidant vitamins on work capacity (Bendich 1991, Van der Beek 1991). Rokitzki et al. (1994) confirmed this opinion when they

reported no effect on physical performance in German elite cyclists after 5 month of supplementation with vitamin E (320 IU/day) despite a substantial increase in the plasma level of this vitamin. Likewise, vitamin E supplementation had no effect on performance in the marathon when ingested prior to competition (Buchman et al. 1999). Itoch et al. (2000) also detected no significant difference in physical performance of runners supplemented with vitamin E (1200 IU/day for 4 weeks) and a placebo group.

The supplemented group also registered a insignificant rise in total work during the last 15 min of the test. After 3 week of the experiment the S group reached a significantly ($p<0,05$) higher work output in comparison to the C group. Significant effects of supplementation with vitamins C, E and β-carotene on work capacity were observed by Snider et al. (1992), Simon-Schnaβ et al. (1988), Shephard (1983) and Keren et al. (1980).

After 3 weeks of supplementation with antioxidant vitamins a significant increase in the concentration of α-tocopherol occurred, while the rise in the level of vitamin C and selenium was insignificant. The level of retinol and γ-tocopherol did not change. This indicates the organism reacts positive to 3 weeks of supplementation but after 6 weeks it adopts to the higher concentration of these vitamins as they are excreted or the bioavailability decreases. The ANOVA indicates that the supplementation influenced significantly the amount of work performed and the concentration of vitamins E and C. Although the evidence on antioxidants and performance is controversial and their effect in sports is probably minor, there is much evidence that antioxidants may indeed prevent some of the exercise-induced damage and possibly the balance in their favor in exercise over the long term. Schroder et al. (2000) indicated that basketball training significantly decreases the level of vitamin C in the organism of athletes, thus supplementation seems justified. Antioxidant supplementation stimulated the biosynthesis of enzymatic proteins – superoxide dismutase (SOD) and catalase (CAT), as their activity increased after 3 and 6 weeks of the experiment. There were no changes in glutathione peroxidase (GSH-Px) activity after antioxidant supplementation. Similarly Tauler et al. (2002) showed a significant increase in SOD and CAT activity in neutrophiles after supplementation with vitamin E

and C (90 days of vitamin E 500 mg/day, β-carotene 30 mg/day and last 15 days vitamin C 1 g/day).

The dosages of antioxidant vitamins used in the presented research did not change the resting creatine kinase (CK) activity, thus the protection of muscle cell membrane was insufficient. The supplementation did not diminish the antioxidant stress as the level o malondialdehyde (MDA) did not change after 3 or 6 weeks of ingesting the vitamins. It is supposed that daily doses of antioxidant vitamins were to small. Many factors related to physical exercise such as mechanical tissue damage, decrease in cellular energetic compounds, acidosis, influx of lymph and presence of soluble muscle proteins in the interstitium, membrane lipid peroxidation stimulate the influx of cellular enzymes into the blood. After extensive contraction of the muscles the CK efflux correlated with the enhanced free radical signal (Faff 2001). Itoch et al. (2000) reported that vitamin E administration (1200 IU per day, for 4 weeks) reduced CK activity following running training during six consecutive days.

## *Conclusions*

In conclusion it can be stated that supplementation with antioxidant vitamins and selenium has a positive effect on physical work capacity evaluated by $VO_2$max and total work performed in the last 15 min of a ergocycle test. Supplementation had not effort on blood antioxidant status and do not supported organism before exercise stress consequences. Supplementation with vitamins used in this study over a period of 3 weeks is recommended for endurance athletes. Since all of the vitamins and selenium present in the Zellschutz products have antioxidant properties, it is difficult to establish which one has the greatest effect on physical work capacity. It seems necessary to conduct further research in this area with particular vitamins at different doses.

## REFERENCES

Aebi H. 1974. *Catalase.* In Methods of Enzymatic Analysis. Ed. H.O.Bergmeyer ss. 673-683.

Antosiewicz J. 1998. *Witamina C w zywieniu sportowców.* Sport Wycz. 1-2: 60-66.

Beaton L.J., Allan D.A., Tarnopolsky M.A., Tiidus P.M., Philips S.M. 2002. *Contraction-induced muscle damage is unaffected by vitamin E supplementation.* Med. Sci. Sports Exerc 34(5): 798-805.

Bendich A. 1991. *Exercise and free radicals: effects of antioxidant vitamins.* Advances in nutrition and top sport. Ed. F. Brouns. Basel and Karger ss 59-78.

Buchman A.L., Killip D., Ou C.N., Rognerud C.L., Pownall H., Dennis K., Dunn J.K. 1999. *Short-term vitamin E supplementation before marathon running: a placebo-controlled trial*. Nutrition 15(4): 278-283.

Buege J.A., Aust S.D. 1978. *Microsomal lipid peroxidation.* In Methods in Enzymology. Ed. S.Fleisher, L.Packer, Academic Press, New York 52:302-310.

Danch A., Drózdz M. 1996. *Uproszczona metoda fluorymetrycznego oznaczenia selenu w materiale biologicznym.* Diagn. Lab. 32: 529-534.

Dawson B., Henry G.J., Goodman C., Gillam I., Beilby J.R., Ching S., Fabian V., Dasig D., Morling P., Kakulus B.A. 2002. *Effect of vitamin C and E supplementation on biochemical and ultrastructural indices of muscle damage after a 21 km run.* Int. J. Sports Med. 23(1): 10-15.

Evans W.J. 2000. *Vitamin E, vitamin C and exercise.* Am.J.Clin.Nutr. 72(2 Suppl.): 647S-652S.

Faff J. 2001. *Effects of the antioxidant supplementation in athletes on the exercise induced oxidative stress.* Biol. Sport 18(1): 3-20.

Flohé L., Gunzler W.A. 1984. Assay of glutathione peroxidase. In Methods in Enzymology. Academ. Press 105:114-120.

Gerster H. 1989. *Review: The role of vitamin C in athletic performance.* J.Amer.College Nutr. 8(6): 636-643.

Haymes E.M. 1991. *Vitamin and mineral supplementation to athletes*. Int.J.Sport Nutr. 1(2): 146-169.

Itoh H., Ohkuwa T., Yamazaki Y., Shimoda T., Wakayama A., Tamura S., Yamamoto T., Sato Y., Miyamura M. 2000. *Vitamin E supplementation attenuates leakage of enzymes following 6 succesive days of running training.* Int. J. Sport Med. 21(5): 369-374.

Jeukendrup A., Saris W.H.M., Brouns F., Kester A.D.M. 1996. *A new validated endurance performance test.* Med.Sci.Sports Exerc. 28(2): 266-270.

Kanter M., Nolte M., Lori A., Holloszy J.O. 1993. *Effects of antioxidant vitamin mixture on lipid peroxidation at rest and postexercise.* J.Appl.Physiol. 74(2): 965-969.

Keren G., Y. Epstein 1980. *The effect of high dosage vitamin C intake on aerobic and anaerobic capacity.* J.Sports Med.Phys.Fitness 20(2): 145-148.

Nieman D.C., Henson D.A., McAnulty S.R., McAnulty L., Swick N.S., Utter A.C., Vinci D.M. Opiela S.I. Morrow J.D. 2002. *Influence of vitamin C supplementation on oxidative and immune changes after an ultramarathon.* J. Appl. Physiol./ 92(5): 1970-1977.

Omaye S.T., Turnbul J.D., Sauberlich H.E. 1979. *Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids.* Methods in Enzymology. 62: 3-11.

Packer L. 1997. *Oxidants, antioxidant nutrients and the athlete.* J.Sports Sciences 15: 353-363.

Peters E.M., Anderson R., Nieman D.C., Ficki H., Jogessar V. 2001. *Vitamin C supplementation attenuates the increases in circulating cortisol, adrenaline and anti-inflamantory polypeptides following ultramarathon running.* Int.J.Sports Med. 22(7): 537-543.

Rokitzki L., Logemann E., Huber G., Keck E., Keul J. 1994. *a-Tocopherol supplementation in racing cyclists during extreme endurance training.* Int.J.Sport Nutr. 4: 253-264.

Schröder H., Navarro E., Tramullas A., Mora J., Galiano D. 2000. *Nutrition antioxidant status and oxidative stress in profesional baskedball players: Effect of a tree compaund antioxidative supplement.* Int.J.Sports Med. 21: 146-150.

Schröder H., Navarro E., Mora J., Galiano D., Tramullas A. 2001. *Effects of a-tocopherol b-carotene and ascorbic acid on oxidative hormonal and enzymatic exercise stress markers in habitual training activity of professional basketball players.* Eur.J.Nutr. 40(4): 178-184.

Shephard R.J. 1983. *Vitamin E and athletic performance.* J.Sports Med.Phys.Fitness. 23(4): 461-470.

Simon-Schnaβ I., Pabst H., Herrligkoffer K.M. 1987. *Der Einfluss von Vitamin E auf leistungsabhängige Parameter beim Höhenbergsteigen.* Dt.Z.f.Sportmed. 38(5): 199-206.

Simon-Schnaβ I.M., Pabst H. 1988. *Influence of vitamin E on physical performance.* Int. Vit.Nutr.Res. 58: 49-54.

Snider. I.P., Bazzarre T.L., Murdoch S.D., Goldfarb A. 1992. *Effects of Coenzyme Athletic System as on ergogenic aid on endurance performance to exhaustion.* Int.J.Sports Med. 2(3): 272-286.

Sobczak A., Skop B., Kula B. 1999. *Simulatenous determination of serum retinol and alpha- and gamma tocopherol levels in type II diabete patients using high-performance liquid chromatography with fluorescence detection.* J.Chromatogr.B.Biomed.Sci.Appl. 730: 265-271.

Takanami Y., Iwane H., Kawai Y., Shimomitsu T. 2000. *Vitamin E supplementation and endurance exercise: are there benefits?* Sports Med. 29(2): 73-83.

Tauler P., Aguilo A., Fuentespina E., Tur J.A., Pons A. 2002. *Diet supplementation with vitamin E, vitamin C and beta-caroten cocktail enhanges basal neutrophil antioxidant enzymes in athletes.* Pflugers Arch. 443(5-6): 791-797.

Thompson D., Williams C., Kingsley M., Nicholas C.W., Lakomy H.K., McArdle F., Jakson M.J. 2001. *Muscle soreness and damage parameters after prolonged intermittent shutle-running following acute vitamin C supplementation.* Int.J.Sports Med. 22(1): 68-75.

Thompson D., Williams C., McGregor S.J., Nicholas C.W., McArdle F., Jakson M.J., Powell J.R. 2001. *Prolonged vitamin C supplementation and recovery from demanding exercise.* Int.J.Sport Nutr.Exerc.Metab. 11(4): 466-481.

Van der Beek E.J. 1991. *Vitamin supplementation and physical exercise performance.* J.Sports Sci. 9(Spec. No): 77-89.