The Effect of Vitamin Supplementation on Antioxidant Enzymes

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Introduction

Free radicals have been implicated in the aetiology of numerous disease states, including cardiovascular disease, cancer and diabetes. Although free radicals occur as a natural consequence of cell metabolism, they are also produced as a result of oxidative stress[1]. Antioxidant vitamins and minerals are thought to provide protection against free radical-induced damage, and recent research has suggested an increased dietary intake of antioxidants is associated with a reduced risk of certain diseases[2]. Conversely, the consumption of low levels of antioxidants in the form of fruit and vegetables has been shown to more than double the incidence of certain cancers[3]. The object of this study was to examine the effect of dietary supplementation with antioxidant tablets on a variety of parameters. Sixteen individuals were studied before and after a 120 day period of vitamin supplementation. Total Antioxidant Status (TAS), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR) were measured, using kits developed by Randox Laboratories Ltd. which provide a rapid and easily automated means of assessment of the antioxidant defences possessed by an individual.

Materials and Methods

Samples

Lithium heparinized, and EDTA blood was obtained from each of 16 normal volunteers, in order to determine a baseline level prior to supplementation. Volunteers were then given one tablet containing antioxidant supplements each day for 60 days. At the end of this period, further blood samples were taken for determination of post-supplementation levels. Antioxidant supplements contained:

Selenium (10 µg)
Vitamin A (450 µg), vitamin C (100 mg), vitamin E (91 mg)
Bioflavonoids (50 mg)

Two ml of whole blood was removed from the lithium heparinized samples and centrifuged at 1000 x g for 10 min. The resultant plasma was removed and retained for the assay of TAS and GR. The remainder of the whole blood was used for the assay of SOD and GPx.
Assays for TAS, SOD, GPx and GR were performed at 37°C using a Cobas Fara II centrafugal analyser (Roche, Switzerland).

Total Antioxidant Status

Total Antioxidant Status (TAS) was measured using a kit supplied by Randox Laboratories Ltd. (Cat. No. NX2332). The plasma sample volume was 5 µl, in a total assay volume of 305 µl. Colour production is measured at 600 nm with a read time of 3 min.

Superoxide dismutase

Superoxide dismutase (SOD) was measured using a kit supplied by Randox Laboratories Ltd. (Cat. No. SD125) using an appropriate whole blood SOD control (Cat. No. SD126). Aliquots of whole blood (0.5 ml) were centrifuged at 2,000 x g, washed x 4 with 0.9% NaCl, and lysed in a total volume of 2 ml of ice-cold double deionised H_2O. The lysate was then diluted 1 in 25 with RANSOD diluting buffer (Cat. No. 366MS). This preparation was used for measurement of SOD. The reaction was measured at 500 nm, using 5 µl of sample in a total reaction volume of 230 µl.

Glutathione peroxidase

Glutathione peroxidase (GPx) was measured using a kit supplied by Randox Laboratories Ltd. (Cat. No. RS505), using the appropriate whole blood control (SC692). Whole blood 50 µl was diluted with 1 ml RANSEL diluting agent and incubated for 5 min. One ml of double-strength Drabkin's solution was added, and assays were performed within 20 min. GPx activity was measured at 340 nm, using a sample volume of 5 µl in a total reaction volume of 285 µl.

Glutathione reductase

Glutathione reductase (GR) was measured using a kit supplied by Randox Laboratories Ltd. (Cat. No. GR2368). The decrease in absorbance is measured at 340 nm. The assay requires a sample volume of 10 µl in a total reaction volume of 310 µl.

Malondialdehyde

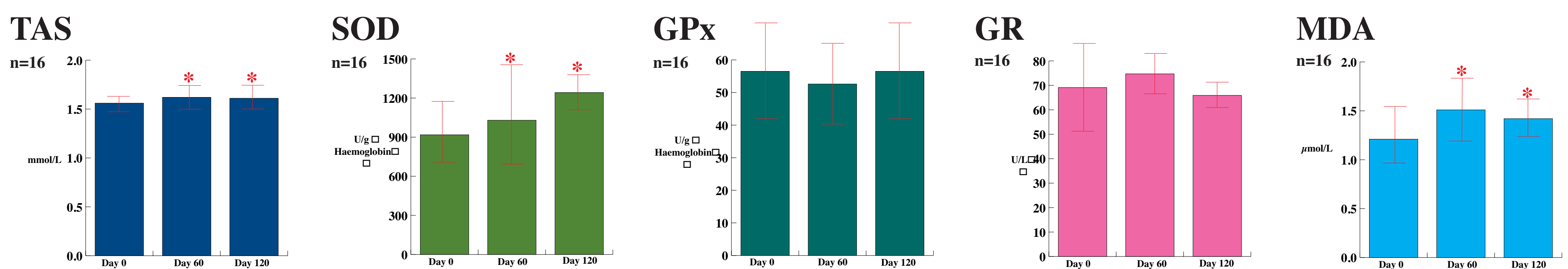
Malondialdehyde (MDA) was assayed as a marker of lipid peroxidation using a colorimetric reaction which uses 1-methyl-2-phenylindole as chromogen. Condensation of one molecule of MDA with 2 molecules of 1-methyl-2-phenylindole under acidic conditions results in the formation of a chromophore with an absorbance maximum at 586 nm.
A 7.6 mM solution of 1-methyl-2-phenylindole (MPI) was prepared immediately prior to use, in 33% methanol in acetonitrile. A 650 µl aliquot of MPI was placed in each test tube, to which was added 200 µl of plasma. The tubes were mixed well, and 150 µl of 10 M HCl was added. After mixing once more, the tubes were sealed, and incubated for 60 min. at 45°C. After incubation, the tubes were chilled on an ice bath, and spun at 10,000 x g for 5 min. to remove debris. The absorbance at 586 nm was measured and subtracted from the blank value, obtained by replacing plasma with water. A calibration graph was prepared using 4 µmol/L, 8 µmol/L, 16 µmol/L and 20 µmol/L of 1,1,3,3-tetramethoxypropane in 20 mM Tris-HCl, buffer, pH 7.4.

Statistics

Statistical significance was assessed using Student's t-test. Results were deemed statistically significant where $p<0.05$.

Results

After 60 days, TAS, SOD and MDA showed a statistically significant increase, with an increase in the mean from 1.56 mmol/L to 1.62 mmol/L for TAS, from 917.9 U/g Hb to 1029.5 U/g Hb for SOD, and from 1.215 µmol/L to 1.513 µmol/L for MDA. No significant difference was noted between pre- and post-supplementation levels of GPx or GR at 60 days. After 120 days, the mean TAS value had remained virtually unchanged at 1.61 mmol/L. SOD levels had further increased to 1242 U/g Hb. MDA levels had also risen slightly, but significantly to 1.42 µmol/L, from a pre-supplementation level of 1.21 µmol/L. Levels of GPx and GR had not undergone significant changes, with the mean levels after 120 days reaching 56.5 U/g Hb and 65.9 U/L respectively.



Comparison of Antioxidant Markers at Day 0, Day 60 and Day 120 of Vitamin Supplementation (16 Subjects)

Marker	Day 0 Mean ± S.D.	Day 60 Mean ± S.D.	Day 120 Mean ± S.D.	p (T-Test) Day 0/Day 60	p (T-Test) Day 0/Day 120
TAS (mmol/L)	1.56 ± 0.074	1.62 ± 0.12	1.61 ± 0.13	0.039*	0.05*
SOD (U/g Hb)	917.9 ± 187.5	1029.5 ± 304.6	1242 ± 110.6	0.031*	0.00005*
GPx (U/g Hb)	56.5 ± 14.5	52.6 ± 12.18	56.5 ± 12.6	0.204 NS	0.393 NS
GR (U/L)	69.1 ± 17.9	74.7 ± 8.31	65.9 ± 7.87	0.135 NS	0.288 NS
MDA (µmol/L)	1.21 ± 0.35	1.51 ± 0.26	1.42 ± 0.15	0.005*	0.005*

* - Statistically Significant (P<0.05) NS - Not Statistically significant

TAS	:	Total Antioxidant Status
SOD	:	Superoxide Dismutase
GPx	:	Glutathione Peroxidase
GR	:	Glutathione Reductase
MDA	:	Malondialdehyde

Discussion

Dietary supplementation with antioxidant vitamins and nutrients resulted in a statistically significant difference in the TAS, SOD and MDA levels of the individuals in the study. The rise in SOD over the 120 day period may be due to the availablility of co-factors, such as copper and zinc, during supplementation. This may in turn contribute to the rise in TAS. In a previous study, no difference in SOD levels was detected after a 30 day supplementation period. This may be due to insufficient time for incorporation into red blood cells, which have a turnover time of 120 days. The increased levels of MDA may be related to peroxidation of the increased intake of vitamin E. No significant changes were noted between pre- and post-supplementation levels of GPx or GR. The results of this study indicate that the Randox Total Antioxidant Status kit can be used to monitor the effects of dietary antioxidant intake. This may then be correlated with the incidence/aetiology of free radical-induced disease.

References

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2. Machlin, L.J. (1993) Toxicology and Industrial Health 9, 383-387.
3. Block, G., Patterson, B. and Subar, A. (1992) Nutr. Cancer. **18**, 1-29.

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