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Effect of different dietary levels of vitamin E on lipid peroxidation in rats

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SUMMARY.- The regulation of normal oxidative balance include the maintenance of adequate levels of dietary antioxidants such as vitamin E. The objective of this investigation was to study the effect of three different dietary levels of vitamin E (normal, *supplemented* 20 times higher and deficient) on plasma and liver lipid peroxidation, assayed by determination of thiobarbituric acid reactive substances (TBARS) and vitamin E in plasma and liver and hepatic reduced glutathione. Administration of dietary vitamin E caused a dose-dependent increase in liver and plasma concentration of this vitamin to 42.11µg/g liver and 29.52 µmol/l respectively, in the supplemented group, and a low concentration of TBARS, 0.67 nmol/mg protein, in liver. The group receiving the diet without vitamin E showed high values of hepatic TBARS, 2.95 nmol/mg protein, and low values of reduced glutathione and reduced concentration of hepatic and plasma vitamin E (1.75 µg/g liver and 3.67 µmol/l, respectively). In conclusion, the vitamin E deficiency alone induces the liver lipid peroxidation in rats, and maintenance of adequate or higher vitamin E levels acts as a protective factor against free radical generation.

RESUMEN: Efectos de diferentes niveles dietéticos de vitamina E en la peroxidación lipídica de ratas.- La regulación del balance oxidativo normal incluye mantener adecuados niveles dietéticos de antioxidantes, como la vitamina E. Esta investigación tuvo por objetivo analizar el efecto de tres cantidades diferentes de vitamina E en la dieta: normal, veinte veces mayor y de deficiencia, en la peroxidación lipídica en plasma e hígado, obtenidas por la determinación de las substancias ácido reactivas de los tiobarbitúricos (TBARS), vitamina E en el plasma, hígado y glutation hepático reducido. La administración de diferentes niveles dietéticos de vitamina E por un período de diez semanas causaron un incremento dosis-dependiente en el hígado y plasma de esta vitamina de 42.11 µg/g y 29.52 µmol/l, respectivamente. El grupo con suplementación tiene baja concentración de TBARS, 0.67 nmol/mg de proteína en el hígado. El grupo que recibió dieta sin vitamina E presentó valores hepáticos superiores de TBARS, 2.95 nmol/mg de proteína y bajos valores de glutation reducido y baja concentración de vitamina E en el hígado y plasma (1.75µg/g y 3.67µmol/ l. respectivamente. Concluyendo, la deficiencia sola de vitamina E induce la peroxidación lipídica hepática en ratas y niveles adecuados o altos de esta vitamina actúan como factores protectores contra la producción de radicales libres.

INTRODUCTION

Adequate levels of nutritional antioxidants such as vitamin E are an essential factor in health maintenance, having an important influence on several processes such as drug metabolism. In human and animal diets the main forms of vitamin E are α-tocopherol and γ-tocopherol, from natural sources. The most biopotent form of this vitamin is R,R,R α-tocopherol (1). Vitamin E protects against free radical generation in a system that involves ascorbic acid and glutathione. Thus, nutritional adequacy of vitamin E and vitamin C is essential, controlling the membrane damage initiated by free radicals (2,3). Oxidative damage caused by reactive oxygen species, referred to as «oxidative stress», reflects a shift in the prooxidant-antioxidant balance, in favor of the prooxidant process. Aging and some diseases such as diabetes, atherosclerosis, and cancer appear to involve reactive oxygen species (4,5,6). Vitamin E acts in this process as the major lipid soluble antioxidant responsible for protecting the polyunsatured fatty acids in membranes against lipid peroxidation, acting as an efficient scavenger of hydroperoxyl radicals in biological systems (7,8). Animal experiments and human epidemiological studies suggest that vitamin E may reduce the risk of cancer, probabilly due to its antioxidant properties. The objective of this study was to examine the effect of different dietary levels of vitamin E (normal, supplemented 20 times higher and deficient) on biochemical indicators of lipid peroxidation

MATERIAL AND METHODS

Animals. Male Wistar rats from the colony of the Faculty of Medicine-USP, Ribeirão Preto Brazil, were used at approximately 60 g of initial body weight. Animals were randomly divided into three groups of six animals each: the first group (control) was kept on a basal diet with normal recommended levels of vitamin E for rodent laboratory animals (9), the second group (supplemented) received a basal diet to which vitamin E was added in an amount 20 times higher the recommended level and the third group (deficient) received a diet containing no vitamin E in a vitamin mix. All nutrients

except vitamin E in the experimental diet were adequate for optimum animal growth (9). Animals were maintained under standardized conditions of light (8:00 am to 8:00 pm), humidity and temperature (22°C) and given diets and water *ad libitum* for a period of ten weeks. At the end of the experiment the animals were killed by heart puncture. Blood was collected in a heparinized syringe, and plasma was obtained after centrifugation at 1.000g for 10 min. Immediately after sacrifice the livers were removed, washed in saline, weighed and frozen in liquid nitrogen and stored at 70° C until biochemical analysis.

Biochemical analysis. Lipid peroxidation is meassured as thiobarbituric acid reactive subtance (TBARS) concentration in plasma and liver of rats. Plasma TBARS were measured by the method of Buege and Aust (10) and liver TBARS by the method of Uchiyaa and Mihara (11). Plasma and liver vitamin A and vitamin E were determined by the HPLC method (12). HPLC separations were perfomed on a 5- µm Shimpack C-18 column (6.0 mm x 15 cm) preceded by a guard C-18 column (4.6 mm x 1 cm) with a mobile phase of acetonitrile: dicloromethane: methanol (70:20:10) at a flow rate of 2 ml/min. Retinol was detected at 325 nm and vitamin E at 292 nm in a photodiode array UV/VIS detector. The concentration of vitamins was calculated from peak area responses using a standard curve prepared by chromatography of known amounts of pure vitamins. Reduced glutathione was measured in the liver samples by a modification of the fluorimetric method of Hu (13). Protein concentration was determined by the method of Lowry et al.(14) and albumin by the bromocresol green method (15).

Statistical analysis. Comparison of group means were done by ANOVA and regression analysis was perfomed among the parameters obtained in this study (16).

RESULTS

The effect of ten weeks period of experimental diet on plasma and liver vitamin A and vitamin E in rats is show in a Table 1. Dietary different levels of vitamin E had no significant effect on level of plasma retinol but liver vitamin A were significantly higher in a control group, compared with rats fed supplemented and deficient diets. Plasma vitamin E concentration differed among groups with supplemented rats, reaching higher values compared with others groups and the deficient group showing the lowest value. A significantly higher accumulation of α-tocopherol was observed in the liver of vitamin E supplemented group, whereas control and deficient group showed no difference in a liver α-tocopherol concentration. Plasma lipid hydroperoxide formation as measured by TBARS did not differ significantly between the experimental groups (Table 2); however, hepatic peroxide levels were higher in the deficient group compared with other two groups. Plasma albumin and reduced glutathione levels were similar in all groups (Table 3). The different levels of dietary vitamin did not cause any significant change in a final body weight and the average food intake per day did not vary among the three experimental groups (data not show). The correlation coeficients among the parameters obtained in this study are presented in Table 4.

DISCUSSION

Regulation of the normal prooxidant-antioxidant state includes the maintenance of adequate levels of antioxidants and the localization of these compounds and enzymes involved in antioxidative process. An adequate diet suppling the dietary levels of antioxidants as vitamins E and C, β-carotene and others micronutrients is an essential factor. The present data show that differents levels of vitamin E content in the diet lead to differnt vitamin E concentration in plasma and liver tissue. Dietary vitamin E deficiency causes a dramatic depletion of a α-tocopherol in plasma, and lower hepatic value. In contrast the supplemented group had higher levels of α-

TABLE 1
Plasma and liver levels of vitamin A and vitamin E in rats submmited to different dietetic levels of vitamin E

Diet	Plasma vitamin A µmol/l	Liver vitamin A µg/g of tisuue
Normal	0.71±0.39 A	1.05±0.44 B
Supplemented	0.78±0.34 A	0.52±0.23 A
Deficient	1.19±0.58 A	0.63±0.29 A

Diet	Plasma vitamin E µmol/l	Liver vitamin E µg/g of tisuue
Normal	20.78±2.49 B	8.35± 6.17 A
Supplemented	29.52±4.26 C	42.11±12.42B
Deficient	3.67±1.36 A	1.75±1.16 A

Mean± SD of six rats per group. Means in the same column with different letters are significantly different ($p<0.05$) as assessed by ANOVA

TABLE 2
Effect of diet on levels of plasma and liver TBARS

Diet	Plasma TBARS µmol/l	Liver TBARS nmol/mg protein
Normal	1,44±0,12 B	0,54±0,06 A
Supplemented	1,09±0.10 A	0,67±0,06 A
Deficient	1,24±0,17 A	2,95±0,27 B

Mean± SD of six rats per group. Means in the same column with different letters are significantly different ($p<0.05$) as assessed by ANOVA test

TABLE 3
Reduced glutathione and albumin in plasma of rats submmited to different dietary levels of vitamin E

Diet	Reduced Glutathione ug/g	Albumin g/100ml
Normal	1396.9±143.43 A	3.79±0.11A
Supplemented	1427.3±319.91 A	3.55±0.07 A
Deficient	1146.8±252.16 A	3.49±0.30 A

Mean± SD of six rats per group. No significant differences ($p<0.05$) as assessed by ANOVA test.

TABLE 4

Regression analysis and correlation coeficients among parameters assessed in this study

	PR	LR	PT	LT	PTB	LTB	ALB	GSH
Plasma Retinol (PR)	1.00	-0.28	-0.44	-0.26	0.26	0.43	-0.61*	-0.038
Liver Retinol (LR)		1.00	-0.28	-0.039	0.35	0.21	0.32	0.31
Plasma Tocopherol (PT)			1.00	0.76*	-0.25	-0.89*	0.32	0.40
Liver Tocopherol (LT)				1.00	-0.59*	-0.53*	-0.09	0.33
Plasma TBARS (PTB)					1.00	-0.11	0.04	-0.13
Liver TBARS (LTB)						1.00	-0.46	-0.51*
Albumin (ALB)							1.00	0.30
Glutathione (GSH)								1.00

*$p<0.05$

tocopherol in plasma and liver. The liver is the one of the major organs that store vitamin E and the concentration obtained in this study correlates well with the plasma values. The liver retinol level also is affected by level of vitamin E in diet, with the control group showing more accumulation of vitamin A. A higher TBARS concentration in liver was found in this study, revealing that vitamin E alone may be responsible for elevation of malondialdehyde in this organ. Table 4 shows a highly negative correlation ($r = -0.89$, $p < 0.05$) between values of hepatic TBARS and plasma vitamin E. Vitamin E levels in plasma of control and supplemented rats correspond to similar values of hepatic TBARS, however the low level of plasma vitamin E in a deficient animals causes a significant increase on hepatic peroxide. In this study the value of circulating vitamin E had a decisive influence on production of lipid hydroperoxide by the rat liver. Vitamin E deficiency induce increased levels of hepatic TBARS but normal or higher vitamin E levels in plasma did protect against the generation of this liver peroxidation product, maintaining the hepatic TBARS at low levels. Liver TBARS levels were inversely correlated with plasma and hepatic vitamin E levels ($p < 0.05$), but the control and supplemented group showed similar levels of liver TBARS, possibly indicating that the protective action of vitamin E against in vivo lipid peroxidation was not dose-dependent in this study, and that the normal dietary level of vitamin E offers the same protection as the higher level, in agreement with reports by others authors (17). Plasma TBARS were inversely correlated with liver concentration of vitamin E ($p < 0.05$). The control group reached higher values when compared with others two groups, this result was unexpected, but determination of plasma malondialdehyde is considered to be a poor index of lipid peroxidation and the method is not specific, since others known and unknown substances produce positive TBARS reaction (17). Others studies that vitamin E was employed as antioxidant demonstrated no significant difference between malondialdehyde or TBARS levels in plasma of rats submmited to supplemented or deficient vitamin E diet (18), but gave a significant diference in hepatic concentration of malondialdehyde, with higher levels of this peroxidation product in rats fed a deficient vitamin E diet (18,19). Deficient vitamin E diet also resulted in a decrease in hepatic levels of cytochrome P450 in association with increase of malondialdehyde concentration in this tissue (20,21). It's generally accepted that cytochrome P450 mediated mixed function oxidases in a detoxification process, with the formation of more polar, biological inactive and readily excretable metabolites (21). Therefore the effect of vitamin E deficiency may decreaee xenobiotic metabolism and thereby increase the toxicity, for the loss in cytochrome P450 activity in liver. Formation of malondialdehyde in oxidative stress also is associated with mutagenesis and carcinogenesis (22) and factors that stimulate lipid peroxidation and malondialdehyde formation include selenium or vitamin E deficiency and accumulation of highly unsaturated acids (22). Application of malonaldehyde in skin of mice resulted in a high incidence of tumors, 12 mg of malonaldehyde applied daily proved toxic, sometimes fatally so (23) some animals in this study also develope carcinomas of their internal organs, includes liver, rectal carcinoma, lung, kidney and rectum metastases (23). Albumin concentration, indicator of hepatocellular function, was similar for three groups studied, revealing that the levels of dietary vitamin E not modify the synthesis of this protein in liver. In this study the deficient group showed lower concentrations of reduced glutahione in relation of control and supplemented group, but the difference was not significant, the values were slightly lower when compared with reported values (24). In conclusion, the results obtained in this study suggest that at the maintenance of adequate levels of vitamin E is indispensable as a protective factor, exerting a significant influence on liver lipid peroxidetion, as assessed by hepatic TBARS, in this group of animals. Vitamin E deficiency alone causes a dramatic increase of TBARS in liver, with possible deleterious consequences.

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