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The effect of α-tocopherol on the lipid peroxidation of mitochondria and microsomes obtained from rat liver and testis

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Abstract

The effect of intraperitoneal administration of α-tocopherol (100 mg/kg wt/24 h) on ascorbate (0.4 mM) induced lipid peroxidation of mitochondria and microsomes isolated from rat liver and testis was studied. Special attention was paid to the changes produced on the highly polyunsaturated fatty acids C20:4 n6 and C22:6 n3 in liver and C20:4 n6 and C22:5 n6 in testis. The lipid peroxidation of liver mitochondria or microsomes produced a significant decrease of C20:4 n6 and C22:6 n3 in the control group, whereas changes in the fatty acid composition of the α-tocopherol treated group were not observed. The light emission was significantly higher in the control than in the α-tocopherol treated group. The lipid peroxidation of testis microsomes isolated from the α-tocopherol group produced a significant decrease of C20:4 n6 , C22:5 n6 and C22:6 n3, these changes were not observed in testis mitochondria. The light emission of both groups was similar. The treatment with α-tocopherol at the dose and times indicated showed a protector effect on the polyunsaturated fatty acids of liver mitochondria, microsomes and testis mitochondria, whereas those fatty acids situated in testis microsomes were not protected during non enzymatic ascorbate-Fe^{2+} lipid peroxidation. The protector effect observed by α-tocopherol treatment in the fatty acid composition of rat testis mitochondria but not in microsomes could be explained if we consider that the sum of C20:4 n6 + C22:5 n6 in testis microsomes is 2-fold than that present in mitochondria. (Mol Cell Biochem **225**: 121–128, 2001)

Key words: lipid peroxidation, α-tocopherol, mitochondria, microsomes, liver, testis

Introduction

In the cells, there are efficient defence systems that control lipid peroxidation and make sure the maintenance of cellular integrity and, therefore, an adequate metabolic and functional activity. Among these defence mechanisms, vitamin E (α-tocopherol) is one of the most important lipid soluble antioxidant systems, because it interferes with the propagation reactions that leads to lipid peroxidation [1]. α-Tocopherol accumulates at those sites within the cell where oxygen radical production is greatest and thus where it is most required: in the membranes of mitochondria and endoplasmic reticulum [2]. If protective mechanisms are altered and free radicals generation exceed antioxidant capacity of the cell, the reactions initiated by these molecules can produce important harmful effects, since they caused alterations of the membrane structures, and finally the irreversible cellular damage [3, 4]. Liver and testis are organs that critically depend upon oxidative metabolism of mitochondria for their function. The role of lipids in the structure and function of the male reproductive system continues to be an interesting and important area of investigation. The predominant lipids of whole testis were reported to be phospholipids, with smaller amounts of acylglycerols (chiefly triacylglycerols) free and esterified cholesterol and even smaller amounts of gangliosides and sulfolipids [5]. The phospholipids of testis and liver are characterized by extremely high proportions of long chain highly polyunsaturated fatty acids. Rat liver phospholipids contain

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esterified C22:6 n3 while C22:5 n6 is the major esterified 22 carbon in testis phospholipids [6]. In addition to the saturated and unsaturated fatty acids commonly found in mammalian tissues, testicular lipids have been shown to be enriched with 20- and 22-carbon polyenes and to contain 24-carbon polyenes [7]. The efficient synthesis of C22:5 n6 may also partly explain why this is the major 22-carbon fatty acid in rat testis [8]. Many studies related with lipid chemistry and metabolism of testicular tissue have led to the suggestion that polyenoic acids, particularly 4,7,10,13,16-eicosapentaenoic acid (C22:5 n6) have an important role in the process of spermatogenesis in the rat [7]. Supplementation with α-tocopherol has been demonstrated to reduce the susceptibility to lipid peroxidation of tissue PUFA [9]. However, none have examined in detail the changes in fatty acid composition produced during lipid peroxidation of selected organelles isolated from rat testis. The aim of this study was to investigate the effect of α-tocopherol on lipid peroxidation of mitochondria and microsomes obtained from rat liver and testis.

Materials and methods

Chemicals

α-Tocopherol, butylated hydroxytoluene (BHT) and phenylmethylsulfonyl fluoride (PMSF) were from Sigma Chemical Co. (St. Louis, MO, USA), Standards of fatty acids methyl esters were from Nu Chek Prep, Inc., Elysian, MN, USA. All other reagents and chemicals were of analytical grade from Sigma.

Animals and membrane preparation

Male Wistar rats, weighing 200–250 g were used. Rats were maintained on a commercial standard pellet diet and tap water *ad libitum*. The diet contained 4% of total lipid with a fatty acid composition of 19.14% palmitic acid C16:0, 0.184% palmitoleic acid C16:1, 4.10% stearic acid C18:0, 19.34% oleic acid C18:1 n9, 51.53% linoleic acid C18:2 n6 and 4.83% linolenic acid C18:3 n3. The diet contained 13.45 μg of α-tocopherol/g determined by HPLC as described previously [10], the same method was used to determine vitamin content in liver and testis homogenates. Two groups of rats were considered, and designated E (α-tocopherol-supplemented), and C (control). All rats were fed commercial rat chow and water *ad libitum*. Group E received one 100 mg/kg body wt i.p. injection of α-tocopherol dissolved in 0.15 M NaCl, 1% Triton X100, 20% ethanol. The control group was injected with the same amount of solution without vitamin E. After 24 h all the rats were sacrificed by cervical dislocation and the liver and testis rapidly removed, cut into small pieces and washed extensively with 0.15 M NaCl. An homogenate of each tissue was prepared in solution A (0.25 M sucrose, 10 mM Tris-HCl pH 7.4, PMSF 0.1 mM), 3 ml of solution per g of tissue, using the potter-Elvejhem homogeneizer. The homogenate was spun at 3,000 × g, pellets were discarded, the supernatant was spun at 20,000 × g for 10 min to obtain liver mitochondria and testis mitochondria.

After the centrifugation, 5 ml of the resultant supernatant was applied to a Sepharose column (1.6 × 12 cm) equilibrated and eluted with 10 mM Tris-HCl (pH 7.4), 0.01% NaN_3. The microsomal fraction appearing in the void volume (12–20 ml) was used and cytosol (30–40 ml) discarded. All operations were performed at 4°C. Microsomes and mitochondria were stored at –83°C and used within a week of its preparation, after one cycle of freezing and thawing. Tissues and membrane preparations were protected from light during the procedures.

Lipid peroxidation of mitochondria and microsomes

Chemiluminescence and lipid peroxidation were initiated by adding ascorbate to mitochondrial or microsomal preparations [11]. Mitochondria and/or microsomes (1 mg of protein) were incubated at 37°C with 0.01 M phosphate buffer (pH 7.4), 0.4 mM ascorbate, final volume 1 ml. Mitochondrial preparations which lacked ascorbate were carried out simultaneously. Membrane light emission was determined over a 120 min period; chemiluminescence was recorded as cpm every 10 min and the sum of the total chemiluminescence was used to calculate cpm/mg protein. A maximal response was obtained between 90–120 min after the addition of ascorbate. Chemiluminescence was measured as counts per min in a liquid scintillation analyzer Packard 1900 TR.

Measurement of fatty acid composition

Mitochondrial or microsomal lipids were extracted with chloroform/methanol (2:1 v/v containing 0.01% BHT as antioxidant) [12]. Fatty acids were transmethylated with 20% F_3B in methanol at 60°C for 3 h. Fatty acids methyl esters were analyzed with a GC-14A gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a packed column (1.80 m × 4 mm id) GP 10% DEGS-PS on 80/100 Supelcoport. Nitrogen was used as a carrier gas. The injector and detector temperatures were maintained at 250°C, the column temperature was held to 200°C during 60 min. The fatty acid methyl esters were identified by comparison of retention times with standard compounds. All compositions were expressed as % by area of total fatty acids.

Protein determination

Proteins were determined by the method of Lowry *et al*. [13] using BSA as standard.

Statistical analysis

Results were expressed as means ± S.D. of 3 independent determinations. Data were evaluated statistically by one-way analysis of variance (ANOVA) and Student's *t*-test. Statistical criterion for significance was selected at different p values and indicated in each case.

Results

Fatty acid composition and α-tocopherol content of dietary lipids

The diet, used in this study is reach in oleic (19.34%) and linoleic acid (51.53%), but do not containing long chain peroxidable fatty acids such as: C20:4 n6 , C22:5 n6 or C22:6 n3 and the content of α-tocopherol/g-food = 13.45 μg/g pellet, is too low compared with the amount injected during the treatment (100 mg/body wt) as a consequence it could be assumed that the fatty acid composition and vitamin E content of the diet are not responsible for the changes observed during non enzymatic ascorbate-Fe^{2+} lipid peroxidation of the liver and testis organelles analysed in this study.

α-Tocopherol content in liver and testis

In liver from the vitamin E-group, the content of vitamin was 17.19 times higher than in the control (19.77 ± 6.76 vs. 1.15 ± 0.94 microgram α-tocopherol/g liver, respectively), while levels in the testis from the vitamin E-group were only 1.74 times higher than in the control (14.34 ± 3.84 vs. 8.21 ± 1.39 microgram α-tocopherol/g testis, respectively).

Effect of α-tocopherol during light emission of rat liver mitochondria and microsomes

When rat liver mitochondria or microsomes obtained from control rats were incubated in the presence of ascorbic acid (0.4 mM), striking variations in the chemiluminescence (light emission) were observed as compared with control liver mitochondria or microsomes incubated without ascorbic acid. However, such variations were not observed when liver mitochondria or microsomes obtained from α-tocopherol treated rats were analysed under similar conditions. Light emission in the absence of ascorbic acid was very low when mitochondrial or microsomal preparations were used (Figs 1 and 2). The times courses of the chemiluminescence of rat liver mitochondria and microsomes isolated from control and vitamin E groups, peroxidized without and with ascorbic acid are shown in Figs 3A, 3B, 4A and 4B respectively. Over the time course studies of rat liver mitochondria, analysis of chemiluminescence demonstrated that the lipid peroxidation process in the presence of ascorbic acid is slow and reach a maximun at near 120 min whereas when rat liver microsomes are used the lipid peroxidation process is rapid and reach a maximun at near 60 min.

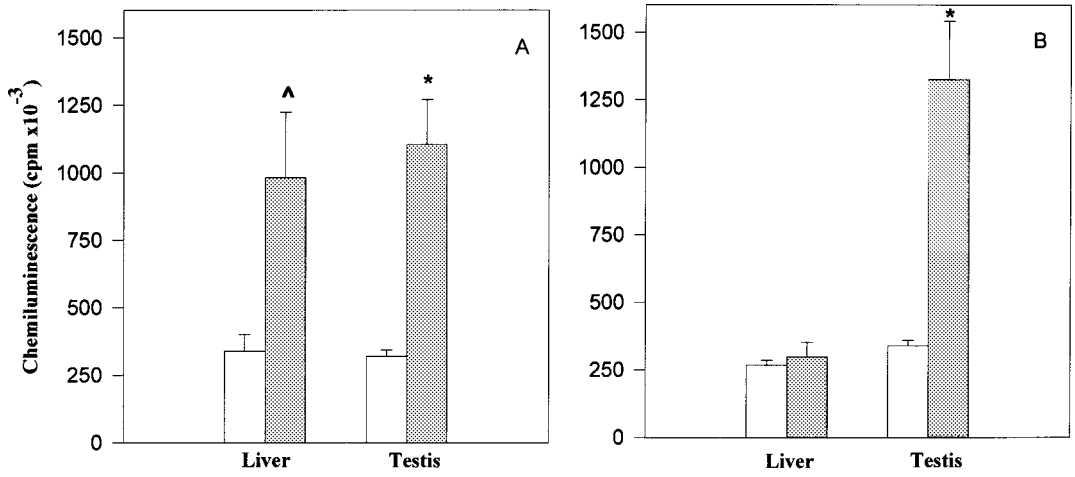

Fig. 1. Light emission produced by rat liver and testis mitochondria, obtained from control (A) and α-tocopherol treated rats (B). Chemiluminescence was determined over a 120 min period and recorded as cpm every 10 min and the sum was used as total chemiluminescence. Without ascorbic acid □, with ascorbic acid ■. Results are expressed as mean ± S.D. of 3 independent experiments. Statistically significant differences between –ascorbic acid and +ascorbic acid groups are indicated by ^p < 0.005; *p < 0.0001.

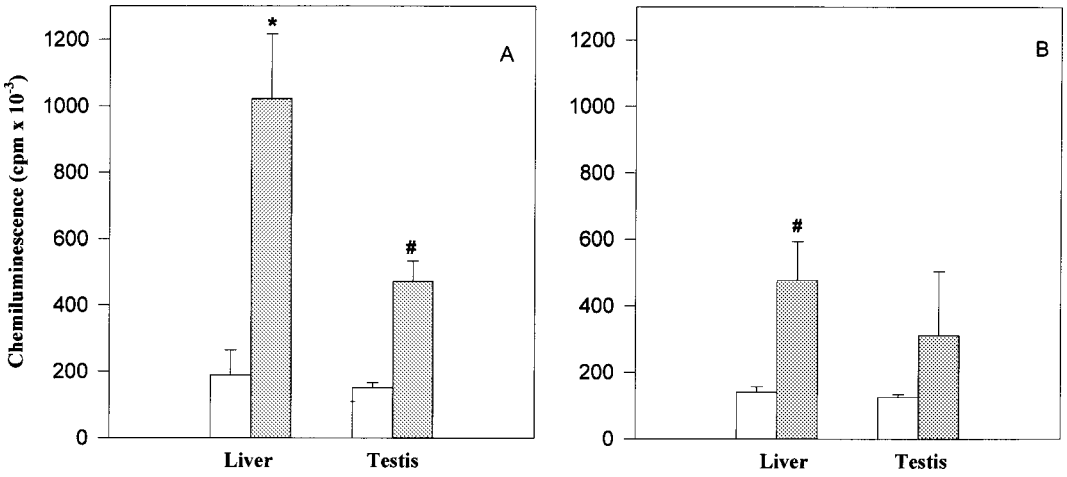

Fig. 2. Light emission produced by rat liver and testis microsomes, obtained from control (A) and α-tocopherol treated rats (B). Chemiluminescence was determined over a 120 min period and recorded as cpm every 10 min and the sum was used as total chemiluminescence. Without ascorbic acid □, with ascorbic acid ▨. Results are expressed as mean ± S.D. of 3 independent experiments. Statistically significant differences between –ascorbic acid and +ascorbic acid groups are indicated by $^{\wedge}p < 0.005$; $^{\#}p < 0.05$; $^{*}p < 0.0001$.

Effect of α-tocopherol during light emission of rat testis mitochondria and microsomes

Chemiluminescence and lipid peroxidation in the presence of ascorbic acid was analysed using testis mitochondria or microsomes (1 mg of protein). No important differences in the chemiluminescence values between control and α-tocopherol treated groups were observed when testis mitochondria or microsomes from both groups were compared (Figs 1 and 2). The times courses of the chemiluminescence of rat

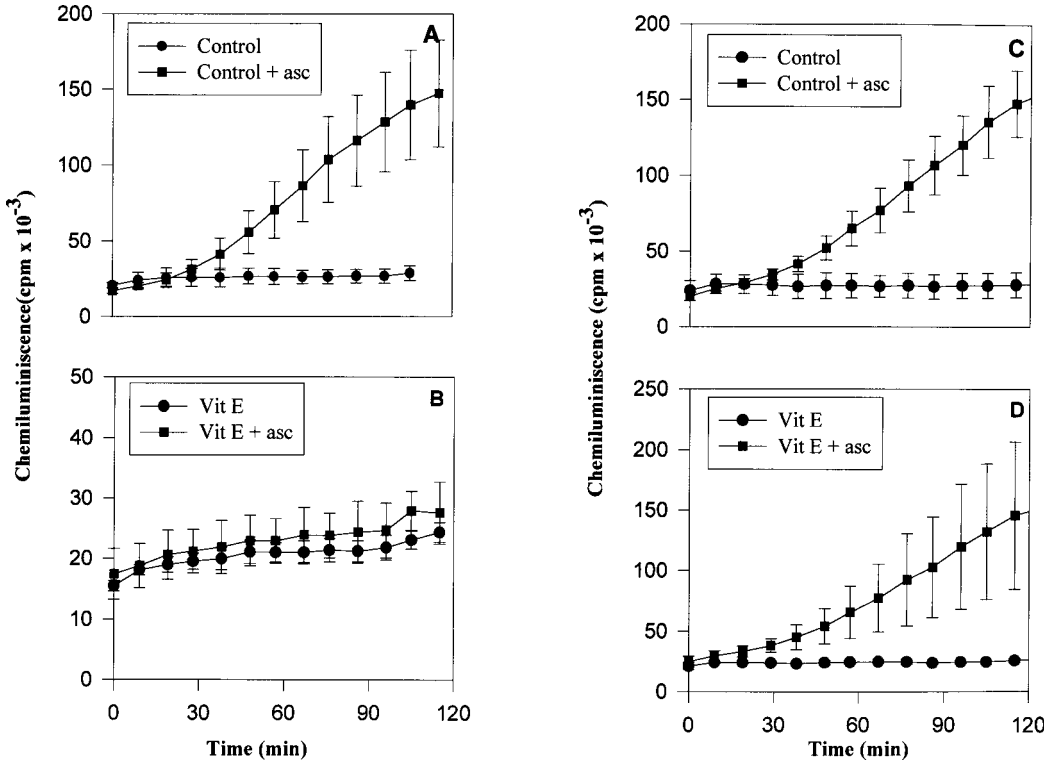

Fig. 3. Ascorbate-Fe^{2+} induced chemiluminescence as a function of time of rat liver mitochondria obtained from control (A) and α-tocopherol treated (B) groups and rat testis mitochondria obtained from control (C) and α-tocopherol treated (D) groups. Without ascorbic acid ●–●, with ascorbic acid ■–■. Data are mean ± S.D. of 3 independent experiments.

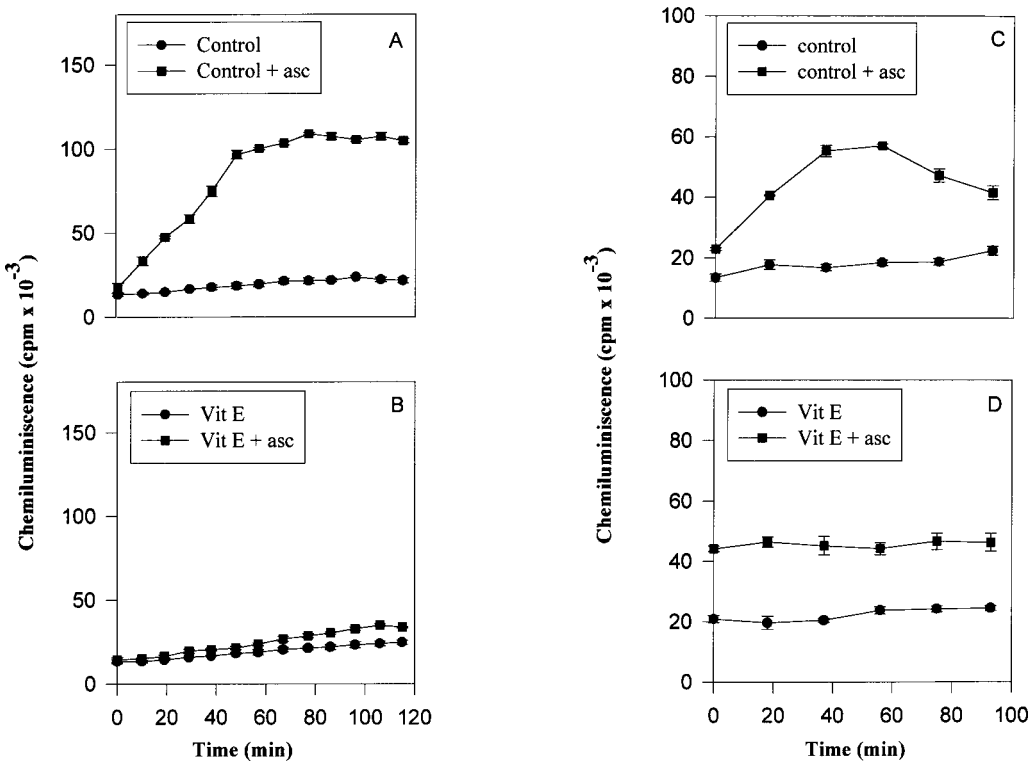

Fig. 4. Ascorbate-Fe^{2+} induced chemiluminescence as a function of time of rat liver microsomes obtained from control (A) and α-tocopherol treated (B) groups and rat testis microsomes obtained from control (C) and α-tocopherol treated (D) groups. Without ascorbic acid ●–●, with ascorbic acid ■–■. Data are mean ± S.D. of 3 independent experiments.

testis mitochondria and microsomes isolated from control and vitamin E groups, peroxidized without and with ascorbic acid are shown in Figs 3C, 3D, 4C and 4D respectively. Over the time course studies of rat testis mitochondria, analysis of chemiluminescence demonstrated that the lipid peroxidation process in the presence of ascorbic acid is slow and reach a maximun at near 120 min whereas when rat testis microsomes are used the lipid peroxidation process is rapid and reach a maximun at near 60 min.

Changes in fatty acid composition of rat liver mitochondria and microsomes during lipid peroxidation

The fatty acid composition of total lipids from rat liver mitochondria and microsomes obtained from control and α-tocopherol groups, peroxidized without and with ascorbic acid (0.4 mM), is shown in Tables 1 and 3. Significant differences in the content of arachidonic acid C20:4 n6 and docosahexaenoic acid C22:6 n3 were observed when control liver mitochondria or microsomes were lipid peroxidized in the presence of ascorbic acid. The fatty acid composition of rat liver mitochondria or microsomes from α-tocopherol group do not showed significant differences in the content of both, C20:4 n6 and C22:6 n3 during lipid peroxidation.

Changes in fatty acid composition of rat testis mitochondria and microsomes during lipid peroxidation

The fatty acid composition of total lipids from rat testis mitochondria and microsomes obtained from control and α-tocopherol groups, peroxidized without and with ascorbic acid, is shown in Tables 2 and 4. Significant differences in the content of arachidonic acid C20:4 n6 and docosapentaenoic acid C22:5 n6 were observed when control mitochondria or microsomes were lipid peroxidized in the presence of ascorbic acid. The fatty acid composition of rat testis microsomes from α-tocopherol group shows significant differences $p < 0.005$ in the content of C20:4 n6 , C22:5 n6 and C22:6 n3 during lipid peroxidation. These changes were not observed in testis mitochondria.

Discussion

Vitamin E is the general term used for all tocopherols and tocotrienols, of which α-tocopherol is the natural and biologically most active form. The antioxidant function of vitamin E is critical for the prevention of oxidation of tissue polyunsaturated fatty acids PUFAs [14]. Intracellular vitamin E is

Table 1. Fatty acid composition (area %) of liver mitochondria obtained from control and vitamin E treated rats peroxidized with and without ascorbic acid

Fatty acid	Control		Vitamin E	
	–ascorbic acid	+ascorbic acid	–ascorbic acid	+ascorbic acid
C16:0	15.40 ± 1.51*	25.76 ± 2.22	15.95 ± 0.49	15.26 ± 1.61**
C16:1 n7	nd	nd	0.29 ± 0.07	0.19 ± 0.025
C18:0	14.78 ± 0.94*	21.62 ± 1.34	23.26 ± 1.73	20.92 ± 1.49
C18:1 n9	7.35 ± 1.68	7.34 ± 2.28	8.56 ± 1.21	8.37 ± 0.10
C18:2 n6	13.54 ± 0.61	17.60 ± 4.22	15.15 ± 3.48	18.21 ± 1.86
C20:3	nd	nd	nd	0.25 ± 0.13
C20:4 n6	23.82 ± 3.76*	9.01 ± 0.46	26.33 ± 2.49	23.81 ± 1.39**
C22:6 n3	5.40 ± 0.54*	1.74 ± 0.01	7.46 ± 1.22	6.58 ± 0.68
Saturated	30.72 ± 0.73*	47.38 ± 2.73	39.21 ± 1.64	36.18 ± 0.60**
Monounsaturated	7.35 ± 1.68	7.34 ± 2.28	8.75 ± 1.20	8.56 ± 0.11
Polyunsaturated	43.11 ± 4.16	29.36 ± 1.32	48.95 ± 1.29	48.76 ± 1.45
Total unsaturated	50.47 ± 5.62	36.70 ± 3.61	57.71 ± 1.44	57.33 ± 1.34
Saturated/unsaturated	0.60 ± 0.06	1.30 ± 0.15	0.67 ± 0.02	0.62 ± 0.006
UI	163.88 ± 19.53	66.97 ± 28.99	189.17 ± 11.86	180.19 ± 7.20

Data shown are given in percentages of total fatty acids contents and are mean ± S.D. of 3 separated experiments. Statistically significant differences in the control group between peroxidized with and without ascorbic acid are indicated by *$p < 0.005$ using Student's *t*-test. Statistically significant differences between control and vitamin E groups peroxidized with ascorbic acid are indicated by **$p < 0.005$. The UI = unsaturation index was calculated as the sum of the percentages by weight of each fatty acid × the number of olefinic bonds.

associated with lipid rich membranes such as mitochondria and endoplasmic reticulum where it plays a very effective role in protecting against membrane lipid peroxidation by reactive lipid peroxyl and alkoxyl radicals [15].

In the early 1930s, Burr and Burr observed that PUFAs were essential for the maintenance of normal testicular function in rats [16]. Since then, the role of lipids in the structure and function of the male reproductive system has been an interesting and important area of investigation [17, 18]. Since the early 1990s, there have been increasing efforts to characterize the lipids of the testis and to describe their metabolism in relation to the development and function of this organ [19]. More of one third of the mitochondrial and microsomal fatty acids from rat liver and testis are polyunsaturated with a prevalence of linoleic C18:2 n6, arachidonic C20:4 n6 and docosahexaenoic acids C22:6 n3 in liver and linoleic C18:2 n6, arachidonic C20:4 n6 and docosapentaenoic acids C22:5 n6 in testis. The diet used in this study do not containg long chain peroxidable fatty acids such as C20:4 n6, C22:5 n6, C22:6 n3 and the content of α-tocopherol/g-food determined by HPLC is too low compared with the amount injected during the treatment (100 mg/body wt) as a consequence it can

Table 2. Fatty acid composition (area %) of testis mitochondria obtained from control and vitamin E treated rats peroxidized with and without ascorbic acid

Fatty acid	Control		Vitamin E	
	–ascorbic acid	+ascorbic acid	–ascorbic acid	+ascorbic acid
C16:0	22.73 ± 4.44	29.90 ± 10.17	29.61 ± 2.81	35.09 ± 4.14
C16:1 n7	0.71 ± 0.001	nd	1.04 ± 0.81	nd
C18:0	7.82 ± 2.18#	15.02 ± 3.52	11.18 ± 3.85	9.53 ± 1.42
C18:1 n9	12.17 ± 4.13	12.53 ± 1.92	13.33 ± 2.65	14.43 ± 1.19
C18:2 n6	15.38 ± 3.69	12.87 ± 6.93	13.07 ± 3.72	18.93 ± 3.30
C20:3	0.39 ± 0.08	nd	0.50 ± 0.012	nd
C20:4 n6	12.63 ± 0.61#	7.27 ± 2.97	12.27 ± 1.33	10.11 ± 3.68
C22:5 n6	11.03 ± 2.08*	1.62 ± 1.021	12.20 ± 2.47	7.26 ± 3.20##
C22:6 n3	1.32 ± 0.25	nd	1.32 ± 0.47	1.28 ± 0.01
Saturated	30.55 ± 2.26#	44.92 ± 6.65	40.79 ± 1.34	44.62 ± 5.07
Monounsaturated	12.41 ± 3.90	12.50 ± 1.92	14.02 ± 3.47	14.43 ± 1.19
Polyunsaturated	42.33 ± 6.08#	21.77 ± 9.42	39.21 ± 5.89	36.74 ± 4.88
Total unsaturated	54.75 ± 2.18#	34.30 ± 7.55	53.24 ± 2.52	51.18 ± 4.02##
Saturated/unsaturated	0.55 ± 0.03*	1.32 ± 0.10	0.76 ± 0.05	0.87 ± 0.15##
UI	161.47 ± 15.49#	86.95 ± 3.92	159.26 ± 19.43	116.70 ± 14.20

Data shown are given in percentages of total fatty acids contents and are mean ± S.D. of 3 separated experiments. Statistically significant differences in the control group between peroxidized with and without ascorbic acid are indicated by *$p < 0.005$. #$p < 0.05$ using Student's *t*-test. Statistically significant differences between control and vitamin E groups peroxidized with ascorbic acid are indicated by ##$p < 0.05$. The UI = unsaturation index was calculated as the sum of the percentages by weight of each fatty acid × the number of olefinic bonds.

Table 3. Fatty acid composition (area %) of liver microsomes obtained from control and vitamin E treated rats peroxidized with and without ascorbic acid

Fatty acid	Control		Vitamin E	
	–ascorbic acid	+ascorbic acid	–ascorbic acid	+ascorbic acid
C16:0	22.19 ± 1.10*	31.62 ± 1.25	16.74 ± 3.25	23.63 ± 0.40**
C16:1 n7	0.41 ± 0.06	nd	nd	0.16 ± 0.01
C18:0	20.18 ± 0.80$^{\#}$	24.78 ± 1.72	23.66 ± 1.78	27.96 ± 1.55
C18:1 n9	12.95 ± 0.02$^{\#}$	9.27 ± 1.67	7.36 ± 0.47***	8.80 ± 0.55
C18:2 n6	13.42 ± 2.45	11.48 ± 0.98	11.74 ± 0.16	13.23 ± 2.22
C20:4 n6	18.96 ± 1.29$^{\#}$	10.72 ± 3.49	23.47 ± 1.86	26.50 ± 2.56**
C22:6 n3	4.39 ± 0.18*	0.62 ± 0.12	6.07 ± 1.17	5.71 ± 0.81**
Saturated	42.37 ± 1.33*	56.40 ± 0.54	40.40 ± 1.94	43.71 ± 10.92
Monounsaturated	13.37 ± 0.04$^{\#}$	9.27 ± 1.67	7.36 ± 0.47***	8.91 ± 0.48
Polyunsaturated	32.39 ± 1.42$^{\#}$	22.20 ± 4.42	35.21 ± 2.02	39.73 ± 4.73**
Total unsaturated	45.75 ± 1.43$^{\#}$	31.47 ± 4.73	42.57 ± 1.64	48.64 ± 5.19**
Saturated/unsaturated	0.93 ± 0.04*	1.83 ± 0.27	0.95 ± 0.08	0.93 ± 0.31**
UI	116.07 ± 2.36	75.11 ± 15.8	124.72 ± 7.36	141.38 ± 15.01**

Data shown are given in percentages of total fatty acids contents and are mean ± S.D. of 3 separated experiments. Statically significant differences in the control group between peroxidized with and without ascorbic acid are indicated by *$p < 0.005$. $^{\#}p < 0.05$ using Student's *t*-test. Statistically significant differences in the vitamin E group between peroxidized with and without ascorbic acid are indicated by ***$p < 0.05$. Statistically significant differences between control and vitamin E groups peroxidized with ascorbic acid are indicated by **$p < 0.05$. The UI = unsaturation index was calculated as the sum of the percentages by weight of each fatty acid × the number of olefinic bonds.

be assumed as a first approximation that the fatty acid composition and vitamin E content of the diet are not responsible for the changes observed during non enzymatic ascorbate-Fe^{2+} lipid peroxidation of the liver and testis organelles analysed in this study. The high concentration of polyunsaturated fatty acids in the membrane of organelles present in these tissues cause susceptibility to lipid peroxidative degradation. This study has examined some of the effects by which ascorbate promotes and α-tocopherol protect lipid peroxidation in mitochondria and microsomes isolated from rat liver and testis. The addition of ascorbate increased lipid peroxidation several-fold over control values (without ascorbic acid) in mitochondria and microsomes from both tissues. Ascorbate-stimulated lipid peroxidation was substantially suppressed in rat liver mitochondria and microsomes isolated from α-tocopherol treated rats but inhibition of light emission was not observed in rat testis mitochondria and microsomes isolated from the same group of rats. It is important to note that the fatty acid composition of rat testis microsomes from α-tocopherol group shows significant differences $p < 0.005$ in the content of C20:4 n6, C 22:5 n6 and C22:6 n3 during lipid peroxidation but these changes were not observed in testis

Table 4. Fatty acid composition (area %) of testis microsomes obtained from control and vitamin E treated rats peroxidized with and without ascorbic acid

Fatty acids	Control		Vitamin E	
	–ascorbic acid	+ascorbic acid	–ascorbic acid	+ascorbic acid
C16:0	33.87 ± 1.53	36.19 ± 2.96	29.31 ± 6.36	32.39 ± 8.48
C16:1 n7	2.35 ± 0.85	0.86 ± 0.01	nd	nd
C18:0	7.62 ± 1.31	10.76 ± 1.36	16.90 ± 5.08	18.84 ± 3.32$^{\#\#}$
C18:1 n9	12.62 ± 0.58	11.84 ± 4.46	10.77 ± 1.47	13.31 ± 2.90
C18:2 n6	11.56 ± 0.81	6.92 ± 1.65	7.65 ± 2.51	7.63 ± 2.79
C20:4 n6	25.29 ± 3.72*	12.00 ± 1.41	21.44 ± 1.00**	12.09 ± 0.88
C22:5 n6	28.84 ± 1.26$^{\#}$	13.49 ± 4.13	27.10 ± 0.61**	14.79 ± 1.13
C22:6 n3	5.91 ± 0.71$^{\#}$	1.25 ± 0.02	5.64 ± 1.53**	0.01 ± 0.00$^{\#\#}$
Saturated	30.20 ± 14.82	46.94 ± 3.45	46.21 ± 2.42	51.23 ± 5.37
Monounsaturated	14.97 ± 0.93	12.41 ± 4.75	10.77 ± 1.47	13.31 ± 2.90
Polyunsaturated	69.63 ± 6.04*	33.24 ± 5.23	50.24 ± 17.60	34.62 ± 1.05
Total unsaturated	84.60 ± 6.81$^{\#}$	45.66 ± 9.87	61.01 ± 18.52	47.93 ± 3.11
Saturated/unsaturated	0.34 ± 0.16$^{\#}$	1.09 ± 0.27	0.87 ± 0.37	1.07 ± 0.14
UI	307.08 ± 30.29$^{\#}$	146.71 ± 31.03	230.88 ± 77.14	151.53 ± 5.14

Data shown are given in percentages of total fatty acids contents and are mean ± S.D. of 3 separated experiments. Statistically significant differences in the control group between peroxidized with and without ascorbic acid are indicated by *$p < 0.005$. $^{\#}p < 0.05$ using Student's *t*-test. Statistically significant differences in the vitamin E group between peroxidized with and without ascorbic acid are indicated by **$p < 0.005$. Statically significant differences between control and vitamin E groups peroxidized with ascorbic acid are indicated by $^{\#\#}p < 0.05$. The UI = unsaturation index was calculated as the sum of the percentages by weight of each fatty acid × the number of olefinic bonds.

mitochondria. The protector effect observed by α-tocopherol treatment in rat testis mitochondria but not in microsomes could be explained if we consider that the sum of C20:4 n6 + C22:5 n6 in testis microsomes is 2-fold than that present in mitochondria. In a previous paper , we have demonstrated similar effects in rat liver microsomes isolated from animals receiving the same doses of vitamin E [10]. It has been suggested that vitamin E may not protect membranes rich in n-3 fatty acids, especially those with five or six double bonds, from lipid peroxidation as efficiently as membranes rich in n-6 fatty acids [20, 21]. If this is the case, mitochondria and microsomes from rat testis would be more protected than the same organelles isolated from rat liver, by vitamin E, but in our experimental conditions the results do not apply to this rule. In conclusion, α-tocopherol at the dose used in this study protect rat liver mitochondria, microsomes and testis mitochondria from lipid peroxidation. The intraperitoneal administration of a single dose of α-tocopherol, 100 mg/kg, is unable to protect rat testis microsomes from lipid peroxidation. Studies in humans in this area of research are scarce. If one searches through the literature, it seems, that for vitamin E there have been studies on its possible therapeutic use in mammalian infertility [22], rather than studies concerning its physiological presence, distribution and role in germ cells and spermatozoa. In this regard Lenzi *et al*. [23] have collected data concerning the presence of vitamin E in rat testis mitochondria and rat epididymal spermatozoa. The aim of their studies was to fill a gap, to examine vitamin consumption by peroxidation of membranes. Mitochondrial membranes of testis cells are richly endowed with α-tocopherol. The amount of vitamin in isolated, intact mitochondria of 12–14 week old rat testis is 0.603 ± 0.04 nmol/mg proteins, a value which is 3 times higher than that, e.g., in liver mitochondria. In immature rat testes (4 weeks old) there are 0.694 ± 0.07 nmol/mg proteins. It remains to be determined how long is needed the α-tocopherol treatment to protect rat testis microsomes from deleterious effects.

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