Dietary Vitamins A and E Affect Differently Lipid Peroxidation in Rat Heart and Testis

Anne M. Melin,* Marie A. Carbonneau, Marie J. Thomas, Annie Perromat, and Michel Clerc

Laboratoire de Biochimie Médicale A, Université de BORDEAUX II, 146 rue Léo Saignat, 33076 Bordeaux Cedex, France

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Summary This study examines the susceptibility to lipid peroxidation of rat heart and testis microsomes, in relation to dietary vitamin A and/or E status. Four groups of rats were fed different levels of the vitamins. After a period of 8 weeks, lipid peroxide levels estimated by thiobarbituric acid method, fatty acid composition and vitamins A and E were measured in serum or in microsomes. In heart, lipid peroxide levels were enhanced in dietary vitamin E deficiency; linoleic acid 18:2 (n-6), arachidonic acid 20:4 (n-6), and docosahexaenoic acid 22:6 (n-3) were significantly decreased. In testis, dietary vitamin A deficiency significantly increased lipid peroxide production and decreased docosapentaenoic acid 22:5 (n-6) and 20:4 (n-6). Supplementation of the diet with both vitamins A and E significantly decreased lipid peroxide production but did not change the fatty acid composition. Induced lipid peroxidation increased in the heart of vitamin E-deficient rats and in the testis of vitamin A-deficient ones. Both in heart and testis, we found a good correlation between spontaneous and induced lipid peroxides and also between lipid peroxides and polyunsaturated to saturated fatty acid ratio. Besides, lipid peroxide production was well correlated with polyunsaturated fatty acids/vitamin A in testis and with polyunsaturated fatty acids/vitamin E molar ratios in heart. Membrane susceptibility to lipid peroxidation varied greatly according to dietary status and organ. Vitamin E seemed to be a more effective antioxidant for heart, and vitamin A, for testis. Supplementation enhanced the beneficial role of each vitamin.

Key Words: vitamin A, vitamin E, lipid peroxidation, rat heart, rat testis

*To whom correspondence should be addressed.
Intracellular lipid peroxidation (LPO) is involved in many pathological disorders [1]. It results in damage to the lipidic components of the cell membranes; among these, polyunsaturated fatty acids (PUFA) undergo oxidative degradation leading to the formation of hydroperoxides and carbonyl compounds that react with thiobarbituric acid (TBA), the so-called lipid peroxides. LPO occurs under physiological conditions, but many enzymatic systems or antioxidant nutrients protect the biological membranes. Vitamin E is considered as an essential cellular antioxidant; it scavenges free radicals produced by peroxidation [2]. α-Tocopherol, the main vitamin E component, inhibits LPO [3] by interrupting the chain-propagation reactions [4]. Recent studies have shown that susceptibility to peroxidation is related to the vitamin E content; Kornbrust and Mavis [5] have reported a high vitamin E level in lung and heart rat microsomes associated with a high resistance to peroxidation. Vitamin A and retinoids play an essential role in cellular proliferation and differentiation [6]; they could act at the nuclear level. Moreover, some researchers have shown that vitamin A can modify the relative lipid composition in the bilayer structure of several subcellular membranes [7]. Vitamin A and some analogs could play a beneficial role in protecting against LPO [8].

Using microsomal preparations of rat heart and testis as a model, we studied the influence of dietary vitamins A and/or E on physiological spontaneous LPO and in vitro non-enzymatic ascorbate-dependent iron-induced LPO. It is now well accepted that some membrane lipid modifications are produced by diet. Vitamin A or E deficiency can be dramatic and results in damaging the cellular membranes. In this study, we determined the extent of LPO and detailed the relationship between diet, lipid peroxide production, and fatty acid profile. Our results suggest that heart and testis have a different sensitivity to vitamin A or E deficiency.

MATERIALS AND METHODS

Male Wistar rats (Charles River, Cleon, France) weighing 50-60 g were divided into four groups. They were housed individually, and the room temperature was maintained at 20-24°C. The basal diet was prepared by INRA (Jouy en Josas, France) as described previously [9] and is shown in Table 1. Group 1 (control) was fed the basal diet supplemented with 5,000 IU/kg vitamin A as retinyl palmitate and 50 IU/kg vitamin E as DL-α-tocopherol. Groups 2 and 3 received the basal diet without vitamin A (−A) or vitamin E (−E), respectively. Group 4 was fed the basal diet supplemented with 50,000 IU/kg vitamin A and 500 IU/kg vitamin E (+A +E). Animals were fed their respective diets for a period of 8 weeks and had free access to water. All groups were fasted for 14 h prior to sacrifice. After decapitation, blood was drawn directly into tubes and centrifuged at 1,500 × g for 10 min. Heart and testis were promptly removed and homogenized as described previously [9]. The microsomal pellets were suspended in 10 mM Tris-HCl, 150 mM KCl buffer, pH 7.45, so as to contain approximately 3-5 mg of

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Table 1. Composition of the basal diet.\(^a\)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount (g/100 g diet)</th>
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<tbody>
<tr>
<td>Casein</td>
<td>22</td>
</tr>
<tr>
<td>Corn starch</td>
<td>44.3</td>
</tr>
<tr>
<td>Sucrose</td>
<td>22.1</td>
</tr>
<tr>
<td>Cellulose</td>
<td>2</td>
</tr>
<tr>
<td>Peanut oil</td>
<td>2.5</td>
</tr>
<tr>
<td>Colza oil</td>
<td>2.5</td>
</tr>
<tr>
<td>Vitamin mixture(^b)</td>
<td>1</td>
</tr>
<tr>
<td>Mineral mixture(^c)</td>
<td>3.5</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.1</td>
</tr>
</tbody>
</table>

\(^a\)The basal diet was deficient in both vitamins A and E. \(^b\)Composition of the vitamin mixture (per kg): thiamin-HCl, 6 mg; riboflavin, 6 mg; pyridoxine-HCl, 7 mg; niacin, 30 mg; calcium pantothenate, 16 mg; folic acid, 2 mg; biotin, 0.2 mg; cyanocobalamin, 0.01 mg; cholecalciferol, 2,500 IU; menadione, 50 IU; and sucrose qs. 1 kg. \(^c\)Composition of the mineral mixture (per kg): calcium phosphate, 17.5 g; sodium chloride, 2.6 g; potassium citrate, 7.7 g; potassium sulfate, 1.82 g; magnesium oxide, 0.84 g; manganese carbonate, 0.12 g; ferric citrate, 0.21 g; zinc carbonate, 0.05 g; cupric carbonate, 0.001 g; potassium iodate, 0.0003 g; sodium selenite, 0.0008 g; chromium potassium sulfate, 0.02 g; and sucrose qs. 1 kg.

protein in 1 ml of suspension.

Vitamins A and E were measured in serum and microsomal fractions by high-performance liquid chromatography (HPLC) by a method described for vitamin A only [10] and adapted as previously described [9]. Values were expressed as pmol/mg of heart and testis microsomal protein or as \(\mu\)mol/liter of serum.

Spontaneous (SP) and induced (IN) LPO was evaluated as lipid peroxides as described by Recknagel et al. [11]. Briefly, 1 ml of 300 mM HEPES, pH 7.45, and 1 ml of distilled water were added to 500 \(\mu\)l of microsomal suspension. Non-enzymatic LPO was induced with 0.5 ml of 0.5 mM ascorbic acid and 0.5 ml of a mixture of 0.5 mM adenosin-diphosphate (ADP), and 5 \(\mu\)M ferrous sulfate. After incubation during 30 min at 37°C and centrifugation at 1,300 \(\times\) g for 10 min at 4°C, 2.5 ml of supernatant were treated by 0.5 ml of 0.6 N HCl and 2 ml of TBA reagent (TBA 120 mM, Tris-HCl 26 mM, pH 7). The mixtures were heated at 100°C for 30 min. Tetraethoxypropane was used as a standard. Lipid peroxides were spectrophotometrically determined at 532 nm, and the LPO level was expressed as nmol of malondialdehyde (MDA) equivalents/mg of heart and testis microsomal protein. Protein was estimated by the method of Lowry et al. [12] using bovine serum albumin as standard.

Total lipids were extracted from 1 ml of microsomal suspension with 5 ml of
hexane: isopropanol (3/2; v/v). Fatty acid composition was determined by gas chromatography from 2 ml of the lipid extract after transformation of the acids into isopropyl esters [13]. Quantification of each fatty acid was expressed as the percentage of the total extract. Polysaturated to saturated (P/S) fatty acid ratio was calculated as the sum of PUFA percentages to the sum of saturated fatty acid percentages. Moreover, PUFA concentrations were quantified with arachidic acid 20:0 used as an internal standard, and the results were expressed as μmol/g microsomal protein or as the PUFA/vitamin A or E (Vit A or E) molar ratios in heart and testis microsomes.

Data are presented as means±SD. Linear regression analyses were used to assess relationships between spontaneous and induced lipid peroxide levels, between lipid peroxide levels and P/S, and between lipid peroxide levels and PUFA/Vit A or E. Statistical analyses were conducted with a statistical software package (Stat-View). For comparison between paired data, the Mann-Whitney test was chosen because of the sample size. A p-value of <0.05 was considered significant.

RESULTS

Over the experimental period of 8 weeks, no difference in body and heart weights (Table 2) was observed in the four groups. Only testis weight slightly decreased, but not significantly, in groups 2 and 3 relative to that of group 1. As shown in Table 3, by comparison with that of the control group 1, the vitamin A level in vitamin A-deficient group 2 was significantly decreased in serum and testis microsomes (p<0.001 and p<0.025, respectively). In vitamin E-deficient group 3, no vitamin E was found in serum, and the level was significantly decreased (p<0.025 and p<0.05, respectively) in heart and testis microsomes. In group 4 supplemented with both vitamins A and E at 10 times the normal intake, we observed a significant (p<0.05) increase in vitamin A level only in testis microsomes. The lipid composition of control rat heart and testis microsomes expressed as μmol/g protein was as follows: total cholesterol, 38.00±5.09 and 54.80±21.00; triglycerides, 24.80±4.56 and 22.40±3.25; phosphatidylcholine, 35.00±4.25 and 38.00±2.10; and phosphatidylethanolamine, 20.00±2.25 and 18.00±1.75.
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Spontaneous lipid peroxidation occurred in control group 1 under normal physiological conditions. We measured LPO markers as lipid peroxides in microsomes: 0.96±0.14 and 0.62±0.09 nmol/mg protein for heart and testis, respectively (Fig. 1). In group 2, lipid peroxides were significantly (p<0.001) enhanced as compared with those of the control group only in testis. In group 3, lipid peroxides increased significantly in both heart (p<0.001) and testis (p<0.01). In group 4, lipid peroxides were significantly decreased (p<0.005); however, the decrease was lower in heart (54.1%) than in testis (62.3%). To study the relative peroxidizability of heart and testis microsomes, we employed non-enzymatic LPO

Table 3. Vitamin A and E concentrations in serum, heart and testis microsomes.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Dietary groups</th>
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<tbody>
<tr>
<td></td>
<td>1 (control)</td>
</tr>
<tr>
<td>Vitamin A</td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>1.59±0.43</td>
</tr>
<tr>
<td>Heart microsomes</td>
<td>0.64±0.27</td>
</tr>
<tr>
<td>Testis microsomes</td>
<td>0.95±0.34</td>
</tr>
<tr>
<td>Vitamin E</td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>13.44±5.60</td>
</tr>
<tr>
<td>Heart microsomes</td>
<td>2.46±0.49</td>
</tr>
<tr>
<td>Testis microsomes</td>
<td>1.94±0.52</td>
</tr>
</tbody>
</table>

*Vitamins A and E are expressed as µmol/liter of serum and as pmol/mg microsomal protein. *Values are means±SD for six animals per group. Significance level for each group compared with control group 1 was studied by the Mann-Whitney test: *p<0.05, **p<0.025, ***p<0.001.

Fig. 1. Lipid peroxides in rat heart and testis microsomes. Rats were fed four different diets for a period of 8 weeks. □, 1 (control); ✹, 2 (-A); □, 3 (-E); ■, 4 (+A+E). Values are means±SD for 6 rats per group. Significance level for each group compared with control group 1 was studied by the Mann-Whitney test: (a, p<0.025; b, p<0.01; c, p<0.005; d, p<0.001). Symbols used are as follows: SP, spontaneous and IN, induced lipid peroxidation (LPO).
induced by an ADP/Fe²⁺ system and stimulated by ascorbate (Fig. 1). Between spontaneous and induced LPO, heart lipid peroxide values were significantly increased in groups 2 and 3 (p<0.025 and p<0.001, respectively); no significant difference was observed either in control group 1 or in supplemented group 4. Testis lipid peroxide values were significantly enhanced both in group 1 (p<0.025) and in groups 2 and 3 (p<0.001). In group 4, the lipid peroxide increase was not significant. As shown in Fig. 2, we plotted induced lipid peroxide versus spontaneous lipid peroxide values in heart and testis microsomes and used linear regression analysis. We found a strong positive correlation (r = 0.97 and r = 0.95, respectively; p<0.001) in both organs. Generally speaking there was no modification in the character of the response observed in induced as compared to spontaneous conditions.

The fatty acid composition of heart and testis microsomal lipids was determined after spontaneous LPO by gas chromatography, as shown in Fig. 3. For dietary control group 1, the most abundant PUFA in heart microsomes were as follows: 18:2 (n-6), 18.33±0.88%; 20:4 (n-6), 18.45±0.99%; and 22:6 (n-3), 7.77±1.39%. In group 2, we observed a significant decrease (p<0.005) in the 18:2 (n-6) content. In group 3, 18:2 (n-6), 20:4 (n-6), and 22:6 (n-3) were depressed significantly (p<0.025, p<0.025, and p<0.05, respectively). The major PUFA in testis microsomes of control group 1 were 22:5 (n-6), 18.91±1.89%; 20:4 (n-6), 13.45±0.66%; and 18:2 (n-6), 4.43±0.62%. We also observed that dietary vitamin A deficiency (group 2) affected testis fatty acid composition. The levels of 20:4 (n-6) and 22:5 (n-6) were significantly decreased (p<0.025 and p<0.05, respectively); all the other PUFA were decreased only slightly. In both heart and testis,
vitamin supplementation (group 4) did not change the profile of the principal fatty acids as compared with that of the control diet (group 1). As shown in Fig. 4, after induced LPO in the heart, the major modifications as compared with the values for spontaneous LPO were a much more marked decrease in 20:4 (n-6) and 22:6 (n-3) in group 3 ($p < 0.005$ and $p < 0.001$, respectively) and a decrease in 18:2 (n-6) and 22:6 (n-3) in group 2 ($p < 0.01$). In testis, it was a marked decrease in 20:4 (n-6) ($p < 0.05$ and $p < 0.025$, respectively) and 22:5 (n-6) ($p < 0.001$ and $p < 0.005$, respectively) in both groups 2 and 3. It is interesting to note that no modification took place in group 4 in either heart or testis. As shown in Fig. 5, an inverse

Fig. 3. Fatty acid composition (area % of major fatty acids) of heart and testis microsomes after spontaneous lipid peroxidation (LPO) in rats fed four different diets. □, 1 (control); ◊, 2 (-A); ▽, 3 (-E); ■, 4 (+A+E). Values are means±SD for 6 rats per group. Significance level for each group compared with control group 1 was studied by the Mann-Whitney test (a, $p < 0.05$; b, $p < 0.025$; c, $p < 0.005$).

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relationship was found for spontaneous or induced lipid peroxides and P/S ratio both in heart and testis microsomes with a significant ($p<0.005$) correlation coefficient ($r = -0.84$ and $-0.85$, respectively, for heart and $r = -0.68$ and $-0.76$, respectively, for testis). In this form, the results show a higher sensitivity of PUFA to spontaneous or induced LPO in heart than in testis. Figure 6 shows the relation between PUFA/Vit A or E molar ratio and lipid peroxide production. Whilst a low ratio could imply a relatively large number of vitamin molecules per fatty acid molecule, a low ratio could also infer a good protection against LPO and a low lipid peroxide production. As we have previously shown, the actual fatty acid content depends very much on the type of diet used and on the organ studied. Moreover, the susceptibility to LPO measured as lipid peroxide production is only well correlated with the PUFA/Vit E molar ratio ($r = 0.88$, $p<0.001$) in heart.

![Graph showing fatty acid composition](image)

Fig. 4. Fatty acid composition (area % of major fatty acids) of heart and testis microsomes after induced lipid peroxidation (LPO) in rats fed four different diets. $\square$, 1 (control); $\triangle$, 2 ($-A$); $\bullet$, 3 ($-E$); $\blacksquare$, 4 ($+A+E$). Values are means ± SD for 6 rats per group. Significance level for each group compared with control group 1 was studied by the Mann-Whitney test (a, $p<0.05$; b, $p<0.025$; c, $p<0.01$; d, $p<0.005$; e, $p<0.001$).

DISCUSSION

It is now well accepted that dietary vitamins produce some membrane lipid modifications [14] such as a decrease in phospholipid content of rat liver microsomes associated with vitamin A deprivation [15]. Our results show that vitamin A deficiency led to slight modifications in fatty acid composition of heart microsomes and to the PUFA/Vit A molar ratio ($r=0.90, p<0.001$) in testis microsomes.

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Among the main PUFA present in heart, only a significant 30% decrease was observed in 18:2 (n-6). As lipid peroxides are highly dependent on microsomal lipids. Among the main PUFA present in heart, only a significant 30% decrease was observed in 18:2 (n-6). As lipid peroxides are highly dependent on

Fig. 6. Correlation between induced lipid peroxide production and PUFA/Vit A or E molar ratio in rat heart (A) and testis (B) microsomes. Data points represent the mean ± SEM from 6 different microsomal preparations for each group. Regression lines were as follows: A: \( y = 4.24 - 0.06x, r = -0.23 \) (a); \( y = -6.23 + 0.12x, r = 0.88 \) (b). B: \( y = -2.09 + 0.06x, r = 0.90 \) (a); \( y = 3.38 - 0.01x, r = -0.06 \) (b).
the amount of double bonds in the carbon chain of fatty acids and as 18:2 (n-6) is slightly implicated in the production of MDA [16], lipid peroxide production was not significantly enhanced and did not reveal a spontaneous LPO. Vitamin A deficiency, which was first associated with morphological and histological alterations in testis [17], greatly affected testicular lipid levels. We observed a significant decrease in PUFA percentages. The highly unsaturated 22:5 (n-6) and 20:4 (n-6) acids seemed to show sensitivity to dietary vitamin A deficiency, decreasing by 14% and 16%, respectively. The 22:5 (n-6) molecule is the major 22-carbon fatty acid in rat testis, and its decrease already has been associated with rat testis degeneration [18]. This decrease in PUFA with multiple double bonds was associated with a highly significant increase in spontaneous lipid peroxides.

Susceptibility to peroxidation was also related to vitamin E content. In heart microsomes during dietary vitamin E deficiency, we observed a significant decrease in 22:6 (n-3), 20:4 (n-6), and 18:2 (n-6). Probably due to the high degree of unsaturation of the two former acids, a significant increase in spontaneous lipid peroxide production was found. It is worthwhile to mention that Eichenberger et al. [19] reported that LPO, measured as lipid peroxides, occurs mainly in (n-3) fatty acids containing five or six double bonds, on the one hand, and that Bruna et al. [16] reported that the oxidation rates of (n-6) acids are higher than those of (n-3) acids, on the other hand. So the lipid peroxide production in heart microsomes may be due to acids of both (n-3) and (n-6) series under our conditions. In agreement with our findings, heart mitochondria [20] were also strongly susceptible to LPO due to their high PUFA content. Additional to its high lipid level, the testis had a PUFA content higher than the heart, with a 20:4 (n-6) level 3-fold higher per g of microsomal protein. However, dietary vitamin E deficiency did not change significantly the PUFA percentage, and the increase in spontaneous lipid peroxide production was lower than that in heart.

Dietary supplementation with both vitamins A and E did not change significantly the fatty acid composition of heart and testis microsomes, but it reduced spontaneous lipid peroxide production. Moderate amounts of the two vitamins (10 times the normal intake) can provide greater protection against LPO by a synergistic interaction than a higher amount of only one vitamin (100 times the normal intake of vitamin A or E) [9]. Similarly, Meydani et al. [21] showed that even at low doses, a dietary combination of vitamin E and selenium decreased in vitro LPO of brain tissue. On the other hand, Arnrich and Arthur [22] reported that excessive amounts of vitamins A and E have a deleterious effect on rats; i.e., the animals presented severe testicular histological damage.

On stimulation of LPO by exogenous addition of ADP-Fe²⁺/ascorbate, a significant decrease in the more unsaturated fatty acids was observed particularly in heart microsomes of group 3 and in testis microsomes of group 2; this induction also significantly enhanced the lipid peroxide production. On the other hand, no significant difference was detected in heart and testis of supplemented group 4.

Moreover, whatever the dietary vitamin status, spontaneous lipid peroxides
were well correlated with induced lipid peroxides, both in heart and testis microsomes. This result may be related to the significant correlation observed between spontaneous or induced lipid peroxide production and the P/S ratio in heart and in testis, as shown in Fig. 5. Although it is now well accepted that lipid peroxide values are not directly correlated with the extent of LPO owing to the indirect and complex nature of the relationship between LPO and lipid peroxide values [23] in natural biological systems, in our experimental model there was a good correlation, principally in heart microsomes. Moreover, we previously showed that for microsomal analyses, there is a good correlation between lipid peroxide values and free MDA determined by a specific HPLC method [24]. This observation indicates that the use of lipid peroxide determination could be taken as an index of LPO in this experimental model system. The correlation between lipid peroxide values and the P/S ratio was lower in testis microsomes than in heart microsomes, probably due to the occurrence of a greater amount of neutral lipids (cholesterol, triglycerides, ...) and/or to the high capacity of testes to produce prostaglandins from 20:4 (n-6) or prostaglandin-like compounds from 22:5 (n-6) [25]. These results suggest that for adequate assessment of LPO, data on fatty acid composition may be required in addition to measurement of the lipid peroxide production [16].

Vitamin A deficiency resulted in a significant decrease in serum and testis microsomal vitamin A ratio. The good correlation between the lipid peroxide production and the PUFA/Vit A molar ratio (Fig. 6) observed in testis suggests the importance of vitamin A in the antioxidant defense system as well as its normal physiological function. Our results on the importance of vitamin A in the inhibition of testis microsomal LPO agree with those of Halevy and Sklan [26] who reported that retinol inhibits enzymatic oxidation of 20:4 (n-6) in bovine seminal vesicles to a greater extent than does α-tocopherol. This oxidation by both cyclooxygenase and lipoxygenase is a free radical-involving reaction, and the inhibitory activity of retinol may be due to its free radical-scavenging action. Moreover, other researchers have observed in analyses both in vivo [27] and in vitro [28] that retinoids can behave as inhibitors of LPO by acting as chain-breaking antioxidants or be effective peroxyl radical scavengers [29, 30]. According to others [31], endogenous vitamin E seems to be effective against LPO, which is particularly deleterious to heart function. Vitamin E deficiency induced a complete absence of vitamin E in serum and a large decrease in its level in heart microsomes. Arkhipenko et al. [32] found that heart was more sensitive than liver to dietary vitamin E deficiency, since the deficiency of a natural antioxidant such as vitamin E was not compensated by activation of other defensive enzymatic systems in the former organ. The good correlation between the lipid peroxide production and the PUFA/Vit E molar ratio observed in heart suggests that vitamin E may play an important role in the protection of its cellular membranous systems. Some in vivo experiments indicate that vitamin E accumulates in membranes containing large amounts of PUFA and that relatively low concentrations.
of vitamin E are required to prevent LPO of a large number of PUFA molecules [33]. Vitamin E can provide protection against LPO by complexing more strongly with the more unsaturated lipids [34] and by interfering with the propagation step of LPO [35].

The susceptibility to LPO may greatly vary depending upon the organ. Vitamin A appears to be a more effective antioxidant in rat testis than vitamin E, whereas vitamin E decreases susceptibility to LPO in rat heart to a greater extent than vitamin A. These results suggest that dietary vitamins A or E have a differential influence on the peroxidation potential of cells. This observation may be partly explained on the grounds that initiation of LPO may proceed differently according to the microsome origin. A role for transition metals such as iron or copper is generally accepted. Nevertheless, it remains unclear whether transition metals may promote LPO directly through the formation of metal-oxo intermediates, or by forming oxygen-centered radicals [36]. Both these processes seem to be of foremost importance in biological systems and will be evaluated in further investigations in our laboratory.

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REFERENCES


