Vitamin E–polyunsaturated lipid relationship in diet and tissues[1]

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ABSTRACT It is firmly established that the requirement for vitamin E is related to the fatty acid composition of the tissue lipids. Unfortunately, the complex relationships between dietary lipid composition and tissue lipid composition are not understood or appreciated by many investigators. Frequently one sees reports of drastic changes made in diet lipids and attempts to measure effects attributable to the change long before the establishment of new tissue equilibria. Data from animal studies are not directly applicable to adult man unless careful attention is paid to the extremely slow approach of certain tissues to equilibrium noted in the experiments of Dayton et al. and others. The kinetics of lipid autoxidation are relatively complex and it is not surprising that attempts to make predictions regarding events in biological systems have not always been successful. When the analogies are correctly stated excellent correlation is noted between lipid autoxidation in vitro and lipid peroxidation in vivo in various studies including those of Green and co-workers. *Am. J. Clin. Nutr.* 27: 952–959, 1974.

In 1961 after rather lengthy delays, arising largely from editorial skepticism, a paper was published, reporting that the polyunsaturated fatty acid (PUFA) content of various tissue lipids, including even the brain, could be modified by dietary lipid (1). This information was not really new when considered in terms of Lebedeff's work (2) on canine adipose tissue in 1882 or Sinclair's studies (3) of the effect of diet on the iodine value of rat tissue phospholipids in 1932. The advent of commercially available gas chromatography equipment at this stage suddenly permitted facile, rapid, detailed determinations of the fatty acid composition of numerous tissues, subcellular fractions (4), and individual lipid classes (5). Less than 10 years later this concept had been so generally accepted that changes in diet lipid composition were being described as producing changes in tissue lipid fatty acid composition by investigators who did not analyze tissue fatty acid composition (6–10). Similarly, data have been presented in relation to a general series of changes in diet lipid even though tissue lipid fatty acid analysis indicated that under the protocol used, significant differences had not actually been produced between groups (11). The modification of tissue lipid PUFA by dietary PUFA is quite complex (12–15), and considerable confusion on this point is evident in the literature. The most frequent error is the failure to reach an equilibrium between tissue lipid PUFA levels and the diet.

The extreme difficulties encountered in attempting to detect or measure the occurrence of lipid peroxidation in vivo have led to the design of experiments intended to test hypotheses based on the kinetics of lipid autoxidation in vitro (6, 16–18). At least one highly qualified investigator (19) has stated that the kinetics of autoxidation in the presence of α-tocopherol are too complex to interpret. Uri (20) has described some of the problems encountered, including the variable effect of concentration on antioxidant efficiency and the nonuniform fate of antioxidant molecules. Controversy has arisen over the interpretation of data from biological systems (13, 18, 21).

This report attempts to clarify various areas of controversy related to the interrelationship of vitamin E and dietary and tissue PUFA.

Modification of tissue lipid fatty acid composition by diet

The tissue lipid fatty acid composition of even the newborn is not completely responsive to the maternal diet and tissue stores, as certain types of fatty acids, particularly *trans* fatty

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acids, are not transported across the placenta (22, 23). In the young, rapidly growing animal (24) most tissue lipids, such as those of muscle, will equilibrate with the diet in 4 to 10 weeks (Table 1). Obviously, a tissue such as the rat testis, that undergoes a rather dramatic change in fatty acid composition concomitant with the onset of spermatogenesis (25, 26), might give confusing data in a poorly designed experiment. The older and fatter the subject the greater will be the delay in attaining equilibrium after a change in diet lipid (27). The liver is perhaps the easiest organ in which to produce a rapid change in PUFA content. With rats approximately 20 weeks old, 20 to 30 weeks were required to approach a new equilibrium in hepatic lipid PUFA after a change in diet lipid (Table 1).

In adult male human subjects fed a diet supplying 60 g/day of safflower oil after consuming a diet containing 60 g/day of coconut oil for 30 months, adipose tissue linoleate levels showed a continuous increase for 58 months (13, 14, 28). With similar subjects fed a diet supplying 60 g/day of beef fat after consuming a diet containing 60 g/day of corn oil for 58 months, erythrocyte linoleate levels were still changing after 52 months with one-third of the total observed change occurring within 3 weeks, one-third between 3 weeks and 10 months, and one-third between 10 and 52 months (29).

One approach to relating vitamin E requirement to the PUFA content of a lipid has been the use of peroxidizability index (30). By this means, the susceptibility to peroxidation of a complex mixture of fatty acids may be described by a single number related to the relative maximum rates of autoxidation of these fatty acids in vitro. Bieri and Poukka (31) found that this approach could be used successfully in correlating erythrocyte hemolysis by peroxide and erythrocyte lipid fatty acid composition.

The essential fatty acid-deficient rat (32) and monkey (33) are known to develop signs of tocopherol deficiency. In such animals, high levels of PUFA may arise from de novo synthesis of eicosatrienoic acids from acetate via oleate and palmitoleate (34). When essential fatty acid-deficient rats were fed graded levels of linoleate (11) for 5 weeks, tissue lipid analyses expressed in terms of peroxidizability index (percent monoenoic acid X 0.025) + (percent dienoic acid X 1) + (percent trienoic acids X 2) + (percent tetraenoic acids X 4) + (percent pentaenoic acids X 6) + (percent

TABLE 1
Changes in tissue PUFA levels after a change in dietary PUFA levels

Experiment A[a] Rat gastrocnemius and quadriceps muscle phospholipids; subject 21 day old weanlings (n = 3–6)

	Time, weeks				
Fatty acids	0	2	4	10	26
20:4, %	11.2	7.1	5.2	4.8	4.8
22:5, %	2.9	4.2	5.2	6.2	6.1

Experiment B[b] Rat liver total lipids; subjects; 130 days old (n = 3)

	Time weeks							
Fatty acids	0	2	4	10	17	36	56	79
18:2, %	35	17	10	8	7	5	5	
18:2, %	27	14	9	6	4	4		4

Experiment C[c] Human adipose tissue; subjects; six adult males

	Time, months							
Fatty acid	0	6	14	22	36	42	47	58
18:2, %	17	25	32	38	43	47	49	51

Experiment D[d] Human erythrocyte lipids; subjects; six adult males

	Time, months							
Fatty acid	0	2/3	2	10	19	27	35	52
18:2, %	15.1	12.0	10.9	9.1	8.3	7.9	6.9	6.1

[a] Commercial weanling rats (Holzman) fed a mixture of linseed oil and beef tallow randomized by transesterification (24). [b] Weanling rats fed *1*) safflower oil or *2*) corn oil for 109 days and then fed beef fat (27). [c] Adult male human subjects fed a diet containing 60 g/day safflower oil after having been fed a diet containing 60 g/day coconut oil for 30 months (13, 14, 28). [d] Adult male human subjects fed a diet containing 60 g/day beef fat after having been fed a diet containing 60 g/day corn oil for 58 months (29).

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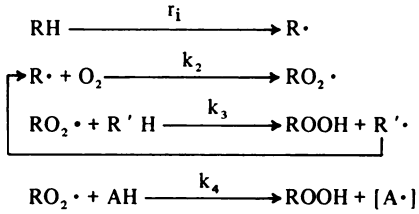
hexaenoic acids X 8), Table 2, clearly indicated that a direct relationship between tissue PUFA and dietary PUFA was not obtained under these conditions. Frequently the decrease in trienoic acids and increase in arachidonate were balanced so that a significant change in susceptibility to peroxidation was not produced by the change in the level of supplementation with linoleate.

No statements regarding the effect of dietary lipid on vitamin E requirement should be accepted without detailed analyses demonstrating the effect of that dietary treatment on tissue lipid PUFA content at the time of the experiment and at the time of attainment of an equilibrium composition.

Kinetics of autoxidation and of peroxidation

Autoxidation in the presence of vitamin E (20) is conventionally described by the sequence of reactions:



If the free radical-initiated, cyclic chain reaction is drawn as a spiral (Fig. 1), several points are conveniently illustrated. The slowest and therefore rate-limiting reaction is the reaction of a peroxy free radical with another fatty acid. The rate (k_3) of this reaction increases with the degree of unsaturation, whereas the rate (k_4) of the competitive termination reaction by which free radicals are withdrawn from the system is not highly sensitive to fatty acid unsaturation. The length, or number of cycles (n) of the spiral is proportional to $k_3[RH]/k_4[AH]$ and increases

TABLE 2
Effect of the level of linoleate supplementation in the essential fatty acid-deficient rat on the PUFA content and peroxidizability index (PI) of various tissue lipids after 5 weeks repletion[a]

	Percent of total fatty acids				
	Monoenes	Dienes	Trienes	Tetraenes	PI
Plasma					
Linoleate, % of diet					
0	48.9	3.7	16.0	1.2	41.7
0.25	44.4	5.7	5.6	4.9	37.6
0.5	42.7	7.8	4.5	13.2	70.7
1.0	43.4	6.6	3.8	15.1	75.1
4.0	33.8	15.2		23.0	108.8
5.0	24.7	28.0		19.8	107.8
Erythrocytes					
Linoleate, % of diet					
0	25.3	1.4	24.4	4.6	69.2
0.25	21.8	2.9	12.8	14.3	86.2
0.50	19.1	2.9	8.4	18.2	103.0
1.0	20.0	3.7	7.2	19.0	94.6
4.0	15.4	7.8	5.5	25.5	121.2
5.0	13.8	7.7	4.3	23.7	111.4
Liver					
Linoleate, % of diet					
0	54.5	2.2	9.7	1.6	29.4
0.25	48.6	4.3	5.8	7.5	47.1
0.50	46.9	4.8	3.5	9.9	52.6
1.0	46.9	5.3		9.8	45.7
4.0	34.1	11.8		16.3	77.8
5.0	30.1	13.1		17.8	84.0

[a] (Adapted from references 11, 30.)

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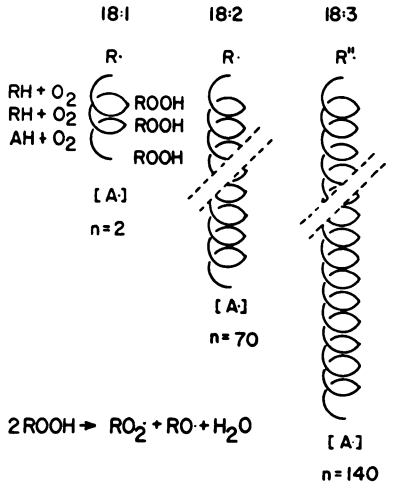

FIG. 1. Effect of increasing the degree of unsaturation on the length of the cyclic chain reaction and the quantity of hydroperoxide produced at optimal concentrations of R-α-tocopherol.

with the degree of unsaturation. As product hydroperoxides break down to form new free radicals, the rate of initiation (r_i) increases and the reaction becomes autocatalytic. The values of n (Fig. 1) describing the length of the cyclic chain reaction are those found experimentally at optimum R-α-tocopherol concentrations in vitro (18).

It has been possible to demonstrate in man and in animals that the vitamin E requirement or the rate of development of a tocopherol-deficiency sign, or both is related to the input of PUFA into the tissue lipids (13). In animals in which somewhat more detailed experiments were possible, the relationship was found to be consistent with the relative susceptibility of the PUFA to autoxidation in vitro (12, 13, 16, 30). This is not the same as demonstrating that lipid peroxidation occurs in vivo. Green and co-workers (6–10) have suggested that the rate of tocopherol oxidation in vivo should be related to the rate of lipid peroxidation in vivo if lipid peroxidation actually occurred in vivo and if vitamin E actually functioned as a lipid autoxidant in vivo. In a large number of experiments of various types, the rate of oxidation of a tracer dose of radioactively labeled α-tocopherol was not found to be particularly influenced by the degree of unsaturation of tissue lipids.

At relatively high tocopherol concentrations, new free radical initiations arise largely from the bimolecular reaction 2 ROOH → RO_2· + RO· + H_2O. At relatively low tocopherol concentrations, such as might occur in vitamin E deficiency, only low concentrations of hydroperoxides may be formed prior to the reaction entering the exponential phase. Under these conditions, the monomolecular reaction ROOH → RO· + ·OH is the source of new free radicals. The rate at which the hydroperoxide breaks up into new free radicals by the monomolecular reaction appears to be inversely related to the degree of unsaturation. At low tocopherol concentrations, the rate of tocopherol oxidation appears to be independent of the degree of fatty acid unsaturation. This has been suggested as a possible explanation of the observations of Green and co-workers (6–10).

The autoxidation of a fatty ester in the presence of R-α-tocopherol (18) has two phases (Fig. 2). Initially, a relatively slow formation of hydroperoxides occurs concomitant with tocopherol oxidation. During the second rapid or exponential phase of lipid autoxidation, there is relatively little tocopherol destruction compared to the quantity of hydroperoxide formed. The termination reaction RO_2· + $AH \overset{k_3}{\rightleftarrows} ROOH + [A\cdot]$ is reversed by the buildup of hydroperoxide and the cyclic chain reaction

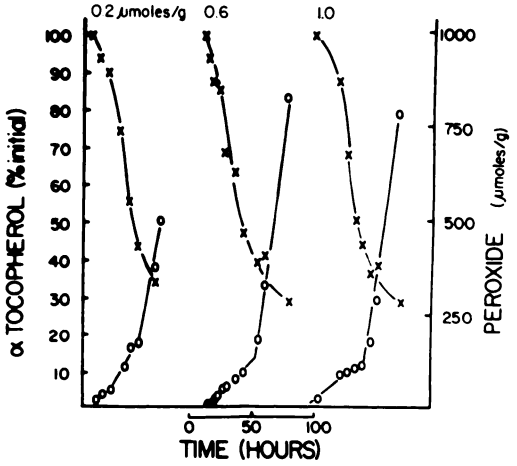

FIG. 2. Tocopherol oxidation during the autoxidation of ethyl linolenate. Peroxide formation (o) and tocopherol oxidation (x). Initial concentration of R-α-tocopherol in micromoles/gram as indicated at top of figure (18).

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proceeds with less net destruction of the residual antioxidant.

At the transition to the exponential phase of autoxidation the ratio of molecules of hydroperoxide formed per molecule of antioxidant destroyed increases by at least an order of magnitude, from approximately 100:1 to several thousand to one in linoleate. The higher the initial concentration of vitamin E the greater the concentration of hydroperoxide that can occur prior to entering the exponential phase of autoxidation.

The reversal of the termination reaction (35) tends to be ignored because with hindered phenols such as 2,4,6-tri-*tert*-butylphenol k_3 forward/k_3 reverse is approximately 30,000. However, with unhindered phenolic antioxidants such as phenol this value may decrease to 0.05. Addition of high concentrations of hydroperoxide has no effect on the inhibited rates of oxygen absorption in the presence of a hindered phenolic antioxidant. The antioxidant properties of vitamin E (Fig. 2) however, closely resemble those of 4-methoxyphenol (35). At high concentrations of 4-methoxyphenol the rate of oxygen uptake is independent of the concentration of the antioxidant, whereas at low concentrations the addition of hydroperoxide accelerates the oxygen uptake.

For this type of system, a concentration of antioxidant can be found that will result in a minimum rate of oxygen uptake. The antioxdant activity of α-tocopherol in vitro is greatest at 0.8 to 1.8 μmoles/g fatty acids, which corresponds closely to normal tissue levels (18).

A study (18) of α-tocopherol oxidation in autoxidizing ethyl linoleate, linolenate, and arachidonate suggested that the reversal of the termination reaction is sensitive to fatty acid structure. The shift to an exponential rate of oxidation occurred at hydroperoxide to residual tocopherol ratios of approximately 160:1, 320:1, and 640:1 in linoleate, linolenate, and arachidonate, respectively.

Studies by DiLuzio and Hartman (25) with ethanol metabolism in vitro and by McCay and co-workers (37, 38) with NADPH and the hepatic microsomal mixed-function oxidase in vitro clearly indicate that tocopherol oxidation precedes damaging levels of lipid peroxidation. The data of Green et al. (6–10), DiLuzio and Hartman (36), and McCay et al. (37, 38) would strongly suggest that if damaging levels of lipid peroxidation were occuring in vivo or in vitro, the reaction would be in the exponential phase and proceeding as if the residual antioxidant were almost without antioxidant activity. Upon addition of high specific activity, labeled tocopherol in trace quantities, tocopherol oxidation would be demonstrable as the reversible termination reaction is inhibited but not prevented. Net production of oxidized tocopherol cannot proceed as rapidly as the production of hydroperoxides, however, because of product buildup favoring the reversal of the termination reaction.

The initial tocopherol oxidation product would be the chromanoxyl free radical (39). Reactions producing tocopheryl quinone or the tocopherol dimer (40) via the quinone methide would be competitive with the reversal of the termination reaction and would ensure the slow production of oxidized tocopherol. The rate of production of oxidized tocopherol under these conditions would be independent of tissue lipid PUFA content.

Prevention or amelioration of liver damage resulting from acute intoxications by lipid antioxidants is consistent with the above description. Green and co-workers (41) showed that in vitamin E-deficient rats simultaneous oral administration of a massive acute dose of carbon tetrachloride (2.0 ml/kg) had no effect on the distribution of ^{14}C-α-tocopherol between unchanged material and metabolites in the liver of rats killed 24 hr later. However, in vitamin E supplemented rats (42) carbon tetrachloride resulted in loss of 50 to 75% of the hepatic α-tocopherol in 24 hr. An average of 35% of the initial hepatic level of α-tocopherol remained unoxidized, regardless of the magnitude of the initial hepatic level of α-tocopherol, despite necrosis of the entire centrilobular region with an outer zone of hydropic degeneration. Ingestion of certain hepatotoxins such as carbon tetrachloride or ethanol appears to initiate rapid lipid peroxidation related to the active metabolism of the intoxicant. The rapid production of a large number of new free radicals would be expected to result in the critical [ROOH]: [AH] ratio being exceeded and the reaction would enter the exponential phase. In 1942, Mason showed that the liver was apparently the only organ capable of gross storage of vitamin E (43). Tissue damage would be prevented or amelio-

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rated if the reaction could be prevented from entering the exponential phase of lipid peroxidation.

Vitamin E cannot prevent the active metabolism of these hepatotoxins and the production of free radicals. An antioxidant minimizes the yield of product peroxide per free radical initiation but does not prevent lipid peroxidation. Increasing the tocopherol cencentration beyond the optimal level normally present in tissue lipids does not further decrease the yield of product peroxide per free radical initiation (Fig. 3). This is a normal observation with unhindered phenolic antioxidants (35). The excess lipid antioxidant prevents the reaction from entering the tissue damaging exponential phase and presumably provides time for such enzymes as glutathione peroxidase to dispose of the product peroxide.

Rather gross decreases in hepatic PUFA levels consistent with the occurrence of lipid peroxidation in vivo have been observed after acute or chronic administration of various hepatotoxins (44). Some confusion has existed, however, since feeding one hepatotoxin, ethionine, was reported to inhibit the fatty acid desaturase system in liver (45, 46). Vesicular dilatation of the hepatic endoplasmic reticulum in the treated animals, however, appears to render the microsomal enzymes somewhat more susceptible to damage during preparation for assay in vitro, and these results are therefore regarded as artefactual in origin (43). Other

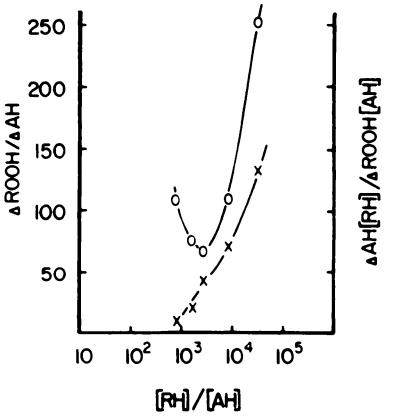

FIG. 3. Effect of concentration on the efficiency of R-α-tocopherol as an antioxidant in ethyl linoleate. Moles peroxide formed per mole of antioxidant oxidized (○) and efficiency of R-α-tocopherol as an antioxidant (x) (18).

experiments appear to indicate that in several mild chronic intoxications there is increased PUFA synthesis and an actual net increase in the phospholipid PUFA content of the liver associated with the "packaging" of the neutral lipid which accumulates in the fatty liver (47).

The interrelationship of vitamin E and polyunsaturated fatty acids in diet and tissue is complex but real, as is invariably demonstrated in reasonable experiments.

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Discussion

J. G. Bieri: I am a little confused in terms of the data you have presented regarding the rate of change of linoleate in the tissues as compared with some of the work that Holman's group has done over the years along similar lines. I was under the impression that most tissues when you add a fair amount of linoleate to the diet, increase rather rapidly for several weeks and reach a fairly stable equilibrium level, though it may continue to increase slightly. Is this in variance with what you are telling us? The problem arises as to how much linoleate you are going to feed. I can see how, if you overwhelm the animal with a tremendous amount of linoleate, you may get changes for a long period as you show, but if you add smaller amounts, isn't the change rather rapid initially and then slow after that?

L. A. Witting: I think this is consistent with what we have shown; if you make a relatively drastic change in diet, it takes a reasonable amount of time to reach an equilibrium. The small changes that we have shown are based on having enough animals and enough samples to show that the further change is actually significant.

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