The Unexpected Role of Vitamin E (α-Tocopherol) in the Peroxidation of Human Low-Density Lipoprotein[1]

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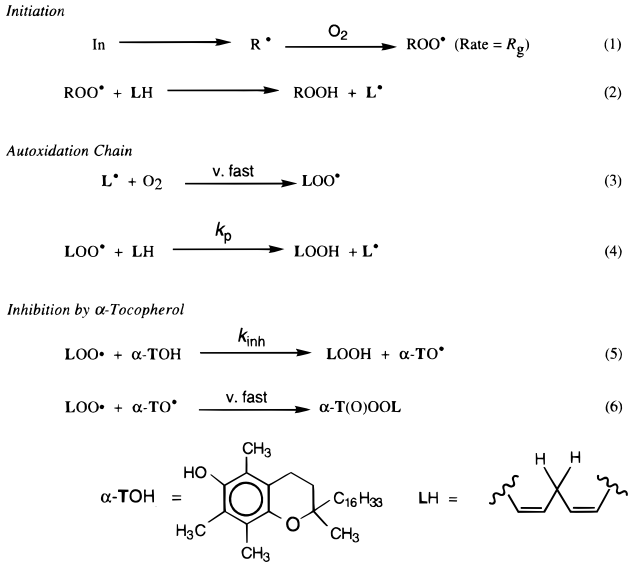
Introduction

Atherosclerosis is the single most important cause of death in Western Society. "Fatty streaks" formed on the interior walls of arteries are the first visible signs of atherosclerosis. These fatty streaks contain peroxidized low-density lipoprotein (LDL)[2] particles, and oxidized LDL is strongly implicated in the initiation and development of atherosclerosis.[3] Antioxidants would therefore be expected to attenuate atheresclerosis, and there are a number of human[4] and animal studies[5] indicating that this is the case. Naturally, there has been a veritable explosion of research into free-radical-induced peroxidation of LDL and its prevention by antioxidants.[6]

By far the major free-radical antioxidant in LDL lipid is α-tocopherol, α-**T**OH, biologically and chemically the most active form of vitamin E.[7] The very high reactivity of α-**T**OH toward peroxyl radicals[7] and the relatively large amount of α-**T**OH present in LDL (vide infra) leads one to expect that LDL peroxidation would be very strongly inhibited. Yet despite this "expectation" and despite scores of experimental studies, there is still no consensus that enrichment of the lipoprotein with α-**T**OH will actually retard the oxidative modification of LDL in vitro.[8]

Our investigations into the kinetics of LDL peroxidation suggest that this lack of agreement arises from an "unrecognized consequence of lipid particle size"[9] endowing α-**T**OH with a *prooxidant* activity that manifests itself in various ways according to experimental conditions (e.g., the type and amount of radical initiator).[10] In this Account we describe (i) how and when α-**T**OH becomes a prooxidant,[11] (ii) the inhibition of α-**T**OH's prooxidant activity by co-antioxidants,[12] (iii) use of a simple deuterium kinetic isotope effect to probe the peroxidation mechanism,[13] and (iv) the implications of our findings for atherosclerosis research.

Scheme 1. α-Tocopherol-Inhibited Peroxidation of Esters of Polyunsaturated Fatty Acids (PUFA = LH)



Background

Radical Scavenging in Homogeneous Systems. The principal elementary reactions involved in α-**T**OH-inhibited lipid peroxidation in a homogeneous system are shown in Scheme 1.[14] Each molecule of α-**T**OH terminates two peroxidation chains (reactions 5 and 6), and the overall kinetic rate law is

$$\mathrm{d[LOOH]}/\mathrm{d}t = R_g k_p\,[\mathrm{LH}]/2k_{inh}[\alpha\text{-TOH}] \qquad (\mathrm{I})$$

where R_g is the rate of generation of the radicals which initiate peroxidation chains. In homogeneous, lipid-like solvents, k_{inh} for α-**T**OH is greater[15] than for any other lipid-soluble biological antioxidant and hence α-**T**OH is the most powerful natural inhibitor of lipid peroxidation.

The trapping of peroxyl radicals by α-**T**OH is much slower in polar solvents; e.g., k_{inh} is 50-fold lower in ethanol than in cyclohexane solvent.[16] Our recent investigations into the role of the solvent in hydrogen atom abstraction reactions[17−20] show that slower trapping in polar solvents is due to hydrogen bonding of the substrate to hydrogen bond acceptor (HBA) solvents. Thus, the reactivity of α-**T**OH in an HBA solvent, **S**, depends on the fraction of the α-**T**OH that is *not* bound to the solvent (Scheme 2).[20] The solvent effect depends solely on the substrate−solvent interaction and is therefore *independent*

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Scheme 2

$$k_{inh} = k_H / K^S[S] \qquad (II)$$

of the reactivity of the attacking radical, a remarkable property that has been confirmed over a very wide (10^{10}-fold!) kinetic range.[18] The importance of this new principle is that if we know a single rate constant for H-abstraction from α-**T**OH by some radical, e.g., a peroxyl radical, we can accurately predict the magnitude of this rate constant (e.g., k_{inh}) in many other solvents.

Radical Scavenging in Nonhomogeneous Systems. Reported rate constants for peroxyl radical trapping by α-**T**OH dispersed in SDS micelles[21] and phospholipid liposomes[22] are lower and very much lower, respectively, than the values reported for any homogeneous system, cf. $k_{inh}/M^{-1}\ s^{-1} = (5 \pm 1) \times 10^4$ (micelles) and 3×10^3 (liposomes) versus 5.1×10^5 (*tert*-butyl alcohol,[16] a good HBA solvent). These results cannot be explained via Scheme 2 with **S** = H_2O for the simple reason that water is not as good an HBA solvent as, say, *tert*-butyl alcohol.[23]

Since the low reactivity of α-**T**OH in aqueous dispersions is not due to hydrogen bonding, it must be a consequence of a physical separation between at least some of the lipid peroxyl radicals and some of the α-**T**OH molecules. Both inter- and intraparticle separations are possibilities. In the inter- case, α-**T**OH in particle **A** obviously cannot capture an **L**OO• in particle **B** until, e.g., **L**OO• diffuses from **A** to **B**. If **B** were completely depleted in α-**T**OH and **L**OO• exit from the particle was slow, then **B** could undergo extensive peroxidation despite the presence of abundant α-**T**OH in **A** (and hence in the overall system).

Thus, the *true* rate constant for the α-**T**OH/**L**OO• reaction 5 in aqueous dispersions of lipid is unknown, and cannot be reliably derived from conventional measurements suitable for a homogeneous system.[24,25] This is true not only for SDS micelles and phospholipid liposomes but also for LDL particles.

Low-Density Lipoprotein (LDL). The "peroxidation profile" of an LDL particle is shown in Figure 1.[26,27] Of obvious importance to LDL peroxidation are (a) *substrate*, ~1450 bisallylic CH_2 groups (**L**Hs) per particle (see Scheme 1), and (b) *antioxidant*, ~6−12 molecules of α-**T**OH per particle and lesser amounts of other real or putative[28] antioxidants (see Figure 1 and below). Of less obvious importance to peroxidation is

(c) *particle size*, Stokes radius ~10 nm, volume 3.8×10^{-24} m^3 with a free lipid volume $V_{lip} \approx 3.2 \times 10^{-24}$ m^3 and hence a free molar volume, $N_A V_{lip} \approx 2 \times 10^3$ $dm^3\ mol^{-1}$. What this means is that an LDL particle is simply too small to hold more than one radical for more than a very brief moment, e.g., ~1 s for a pair of α-**T**O• radicals, ~1 ms for a pair of **L**OO• radicals, and ~1 μs for an **L**OO• (**R**OO•) and α-**T**O• combination.[29]

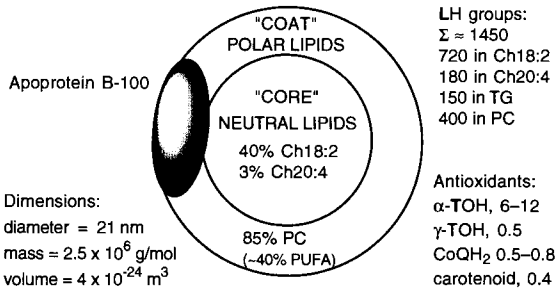


FIGURE 1. Schematic of an LDL particle. Each particle is a molecular assembly containing a protein moiety (apoprotein B-100, which intercalates and stabilizes the structure), a ***core*** of neutral lipids (cholesteryl and triglyceryl (TG) esters and free cholesterol), and a ***coat*** of polar lipids (mainly phosphatidylcholine (PC) diesters). Ch18:2 and Ch20:4 refer to the cholesteryl esters of linoleic and arachidonic acids, respectively.

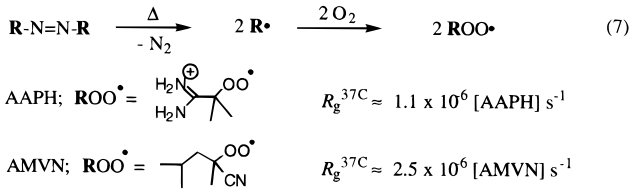
In our studies (unlike many others) the LDL was very rapidly isolated. It contained ≤1 LOOH molecule per 500 particles[30] and retained most of its associated coenzyme Q_{10} in its reduced form, $CoQH_2$, at the start of reaction (vide infra).

LDL Peroxidation

Theoretical Expectations. It can be seen from Scheme 1 (or eq I) that lipid peroxyl radicals partition between chain-terminating attack on α-**T**OH (reaction 5) and chain-propagating attack on **L**H (reaction 4) in the ratio k_{inh}[α-**T**OH]/k_p[**L**H]. For example, when k_{inh}[α-**T**OH] = k_p[**L**H], 50% of the **L**OO• will be regenerated (via reactions 4 and 3) so that any peroxidation that does occur does not involve a radical chain reaction, or, in other words, in this situation the chain length, ν, would be 1.0. While there is some uncertainty regarding the effective magnitude of k_{inh} in LDL (vide supra), it probably lies between the value found in a saturated hydrocarbon solvent and the value estimated in water, viz. $k_{inh} \approx (68-8) \times 10^5\ M^{-1}\ s^{-1}$. Therefore, since $k_p \approx 100\ M^{-1}\ s^{-1}$ per **L**H moiety,[31] one molecule of α-**T**OH should be able to protect between 68 000 and 8000 **L**H mioeties. The [**L**H]/[α-**T**OH] ratio in LDL is roughly 1450/9 = 160 (Figure 1), and so it would appear that LDL is massively overprotected against peroxidation.

Experiments in which LDL was "oxidatively modified" using air and copper ions (usually >5 Cu^{II} ions per LDL particle) lent support to the above analysis in that relatively little **L**OOH was formed until the α-**T**OH (and other endogenous antioxidants) had been consumed.[32] Naturally this type of experiment led to the view that α-**T**OH is a powerful antioxidant for LDL, just as it is for bulk lipids. *Unfortunately*, there was a very poor correlation between the α-**T**OH content of LDL and the duration of the induction (or lag) period preceding lipid peroxidation.[6] This little inconsistency led to the invention of a new variable, "*unknown antioxidants*" in the LDL. Naturally, these have remained unknown because they simply do not exist!

Scheme 3



Practical Reality. In 1990 two independent studies reported on the peroxidation of LDL exposed to organic peroxyl radicals (**R**OO•) generated at a steady rate (R_g) by water-soluble and lipid-soluble azo compounds (2,2′-azobis(2-amidinopropane hydrochloride), AAPH, and 2,2′-azobis(2,4-dimethylvaleronitrile), AMVN, respectively)[33,34] (Scheme 3).

Both studies showed that LDL peroxidation occurred at much the same rate in the formally α-**T**OH inhibited period of peroxidation as in the subsequent uninhibited (α-**T**OH consumed) period. Both studies also noted that the minor quinolic antioxidant, ubiquinol-10 ($CoQH_2$), was consumed *before* α-**T**OH.

A more detailed investigation of the early period of peroxidation[35] revealed that the disappearance of $CoQH_2$ corresponded closely with the onset of a rapid oxidation phase.[36] In the presence of vitamin C (ascorbate, $AscH^-$), the $CoQH_2$ was still consumed first but now peroxidation was inhibited until the ascorbate was also depleted.

Even more intriguing was that peroxidation in the formally inhibited phase of LDL peroxidation was being propagated in a radical *chain*. Up to 100 **L**OOH molecules were formed per initiating radical, **R**OO•, regardless of whether the peroxidation was induced by AAPH, AMVN, or (the oxidants generated by) stimulated neutrophils (scavenger cells).[35]

These results were contrary to expectations for a peroxyl-mediated, α-**T**OH-inhibited peroxidation (Scheme 1). That is, being 10-fold *less* reactive than α-**T**OH toward peroxyls in solution,[37] $CoQH_2$ was *expected* to be consumed after α-**T**OH and to have little effect on the peroxidation rate. Water-soluble vitamin C was also *expected* to have little or no effect on the peroxidation rate in AMVN-initiated LDL since it is known to be a very poor inhibitor of peroxidation induced by lipid-soluble **R**OO• radicals in micelles and liposomes.[38] And we certainly did *not expect* a radical chain in tocopherol-containing LDL.[39]

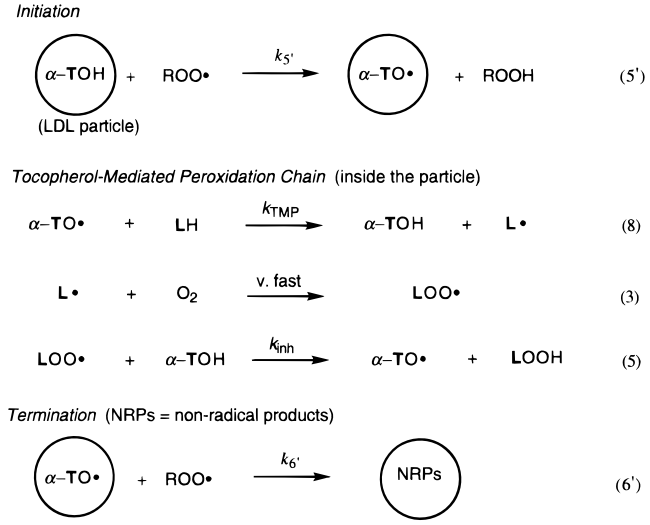
Tocopherol-Mediated Peroxidation (TMP). These inconsistencies forced us to reconsider the fate of α-**T**O• radicals, in particular, to consider whether the known, but very slow, chain-transfer reaction of α-**T**O• with lipid

$$\alpha\text{-}\mathbf{T}O^{\bullet} + \mathbf{L}H \xrightarrow{0.1\ M^{-1}\ s^{-1}} \alpha\text{-}\mathbf{T}OH + \mathbf{L}^{\bullet} \qquad (8)$$

was contributing to peroxidation by regenerating **L**OO• radicals. This would help explain the high effectiveness of vitamin C since it was known that vitamin C ($AscH^-$) could reduce α-**T**O• in PC liposome dispersions.[38]

$$(AscH^-)_{aq} + (\alpha\text{-}\mathbf{T}O^{\bullet})_{lipid} \rightarrow (Asc^{\bullet -})_{aq} + (\alpha\text{-}\mathbf{T}OH)_{lipid} \qquad (9)$$

Scheme 4. Tocopherol-Mediated Peroxidation (TMP)



One can readily see that reaction 8 followed by coupling of the **L**• radical with oxygen and reaction of the resulting **L**OO• with α-**T**OH constitutes a *potential* radical chain—a tocopherol-mediated peroxidation (TMP) chain (Scheme 4)—but is it a plausible one? The problem here is that reaction 8 (chain transfer) is so slow that an α-**T**O• radical would need to "wait around" for ∼30 s between propagation steps ($k_{TMP}[\mathbf{L}H] \approx 0.03\ s^{-1}$). At first sight this seemed highly unlikely in the face of chain-terminating reactions with rate constants of up to $3 \times 10^8\ M^{-1}\ s^{-1}$ (reaction 6) in a *homogeneous system*.[40]

However, LDL is not homogeneous and diffusion of lipid-soluble compounds *between* LDL particles is known to be slow.[41,42] It occurred to us that once an α-**T**O• radical was formed in an LDL particle (reaction 5′) it might remain trapped within that particle and thereby be forced to "wait around" for a relatively long time for a *second* **R**OO• radical to strike the particle (reaction 5′). Simple calculation revealed a "waiting period" on the order of several minutes for our reaction conditions; e.g., for a typical LDL + AAPH experiment, $[LDL]/R_g \approx 1\ \mu M/1\ nM\ s^{-1} = 1000$ s or 17 min. This is, indeed, ample time for several **L**OOH molecules to be generated via the TMP chain in Scheme 4.

Models of TMP Kinetics. To prove the existence of TMP in LDL, we first needed to know what to look for in our experimental data. Kinetic models were developed from three basic premises: (i) no transfer of radicals *between* particles, (ii) a one-radical-per-particle limit (vide supra, particle size), and (iii) α-**T**OH retains its "normal" high radical scavenging activity in LDL.

(a) Simple Emulsion Polymerization Model. The third premise immediately told us that the vast majority of **L**OOH formed in the presence of α-**T**OH *must be* formed via reaction 5 (because a **L**OO• radical is ∼200-fold more likely to react with α-**T**OH than with **L**H in the LDL particle). Kinetic analysis of the resulting TMP-only system[43] yielded what we thought was a very unusual prediction: at steady state exactly half of the particles would contain one radical each and the other half none

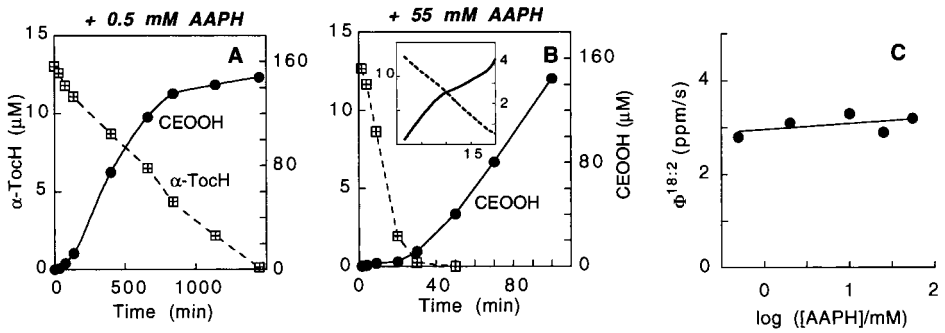


FIGURE 2. Consumption of α-**T**OH and formation of cholesteryl ester hydroperoxide (CEOOH) in LDL initiated with various concentrations of AAPH. R_p^{max} and the fractional rates of peroxidation ($\Phi = R_p^{max}/[\mathbf{L}H]$) were calculated from maximum slopes of the CEOOH vs time plots. Fractional peroxidation rates were nominally uniform between lipid classes and were proportional to the number of bisallylic methylene groups per lipid molecule (so, e.g., cholesteryl arachidonate, Ch24:4, peroxidized 3 times faster than cholesteryl linoleate, Ch18:2, $\Phi^{24:4} \approx 3\Phi^{18:2}$).

(i.e., [α-**T**O•] = [LDL]/2), and consequently the *peroxidation rate would be independent of the rate of radical generation*

$$R_p = -\mathrm{d}[\mathbf{L}H]/\mathrm{d}t\ (\approx \mathrm{d}[\mathbf{L}OOH]/\mathrm{d}t) = [\alpha\text{-}\mathbf{T}O^\bullet][\mathbf{L}H]k_{TMP} = ([LDL]/2)[\mathbf{L}H]k_{TMP} \quad \text{(III)}$$

In the event, the experimental rate of α-**T**OH-inhibited peroxidation was remarkably insensitive to the concentration of initiator (see Figure 2C). We had, in fact, uncovered the first example of *emulsion polymerization kinetics*[44,45] in a nonpolymer system.

(b) Modified Emulsion Polymerization Model. Figure 2A demonstrates that eq III is not consistent with experiment since the oxidation rate (R_p) clearly becomes slower as the α-**T**OH is consumed. Further experiments showed that R_p was faster in α-**T**OH-enriched LDL.[11] This *prooxidant* activity of α-**T**OH could only be due to a high *phase-transfer* activity of α-**T**OH. That is, instead of particles being uniformly reactive to initiating radicals (as is probably true for emulsion polymerizations), the likelihood of a particle reacting with an incoming **R**OO• radical must (by premise iii) be dominated by α-**T**OH in nonperoxidizing particles ($[\alpha\text{-}\mathbf{T}OH]k_{ROO^\bullet/\alpha\text{-}TOH} \approx 6 \times 10^3\ s^{-1}$) and by α-**T**O• in peroxidizing particles ($[\alpha\text{-}\mathbf{T}O^\bullet]k_{ROO^\bullet/\alpha\text{-}TO^\bullet} \approx 2 \times 10^5\ s^{-1}$).[46] Thus, assuming (i) *all* initiation occurred via reaction 5′ (i.e., none via reaction 2) and (ii) *all* termination occurs via reaction 6′, we found that a steady-state radical population required[47]

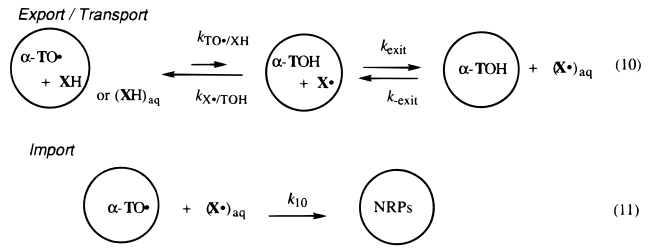
$$k_{5'}[\alpha\text{-}\mathbf{T}OH][\mathbf{R}OO^\bullet] = 2k_{6'}[\alpha\text{-}\mathbf{T}O^\bullet][\mathbf{R}OO^\bullet] \quad \text{(IV)}$$
(*rate of initiation*) (*rate of termination*)

That is, [α-**T**O•] = ($k_{5'}/2k_{6'}$)[α-**T**OH], and therefore

$$R_p^{inh} = k_{TMP}[\mathbf{L}H][\alpha\text{-}\mathbf{T}OH](k_{5'}/2k_{6'}) \quad \text{(V)}$$

(see Scheme 4). Equation V is consistent with the "sigmoid" shapes of the **L**OOH (CEOOH) vs *time* plots (Figure 2). After an early maximum in R_p, the rate falls as α-**T**OH is consumed, reaching a minimum just as the last few percent of α-**T**OH disappears, before accelerating again in the post-tocopherol period. Experimental data for AAPH- and AMVN-initiated native LDL yielded values of

Scheme 5. Radical Anti-TMP Mechanisms



$k_{5'}/k_{6'}$ = 0.03 and 0.006, respectively, which may be compared with $k_{ROO^\bullet/\alpha\text{-}TOH}/k_{ROO^\bullet/\alpha\text{-}TO^\bullet}$ = 0.005 obtained using AMVN in an organic solution of α-**T**OH.[40]

Co-antioxidants for LDL. *Plasma Antioxidants.* We tested the effect of some of the conditions and compounds found in blood plasma on azo-initiated peroxidation. It became clear from these experiments that TMP in LDL was strongly inhibited in plasma by $CoQH_2$, ascorbate, and bilirubin,[48] whereas other components such as free fatty acids, proteins, thiols, amino acids, and carotenoids, individually or in combination, had little or no effect on the peroxidation rate during the formal α-**T**OH-inhibited period. Lowering the oxygen tension from 150 mmHg (air) to a more physiological 15 mmHg also had negligible effect on the lipid peroxidation rate in tocopherol-containing LDL (cf. ref 49).

The putative plasma antioxidant, urate, proved interesting since it had a marked *prooxidant* effect on AAPH-initiated LDL. That is, addition of urate at its blood plasma concentration (~0.3 mM) *increased* the rate of **L**OOH formation by ~50% while slowing the rate of α-**T**OH consumption by ~90%. Presumably, urate—as a good scavenger of aqueous peroxyl but not α-**T**O• radicals[50]—was merely prolonging the effect of TMP by shielding the α-**T**OH from initiating radicals.

Anti-TMP Mechanisms. Since α-**T**O• radicals drive peroxidation in tocopherol-containing LDL, *any* mechanism that reduces the rate of formation of α-**T**O• radicals or increases their destruction once formed will retard lipid peroxidation. Three possible avenues for inhibiting TMP are open; they have been named radical export, interparticle radical transport, and radical import (Scheme 5).

Radical export is the irreversible transfer of a radical center from the LDL lipid to the aqueous phase. Ascorbate in the aqueous phase does this by rapidly reducing α-**T**O$^{\bullet}$ (reaction 9) to yield α-**T**OH and the water-soluble, strongly reducing, ascorbyl radical. Even micromolar amounts of ascorbate prevented TMP from occurring in LDL. Ubiquinol-10 ($CoQH_2$) also reacts rapidly with α-**T**O$^{\bullet}$ (reaction 12), but now radical export must occur indirectly

$$\alpha\text{-}\mathbf{T}O^{\bullet} + CoQH_2 \rightarrow \alpha\text{-}\mathbf{T}O^{\bullet} + CoQH^{\bullet}/(CoQ^{\bullet -} + H^{+}) \quad (pK_a = 5.4) \quad (12)$$

since the ubisemiquinone radical ($CoQH^{\bullet}$) will remain "tied" to its LDL particle by its long (C50) alkyl tail. We have proposed that radical export takes place via the reaction of $CoQH^{\bullet}$ (or $CoQ^{\bullet -}$) with oxygen.

$$(CoQH^{\bullet}/CoQ^{\bullet -}) + O_2 \rightarrow CoQ + (HOO^{\bullet}/O_2^{\bullet -})_{aq} \quad (13)$$

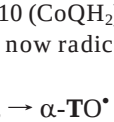
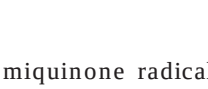
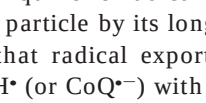
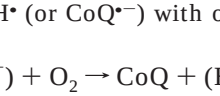
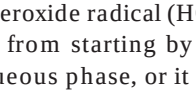
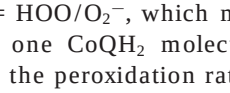
The resulting superoxide radical ($HOO^{\bullet}/O_2^{\bullet -}$) may prevent oxidation chains from starting by reacting with another radical in the aqueous phase, or it may terminate a chain (reaction 11, **X** = HOO/O_2^{-}, which may help to explain why as little as one $CoQH_2$ molecule per three LDL particles reduces the peroxidation rate[35]).

Interparticle radical transport, when enhanced, will inhibit TMP by shortening the lifetime of the LDL-trapped α-**T**O$^{\bullet}$ radicals (Scheme 5). Millimolar amounts of many natural and synthetic compounds and, remarkably, even the "prooxidant" *tert*-butyl hydroperoxide slowed the rate of TMP in LDL. To be really effective at low (i.e., micromolar) concentrations, however, a radical "shuttling" anti-TMP agent (**X**H) needs to (a) *react rapidly* with $(\alpha\text{-}\mathbf{T}O^{\bullet})_{LDL}$, (b) yield an antioxidant radical, **X**$^{\bullet}$, that *diffuses rapidly* to other particles, and (c) yield an **X**$^{\bullet}$ radical that *terminates rapidly* with $(\alpha\text{-}\mathbf{T}O^{\bullet})_{LDL}$ (i.e., reaction 11 rather than reacting with α-**T**OH to reinitiate TMP, reaction −10).[50] The role of each of these factors was examined in an extensive study of potential co-antioxidants (excerpted in Table 1).[12] In brief, all else being equal, the most reactive phenols in our study give the best inhibition (cf. factor a). For phenols with identical chemical reactivities, those with the greatest water solubility afforded the greatest inhibition of TMP (cf. factor b). Thus, in a series of α-**T**OH homologues, the co-antioxidant effectiveness decreased as the length of the alkyl tail (**R**) attached to the chromanol headgroup increased (see Table 2).

The third factor (selectivity for termination rather than reinitiation) is illustrated by the high co-antioxidant effectiveness of quinols compared to phenols (Table 1) since, in contrast to phenoxyls, semiquinone radicals (**X**$^{\bullet}$ = H**Q**$^{\bullet}$) are strongly reducing species that react very rapidly with α-**T**O$^{\bullet}$ (e.g., cf. $k_{\mathbf{T}O^{\bullet}/CoQH^{\bullet}} = 10^9$ M^{-1} s^{-1} and $k_{\mathbf{T}O^{\bullet}/BHT^{\bullet}} = 10^5$ M^{-1} s^{-1}).

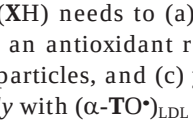
Radical import might be varied either to enhance chain termination (reaction 11) or to lower the reactivity of LDL particles toward initiating radicals. The most obvious way to achieve the latter goal is to remove α-**T**OH from the LDL, and indeed, a recent study by Stocker *et al.*[51] shows

Table 1. Co-antioxidant Effectiveness of Some Compounds Added to AAPH− or AMVN-Initiated LDL at a Concentration Equal to That of the Endogenous α-TOH (10 μM)

Compound	$(1 - R_p^{with} / R_p^{without})$, % [a]	
	AAPH	AMVN
Ascorbic Acid		
AsH$^-$ 	99.8	99.9
Phenols		
BHT 	90	95.2
DBHA 	97	95.3
ED 	- 3	- 11
Quinols and catechols		
TBHQ 	99.9	99.9
2HED 	98.1	99.8

[a] Peroxidation rates (R_p) at 37 °C calculated from the time point corresponding to 20% consumption of α-**T**OH in the unsupplemented (without) sample (see ref 12).

Table 2. Co-antioxidant Effectiveness of α-TOH Homologues Added to AAPH-Initiated LDL at a Concentration Equal to That of the Endogenous α-TOH (10 μM)

 , R =	Co antioxidant effectiveness $(1 - R_p^{with} / R_p^{without})$, % [a]
CO_2H (Trolox)	99.0
CH_2OH	99.2
CH_3	95.4
n-C_6H_{11}	80.9
n-$C_{11}H_{23}$	35.6
n-$C_{13}H_{27}$	0
n-$C_{15}H_{31}$	- 7

[a] See Table 1 footnote *a*.

that peroxide-free, tocopherol-depleted LDL is virtually inert to AAPH-initiated peroxidation!

Another approach to lowering LDL's reactivity toward initiating radicals is to convert the endogenous α-**T**OH into its deuterated form, α-**T**OD.[13] In a homogeneous system this will accelerate autoxidation by about a factor of 4 since α-**T**OD is about 4-fold less reactive than α-**T**OH toward **L**OO$^{\bullet}$ [53] (eq I). However, in LDL reaction 5′ will be slower with α-**T**OD than with α-**T**OH, and therefore the rate of peroxidation was predicted to be slower (eq V). In the event, the AAPH-initiated peroxidation of LDL was found to be 2.5 times *slower* in D_2O buffer than in H_2O buffer.[54,55] The implied deuterium kinetic isotope

effect, DKIE = $k_{ROO^{\bullet}/TOH}/k_{ROO^{\bullet}/TOD} \approx 2.5$, is lower than the DKIE in a nonpolar solvent.[53]

Transition Metal-Induced Peroxidation. Most in vitro studies of LDL oxidation have used copper ions and air as the oxidant. Despite extensive studies, the exact mechanism for this oxidation remains uncertain.[56] However, it now seems clear that α-**T**OH plays a crucial role in the early stages of oxidation since tocopherol-depleted, peroxide-free LDL is relatively inert to oxidation by copper ions (and, for that matter, iron ions, *lipoxygenase*, *horseradish peroxidase*, AAPH, and even radiolytically generated hydroxyl radicals![51]). Thus, in fresh rapidly isolated LDL the initial radical-generating reaction is undoubtedly reduction of (lipoprotein associated[57]) Cu^{2+} to Cu^{1+} by α-**T**OH.[58,59]

$$Cu^{2+} + \alpha\text{-}\mathbf{T}OH \rightarrow Cu^{1+} + \alpha\text{-}\mathbf{T}O^{\bullet} + H^{+} \qquad (14)$$

The lack of any definite relationship between the (so-called) *lag phase* in copper ion-initiated oxidations and the tocopherol content of the lipoprotein[6] supports the notion that reaction 14 plays a dominant role in early peroxidation. Thus, since the rate of initiation will be *proportional* to the concentration of the major *antioxidant*, α-**T**OH (per reaction 14), sample to sample variation in the lag period in copper ion-induced LDL oxidation is not expected to correlate with the α-**T**OH /LDL ratio.[60]

The relative importance of the TMP ensuing from reaction 14 depends on how much copper is used. Large amounts of copper consume α-**T**OH so quickly that very little **L**OOH is produced in the inhibited period, whereas slower initiation (e.g., with less than two copper ions per LDL particle) leads to substantial peroxidation in the "inhibited period".[61] Under very mild oxidizing conditions (such as those produced by a common cell culture medium, F-10) over 50% of the PUFA lipid in native LDL may be oxidized *before* the tocopherol is consumed,[26] a clear illustration of the importance of TMP in vitro if not in vivo.

TMP and Atherosclerosis

According to the oxidized lipoprotein hypothesis the initiating event for atherosclerosis is a modification of LDL by oxidants in the artery wall. This "oxidatively modified LDL" is recognized and scavenged by macrophages, transforming them into lipid-laden "foam" cells that deposit as fatty streaks and eventually plaque on the artery wall.[62]

The role of α-**T**OH in this in vivo process is hard to define since the conditions and agents that modify LDL are unknown.[58] Our in vitro experiments have demonstrated that the lipid in LDL can be substantially oxidized *in the presence of* α-**T**OH. It is therefore tempting to ascribe an in vivo TMP process to the finding that in plaque containing normal amounts of vitamin E, "Approximately 30% of plaque Ch18:2 was oxidized...".[63] Unfortunately, however, this may be an oversimplification since the coexistence of oxidized lipid and antioxidant in plaque extracts may merely reflect the localized nature of peroxidation; i.e., peroxidation may occur in sites that are sequestered from replenishment of endogenous antioxidants such as ascorbate. Indeed, lipid peroxidation may even be a *result* of scavenger-cell ingestion rather than its primary cause; i.e., the LDL modification leading to its uptake by macrophages may have been due to a more protein-specific reaction (e.g., with neutrophil-derived chlorine/hypochlorite[64]), with lipid peroxidation occurring subsequently in the macrophage vacuole (possibly initiated by tyrosyl radicals[65] and sequestered from ascorbate).

Outlook

Careful in vitro kinetic studies revealed that in LDL the powerful antioxidant, α-**T**OH, can become a powerful prooxidant—particularly under mildly oxidizing conditions which consume α-**T**OH very slowly. It is reasonable to expect that the oxidizing conditions in vivo will be even milder than any applied in vitro. However, this does not necessarily mean that TMP must be important in vivo because vitamin C, ubiquinol, and many other natural and synthetic compounds can eliminate TMP—provided they have physical access to LDL/α-**T**O$^{\bullet}$ particles. The prime research challenge today is to determine the importance of TMP in vivo and the magnitude of its contribution to atherosclerosis. Until answers to these problems are forthcoming, we simply recommend an adequate daily intake of both vitamins E and C as being a risk-free, cheap, and reasonable way to avert a possible heart attack or stroke.

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