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VITAMIN E: ANTIOXIDANT ACTIVITY, BIOKINETICS, AND BIOAVAILABILITY

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INTRODUCTION

In recent years there has been a growing realization that harmful effects can result from free radical processes occurring in biological systems (4, 87).

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With this realization and with a better understanding of the function of vitamin E, there has been a renewed interest in α-tocopherol, the most potent biological (33, 36) and antioxidant form of the vitamin (16, 18, 20, 22). This interest is reflected, for example, in current studies that seek to determine whether or not vitamin E may reduce ischemia-reperfusion injury (77, 78, 104) or impede the formation of oxidized low density lipoproteins (LDL) and their postulated atherosclerotic effects (93).

To fully understand and to begin to make use of the potential of vitamin E requires not only an understanding of its antioxidant properties at the molecular level but also an understanding of the kinetics and mechanism of its distribution in organs and within tissues (i.e. biokinetics), together with a knowledge of which forms of the vitamin provide the most effective means of delivery (i.e. bioavailability).

ANTIOXIDANT ACTIVITY

Free Radicals and Oxygen: Lipid Peroxidation and Antioxidants

The free radicals of most significance in systems living in aerobic environments are oxygen (or oxy) radicals. These include superoxide anion $O_2^{\bullet -}$, its conjugate acid HOO• (the simplest form of peroxyl radical), lipid alkoxyl and peroxyl radicals (derived from polyunsaturated fatty acids), and the very reactive hydroxyl radical HO•. Although all of these radicals are involved in some aspect of free radical activity in vivo, the peroxyl radical has special significance because of its involvement in lipid peroxidation, the most common outward indicator of the operation of free radical processes in living systems.

Lipid peroxidation (or autoxidation) is a chain reaction that proceeds in three stages. In the initiation phase (Reaction I), carbon-centered lipid radicals, R•, are produced by the abstraction from, or addition to, a polyunsaturated fatty acid, RH, of an oxy radical generated elsewhere (e.g. by the decomposition of a hydroperoxide, ROOH).

Initiation: production of R• (carbon-centered radical). 1.

In the propagation phase (Reactions 2 and 3), the carbon-centered radical reacts rapidly with molecular oxygen to form a peroxyl radical (ROO•), a chain-carrying radical that is able to attack another polyunsaturated lipid molecule. Although the initial peroxyl radical is converted to a hydroperoxide (ROOH), this process produces a new carbon centered radical, which is rapidly converted by Reaction 2 into another peroxyl radical.

Propagation: $R\bullet + O_2 \rightarrow ROO\bullet$ (fast), 2.

$ROO\bullet + RH \rightarrow ROOH + R\bullet$ (H atom abstraction). 3.

The propagative process continues and can become a runaway process, consuming valuable polyunsaturated fat and producing a corresponding quantity of hydroperoxide (ROOH). The chain reaction does not stop until the chain-carrying peroxyl (ROO•) meets and combines with another peroxyl radical to form inactive products (Reaction 4).

Termination: $ROO\bullet + ROO\bullet \rightarrow$ inactive products. 4.

Ideally, this undesirable process can be prevented altogether by stopping the initial production of radicals. Compounds that do this are called preventive antioxidants (21). Catalase and glutathione peroxidase, for example, convert the free radical precursors hydrogen peroxide (H_2O_2) and fatty acid hydroperoxides (ROOH), respectively, into harmless products. Ceruloplasmin is another type of preventive antioxidant that inhibits transition metal ion-catalyzed production of radicals from hydroperoxides by sequestration of the metal ion.

In the event that peroxyl radicals (ROO•) do form, the action of a chain-breaking antioxidant is required to inhibit propagation. Typically, inhibitors are sterically hindered phenols, of which α-tocopherol is a special example (Figure 1). α-Tocopherol (α-TOH) short-circuits the destructive propagative cycle and can intercept the peroxyl radical (ROO•) more rapidly than can polyunsaturated fatty acids. The α-TOH donates its phenolic hydrogen atom to the radical and converts it to a hydroperoxide product (Reaction 5). The tocopheroxyl radical (α-TO•) that is formed is sufficiently stable to be unable to continue the chain and, instead, is removed from the cycle by reaction with another peroxyl radical to form inactive, nonradical products (Reaction 6):

Inhibition: $\alpha\text{-TOH} + ROO\bullet \rightarrow \alpha\text{-TO}\bullet + ROOH$, 5.

$\alpha\text{-TO}\bullet + ROO\bullet \rightarrow$ inactive products. 6.

The dynamic interplay of these factors is illustrated in Figure 2. Although polyunsaturated fat is a very vulnerable and the most probable target, it should be remembered that proteins, too, can and do undergo free radical peroxidative attack (37, 107).

The rate at which phenolic antioxidants react with peroxyl radicals (Reaction 5) is a direct measure of their antioxidant efficiency. It has been determined, after a comprehensive survey, that α-tocopherol is one of the most

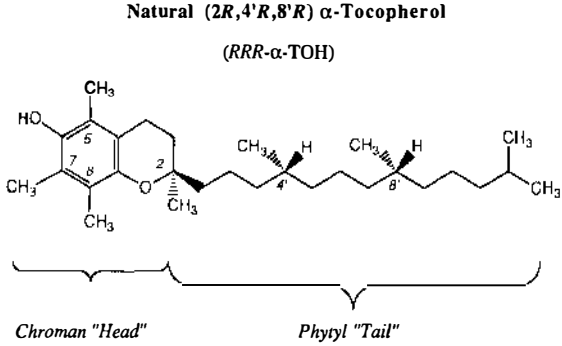

Figure 1 *RRR*-α-tocopherol showing the chroman ring and the stereochemistry of the phytyl tail.

efficient chain-breaking antioxidants available (16–18). For example, it reacts approximately 200 times faster with a peroxyl radical than does the commercial antioxidant, butylated hydroxytoluene (BHT) (16). Furthermore, the chroman head group (see Figure 1) is entirely responsible for the near-optimal antioxidant properties of α-tocopherol; the phytyl tail has been shown to have no influence on antioxidant activity (16, 18, 20, 22).

Because α-tocopherol can compete for peroxyl radicals much faster than can polyunsaturated fatty acids, a small amount of α-tocopherol is able to protect a large amount of polyunsaturated fat. Concentrations of α-tocopherol in biological membranes are approximately one part per 1000 lipid molecules (25). Of course, in protecting against peroxidation, vitamin E is consumed eventually. Therefore, unless it is regenerated via the tocopheroxyl radical by vitamin C, for example, which has been shown to be feasible in vitro (34, 83, 85) (however, see below), or by some other means (40, 86, 88), vitamin E will need to be replenished either directly through the diet or from reserves elsewhere.

The importance of vitamin E for protecting the integrity of lipid structures (especially membranes) in vivo is underscored by the finding that it is the only major lipid-soluble, chain-breaking antioxidant that has been found in plasma, red cells, and tissues (24, 25, 29, 30). This finding holds true even in the plasma of children with chronic, severe vitamin E deficiency (55). Although β-carotene has chain-breaking antioxidant activity also, it is less efficient than vitamin E and is expected to be important only in regions of very low oxygen partial pressure (19).

Demonstrations of the effectiveness of vitamin E in lessening the effects of lipid peroxidation in living systems are rare and difficult to obtain. However, Lemoyne et al (70, 71) have shown that pentane, a minor product released during the peroxidation of polyunsaturated fat, is reduced in the breath of

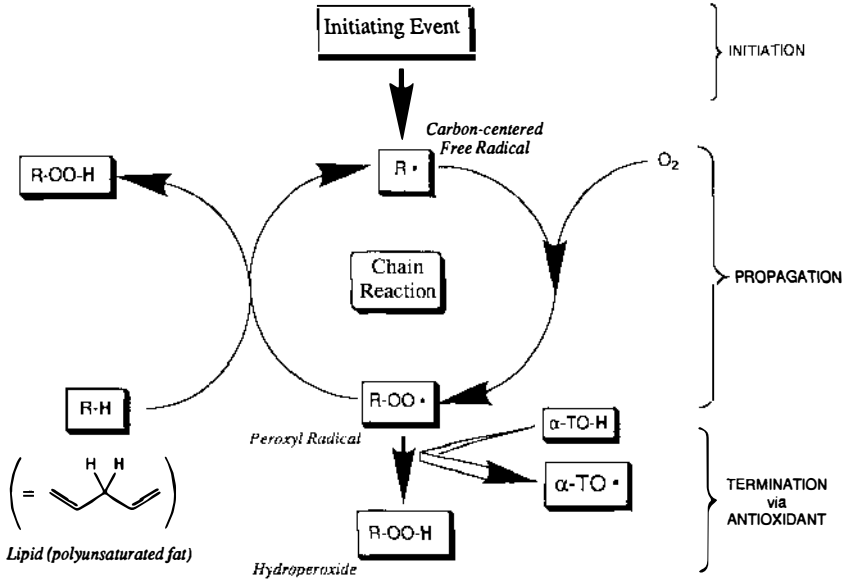

Figure 2 Scheme showing the three phases of the free radical chain mechanism of lipid peroxidation. The RH group contains the unsaturated unit common to all polyunsaturated acids.

humans supplemented with vitamin E. This strongly suggests that vitamin E prevents the peroxidation of polyunsaturated fat in vivo.

BIOKINETICS AND BIOAVAILABILITY

While the chroman group of vitamin E is responsible for the antioxidant activity of the molecule, the phytyl group largely determines the kinetics of transport to, and retention within, membranes. For example, addition of α-tocopherol in nonperoxidizable dimyristoyl phosphatidyl choline liposomes does not prevent the oxidation of either liposomes containing polyunsaturated fatty acids (soybean lecithin) or rabbit red blood cells (82, 84). By contrast, when the nonperoxidizable liposomes contain an analog of α-tocopherol, in which the phytyl group is replaced with a methyl group, the analog exchanges with the peroxidizable liposomes, or red blood cells, and prevents their oxidation.

Until quite recently, studies on the intestinal absorption, transport, and uptake into tissues of vitamin E in vivo have been done either using radiolabeled tocopherol (38, 39) or, less satisfactorily, using large doses of the unlabeled vitamin (5, 72, 103). Both of these methods have serious shortcomings. Radiolabeled vitamin E is not suitable for general use with

humans and, because of its tendency to decompose, must be purified immediately before use. The use of unlabeled vitamin E suffers from the disadvantages that, because large doses must be used in order to distinguish any change relative to background levels, the dose may not be physiologically relevant and, furthermore, it is usually necessary to use a relatively large number of subjects or animals so as to minimize the effects of individual variation.

New Methodology: Deuterated Vitamin E

In an effort to make biokinetic and bioavailability studies of vitamin E more convenient and accessible, our research groups have developed and are applying the technique of stable isotope labeling and gas chromatography-mass spectrometry (GC-MS) to the measurement of absorption, transport, uptake, and retention of tocopherols in humans and laboratory animals. In particular, we have taken advantage of the availability of γ and δ-tocopherols and have adapted existing methylation techniques for converting these forms into α-tocopherol to produce, on a laboratory scale, tens of grams of the natural stereoisomer 2*R*,4'*R*,8'*R*-α-tocopherol (*RRR*-α-TOH), containing three or six atoms of deuterium per molecule that are located in nonlabile, aromatic methyl positions (53, 54) (Figure 3). Deuterated tocopherols may be ingested safely because deuterium is a stable isotope and has no deleterious effects. Furthermore, the deuterated tocopherols do not undergo any measurable, metabolically mediated exchange at the positions of substitution (G. Burton, unpublished results).

The relative amounts of deuterated (d_3- and d_6-α-TOH) and nondeuterated α-tocopherol (d_0-α-TOH) present in plasma, red cell, and tissue lipid extracts are determined by silylating the tocopherol fraction purified by high-pressure liquid chromatography (HPLC) and by injecting the tocopheryl silyl ethers (see Figure 3) into a commercially available, bench-top type of gas chromatograph–mass spectrometer (53). As the α-tocopheryl silyl ether emerges from the gas chromatograph and enters the mass spectrometer, it is resolved simultaneously into its various component parent ion masses (Figure 4). The absolute concentration of each tocopherol is readily determined by relating the peak area of its parent ion to that of a d_9-α-TOH internal standard (Figure 3), added in known amount to the sample just prior to extraction.

SENSITIVITY AND LIMITATIONS Although it is possible to detect as little as 40 pg and we have successfully measured uptake into human heart tissue from biopsy samples as small as 1 mg (104), normal processing requires at least 100 ng (ca 0.2 nmol) of α-tocopherol in the final, concentrated 50-μl volume from which 1 μl is drawn for analysis by gas chromatography-mass spectrometry (GC-MS).

Sometimes, correction for naturally occurring isotopes can limit the reliability of deuterated tocopherol measurements and, therefore, the range of

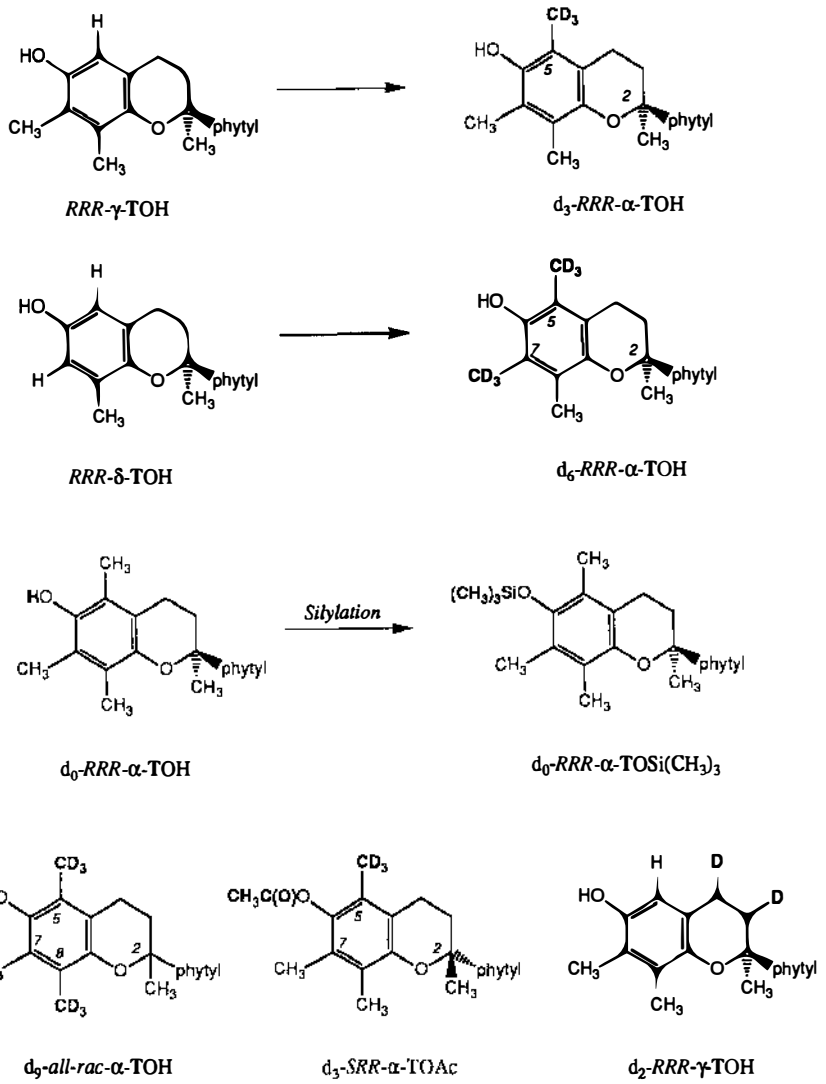

Figure 3 Scheme showing synthesis of d_3- and d_6-*RRR*-α-TOH from *RRR*-γ-TOH and *RRR*-δ-TOH, respectively; derivatization by silylation for GC-MS; d_9 internal standard; d_3-*SRR*-α-TOAc and d_2-*RRR*-γ-TOH used with d_6-*RRR*-α-TOAc in competitive uptake studies.

applicability of the technique. For example, when d_0-α-TOH and d_3-α-TOH are analyzed as their silyl ethers, the contribution to the d_3-α-TOSi$(CH_3)_3$ parent ion peak by natural abundance isotopes (^{13}C, ^{29}Si, ^{30}Si) present in d_0-α-TOSi$(CH_3)_3$ is 2.37% of the peak area of the unlabeled parent ion. Limiting situations most commonly arise when the amount of d_3-α-TOSi$(Ch_3)_3$ is low (<5%) compared to the amount of d_0-α-TOSi$(CH_3)_3$.

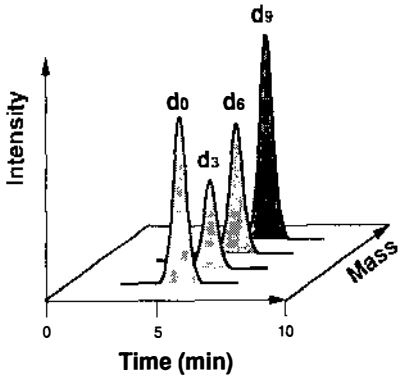

Figure 4 Depiction of the resolution by mass spectrometry of a mixture of deuterated tocopherols into their components after they emerge simultaneously from the gas chromatograph.

Under these circumstances, the contribution by natural abundance isotopes to the d_3 peak area from the d_0 silyl ether becomes comparable to the contribution to the peak area by the d_3 silyl ether itself. Therefore, when designing studies in which the amount of the trideuterated form is expected to be low compared to unlabeled α-tocopherol, it is better, if possible, to use d_6-α-tocopherol, because then no corrections are necessary. If this is not possible, another alternative is to analyze tocopherol as the free phenol instead of the silyl ether, because the absence of silicon substantially reduces the correction to the measured d_3-α-TOH peak from 2.37 to 0.63% of the d_0-α-TOH peak area. The drawback of this approach is that underivatized peaks tend to tail, which affects the accuracy of peak area integration; furthermore, in our experience repeated use of this approach seems to lead to a much more rapid decline of GC-MS performance.

Biokinetic Studies

LONG-TERM STUDIES The availability of multi-gram amounts of deuterated tocopherol has permitted us to conduct studies to measure the long-term uptake of vitamin E supplied continuously in the diet. In the first application of deuterated vitamin E, a group of weanling male rats, maintained for four weeks on a diet containing 36 mg kg^{-1} of the acetate of d_0-α-TOH (d_0-α-TOAc) as the only source of vitamin E, were switched to a diet in which the d_0-α-TOAc was replaced with d_3-α-TOAc at the same level (53). The relative amounts of d_0-α-TOH and d_3-α-TOH in blood and tissues were determined periodically by GC-MS over the following two months. Rates of turnover were estimated by interpolating from the data the time at which the amount of "new" d_0-α-TOH became equal to the "old" d_0-α-TOH remaining in each tissue (i.e. the equalization times, $t_{1:1}$). The equalization times showed a tenfold range in values, with turnover most rapid in plasma, red blood cells, spleen, and liver and slowest in spinal cord.

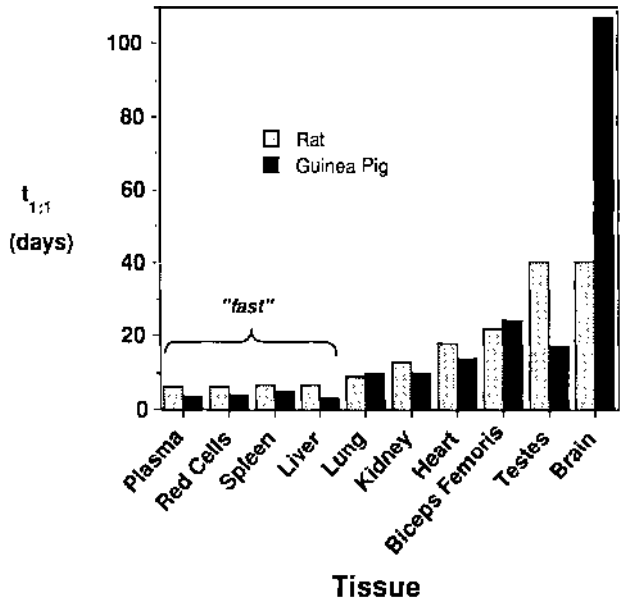

Figure 5 Comparison of times to reach equal amounts of deuterated and unlabeled α-tocopherols (equalization times, $t_{1:1}$) in tissues of rats and guinea pigs placed on a diet containing deuterated α-tocopherol as the only source of vitamin E.

Subsequently, we conducted a similar study with weanling male guinea pigs (26). In Figure 5, the $t_{1:1}$ values for the same tissues are compared for the two types of animals. Most tissues can be assigned to one or the other of two broad classes. Turnover is fast in plasma, red blood cells, liver, and spleen and is faster in the guinea pig than in the rat, but there is little difference between $t_{1:1}$ values within the same animal type. In the slower tissues, which include heart, testis, muscle (biceps femoris), brain, and spinal cord (not shown), turnover shows much more variability, both between and within animal types. In particular, turnover in guinea pig brain, which has the largest $t_{1:1}$ by far, is almost three times slower than in rat brain. Possibly, these animal-specific differences in slow tissue turnover reflect the underlying factor(s) responsible for the bewilderingly diverse range of symptoms associated with vitamin E deficiency.

The reluctance of neurological tissues (brain, cord, and nerve) to lose α-tocopherol compared to other tissues also has been demonstrated very clearly by Goss-Sampson et al (41), who compared the decline, over a period of 52 weeks, of α-tocopherol levels in weanling rats, fed a vitamin E–deficient diet, with the corresponding levels in control rats. The depletion of vitamin E was not accompanied by a compensating increase in the activity of

the superoxide dismutase and glutathione peroxidase antioxidant systems; tests of neurological function, conducted concurrently, indicated abnormalities after 40 weeks on the vitamin E–deficient diet.

Measuring turnover by continuous dietary feeding of deuterated α-tocopherol has recently shown that manipulation of the dietary level of vitamin C does not affect the net rate of loss, over a period of six weeks, of unlabeled vitamin E from the plasma, red blood cells, and selected tissues of young, male guinea pigs (26). This finding suggests that, in guinea pigs at least, vitamin C does not regenerate vitamin E to any significant extent under normal physiological conditions, despite its well-known ability to do so in vitro (34, 83, 85) (see above).

SINGLE DOSE STUDIES Bjørneboe et al. (6, 7, 9) have conducted a series of studies, using radiolabeled all-racemic *(all-rac)* α-tocopherol, which provide valuable information on intestinal absorption and tissue uptake in the rat. The first study (6) confirmed that α-tocopherol, when given in soybean oil, is primarily absorbed into the lymphatic pathway (15±9% of the dose) in the form of chylomicrons, and the bulk of the absorbed dose (> 92%) is taken up by the liver, skeletal muscle, and adipose tissue. When lymph containing labeled α-tocopherol was injected intravenously into rats, it was rapidly cleared from the plasma (ca 12 min) (7); after 24 h about 11% of the dose was found in the liver while about 14% of the radioactivity was recovered in the bile, of which only 13% was unchanged α-tocopherol. In another study, it was shown that long-term feeding of ethanol to rats reduced liver α-tocopherol by 25% (9). The appearance of α-tocopherol in rat bile after intravenous injection of lymph containing labeled α-tocopherol has been shown also by Lee-Kim et al (69). Again, it was found that unchanged α-tocopherol was a very small fraction of the total radioactivity in the bile, which would indicate the presence of a large amount of tocopherol metabolite(s). This study also showed that the enterohepatic circulation of α-tocopherol is negligible.

Most of the single dose studies using deuterated vitamin E have been done on humans. The findings of these studies will be described below in the section on lipoprotein studies.

Bioavailability Studies: The Competitive Uptake Method

A valuable feature of the deuterated vitamin E technique is the availability of α-tocopherols, which are substituted with different amounts of deuterium (e.g. d_3, d_6, etc.), and the ease with which these deuterated forms can be analyzed. This makes it possible to evaluate, within the same subject, the simultaneous, competitive uptake of two (or even three) different forms of

α-tocopherol labeled with different amounts of deuterium. That is, the subject acts as his or her own control.

ACETATE VERSUS FREE PHENOL The relative bioavailabilities of α-tocopherol and α-tocopheryl acetate are of interest because the free form occurs naturally in food, whereas the ester, which is much more air-stable than the phenol, is the form most commonly used in vitamin E supplements. Tocopheryl acetate is inactive, both biologically and as an antioxidant, and first must be hydrolyzed to the phenol in the gut before absorption of the vitamin can occur. Previously, the phenol had been reported to be only about half as potent as the acetate in the rat fetal gestation-resorption assay (46, 106). Because we found this to be a surprising result, we administered an equimolar mixture of d_6-*RRR*-α-TOH and d_3-*RRR*-α-TOAc to two vitamin E–deficient and two normal male rats under the same conditions that were used in the resorption bioassay (i.e. four consecutive daily oral doses in tocopherol-stripped corn oil), and we measured the relative amounts of deuterated *RRR*-α-TOH taken up from the two vitamin E forms by each rat (23). The phenol/"acetate" (d_6/d_3) ratios in the blood and the tissues after sacrifice on the fifth day were in good agreement with the bioassay result, and they were remarkably similar for all four animals (0.49 ± 0.05), whether or not they were vitamin E deficient, which indicates that vitamin E status is not important for determining relative absorption and transport of the two forms.

In contrast to the traditional fetal resorption assay technique (the vitamin is administered in tocopherol-stripped corn oil), when the deuterated tocopherol mixture was administered in an aqueous bolus of laboratory food to five rats, the mean d_6/d_3 ratio in the blood and tissues 24 h later was 1.06 ± 0.11. The relative bioavailability therefore shows a strong dependence upon the vehicle in which the vitamin E is delivered. These experiments suggest that the relative biopotency measured by the resorption assay would yield a value close to one if the compounds were administered in an aqueous bolus of food, rather than in oil.

When a single oral dose of an equimolar mixture of the same deuterated phenol-acetate pair (ca 50 mg of each) was given to five adult humans with an evening meal, the mean phenol/"acetate" ratio measured in the plasma and red cells over the ensuing two days was also very close to 1.0 (23). Slightly higher concentrations of the vitamin have been reported in the plasma of humans given large doses of unlabeled *RRR*-α-TOH, compared to subjects given *RRR*-α-TOAc (51). A similar finding has been reported for the plasma and tissues of sheep given the vitamin in their diet over a period of 28 days (50). Thus it appears that both the acetate and the phenol forms of vitamin E are equally well absorbed and transported into plasma.

STEREOISOMERS: *RRR*-VERSUS *SRR*-α-TOCOPHERYL ACETATE α-Tocopherol has three chiral centers in the phytyl tail, making eight stereoisomeric forms possible. Naturally occurring α-tocopherol exists only as the *RRR*-form (Figure 1), whereas the synthetic, all-racemic form (*all-rac*-α-TOH) is a mixture of approximately equal amounts of all eight stereoisomers. It is well known that natural *(RRR)* vitamin E is more potent, biologically, than the synthetic *(all-rac)* form (33), as determined by the fetal resorption assay (105, 106) or the plasma pyruvate kinase assay (73). Furthermore, it has been shown that all seven unnatural stereoisomers have lower activities than the *RRR*-form in the resorption bioassay (105). The simplest explanation for this behavior is that the differences in the stereochemistry of the phytyl tail influence the bioavailabilities of these compounds.

We have begun to explore this hypothesis by means of the competitive uptake method, using suitably deuterated stereoisomers. However, preparing and analyzing differently deuterated forms of the eight stereoisomers is a formidable synthetic and analytical challenge. Instead, we have used deuterated froms of *RRR*- and 2*S*,4'*R*,8'*R*-α-tocopherols (i.e. d_6-*RRR*-α-TOAc and d_3-*SRR*-α-TOAc, respectively), stereoisomers that differ only at the 2-position where the chroman ring and the phytyl tail are joined (see Figures 1 and 3) (53). Thus, competitive uptake studies with this pair of compounds provide direct evidence of the importance of the stereochemistry of the ring-tail junction. There is evidence that the 2-position is the major determinant of differences between α-tocopherol stereoisomers: Bioassays (73, 105, 106) have shown that 2-*ambo*- (an approximately 1:1 mixture of *RRR*- and *SRR*-α-tocopherols) and *all-rac*-α-tocopheryl acetates have very similar biopotencies.

The study of the competitive uptake of d_6-*RRR*-α-TOAc and d_3-*SRR*-α-TOAc in male rats was conducted using long-term dietary feeding of the deuterated tocopherols, as described above (53). The sole dietary source of vitamin E was a 1:1 mixture of d_6-*RRR*-αTOAc and d_3-*SRR*-α-TOAc (18 mg of each per kilogram of diet), and the relative amounts of d_3- and d_6-α-TOH appearing in plasma, red cells, and tissues were determined over a five-month period. Initially, the liver contained twice as much d_3-*SRR*-α-TOH as d_6-*RRR*-α-TOH, but over the following three weeks the difference in the amounts of the two forms decreased and eventually there was a slight excess of d_6-*RRR*-α-TOH. In contrast, plasma, red cells, and all other organs showed an immediate preference for the d_6-*RRR*-α-TOH. The d_6-*RRR*-α-TOH/d_3-*SRR*-α-TOH ratio increased with time in all of the tissues until reaching, within about two months, a plateau (except brain and spinal cord). The rate of increase and the final ratio of the two stereoisomers depended on the specific tissue concerned. The final ratios of some of the tissues and fluids examined were 2.7 (plasma), 1.2 (liver), 2.6 (lung), 1.7 (biceps femoris), 1.8 (kidney),

3.6 (red blood cells), 1.9 (heart), 1.6 (inguinal white adipose tissue), 1.4 (skin), and 2.0 (testis).

Of special interest are the ratios for brain and spinal cord, which showed no sign of leveling off after five months and reached values of 5.3 and 3.7, respectively. The strong contrast at early time-points between the liver (*RRR/SRR* <1) and the other tissues (*RRR/SRR* > 1) suggests that in the transit from the gut to the tissues, the liver is a major point of discrimination between these two tocopherols. This aspect is discussed in more detail below in the next section.

The tissue specificity and time dependence of the relative bioavailabilities of the *RRR*- and *SRR*-α-tocopheryl acetates strongly suggest that a single number, traditionally determined by the rat fetal gestation-resorption assay, cannot always express adequately the relative potencies of different forms of tocopherol. *SRR*-α-tocopheryl acetate itself has been determined to be 0.31 times as potent as *RRR*-α-tocopheryl acetate (i.e. *RRR/SRR* = 3.2) in the fetal resorption assay (105). Whether or not this result will correlate with an *RRR/SRR* bioavailability ratio measured by competitive uptake into rat uterine, fetal, or some other, presently unidentified, critical tissue has yet to be determined.

The competitive uptake of *SRR*- and *RRR*-α-tocopheryl acetates has been developed into a tool for probing the details of absorption and secretion of vitamin E in vivo and will be useful in determining nutritional bioavailability standards of various forms of vitamin E. Examples of the application of this tool in human studies are provided and discussed in the next section.

Lipoprotein Studies: Human Aspects

Vitamin E is transported in plasma only in the lipoproteins, and its concentration is related to the concentration of plasma lipids (52). Although plasma carrier proteins have been described for other fat-soluble vitamins, such as the retinol-binding protein (60), no plasma carrier proteins have been discovered for vitamin E. Even though vitamin E is present in all of the lipoprotein fractions, it is the low and high density lipoproteins (LDL and HDL, respectively) that are the major vehicles for transport in human plasma (3, 11, 43).

Lipoproteins have a hydrophilic surface of a cholesterol/phospholipid monolayer, studded with apolipoproteins (apos), and a core of neutral lipid (predominantly composed of triglycerides and cholesteryl esters), and they are characterized by size (flotation densities), apo distribution, and electrophoretic mobility (47). Chylomicrons, synthesized by the intestine for transport of dietary fats to the liver, are very large, light particles that have only 2% protein and a triglyceride-rich core. Newly synthesized chylomicrons contain apo AI (28 kd), apo AII (17 kd), apo A-IV (46 kd), and apo B-48 (48% of the molecular weight of apo B-100). Very low density lipoproteins,

VLDL, are triglyceride-rich particles secreted by the liver and contain apo B-100 (512 kd), apo CI (5.8 kd), apo CII (9.1 kd), apo C-III (8.8 kd), and apo E (35 kd).

Apo Bs are required for secretion of triglyceride-rich lipoproteins. Patients with a genetic deficiency in apo B illustrate the requirement for this protein in the assembly of triglyceride-rich lipoproteins because these people do not secrete chylomicrons or VLDL (65). Apo B-48 is required for the secretion of chylomicrons by the intestine while apo B-100 is required for the secretion of VLDL by the liver. These requirements were demonstrated in a patient who secreted chylomicrons, but not VLDL (75).

The remnants of VLDL catabolism are LDL, which contain apo B-100 as the only apolipoprotein. Both apo B-100 and apo E contain specific regions recognized by the LDL receptor (74). During the lipolysis of chylomicrons and VLDL by lipoprotein lipase in the circulation, apos A, C, and E undergo exchange with high density lipoprotein, HDL. HDL contain 40% protein, as apo A-I, A-II, C, and E, and have a cholesteryl ester-rich core.

There have been relatively few investigations of how vitamin E is delivered to tissues. Various studies have documented that vitamin E can exchange between lipoproteins and red blood cells (RBC), between plasma and RBC, and between artificial liposomes and lipoproteins or RBC (10, 31, 76). α-Tocopherol, which exchanges more slowly than cholesterol but much more rapidly than triglyceride, does not require a transfer protein for exchange to occur (42).

One route by which at least some tissues probably receive significant amounts of vitamin E is via the LDL receptor mechanism. The LDL receptor recognizes apo B on plasma LDL and is expressed on the surface of cells requiring cholesterol (14). Studies in vitro have demonstrated that, following incubation with LDL, the tocopherol content of cells with LDL receptors is increased compared with receptor-negative cells (97). Presumably, tissues that take up LDL in vivo by the LDL receptor will also take up vitamin E, along with the other lipids in the LDL particle (97). This mechanism would simultaneously deliver to cells peroxidizable lipids, such as linoleic acid, in the form of cholesteryl esters, and the antioxidant, vitamin E. Tissues with relatively high levels of LDL receptors, such as the adrenal gland, may obtain most of their tocopherol in this way.

Another mechanism available for vitamin E uptake involves lipoprotein lipase, which is present in the circulation on the endothelial lining of capillary walls and hydrolyzes triglycerides in chylomicrons and VLDL to free fatty acids and monoglycerides (81). In vitro, this enzyme functions as a transfer protein for vitamin E during lipolysis of triglyceride-rich lipoproteins and emulsions (100). Addition of lipoprotein lipase to chylomirons, or to a triglyceride emulsion containing vitamin E, transfers both α- and γ-

tocopherols to cells in culture. The lipase must bind to the cell surface in order for this transfer to occur. Patients with familial hyperlipoproteinemia (Type I), who have elevated plasma triglycerides (> 1000 mg dl^{-1} compared to 100 mg dl^{-1} in normal subjects) because of a lack of lipoprotein lipase, also have elevated levels of vitamin E in their chylomicron and VLDL fractions (62, 100). It is likely that tissues with high levels of lipoprotein lipase activity, such as adipose tissue and muscle, might transfer significant amounts of vitamin E by this mechanism. The brain also has been reported to have lipoprotein lipase activity (35), which might serve to mediate some vitamin E transfer into this organ.

The first documented cases of vitamin E deficiency in humans were in patients with abetalipoproteinemia, who lack apo B-containing lipoproteins (61, 64). These patients develop severe neurologic abnormalities during the first two decades of life because of vitamin E deficiency that results from their inability to transport sufficient amounts of the dietary vitamin. Pharmacological supplements of vitamin E (100 mg per kg body weight per day) can prevent the neurologic disease from occurring in infants and can prevent further deterioration in adults (12, 13, 79). Tocopherol measurements of the peripheral nerves of patients with abetalipoproteinemia and vitamin E deficiency have demonstrated that depletion of tocopherol precedes histologic damage and that nerve tocopherol levels are correlated with adipose tissue tocopherol (102). Thus, adipose tissue levels can be used to assess the adequacy of vitamin E supplementation in those patients in whom plasma levels of vitamin E remain at one tenth of normal. Such studies have demonstrated that adequate amounts of vitamin E can be delivered to the tissues of patients with abetalipoproteinemia (63) and that HDL, which are the only lipoproteins present in the plasma of these patients, can transport some tocopherol to tissues if sufficient amounts of vitamin E are consumed.

Ironically, elevated levels of plasma lipid can mask inadequate vitamin E levels. This is especially true in patients with cholestatic liver disease. These patients can have such high lipid levels that the concentration of plasma vitamin E can lie within the normal range, yet the vitamin E/lipid ratio falls into the deficient range (89, 91). Children with this disease develop severe neurologic abnormalities that are probably due to inadequate delivery of tocopherol to the affected tissues. Studies in obese and hyperlipidemic rats have demonstrated that transfer of tocopherol to tissues is impaired in hyperlipidemic animals, whether or not they are obese. That is, the amount of tocopherol in the tissues is not as high as in the tissues of normolipidemic animals with similar levels of plasma tocopherol (28).

BIOKINETICS OF DEUTERATED *RRR*-α-TOCOPHEROL IN HUMAN LIPOPROTEINS In the first application of deuterated vitamin E to the study of the

transport of tocopherol in human lipoproteins, 50–150 mg of d_n-*RRR*-α-TOAc (n = 3 or 6) were consumed with a meal, and the concentrations of d_n-*RRR*-α-TOH and d_0-*RRR*-α-TOH were determined in plasma samples obtained at intervals for up to 26 h afterward (96). The deuterated tocopherol, expressed as a percentage of the total tocopherol, was highest at the early time points in the chylomicron fractions, which indicates that, like other dietary lipids, the deuterated vitamin E was absorbed from the intestinal lumen and secreted in chylomicrons. During the period of chylomicron catabolism and lipoprotein remodeling, the deuterated tocopherol also appeared in the other lipoprotein fractions and thus demonstrated the rapid exchange between lipoproteins in vivo.

Chylomicron remnants are rapidly sequestered by the liver where they are hydrolyzed by hepatic triglyceride lipase and are internalized via the apo E-mediated mechanism (48, 49, 74). The free fatty acids released from the chylomicron remnants are re-esterified to triglycerides by the liver, and these triglycerides are secreted in newly assembled VLDL. Studies carried out in intact rats (32), as well as in isolated rat hepatocytes (8, 32), have demonstrated that tocopherol is secreted from hepatocytes in nascent VLDL. Our study also showed that, following its appearance in chylomicrons, the VLDL become enriched in deuterated tocopherol and then, during the subsequent catabolism of VLDL, the LDL and HDL also become enriched. The latter fractions ultimately bear the largest proportion of the deuterated tocopherol.

Deuterated vitamin E has been used in a similar way to investigate the etiology of a very rare disease (9 patients have been reported worldwide), familial isolated vitamin E deficiency (101). Patients with this disease have extremely low levels of plasma tocopherol when consuming a normal diet and eventually develop neurological abnormalities characteristic of vitamin E deficiency, yet they do not have lipid malabsorption or any other disease known to cause vitamin E deficiency (15, 45, 66–68, 92, 108). Administration of supplemental vitamin E (800–1200 IU day^{-1}) restores the plasma levels to normal and reverses, or at least prevents, further progression of the neurologic disease.

When 4 patients and 6 controls were each given d_6-*RRR*-α-TOAc (ca 15 mg) with a meal, both groups had equal plasma concentrations of d_6-*RRR*-α-TOH during the first 12 h; however, by 24 h the level had become significantly lower in the patients (101). Isolation of the plasma lipoproteins demonstrated that the chylomicrons from the two groups contained essentially equal concentrations of d_6-*RRR*-α-TOH at all time points, but by 24 h the levels in the VLDL of the patients had become significantly lower than in controls. Figure 6, which shows representative data from one patient and one control subject, illustrates the speed with which the tocopherol disappeared from the VLDL fraction in the patient. Furthermore, the d_6-*RRR*-α-TOH was found to

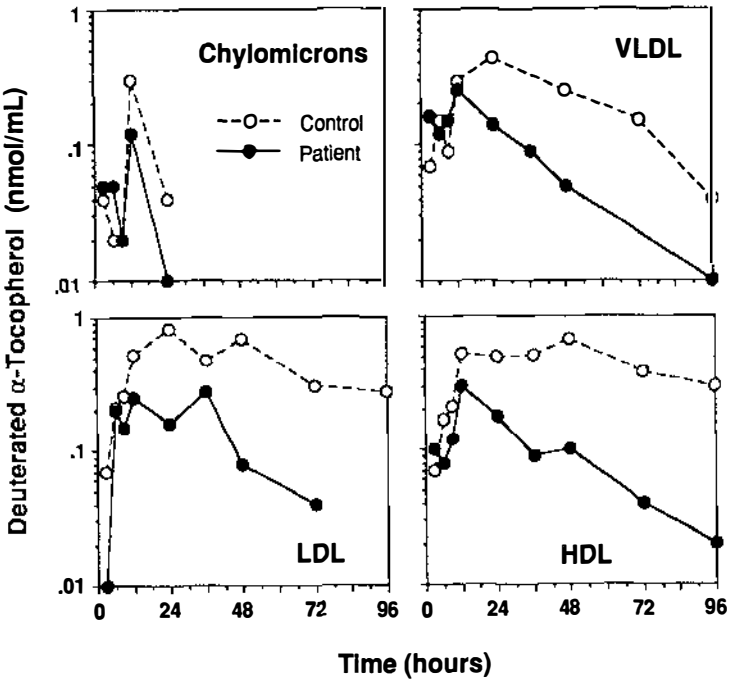

Figure 6 Concentrations of d_6-*RRR*-α-TOH in lipoprotein fractions (nmol ml^{-1} plasma) after ingestion of a single dose of 15 mg of d_6-*RRR*-α-TOAc by a patient with familial isolated vitamin E deficiency and by a control subject (101).

decrease at a significantly faster rate in the plasma and the isolated lipoproteins (except chylomicrons) of the patients compared to the control subjects. On the basis of these results, it has been suggested (101) that the patients lack, or have a defective liver tocopherol transfer protein (see below), and hence the secretion of α-tocopherol in nascent VLDL is impaired. For this reason, these patients may be unable to maintain normal concentrations of plasma vitamin E.

BIOAVAILABILITY STUDIES USING COMPETITIVE UPTAKE In order to gain a better understanding of the mechanisms of absorption and lipoprotein transport, a competitive bioavailability technique has been developed and used to compare the behavior of tocopherols in normal subjects with their behavior in patients who have defined metabolic abnormalities of lipid metabolism. That is, each subject is given a dose of a 1:1 mixture of two tocopherols, and the relative uptake and transport of the two forms in the normal subject and in the patient is monitored.

γ-Tocopherol versus α-tocopherol It has been observed that after the administration of supplemental vitamin E to humans, the increase in α-tocopherol in plasma is accompanied by a decrease in γ-tocopherol (1, 44). This finding has been exploited to develop a competitive bioavailability technique using large doses of a mixture of unlabeled α- and γ-tocopherols to probe the mechanism of tocopherol uptake. The results indicate that the liver exerts a strong preference for secretion of α-tocopherol into the plasma (98).

Following the consumption by normal humans of a mixture of *all-rac*-α-TOH (1 g) and *RRR*-γ-TOH (1 g) with a meal, both tocopherols were found to increase similarly in the plasma up to 12 h, but by 24 h γ-TOH had decreased precipitously while α-TOH remained almost unchanged. Patients with lipoprotein lipase deficiency, in whom chylomicrons are catabolized slowly, also showed similar increases of both tocopherols over the first 12 h but, in contrast to the controls, both tocopherols remained elevated at 24 h. However, a patient with dysbetalipoproteinemia (type III hyperlipoproteinemia), in whom VLDL are catabolized slowly owing to an abnormal apolipoprotein E phenotype, exhibited an uptake pattern similar to that of normal subjects. Thus, both tocopherols are absorbed and secreted from the intestine in chylomicrons, but subsequent to chylomicron catabolism α-tocopherol becomes preferentially incorporated into the VLDL secreted from the liver.

SRR-*versus* RRR-*α-tocopheryl acetate* Recently, we have explored, in a similar way, the use of much smaller doses of a mixture of deuterated *RRR*- and *SRR*-α-tocopheryl acetates. Our earlier study of the long-term relative uptake of these two tocopherols in rats showed significant discrimination in favor of the natural *RRR* form (53), as has already been discussed.

When humans were given a single dose of a 1:1 mixture of d_3-*SRR*-α-TOAc and d_6-*RRR*-α-TOAc (50–75 mg of each), both tocopherols were secreted in approximately equal amounts in the chylomicrons, but by 9 h the VLDL, LDL, and HDL fractions had become preferentially enriched in d_6-*RRR*-α-TOH, as the semi-log plots from a representative subject show in Figure 7 (95). In all of the four subjects studied, the slopes of the approximately first-order, semi-log plots of the decline of the deuterated/total tocopherol concentration ratio during post-absorption (up to 72 h) were at least twice as fast for d_3-*SRR*-α-TOH as for d_6-*RRR*-α-TOH in the VLDL, LDL, and HDL fractions. The finding that chylomicrons contained similar amounts of d_3-*SRR*-α-TOH and d_6-*RRR*-α-TOH while VLDL were preferentially enriched in d_6-*RRR*-α-TOH is consistent with the α- vs γ-tocopherol competitive uptake results. These findings point to a mechanism that discriminates in favor of the natural α-tocopherol during secretion from the liver but that does not exist or operate during absorption from the intestine.

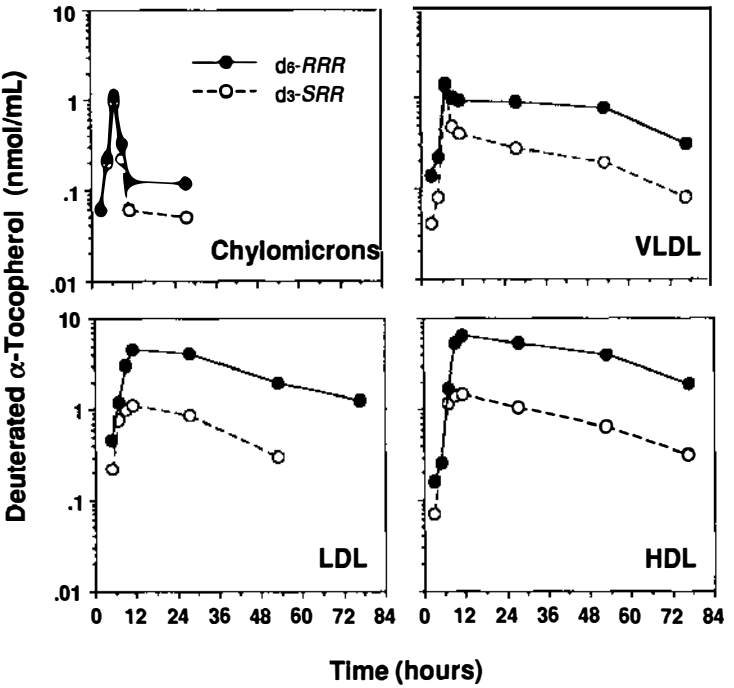

Figure 7 Concentrations of d_3-*SRR*-α-TOH and d_6-*RRR*-α-TOH in lipoprotein fractions (nmol ml^{-1} plasma) after ingestion of a single dose of a mixture of 75 mg each of d_3-*SRR*-α-TOAc and d_6-*RRR*-α-TOAc by a normal human (95).

One difficulty in establishing the hypothesis that the liver secretes nascent VLDL enriched in natural α-tocopherol has been that the VLDL isolated by ultracentrifugation at $d < 1.006$ g/ml contain equal concentrations of d_3-*SRR*-α-TOH and d_6-*RRR*-α-TOH during the first 6–9 h; furthermore, the concentrations of d_6-*RRR*-α-TOH in the VLDL isolated from patients with familial isolated vitamin E deficiency during the initial 9 h of the study are equal to those of the control subjects. We have suggested that these findings might be due to our inability to separate VLDL from the chylomicron remnants, which contain newly absorbed tocopherols. Alternatively, if tocopherols, along with other surface components, are transferred to HDL during chylomicron catabolism, then HDL might transfer excess tocopherols back to circulating VLDL [HDL have been shown to readily transfer tocopherol to VLDL in vitro (76)]. Although both explanations are plausible, we have sought a direct test of the hypothesis that the liver secretes nascent VLDL preferentially enriched in natural α-tocopherol.

A primate liver perfusion system for studying nascent lipoprotein secretion by the liver has been devised by Johnson et al (57). Monkeys are fed diets

with 40% fat, either as polyunsaturated or saturated fat, and with or without added cholesterol to modify lipoprotein production. At sacrifice the livers are removed, rinsed, then perfused for 90 min to remove any adherent plasma lipoproteins. The perfusate solution is then replaced and fresh perfusate is recirculated through the liver for 4 h. The lipoproteins isolated from the 4-h perfusate include VLDL, discoidal HDL, and d > 1.006 apolipoprotein B-containing lipoproteins that are deficient in core lipids and have redundant surface components (56–59).

When monkeys were fed a single dose of a 1:1:1 mixture of d_3-*SRR*-α-TOAc, d_6-*RRR*-α-TOAc, and d_2-*RRR*-γ-TOH (see Figure 3), it was found that the nascent VLDL secreted by the liver, as isolated from the perfusate, were preferentially enriched in d_6-*RRR*-α-TOH (94). In a companion study (94), the appearance of the deuterated tocopherols in the plasma lipoprotein fractions was followed in monkeys that were dosed in an identical manner. The results were essentially the same as those obtained earlier in the human studies using the α/γ and *RRR/SRR* pairs of tocopherols. Thus, the chylomicron fraction was found to contain equal amounts of all three tocopherols during the first 9 h, as did the plasma and the other lipoprotein fractions isolated at earlier time points (3–6 h). By 24 h the plasma obtained from all five of the monkeys studied was preferentially enriched in d_6-*RRR*-α-TOH.

In summary, several lines of evidence, when taken together, strongly implicate the liver and rule out the intestine as being responsible for the progressive, preferential enrichment of *RRR*-α-TOH in human, monkey, and rat plasma and rat tissues (except liver). (*a*) Rats given α-tocopherol supplements added to soybean oil absorb both the supplemental α-tocopherol and the γ-tocopherol present in the oil, and even the presence of a 50-fold excess of α-tocopherol has no effect on the amount of γ-tocopherol secreted into chylomicrons (99). (*b*) In rats given a single dose of a 1:1 mixture of d_3-*SRR*-α-TOAc and d_6-*RRR*-α-TOAc (or d_3-*SRR*-α-TOH and d_6-*RRR*-α-TOH), only 22–26% more d_6-*RRR*-α-TOH than d_3-*SRR*-α-TOH was collected in the thoracic duct lymph, whereas in noncannulated rats the plasma showed a progressive increase of the d_6-*RRR*- over the d_3-*SRR*-form (H. Zahalka, G. Burton, and K. Ingold, unpublished results). When rats were fed continuously a diet containing a 1:1 mixture of d_3-*SRR*-α-TOAc and d_6-*RRR*-α-TOAc, the liver, unlike any other tissue, showed a preferential accumulation of d_3-*SRR*-α-TOH over d_6-*RRR*-α-TOH during the first 2–3 weeks of the study (53). (*c*) When patients with lipoprotein lipase deficiency were given a 1:1 mixture of α- and γ-tocopherols, the concentration of both tocopherols increased similarly in the triglyceride-rich lipoprotein fractions during the first 24 h (98). (*d*) Chylomicrons, as well as other lipoprotein fractions and plasma, isolated from normal human subjects during the first 6–9 h following the oral administration of a 1:1 mixture of d_3-*SRR*-α-TOAc and d_6-*RRR*-α-

TOAc contained equal amounts of both tocopherols (95). (*e*) Essentially the same result was obtained with monkeys fed a 1:1:1 mixture of d_3-*SRR*-α-TOAc, d_6-*RRR*-α-TOAc, and d_2-*RRR*-γ-TOH (94).

A tocopherol-binding protein has been found in rat liver (2, 27), but not in intestine (27), and has been reported to transfer tocopherol between liposomes and mitochondria (80). This protein may play a role in the discrimination shown by the liver (53). The function of the tocopherol-binding protein may be to transfer *RRR*-α-tocopherol, during the assembly of VLDL, from lysosomes containing chylomicron remnants to the golgi containing the nascent lipoprotein particles (101). It is tempting to suggest that the decline of *SRR*-α-tocopherol in normal subjects (Figure 6) and the decline of *RRR*-α-tocopherol in the patient with the familial isolated vitamin E deficiency (Figure 7) are similar because the patients are lacking or are deficient in the same protein that we suggest is responsible for the preferential incorporation of *RRR*-α-TOH in VLDL and the maintenance of normal plasma tocopherol levels.

CONCLUSION

α-Tocopherol is a superb chain-breaking antioxidant that appears to be the only major lipid-soluble antioxidant of its kind in mammalian membranes and lipoproteins. It is absorbed rather inefficiently from the gut into the lymphatic system and is transported from the intestine in chylomicrons. During chylomicron catabolism by lipoprotein lipase, vitamin E can be transferred to other lipoproteins and to the tissues. Tocopherol reaches the liver by uptake of chylomicron remnants. It is secreted from the liver into the plasma in VLDL, the metabolism of which effects transfer to LDL and HDL, as well as to tissues. The liver shows a strong preference for secretion of natural *RRR*-α-tocopherol.

In this review, we have shown that deuterated tocopherols provide an effective means for elucidating various aspects of vitamin E transport, especially when use is made of the competitive uptake method. These techniques will be especially important in determining vitamin E requirements in the future.

Studies of the biokinetics and bioavailability of vitamin E have shown that vitamin E turnover is slowest in neural and brain tissues. It is therefore interesting to note that vitamin E plays a very important role in maintaining neurological function in man (90). The mechanism by which vitamin E performs this function, however, and the connection to its antioxidant activity remain to be determined. Further research will help us better understand the role of vitamin E in the maintenance of health and normal nerve function and

should increase our appreciation of its ability to minimize destructive processes associated with degenerative diseases.

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Literature Cited

1. Baker, H., Handelman, G. J., Short, S., Machlin, L. J., Bhagavan, H. N., et al. 1986. Comparison of plasma α- and γ-tocopherol levels following chronic oral administration of either all-rac-α-tocopheryl acetate or RRR-α-tocopheryl acetate in normal adult male subjects. *Am. J. Clin. Nutr.* 43:382–87
2. Behrens, W. A., Madere, R. 1982. Transfer of α-tocopherol to microsomes mediated by a partially purified liver α-tocopherol binding protein. *Nutr. Res.* 2:611–18
3. Behrens, W. A., Thompson, J. N., Madere, R. 1982. Distribution of alpha tocopherol in human plasma lipoproteins. *Am. J. Clin. Nutr.* 35:691–96
4. Belch, J. J. F., Chopra, M., Hutchison, S., Lorimer, R., Sturrock, R. D., et al. 1989. Free radical pathology in chronic arterial disease. *Free Radical Biol. Med.* 6:375–78
5. Bieri, J. G. 1972. Kinetics of tissue alpha tocopherol depletion and repletion. *Ann. NY Acad. Sci.* 203:181–91
6. Bjørneboe, A., Bjørneboe, G.-E. A., Bodd, E., Hagen, B. F., Kveseth, N., Drevon, C. A. 1986. Transport and distribution of α-tocopherol in lymph, serum and liver cells in rats. *Biochim. Biophys. Acta* 889:310–15
7. Bjørneboe, A., Bjørneboe, G.-E. A., Drevon, C. A. 1987. Serum half-life, distribution, hepatic uptake and biliary excretion of α-tocopherol in rats. *Biochim. Biophys. Acta* 921:175–81
8. Bjørneboe, A., Bjørneboe, G.-E. A., Hagen, B. F., Nossen, J. O., Drevon, C. A. 1987. Secretion of α-tocopherol from cultured rat hepatocytes. *Biochim. Biophys. Acta* 922:199–205
9. Bjørneboe, G.-E. A., Bjørneboe, A., Hagen, B. F., Mørland, J., Drevon, C. A. 1987. Reduced hepatic α-tocopherol after long-term administration of ethanol to rats. *Biochim. Biophys. Acta* 918: 236–41
10. Bjornson, L. K., Gniewkowski, C., Kayden, H. J. 1975. A comparison of the exchange of α-tocopherol and of free cholesterol between rat plasma lipoproteins and erythrocytes. *J. Lipid Res.* 16:39–53
11. Bjornson, L. K., Kayden, H. J., Miller, E., Moshell, A. N. 1976. The transport of alpha tocopherol and beta carotene in human blood. *J. Lipid Res.* 17:343–51
12. Brin, M. F., Nelson, J. S., Roberts, W. C., Marquardt, M. D., Suswankosai, P., Petito, C. K. 1983. Neuropathology of abetalipoproteinemia: A possible complication of the tocopherol (vitamin E)-deficient state. *Neurology* 33(Suppl. 2):142–43
13. Brin, M. F., Pedley, T. A., Emerson, R. G., Lovelace, R. E., Gouras, P., et al. 1986. Electrophysiological features of abetalipoproteinemia: functional consequences of vitamin E deficiency. *Neurology* 36:669–73
14. Brown, M. S., Goldstein, J. L. 1986. A receptor-mediated pathway for cholesterol homeostasis. *Science* 232:34–47
15. Burck, U., Goebel, H. H., Kuhlendahl, H. D., Meier, C., Goebel, K. M. 1981. Neuromyopathy and vitamin E deficiency in man. *Neuropediatrics* 12:267–78
16. Burton, G. W., Doba, T., Gabe, E. J., Hughes, L., Lee, F. L., et al. 1985. Autoxidation of biological molecules. 4. Maximizing the antioxidant activity of phenols. *J. Am. Chem. Soc.* 107:7053–65
17. Burton, G. W., Hughes, L., Ingold, K. U. 1983. Antioxidant activity of phenols related to vitamin E. Are there chain-

breaking antioxidants better than α-tocopherol? *J. Am. Chem. Soc.* 105: 5950–51
18. Burton, G. W., Ingold, K. U. 1981. Autoxidation of biological molecules. I. The antioxidant activity of vitamin E and related chain-breaking phenolic antioxidants in vitro. *J. Am. Chem. Soc.* 103:6472–77
19. Burton, G. W., Ingold, K. U. 1984. β-Carotene: an unusual type of lipid antioxidant. *Science* 224:569–73
20. Burton, G. W., Ingold, K. U. 1986. Vitamin E: applications of the principles of physical organic chemistry to the exploration of its structure and function. *Acc. Chem. Res.* 19:194–201
21. Burton, G. W., Ingold, K. U. 1989. Mechanisms of antioxidant action: preventive and chain-breaking antioxidants. In *Handbook of Free Radicals and Antioxidants in Biomedicine*, ed. J. Miquel, A. T. Quintanilha, H. Weber, pp. 29–43. Boca Raton, Fla: CRC Press
22. Burton, G. W., Ingold, K. U. 1989. Vitamin E as an in vitro and in vivo antioxidant. *Ann. NY Acad. Sci.* 570:7–22
23. Burton, G. W., Ingold, K. U., Foster, D. O., Cheng, S. C., Webb, A., et al. 1988. Comparison of free α-tocopherol and α-tocopheryl acetate as sources of vitamin E in rats and humans. *Lipids* 23:834–40
24. Burton, G. W., Joyce, A., Ingold, K. U. 1982. First proof that vitamin E is major lipid-soluble, chain-breaking antioxidant in human blood plasma. *Lancet* 2:327
25. Burton, G. W., Joyce, A., Ingold, K. U. 1983. Is vitamin E the only lipid-soluble, chain-breaking antioxidant in human blood plasma and erythrocyte membranes? *Arch. Biochem. Biophys.* 221:281–90
26. Burton, G. W., Wronska, U., Stone, L., Foster, D. O., Ingold, K. U. 1990. Biokinetics of dietary RRR-α-tocopherol in the male guinea pig at three dietary levels of vitamin C and two levels of vitamin E. Evidence that vitamin C does not "spare" vitamin E in vivo. *Lipids*. 25:In press
27. Catignani, G. L., Bieri, J. G. 1977. Rat liver α-tocopherol binding protein. *Biochim. Biophys. Acta* 497:349–57
28. Catignani, G. L., Fuller, P. A. 1982. Tissue α-tocopherol levels in normal, obese, and hyperlipidemic rats. *Ann. NY Acad. Sci.* 393:167–68
29. Cheeseman, K. H., Burton, G. W., Ingold, K. U., Slater, T. F. 1984. Lipid peroxidation and lipid antioxidants in normal and tumor cells. *Toxicol. Pathol.* 12:235–39
30. Cheeseman, K. H., Emery, S., Maddix, S. P., Slater, T. F., Burton, G. W., Ingold, K. U. 1988. Studies on lipid peroxidation in normal and tumour tissues. *Biochem. J.* 250:247–52
31. Cheng, S. C., Burton, G. W., Ingold, K. U., Foster, D. O. 1987. Chiral discrimination in the exchange of α-tocopherol stereoisomers between plasma and red blood cells. *Lipids* 22:469–73
32. Cohn, W., Loechleiter, F., Weber, F. 1988. α-Tocopherol is secreted from rat liver in very low density lipoproteins. *J. Lipid Res.* 29:1359–66
33. Diplock, A. T. 1985. Vitamin E. In *Fat-Soluble Vitamins*, ed. A. T. Diplock, pp. 154–224. Lancaster, Pa: Technomic Publ.
34. Doba, T., Burton, G. W., Ingold, K. U. 1985. Antioxidant and co-antioxidant effect of vitamin C. The effect of vitamin C, either alone or in the presence of vitamin E or a water-soluble vitamin E analog, upon the peroxidation of aqueous multilamellar phospholipid liposomes. *Biochim. Biophys. Acta* 835: 298–303
35. Eckel, R. H., Robbins, R. J. 1984. Lipoprotein lipase is produced, regulated and functional in rat brain. *Proc. Natl. Acad. Sci. USA* 81:7604–7
36. Farrell, P. M. 1980. Deficiency states, pharmacological effects, and nutrient requirements. In *Vitamin E: A Comprehensive Treatise*, ed. L. J. Machlin, pp. 520–620. New York: Dekker
37. Fraga, C. G., Zamora, R., Tappel, A. L. 1989. Damage to protein synthesis concurrent with lipid peroxidation in rat liver slices: effect of halogenated compounds, peroxides and vitamin E. *Arch. Biochem. Biophys.* 270:84–91
38. Gallo-Torres, H. E. 1980. Absorption. See Ref. 36, pp. 170–92
39. Gallo-Torres, H. E. 1980. Transport and metabolism. See Ref. 36, pp. 193–267
40. Gibson, D. D., Hawrylko, J., McCay, P. B. 1985. GSH-dependent inhibition of lipid peroxidation: properties of a potent cytosolic system which protects cell membranes. *Lipids* 20:704–11
41. Goss-Sampson, M. A., MacEvilly, C. J., Muller, D. P. R. 1988. Longitudinal studies of the neurobiology of vitamin E and other antioxidant systems, and neurological function in the vitamin E deficient rat. *J. Neurol. Sci.* 87:25–35
42. Granot, E., Tamir, I., Deckelbaum, R. J. 1988. Neutral lipid transfer protein does not regulate α-tocopherol transfer

between human plasma lipoproteins. *Lipids* 23:17–21
43. Haga, P., Ek, J., Kran, S. 1982. Plasma tocopherol levels and vitamin E/beta lipoprotein relationships during pregnancy and in cord blood. *Am. J. Clin. Nutr.* 36:1200–4
44. Handelman, G. J., Machlin, L. J., Fitch, K., Weiter, J. J., Dratz, E. A. 1985. Oral α-tocopherol supplements decrease plasma γ-tocopherol levels in humans. *J. Nutr.* 115:807–13
45. Harding, A. E., Matthews, S., Jones, S., Ellis, C. J. K., Booth, I. W., Muller, D. P. R. 1985. Spinocerebellar degeneration associated with a selective defect of vitamin E absorption. *New Engl. J. Med.* 313:32–35
46. Harris, P. L., Ludwig, M. I. 1949. Vitamin E potency of α-tocopherol and α-tocopherol esters. *J. Biol. Chem.* 180: 611–15
47. Havel, R. J. 1987. Origin, metabolic fate, and function of plasma lipoproteins. In *Hypercholesterolemia and Atherosclerosis, Pathogenesis and Prevention*, ed. D. Steinberg, J. M. Olefsky, pp. 117–41. New York: Livingston
48. Havel, R. J., Hamilton, R. L. 1988. Hepatocytic lipoprotein receptors and intracellular lipoprotein catabolism. *Hepatology* 8:1689–1704
49. Herz, J., Hamann, U., Rogne, S., Myklebost, O., Guasepohl, H., Stanley, K. K. 1988. Surface location and high affinity for calcium of a 500-kd liver membrane protein closely related to the LDL-receptor suggest a physiological role as lipoprotein receptor. *EMBO J.* 7:4119–27
50. Hidiroglou, N., McDowell, L. R., Pastrana, R. 1988. Bioavailability of various vitamin E compounds in sheep. *Int. J. Vitam. Nutr. Res.* 58:189–97
51. Horwitt, M. K., Elliott, W. H., Kanjananggulpan, P., Fitch, C. D. 1984. Serum concentrations of α-tocopherol after ingestion of various vitamin E preparations. *Am. J. Clin. Nutr.* 40:240–45
52. Horwitt, M. K., Harvey, C. C., Dahm, C. H., Searcy, M. T. 1972. Relationship between tocopherol and serum lipid levels for determination of nutritional adequacy. *Ann. NY Acad. Sci.* 203:223–36
53. Ingold, K. U., Burton, G. W., Foster, D. O., Hughes, L., Lindsay, D. A., Webb, A. 1987. Biokinetics of and discrimination between dietary RRR- and SRR-α-tocopherols in the male rat. *Lipids* 22:163–72
54. Ingold, K. U., Hughes, L., Slaby, M., Burton, G. W. 1987. Synthesis of 2R,4'R,8'R-α-tocopherols selectively labelled with deuterium. *J. Label. Compd. Radiopharm.* 24:817–31
55. Ingold, K. U., Webb, A. C., Witter, D., Burton, G. W., Metcalfe, T. A., Muller, D. P. R. 1987. Vitamin E remains the major lipid-soluble, chain-breaking antioxidant in human plasma even in individuals suffering severe vitamin E deficiency. *Arch. Biochem. Biophys.* 259:224–25
56. Johnson, F. L., Babiak, J., Rudel, L. L. 1986. High density lipoprotein accumulation in perfusates of isolated livers of African green monkeys. Effects of saturated versus polyunsaturated dietary fat. *J. Lipid Res.* 27:537–48
57. Johnson, F. L., St. Clair, R. W., Rudel, L. L. 1983. Studies on the production of low density lipoproteins by perfused livers from nonhuman primates. *J. Clin. Invest.* 72:221–36
58. Johnson, F. L., St. Clair, R., W., Rudel, L. L. 1985. Effects of the degree of saturation of dietary fat on the hepatic production of lipoproteins in the African green monkey. *J. Lipid Res.* 26:403–17
59. Johnson, F. L., Swift, L. L., Rudel, L. L. 1987. Nascent lipoproteins from recirculating and nonrecirculating liver perfusions and from the hepatic Golgi apparatus of African green monkeys. *J. Lipid Res.* 28:549–64
60. Kanai, M., Raz, A., Goodman, D. S. 1968. Retinol-binding protein and the regulation of vitamin A transport. *J. Clin. Invest.* 47:2025–44
61. Kayden, H. J. 1967. Vitamin E deficiency in patients with abetalipoproteinemia. In *Vitamine A,E und K. Klinische und physiologisch-chemische probleme*, ed. H. F. von Kress, K. U. Blum, pp. 301–8. Freien Univ. Berlin: F. K. Schattauer Verlag
62. Kayden, H. J. 1983. Tocopherol content of adipose tissue from vitamin E deficient humans. In *Biology of Vitamin E*, ed. R. Porter, J. Whelan, pp. 70–91. London: Ciba Found. Symp.
63. Kayden, H. J., Hatam, L. J., Traber, M. G. 1983. The measurement of nanograms of tocopherol from needle aspiration biopsies of adipose tissue: normal and abetalipoproteinemic subjects. *J. Lipid Res.* 24:652–56
64. Kayden, H. J., Silber, R. 1965. The role of vitamin E deficiency in the abnormal autohemolysis of acanthocytosis. *Trans. Assoc. Am. Physicians* 78:334–41
65. Kayden, H. J., Traber, M. G. 1986. Clinical, nutritional and biochemical consequences of apolipoprotein B deficiency. In *Lipoprotein Deficiency Syn-*

dromes, ed. A. Angel, J. Frohlich, pp. 67–81. New York: Plenum
66. Kohlschutter, A., Hubner, C., Jansen, W., Lindner, S. G. 1988. A treatable familial neuromyopathy with vitamin E deficiency, normal absorption, and evidence of increased consumption of vitamin E. *J. Inherit. Metab. Dis.* 11:149–52
67. Krendel, D. A., Gilchrest, J. M., Johnson, A. O., Bossen, E. H. 1987. Isolated deficiency of vitamin E with progressive neurologic deterioration. *Neurology* 37:538–40
68. Laplante, P., Vanasse, M., Michaud, J., Geoffroy, G., Brochu, P. 1984. A progressive neurological syndrome associated with an isolated vitamin E deficiency. *Can. J. Neurol. Sci.* 11:561–64
69. Lee-Kim, Y. C., Meydani, M., Kassarjian, Z., Blumberg, J. B., Russell, R. M. 1988. Enterohepatic circulation of newly administered α-tocopherol in the rat. *Int. J. Vitam. Nutr. Res.* 58:284–91
70. Lemoyne, M., Van Gossum, A., Kurian, R., Jeejeebhoy, K. N. 1988. Plasma vitamin E and selenium and breath pentane in home parenteral nutrition patients. *Am. J. Clin. Nutr.* 48:1310–15
71. Lemoyne, M., Van Gossum, A., Kurian, R., Ostro, M., Axler, J., Jeejeebhoy, K. N. 1987. Breath pentane analysis as an index of lipid peroxidation: a functional test of vitamin E status. *Am. J. Clin. Nutr.* 46:267–72
72. Machlin, L. J., Gabriel, E. 1982. Kinetics of tissue alpha-tocopherol uptake and depletion following administration of high levels of vitamin E. *Ann. NY Acad. Sci.* 393:48–60
73. Machlin, L. J., Gabriel, E., Brin, M. 1982. Biopotency of α-tocopherols as determined by curative myopathy bioassay in the rat. *J. Nutr.* 112:1437–40
74. Mahley, R. W. 1988. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science* 240:622–30
75. Malloy, M. J., Kane, J. P., Hardman, D. A., Hamilton, R. L., Dalal, K. B. 1981. Normotriglyceridemic abetalipoproteinemia; absence of the B-100 apolipoprotein. *J. Clin. Invest.* 67:1441–50
76. Massey, J. B. 1984. Kinetics of transfer of α-tocopherol between model and native plasma lipoproteins. *Biochim. Biophys. Acta* 793:387–92
77. Massey, K. D., Burton, K. P. 1989. α-Tocopherol attenuates myocardial membrane-related alterations resulting from ischemia and reperfusion. *Am. J. Physiol.* 256:H1192–99
78. Mickle, D. A. G., Lee, R.-K., Weisel, R. D., Birnbaum, P. L., Wu, T.-W., et al. 1989. Myocardial salvage with Trolox and ascorbic acid for an acute evolving infarction. *Ann. Thorac. Surg.* 47:553–57
79. Muller, D. P. R., Lloyd, J. K. 1982. Effect of large oral doses of vitamin E on the neurological sequelae of patients with abetalipoproteinemia. *Ann. NY Acad. Sci.* 393:133–44
80. Murphy, D. J., Mavis, R. D. 1981. Membrane transfer of α-tocopherol. *J. Biol. Chem.* 256:10464–68
81. Nelsson-Ehle, P., Garfinkel, A. S., Schotz, M. C. 1980. Lipolytic enzymes and plasma lipoprotein metabolism. *Annu. Rev. Biochem.* 49:667–93
82. Niki, E., Kawakami, A., Saito, M., Yamamoto, Y., Tsuchiya, Y., Kamiya, Y. 1985. Effect of phytyl side chain of vitamin E on its antioxidant activity. *J. Biol. Chem.* 260:2191–96
83. Niki, E., Kawakami, A., Yamamoto, Y., Kamiya, Y. 1985. Oxidation of lipids. VIII. Synergistic inhibition of oxidation of phosphatidylcholine liposome in aqueous dispersion by vitamin E and vitamin C. *Bull. Chem. Soc. Jpn.* 58:1971–75
84. Niki, E., Komuro, E., Takahashi, M., Urano, S., Ito, E., Terao, K. 1988. Oxidative hemolysis of erythrocytes and its inhibition by free radical scavengers. *J. Biol. Chem.* 263:19809–14
85. Packer, J. E., Slater, T. F., Willson, R. L. 1979. Direct observation of a free radical interaction between vitamin E and vitamin C. *Nature* 278:737–38
86. Packer, L., Maguire, J. J., Mehlhorn, R. J., Serbinova, E., Kagan, V. E. 1989. Mitochondria and microsomal membranes have a free radical reductase activity that prevents chromanoxyl radical accumulation. *Biochem. Biophys. Res. Commun.* 159:229–35
87. Poli, G., Biasi, F., Chiarpotto, E., Dianzani, M. U., De Luca, A., Esterbauer, H. 1989. Lipid peroxidation in human diseases: evidence of red cell oxidative stress after circulatory shock. *Free Radical Biol. Med.* 6:167–70
88. Scholich, H., Murphy, M. E., Sies, H. 1989. Antioxidant activity of dihydrolipoate against microsomal lipid peroxidation and its dependence on α-tocopherol. *Biochim. Biophys. Acta* 1001:256–61
89. Sokol, R. J. 1988. Vitamin E deficiency and neurologic disease. *Annu. Rev. Nutr.* 8:351–73
90. Sokol, R. J. 1989. Vitamin E and

neurologic function in man. *Free Radical Biol. Med.* 6:189–207

91. Sokol, R. J., Heubi, J., Iannaccone, S. T., Bove, K. E., Balistreri, W. F. 1984. Vitamin E deficiency with normal serum vitamin E concentrations in children with chronic cholestasis. *New Engl. J. Med.* 310:1209–12
92. Sokol, R. J., Kayden, H. J., Bettis, D. B., Traber, M. G., Neville, H., et al. 1988. Isolated vitamin E deficiency in the absence of fat malabsorption—familial and sporadic cases: Characterization and investigation of causes. *J. Lab. Clin. Med.* 111:548–59
93. Steinberg, D., Parthasarathy, S., Carew, T. E., Khoo, J. C., Witztum, J. L. 1989. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. *New Engl. J. Med.* 320:915–24
94. Traber, M., Rudel, L., Burton, G., Hughes, L., Ingold, K., Kayden, H. 1990. Nascent VLDL from liver perfusions of cynomologous monkeys are preferentially enriched in RRR- compared with SRR-α tocopherol. Studies using deuterated tocopherols. *J. Lipid Res.* 31:In press
95. Traber, M. G., Burton, G. W., Ingold, K. U., Kayden, H. J. 1990. Both *RRR*- and *SRR*-α-tocopherols are secreted without discrimination in human chylomicrons, but *RRR*-α-tocopherol is preferentially secreted in very low density lipoproteins. *J. Lipid Res.* 31:In press
96. Traber, M. G., Ingold, K. U., Burton, G. W., Kayden, H. J. 1988. Absorption and transport of deuterium-substituted 2R,4'R,8'R-α-tocopherol in human lipoproteins. *Lipids* 23:791–97
97. Traber, M. G., Kayden, H. J. 1984. Vitamin E is delivered to cells via the high affinity receptor for low density lipoprotein. *Am. J. Clin. Nutr.* 40:747–51
98. Traber, M. G., Kayden, H. J. 1989. Preferential incorporation of α-tocopherol vs γ-tocopherol in human lipoproteins. *Am. J. Clin. Nutr.* 49:517–26
99. Traber, M. G., Kayden, H. J., Green, J. B., Green, M. H. 1986. Absorption of water miscible forms of vitamin E in a patient with cholestasis and in rats. *Am. J. Clin. Nutr.* 44:914–23
100. Traber, M. G., Olivecrona, T., Kayden, H. J. 1985. Bovine milk lipoprotein lipase transfers tocopherol to human fibroblasts during triglyceride hydrolysis in vitro. *J. Clin. Invest.* 75:1729–34
101. Traber, M. G., Sokol, R. J., Burton, G. W., Ingold, K. U., Papas, A. M., et al. 1990. Impaired ability of patients with familial isolated vitamin E deficiency to incorporate α-tocopherol into lipoproteins secreted by the liver. *J. Clin. Invest.* 85:397–407
102. Traber, M. G., Sokol, R. J., Ringel, S. P., Neville, H. E., Thellman, C. A., Kayden, H. J. 1987. Lack of tocopherol in peripheral nerves of vitamin E-deficient patients with peripheral neuropathy. *New Engl. J. Med.* 317:262–65
103. Vatassery, G. T., Brin, M. F., Fahn, S., Kayden, H. J., Traber, M. G. 1988. The effect of high doses of dietary vitamin E upon the concentrations of vitamin E in several brain regions, plasma, liver and adipose tissue of rats. *J. Neurochem.* 51:621–23
104. Weisel, R. D., Mickle, D. A. G., Ingold, K. U., Burton, G. W. 1989. Prevention of free radical injury during coronary revascularization. *FASEB J.*3:A940
105. Weiser, H., Vecchi, M. 1982. Stereoisomers of α-tocopheryl acetate. II. Biopotencies of all eight stereoisomers, individually or in mixtures, as determined by rat resorption-gestation tests. *Int. J. Vitam. Nutr. Res.* 52:351–70
106. Weiser, H., Vecchi, M., Schlachter, M. 1986. Stereoisomers of α-tocopheryl acetate. *Int. J. Vitam. Nutr. Res.* 56:45–56
107. Yamamoto, Y., Niki, E., Eguchi, J., Kamiya, Y., Shimasaki, H. 1985. Oxidation of biological membranes and its inhibition. Free radical chain oxidation of erythrocyte ghost membranes by oxygen. *Biochim. Biophys. Acta* 819: 29–36
108. Yokota, T., Wada, Y., Furukawa, T., Tsukagoshi, H., Uchihara, T., Watabiki, S. 1987. Adult-onset spinocerebellar syndrome with idiopathic vitamin E deficiency. *Ann. Neurol.* 22:84–87