Journal of Parenteral and Enteral Nutrition
Volume XX Number X
Month 201X 1–10

DOI: 10.1177/0148607114547537
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# Distribution of Tocopherols and Tocotrienols in Guinea Pig Tissues Following Parenteral Lipid Emulsion Infusion

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## Abstract

*Background:* Tocopherols and tocotrienols possess vitamin E activity and function as the major lipid-soluble antioxidants in the human body. Commercial lipid emulsions are composed of different oils and supply different amounts of vitamin E. The objective of this study was to measure all 8 vitamin E homologs within 4 different commercial lipid emulsions and evaluate their distribution in guinea pig tissues. *Materials and Methods*: The distribution of vitamin E homologs within plasma and guinea pig tissues was determined using a high-performance liquid chromatography (HPLC) system. Lipid hydroperoxides in lipid emulsions were determined using a commercial kit (Cayman Chemical Company, Ann Arbor, MI), and malondialdehyde tissue levels were determined using an HPLC system. *Results*: The lipid emulsions contained variable amounts of tocopherols, which were significantly different between emulsions. Tocotrienols were present at very low concentrations (≤0.3%). We found no correlation between the amount of vitamin E present in the lipid emulsions and lipid peroxidation. Hydroperoxides were the lowest with an olive oil–based emulsion and highest with a fish oil emulsion. The predominant vitamin E homolog in guinea pig tissues was α-tocopherol. No tissues had detectable levels of tocotrienols. Vitamin E levels (primarily α-tocopherol and γ-tocopherol) were highly variable among organ tissues. Plasma levels were a poor reflection of most tissue levels. *Conclusion*: Vitamin E levels within different lipid emulsions and plasma/tissues are highly variable, and no one tissue or plasma sample serves as a good proxy for levels in other tissues. All study emulsions were well tolerated and did not significantly increase systemic lipid peroxidation. (*JPEN J Parenter Enteral Nutr*. XXXX;xx:xx-xx)

## Keywords

tocopherol; tocotrienol; vitamin E; lipid emulsions; lipid peroxidation; tissues

## Clinical Relevancy Statement

Parental lipid emulsions are a source of calories and essential fatty acids for acute and chronically ill patients. These emulsions, in addition to fatty acids, also contain variable amounts of vitamin E homologs. The present study investigated the extent of lipid oxidation in different lipid emulsions and the distribution of vitamin E homologs in lipid emulsions and guinea pig tissues after infusion. The findings of this study will provide clinicians with levels of vitamin E in different lipid emulsions, information on peroxidation within lipid emulsions, and information on tissue levels of vitamin E and systemic peroxidation following infusion of lipid emulsions.

## Introduction

*Vitamin E* is the generic term for a family of tocopherol and tocotrienol homologs.[1-3] In nature, 8 substances have been found to possess vitamin E activity. These substances include α, β, γ, and δ tocopherols and α, β, γ, and δ tocotrienols. All of these compounds feature a chromanol ring with a hydroxyl group that can donate a hydrogen atom to reduce free radicals and a hydrophobic side chain that allows for penetration of the compounds into biological membranes. Tocotrienols differ from tocopherols by the presence of 3 double bonds on the hydrophobic side chain. Due to their lipid solubility, vitamin E homologs co-locate within cellular lipid compartments. Vitamin E homologs act as the primary antioxidants for circulating lipids and lipid organelles such as cell surface membranes,

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Financial disclosure: This study was supported by a grant from Baxter Healthcare Corporation, Deerfield, Illinois.

Conflict of interest: Dr Zaloga and Mr Pavlina are or were employees of Baxter Healthcare. None of the other authors declare any conflict of interest.

Received for publication May 23, 2014; accepted for publication July 24, 2014.

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endoplasmic reticulum, and mitochondrial membranes. Thus, vitamin E functions as the primary chain-breaking lipid-soluble antioxidant in the body, protecting polyunsaturated fatty acids (PUFAs) from free radical oxidative damage.[4] The content of α-tocopherol in animal tissues has previously been reported.[5-9] However, the content of all tocopherols and tocotrienols in various tissues has not been determined. Thus, a major objective of this study was to quantify and compare tissue contents of the 8 vitamin E homologs across different tissues in animals receiving a nutritionally complete oral diet or a fat-free oral diet supplemented with intravenous (IV) lipid emulsion and to determine if one tissue (particularly plasma, red blood cell [RBC], or adipose tissue) could serve as a proxy for levels in other tissues.

Various lipid emulsions are marketed for use with parenteral nutrition (PN). These emulsions are based on different oil sources that include soybean oil, olive oil, medium-chain triglycerides (from palm or coconut oils), and fish oil. Lipid-rich plant sources and vegetable oils are the main natural sources of vitamin E. However, levels of the various vitamin E homologs differ substantially between the oils.[10,11] The content of the various vitamin E homologs in commercial lipid emulsions and the relationship between vitamin E content and lipid peroxidation of the emulsions have not been determined and are another major objective of this study. In addition to vitamin E protecting the lipid emulsions from peroxidative damage, lipid emulsions also supply vitamin E for tissue utilization. Intake of high concentrations of PUFAs are known to increase requirements for vitamin E and may contribute to production of lipid peroxides (especially in patients with underlying oxidative stress).[12] It is unclear whether the quantities of vitamin E within commercial lipid emulsions provide a significant contribution to vitamin E tissue content and are sufficient to limit production of lipid peroxides in vivo following lipid infusion. In this study, we also sought to determine the effects of infusion of fatty acids in the form of triglycerides from different lipid emulsions upon tissue vitamin E levels. We were particularly interested in determining whether tissue vitamin E content (ie, distribution, homolog quantities) was diminished during infusion of triglycerides with high PUFA content.

This is the first study to evaluate the content of all vitamin E homologs in commercial lipid emulsions and tissues and to evaluate the effects of IV infusion of triglycerides on tissue levels of all vitamin E homologs. This study was performed using a guinea pig model, since multiple-organ analysis of vitamin E homologs cannot be performed safely in humans. We selected the guinea pig as an animal model because the lipoprotein profile of guinea pigs, as opposed to other rodents, more closely resembles that of the human in terms of the low-density lipoprotein cholesterol and high-density lipoprotein cholesterol constituents.[13] Furthermore, guinea pigs share with humans several other characteristics of lipid metabolism.[14-18]

## Materials and Methods

### *Lipid Emulsions*

Four commercial lipid emulsions were chosen for infusion studies, based on their different oil (ie, fatty acid) compositions. We chose a predominant ω-6 PUFA emulsion based on soybean oil (SO; Intralipid, Fresenius Kabi, Bad Homburg, Germany), a predominant ω-9 monounsaturated fatty acid emulsion based on olive oil (OO; ClinOleic, Baxter Healthcare, Deerfield, IL), an ω-3 PUFA predominant emulsion based on fish oil (FO; Omegaven, Fresenius Kabi), and a mixture of soybean, olive, fish, and medium-chain triglyceride (MCT) oils (MIX; SMOFlipid, Fresenius Kabi). The FO emulsion was supplied as a 10% emulsion (10 g/100 mL), while the other emulsions were supplied as 20% (20 g/100 mL) emulsions.

### *Animals*

Hartley guinea pigs, with their jugular vein catheterized, were purchased from Charles River Laboratories International (Wilmington, MA). All guinea pigs were housed under controlled temperature and humidity in the Methodist Research Institute Animal Care Facility, with a 12-hour light-dark cycle. Animals were provided with food and water ad libitum. The protocol for these studies was approved (Protocol 2010-17) by the Methodist Research Institute's Animal Research Committee (Animal Welfare Assurance No. A3772-010) and strictly followed the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health publication No. 85-23, revised 1996).

### *Infusion Studies*

For lipid emulsion infusion studies, guinea pigs were placed on the study diet (Table 1; 30% proteins, 53% carbohydrates, and 15% fat; Research Diets, New Brunswick, NJ) for a week prior to treatment with the lipid emulsions. Average consumption of the animals was 20 g of diet per day. After a week on the study diet, guinea pigs scheduled to receive the lipid infusions were fed a fat-free diet (Table 1; 36% protein, 64% carbohydrate, and 0% fat; Research Diets) and treated with lipid emulsion infusions or saline (5/group) for 10 days. The fat-free diet was identical in nutrient content to the study diet except that it contained no lipid (lipid was given intravenously). The study diet and fat-free diet used well-defined protein, carbohydrate, lipid, and vitamin sources. The animals were administered, via the jugular vein, 5 mL of lipid emulsion or saline over a 1-hour period using syringe pumps daily for 10 consecutive days. Thus, animals were infused with 1 g of lipid (20% emulsions; approximately 2.5 g/kg/d) or 0.5 g of lipid (10% emulsions; ~1.25 g/kg/d). At the end of the 10-day study period, animals were fasted overnight. Blood was then collected (5 mL) and animals were euthanized. Organs (liver, lungs, kidneys, heart,

**Table 1.** Composition of Guinea Pig Diets.

| Characteristic | Study Diet | | Fat-Free Diet | |
|---|---|---|---|---|
| | g | kcal (%) | g | kcal (%) |
| Proteins | 265 | 1060 (30) | 265 | 1060 (36) |
| Carbohydrates | 470 | 1880 (53) | 470 | 1880 (64) |
| Fat | 60 | 540 (15) | 0 | 0 |
| Ingredients | | | | |
| Casein, 80 mesh | 260 | 1040 | 260 | 1040 |
| L-methionine | 5 | 20 | 5 | 20 |
| Corn starch | 250 | 1000 | 250 | 1000 |
| Maltodextrin 10 | 35 | 140 | 35 | 140 |
| Sucrose | 185 | 740 | 185 | 740 |
| Cellulose BW200 | 110 | 0 | 170 | 0 |
| Soybean oil | 25 | 225 | 0 | 0 |
| Olive oil | 35 | 315 | 0 | 0 |
| Mineral Mix S20001 | 82 | 0 | 82 | 0 |
| Vitamin Mix V20001 | 11 | 44 | 11 | 44 |
| Choline bitartrate | 2 | 0 | 2 | 0 |
| Vitamin E, μg/g[a] | | | | |
| α-Tocopherol | 61.9 ± 2.0 | | 64.6 ± 5.6 | |
| β-Tocopherol | 0.2 ± 0.0 | | ND | |
| γ-Tocopherol | 19.8 ± 0.5 | | 0.02 ± 0.0 | |
| δ-Tocopherol | 6.6 ± 0.0 | | 0.2 ± 0.0 | |
| **Total** | **1000** | **3524** | **1000** | **2984** |

[a]Tocopherol concentrations (μg/g diet) expressed as mean ± SD. Tocotrienols were not detected (ND).

and epididymal adipose tissues) were harvested and snap-frozen in liquid nitrogen for biochemical analysis. Plasma and RBCs were obtained from the blood by centrifugation. All tissue samples were stored at –80°C until analyzed.

## *Tissue Processing*

Guinea pig tissues (liver, heart, lungs, kidneys, RBCs, and plasma) were removed from –80°C and allowed to thaw on ice. A small sample (~200 mg) of the tissue was transferred to a tube (100 × 16 mm) and placed on ice. Cold phosphate-buffered saline (PBS; 2 mL) was added to the tube, and the tissues were homogenized for 30 seconds with an Omni homogenizer (Omni International, Kennesaw, GA) equipped with disposable Omni-Tips. Guinea pig tissue homogenates or plasma containing all *rac*-α-Tocol, an internal standard, were extracted using 800 μL of methanol and 2 mL of hexane containing 0.05% 2,6-di-tert-butyl-4-methylphenol (BHT). These samples were vortexed and centrifuged at 1400 *g* for 20 min, and the nonaqueous layer was analyzed using high-performance liquid chromatography (HPLC). For vitamin E homolog analysis in adipose tissues, the tissue homogenization method was modified. Adipose tissue (50 mg), α-Tocol (50 μg/mL in hexane), 800 μL of methanol, 80 μL of distilled water, and 1 mL of hexane (0.01% BHT) were combined with ~250 mg of stainless steel beads (blend of 0.9–2.0 mm diameter; Next Advance, Averill Park, NY) and for homogenization by placing into a Bullet Blender (Next Advance) for 20 minutes at maximum speed. Samples were vortexed and centrifuged at 15,000 *g* for 20 minutes to separate phases. The hexane layer was collected and dried, and the residual material was reconstituted in 200 μL of hexane for HPLC analysis.

## *Vitamin E Measurement*

Vitamin E content of the tissues was determined by using methods that we previously established.[19] Stocks of a standard mixture containing all 8 vitamin E homologs (200 μg/mL for each compound) were diluted in hexane to generate standard curves for the analysis of the vitamin E homologs. The standard dilutions also contained all *rac*-α-Tocol (internal standard) at 5 μg/mL or 1 μg/mL. A standard curve was generated (triplicate runs) for high (0.781–50 μg/mL) and low (0.012–0.781 μg/mL) vitamin E concentrations using optimized HPLC conditions, and the concentration of each vitamin E homolog was calculated using the equation $Y = aX + b$, where $Y$ = concentration of the vitamin E homolog to be determined (μg/mL), $X$ = ratio of peak area of the vitamin E homolog ($A_{(Sample)}$)/peak area of corresponding internal standard ($A_{standard}$)), $a$ = slope of the standard curve, and $b$ = intercept of the standard curve.

For vitamin E analyses of the lipid emulsions (in triplicate), 200 μL was placed into 10 × 13–mm Pyrex tubes with Teflon-lined screw caps. To these tubes, 20 μL (for low vitamin E concentrations) or 40 μL (for high vitamin E concentrations) of internal standard (10 or 250 μg/mL, in hexane), 800 μL of methanol, and then 2000 μL of hexane were added. Tubes were vortexed for 1 minute and then centrifuged at 1400 *g* for 20 minutes to separate the aqueous and nonaqueous layers (2 mL). For high vitamin E concentrations, 200 μL of the top layer (nonaqueous layer) was transferred to the HPLC sample vial for HPLC analysis. For low vitamin E concentrations, the entire top layer was transferred to a clean glass tube and dried under $N_2$ flow. The residues were reconstituted in 200 μL of hexane and then transferred to the HPLC sample vials for analysis.

The HPLC system (Shimadzu, Kyoto, Japan) consisted of an LC-20AT pump, a SIL-20AC autosampler, and a DGU-20A degasser and was equipped with an RF-10A fluorescence detector and an SPD-M20A diode array detector. The wavelengths of the detector were set at 292 nm for excitation and 330 nm for emission for the identification and quantification of the vitamin E homologs. A pinnacle DB silica normal phase column (100 × 2.1 mm, 1.9 μm; Restek, Bellefonte, PA, USA) was used. The isocratic mobile phase contained 2% of 1, 4-dioxane and 98% of n-hexane. The flow rate was adjusted to 300 μL/min. Tocopherol and tocotrienol peaks were identified by comparing their retention time with the reference standards. Concentrations of the vitamin E homologs were calculated using the external standard equations as described above.

### Malondialdehyde Measurement

Malondialdehyde (MDA) is formed through the interaction of reactive oxygen species with PUFAs and is used as an indicator of oxidative stress. MDA was measured in guinea pig plasma and liver and in the study lipid emulsions. Commonly, MDA is measured spectrometrically via its reactivity with the chromogen, 2-thiobarbiturate. The test is also named the thiobarbituric acid reactive substances (TBARS) assay. However, this test is not specific for MDA,[20] since other related compounds have been shown to react. The TBARS assay, therefore, is not suitable for many biological samples.[21] During the present studies, quantification of MDA from guinea pig plasma and liver was performed using an HPLC technique with some modifications[22,23] specific for MDA. An aliquot of 200 µL of sample (plasma or liver homogenate) was placed in a 1.5-mL Eppendorf tube, and 50 µL of 6M NaOH were added. Alkaline hydrolysis of protein-bound MDA was achieved by incubating this mixture in a 60°C water bath for 30 minutes, vortexing, and repeating the 30-minute heating period. The protein was precipitated with 125 µL of 35% (v/v) perchloric acid, and the mixture was centrifuged at 2800 *g* for 10 minutes. A 200-µL volume of supernatant was transferred to a 1.5-mL Eppendorf tube and mixed with 25 µL of 2,4-dinitrophenylhydrazone (DNPH) prepared as a 5-mM solution in 2M hydrochloric acid. This reaction mixture was incubated for 30 minutes at room temperature and protected from light. Then, 20 µL of this reaction mixture was injected onto a Shimadzu LC-20AT HPLC system equipped with a multi-wavelength diode array detector (DAD), an SIL-20ACHT autosampler, and a Restek pinnacle DB C18 column (2.1 × 100 mm, 1.9 µm). Samples were isocratically eluted with a mixture of 0.2% (v/v) acetic acid in deionized water and acetonitrile (62:38, v/v) at a flow rate of 0.3 mL/min at room temperature. Chromatograms were acquired at 310 nm. Data were analyzed with Shimadzu's GC solutions software. The concentration of MDA in plasma and liver was calculated from a reference calibration curve, which was processed using the same protein hydrolysis and DNPH derivatization as the serum and liver homogenate. For MDA analysis in lipid emulsions, an aliquot of 20 µL of lipid emulsion, 180 µL of acetonitrile, and 50 µL of 6M NaOH was placed in a 1.5-mL Eppendorf tube. The alkaline hydrolysis, DNPH derivatization, and HPLC analysis were performed as described above.

### Lipid Hydroperoxides Measurement

Lipid hydroperoxides were assayed in freshly drawn samples from each unopened emulsion container. Lipids were extracted in chloroform, and the concentration of lipid hydroperoxides was determined using a kit as per the manufacturer's protocol (Cayman Chemical Company, Ann Arbor, MI).

### Calculation of α-Tocopherol Equivalents

The α-tocopherol equivalents represent a measure of total vitamin E activity. Measured α-tocopherols equivalents reflect the amount of α-tocopherol activity that is present based on the different vitamin E homologs. The α-tocopherol equivalents (in µg) in the study lipid emulsions were calculated as $(1 \times \alpha\text{-T} + 0.5 \times \beta\text{-T} + 0.1 \times \gamma\text{-T} + 0.01 \times \delta\text{-T} + 0.3 \times \alpha\text{-T}_3 + 0.05 \times \beta\text{-T}_3)$, where T represents tocopherols and $T_3$ represents tocotrienols. The requirement for vitamin E has been shown to vary as a function of the PUFA content of the diet.[11] As a result, formulas have been developed to determine the amount of α-tocopherol equivalents that are required to protect unsaturated fatty acids with different numbers of double bonds from peroxidation. We calculated the requirements for α-tocopherol equivalents (in µg) in the study lipid emulsions as $(0.09 \times G1 + 0.6 \times G2 + 0.9 \times G3 + 1.2 \times G4 + 1.5 \times G5 + 1.8 \times G6)$, where Gn is mg of unsaturated fatty acid with n double bonds.[12] The more double bonds a fatty acid contains, the more α-tocopherol is required. The measured α-tocopherol equivalents are directly compared with the calculated requirements for each lipid emulsion. The α-tocopherol equivalents are useful for comparing diets or oils (including lipid emulsions) enriched with PUFA.

### Materials

Tocopherols (α, β, γ, and δ homologs) were purchased from Calbiochem (San Diego, CA). Tocotrienols (α, β, γ, and δ homologs) were purchased from Davos Life Science Pte Ltd (Singapore). All *rac*-α-Tocol was purchased from Matreya LLC (Pleasant Gap, PA). The purity of all reference standards was at least 95%. The lipid hydroperoxidation kit was purchased from Cayman Chemical Company. Hexane (CHROMASOLV; for HPLC, ≥97.0%), methanol (anhydrous, 99.8%), BHT (≥99.0%), DNPH, MDA, and all other reagents were purchased from Sigma-Aldrich (St. Louis, MO).

### Statistical Analysis of Data

The data are reported as mean ± SD unless stated otherwise. All comparisons are made by 1-way analysis of variance (ANOVA) with Tukey's post hoc test using SPSS version 20 software (SPSS, Inc, an IBM Company, Chicago, IL). Analyses were done on the normalized data (not adjusted for interdependence) using R (version 1.15.1) software (R Foundation for Statistical Computing, Vienna, Austria)[24] from means and standard deviations. Mean differences were compared using the studentized range with Tukey's honestly significant difference. All significant values are reported at $P < .05$.

## Results

We analyzed the tocopherol and tocotrienol content of the 4 commercially available lipid emulsions used in this study (Table 2). Tocopherols (α, β, γ, and δ) were the predominant vitamin E homologs for all emulsions, with tocotrienol content ≤0.3%.

The study lipid emulsions had varying quantities of PUFAs, with mean concentrations ranging from 49–121 mg/mL (Table 3). We measured the fatty acid content of the study lipid

**Table 2.** Tocopherol and Tocotrienol Content of Lipid Emulsions (μg/mL, Mean ± SD).[a]

| Vitamin E | SO | OO | FO | MIX |
|---|---|---|---|---|
| α-T | 21.02 ± 0.21 | 32.03 ± 0.67 | 230.12 ± 0.78 | 164.50 ± 2.66 |
| β-T | 3.76 ± 0.74 | 0.58 ± 0.11 | ND | 1.46 ± 0.14 |
| γ-T | 107.54 ± 0.85 | 13.97 ± 0.03 | 0.15 ± 0.00 | 29.24 ± 0.62 |
| δ-T | 32.99 ± 0.15 | 10.51 ± 0.03 | 0.01 ± 0.00 | 10.71 ± 0.13 |
| α-T3 | ND | ND | 0.67 ± 0.20 | ND |
| β-T3 | ND | ND | 0.03 ± 0.00 | ND |
| γ-T3 | ND | ND | ND | ND |
| δ-T3 | 0.02 ± 0.00 | 0.01 ± 0.00 | ND | 0.03 ± 0.04 |
| ΣT | 165.31 ± 1.95 | 57.09 ± 0.83 | 230.28 ± 0.78 | 205.91 ± 3.56 |
| ΣT3 | 0.02 ± 0.00 | 0.01 ± 0.00 | 0.70 ± 0.21 | 0.03 ± 0.04 |
| Total | 165.33 ± 1.95 | 57.93 ± 0.86 | 230.98 ± 0.98 | 205.94 ± 3.59 |
| %T | 99.99 | 98.55 | 99.70 | 99.99 |
| %T3 | 0.01 | 0.02 | 0.30 | 0.01 |

FO, fish oil; MIX, mixture of soybean, olive, fish, and medium-chain triglyceride oils; ND, not detected; OO, olive oil; SO, soybean oil; T, tocopherol; T3, tocotrienol.
[a]Data are the average of triplicate samples.

emulsions, using a technique described in an earlier publication.[9] We evaluated 3 calculations reported in the nutrition literature for estimating the vitamin E requirements for protecting fatty acids from peroxidation in relation to PUFA intake. Harris and Embree[25] suggest a requirement of 0.6 mg of α-tocopherol/g of PUFA to optimally minimize lipid peroxidation. The Institute of Medicine[26] suggests a requirement of 0.4 mg α-tocopherol/g PUFA. Valk and Hornstra[12] introduced the concept of α-tocopherol equivalents to express vitamin E activity. The α-tocopherol equivalents reflect the antioxidant potential of all vitamin E homologs (not just α-tocopherol) contained within the lipid emulsions. Requirements for α-tocopherol equivalents are calculated using a formula, which weights the oxidation potential of the number of double bonds in the fatty acid molecule (see Materials and Methods). These calculated vitamin E requirements were compared with the actual vitamin E contents (expressed as equivalents) as analyzed during the present investigation (Table 3).

Calculated antioxidant requirements for α-tocopherol equivalents were higher than the requirements calculated based on use of fixed values for all PUFAs (ie, 0.4–0.6 mg α-tocopherol/g PUFA) (Table 3). The higher requirements using the Valk and Hornstra[12] method reflect the enrichment of the emulsions with highly unsaturated fatty acids. Thus, FO had the highest requirements for α-tocopherol equivalents despite having lower PUFA content than did SO. OO with the lowest PUFA content had the lowest α-tocopherol requirements. For the SO-based lipid emulsion, measured vitamin E antioxidant content (α-tocopherol equivalents) was lower than calculated requirements (Table 3). The OO lipid emulsion contained α-tocopherol equivalents that approximated requirements. The fish oil–containing lipid emulsions (MIX, FO) contained α-tocopherol equivalents in excess of calculated requirements.

In addition to calculating vitamin E antioxidant requirements for the lipid emulsions, we also measured hydroperoxides within the lipid emulsions and correlated the levels with the vitamin E content of the emulsions (Figure 1A). The goal was to determine if the measured α-tocopherol equivalents were adequate for preventing or minimizing lipid peroxidation during formulation and storage of the lipid emulsions. There was a large range of values for lipid hydroperoxides when comparing the different lipid emulsions, with OO (containing predominantly monounsaturated fatty acids) containing the lowest and FO containing the highest levels. The 2 fish oil–containing emulsions (FO, MIX) had similar levels of the vitamin E homologs but markedly different lipid hydroperoxide levels. We further investigated whether the lipid emulsions with higher unsaturation indexes (ie, more double bonds) had higher lipid hydroperoxidation (Figure 1B). Interestingly, the lowest lipid peroxidation occurred in OO and MIX despite markedly different vitamin E contents but similar unsaturation indexes. The highest lipid peroxidation occurred in SO and FO, emulsions with the highest unsaturation index. In addition to hydroperoxides, we also measured MDA levels in the 4 study lipid emulsions. Similar to levels of hydroperoxides, MDA levels in the emulsions were lowest for OO and highest for FO (Figure 2).

Next, we measured vitamin E levels in plasma and tissues of guinea pigs on the study diet (15% fat energy) and following infusion of the lipid emulsions into the animals receiving the fat-free diet. The nutrition contents of the study and fat-free diets are listed in Table 1. The total vitamin E content of the fat-free diet was lower than the content of the study diet. The difference reflects the vitamin E content of the dietary lipids. α-Tocopherol was the predominant vitamin E homolog in plasma and tissues of animals receiving the oral study diet and IV lipid infusions (Table 4). None of the tissues had detectable levels of tocotrienols; therefore, the data for tocotrienols are not reported. Vitamin E content (primarily α-tocopherol) varied greatly between tissues. In animals receiving the study diet, levels were highest in lungs (26.86 ± 5.54 μg/g), liver (20.01 ± 5.26 μg/g), and RBCs (20.20 ± 1.01 μg/g protein). Levels were intermediate in adipose tissue (10.07 ± 3.92 μg/g) and lowest in heart (3.48 ± 0.73 μg/g), kidney (2.37 ± 0.71 μg/g), and plasma (3.13 ± 1.6 μg/mL). Similar results were found following lipid infusion. Interestingly, lungs had higher vitamin E levels than any other organ studied.

In general, vitamin E levels (primarily α-tocopherol) were maintained at baseline levels or slightly increased with all 4 lipid emulsions (Table 4). The few exceptions were an increase in total tocopherol content in adipose tissue with SO, in lungs with FO, and in kidney with MIX. γ-Tocopherol and/or δ-tocopherol levels were significantly increased in most tissues following infusion of SO, which contained the highest levels of these tocopherols (Table 2). The increase in total tocopherol content of lungs with FO resulted from a significant increase in α-tocopherol.

**Table 3.** Calculated Requirements and Measured α-Tocopherol Content (Mean ± SD).

| Lipid Emulsion | PUFA,[a] mg/mL (%)[b] | Calculated α-Tocopherol Requirements Based on Equivalents,[c] μg/mL | Calculated α-Tocopherol Requirements,[d] μg/mL | Calculated α-Tocopherol Requirements,[e] μg/mL | Measured α-Tocopherol Content,[f] μg/mL | Measured α-Tocopherol Equivalents,[g] μg/mL |
|---|---|---|---|---|---|---|
| SO | 120.42 ± 0.73 (60.2) | 80.23 ± 0.54 | 72.25 ± 0.44 | 48.17 ± 0.33 | 21.02 ± 0.21 | 33.98 ± 0.67 |
| OO | 39.93 ± 0.06 (20.0) | 36.48 ± 0.20 | 23.96 ± 0.03 | 15.97 ± 0.03 | 32.03 ± 0.67 | 33.82 ± 0.72 |
| MIX | 49.19 ± 0.36 (24.6) | 45.33 ± 0.39 | 29.51 ± 0.22 | 19.68 ± 0.14 | 164.50 ± 2.66 | 168.26 ± 2.80 |
| FO | 55.87 ± 1.26 (55.9) | 85.92 ± 1.82 | 33.52 ± 0.75 | 22.35 ± 0.5 | 230.12 ± 0.78 | 230.15 ± 0.78 |

FO, fish oil; MIX, mixture of soybean, olive, fish, and medium-chain triglyceride oils; ND, not detected; OO, olive oil; PUFA, polyunsaturated fatty acid; SO, soybean oil.
[a]Sum of PUFAs containing 2–6 double bonds; mean ± SD.
[b]Percent (%) PUFA using mean PUFA/total fatty acids concentrations of emulsions.
[c]Calculated requirements for α-tocopherol equivalents (μg/mL) = (0.09 × G1 + 0.6 × G2 + 0.9 × G3 + 1.2 × G4 + 1.5 × G5 + 1.8 × G6), where Gn (in mg/mL) represents fatty acids with n double bonds; based on Valk and Hornstra.[12]
[d]Calculated requirements based on 0.6 mg α-tocopherol/g PUFA; based on Harris et al.[10]
[e]Calculated requirements based on 0.4 mg of α-tocopherol/g PUFA; based on Institute of Medicine.[26]
[f]Current high-performance liquid chromatography–based analysis.
[g]Measured α-tocopherol equivalents (μg/mL) = (1 × α-T + 0.5 × β-T + 0.1 × γ-T + 0.01 × δ-T + 0.3 × $\alpha$-$T_3$ + 0.05 × $\beta$-$T_3$) (all in μg/mL).

To determine whether tissue and/or plasma levels of vitamin E were adequate to protect from lipid peroxidation during in vivo infusion of the lipid emulsions, we also investigated lipid peroxidation in plasma and liver of the animals receiving 10-day infusions of the 4 study lipid emulsions (Table 5). Lipid peroxidation was assessed using MDA levels. Analysis of plasma and liver samples indicated that these lipid emulsions were well tolerated and that there were no significant differences between MDA levels in plasma or liver tissues of control (diet) animals or in animals following infusion of the lipid emulsions. However, there was a trend for higher MDA levels in liver in the animals receiving FO and MIX, both containing the highest quantities of highly unsaturated long-chain fatty acids.

## Discussion

Vitamin E homologs have many different biological activities; however, the primary function is as a lipid antioxidant.[1] Humans absorb all forms of vitamin E through the intestine as components of chylomicrons, but the body preferentially accumulates α-tocopherol[1,27,28] due to the presence of a specific α-tocopherol transfer protein. Serum concentrations of vitamin E in humans receiving enteral diets depend largely on liver uptake of α-tocopherol following absorption (which is dependent on fat absorption and production of bile). The liver uptakes the various vitamin E homologs but preferentially incorporates only α-tocopherol into circulating lipoproteins via the hepatic α-tocopherol transfer protein.[29] The other forms of vitamin E are metabolized and excreted in the bile.[30] Vitamin E is delivered to the tissues as a component of lipoproteins. However, parenteral lipid emulsions are administered into the central vascular circulation and bypass first-pass hepatic extraction. The lipid globules of parenteral lipid emulsions acquire apoproteins during circulation and resemble lipoproteins. Thus, levels of vitamin E in the blood and tissues may not reflect levels seen with enteral administration. Because of the difference in metabolism of the enteral and parenteral compounds, tissue distribution may also be different.

We were particularly interested in the changes in vitamin E levels that might occur with infusion of different lipid emulsions containing different quantities of PUFAs (especially the highly unsaturated fatty acids docosahexaenoic acid and eicosapentaenoic acid). Our results indicate that α-tocopherol predominates over the other vitamin E homologs in all tissues in animals receiving study diets and following lipid emulsion infusion. This result is similar to humans, in whom α-tocopherol represents the primary circulating and tissue form of vitamin E.[1,4] Interestingly, tocotrienols were not detected in any of the tissues. Thus, despite the purported properties of tocotrienols, including their antioxidant, anticancer, cardioprotective, and neuroprotective effects,[31,32] we are unable to speculate on any relevance of tocotrienols for health and disease development relative to our experimental outcomes. Levels of tocopherols varied greatly between organ tissues from a mean level of 2.37 μg/g in kidney to 50.71 μg/g in lungs (Table 4). It remains unclear why tocopherol content is so variable between different tissues and how tissue content is regulated. Our results indicate that no one tissue is a good proxy for tocopherol levels in other tissues.

γ-Tocopherol levels were low in all tissues except for the RBCs, where it represented 14.8%–21.6% of total tocopherols. γ-Tocopherol levels increased in most organs in the animals receiving the SO lipid emulsion (which is high in γ-tocopherol). RBC levels of γ-tocopherol remained higher than those in other organs following infusion of the lipid emulsions. It is unclear why or how the RBCs accumulated higher amounts of γ-tocopherol than other organs and whether the content of

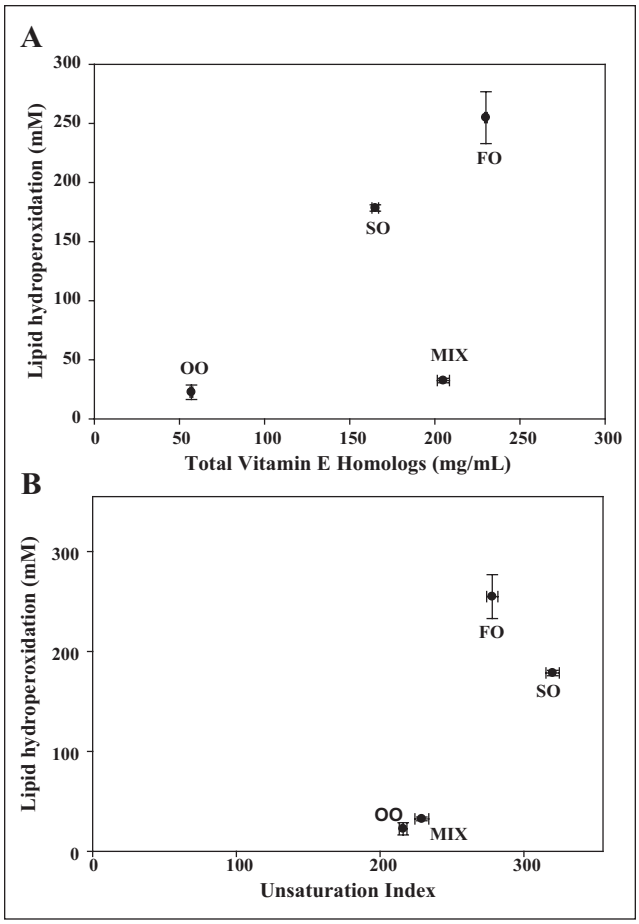


**Figure 1.** Relationship between vitamin E contents and fatty acid unsaturation index of lipid emulsions with lipid hydroperoxidation. The quantities of total vitamin E homologs were assayed by high-performance liquid chromatography, whereas the unsaturation index was calculated by totaling the number of double bonds × mg of fatty acids/mL of lipid emulsion as reported earlier.[43] Lipid hydroperoxidation was assayed using a commercially available kit (Cayman Biochemical Company, Ann Arbor, MI). The values are mean ± SD for 3 determinations. FO, fish oil; MIX, mixture of soybean, olive, fish, and medium-chain triglyceride oils; OO, olive oil; SO, soybean oil.

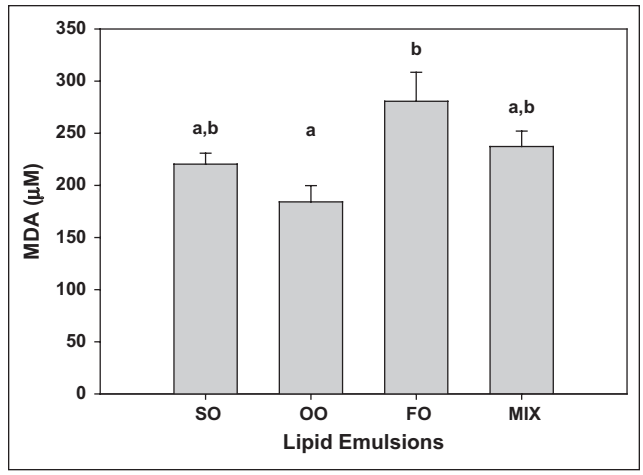


**Figure 2.** Concentration of malondialdehyde (MDA) in lipid emulsions. The concentration of MDA was assayed using a high-performance liquid chromatography system as described in the text. The values are mean ± SD for 3 determinations. The data were analyzed using 1-way analysis of variance with Tukey's post hoc test using SPSS version 20 software (SPSS, Inc, an IBM Company, Chicago, IL). Bars labeled with different characters indicate a significant difference at $P < .05$. FO, fish oil; MIX, mixture of soybean, olive, fish, and medium-chain triglyceride oils; OO, olive oil; SO, soybean oil.

γ-tocopherol has physiologic significance. However, we speculate that γ-tocopherol contributes to the antioxidant activity within the RBCs, protecting it from hemolysis.

We compared our vitamin E tissue measurements with those of representative studies from the published literature using the guinea pig.[5-9] Most published studies measured only α-tocopherol levels. We could find no studies that measured all 8 vitamin E homologs in animal tissues (only 1 study quantitated the tocopherol homologs), and none of the studies evaluated vitamin E tissue levels following IV lipid infusion. We were not able to directly compare tissue α-tocopherol levels between studies due to differences in dietary intakes, form of vitamin E used in the diet, ages of the animals, physiologic state of the animals, tissues analyzed, timing of tissue sampling in relationship to dietary intake, and assays used. Overall, our study was consistent with the published literature in its demonstration of the variability in tissue levels of α-tocopherol.[5-9] Considering the differences in study diets and procedures, our values fall within the ranges of values found in these cited studies. The study diet that we used in our investigation contained 88.5 mg of total tocopherols/kg diet (61.9 mg α-tocopherol/kg diet), which falls within the amounts in the cited studies. What is clear from our study and the cited studies is that vitamin E levels are highly variable in different tissues and that no one tissue reflects values of the other tissues. In particular, plasma appears to be a poor proxy for most other tissues.

Similar to diet, the natural vitamin E contents of the lipid emulsions are significantly affected by the oil species used to make the emulsions. The content of vitamin E homologs in oils is also influenced by harvesting, processing, and storage. Furthermore, total vitamin E and α-tocopherol content vary greatly (ie, 0.20–2.25 mg/100 g) between different species of fish,[33] likely reflecting their different diets. We chose 4 commercial lipid emulsions for in vivo study based on their differences in oil composition. We chose a lipid emulsion based on SO (the most common lipid emulsions used throughout the world today and high in ω-6 PUFAs), one based on OO (high in ω-9 monounsaturated fatty acids), one based on FO (high in ω-3 PUFAs), and one containing a mixture of the oils (MIX; soybean/olive/fish/MCT oils). To our knowledge, this is the

**Table 4.** Concentration of Vitamin E Homologs in Guinea Pig Tissues Following a 10-Day Infusion of Lipid Emulsions.

| Characteristic | Transition Diet | SO | OO | FO | MIX |
|---|---|---|---|---|---|
| Plasma (μg/mL ± SD) | | | | | |
| α-T | 3.00 ± 1.56[a,b] | 1.84 ± 0.68 | 3.00 ± 0.92 | 1.48 ± 0.47 | 3.72 ± 0.90 |
| β-T | 0.02 ± 0.00[a] | 0.03 ± 0.01 | 0.02 ± 0.00 | 0.01 ± 0.00 | 0.01 ± 0.00 |
| γ-T | 0.08 ± 0.02[a] | 0.08 ± 0.05[a] | 0.03 ± 0.01[b] | 0.02 ± 0.00[b] | 0.03 ± 0.01[b] |
| δ-T | 0.03 ± 0.01[a] | 0.01 ± 0.00[a] | ND | ND | 0.01 ± 0.01[a] |
| Total | 3.13 ± 1.60[a,b] | 1.96 ± 0.74[a,b] | 3.05 ± 0.94[a,b] | 1.51 ± 0.47[a] | 3.77 ± 0.92[b] |
| RBC (μg/mg protein ± SD) | | | | | |
| α-T | 16.17 ± 0.55[a] | 17.81 ± 2.00[a,b] | 20.17 ± 3.80[a,b] | 22.53 ± 4.05b | 17.09 ± 0.90[a] |
| β-T | ND | 1.14 ± 0.06 | ND | ND | ND |
| γ-T | 3.48 ± 0.39[a] | 5.22 ± 2.18[a] | 3.57 ± 0.33[a] | 4.47 ± 1.61[a] | 4.64 ± 0.93[a] |
| δ-T | 0.55 ± 0.08[a] | ND | 0.39 ± 0.10[a] | ND | ND |
| Total | 20.20 ± 1.01[a] | 24.17 ± 4.24[a] | 24.13 ± 4.24[a] | 26.99 ± 5.67[a] | 21.73 ± 1.83[a] |
| Liver (μg/g tissue ± SD) | | | | | |
| α-T | 19.00 ± 4.93[a,b] | 11.98 ± 3.81[b] | 16.77 ± 5.71[a,b] | 18.71 ± 5.01[a,b] | 21.34 ± 7.18[a] |
| β-T | 0.20 ± 0.05[a,c] | 0.28 ± 0.04[a,b] | 0.18 ± 0.03[c] | ND | 0.27 ± 0.11[a,b] |
| γ-T | 0.62 ± 0.21[a] | 1.35 ± 0.38[b] | 0.42 ± 0.21[a] | ND | 0.53 ± 0.17[a] |
| δ-T | 0.19 ± 0.07[a] | 0.17 ± 0.11[a] | 0.51 ± 0.33[a] | ND | 0.06 ± 0.03[a] |
| Total | 20.01 ± 5.26[a] | 13.78 ± 4.33[a] | 17.88 ± 6.28[a] | 18.74 ± 5.01[a] | 22.20 ± 7.48[a] |
| Heart (μg/g tissue ± SD) | | | | | |
| α-T | 2.95 ± 0.50[a,b] | 3.29 ± 0.69[a,b] | 2.60 ± 0.87[a] | 3.24 ± 0.72[b] | 5.06 ± 2.42[a] |
| β-T | ND | 0.25 ± 0.05 | ND | ND | 0.28 ± 0.23 |
| γ-T | 0.44 ± 0.16[a] | 1.36 ± 0.60[b] | 0.31 ± 0.13[a] | 0.23 ± 0.09[a] | 0.37 ± 0.20[a] |
| δ-T | 0.09 ± 0.07[a] | 0.67 ± 0.22[b] | 0.07 ± 0.02[a] | ND | 0.13 ± 0.08[a] |
| Total | 3.48 ± 0.73[a] | 5.58 ± 1.57[a] | 2.98 ± 1.03[a] | 3.47 ± 0.80[a] | 5.85 ± 2.93[a] |
| Lungs (μg/g tissue ± SD) | | | | | |
| α-T | 25.95 ± 5.36[a] | 29.74 ± 7.00[a] | 33.24 ± 5.23[a] | 48.12 ± 6.30[b] | 35.77 ± 3.90[a] |
| β-T | 0.24 ± 0.04[a] | ND | 0.61 ± 0.53[a] | 0.53 ± 0.69[a] | 0.22 ± 0.08[a] |
| γ-T | 0.47 ± 0.08[a] | ND | 0.87 ± 0.36[a] | 1.94 ± 3.33[a] | 1.00 ± 0.27[a] |
| δ-T | 0.19 ± 0.06[a] | 1.37 ± 0.27[b] | 0.24 ± 0.12[a] | 0.13 ± 0.10[a] | 0.27 ± 0.06[a] |
| Total | 26.86 ± 5.54[a] | 31.11 ± 7.27[a] | 34.97 ± 6.25[a] | 50.71 ± 10.42[b] | 37.26 ± 4.32[a] |
| Kidney (μg/g tissue ± SD) | | | | | |
| α-T | 1.96 ± 0.65[a] | 2.70 ± 0.87[a,b] | 3.04 ± 0.56[a,b] | 4.26 ± 1.44[b] | 3.97 ± 1.25[a,b] |
| β-T | 0.14 ± 0.01[a] | 0.17 ± 0.02[b] | 0.10 ± 0.01[a,c] | 0.11 ± 0.01[a,d] | 0.15 ± 0.03[a,b] |
| γ-T | 0.22 ± 0.02[a] | 1.07 ± 0.46[b] | 0.26 ± 0.06[a] | 0.18 ± 0.03[a] | 0.39 ± 0.06[a] |
| δ-T | 0.05 ± 0.03[a] | 0.34 ± 0.08[b] | 0.06 ± 0.01[a] | ND | 0.11 ± 0.03[a] |
| Total | 2.37 ± 0.71[a] | 4.28 ± 1.43[a,b] | 3.46 ± 0.64[a,b] | 4.56 ± 1.50[a,b] | 4.63 ± 1.37[b] |
| Adipose tissues (μg/g tissue ± SD) | | | | | |
| α-T | 9.10 ± 3.41[a] | 13.19 ± 3.30[a] | 12.20 ± 6.44[a] | 13.14 ± 6.86[a] | 8.64 ± 4.79[a] |
| β-T | ND | 0.16 ± 0.08[a] | ND | ND | 0.06 ± 0.03[a] |
| γ-T | 0.34 ± 0.24[a] | 5.86 ± 2.46[b] | 1.15 ± 0.37[a,c] | 0.68 ± 0.29[a,c] | 1.15 ± 0.15[c] |
| δ-T | 0.63 ± 0.27[a] | 3.80 ± 0.99[b] | 1.00 ± 0.21[a] | 0.70 ± 0.21[a] | 1.17 ± 0.17[c] |
| Total | 10.07 ± 3.92[a] | 23.01 ± 6.84[b] | 14.36 ± 7.03[a,b] | 14.52 ± 7.36[a,b] | 11.01 ± 5.14[a,c] |

FO, fish oil; MIX, mixture of soybean, olive, fish, and medium-chain triglyceride oils; ND, not detected; OO, olive oil; SO, soybean oil. Values with different superscripted letters exhibit significant differences at $P < .05$.

first study to report the content of all 8 vitamin E homologs in commercial lipid emulsions based on different oils. Our results indicate that tocopherols were the predominant forms of vitamin E in the lipid emulsions. Tocotrienol content of the emulsions was not detectable or very low. α-Tocopherol predominated in OO, FO, and MIX emulsions while γ-tocopherol predominated in the SO emulsion. The total vitamin E content of the lipid emulsions varied from a mean of 57.93–230.98 μg/mL (Table 2).

In addition to variations in vitamin E content, lipid emulsions also vary in their content of saturated, monounsaturated, and PUFAs. The PUFAs are susceptible to peroxidation during formulation and storage. Vitamin E is well known to limit peroxidation within lipid emulsions and is used by manufacturers

**Table 5.** Malondialdehyde (MDA) Concentrations in Plasma and Liver of Guinea Pigs Following a 10-Day Lipid Emulsion Infusion.

| Characteristic | Plasma, μM, Mean ± SD | Liver, μg/g Tissue, Mean ± SD |
|---|---|---|
| Control (diet) | 5.16 ± 0.45 | 284.05 ± 128.60 |
| SO | 5.13 ± 0.50 | 311.93 ± 99.81 |
| OO | 5.16 ± 0.55 | 295.74 ± 91.27 |
| FO | 4.95 ± 0.66 | 447.80 ± 146.36 |
| MIX | 6.13 ± 1.19 | 331.18 ± 144.76 |

FO, fish oil; MIX, mixture of soybean, olive, fish, and medium-chain triglyceride oils; OO, olive oil; SO, soybean oil.

to protect the PUFAs from peroxidation. Despite the different levels of these lipid antioxidants, lipid hydroperoxides were highest with FO (highest in vitamin E content) and lowest with OO (lowest in vitamin E content) and MIX (high in vitamin E content). Lipid hydroperoxides were intermediate with SO (intermediate in vitamin E content) (Figure 1). MDA levels in the lipid emulsions were similar to hydroperoxide content, with the lowest levels measured in OO and the highest levels in FO; MIX and SO had intermediate MDA levels (Figure 2). Overall, there was no direct relationship between vitamin E content and lipid peroxidation in the lipid emulsions. These results support the complex nature of lipid peroxidation within lipid emulsions, which relates to many factors inclusive of the vitamin E content, unsaturation of the fatty acids, other components of the emulsions, and the container. It is also important to note that high levels of vitamin E can increase in vitro lipid peroxidation.[34-37]

We compared measured α-tocopherol equivalents of the lipid emulsions (based on content of the vitamin E homologs) and compared the results with calculated requirements (using the method of Valk and Hornstra[12]) (Table 3). FO and MIX contained greater measured content than did calculated requirements, OO had content approximating requirements, and SO contained lower content than calculated requirements. Interestingly, despite the excess of vitamin E in the FO and MIX emulsions, their hydroperoxide and MDA contents were higher than the other emulsions. OO with measured content approximating calculated requirements had the lowest levels of lipid peroxidation. It is unclear whether the higher levels of the vitamin E homologs contributed to peroxidation.

Oxidant stress is implicated in the pathophysiology of many human diseases. Antioxidants may decrease oxidant damage and modify disease development and progression. Vitamin E is the primary lipid-soluble antioxidant in the human body.[1] The structure of vitamin E, with its hydrophobic side chain, makes it unique and indispensable in protecting cell membranes from oxidant damage.[38,39] Due to its preferential accumulation in the body, α-tocopherol arguably is the most important vitamin E homolog believed to modify the course of many oxidative diseases.[40,41]

The various vitamin E homologs have been proposed to have numerous physiologic effects within the body that include modulation of oxidation, cell proliferation and apoptosis, inflammation, cytokine production, immune cell functions, cholesterol metabolism, and platelet function. A discussion of the potential functions of vitamin E in humans is beyond the scope of this report. However, most evidence for vitamin E actions upon various cellular processes relates to its antioxidant functions and protection of PUFAs within cell membranes.[4]

We did not detect significant decreases in tocopherol content of tissues following infusion of the lipid emulsions with high content of PUFAs (ie, SO, FO, MIX). We also failed to detect increased levels of MDA in plasma or liver tissues following 10 days of lipid emulsion infusion. We infused 1.25–2.5 g of triglycerides/kg/d into the animals. These amounts fall within the quantities recommended by the American Society for Parenteral and Enteral Nutrition safe practices guidelines, which state that lipid should be administered at levels to meet 15%–30% of energy and be limited to 2.5 g/kg/d.[42] Thus, for the SO, OO, and MIX emulsions, we administered the maximal amount recommended for humans. However, it should be noted that guinea pigs have energy requirements (carbohydrate and lipid) that are higher than human requirements (due to their higher basal metabolic rates). Our results suggest that infusion of lipid emulsions does not induce significant oxidative stress in these tissues. It should be noted that the animals were free of disease and injuries (other than the recent surgery to place central venous catheters). Results may differ in animals with underlying inflammatory disease, malignant disease, traumatic or burn injuries, or other disease that predisposes to oxidative stress. The animals in this study received oral nutrition in addition to parenteral lipids, and results may also vary in animals receiving only PN.

In conclusion, commercial lipid emulsions contained variable amounts of tocopherols that were significantly different between emulsions. α-Tocopherol predominated in the OO, FO, and MIX emulsions, while γ-tocopherol predominated in the SO emulsion. Tocotrienols were present in the emulsions at very low concentrations (≤0.3%). However, there was no correlation between the amount of vitamin E present in the lipid emulsions and in vitro lipid peroxidation within the emulsions. Some lipid emulsions with higher concentrations of vitamin E contained higher levels of hydroperoxides. Hydroperoxides were the lowest with the OO emulsion and highest with the FO emulsion. The predominant vitamin E homolog in guinea pig tissues of animals receiving oral diets and following IV lipid infusion was α-tocopherol. None of the tissues had detectable levels of tocotrienols. Levels of vitamin E (primarily α-tocopherol and γ-tocopherol) were highly variable between organ tissues, and no one tissue reflected the contents of the other tissues. In particular, plasma levels were a poor reflection of most tissue levels. Overall, vitamin E content of tissues was maintained at baseline (diet) levels or slightly increased following infusion of SO, OO, FO, and MIX lipid emulsions, and

none of the tissues demonstrated increased MDA levels in plasma or liver compared with control animals (even after infusion of highly unsaturated fatty acids in fish oil).

## Acknowledgments

We thank Elaine Bammerlin for providing the editorial assistance and Dr Cary Mariash for the statistical analysis of data.

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