

## AN ABSTRACT OF THE THESIS OF

Heather D. Vaule for the degree of Master of Science in Nutrition and Food Management presented on July 18, 2001. Title:  $\alpha$ -Tocopherol is Specifically Delivered to Human Skin; Studies using Deuterium-Labeled  $\alpha$ -Tocopherol.

Abstract approved: \_\_\_\_\_

Maret G. Traber

The relative enrichment of skin sebaceous gland lipids with deuterium-labeled  $\alpha$ -tocopherol was compared with plasma enrichment to evaluate the delivery of vitamin E to skin. For the first week of this study, each subject consumed a daily dose of deuterated vitamin E (150 mg of an equimolar mixture of *RRR*- $\alpha$ -[5-(C<sup>2</sup>H<sub>3</sub>)]- (d<sub>3</sub>) and *all rac*- $\alpha$ -[5,7-(C<sup>2</sup>H<sub>3</sub>)<sub>2</sub>]- (d<sub>6</sub>) tocopheryl acetates) with breakfast. Blood was drawn and skin lipids were collected daily for two weeks, then every other day for the following two weeks. Labeled and unlabeled vitamin E analysis was carried out using liquid chromatography and mass spectrometry (LC/MS). Skin cholesterol, plasma cholesterol and triglycerides were measured to evaluate changes in vitamin E levels relative to lipid content. While d<sub>3</sub> and d<sub>6</sub>- $\alpha$ -tocopherols were found in plasma 24 h after the first dose, d<sub>3</sub>- $\alpha$ -tocopherol was only detected in the skin sebaceous gland secretions after 1 week of supplementation. This data suggests a skin-mediated delivery system for vitamin E

into skin lipid secretions. This finding is also supported by the observation that the ratio of  $\alpha$ -to  $\gamma$ -tocopherol was greater in the skin than in the plasma.

$\alpha$ -Tocopherol is Specifically Delivered to Human Skin;  
Studies using Deuterium-Labeled  $\alpha$ -Tocopherol

By  
Heather D. Vaule

A THESIS

Submitted to  
Oregon State University

in partial fulfillment of  
the requirements for the  
degree of

Master of Science

Presented July 18, 2001  
Commencement June 2002

Master of Science thesis of Heather D. Vaule presented on July 18, 2001

APPROVED

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Major Professor, representing Nutrition and Food Management

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Department Head of Nutrition and Food Management

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Dean of Graduate School

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Heather D. Vaule, Author

## ACKNOWLEDGEMENTS

This study was funded by a grant from Nestlé. The deuterated vitamin E capsules were a gift from the Natural Source Vitamin E Association (NSVEA), and were synthesized by Eastman Kodak, Rochester, NY. The d<sub>9</sub>-*all rac*- $\alpha$ -tocopheryl acetate used for our internal standard was provided by Dr. Carolyn Good of General Mills (Minneapolis, MN) and was synthesized by Isotec Inc. (Miamisburg, OH). Vitamin E standards were gifts from James Clark of Cognis Nutrition and Health, LaGrange, IL.

## **CONTRIBUTION OF AUTHORS**

Dr. Maret G. Traber was involved in the design, analysis, and writing of this thesis. Scott W. Leonard was involved in the method design and data collection for the study. Both assisted in the interpretation of data.

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## **$\alpha$ -Tocopherol is Specifically Delivered to Human Skin; Studies using Deuterium-Labeled $\alpha$ -Tocopherol**

### **SPECIFIC AIMS AND HYPOTHESIS**

Does human skin contain a vitamin E-mediated defense mechanism to protect against oxidative injury? If so, the mechanism could involve the  $\alpha$ -tocopherol transfer protein in association with lipid secretion by the sebaceous glands in the skin. The hypothesis for this study is that vitamin E is secreted onto the surface of the skin in the sebum where it may be absorbed through the superficial layers into the stratum corneum and then into the "live" dermal layers to provide protection to the skin from oxidative stressors. This study sought to support the hypothesis that an  $\alpha$ -tocopherol regulatory mechanism exists in skin.

The specific aims of this study were to a) measure vitamin E in facial skin secretions relative to plasma concentrations and b) to follow the kinetics of vitamin E delivery to skin using stable isotope-labeled vitamin E.

## LITERATURE REVIEW

### INTRODUCTION

This study investigated the hypothesis that dietary vitamin E is delivered to the skin in sebum. The enrichment of human skin lipids with vitamin E following supplementation with deuterium labeled  $\alpha$ -tocopherol was evaluated. To our knowledge, this is the first study to use stable-isotope labeled vitamin E to investigate delivery of dietary vitamin E to skin secretions.

Facial skin is exposed to a variety of environmental oxidants and therefore requires antioxidant protection. These oxidants may play key roles in skin cancer, aging and other skin diseases. Vitamin E is the most potent lipid soluble antioxidant *in vivo* (1) and therefore may have an important role in skin protection.

Topical protection of skin by vitamin E *in vitro* and *in vivo* has been thoroughly investigated, yet very few studies have investigated delivery of dietary vitamin E to skin.

### VITAMIN E

Vitamin E is a lipid soluble antioxidant that is classified as a vitamin because it is required by humans (2). Within the body, vitamin E is a constituent of lipids, lipoproteins and cell membranes (2). It intercalates into the lipid bilayers of

cell membranes, where it terminates free radical chain reactions and confines membrane damage (3). As a result of its ability to maintain cellular integrity and protect against oxidants, vitamin E has become a popular dietary supplement as well as a component of many cosmetic products.

### **Vitamin E Requirements and Dietary Sources**

The 2000 recommended dietary allowance (RDA) for vitamin E is 15 mg  $\alpha$ -tocopherol (specifically the 2*R* forms, see below) daily for both men and women; the tolerable upper intake level (UL) for adults is set at 1000 mg/day of any form of supplemental  $\alpha$ -tocopherol (4).

Vitamin E can be readily obtained from dietary sources. The richest being edible vegetable oils (5).  $\alpha$ -Tocopherol is especially high in wheat germ oil, safflower oil, and sunflower oil (5). Soybean and corn oils contain predominantly  $\gamma$ -tocopherol, as well as some tocotrienols (5). Unprocessed cereal grains, nuts and animal fat are also good sources of vitamin E (5).

### **Forms and Structures**

Eight different molecules have vitamin E antioxidant activities. These include four tocopherols and four tocotrienols, that have similar chromanol structures: trimethyl ( $\alpha$ ), dimethyl ( $\beta$  or  $\gamma$ ), and monomethyl ( $\delta$ ) (Appendix A). Tocotrienols differ from tocopherols, as they have an unsaturated side chain (2).

The chromanol head group is responsible for the antioxidant activity whereas the transport between and the retention within membranes and lipoproteins are affected largely by the phytyl tail (6). Of all the naturally occurring vitamin E forms, the human body prefers *RRR*- $\alpha$ -tocopherol (2). Studies with stable isotope labeled vitamin E were instrumental in defining this preference for *RRR*- $\alpha$ -tocopherol (2), (7).

Chemically synthesized  $\alpha$ -tocopherol is not identical to the naturally occurring  $\alpha$ -tocopherol (2). The naturally occurring form is present only as 1 stereoisomer, *RRR*- $\alpha$ -tocopherol, while the chemically synthesized all racemic form contains 8 different stereoisomers as a result of the 3 chiral centers in the phytyl tail (Appendix B). The most important chiral center is at the junction between the phytyl tail and the chromanol ring, the 2-position, with preference given to the 2*R* isomers by the  $\alpha$ -tocopherol transfer protein (8).

### **Deuterium-Labeled Tocopherols**

Deuterated vitamin E is not radioactively labeled material, rather it is tagged with a stable isotope of hydrogen (deuterium) that has no recognized harmful effects in humans (9). The deuteriums replace hydrogens on the methyl groups of the chromanol ring. An advantage inherent in the use of deuterated tocopherols is that, unlike radiolabeled vitamin E, humans may ingest the stable isotope-labeled compounds. The human body does not appear to be able to distinguish between deuterium-labeled and non-labeled forms of vitamin E (9). Furthermore, as the

deuterium appears not to undergo any measurable, metabolically mediated exchange, deuterated tocopherols have been touted for use in human studies (6). These stable-isotope labeled vitamin E forms have been used extensively in humans to study absorption and plasma transport (2).

### **Vitamin E Absorption and Lipoprotein Transport**

Intestinal absorption of vitamin E is dependent upon the mechanisms for lipid absorption from the digestive tract; efficient emulsification, and solubilization within mixed bile salt micelles (10).  $\alpha$ -Tocopheryl acetate is inactive as an antioxidant and requires enzymatic hydrolysis to produce the active antioxidant  $\alpha$ -tocopherol.  $\alpha$ -Tocopheryl acetate, a common form in vitamin E supplements, taken orally, is quantitatively hydrolyzed by pancreatic esterases to  $\alpha$ -tocopherol in the gut (11, 12). Then the uptake of vitamin E by the enterocyte takes place by passive diffusion. The process is non-saturable, non-carrier-mediated, unaffected by metabolic inhibitors and does not require energy (10). Within the enterocyte, vitamin E is incorporated into chylomicrons, secreted into the intracellular spaces and lymphatics and secreted into the bloodstream (10). It is not until vitamin E has reached the liver that any discrimination occurs between the various dietary vitamin E forms (10).

The *2R*- $\alpha$ -tocopherols are preferentially secreted from the liver. The liver  $\alpha$ -tocopherol transfer protein ( $\alpha$ -TTP) regulates plasma vitamin E; and in humans, a genetic defect in  $\alpha$ -TTP results in severe vitamin E deficiency (2).  $\alpha$ -TTP

facilitates the secretion of  $\alpha$ -tocopherol from the liver into the plasma in very low density lipoproteins (VLDL). In the circulation VLDL are catabolized as a result of various lipases to IDL and eventually LDL. These lipoproteins have a hydrophobic core of triglycerides and cholesteryl esters surrounded by phospholipids and protein. Their densities are inversely proportional to their lipid contents, thus VLDL contains the greatest amount of lipid. For this reason plasma vitamin E is often expressed as a ratio of vitamin E per cholesterol or per total lipid.

VLDL, IDL and LDL all contain an apoprotein called apoprotein B (apo B-100). This protein is recognized by LDL receptors for uptake. Through this pathway vitamin E is able to reach tissues throughout the body (13).

Vitamin E is transported non-specifically in the blood by all of the plasma lipoproteins (7). There is no evidence for the existence of a specific vitamin E plasma carrier protein. One of the advantages of vitamin E in the lipoproteins is polyunsaturated fatty acids (PUFAs) and other lipids, susceptible to oxidation are protected from free-radicals in the circulation (10).

The apparent half-life of RRR- $\alpha$ -tocopherol in plasma of normal subjects is approximately 48 hours (14). Normal plasma vitamin E concentrations in humans range from 11 to 37  $\mu\text{mol/L}$  (10). When plasma lipids are taken into account the lower limits of normal are 1.6  $\mu\text{mol } \alpha\text{-tocopherol/mmol lipid}$  or 2.5  $\mu\text{mol } \alpha\text{-tocopherol/mmol cholesterol}$  (10).

While it is unknown how vitamin E specifically is delivered to skin there are two major routes by which tissues are hypothesized to acquire vitamin E. One,



by lipoprotein lipase-mediated lipoprotein catabolism (15) and/or two, by LDL receptor-mediated uptake (13). However, it is beyond the scope of this study to establish which routes may be important in the skin.

### **Oxidative Stress and Vitamin E Function**

Vitamin E is the most potent, naturally occurring nonenzymatic, lipid-soluble antioxidant in human tissue (16). The role of vitamin E as the major membrane-linked radical scavenger in the lipid environment is thought to be unique (16). Vitamin E has multiple functions. As an antioxidant and free radical scavenger vitamin E is involved in the maintenance of the integrity of cellular and subcellular membranes, heme synthesis, and mitochondrial metabolism (17). In addition, vitamin E stabilizes lysosomes, interacts with eicosanoids to reduce prostaglandin E<sub>2</sub> synthesis, and increases IL-2 production, resulting in anti-inflammatory and immunostimulatory effects (18). Of these, the principle function of vitamin E is its antioxidant activity to maintain membrane integrity (4, 19).

Vitamin E exists in biological membranes in a low molar ratio to unsaturated phospholipids—approximately 1 molecule per 1000 to 2000 membrane phospholipid molecules (20). Vitamin E protects polyunsaturated fatty acids (PUFAs) within membrane phospholipids and plasma lipoproteins (4).

Within cellular systems, reactive oxygen radicals are generated in numerous physiological and pathological processes including cellular respiration, inflammation, excessive physical activity, nutritional imbalances, as well as

chemically or physically induced damage caused by mediators such as alcohol, chloroform, paraquat, cigarette smoke, ozone or ultraviolet radiation (16) (Appendix C). The membranes of tissue cells and intracellular organelles contain phospholipids that could spontaneously oxidize unless protected by antioxidants (18). Damage may occur when a lipid with double bonds, e.g. PUFA, is exposed to ultraviolet irradiation, and then loses an electron to form a lipid radical. Then in the presence of molecular oxygen, the lipid radical is transformed to a lipid peroxy radical. The lipid peroxy radical is again able to attack unsaturated lipids with double bonds thereby forming another lipid radical and a lipid hydroperoxide. This cycle is termed a radical chain reaction. Lipid peroxy radicals can be generated in membranes at the rate of 1-5 nmol/mg of membrane protein per minute, yet destructive oxidation of membrane lipids does not normally occur, nor is vitamin E rapidly depleted. Without vitamin E or another antioxidant to slow, stop, or prevent the radical formation cycle, damage to tissue components may readily occur. When cell membrane peroxidation occurs, free radicals are released that destroy cells and cause lysosomal enzyme leakage, allowing formation of autoimmune antibodies and more cell destruction (18).

In plasma and red blood cells, vitamin E is the main lipid-soluble antioxidant that protects cell membrane lipids from peroxidation. Peroxy radicals can react 1000 times faster with vitamin E than with polyunsaturated fatty acids (PUFA) (21). The phenolic hydroxyl group of  $\alpha$ -tocopherol reacts with the peroxy radical to form the corresponding hydroperoxide and the tocopheroxyl radical. In

this way vitamin E acts as a chain-breaking antioxidant, preventing further autooxidation of lipids, acids, or other organic compounds (2, 19).

The low-energy tocopheroxyl radical can then react with other antioxidants (16).

Vitamin E is not consumed in this reaction due to an event called, “vitamin E recycling”, where the ability to be an antioxidant is continuously restored by other antioxidants such as vitamin C, ubiquinol, and thiols such as glutathione (20).

Unfortunately, this process may deplete these other antioxidants. For this reason, cosupplementation with other antioxidants has been suggested (22).

## **SKIN**

Skin is a tissue that is constantly exposed to stresses from a wide array of sources (20). More than other tissues, the skin is exposed to numerous environmental, chemical and physical agents, such as ultraviolet light, air pollutants, and chemical oxidants that cause oxidative stress (23). Some of the results of this exposure are erythema, edema, skin thickening, wrinkling, and an increased incidence of skin cancer or precursor lesions (16). Thus, skin is a primary defensive barrier for the body.

As an organ, the skin is composed of several types of cells, which are in different layers: epidermis, dermis, and hypodermis. The most exterior layer of the epidermis is the stratum corneum, a fully keratinized layer of cells that is bound together by lipids. This is a structure containing dead cells in a matrix of lipids, commonly described as “bricks and mortar” (24). The next layer, stratum

granulosum is responsible for producing the lipids found in the stratum corneum. The site of active protein synthesis (keratin) is in the stratum spinosum. Lastly, the stratum basale, or the horny layer, is a single layer of cuboidal cells that separates the epidermis from the dermis. The dermis is the next segment of skin with only two layers, the papillary dermis and the reticular dermis (25). The dermis is a layer of fibroblasts that also contains nerves, sebaceous glands, and blood vessels. Below the dermis is the hypodermis or subcutaneous layer. This layer contains the root of hair follicles and sweat glands. The subcutaneous layer contains adipose tissue and attaches the dermis to underlying tissues of the body (25) (Appendix D).

### **Sebaceous Glands and Sebum Secretion**

The sebaceous gland is unique to mammalian skin and is most abundant in humans. The gland is a collection of lobules of various sizes and shapes that open into a system of ducts, together forming the main excretory duct, which opens into the pilary canal inside the hair follicle (26). On the forehead and face, the sebaceous glands achieve maximal size, secreting into enlarged pilary canals occupied by attenuated vellus hairs. These structures are referred to as sebaceous follicles, which secrete sebum (27).

Sebum is the first line of defense of the skin against its environment and has significant protective and other biological functions (24). Sebum is a thin film of emulsified lipids that spread rather evenly over the entire upper layer of the epidermis (28). The lipid components of this surface film are derived mainly from

the normal secretions of the sebaceous glands. Sebum also contains fatty acids, cholesterol and dead cells (29). Squalene, wax esters and triglyceride are the major lipid components of sebum (30). Squalene is the main component (about 13%) of skin surface lipid, and has an absorption band that coincides with the erythema curve over the range of 290nm-320nm. For this reason, squalene is easily peroxidized by UV irradiation (30).

Along with the film of lipids, keratinizing epithelium protects humans from water loss and noxious physical, chemical and mechanical insults (24). Formation of the epidermal barrier requires delivery of lamellar body contents to the stratum corneum (31). The lamellar bodies are enriched in a mixture of polar lipids and a family of hydrolytic enzymes, which together mediate barrier function (31). Acute barrier disruption leads to immediate secretion of the contents of preformed lamellar bodies from the outermost layer of granular cells (31).

Physiologically, the amount of sebum delivered to the skin surface depends primarily on three factors; the number of sebaceous gland cells per unit area, surface skin temperature, and the emulsifying action of sweating (32). The average time between synthesis of sebum and its excretion onto the skin is approximately 8 days (32).

The stratum corneum regulates the epidermal lipid and DNA-metabolic responses to a variety of exogenous insults. Various signaling mechanisms, including changes in levels of epidermal cytokines and growth factors, are potential candidates to mediate these metabolic responses (33). These signaling molecules

may be generated not in response to permeability barrier requirements, but as an unavoidable consequence of the epidermal injury that accompanies all types of acute barrier abrogation (33). The formation and maintenance of skin barrier function is never ending, it is the product of the highly organized and regulated process of epidermal differentiation (34). Defects in structural components, either protein or lipid, or the enzymes responsible for their synthesis, processing, or assembly can disrupt the barrier or alter the process of renewal (34).

### **Vitamin E and Skin Protection**

Routes of absorption of tocopherol by skin are from stratum corneum into the epidermis and then into dermis or through the hair follicles, by way of pilosebaceous canals and into the outer root sheaths, and eventually into dermal tissue and connective tissue sheaths (11). Presumably, dietary vitamin E could be excreted from the sebaceous gland in the sebum and then follow the pathways of exogenous vitamin E. A study by Theile, Weber, and Packer (23) examined sebaceous gland secretion of vitamin E in humans as a route of delivery to skin and found that vitamin E correlates well with cosecreted squalene levels. Kramer-Stickland et al. (12) found more vitamin E in the extract of skin secretions than in the epidermis, in both irradiated and non-irradiated mice.

By preventing lipid peroxidation of the sebum and epidermal lipids, vitamin E can protect the skin from damage (35-38). A decrease in lipid radical generation may reduce membrane and protein damage by limiting the formation of Schiff

bases (39). It appears that  $\alpha$ -tocopherol is recruited to the epidermis in response to repeated UVB exposure and that some of the epidermal  $\alpha$ -tocopherol is consumed by reactions with oxidants (38). Since vitamin E absorbs UV radiation at 294 nm,  $\alpha$ -tocopherol may act as a sunscreen in the epidermis to prevent direct DNA damage (37). Vitamin E may also prevent ornithine decarboxylase induction, lipid peroxidation and immunosuppression by UV-B irradiation (37). As well, vitamin E may reduce malonyl dialdehyde (MDA) production in the skin (37). MDA is the end product formed by oxygenated free radical-induced peroxidation of unsaturated fatty acids. Endogenous  $\alpha$ -tocopherol plays a role in preventing UV-radiation-induced skin damage by preventing lipid peroxidation. A physiological adaptive response mobilizes diet derived  $\alpha$ -tocopherol to the epidermis in response to chronic UVB irradiation in mice (40).

In studies of vitamin E supplementation, there have been attempts to correlate between dietary intakes and plasma  $\alpha$ -tocopherol concentrations. Studies in humans found that  $\alpha$ - and  $\gamma$ -tocopherol concentrations in plasma and skin punch samples were significantly correlated; and, thus skin concentrations could be estimated from plasma concentrations (41). Yet, no significant association between supplementation or serum  $\alpha$ -tocopherol levels and the risk of subsequent skin cancers (malignant melanoma, basal and squamous cell skin cancers) have been determined (42-45). Potentially this finding is related to the lack of a relationship between diet and plasma  $\alpha$ -tocopherol levels in epidemiological studies (46).

In human supplementation studies with vitamin E, significant increases in levels of  $\alpha$ -tocopherol in both serum and skin have been found (3). As well, there are positive effects on erythema (47, 48) and dermatological conditions (22). An investigation of dermatological conditions in response to supplementation with vitamins E and C or a combination of both found significant decreases in serum lipoperoxides for all groups and sebum lipoperoxide in combination group (22). In rats fed a vitamin E deficient diet, skin lipid peroxides were increased (35). Skin does accumulate  $\alpha$ -tocopherol during supplementation of the diet with vitamin E (37). A dose-dependent increase of skin levels of  $\alpha$ -tocopherol was evident in the ventral skin of dorsally UV-B-irradiated mice (37). Dietary *RRR*- $\alpha$ -tocopheryl acetate reduced skin cancer incidence in a dose-dependent manner in UV-B-irradiated C3H/HeN mice (37).

Both topical and dietary vitamin E can afford a degree of protection against at least some of the damaging effects of solar radiation, yet the protection does not, however, appear to be specifically confined to either DNA or lipid moieties (49). All racemic- $\alpha$ -tocopherol topically applied (39), and both dietary and topical vitamin E (49), reduced UV-induced damage (sunburn-associated erythema, edema, and skin sensitivity in mice, tumors, skin wrinkling) (18) to mice epidermis. Oral and topical vitamin E reduces skin photoaging effects, skin cancer formation, and immunosuppression induced by UVR in mice (18).



## SUMMARY

The main gap identified in the literature indicates that there are few *in vivo* studies evaluating delivery of orally consumed vitamin E to skin and even fewer studies of this type using human subjects. In contrast, there are a plethora of studies using topically applied vitamin E to skin and its subsequent effects.

The present study is important because vitamin E, an antioxidant, is a potent protector of the skin from oxidative stress and the efficacy of the delivery of dietary vitamin E to the skin is unknown. Based on its vitamin E content, sebum seems to be an important route for delivery of vitamin E to the skin surface (23).

**$\alpha$ -TOCOPHEROL IS SPECIFICALLY DELIVERED TO  
HUMAN SKIN; STUDIES USING DEUTERIUM-LABELED  
 $\alpha$ -TOCOPHEROL**

Heather Vaule<sup>1</sup>, Scott W. Leonard<sup>2</sup> and Maret G. Traber<sup>1,2,3</sup>

<sup>1</sup>Department of Nutrition and Food Management, <sup>2</sup>Linus Pauling Institute, Oregon  
State University, Corvallis OR 97331 and the <sup>3</sup>Department of Internal Medicine,  
University of California, Davis, School Of Medicine, Sacramento, California 95817

Address for Correspondence:

Maret G. Traber, Ph.D.

Department of Nutrition and Food Management

Linus Pauling Institute

571 Weniger Hall

Oregon State University

Corvallis, OR 97331-6512

maret.traber@orst.edu

## ABSTRACT

The relative enrichment of skin sebaceous gland lipids with deuterium-labeled  $\alpha$ -tocopherol was compared with plasma enrichment to evaluate the delivery of vitamin E to skin. For the first week of this study, each subject consumed a daily dose of deuterated vitamin E (150 mg of an equimolar mixture of *RRR*- $\alpha$ -[5-(C<sup>2</sup>H<sub>3</sub>)]- (d<sub>3</sub>) and *all rac*- $\alpha$ -[5,7-(C<sup>2</sup>H<sub>3</sub>)<sub>2</sub>]- (d<sub>6</sub>) tocopheryl acetates) with breakfast. Blood was drawn and skin lipids were collected daily for two weeks, then every other day for the following two weeks. Labeled and unlabeled vitamin E analysis was carried out using liquid chromatography and mass spectrometry (LC/MS). Skin cholesterol, plasma cholesterol and triglycerides were measured to evaluate changes in vitamin E levels relative to lipid content. While d<sub>3</sub> and d<sub>6</sub>- $\alpha$ -tocopherols were found in plasma 24 h after the first dose, d<sub>3</sub>- $\alpha$ -tocopherol was only detected in the skin sebaceous gland secretions after 1 week of supplementation. This data suggests a skin-mediated delivery system for vitamin E into skin lipid secretions. This finding is also supported by the observation that the ratio of  $\alpha$ -to  $\gamma$ -tocopherol was greater in the skin than in the plasma.

## INTRODUCTION

More than other tissue, the skin is exposed to numerous environmental, chemical, and physical agents, such as ultraviolet light, air pollutants, and chemical oxidants that cause oxidative stress (23). Exposure to these insults can result in erythema, edema, skin thickening, wrinkling, and an increased incidence of skin cancer or precursor lesions (16). Oxidants may play key roles in skin cancer, aging and other skin diseases. Thus, skin is a primary defensive barrier for the body. Facial skin is exposed to a variety of environmental oxidants (such as oxygen, ozone and ultraviolet irradiation) and therefore facial skin especially requires antioxidant protection. Because vitamin E is the most potent lipid soluble antioxidant in vivo (1), it is thought to play an important role in skin protection.

Sebum is the first line of defense for the skin against its environment and has significant protective and other biological functions (24). Sebum is a thin film of emulsified lipids that spread rather evenly over the entire upper layer of the epidermis (28). The lipid components of this surface film are derived mainly from the normal secretions of the sebaceous glands. Sebum also contains fatty acids, cholesterol and dead cells (29). Squalene, wax esters and triglyceride are the major lipid components of sebum (30). Squalene is the main component (about 13% ) of skin surface lipid, and has an absorption band that coincides with the erythema curve over the range of 290nm-320nm. For this reason, squalene is easily peroxidized by UV irradiation (30).

Along with the film of lipids, keratinizing epithelium protects humans from water loss and noxious physical, chemical and mechanical insults (24). Formation of the epidermal barrier requires delivery of lamellar body contents to the stratum corneum (31). The lamellar bodies are enriched in a mixture of polar lipids and a family of hydrolytic enzymes, which together mediate barrier function (31). Acute barrier disruption leads to immediate secretion of the contents of preformed lamellar bodies from the outermost layer of granular cells (31).

Physiologically, the amount of sebum delivered to the skin surface depends primarily on three factors; the number of sebaceous gland cells per unit area, surface skin temperature, and the emulsifying action of sweating (32). The average time between synthesis of sebum and its excretion onto the skin is approximately 8 days (32).

Topical protection of skin by vitamin E in vitro and in vivo has been thoroughly investigated, yet very few studies have investigated delivery of dietary vitamin E to skin. Vitamin E acts as a chain-breaking antioxidant, stabilizes lysosomes, interacts with eicosanoids to reduce prostaglandin E<sub>2</sub> synthesis and increases IL-2 production, resulting in anti-inflammatory and immunostimulatory effects (18). Vitamin E, an antioxidant, is a potent protector of the skin from oxidative stress and the efficacy of the delivery of dietary vitamin E to the skin is unknown. Based on its vitamin E content, sebum seems an important route for delivery of vitamin E to the skin surface (23).

This study sought to support the hypothesis that an  $\alpha$ -tocopherol regulatory mechanism exists in skin. The specific aims of this study were to a) measure vitamin E in facial skin secretions and b) to follow the kinetics of vitamin E delivery to skin using stable isotope-labeled vitamin E.

## MATERIALS AND METHODS

### Subjects

The Oregon State University Institutional Review Board for the Protection of Human Subjects approved the protocol for this study (Appendix E). Subjects ( $n = 6$ ) were males between 19-33 years of age. Each gave written, informed consent. The subjects' serum cholesterol, triglyceride and glucose concentrations were within normal ranges. Subject characteristics are shown in **Table 1**.

### Materials

*RRR*- $\alpha$ -5-(CD<sub>3</sub>)-tocopheryl acetate ( $d_3$ -*RRR*- $\alpha$ -T) and *all rac*- $\alpha$ -5,7-(CD<sub>3</sub>)<sub>2</sub> tocopheryl acetate ( $d_6$ -*all rac*- $\alpha$ -T) capsules were a gift from the Natural Source Vitamin E Association (NSVEA), and were synthesized by Eastman Kodak, Rochester, NY. The  $d_3$ -*RRR*- and  $d_6$ -*all rac*- $\alpha$ -Ts were encapsulated in a gelatin capsule as nominal mixtures 1:1 in 150 mg quantities diluted with  $\alpha$ -tocopherol-stripped corn oil (USB Corporation, Cleveland, OH). The actual ratio of  $d_3$ -*RRR*- to  $d_6$ -*all rac*- $\alpha$ -tocopherol was determined by GS/MS to be 0.98 (50). The  $d_9$ -*all rac*- $\alpha$ -tocopherol was provided by Dr. Carolyn Good of General Mills (Minneapolis, MN) and was synthesized by Isotec Inc. (Miamisburg, OH).

Subject	Age (y)	Weight (kg)	Height (cm)	BMI (kg/m <sup>2</sup> )
A	25	133.9	190.5	37
B	25	83.5	108.3	26
C	19	77.2	185.4	22
D	32	70.4	177.8	22
E	33	79.0	180.3	24
F	31	89.9	185.4	27
Mean	28	89.0	171.3	26
±Std. Dev.	5	23.0	31.2	6

Table. Subject characteristics



Standards including unlabeled ( $d_0$ ),  $d_6$ -*RRR*- and  $d_3$ -*all rac*- $\alpha$ -tocopheryl acetate were gifts from James Clark of Cognis Nutrition and Health, LaGrange, IL.

HPLC-grade methanol, hexane, and ethanol were obtained from Fisher (Fair Lawn, NJ). Non-labeled  $\gamma$ -tocopherol, ascorbic acid, potassium hydroxide (KOH), butylhydroxy toluene (BHT), and lithium perchlorate were from Sigma (St. Louis, MO).

### **Vitamin E Administration, Blood Drawing, and Plasma Handling**

Each subject consumed a capsule containing deuterium labeled  $\alpha$ -tocopheryl acetates (150 mg) daily during the first week of the study. The capsule was consumed with a standard breakfast of a bagel with 3 tablespoons cream cheese and an 8 ounce glass of orange juice. Each morning before the subjects consumed breakfast (about 8 AM), a fasting blood sample of 15-ml of blood was drawn from the antecubital vein of each subject into EDTA-containing tubes (purple top, Becton-Dickinson, Franklin Lakes, NJ). A sebum sample was also collected (see below). Blood was stored on ice less than 30 minutes and separated by centrifugation to isolate the plasma, which was stored at  $-70^\circ\text{C}$  until analyzed within 1 week's time. The samples that were taken the first day of the study, prior to supplementation, constitute the baseline data for the subjects. The sampling continued daily during the second week of the study; during weeks 3 and 4 of the study, subjects were tested every other day. The study lasted 4 weeks and included 19 sampling sessions.

## **Sebum Collection**

An experimenter, wearing gloves to prevent contamination of the sample, collected all samples. Each subject's forehead was gently swabbed with an alcohol wipe (Professional Disposables Inc.'s Alcohol Prep Pads - 70% isopropyl alcohol, Orangeburg, NY) covering the entire area several times. The wipe was immediately placed into a screw cap glass tube containing 1% ascorbic acid in ethanol. On the day of collection, these samples were analyzed for vitamin E content (see below).

## **Extraction of Plasma and Sebum Labeled and Unlabeled Tocopherols**

Plasma vitamin E was extracted using a modification of the method by Podda et al. (51). In brief, 0.1 ml plasma was added to a 10-ml screw cap containing 2 ml 1% ascorbic acid in ethanol and a known amount of  $d_9$ - $\alpha$ -tocopheryl acetate (as the internal standard). The sample was mixed, then 1 ml water and 0.3 ml saturated potassium hydroxide (KOH) were added and mixed. The tubes were incubated in a 70° C water bath for 30 min. After cooling on ice, 25  $\mu$ L 0.1% (w/v) BHT, 1 ml 1% ascorbic acid in water, and 2-ml hexane were added. The samples were then mixed by inversion, the upper hexane portion collected and a known volume re-suspended, and its tocopherol contents analyzed using liquid chromatography / mass spectrometry (LC/MS) using a modification (see below) of a method developed in our laboratory (52).

Vitamin E was extracted from sebum samples using a similar procedure (51). Briefly, alcohol wipes with sebum samples were added to screw cap tubes containing 4 ml 1% ascorbic acid in ethanol and  $d_9$   $\alpha$ -tocopherol internal standard. Next, 2-ml water, and 0.6 ml saturated KOH, were added. The tubes were incubated in a 70° C water bath for 30 min. After cooling on ice, 25  $\mu$ L 0.1% (w/v) BHT, 2 ml 1% ascorbic acid in water, and 3-ml hexane were added. The samples were then extracted, re-suspended, and analyzed using LC/MS (see below)

### **LC/MS Method**

For LC/MS analysis of the vitamin E extracts a method developed in our laboratory (52) was used with the exception that different equipment was used. A Waters Alliance LC/MS system, which consists of a Waters 2690XE Separations Module and a Waters ZMD MS detector, single quadrupole mass spectrometer configured for Z-spray API LC/MS was used in this study. The samples were analyzed using atmospheric pressure chemical ionization in negative mode (APCI-). The corona discharge electrode was set to 5000 V and the probe temperature was set to 500° C. The curtain gas (nitrogen) was set to 0.6 L/min, the nebulizer gas (air) at 80 npsi, and the auxiliary gas (air) at 1 L/min. The orifice plate voltage was +55V. In brief, the method for quantification of deuterium labeled and unlabeled tocopherols consisted of the separation of  $\alpha$ - and  $\gamma$ -tocopherols with a 75-mm  $C_{18}$  reverse phase column and 100% methanol at 1 ml/min for 4 minutes. Each tocopherol was detected at its mass to charge ( $m/z$ ) of

M-1 using single ion recording and the Micromass MassLynx NT version 3.4 software. Sample concentrations were determined by calibration curves and the MassLynx software integrated peak areas.

### **Plasma Triglycerides and Cholesterol and Sebum Cholesterol**

Plasma triglycerides and cholesterol were determined using the respective Sigma Kits (St. Louis MO). Cholesterol was analyzed in extracts of sebum vitamin E samples using the Amplex Red Cholesterol Assay kit (Molecular Probes Eugene, OR).

### **Mathematical and Statistical Analysis**

Percent  $d_3$  was calculated for each subject as the plasma or sebum  $d_3$ - $\alpha$ -tocopherol concentration divided by the sum of the  $d_3$ ,  $d_6$  and  $d_0$ - $\alpha$ -tocopherol concentrations times 100. Analysis of variance with repeated measures was used to determine the statistical significance of the differences in  $\alpha$ - to  $\gamma$ -tocopherol ratios in the plasma compared to the skin. Statistical comparisons were performed using StatView (SAS Institute, Cary, NC).

## RESULTS

### Plasma Deuterated and Unlabeled $\alpha$ -Tocopherol Concentrations

Subjects were supplemented with 150 mg deuterated tocopheryl acetates daily for the first 7 days of the study. Each subject's vitamin E plasma measurements included unlabeled  $\alpha$ - and  $\gamma$ -tocopherols and  $d_3$ - and  $d_6$ - $\alpha$ -tocopherols (Appendix G shows each individual's plasma tocopherol concentrations).

The subjects' unlabeled  $\alpha$ - and  $\gamma$ -tocopherol ( $d_0$ ) concentrations were fairly consistent throughout the study (**Figure 1A**). The average plasma  $d_0$ - $\alpha$ -tocopherol was  $22.9 \pm 2.7 \mu\text{mol/L}$ ;  $\gamma$ -tocopherol averaged  $1.74 \pm 0.35 \mu\text{mol/L}$ . (Intersubject variability precluded any statistically significant changes in these parameters over time.)

Subjects' deuterated  $\alpha$ -tocopherols ( $d_3$  and  $d_6$ ) peaked by the end of the first week then followed a steady decline after supplementation ceased until about day 21 (**Figure 1B**).  $d_3$ - $\alpha$ -tocopherol peaked at an average of  $3.79 \pm 0.87 \mu\text{mol/L}$ , around day 8 while  $d_6$ - $\alpha$ -tocopherol peaked about  $2.68 \pm 0.72 \mu\text{mol/L}$ . These data suggest that all of the subjects were compliant and consumed the deuterated tocopherols.

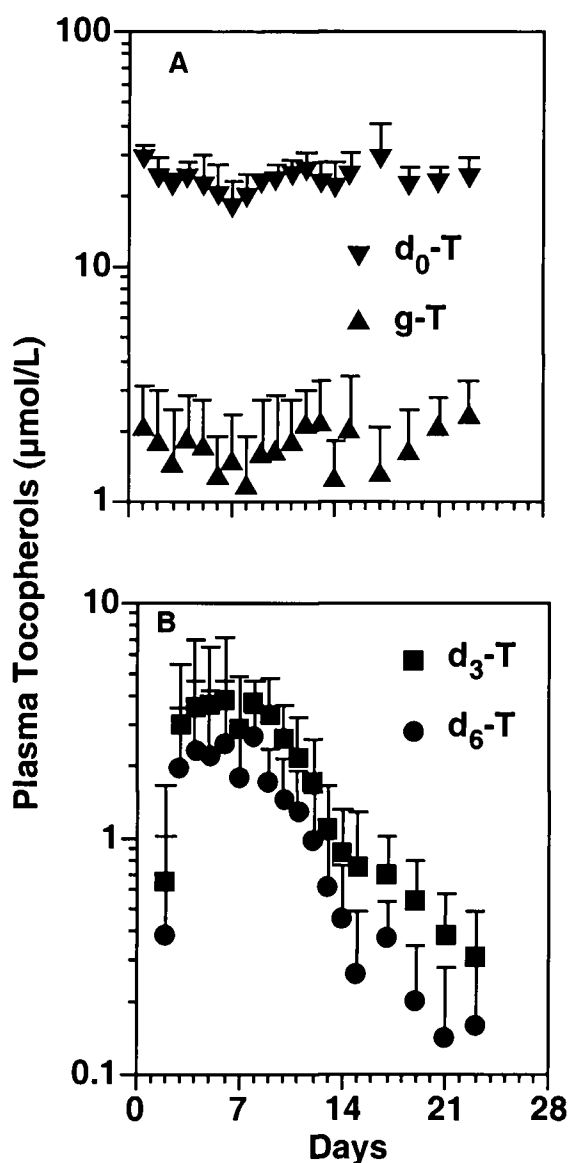


Figure 1. Plasma unlabeled (A) and labeled (B) tocopherols. Each subject consumed a daily dose of 150 mg of a 1:1 *RRR*- $\alpha$ -[5-( $C^2H_3$ )]- ( $d_3$ ) and *all rac*- $\alpha$ -[5,7-( $C^2H_3$ )<sub>2</sub>]- ( $d_6$ ) tocopheryl acetates with breakfast. Shown in (A) are the mean + standard deviations plasma concentrations of  $d_0$ - $\alpha$ -( $d_0$ -T) and (B)  $\gamma$ -tocopherols (g-T) and in (B)  $d_3$  ( $d_3$ -T) and  $d_6$ - $\alpha$ -tocopherols ( $d_6$ -T).

### **Plasma Deuterated and Unlabeled $\alpha$ -Tocopherol Concentrations Corrected for Lipid Concentrations**

Subjects' plasma concentrations of cholesterol, triglycerides and total lipids are shown in **Figure 2**. Concentrations were within the normal range for all subjects and did not vary widely during the study. Plasma vitamin E concentrations were expressed per cholesterol ( $\mu\text{mol}/\text{mmol}$ ), per triglycerides ( $\mu\text{mol}/\text{mmol}$ ), and per total lipids ( $\mu\text{mol}/\text{mmol}$ ) (**Figure 3**). No appreciable changes in the patterns observed for plasma vitamin E concentrations were discerned.

### **Plasma Percent $\text{d}_3$ - $\alpha$ -Tocopherol**

Plasma percent  $\text{d}_3$  was calculated for each subject and the mean + standard deviation is shown in **Figure 4** (Appendix H shows percent  $\text{d}_3$  for each individual subject). All subjects' plasma percent  $\text{d}_3$  peaked in the first week and showed a steady decline once supplementation ceased. Average percent  $\text{d}_3$  curves show a textbook increase, peak at the last day of supplementation and a steady decline. This pattern is similar to that described by Burton et al (9), who used a similar supplementation protocol, albeit a higher deuterated vitamin E dosage (150 mg each). The average exponential rate of disappearance for all subjects estimated

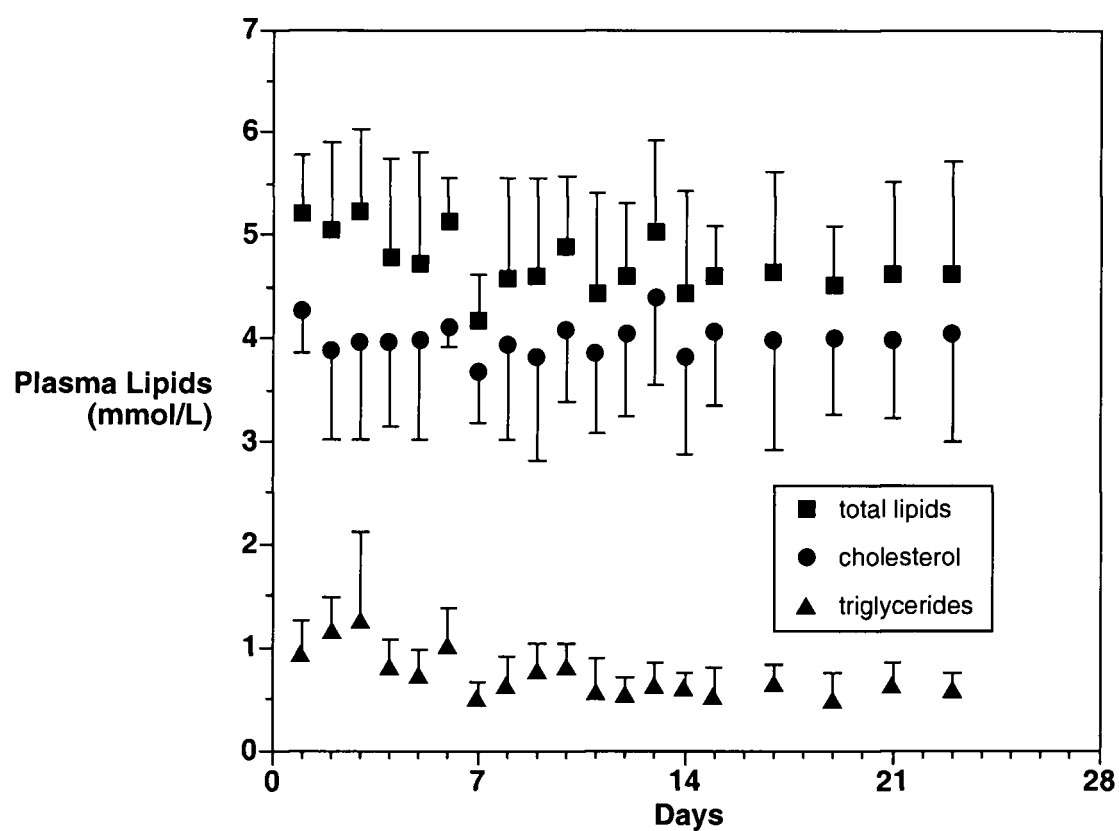


Figure 2. Plasma lipids

Mean and standard deviations of total lipids, cholesterol and triglycerides of subjects during the course of the study.



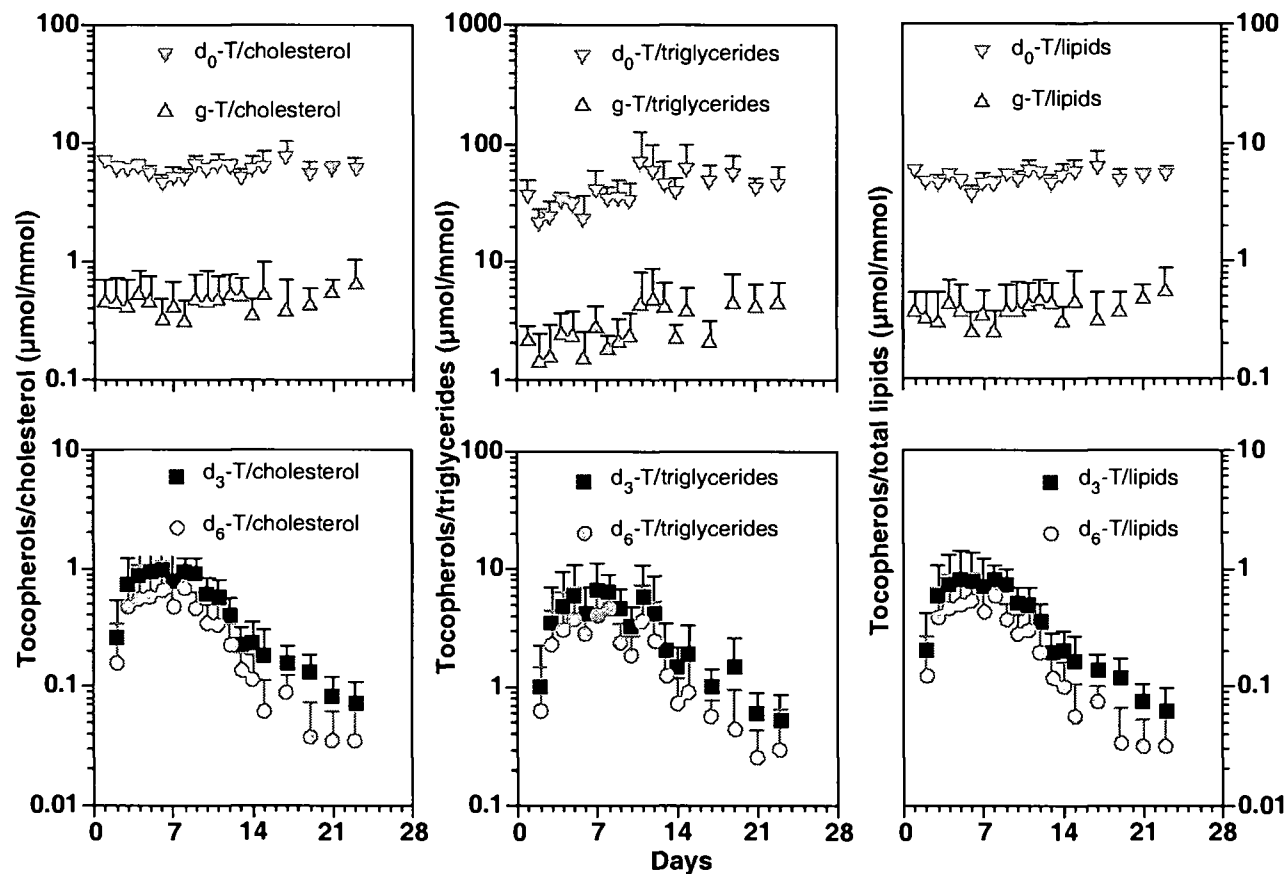


Figure 3. Plasma unlabeled and labeled tocopherols expressed per lipids  
 Shown are the mean + standard deviations of the plasma concentrations of  $d_0$ - $\alpha$  ( $d_0$ -T) and  $\gamma$ -tocopherols (g-T) in the upper panels and in the lower panels  $d_3$  ( $d_3$ -T) and  $d_6$ - $\alpha$ -tocopherols ( $d_6$ -T). Plasma tocopherol concentrations are expressed as per cholesterol in the left panel, per triglycerides in the middle panel, or per total lipids in the right panel.

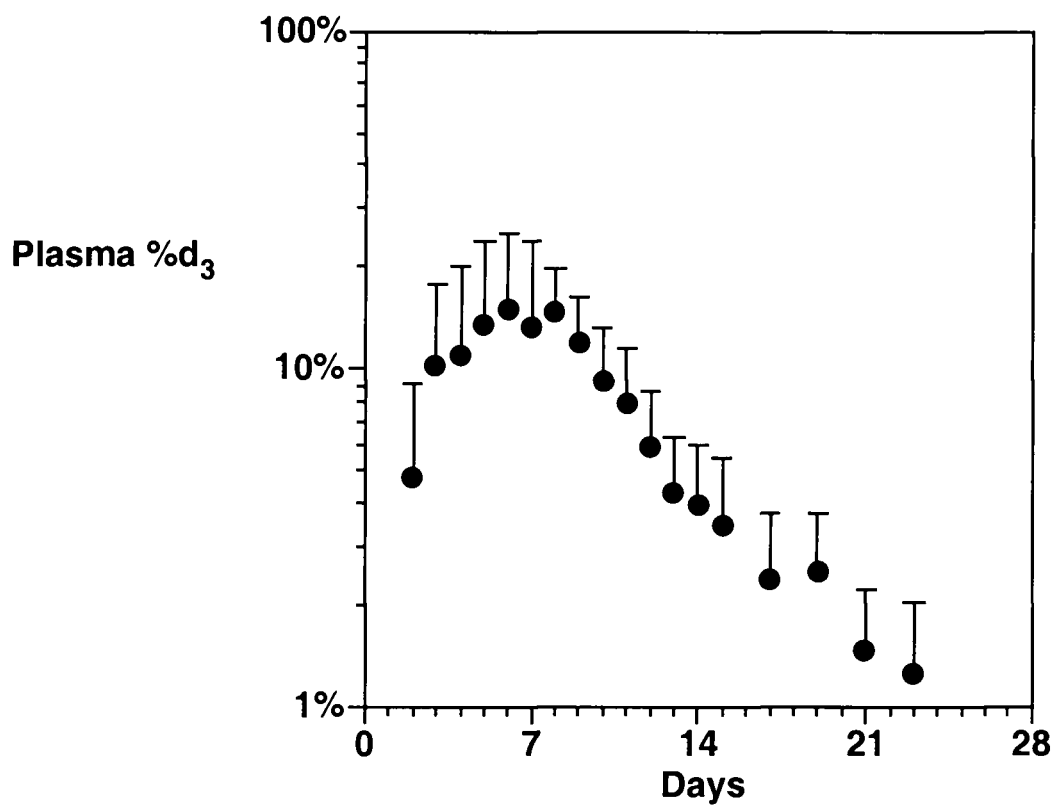


Figure 4. Plasma %  $d_3$   $\alpha$ -tocopherol

Each subject consumed a daily dose of 150 mg of *RRR*- $\alpha$ -[5-( $C^2H_3$ )]- ( $d_3$ ) and *all rac*- $\alpha$ -[5,7-( $C^2H_3$ )<sub>2</sub>]- ( $d_6$ ) tocopheryl acetates) with breakfast for 7 days. Shown are the mean + standard deviation of the %  $d_3$ - $\alpha$ -tocopherol averaged for all subjects (A-F).

from the peak percent  $d_3$  (day 8) through the last day of the study (day 23) was -  $0.1945 \pm 0.056$  pools per day.

### **Skin Secretion of Deuterated Vitamin E in Response to Supplementation**

Since sebum secretion can be altered by a variety of individual characteristics as well as environmental factors and differences in daily collection techniques, sebum vitamin E levels were expressed per cholesterol to investigate the impact of vitamin E supplementation. (Each subject's skin sebum tocopherols are shown in Appendix I). As seen in Figure 5, cholesterol (Figure 5A), as well as  $d_0$ - and  $\gamma$ -tocopherols (Figure 5B) were readily detected in the sebum. It should also be noted that  $d_6$ - $\alpha$ -tocopherol was detected in plasma but was not detected in the sebum. Around day 8  $d_3$ - $\alpha$ -tocopherol was found in sebum samples in our subjects. The appearance of  $d_3$ - $\alpha$ -tocopherol (Figure 5B) in the sebum after 8 days remained sporadic and was lower than  $\gamma$ -tocopherol concentrations (both reported per cholesterol). Over the next two weeks  $d_3$ - $\alpha$ -tocopherols were usually detectable until day 21 when the values dropped below the level of detection.

Each subject responded differently to the deuterated vitamin E supplementation; three patterns in appearance of  $d_3$ - $\alpha$ -tocopherol in sebum were found (**Figure 6**). In two subjects (A and F), percent  $d_3$  peaked at day 8 and decreased until none was detected on day 21. In contrast,  $d_3$ - $\alpha$ -tocopherol was detected at day 8 in two other subjects (B and E), then the percent  $d_3$  increased up to around day 14 followed by a decrease until none was detected on day 21. In the remaining two subjects (C and D),  $d_3$ - $\alpha$ -tocopherol was detected at day 8; but the percent  $d_3$  continued to increase up to day 21.

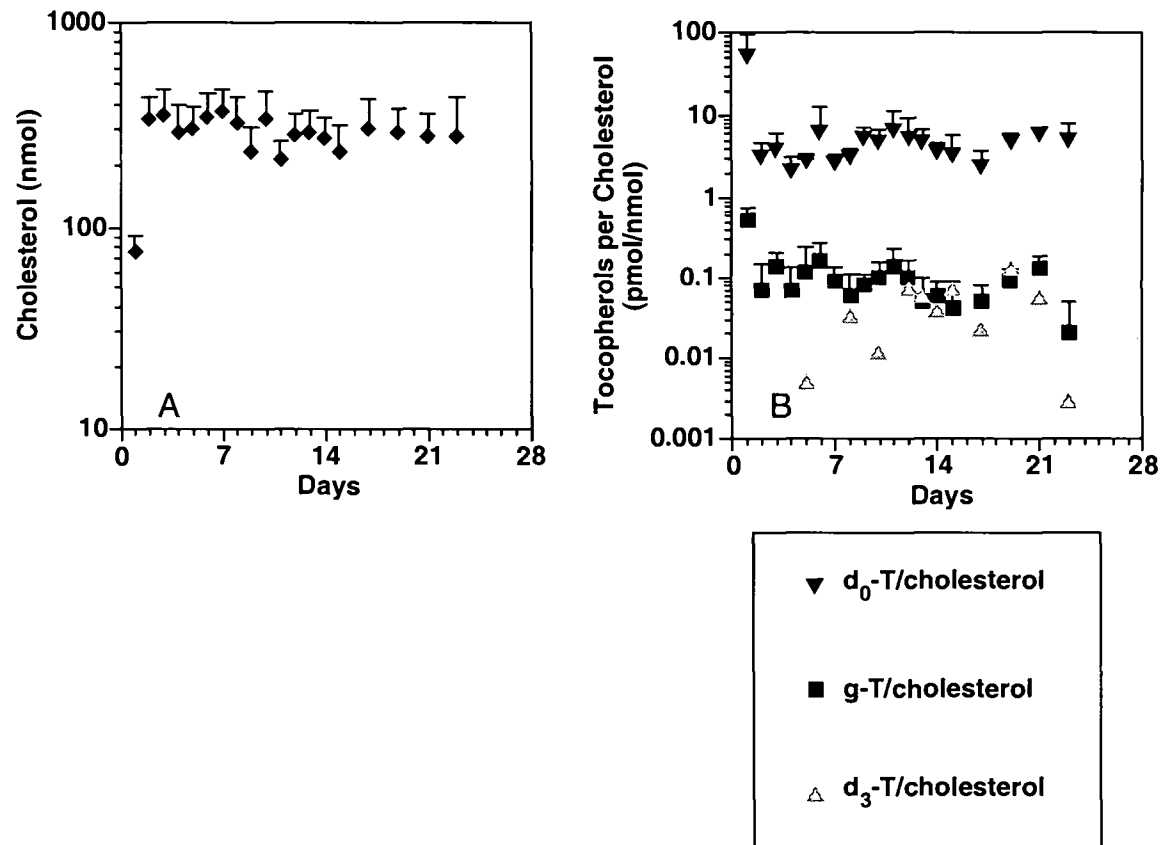


Figure 5. Sebum cholesterol (A) and tocopherols (B)  
 Shown are the mean + standard deviations in sebum secretions collected during the study. Tocopherols ( $\gamma$ -tocopherol (g-T),  $d_0$  ( $d_0$ -T) and  $d_3$ - $\alpha$ -tocopherols ( $d_3$ -T)) are reported per cholesterol.

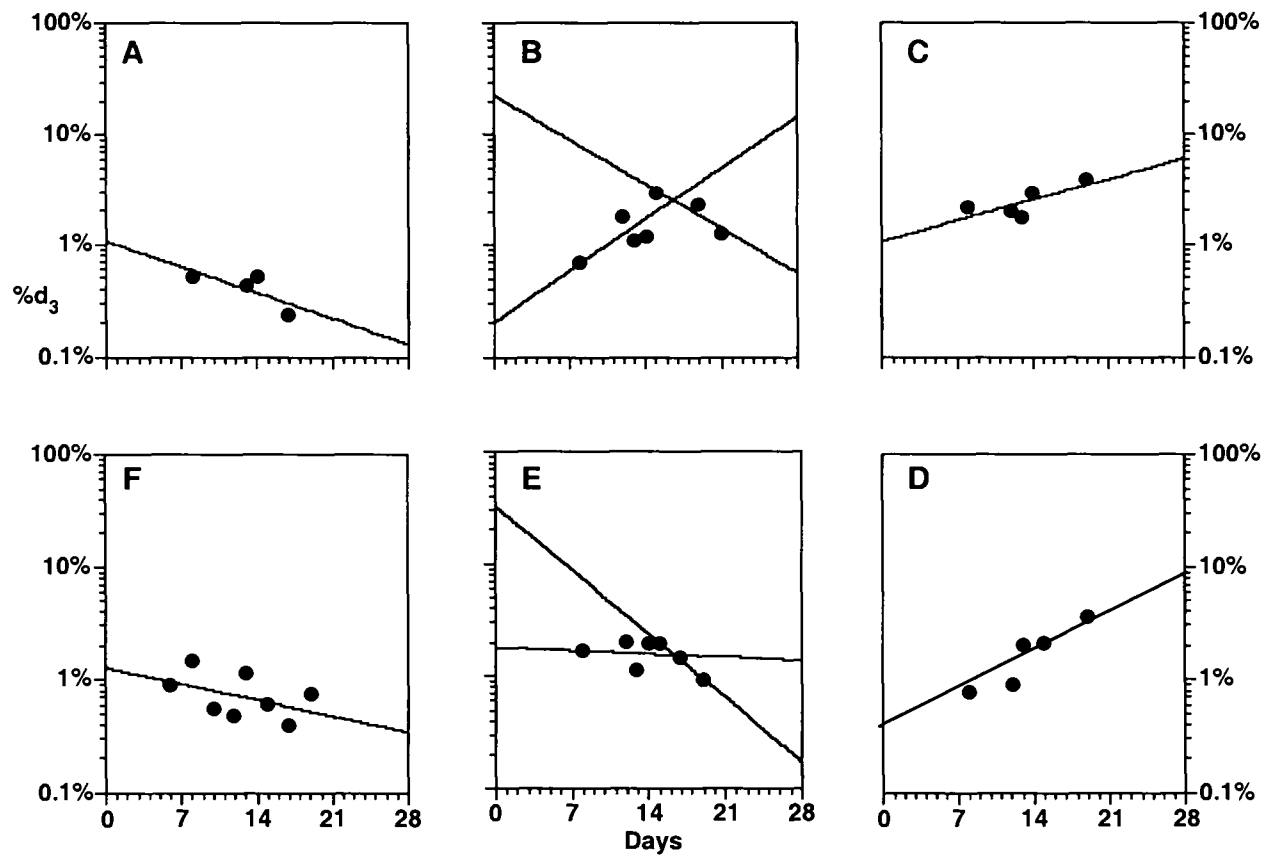


Figure 6. Sebum percent  $d_3$ - $\alpha$ -tocopherol (%  $d_3$ ) showing three patterns. Subjects A and F, B and E, C and D show differing responses to deuterated vitamin E supplementation.

### **Comparisons between Skin and Plasma Percent d<sub>3</sub>- $\alpha$ -Tocopherol**

The average plasma percent d<sub>3</sub> peaked at day 8 (the end of the supplementation), while sebum percent d<sub>3</sub> only just appeared at day 8 and increased on average until day 19 (**Figure 7**). Exponential rates were calculated from these average data. The plasma percent d<sub>3</sub> decreased from day 8 at a rate of  $-0.162$ , while sebum percent d<sub>3</sub> increased at a rate of  $0.062$  (Figure 7). On day 19 the plasma and skin curves bisect, suggesting that it takes approximately two weeks for the sebum secretions to equilibrate with plasma vitamin E.

The plasma and skin total  $\alpha$ - to  $\gamma$ -tocopherol ratios were also calculated for each subject. The ratio of  $\alpha$  to  $\gamma$  in the skin compared with the plasma was consistently higher in almost all subjects (**Figure 8**). The mean plasma  $\alpha$ - to  $\gamma$ -tocopherol ratio was  $5.6 \pm 2.7$  and the mean skin ratio was  $22.7 \pm 7.25$  ( $p < 0.01$ ). To normalize the variations between subjects, an analysis of variance (ANOVA) with repeated measures was carried out on logarithmic transformed data.

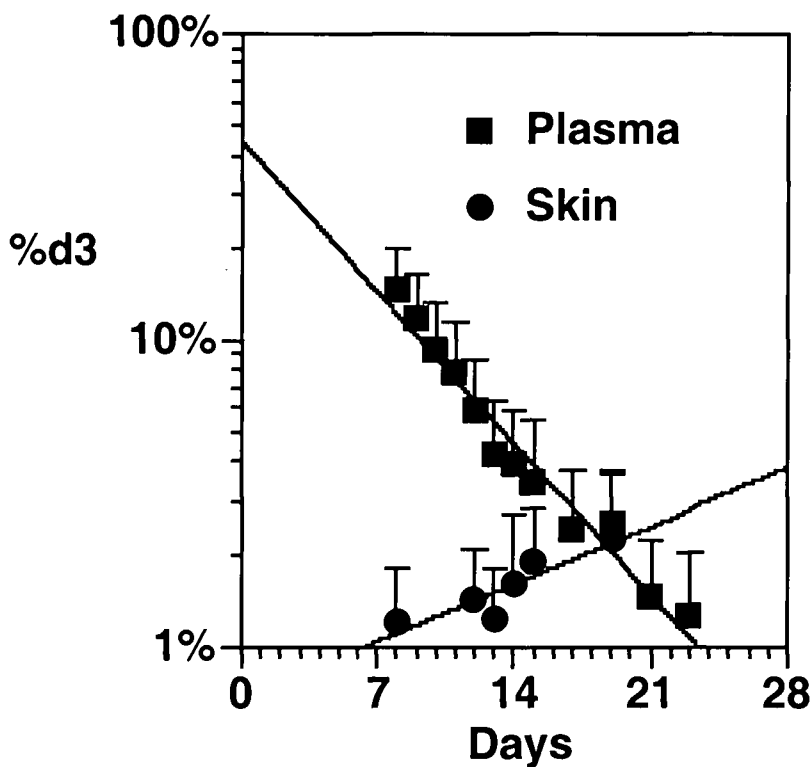


Figure 7. Percent  $d_3$ - $\alpha$ -tocopherol in plasma compared with sebum  
 Comparison of the appearance of  $d_3$ - $\alpha$ -tocopherol in plasma and sebum in subjects supplemented with deuterated vitamin E as described in figure 1. Curve fits were estimated using the average percent  $d_3$  in plasma and skin for all subjects.  
 Plasma  $F(x)=0.447 e(-0.162x)$ ,  $R^2=0.962$   
 Skin  $F(x)=0.0067 e(0.0621x)$ ,  $R^2=0.824$ .

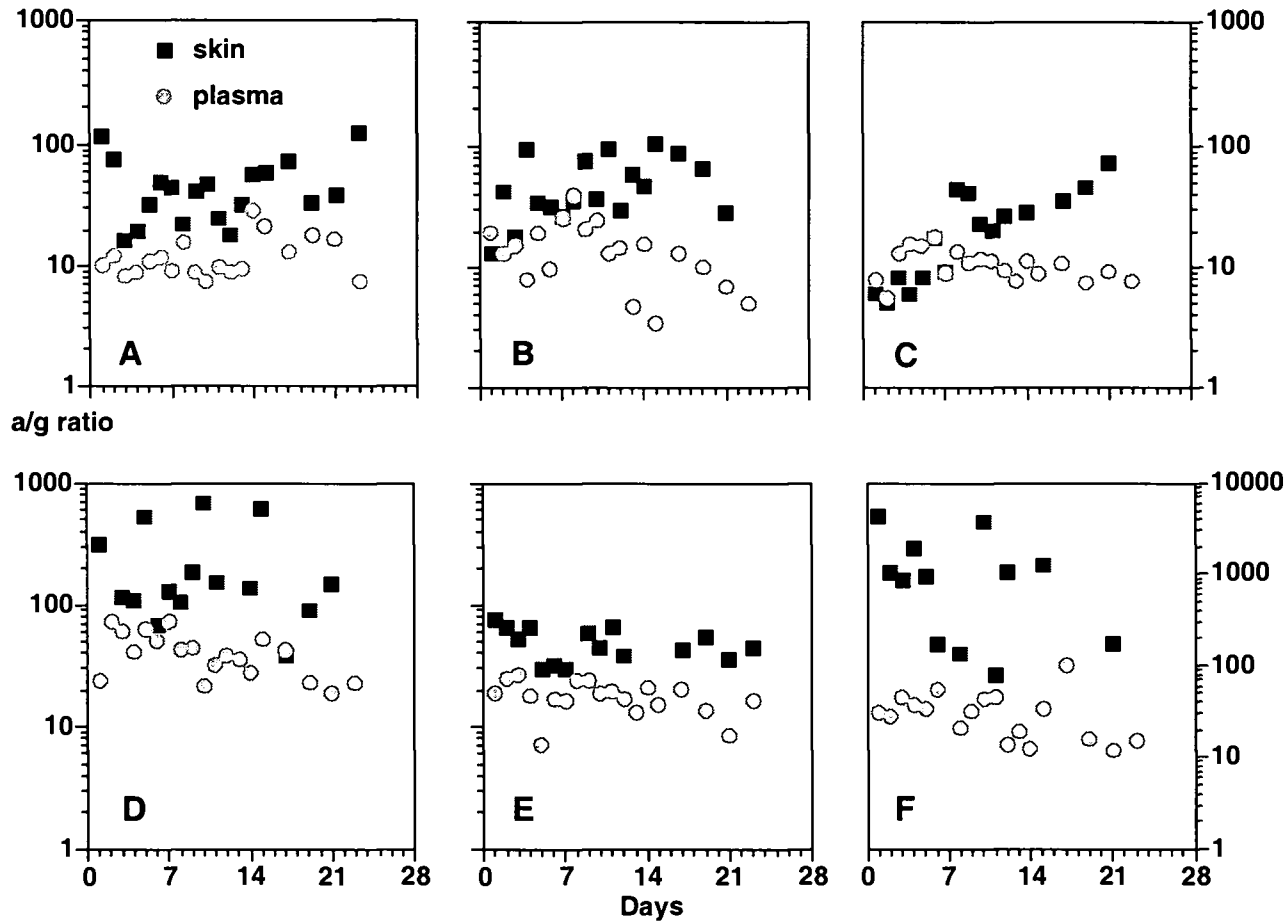


Figure 8. Plasma and skin  $\alpha$ - to  $\gamma$ -tocopherol ratios  
 Plasma (circles) and skin (squares)  $\alpha$ - to  $\gamma$ -tocopherol ratios are shown for each subject. Note the change in y-axis for subject F.



## DISCUSSION

This study reports that the delivery of dietary  $\alpha$ -tocopherol to skin takes approximately 7 days to be detectable in subjects supplemented with 150 mg 1:1  $d_3$ -*RRR*- $\alpha$ - and  $d_6$ -*all rac*- $\alpha$ -tocopheryl acetates consumed daily for 7 days with a meal.  $d_3$ - $\alpha$ -Tocopherol was detected in the sebaceous gland secretions by day 7 of the study and for most subjects peaked one week later. Thus, newly absorbed vitamin E can be detected in the skin secretions, but this was much longer than it took for plasma  $d_3$ - $\alpha$ -tocopherol to be detected.

Plasma vitamin E levels indicate that the supplemental doses were absorbed and the characteristic increases to a plateau during the first week were apparent. The plasma deuterated tocopherols then declined exponentially until the end of the study. This pattern is similar to that described by Burton et al. (9). They found that after supplementing subjects for 8 days with 300 mg of the same mixture of deuterated tocopherol acetates (1:1) that plasma concentrations peaked at 1 week with  $d_3$ - $\alpha$ -tocopherol at 25  $\mu\text{mol/L}$  and  $d_6$ - $\alpha$ -tocopherol at 5  $\mu\text{mol/L}$ ,  $\gamma$ -tocopherol at about 4  $\mu\text{mol/L}$  and  $d_0$ - $\alpha$ -tocopherol at about 19  $\mu\text{mol/L}$ . Our dose and subsequent peaks were lower; we found that plasma concentrations peaked at 1 week with  $d_3$ - $\alpha$ -tocopherol at 4  $\mu\text{mol/L}$  and  $d_6$ - $\alpha$ -tocopherol at 3  $\mu\text{mol/L}$ ,  $\gamma$ -tocopherol at about 2  $\mu\text{mol/L}$  and  $d_0$ - $\alpha$ -tocopherol at about 23  $\mu\text{mol/L}$ . Roxborough et al. (53) in a similar supplementation study of 75-mg

$d_6$ - $\alpha$ -tocopherol found 0.3-12.4  $\mu\text{mol/L}$  in the plasma by 12 hours, indicating the wide variability in plasma response to the deuterated vitamin E. Our deuterated  $\alpha$ -tocopherol concentrations are within these ranges. The  $\alpha$ - and  $\gamma$ -tocopherol concentrations found in our subjects were also similar to those in previous studies; mean plasma  $\alpha$ -tocopherol levels of 28.6  $\mu\text{mol/L}$  in control subjects (44), and in young men to be 19.7  $\mu\text{mol/L}$  (mean age of 31.2 and BMI of 26.0) (46). Together, these data suggest that our supplement protocol and plasma analysis methods were appropriate and accurate when compared to similar supplementation studies.

In the literature, there are very few articles that investigate dietary vitamin E and skin lipid secretions. Theile et al (23) measured sebaceous gland secretion of vitamin E as a route of delivery to skin. Using sebutape patches for sebum collection, they found approximately 55 pmol  $\alpha$ -tocopherol per tape from forehead secretions, and 5 pmol  $\gamma$ - $\alpha$ -tocopherol (23). Cheek sebaceous gland secretions had greater vitamin E concentrations than did samples collected from the arm.

Plasma and sebum  $\alpha$ - to  $\gamma$ -tocopherol ratios were calculated and were found to be much higher in sebum than in plasma. Plasma ratios were approximately 10:1 in our subjects. Handelman et al (54) reported plasma ratios of about 7, while Baker et al (55) found  $\alpha$ - to  $\gamma$ -tocopherol ratios of 8 to 10 prior to supplementation. Both studies also showed a decrease in the  $\gamma$ - to  $\alpha$ -tocopherol ratio following supplementation of  $\alpha$ -tocopherols. One would anticipate that the ratio of  $\alpha$ - to  $\gamma$ -tocopherol would be the same in the skin as the plasma. Surprisingly, in our study, the sebum  $\alpha$ - to  $\gamma$ -tocopherol ratios were 10 fold greater than in the plasma.

This finding is further surprising in that the sensitivity of the LC/MS is greater for  $\gamma$ - than  $\alpha$ -tocopherol (Leonard, S. W. and Traber M. G. unpublished finding).

Taken together these data suggest the probability for an  $\alpha$ -TTP-like mechanism in the skin that preferentially secretes  $\alpha$ -tocopherol into the sebum. Currently, we do not know why the skin secretions are so much slower than the plasma for delivery of vitamin E, but this may be related to tissue turnover and the general physiology of sebum production [Downing, 1982 #30].

Further research will hopefully make these preliminary findings clearer.

The detection of  $\alpha$ -TTP-like protein in the sebaceous gland would be very exciting in support of this data.

## CONCLUSIONS

Since vitamin E is a potent antioxidant and the skin is readily exposed to oxidative stressors it is physiologically significant to understand how protection may occur. The efficacy and mechanism of dietary vitamin E delivery to the skin has remained unknown, but this study has revealed that there is a greater secretion of  $\alpha$ -tocopherol compared with  $\gamma$ -tocopherol in the skin secretions. Future research should be directed toward elucidating this delivery mechanism. Studies designed to determine if there is a preference for the naturally occurring *RRR*- $\alpha$ -tocopherol in the skin could help settle the debate of natural versus synthetic (all racemic) vitamin E for studies. Smokers versus non-smokers' responses to supplementation in skin sebum would elucidate the impact of oxidative stress (smoking) on skin antioxidant delivery systems. Women compared to men and younger versus older individuals would also make very good supplementation studies and provide the scientific community more knowledge on the benefits of dietary supplementation of vitamin E for skin protection. Lastly, labeled  $\gamma$ -tocopherols, *RRR*-, and *SRR*- $\alpha$ -tocopherols used in a supplementation study could better define this regulatory mechanism in the skin.

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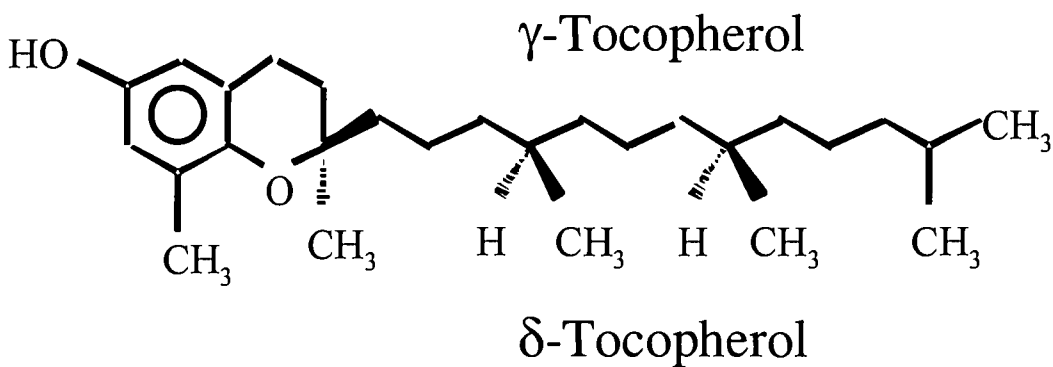
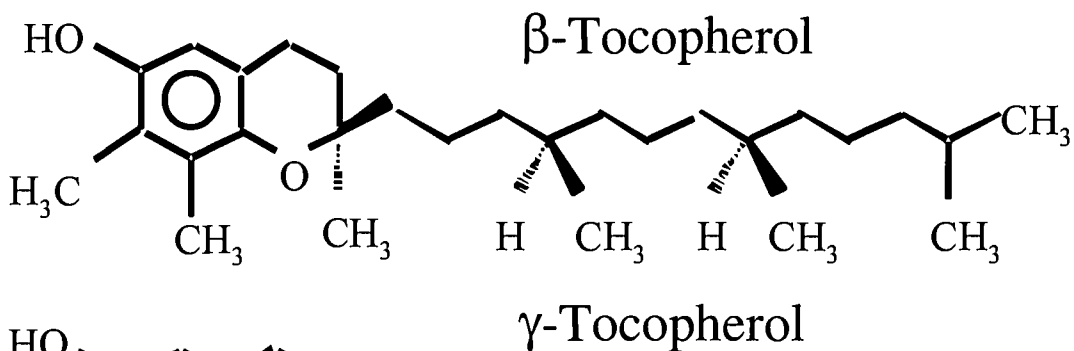
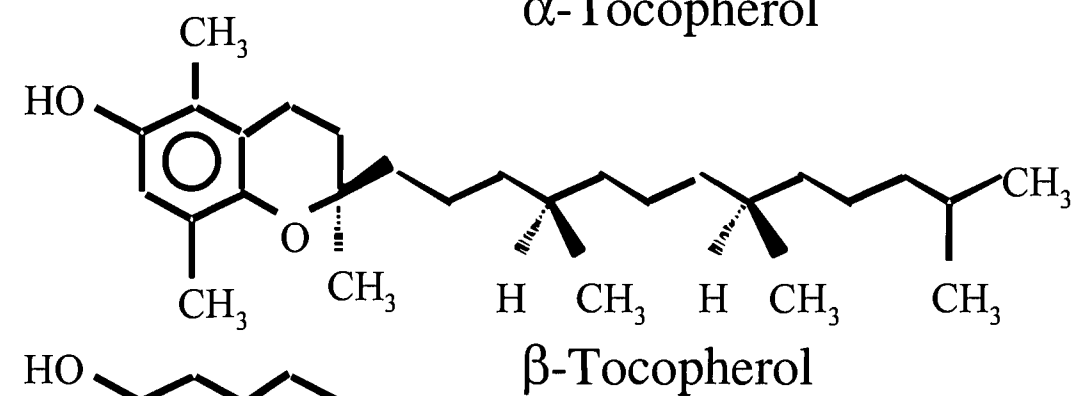
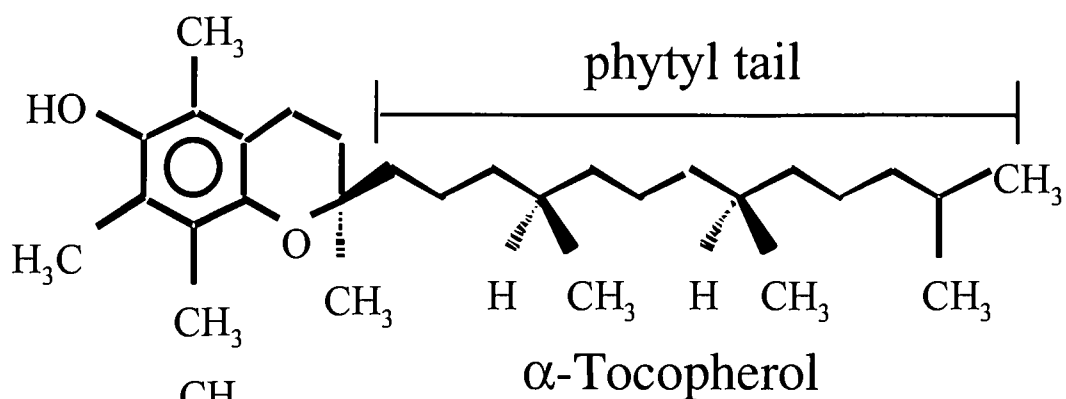
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## **APPENDICES**

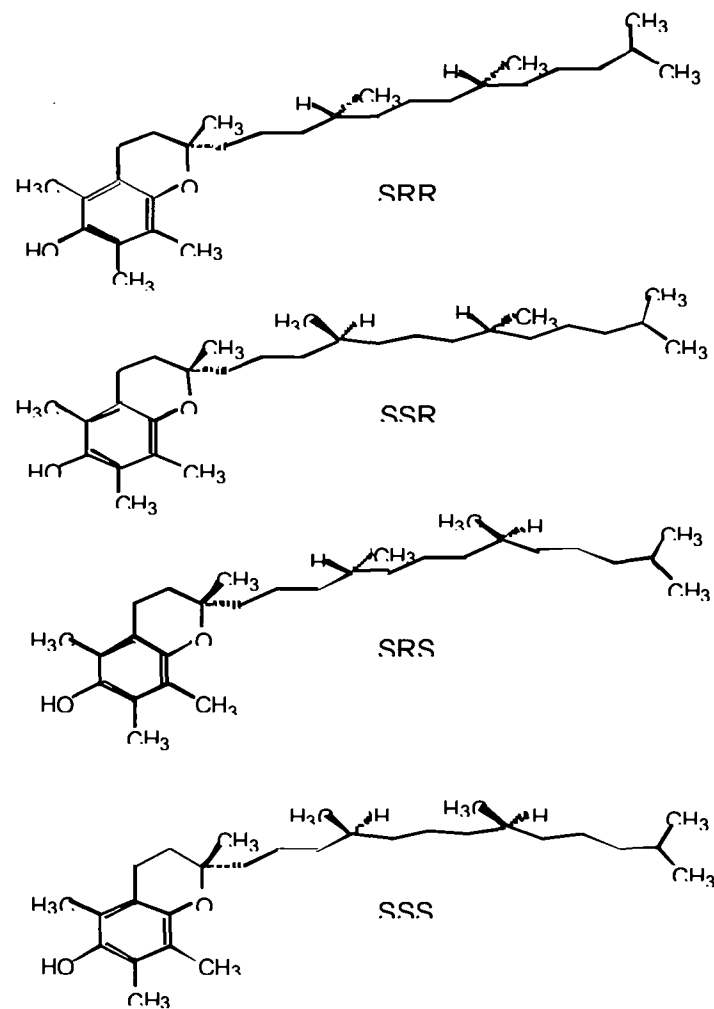
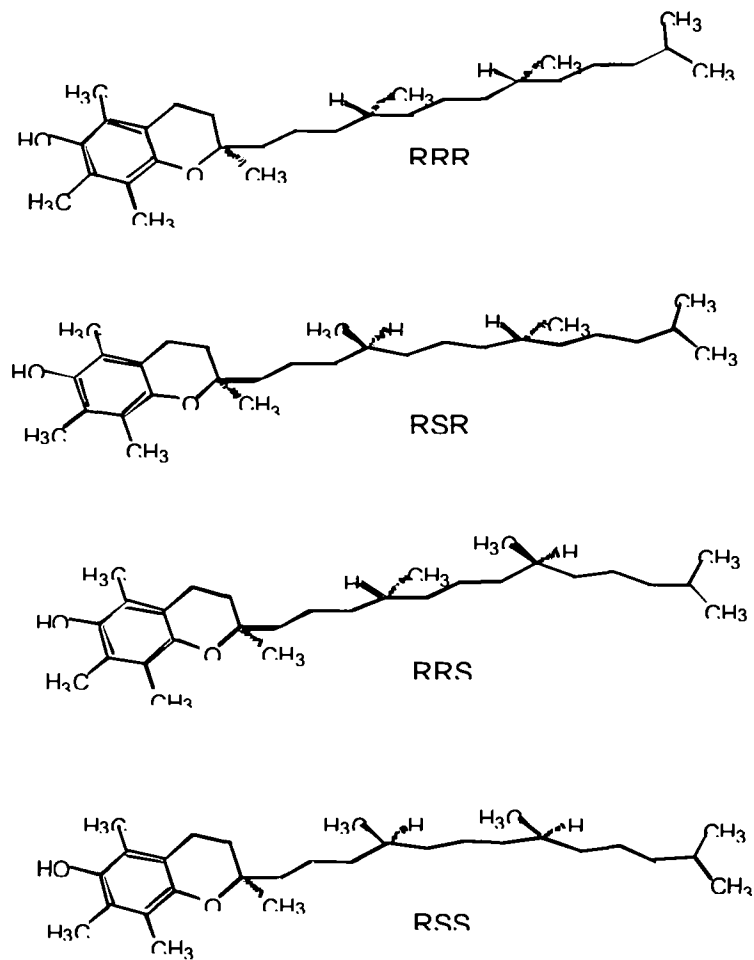
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## APPENDIX A. NATURALLY OCCURING TOCOPHEROLS



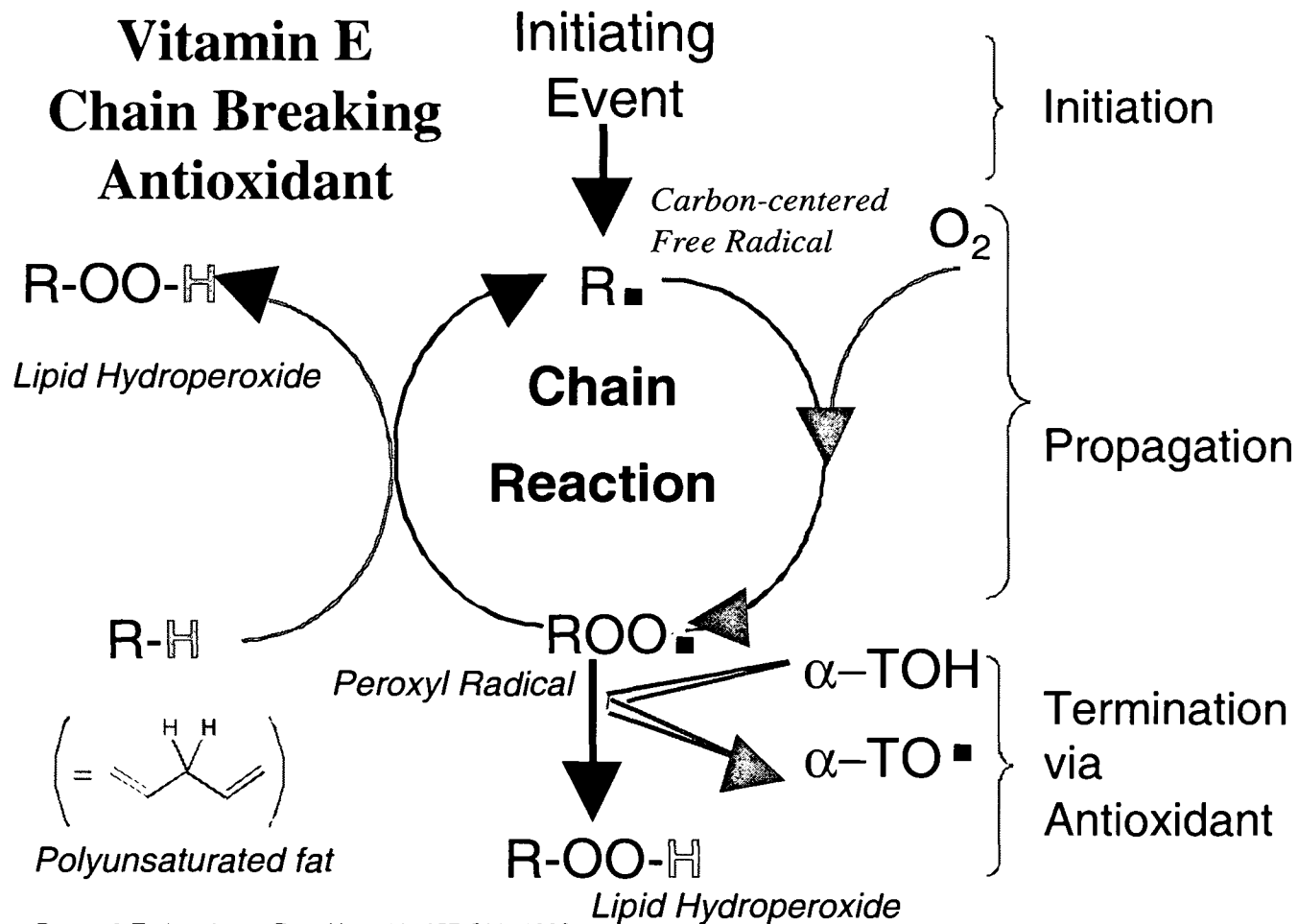
**APPENDIX B. SYNTHETIC  $\alpha$ -TOCOPHEROL  
STEREoisomers**



Appendix B. Synthetic  $\alpha$ -Tocopherol Stereoisomers  
Adapted from Brigelius-Flohé and Traber 1999.

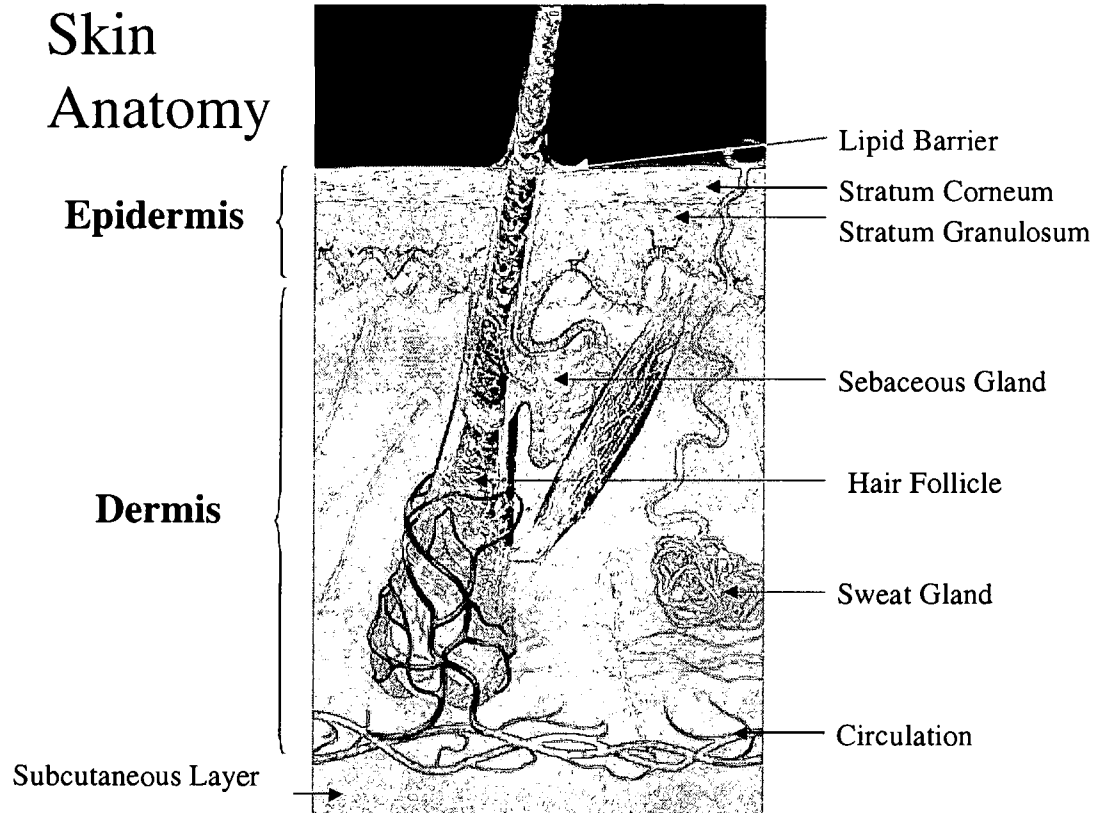
## **APPENDIX C. VITAMIN E: CHAIN BREAKING ANTIOXIDANT**





Burton & Traber Annu. Rev. Nutr. 10: 357-382; 1990

Appendix C. Vitamin E: Chain Breaking Antioxidant

**APPENDIX D. DIAGRAM OF SKIN**

Adapted from the Skin Care Forum at [www.scf-online.com/english/24\\_e/images24\\_e/sebum\\_1\\_24.jpg](http://www.scf-online.com/english/24_e/images24_e/sebum_1_24.jpg).

## APPENDIX E. OSU COMMITTEE FOR THE PROTECTION OF HUMAN SUBJECTS - APPROVAL

RESEARCH OFFICE



**OREGON  
STATE  
UNIVERSITY**

312 Kerr Administration Bldg.  
Corvallis, Oregon  
97331-2140

Telephone  
541-737-8008  
Fax  
541-737-3093  
Internet  
Laura.Lincoln@orst.edu

Principal Investigator:

The following project has been approved for continuation under the guidelines of Oregon State University's Committee for the Protection of Human Subjects and the U.S. Department of Health and Human Services:

Principal Investigator(s):     Maret Traber

Student's Name (if any):

Department:                     Linus Pauling Institute

Source of Funding:             Nestle

Project Title:                   Regulation of Skin Vitamin E by Oxidative  
Stress (continuation)

Comments:                       This approval for continuation is valid for one  
year from the date of this letter.

Sincerely,

Warren N. Suzuki, Chair  
Committee for the Protection of Human Subjects  
(Education, x7-6393, suzukiw@orst.edu)

Date: 04/14/00

## APPENDIX F. CONSENT FORM

Page 1 of 4

LINUS PAULING INSTITUTE



OREGON STATE UNIVERSITY  
571 Weniger Hall, Corvallis, Oregon 97331-6512  
Telephone 541-737-7977, Fax 541-737-5077

July 26, 2001

### CONSENT TO PARTICIPATE IN A RESEARCH STUDY

---

**A. TITLE OF THE RESEARCH PROJECT:**Regulation of Human Skin Vitamin E by Oxidative Stress

---

**B. INVESTIGATORS**

Maret G. Traber, Ph.D.  
Associate Professor  
Department of Nutrition and Food  
Management  
Linus Pauling Institute  
571 Weniger Hall  
Oregon State University  
Corvallis, OR 97330  
Phone: 541-737-7977  
Fax: 541-737-5077  
email: maret.traber@orst.edu

Heather Vaule  
Graduate Student

541-737-8004

---

**C. PURPOSE OF THE RESEARCH PROJECT:**

You are being asked to participate in a research study. We hope to learn whether smoking cigarettes destroys the bodies' supply of vitamin E. For this study we need both smokers and non-smokers. If you do not smoke, we do not want you to smoke. If you do smoke, or have smoked in the past, you will be asked to discontinue smoking for 1 h prior to blood drawing. You are one of 20 subjects to be enrolled in this study.

---

**D. PROCEDURES****1. PRE-STUDY SCREENING.**

You have been asked to come to this pre-study screening to learn about our study and to provide a fasting blood sample. If you have eaten anything besides water for the past 12 h you are not eligible to donate blood. After you have read this form and signed it, we will measure your blood pressure, your height and weight, and a blood sample will be drawn to measure your blood cell count and whether you have normal levels of blood cholesterol and enzymes. If you have normal blood chemistry and are healthy and fit our study criteria, we will call you within 1 week to invite you to participate in our vitamin E study and we will assign you to a participation group.

Participant's Initials \_\_\_\_\_

## APPENDIX F.    CONSENT FORM - CONTINUED

Page 2 of 4

### 2. WHAT PARTICIPANTS WILL DO DURING THE STUDY.

We will invite you to the Linus Pauling Institute room 573 Weniger Hall.

Every day for 1 week, we will provide to you a capsule to eat at the end of your breakfast. This capsule contains vitamin E that has been specially tagged with deuterium to make it heavier, so we can measure it. The deuterium NOT radioactive and is not harmful in anyway. Except for the label it is identical to the vitamin E you purchase at the drug store.

Every morning for the first two weeks, we will ask you to return to the Linus Pauling Institute early in the morning (about 7 AM) after fasting 12 h. We will then take a blood sample from a vein in your arm. A needle will be inserted in the vein and 15 ml (1 tbs.) blood removed. We will then provide you with a breakfast. We will also place four pieces of tape on your forehead and ask you to wear this tape for 1 h. The tape will collect skin secretions, which we will analyze. The tape is not very sticky, so will not hurt when it is removed. We will ask you not to wear make up or apply skin creams to your forehead during the study.

Then we will ask you to come to the Linus Pauling Institute in the morning twice a week for the two weeks for blood drawing and skin oil analysis. (A total of 19 blood samples will be taken). The total blood drawn will be about a cup.

### CALENDAR

Week	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
1		<ul style="list-style-type: none"> <li>• blood draw</li> <li>• skin oils</li> <li>• breakfast with vitamin E</li> </ul>	<ul style="list-style-type: none"> <li>• blood draw</li> <li>• skin oils</li> <li>• breakfast with vitamin E</li> </ul>	<ul style="list-style-type: none"> <li>• blood draw</li> <li>• skin oils</li> <li>• breakfast with vitamin E</li> </ul>	<ul style="list-style-type: none"> <li>• blood draw</li> <li>• skin oils</li> <li>• breakfast with vitamin E</li> </ul>	<ul style="list-style-type: none"> <li>• blood draw</li> <li>• skin oils</li> <li>• breakfast with vitamin E</li> </ul>	<ul style="list-style-type: none"> <li>• blood draw</li> <li>• skin oils</li> <li>• breakfast with vitamin E</li> </ul>
2	<ul style="list-style-type: none"> <li>• blood draw</li> <li>• skin oils</li> <li>• breakfast with vitamin E</li> </ul>	<ul style="list-style-type: none"> <li>• blood draw</li> <li>• skin oils</li> <li>• breakfast</li> </ul>	<ul style="list-style-type: none"> <li>• blood draw</li> <li>• skin oils</li> <li>• breakfast</li> </ul>	<ul style="list-style-type: none"> <li>• blood draw</li> <li>• skin oils</li> <li>• breakfast</li> </ul>	<ul style="list-style-type: none"> <li>• blood draw</li> <li>• skin oils</li> <li>• breakfast</li> </ul>	<ul style="list-style-type: none"> <li>• blood draw</li> <li>• skin oils</li> <li>• breakfast</li> </ul>	<ul style="list-style-type: none"> <li>• blood draw</li> <li>• skin oils</li> <li>• breakfast</li> </ul>
3	<ul style="list-style-type: none"> <li>• blood draw</li> <li>• skin oils</li> <li>• breakfast</li> </ul>	<ul style="list-style-type: none"> <li>• blood draw</li> <li>• skin oils</li> <li>• breakfast</li> </ul>		<ul style="list-style-type: none"> <li>• blood draw</li> <li>• skin oils</li> <li>• breakfast</li> </ul>		<ul style="list-style-type: none"> <li>• blood draw</li> <li>• skin oils</li> <li>• breakfast</li> </ul>	
4		<ul style="list-style-type: none"> <li>• blood draw</li> <li>• skin oils</li> <li>• breakfast</li> </ul>		<ul style="list-style-type: none"> <li>• blood draw</li> <li>• skin oils</li> <li>• breakfast</li> </ul>			

Participant's Initials \_\_\_\_\_

## APPENDIX F. CONSENT FORM – CONTINUED

Page 3 of 4

### 3. FORESEEABLE RISKS OR DISCOMFORTS.

Risks of blood drawing include some discomfort, bruising and rarely infection. Skin oil removal by taping is not harmful and does not involve any discomfort.

### 4. BENEFITS TO BE EXPECTED FROM THE RESEARCH.

Results of this study will increase our knowledge about how cigarette smoking affects skin antioxidant nutrients. You will not be monetarily compensated for the initial screening blood draw, but you will learn of your blood testing results as part of our screening study. You will receive breakfasts as indicated in the calendar. You will be paid for the samples of blood and skin oils we take during the study.

---

### E. CONFIDENTIALITY

The results from this study will be published in the scientific literature. The identity of the subjects will be kept confidential; only a code will be published. Absolute confidentiality cannot be guaranteed, since research documents are not protected from subpoena.

---

### F. COMPENSATION

You will receive \$10 per blood draw and skin oil sample; a total of \$200 (including a \$20 bonus) if all of the blood and skin oil samples are obtained. You will be paid only for the blood draws and skin oil samples you have given.

---

### G. COSTS

I understand that Oregon State University does not provide a research subject with compensation or medical treatment in the event the subject is injured as a result of participation in the research project.

---

### H. VOLUNTARY PARTICIPATION STATEMENT

You may change your mind about being in the study and quit after the study has started. Your participation is completely voluntary and you may either refuse to participate or withdraw from the study at any time without penalty or loss of benefits to which you are otherwise entitled. If you withdraw from the study before it is completed, the amount of money will be less than the full amount.

---

### I. PRINCIPAL INVESTIGATOR'S DISCLOSURE OF PERSONAL OR FINANCIAL INTERESTS IN THE RESEARCH STUDY AND SPONSOR

Your investigators have NO financial interest in this research.

---

### J. QUESTIONS

If you have any questions, please ask us now. If you have any additional questions later concerning the research study or specific procedures, contact Prof. Traber (541-737-7077), who will answer them. Any other questions can be directed to IRB Coordinator, OSU Research Office 541-737-8008.

Participant's Initials \_\_\_\_\_

**APPENDIX F. CONSENT FORM – CONTINUED**

Page 4 of 4

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**K. CONSENT**

**YOUR SIGNATURE, BELOW, WILL INDICATE THAT YOU HAVE DECIDED TO VOLUNTEER AS A RESEARCH SUBJECT AND THAT YOU HAVE READ AND UNDERSTOOD THE INFORMATION PROVIDED ABOVE.**

Signature of participant or legal representative \_\_\_\_\_ Date \_\_\_\_\_

Subject's Printed name \_\_\_\_\_

Subject's Present address \_\_\_\_\_

\_\_\_\_\_

Subject's phone number \_\_\_\_\_

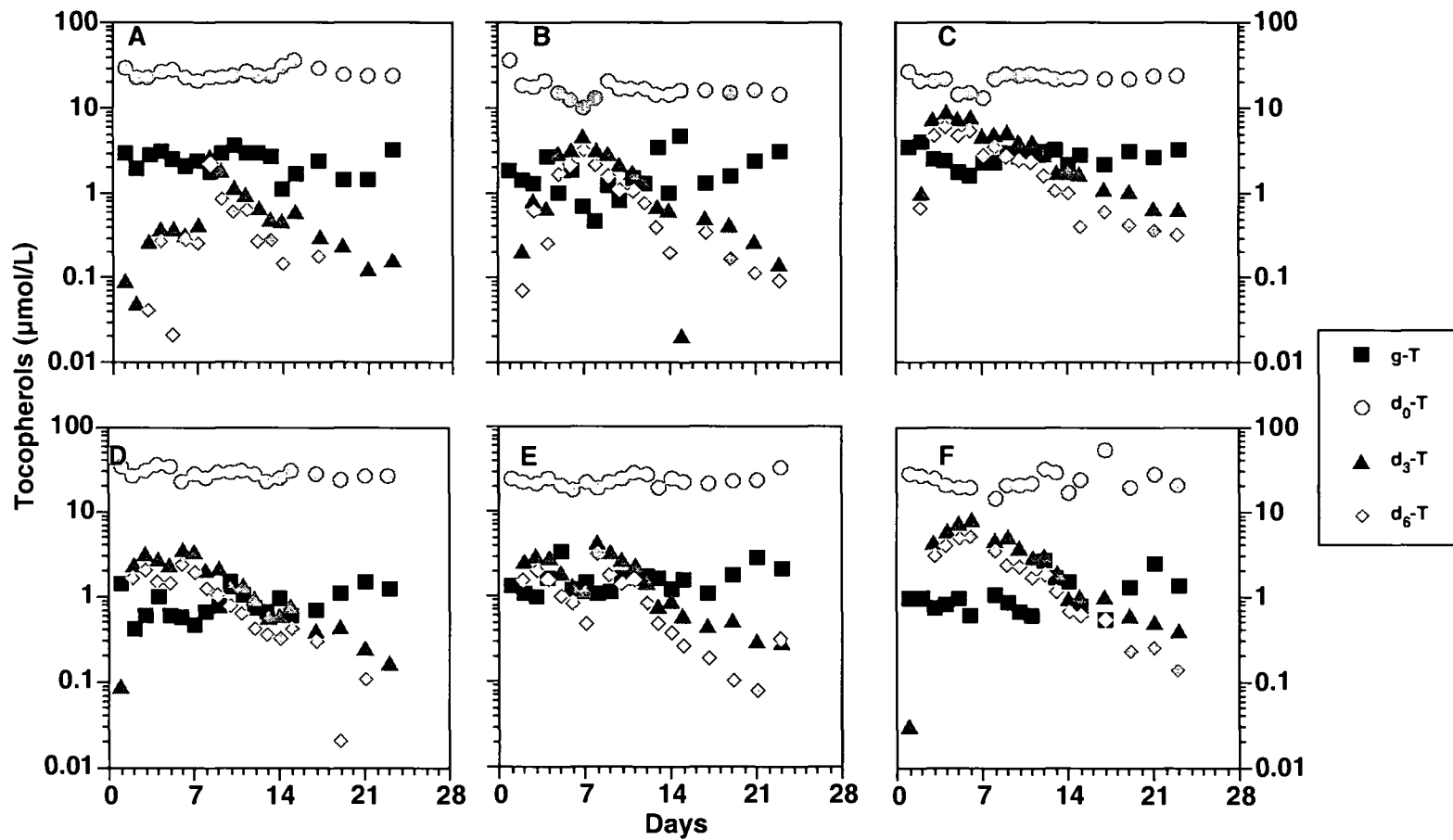
Signature of Investigator \_\_\_\_\_ Date \_\_\_\_\_

**You will be given a signed and dated copy of this form to keep.**

Participant's Initials \_\_\_\_\_

## **APPENDIX G. INDIVIDUAL PLASMA TOCOPHEROL CONCENTRATIONS**

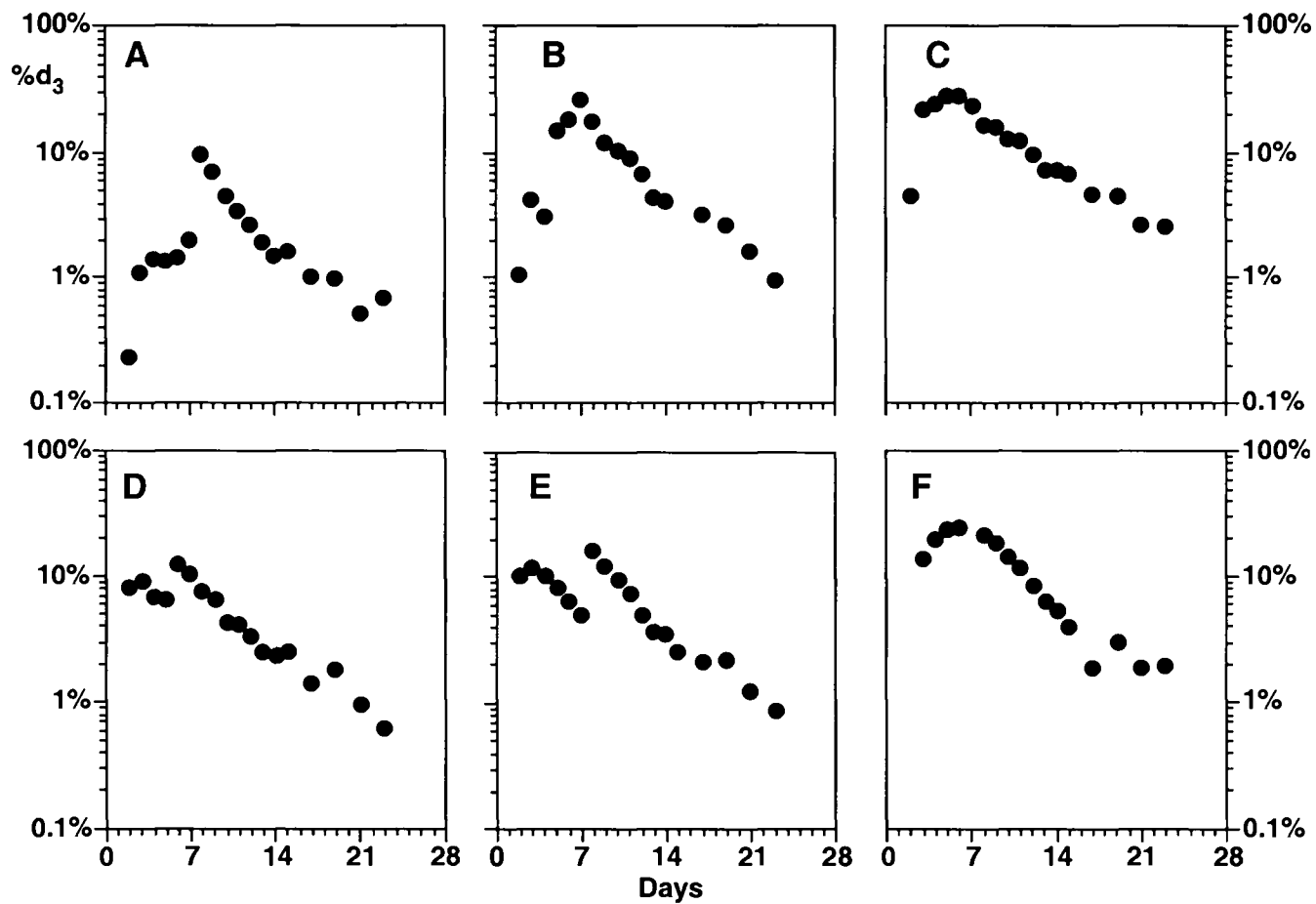




#### Appendix G. Individual plasma tocopherol concentrations

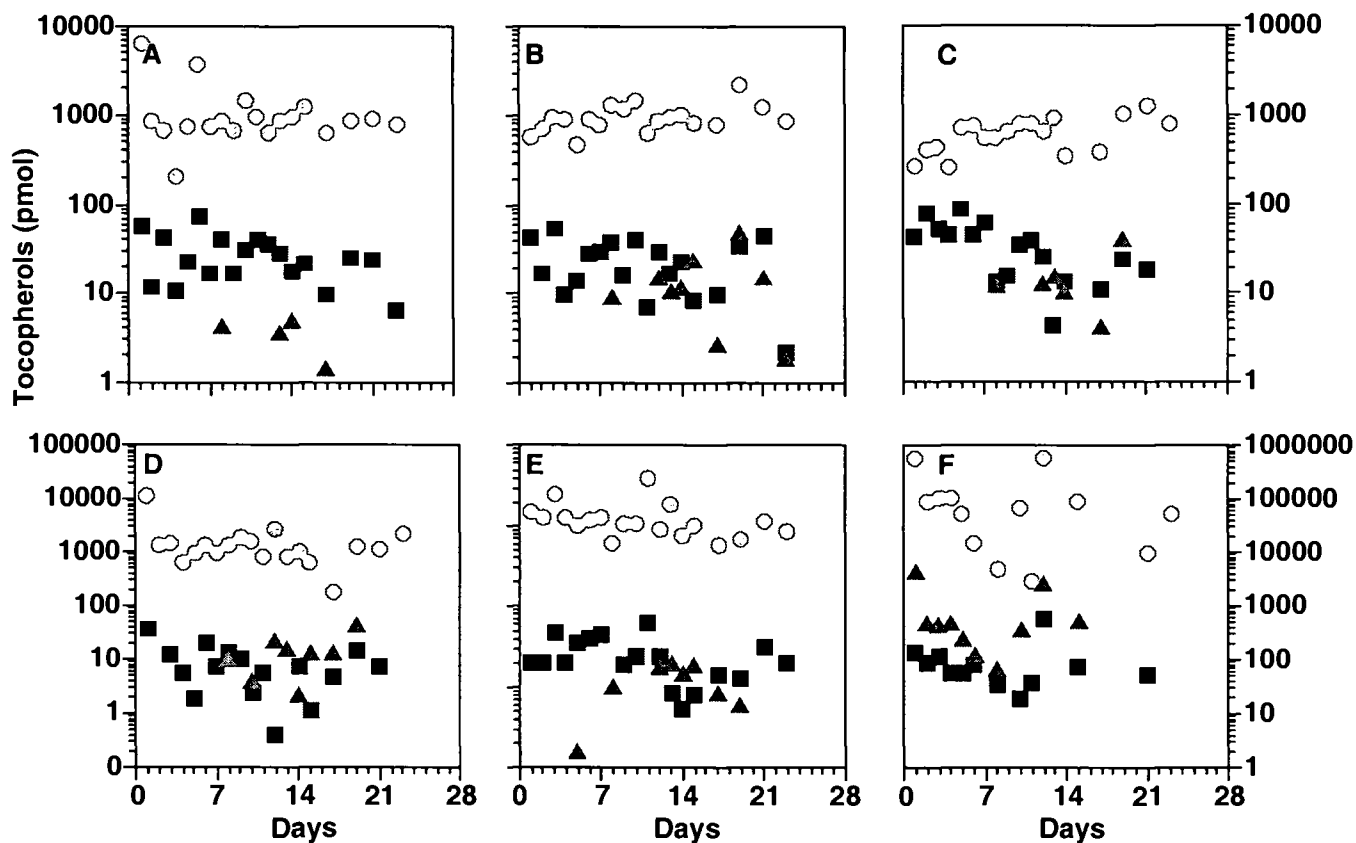
Each subject consumed a daily dose of 150 mg of a 1:1 *RRR*- $\alpha$ -[5-(C<sup>2</sup>H<sub>3</sub>)]-(d<sub>3</sub>) and *all rac*- $\alpha$ -[5,7-(C<sup>2</sup>H<sub>3</sub>)<sub>2</sub>]- (d<sub>6</sub>) tocopheryl acetates with breakfast for 7 days. Shown are the plasma  $d_0$ - $\alpha$ -tocopherol (T) (circles),  $\gamma$ -T (squares),  $d_3$  (triangles) and  $d_6$  (diamonds) in the 6 subjects (A-F).

**APPENDIX H. INDIVIDUAL SUBJECTS' PLASMA  
PERCENT d<sub>3</sub> α-TOCOPHEROL**



Appendix H. Individual subjects' plasma percent  $d_3$   $\alpha$ -tocopherol  
 Each subject consumed a daily dose of 150 mg of *RRR*- $\alpha$ -[5-( $C_2H_3$ )]-( $d_3$ ) and *all rac*- $\alpha$ -[5,7-( $C_2H_3$ )<sub>2</sub>]-( $d_6$ ) tocopheryl acetates with breakfast. Shown are the percent  $d_3$  for each subject (A-F).

## **APPENDIX I.    INDIVIDUAL SUBUM TOCOPHEROLS**



#### Appendix I. Individual sebum tocopherols

Each subject consumed a daily dose of 150 mg of a 1:1 *RRR*- $\alpha$ -[5-(C<sup>2</sup>H<sub>3</sub>)]-(d<sub>3</sub>) and *all rac*- $\alpha$ -[5,7-(C<sup>2</sup>H<sub>3</sub>)<sub>2</sub>]- (d<sub>6</sub>) tocopheryl acetates with breakfast for 7 days. Shown are the sebum d<sub>0</sub>- $\alpha$ -tocopherol (T) (circles),  $\gamma$ -T (squares), and d<sub>3</sub> (triangles) in the daily collections from each of the 6 subjects (A-F). Note the maximum y-axis for subjects B & E was 10,000.