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Maret G. Traber

In rats, sesame lignans increase plasma γ-tocopherol concentrations and inhibit γ-tocopherol degradation to its metabolite γ-carboxyethylhydroxychroman (γ-CEHC). To test if sesame lignan consumption inhibits γ- tocopherol metabolism in humans, muffins prepared with either corn oil (control) or sesame oil and an equimolar mixture of deuterium labeled d_6-α- and d_2-γ- tocopheryl acetates were administered to male (n=5) and female (n=5) volunteers. Tocopherol and CEHC concentrations were followed for 72 h. Sesame lignan consumption significantly increased plasma d_2-γ-TOH concentrations ($p<0.05$). In men, sesame lignans increased plasma d_2-γ-tocopherol areas under the curve (AUC; sesame oil: 34.3 ± 4.6; corn oil: 28.9 ± 3.3 μmol/L·h, $p<0.01$) and reduced d_2-γ-CEHC AUCs ($p<0.05$). In men, differences in urinary d_2-γ-CEHC AUCs did not reach statistical significance (AUCs for 24 h, corn oil: 11.2 ± 3.0 μmol/g creatinine·h; vs sesame oil: 5.0 ± 1.5). In women, sesame lignan consumption did not alter plasma tocopherol CEHC concentrations but reduced urinary d_2-γ-CEHC excretion (AUCs for 24 h, corn oil: 19.3 ± 4.9 μmol/g creatinine·h; and sesame oil: 7.7 ± 2.0, $p <$

0.05). These data suggest that sesame lignans alter γ-tocopherol metabolism differently in men and women. Further research is needed to assess the mechanism involved in these differences.

Sesame Oil Increases Plasma γ-Tocopherol and Inhibits γ-Tocopherol Metabolism in Humans

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Sangeun Lee, Author

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CONTRIBUTION OF AUTHORS

Drs. Jan Frank, Jeffrey K. Atkinson, and Maret G. Traber were involved with the design the study. Dr. Jan Frank and Scott W. Leonard participated in the sample collection and analysis. Drs. Jan Frank, Afaf Kamal-Eldin, and Maret G. Traber assisted in the writing, editing, and reviewing of the manuscript.

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INTRODUCTION

Hypothesis

Sesame oil (containing the lignans, sesamin and sesamolin) consumption inhibits tocopherol ω-hydroxylase activity, which reduces the metabolism of γ-tocopherol. Sesame oil consumption will increase plasmą γ-tocopherol concentrations and decrease plasma and urinary γ-tocopherol metabolite 2,7,8-trimethyl-2-(β-carboxyethyl)-6-hydroxychromaṇ γ-CEHC) concentrations. See Appendix A : Tocopherol ω-Hydroxylase Activity.

Specific Aims

AIM 1: Determine the inhibitory effect of sesame oil consumption on plasma vitamin E metabolism.

Plasma deuterium-labeled vitamin E (α-[5,7-(C^2H_3)$_2$]tocopherol; d_6-α-tocopherol, and γ-[3,4-^{2}H] tocopherol; d_2-γ-tocopherol) and unlabeled vitamin E concentrations and

their metabolites (α-CEHC and γ-CEHC) will be measured in subjects following consumption of deuterated vitamin E (α-[5,7-(C^2H_3)$_2$] tocopheryl acetate; d_6-α-tocopheryl acetate, and γ-[3,4-^{2}H] tocopheryl acetate; d_2-γ-tocopheryl acetate) and corn oil muffins for breakfast (control group) and then after a washout period, consumption of deuterated vitamin E and sesame oil muffins (sesame group).

AIM 2: Verify the inhibitory effect of sesame oil consumption on vitamin E metabolism by determination of urinary excretion of vitamin E metabolites.

Deuterium-labeled and unlabeled α-CEHC and γ-CEHC will be measured in urine. Then the concentrations in the corn oil and the sesame oil groups will be compared.

LITERATURE REVIEW

Overview

The National Health and Nutrition Examination Survey (NHANES) III reported that approximately 40% of the U.S. population is taking vitamin or mineral supplements (1). Vitamin E is one of the most popular supplements in the U.S.(2).

Vitamin E is a lipid soluble vitamin with a chain reaction breaking antioxidant function. Most studies in regard to human disease have been done with α-tocopherol (3-6). However, γ-tocopherol has recently received more attention because of the unique benefits of γ-tocopherol on human health (7-9). This proposal will describe background information about vitamin E and about sesame lignans, which have been reported to alter vitamin E metabolism, and thus, hypothetically, increase vitamin E concentrations.

Vitamin E

Antioxidant properties and structure of vitamin E

Vitamin E, discovered in 1922 by Evans and Bishop (10), is a lipid soluble

vitamin with a chain reaction breaking antioxidant function in plasma and tissues (11). Plants synthesize eight different vitamin E forms, including: α-, β-, γ-, and δ-tocopherols and α-, β-, γ-, and δ-tocotrienols (12). All tocopherols and tocotrienols consist of a chromanol ring and a varying number of methyl groups on the chromanol ring. The difference between tocopherols and tocotrienols is that tocopherols have a saturated tail but tocotrienols have an unsaturated tail.

Vitamin E is a peroxyl radical scavenger and protects polyunsaturated fatty acids within membranes and plasma lipoproteins (12). The phenolic hydroxyl group of tocopherols reacts with the peroxyl radicals to form the lipid hydroperoxide and the tocopheroxyl radical (13). The tocopheroxyl radical can be recycled to its unoxidized form by other antioxidants such as vitamin C, and ubiquinol, and thiol like glutathione (13). In addition, vitamin E has 1000 times faster reactivity to peroxyl radicals than do polyunsaturated fatty acids (14). Therefore, vitamin E may effectively prevent propagation of lipid peroxidation.

The chemical synthesis of α-tocopherol results in an equal mixture of eight different stereoisomers (*RRR*, *RSR*, *RRS*, *RSS*, *SRR*, *SSR*, *SRS*, and *SSS*) (13). Among the eight stereoisomers of α-tocopherol, *RRR*-α-tocopherol and the other 2*R*-stereoisomers are maintained in human plasma and tissues (12). The *RRR*-stereoisomer has approximately twice the availability of the all rac forms (15). Thus, the consumption of 15 mg/day of *RRR*-α-tocopherol or 2*R*-stereoisomeric forms of α-tocopherol can meet the current RDA, 15 mg/day of α-tocopherol, while 30 mg/day of all rac-α-tocopherol should be consumed (12).

The absorption and regulation of vitamin E

Vitamin E absorption begins when micelles are formed in the intestine lumen. Biliary and pancreatic secretions are required for micell formation, a process that allows vitamin E uptake into enterocytes. In the enterocyte, vitamin E is incorporated into chylomicron that enters into blood stream through lymph. Vitamin E studies have reported that the discrimination between *RRR*- and *SRR*-α-tocopherols, α-and γ-tocopherols, or *RRR*- and all rac-α-tocopherols does not occur during intestinal absorption and secretion in chylomicrons (16, 17).

In the circulation, chylomicrons are catabolized to chylomicron remnants by lipoprotein lipase, allowing non-specific transfer of vitamin E both to tissues and to other circulating lipoproteins (13). Chylomicron remnants containing vitamin E are taken up by the liver via mechanisms mediated by lipoprotein receptors (18, 19).

RRR α-tocopherol is preferentially secreted from the liver in nascent very low density lipoproteins (VLDLs) in a process mediated by the hepatic α-tocopherol transfer protein (α-TTP) (16, 20). ATP-binding cassette protein A1 has been proposed (ABCA1) to transfer α-tocopherol to α-TTP (21). The different affinities of α-TTP for various vitamin E forms cause the discrimination between them, resulting in the preferential plasma enrichment with α-tocopherol. Plasma α-tocopherol concentrations in humans are about 5 times higher than γ-tocopherol (α-tocopherol, 11 to 37 μmol/L; γ-tocopherol, 2 to 5 μmol/L) and tocotrienol concentrations are a less than 1 μmol/L (22). Thus, liver is critically responsible for the regulation of plasma vitamin E concentrations.

Metabolism and excretion

Vitamin E initially undergoes ω-hydroxylation by cytochrome P450 and then passes through several steps of β-oxidation of the liver phytyl tail (23, 24), resulting in vitamin E metabolites comprised of a shortened phytyl tail and intact chromanol ring (25). Vitamin E metabolites in human urine include both 2,5,7,8-tetramethyl-2-(2'-carboxyethyl)-6-hydroxychriman (α-CEHC) derived from α-tocopherol or α-tocotrienol, and 2,7,8-trimethyl-2-(2'carboxyethyl)-6-hydroxychroman (γ-CEHC) derived from γ-tocopherol or γ-tocotrienol (13). CEHCs have been also found in bile, as well as the urine, of rats (26) and bile was suggested a major route for CEHC excretion (13). CEHC can be sulfated or glucuronidated (27-29). However, it remains unknown as to where these metabolites are formed.

γ-CEHC, a water soluble metabolite of γ-tocopherol, was discovered by Wechter et al. in 1996 as an endogenous natriuretic factor which increases sodium excretion in human urine (30). Due to these findings, γ-CEHC was suggested to aid in the prevention of hypertension and coronary heart disease (31).

The metabolism of α- and γ-tocopherols to α- and γ-CEHCs occurs through a cytochrome P450 (CYP) dependent ω-oxidation (23, 24). Sontag and Parker reported that tocopherol ω-hydroxylase demonstrates higher catalytic activities for γ-tocopherol than for α-tocopherol, and that CYP 4F2 is involved in ω-oxidation of tocopherol in human HepG2 cells (24). On the other hand, CYP 3A has also been suggested to be involved in the tocopherol ω-oxidation process (23, 32, 33).

Various forms of vitamin E have been shown to bind to a nuclear receptor, the pregnane X receptor (PXR) (34). Hypothetically, as a PXR ligand, vitamin E could modify PXR which could up-regulate cytochrome p450 (CYP 3A) activity (35). Vitamin E has been shown to modulate CYP 3A expression. In mice fed diets with varying α- and γ-tocopherol contents, liver α-tocopherol modulated Cyp3a protein expression (35). In rats receiving daily subcutaneous α-tocopherol injections, CYP3A, CYP2B, and CYP2C, which are key xenobiotic metabolizing enzymes, as well as p-glycoprotein involved in biliary xenobiotic excretion, were highly increased, but α-TTP and CYP4F were nor altered (36). This study suggested that extremely high hepatic α-tocopherol concentrations change hepatic proteins involved in metabolism and disposition of xenobiotic agents and supported that high dose of vitamin E may not be accumulated up to the toxic level in vivo.

In addition, Schultz et al. reported that increasing doses of supplemental vitamin E in humans result in increasing urinary excretion of the α-CEHC metabolite (37). As compared with *RRR*-α-tocopherol, all rac-α-tocopherol and other vitamin E forms (including γ-tocopherol and tocotrienols) disappear faster from plasma and are excreted as CEHCs to a greater extent in urine (27, 38, 39). Traber et al. have suggested that vitamin Es are metabolized similarly to xenobiotics in the liver (40).

γ-Tocopherol's bioavailability

γ-Tocopherol is the most abundant form of vitamin E in the American diet, but γ-

tocopherol levels in plasma and tissues are remarkably low (22). The half-life of γ-tocopherol disappearance in plasma is about 15 h (41). The fact that α-TTP has a binding pocket in the structure (20, 42) that preferentially binds only α-tocopherol is a important factor for the low plasma γ-tocopherol concentration. Hosomi et al. have demonstrated that the number of methyl groups and the stereochemistry of the phytyl tail at the point (position 2) on the chromanol ring determine the affinity of the α-TTP for α-tocopherol (43). The study implies that γ-tocopherol structural feature is the critical factor affecting γ-tocopherol bioavailability (43, 44). See Appendix B: Structure of Tocopherols.

Additionally, up to 50% of γ-tocopherol is metabolized to γ-CEHC (27). Sontag and Parker reported that tocopherol ω-hydroxylase demonstrates higher catalytic activities for γ-tocopherol than for α-tocopherol in human HepG2 cell (24). A recent study demonstrated that structural features of vitamin E, the number and position of methyl groups on chromanol ring and saturation of side chain, influence ω-hydroxylation by cellular and microsomal tocopherol-ω-hydroxylase (44). The presence of methyl groups at carbon 5 decreases ω-hydroxylase activity while unsaturation of the side chain increases ω-hydroxylase activity (44). Therefore, the activity of ω-hydroxylase is higher toward γ-tocopherol without the methyl groups at carbon 5 or toward the tocotrienols (44). These findings indicate that the unique structure of γ-tocopherol may be responsible for its lower bioavailability.

γ-Tocopherol's physiologic effect on human health

α-Tocopherol is the only form to meet the human requirement of vitamin E and demonstrated to prevent vitamin E deficiency symptoms in humans (12). However, γ-tocopherol has recently received more attention because of the unique benefits of γ-tocopherol on human health (7-9).

γ-Tocopherol and its metabolites have shown anti-inflammatory activity (45). γ-Tocopherol and its metabolites directly inhibit cyclooxygenase 2 activity and the generation of prostaglandin E_2 (PGE_2) in macrophages and human epithelial cells to a greater extent than does α-tocopherol (45). γ-Tocopherol increases nitric oxide generation and endothelial nitric oxide synthase activity and only γ-tocopherol increased endothelial nitric oxide synthase protein expression in rats (46). Since nitric oxide is a potent vasodilator, the nitric oxide generation increased by γ-tocopherol can help to regulate blood pressure. In insulin resistant rats, γ-tocopherol attenuated the formation of neointima lesions induced by vascular injury (47). Moreover, γ-tocopherol is a superior scavenger of reactive nitrogen species (RNS) which are involved in the pathology of atherosclerosis, coronary heart disease, and cancer, forming 5 nitro-γ-tocopherol (48). γ-tocopherol lacks a methyl group on C-5 position on the chromanol ring and therefore can trap RNS efficiently (48, 49). Importantly, a study in cigarette smokers, who are under nitrosative stress, showed plasma 5 nitro-γ-tocopherol concentrations are higher than in non-smokers (50).

The anti-inflammatory and antioxidant effect of γ-tocopherol may have beneficial

effects relative to atherosclerosis and coronary heart disease in humans. γ-Tocopherol supplementation in rats decreased arterial superoxide anion generation, lipid peroxidation and low density lipoprotein (LDL) oxidation, and increased endogenous superoxide dismutase (SOD) activity; furthermore, it potently decreased platelet aggregation and thrombosis (51). Several epidemiologic studies have shown that γ-tocopherol concentrations in blood are inversely associated with increased morbidity and mortality due to coronary heart disease (9, 52, 53). In U.S. postmenopausal women, dietary intake of vitamin E (mainly γ-tocopherol) was significantly inversely associated with increased risk of death by coronary heart disease (5).

In epidemiologic studies, γ-tocopherol has been shown to decrease prostate cancer risk (54, 55). Helzsouer et al (54) reported that the risk of prostate cancer is a 5-fold lower in men in the highest quintile of plasma γ-tocopherol intakes compared with those in the lowest quintile. However, a recent study failed to show the association between dietary vitamin E and prostate cancer (56). It seems that the effect of γ-tocopherol in prostate cancer prevention is not yet clear. Therefore, well-designed clinical studies are required to test the direct effect of γ-tocopherol on prostate cancer through a single intervention of γ-tocopherol or exact estimation of dietary γ-tocopherol consumption in separate from α-tocopherol consumption.

In addition to γ-tocopherol, γ-CEHC increases sodium excretion in human urine as an endogenous natriuretic factor (30, 31). Therefore, γ-tocopherol potentially improves blood pressure and decreases blood vessel injury.

Sesame Lignans

Sesamin and sesamolin

Sesame (*sesame indicum*) has been part of the human diet since ancient times. Sesame oil is one of the major dietary oils in Asian countries. Sesame seeds and oil contain several kinds of sesame lignans that may contribute to improved human health. Sesamin and sesamolin are the most abundant lignans of sesame seeds and the major fat soluble lignans (57). Sesamin and sesamolin are comprised of benzene and furofuran rings. See Appendix C: Major Sesame Lignans. The structural difference between them is that sesamolin contains an oxygen between its benzene and furofuran rings (58). Sesamin is absorbed via the lymph, incorporated into the liver, and then transported to the other tissues such as lung, heart, kidney, and brain (59). Sesamin is removed from serum and tissue within 24 hours after oral administration in rats (59), sesamin metabolite is mostly excreted and disappeared in urine within 24 hours (60).

Although all sesame lignans have not been fully identified due to lack of authentic compounds or related spectral data, sesamin and sesamolin are the only lignans detected in the sesame oil portion (57). Sesamin and sesamolin were excreted in feces, while sesamin or other sesame lignans were not detected in the urine of rats fed a sesame seed diet (57, 61). However, a number of sesamin and sesamolin metabolites were detected in urine in vitro and vivo (57, 60, 61). Sesamin metabolites were also detectable in bile after the hydroxylation in the rat liver in vitro (62). These facts support the hypothesis

that sesamin may be metabolized in either the intestine or liver.

Studies in vivo and vitro have proposed that the metabolic pathway of sesamin and sesamolin are partially metabolized in the liver to hydroxylated metabolites and then may be excreted in bile. Furthermore, the metabolites may be metabolized to the mammalian lignans by the intestinal microbiota. In vitro, sesamin was converted by fermentation with human fecal microbiota to enterodiol (ED) and enterolactone (EL) forms. These lignans have been shown to have estrogenic and antiestrogenic activities (57). In healthy humans, the consumption of sesame seed increased plasma ED and EL concentrations; EL was the major metabolite of sesamin (63). In addition, Nakai et al. reported that sesamin is metabolized by cytochrome P450 in rat liver which results in conversion of the methylenedioxyphenyl to dihydrophenyl (catechol) moiety in structures s (62). The dihydrophenyl (catechol) moiety has been reported to possess strong radical scavenging activities (62). In this study, after oral administration of sesamin substantial amounts of these same metabolites were also found in rat bile as glucuronic acid and/or sulfic acid conjugates (62).

Adverse effects of sesame lignans have not been reported. Recent studies have shown that sesamin and sesamolin are not detectable in urine, and that only their metabolites are excreted in urine and are cleared from plasma within 24 hours (57, 60). These data support that the consumption of sesamin and sesamolin are safe for humans. However, sesame lignans have shown some interactions with vitamin E (32, 33, 64, 65), fish oil rich in PUFA (66-68), and phytosterols. With respect to the safety issue; therefore, it would be recommended to consume foods containing sesame seeds or oil, rather than taking high dose purified sesame lignans supplements.

Antioxidative function of sesame lignans

Studies in experimental animal models have suggested that sesame lignans may have an antioxidant function. Sesamin and sesamolin have inhibitory effects on the lipid peroxidation activity of rat liver (61). Sesamin metabolites exhibited potent radical scavenging activities in rat liver (62) and reduced xanthine/xanthin oxidase-induced superoxide production (69). In addition, sesamolin and its metabolites including sesamol and sesamolinol possess strong antioxidative activity in rat liver and kidney (61). Besides sesamin and sesamolin, sesaminol also demonstrated the antioxidant properties on the in vitro oxidative modification of human low-density lipoprotein (LDL); furthermore, it was a more effective scavenger than either α-tocopherol or probucol in reducing the peroxyl radicals derived from 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH) (70). The findings suggest the potential effect of sesaminol to protect LDL against lipid peroxidation.

In addition to decrease lipid peroxidation and generation of reactive oxidative species, sesame oil increased the activities of antioxidative enzymes such as glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase in rodents under various conditions of oxidative stress (71-74). A study in hypertensive patients indicated that sesame oil consumption remarkably reduced oxidative stress and simultaneously increased GPx, SOD, and catalse activities (75). These results support the hypothesis that sesame consumption may help to enhance antioxidant defense system in humans.

Influence of sesame lignans on lipid metabolism

Sesame lignans have been shown influence in lipid metabolism in vitro and vivo, which leads to inhibition of fatty acid and cholesterol synthesis, and activation of fatty acid β-oxidation in peroxisomes and mitochondria.

Sesamin has been reported to inhibit Δ5 desaturase activity, an enzyme that converts dihomo γ-linolenic acid (DGLA, 20:3, n-6) to arachidonic acid (AA, 20:4, n-6) (76). The inhibition of Δ5 desaturase activity results in accumulation of dihomo γ-linolenic acid whereas arachidonic acids are decreased, which also reduces the formation of pro-inflammatory mediators including prostaglandin (PG)E2, TNF-alpha, IL-6 and IL10 in mice (77). Thus, these studies imply that sesame lignans may affect the inflammatory pathway.

Animal studies have suggested that sesame lignans reduce cholesterol levels by both by inhibiting absorption and by decreasing synthesis of cholesterol (78, 79). Sesamin supplementation significantly reduced the concentration of serum cholesterol in rats fed a cholesterol-enriched diet; moreover, a significant reduction in the activity of liver microsomal 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase), the rate limiting enzyme of cholesterol synthesis in liver was observed (79). Additionally, sesamin can play a role as a transcriptional factor that regulates gene expression, sterol regulatory element binding proteins (SREBPs) which are membrane-bound transcriptional factors of the basic-helix-loop-helix-leucine zipper family, relating to cholesterol biosynthesis and LDL receptors, as well as fatty acid synthesis (78, 80).

SREBP-1 is mainly involved in the gene expression of enzymes in fatty acid synthesis and SREBP-2 regulates the gene expression of enzymes involved in cholesterol synthesis and the LDL receptor (80, 81). Dietary sesamin remarkably decreased not only mRNA of HMG-CoA reductase and LDL receptor, but also mRNA level and protein content of SREBP-1 in rat liver (78).

Furthermore, sesame lignans increase peroxisomal and microsomal hepatic fatty acid oxidation through increased gene expression of hepatic fatty acid oxidation enzymes in vivo in animal models (66-68, 82, 83). The mechanism of peroxisome proliferators-activated receptorα (PPARα) regulation of gene transcription has been proposed (80, 84), which is that PPAR binds DNA at direct repeats as a heterodimer with retinoid X receptor (RXR). In the unliganded state, this complex binds co-repressor proteins while in the liganded state, the co-repressor complex is replaced by a co-activator complex. This leads to a conformational change and promotes gene activation. These findings indicate that sesamin or other sesame lignans may act as a ligand for SREBPs and PPARs.

Physiologic effects of sesame lignans

The consumption of sesame seed or pure sesame lignans has been shown in vitro and in vivo to have diverse physiological functions, which may include antihypertensive and hypocholesterolemic effects.

Consumption of sesame lignans or sesame oil has been shown to lower blood

pressure in several types of hypertensive animals and humans. A clinical trial in hypertension patients on treatment with nifedipine, an antihypertensive drug has demonstrated that the group that consumed dietary sesame oil had significantly lowered blood pressure compared with a group with nifedipine alone or other dietary oils (75). This study indicates that sesame oil may have potential effects on drug metabolism in humans. Sesamin metabolites containing a dihydroxyphenyl (catechol) structures have potent radical scavenging activities in vitro (62). It has been suggested that sesamin metabolites modulate the vascular tone and contribute to the in vivo antihypertensive effect of sesamin by inducing an endothelial nitric oxide-dependent vasorelaxation (69). The study suggests that the enhancement of endothelium-dependent vasorelaxation induced by sesamin metabolites is one of the possible mechanisms of antihypertensive effects of sesamin (69).

Sesame lignans may affect blood lipids as well as lipid metabolism, acting a hypocholestrolemic agent. The absorption of lymphatic cholesterol and fatty acids was highly inhibited and liver cholesterol levels were significantly lower in rats fed sesame oil diet (85, 86). Furthermore, the sesame oil diet significantly decreased levels of serum total cholesterol and LDL-cholesterol in rats (86). Sesamin supplements had similar effects on reducing the absorption of lymphatic and serum cholesterol in rats; moreover, a significant reduction in the activity of liver microsomal HMG-CoA reductase was observed (79). Animal studies have demonstrated that dietary sesame lignans may decrease triacylglycerols (TG) and cholesterol concentrations in blood and liver presumably through decreasing HMG-CoA reductase and LDL receptor mRNA levels

(78). A recent study in postmenopausal women also showed that the consumption of dietary sesame seed powder reduces plasma total cholesterol, LDL cholesterol, and the ratio of LDL to HDL cholesterol (65). Another study with hypertensive patients demonstrated that total cholesterol, LDL-cholesterol and triglyceride decreased, while HDL-cholesterol was elevated by sesame oil consumption (75). These findings support that sesame consumption may inhibit the absorption and synthesis of cholesterol, which can improve blood lipids levels in humans.

Sesame and Vitamin E (γ-Tocopherol)

A number of studies in vitro and vivo have shown that the consumption of sesame seed or pure sesame lignan affects γ-tocopherol metabolism, resulting in increased plasma γ-tocopherol concentrations (24, 32, 65, 87, 88). In rat studies, dietary supplementation with sesame seeds or pure sesame lignans dramatically increased blood and tissue γ-tocopherol concentrations (33, 64, 89-92). Additionally, urinary excretion of γ-CEHC in rats fed sesame lignans significantly decreased (33). The effect of sesame on γ-tocopherol has been studied in humans. Women who ate unrefined sesame oil (22.5 g/d) for 4 weeks demonstrated a 42% increase in serum γ-tocopherol concentrations (88). Postmenopausal women who consumed sesame powder (50 g/day) for 5 wk also had increased serum γ-tocopherol concentrations (65).

The cytochrome P450 (CYP), a superfamily of heme-thiolate proteins is responsible for the detoxification of foreign compounds or xenobiotic chemicals such as drugs and carcinogens, as well as for metabolism of endogenous compounds such as steroids, bile acids, and fat soluble vitamins (93). In vitro studies in HepG2 cells and primary rat hepatocytes have suggested that CYP enzymes mediate ω-hydroxylation of the tocopherol side chain (24, 32). Ketoconazole and sesamin, the inhibitors of CYP enzyme activity, inhibited α- and γ-tocopherol metabolism (24, 32). Rifampicin, an inducer of CYP3A increased *all rac* α-tocopherol metabolism to α-CEHC up to 5 fold in HepG2 cells, while clofibrate, an inducer of peroxysomal β-oxidation did not (23). In addition, several in vitro and in vivo studies have reported the detection of tocopherol

metabolites (CEHC) and intermediate precursors of CEHCs (carboxymethylbutyl-hydroxychromans : CMBHC) both in hepatocytes (23, 32, 36) and in human urine (94). Thus, these studies support the concept that CYP enzymes are necessary for ω-oxidation in tocopherol metabolism.

CYPs involved in vitamin E metabolism are not entirely defined but several in vitro studies have proposed specific ω-hydroxylases including CYP3A and CYP4F (24, 32). In mice, Cyp3a was correlated with liver α-tocopherol, but Cyp4f protein expression was not changed by high γ-tocopherol diet or genotype (T*tpa*$^{-/-}$, $^{+/-}$, $^{+/+}$) (35). Vitamin E is suggested to serve as a ligand for PXR and modify cytochrome p450 (CYP 3A) activity mediated by PXR (40).Therefore, CYP3a seems to be promising ω-hydroxylase relating to vitamin E metabolism.

Parker et al (32) demonstrated that sesamin, a major lignan of sesame seed, inhibits CYP3A-dependent metabolism of tocopherols and increases tissue tocopherol concentrations in rats. While sesame seed or pure sesame lignan consumption increased plasma and tissue γ-tocopherol concentrations (33, 64, 89-92), urinary excretion of γ-CEHC in rats fed sesame lignans significantly decreased (33). Based on those studies, sesame lignans such as sesamin and sesamolin, have also been suggested to inhibit tocopherol ω-hydrolylase activity and thereby increase plasma tocopherol concentrations (33, 89-92, 95).

Therefore, sesame seed or oil consumption might increase plasma γ-tocopherol concentrations and thereby improve the bioavailability in humans. Increased γ-tocopherol levels would reduce oxidative damage by reactive nitrogen species and act as

an antioxidant.

SESAME OIL INCREASES PLASMA γ-TOCOPHEROL AND INHIBITS γ-TOCOPHEROL METABOLISM IN HUMANS

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ABSTRACT

In rats, sesame lignans increase plasma γ-tocopherol concentrations and inhibit γ-tocopherol degradation to its metabolite γ-carboxyethylhydroxychroman (γ-CEHC). To test if sesame lignan consumption inhibits γ- tocopherol metabolism in humans, muffins prepared with either corn oil (control) or sesame oil and an equimolar mixture of deuterium labeled d_6-α- and d_2-γ-tocopheryl acetates were administered to male (n=5) and female (n=5) volunteers. Tocopherol and CEHC concentrations were followed for 72 h. Sesame lignan consumption significantly increased plasma d_2-γ-TOH concentrations ($p<0.05$). In men, sesame lignans increased plasma d_2-γ- tocopherol areas under the curve (AUC; sesame oil: 34.3 ± 4.6; corn oil: 28.9 ± 3.3 μmol/L·h, $p<0.01$) and reduced d_2-γ-CEHC AUCs ($p<0.05$). In men differences in urinary d_2-γ-CEHC AUCs in men did not reach statistical significance (AUCs for 24 h, corn oil: 11.2 ± 3.0 μmol/g creatinine·h; vs sesame oil: 5.0 ± 1.5). In women, sesame lignan consumption did not alter plasma tocopherol or CEHC concentrations but reduced urinary d_2-γ-CEHC excretion (AUCs for 24 h, corn oil: 19.3 ± 4.9 μmol/g creatinine·h; and sesame oil: 7.7 ± 2.0, $p < 0.05$). These data suggest that sesame lignans alter γ-tocopherol metabolism differently in men and women. Further research is needed to assess the mechanism involved in these differences.

INTRODUCTION

National Health and Nutrition Examination Survey (NHANES) III reported that approximately 40% of the U.S. population is taking vitamin or mineral supplements. Vitamin E, discovered in 1922 by Evans and Bishop, is one of the most popular supplements in the U.S. (1, 2, 10). Vitamin E is a lipid soluble vitamin with a chain reaction breaking antioxidant function. It is a mixture of α-, β-, γ-, and δ- tocopherol and tocotrienols (12). γ-Tocopherol is the most prevalent form of vitamin E in the American diet. α-Tocopherol, however, is the only form to meet the human requirement of vitamin E and to prevent vitamin E deficiency symptoms in humans (12). Thus, most studies of vitamin E have been done with α-tocopherol in regard to human disease (3-6). However, γ-tocopherol has recently received more research attention because of the unique benefits of γ-tocopherol on human health (7-9).

γ-Tocopherol inhibits cyclooxygenase 2 activity and the generation of prostaglandin E_2 (PGE_2) in macrophages and human epithelial cells to a greater extent than does α-tocopherol (45). α-Tocopherol has been demonstrated to increase nitric oxide generation and endothelial nitric oxide synthase activity as much as γ-tocopherol; however, only γ-tocopherol showed an increase of endothelial nitric oxide synthase protein expression in rats (46). Moreover, γ-tocopherol is a superior scavenger of reactive nitrogen species, forming 5 nitro-γ-tocopherol (48). In cigarette smokers undergoing nitrosative stress, plasma 5 nitro-γ-tocopherol concentration has been shown to be higher than that in non-smokers (50).

γ-Tocopherol is metabolized to 2, 7, 8-trimethyl-2-(β-carboxyethyl)-6-hydroxychroman (γ-CEHC) and excreted in urine in humans (27). γ-CEHC, a water soluble metabolite of γ-tocopherol, was discovered by Wechter et al in 1996 as an endogenous natriuretic factor which increases sodium excretion in human urine (30, 31). Due to these findings, γ-CEHC was suggested to aid in the prevention of hypertension and coronary heart disease (31). CEHCs were also found in bile as well as the urine of rats (26) and bile was suggested a major route for CEHC excretion (13).

The metabolism of α- and γ-tocopherol to α- and γ-CEHC occurs through cytochrome P450 dependent ω-oxidation followed by β-oxidation of the phytyl tail (23, 24, 96). Furthermore, Sontag and Parker reported that tocopherol ω-hydroxylase demonstrates higher catalytic activities for γ-tocopherol than for α-tocopherol, and that CYP 4F2 is involved in ω-oxidation of tocopherol in human HepG2 cell (24). On the other hand, CYP 3A has also been suggested to be involved in tocopherol ω-oxidation process (23, 32, 33). As a PXR ligand, vitamin E could modify cytochrome p450 (CYP 3A) expression modulated by PXR (40). In mice fed high γ-tocopherol, α-tocopherol modulated Cyp3a protein expression, but not Cyp4f, regardless of diet or mouse genotype (35). Parker et al (32) demonstrated that sesamin, a major lignan of sesame seed, inhibits CYP3A-dependent metabolism of tocopherols and increases tissue tocopherol concentrations in rats. While sesame seed or pure sesame lignan consumption increased plasma and tissue γ-tocopherol concentrations (33, 64, 89-92), urinary excretion of γ-CEHC in rats fed sesame lignans significantly decreased (33). Based on those studies (33, 89-92, 95), sesame lignans such as sesamin and sesamolin

have also been attributed to inhibit tocopherol ω-hydrolylase activity and thereby increase plasma tocopherol concentration.

Although CYPs involved in vitamin E metabolism are not entirely understood, studies (65, 87, 88) in human subjects who consumed sesame supported the view that tocopherol metabolism, especially γ-tocopherol, is mediated by cytochrome p450 dependent ω-oxidation. Similar to animal studies, sesame consumption increased human plasma γ-tocopherol concentrations (87). Women who ate unrefined sesame oil (22.5 g/d) for 4 weeks demonstrated a 42% increase in seruṃ γ-tocopherol concentrations, but α-tocopherol concentrations were not changed (88). Postmenopausal women who consumed sesame powder (50 g/day) for 5 wk also had increased serum γ-tocopherol concentrations (65). Clearly, sesame has been shown to increase γ-tocopherol concentrations in blood and tissues in both humans and animals. To our knowledge, however, there is no mechanistic study to investigate how the metabolism of tocopherols is altered by sesame in humans using deuterium labeled tocopherols.

We hypothesized that sesame lignans would rapidly increase plasma γ-tocopherol concentrations by inhibiting cytochrome P450 dependent ω-hydroxylase, and decrease γ-CEHC excretion into plasma urine. Therefore, in the present study, we quantified the inhibition of deuterium labeled vitamin E metabolism by a single dose of sesame oil lignans (94 mg sesamin, 42 mg sesamolin) during 72 hours. See Appendix D : Deuterium-Labeled Tocopherols.

MATERIALS AND METHODS

Subjects

This study protocol was approved by the Institutional Review Board for the protection of human subjects at Oregon State University. All subjects were recruited in Corvallis, Oregon and they signed an informed consent form prior to participation in the study. Subjects (5 women and 5 men) were healthy non-smokers aged 18-35 years who did not take any vitamins or dietary antioxidant supplements at least 3 weeks prior to the study. Their physical activity was restricted less than 5 h in weekly. Routine serum blood chemistry profiles (**Table 1**), including lipid panels, were performed at Good Samaritan Hospital to evaluate the health status on all of the subjects (Corvallis, OR). All subjects met normal criteria of serum blood chemistries and lipids (Table 1).

Materials

α-[5,7-(C^2H_3)2] tocopheryl acetate (d_6-α-tocopheryl acetate) was a gift from Dr. James Clark of Cognis Nutrition and Health. γ-[3,4-^{2}H] tocopheryl acetate (d_2-γ-tocopheryl acetate) was prepared from γ-tocopherol labeled with two deuterium atoms, as described (97), by Dr. Jeffrey Atkinson at Brock University, Canada. Capsules contained a 1:1 equimolar mixture of d_6-α- and d_2-γ-tocopherols which were

Table 1. Subject Characteristics

Parameter	Men (n = 5)	Women (n = 5)	Total (n = 10)
Age (y)	30.6 ± 5.8	25.4 ± 4.0	28.0 ± 5.9
BMI (kg/m^2)	24.9 ± 2.3	23.7 ± 1.3	24.4 ± 2.1
Plasma Concentrations			
α-Tocopherol (μmol/L)	18.3 ± 2.4	19.4 ± 3.4	18.9 ± 2.9
γ-Tocopherol (μmol/L)	3.52 ± 1.47 *	1.76 ± 0.54	2.64 ± 1.40
Total Cholesterol (mmol/L)	4.97 ± 0.24 *	4.40 ± 0.48	4.68 ± 0.47
HDL (mmol/L)	1.31 ± 0.25 *	1.67 ± 0.35	1.49 ± 0.34
LDL (mmol/L)	3.14 ± 0.28 *	2.40 ± 0.37	2.77 ± 0.50
VLDL (mmol/L)	0.52 ± 0.18	0.33 ± 0.14	0.42 ± 0.19
Triglycerides (mmol/L)	1.12 ± 0.41	0.72 ± 0.32	0.92 ± 0.41

All data are shown as mean ± SD.

*, $p < 0.05$ for unpaired comparison of men with women.

approximately 50 mg each. The d_6-α- to d_2-γ-tocopherol molar ratio was determined by liquid chromatography/mass spectrometry (LC/MS) to be 0.98. The internal standard, α-[5,7,8-$(C^2H_3)_3$] tocopheryl acetate (d_9-α-tocopheryl acetate), was provided by Dr. Carolyn Good of The Bell Institute of Health and Nutrition, and was synthesized by Isotec, Inc. (Miamisburg, OH, USA). Standards of α-CEHC and γ-CEHC (LLU-α) were gifts from W.J. Wechter of Loma Linda University.

HPLC-grade methanol, hexane, ethanol, and glacial acetic acid were obtained from Fisher (Fair Lawn, NJ). Unlabeled γ-tocopherol (d_0-γ-tocopherol), trolox, ascorbic acid, potassium hydroxide (KOH), butylhydroxy toluene (BHT), lithium perchlorate, trolox, and β-glucuronidase (type H-1, contains minimum 300,000 U/g β-glucuronidase

activity and minimum 10,000 U/g sulfatase activity) were from Sigma-Aldrich (St. Louis, MO, USA). Diethyl ether was obtained from Mallinckrodt Baker, Inc. (Phillipsburg, NJ, USA).

Study Design

This study used a randomized crossover design. Volunteers (n=10) consumed a breakfast with muffins prepared with either corn oil or sesame oil with equalized concentrations of α- and γ-tocopherols and a capsule containing deuterated tocopherols with a 4 week washout between trials. Sesame seed oil or corn oil was incorporated into a muffin administered as part of a standardized breakfast. After 4 weeks washout period, the trial was repeated as described above. If the subject consumed a sesame oil-containing muffin in the first trial, then a placebo (corn oil) muffin was administered the second time, and vice-versa.

On the first day of the trial, blood samples were drawn before breakfast (at 0 h, baseline) from the antecubital vein into evacuated tubes containing 0.05 mL 15 % (wt/vol) EDTA (Becton-Dickinson, Franklin Lakes, NJ, USA). The breakfast was ≈414 kcal with ≈27 % fat calories. Following eating the muffin, the subjects consumed a capsule containing ≈50 mg each of an equimolar ratio of d_6-α- and d_2-γ-tocopheryl acetates. After consuming the breakfast including muffins and the deuterated tocopherol capsule, blood samples were obtained at 3, 6, 9, 12, 24, 36, 48 and 72 h. Plasma was

separated by centrifugation at 4 °C for 15 min at 500 x g (model TJ-6; Beckman Coulter, Palo Alto, CA, USA) and stored at -80 °C. Urine samples were collected in 6 h intervals over 24 h for 3 days. Subjects completed diet records for the first 24 h of each trial; vitamin E intakes were similar for both men and women and for each group for sesame and corn oil treatments (Data not shown). See Appendix E : Experimental Design.

Measurement of Plasma Tocopherols

Plasma labeled and unlabeled α- and γ-tocopherols were extracted as described (98) and measured by LC/MS using negative atmospheric pressure chemical ionization (APCI) and a Waters (Milford, MA, USA) 2690 Seperation Module (99).

Measurement of Plasma and Urinary CEHCs

Plasma and urinary CEHCs were extracted using a modified method of Lodge et al. (38, 100). Samples were analyzed using a Micromass (Manchester, UK) ZQ 2000 single-quadrupole mass spectrometer with an electrospray ionization probe, set to negative ionization mode. Deuterium labeled CEHCs are not commercially available; thus, quantitation was performed using an internal standard of Trolox. Sample CEHC concentrations were calculated from the peak area of the corresponding ion to that of the Trolox peak.

Measurement of Plasma Lipids and Urinary Creatinine

Plasma triglycerides and total cholesterol were determined using the respective ThermoDMA Kits (Louisville, CO, USA). Urinary creatinine was measured spectrophotometrically at a wavelength of 500 nm after reaction with picric acid based on the Jaffé reaction.

Mathematical and Statistical Analyses

The maximum tocopherol and CEHC concentrations (C_{max}) and the time of maximum concentration (T_{max}) were estimated by visually inspecting the data. Fractional disappearance rates (FDRs) were estimated from d_6-α- and d_2-γ-tocopherol concentrations using the linest function of Excel on logarithmically transformed concentrations versus time as described previously (101). FDRS were estimated separately for each individual by fitting a line from plasma concentration data transformed to natural logarithm (In) from T_{max} to 72 h.

The area under the curves (AUC) of plasma tocopherols and CEHCs concentrations were calculated according to the trapezoidal rule for the time points from 0 to 72 h. AUCs of urinary CEHCs excretion were calculated from 0 to 24 h. Data are expressed as means ± SE unless otherwise noted. Statistical analyses were performed with InState (version 4.0; GraphicPad Sofeware, Inc., San Diego, CA) by the unpaired Student's *t*-test or paired Student's t-test with one-tail for comparing differences between

the corn oil and the sesame oil trials, and between genders in the same trial. Results were considered to be statistically significant at the 95 % confidence level ($p < 0.05$).

RESULTS

Breakfast Vitamin E and Sesame Contents

Subjects consumed an equimolar mixture of approximately 50 mg each d_6-α- and d_2-γ-tocopheryl acetates following a breakfast with two muffins prepared with either corn or sesame oils (participants repeated the trial with the opposite muffin after an appropriate washout period). Corn and sesame oils used for the preparation of the muffins contained equalized concentrations of α-tocopherol (17.9 mg/100 g corn oil; 15.6 mg/100 g sesame oil) and γ- tocopherols (84.9 mg/100 g corn oil; 84.0 mg/100 g sesame oil). The breakfast provided, in addition, 0.6 mg α-tocopherol. The two muffins made with either oil contained approximately 13.0 mg α-tocopherol and 2.5 mg γ-tocopherol. The two sesame oil muffins provided 135.4 mg sesame lignans (sesamin 93.8 mg; sesamolin, 41.6 mg). There were no statistically significant differences between dietary intakes of vitamin E during each of the trials or between genders.

Plasma Unlabeled and Labeled α- and γ-Tocopherol Concentrations

Plasma unlabeled α-and unlabeled γ-tocopherol concentrations during the two trials are shown in **Figure 1A.** Muffin consumption did not change plasma d_0-α-

tocopherol concentrations. After 6 h, plasma d_0-γ-tocopherol concentrations were significantly increased in both trials ($p < 0.05$), which was likely due to the γ-tocopherol content of the muffins.

Plasma d_6-α- and d_2-γ-tocopherol concentrations responded differently to sesame oil consumption. Sesame oil consumption did not alter the d_6-α-tocopherol concentrations; however, d_2-γ-tocopherol concentrations were maintained at higher concentrations during the sesame oil trial ($p < 0.05$) such that at 72 h d_2-γ-tocopherol concentrations were significantly higher following sesame oil consumption ($p = 0.005$, **Figure 1B**). Additionally, men and women had similar d_6-α-tocopherol concentrations throughout the study (data not shown). In men, sesame oil consumption significantly increased maximal plasma d_2-γ-tocopherol concentration ($p < 0.05$) and shifted the curve of d_2-γ-tocopherol concentrations to the right, but a similar effect of sesame was not obtained in women (**Figure 2**).

Plasma area under the curve (AUC) of d_6-α-tocopherol concentrations (corn oil: 179 ± 15 μmol/L·h vs sesame oil: 177 ± 16) was unaffected by sesame oil consumption. Similarly, plasma d_6-α-tocopherol C_{max} and T_{max} were not influenced by the consumption of sesame oil muffins. However, in both trials women's plasma d_6-α-tocopherol fractional disappearance rates (FDRs) were faster than those of men (corn oil: women, 0.024 ± 0.001 pools/h vs men, 0.019 ± 0.001, $p < 0.01$; and sesame oil: women, 0.026 ± 0.004 vs men, 0.017 ± 0.001, $p < 0.05$, **Table 2**).

In contrast to α-tocopherol, plasma γ-tocopherol concentrations were altered by sesame oil consumption. In men, d_2-γ-tocopherol AUCs increased during the sesame oil

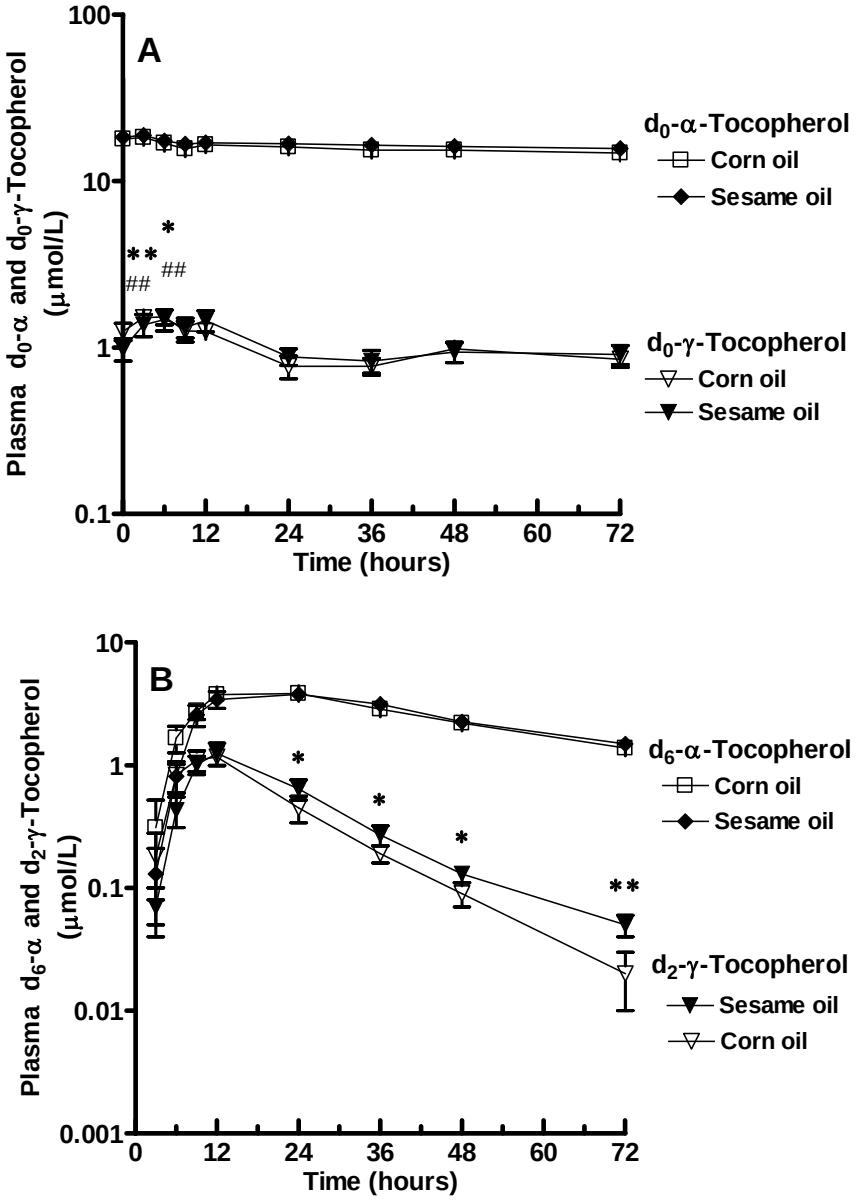

Figure 1. Plasma Concentrations of Labeled and Unlabeled Tocopherols

Plasma concentrations of unlabeled (A) and labeled (B) α- and γ-tocopherol (mean ± SE) during corn oil and sesame oil trials. (A) *, $p < 0.05$ and **, $p < 0.01$ for paired comparison of concentration at 3 h and 6 h with baseline within the corn oil trial. $^{\#}$, $p < 0.05$ and $^{\#\#}$, $p < 0.01$ for paired comparison of concentration at 3 h and 6 h with baseline within the sesame oil trial. (B) *, $p < 0.05$ and **, $p < 0.01$ for paired comparison of concentration between the sesame oil and the corn oil trial

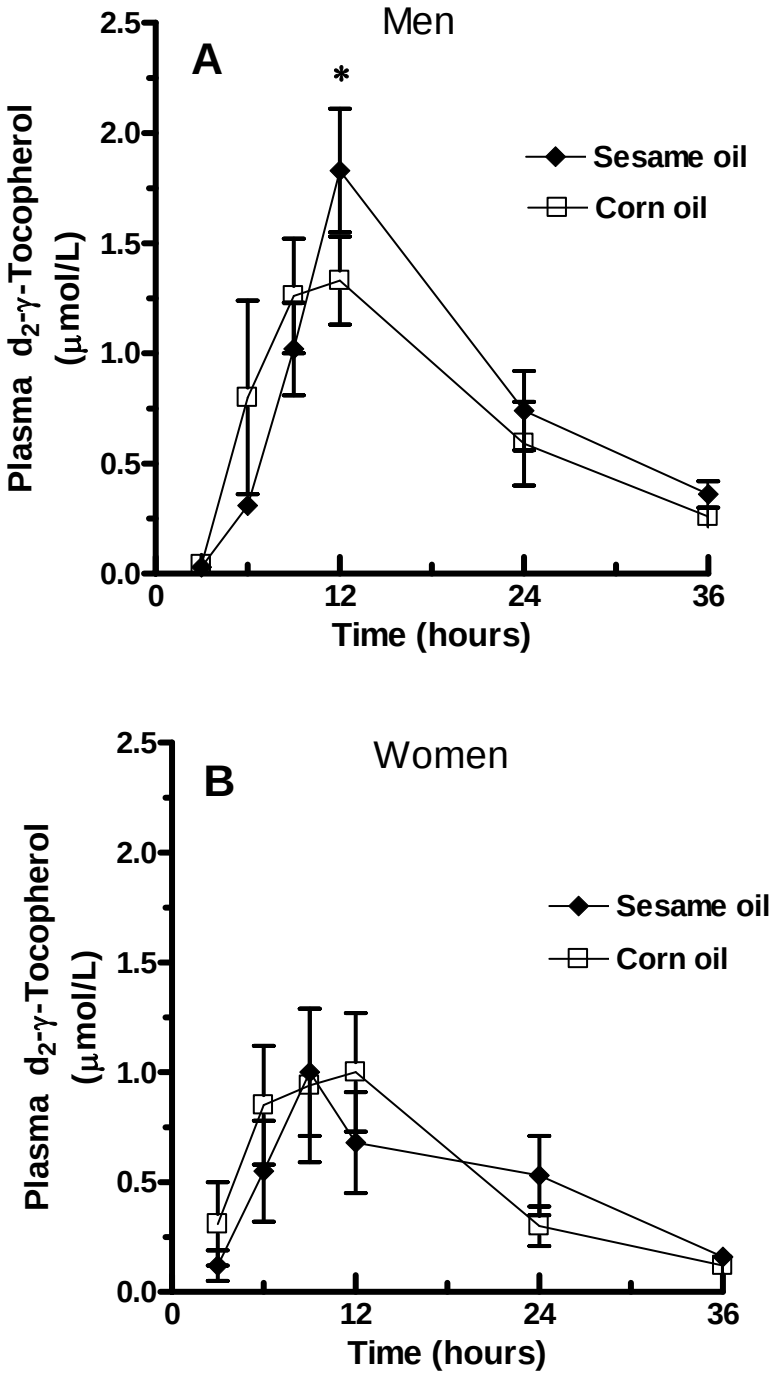

Figure 2. Plasma d_2-γ-Tocopherol Concentrations

Sesame oil increased maximum plasma d_2-γ-tocopherol concentrations (mean ± SE) in men. *, $p < 0.05$ for paired comparisons of concentration between trials within gender.

Table 2. Plasma Labeled α- and γ-Tocopherol, and γ-CEHC Kinetic Parameters

Plasma	d_6-α-Tocopherol		d_2-γ-Tocopherol		d_2-γ-CEHC	
	Corn	**Sesame**	**Corn**	**Sesame**	**Corn**	**Sesame**
Cmax (μM)						
Men (n=5)	4.3 ± 0.6	4.3 ± 0.4	1.8 ± 0.3	2.0 ± 0.2	0.21 ± 0.04	0.10 ± 0.03 *
Women (n=5)	4.2 ± 0.5	4.8 ± 0.4	1.3 ± 0.3	1.3 ± 0.2 $^{+}$	0.18 ± 0.05	0.29 ± 0.08^{+}
Tmax (hour)						
Men (n=5)	19 ± 3	17 ± 3	10 ± 1	11 ± 1	14 ± 2	22 ± 2 *
Women (n=5)	16 ± 3	16 ± 3	10 ± 2	16 ± 3 *	13 ± 3	19 ± 5 *
FDR (pools/h)						
Men (n=5)	0.019 ± 0.001	0.017 ±0.001	0.062 ± 0.004	0.061 ± 0.004	0.049 ± 0.007	0.034 ± 0.002 *
Women (n=5)	0.024 ± 0.001 $^{++}$	0.026 ± 0.004 $^{+}$	0.100 ± 0.017^{+}	0.081 ± 0.013	0.045 ± 0.007	0.044 ± 0.005

All data are shown as mean ± SE.

*, $p < 0.05$ and **, $p < 0.01$ for paired comparison of the corn oil with the sesame oil trials within each gender.

$^{+}$, $p < 0.05$ and $^{++}$, $p < 0.01$ for unpaired comparison of men with women within each test oil group.

trial (34.3 ± 4.6 μmol/L·h) as compared with the corn oil trial (28.9 ± 3.3, $p = 0.01$), but a similar effect was not observed in women. d_0-γ-tocopherol AUCs during the sesame were greater in men (94.5 ± 10.7 μmol/L·h) than in women (53.6 ± 6.3, $p = 0.006$), as were the d_2-γ-tocopherol AUCs (men: 34.3 ± 4.6 vs women: 20.9 ± 3.1, $p < 0.05$). Overall, men had higher AUCs of plasma γ-tocopherol concentrations than do women during both trials. Therefore, these findings suggest that men may respond more sensitively to sesame oil consumption than do women.

Men have higher d_2-γ-tocopherol C_{max} than do women (Table 2). During the sesame oil trial compared with the corn oil trial, women's d_2-γ-tocopherol T_{max} was prolonged about 6 hours ($p < 0.05$) in spite of similar C_{max}s between the corn oil and the sesame oil trial. Plasma d_2-γ-tocopherol disappeared faster in women than in men (corn oil: women vs men, $p < 0.03$; and sesame oil: $p = 0.09$). The faster FDR for γ-tocopherol in women has been reported in a study using deuterium labeled α- and γ- tocopherols by Leonard et al (38).

Plasma Unlabeled and Labeled γ-CEHC Concentrations

Plasma d_0-γ-CEHC and d_2-γ-CEHC concentrations were followed up to 72 h after consuming sesame oil muffins compared with corn oil muffins (**Figure 3**). Sesame oil consumption significantly decreased men's plasma d_0-γ-CEHC AUCs (corn oil: 14.7 ± 2.5 μmol/L·h vs sesame oil: 9.6 ± 0.3, $p < 0.05$). In men, but not in women, sesame oil consumption reduced the plasma d_2-γ-CEHC concentration (**Figure 4**). Also, sesame oil

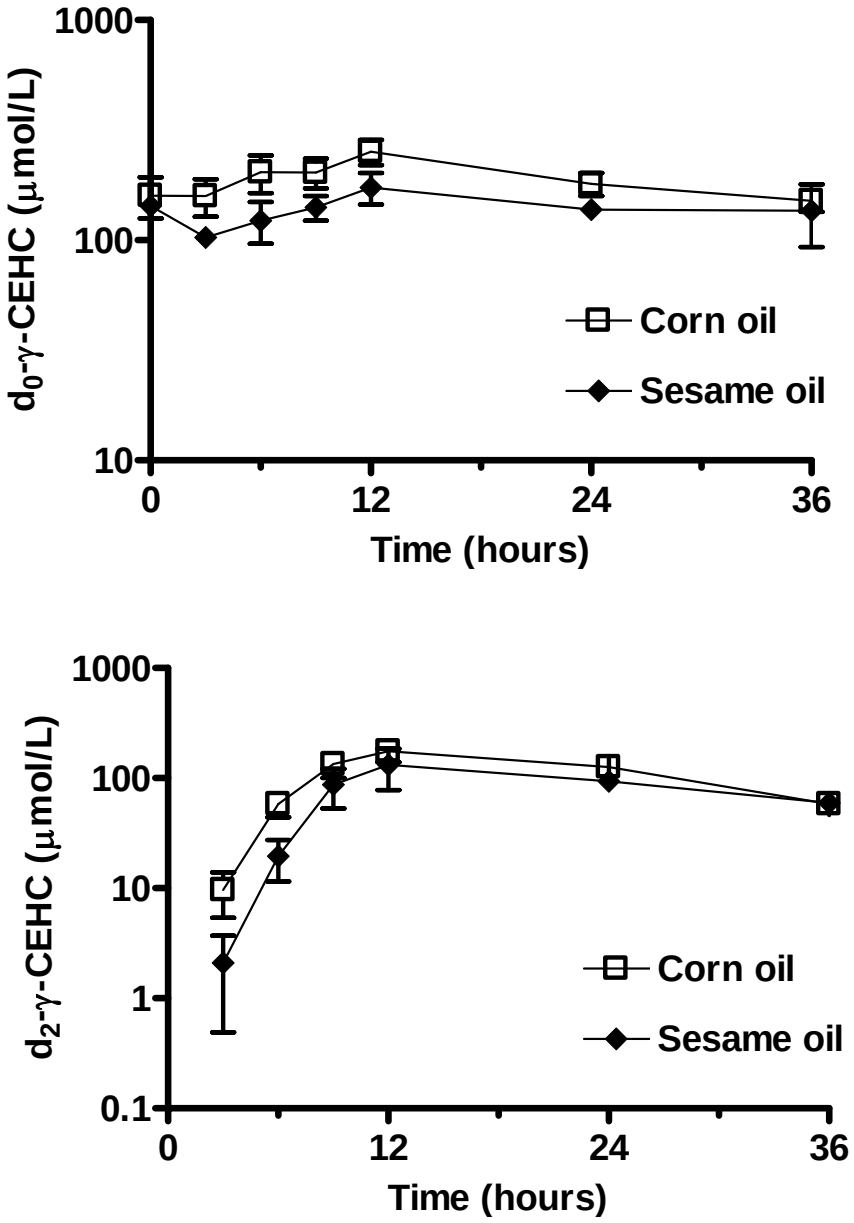

Figure 3. Plasma d_0- and d_2-γ-CEHC Concentrations

Plasma concentrations of d_0- and d_2-γ-CEHC (mean ± SE) during the corn oil and the sesame oil trials. d_0-α-CEHC and d_6-α-CEHC of most subjects were undetectable.

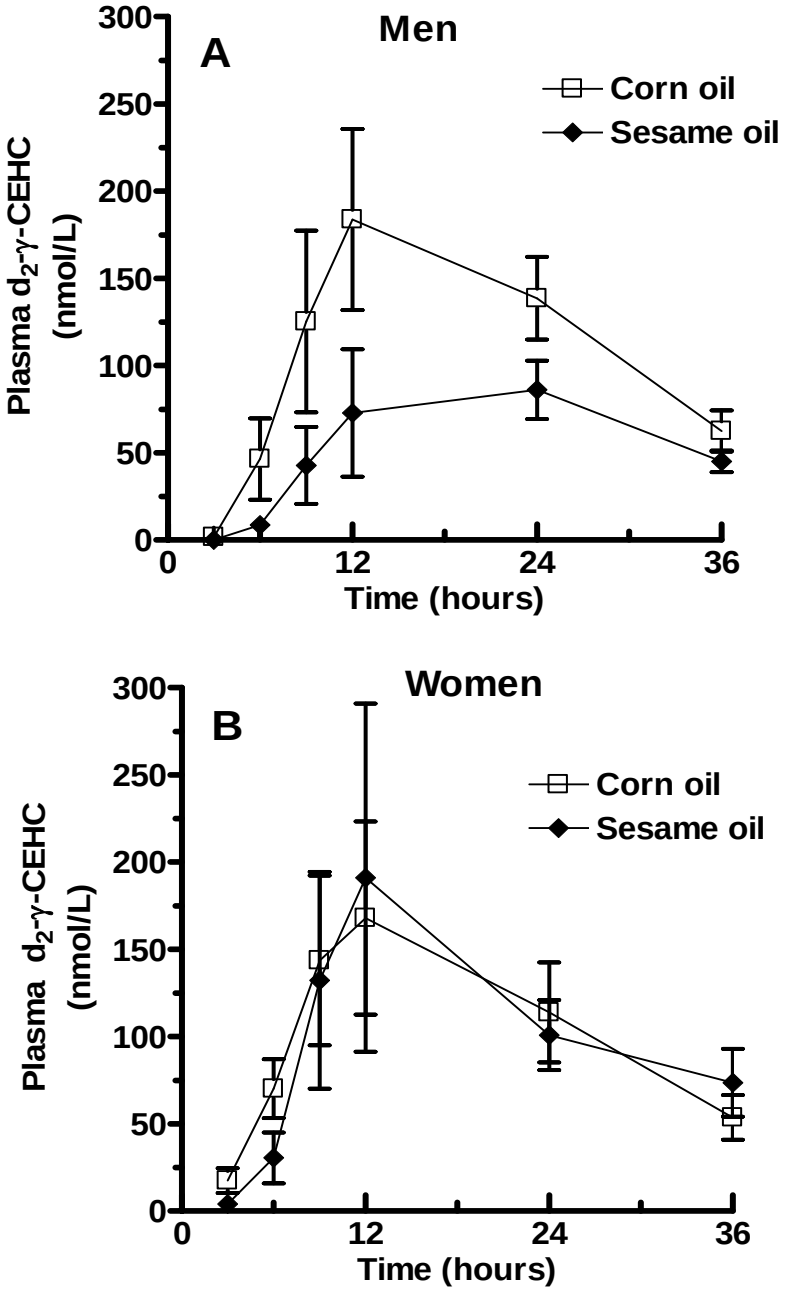

Figure 4. Plasma d_2-γ-CEHC Concentration

Sesame oil decreased plasma d_2-γ-CEHC concentration (mean ± SE) in men.

consumption significantly reduced d_2-γ-CEHC AUC (corn oil: 5.0 ± 1.0 μmol/L·h vs sesame oil: 2.6 ± 0.8, $p < 0.05$) and lowered plasma d_2-γ-CEHC C_{max} in men ($p < 0.05$, Table 2). Overall men's d_2-γ-CEHC AUC and C_{max} for the corn oil trial were nearly double those for the sesame oil trial. Moreover, sesame oil consumption prolonged T_{max} for men ($p < 0.05$). In women, no statistically significant differences in AUCs (corn oil: 4.8 ± 1.0 μmol/L·h vs sesame oil: 4.9 ± 0.8) and in C_{max}s between two trials were observed; however, sesame also delayed d_2-γ-CEHC T_{max}s for women ($p < 0.05$, Table 2).

Plasma Labeled γ-Tocopherol and γ-CEHC FDR and AUC

Women's and men's plasma d_2-γ-tocopherol and d_2-γ-CEHC concentrations appear to respond differently to sesame oil intakes. For example, in women, d_2-γ-tocopherol and d_2-γ-CEHC FDRs were not different regardless of treatments, while in men, d_2-γ-tocopherol FDRs were not different between trials, but d_2-γ-CEHC FDRs were slower during the sesame oil trial compared with the corn oil trial ($p < 0.05$, Table 2). Indeed, plasma d_2-γ-CEHC FDRs for men decreased 31 % by sesame oil intakes ($p < 0.05$, Table 2). In addition, men's (but not women's) plasma ratio of d_2-γ-tocopherol to d_2-γ-CEHC FDR ratios were higher in the sesame oil trial (1.80 ± 0.14) compared with the corn oil trial (1.29 ± 0.06, $p < 0.02$). During the sesame oil trial, plasma d_2-γ-tocopherol AUCs for men were higher compared the corn oil trial; on the contrary,

men's plasma d_2-γ-CEHC AUCs were lower. However, similar effects of sesame on plasma d_2-γ-tocopherol and d_2-γ-CEHC AUCs were not obtained in women (**Figure 5**). These findings suggest that the sesame oil consumption alters men's metabolism of d_2-γ-tocopherol to d_2-γ-CEHC in plasma.

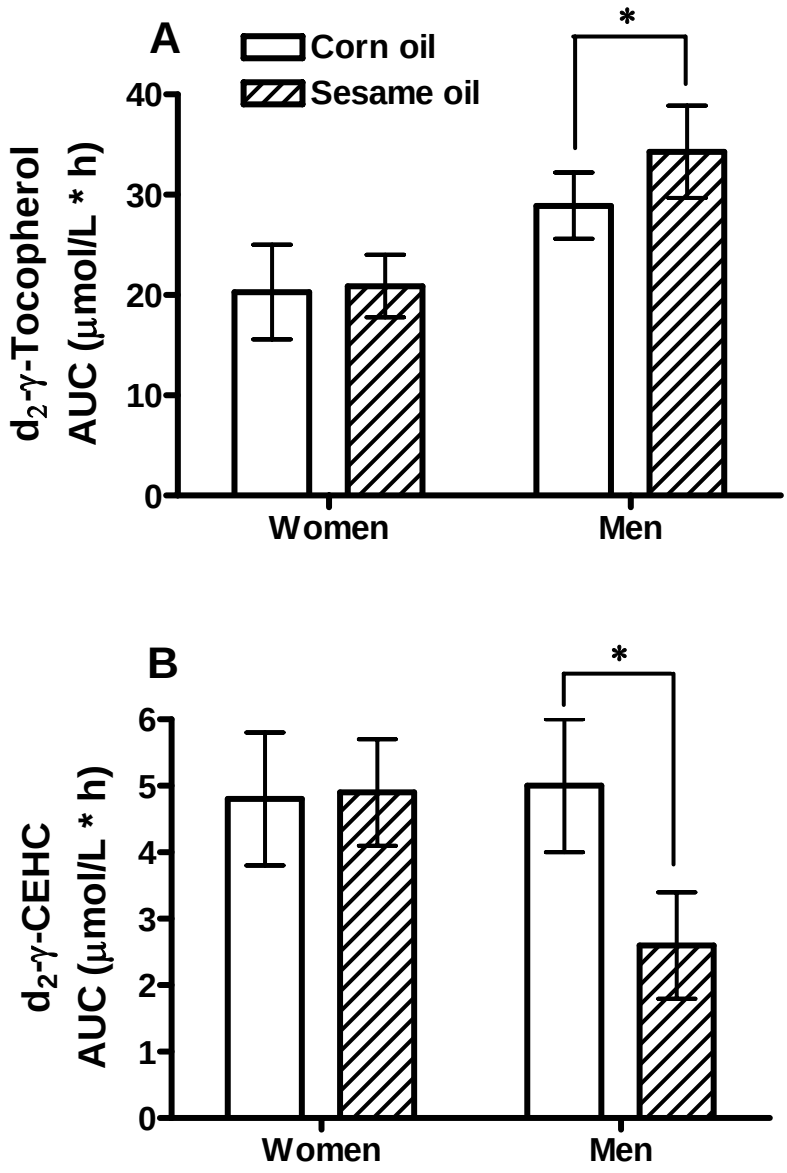

Figure 5. Area Under the Curve of Plasma d_2-γ-Tocopherol and d_2-γ-CEHC.

In men not women, plasma d_2-γ-tocopherol Area under the curve (AUC) for 72 h (mean ± SE) (A) increased, while AUC of plasma d_2-γ-CEHC for 72 h (B) decreased during sesame trial as compared with the corn oil trial.

Urinary CEHC Excretion

In both men and women, urinary d_2-γ-CEHC excretion during trials peaked prior to 24 hours after dosing (**Figure 6**). Sesame consumption significantly decreased urinary d_2-γ-CEHC excretion at 6-12 and 12-24 hours in women. A similar observation was obtained in men, but was not statistically significant. Sesame consumption significantly decreased AUCs of urinary d_2-γ-CEHC excretion up to 24 h in women (corn oil: 19.3 ± 4.9 μmol/g creatinine·h; and sesame oil: 7.7± 2.0, $p < 0.05$, **Figure 7**), no statistically significant differences were observed in men (corn oil: 11.2 ± 3.0 μmol/g creatinine·h; and sesame oil: 5.0 ± 1.5, $p = 0.07$). Again these findings support the hypothesis that sesame oil intake reduced the metabolism of γ-tocopherol. In men (but not women), the AUCs of total excreted γ-CEHC (d_0-γ-CEHC plus d_2-γ-CEHC) were also smaller with the sesame oil muffins (65.9 ± 12.1 μmol/g creatinine·h) than with corn oil muffins (95.4 ± 13.6 μmol/g creatinine·h, $p < 0.024$).

d_6-α-CEHC excreted was undetectable in urine. However, urinary d_0-α-CEHC AUCs in women (corn oil: 37.8 ± 8.8 μmol/g creatinine·h; sesame oil: 30.8 ± 6.2) were greater than those in men (corn oil: 15.6 ± 2.9 μmol/g creatinine·h; sesame oil: 9.4 ± 1.7) during both trials ($p < 0.05$).

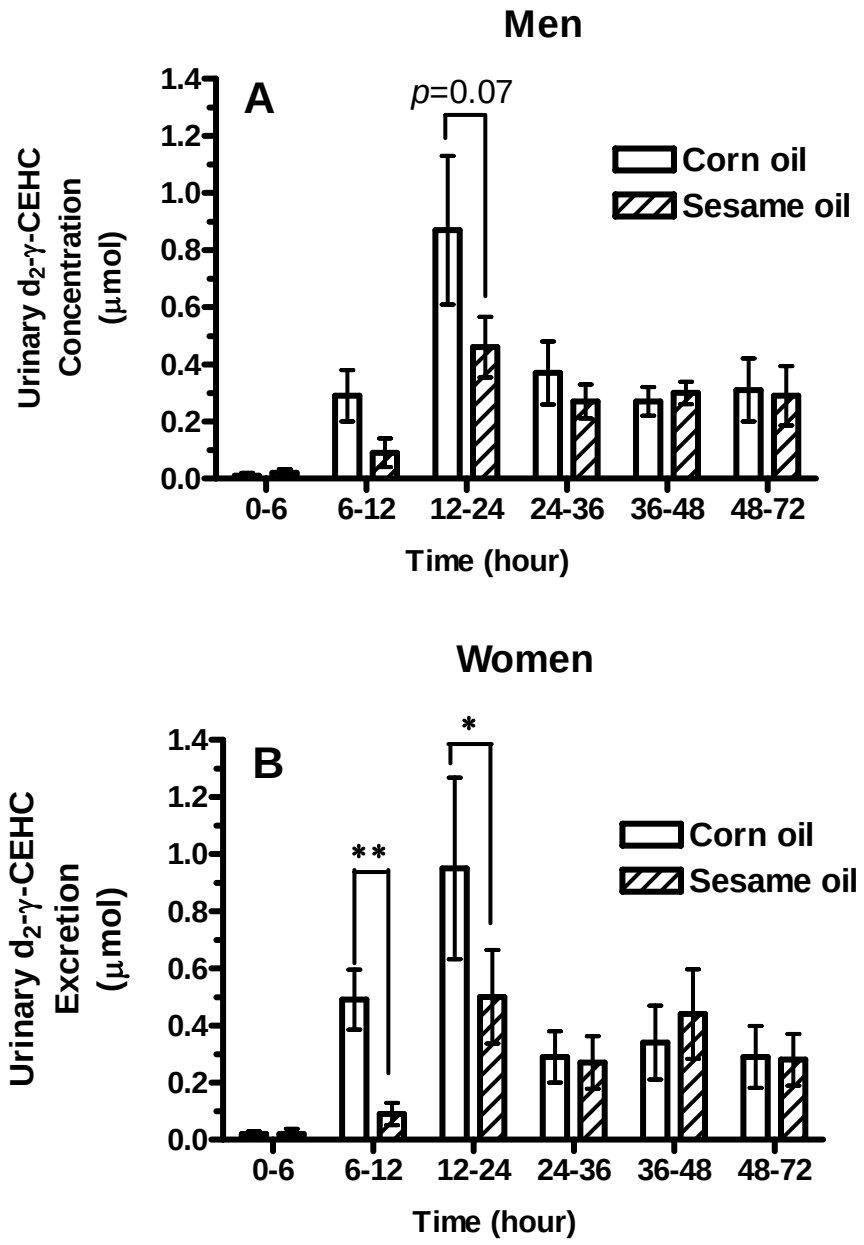

Figure 6. Urinary d_2-γ-CEHC Excretion

Urinary d_2-γ-CEHC peaks prior to 24 hours of labeled tocopherols dosing (mean ± SE). Urine volume was accounted for calculating urinary d_2-γ-CEHC excretion. *, $p < 0.05$ and **, $p < 0.01$ for paired comparison of concentration between trials within each gender.

Figure 7. Area Under the Curve of Urinary d_2-γ-CEHC Excretion

Area under the curve (AUC) of urinary d_2-γ-CEHC excretion for 24 hours (mean ± SE). AUC of urinary d_2-γ-CEHC were decreased during sesame trial as compared with the corn oil trial ($p < 0.05$). *, $p < 0.05$ for paired comparison of concentration between trials within each gender.

DISCUSSION

We expected that sesame oil consumption would increase plasma d_2-γ-tocopherol concentrations, decrease plasma d_2-γ-CEHC concentrations, and decrease urinary d_2-γ-CEHC excretion. In the present study, we demonstrated that sesame oil consumption altered d_2-γ-tocopherol metabolism differently depending on gender. In men, sesame oil consumption significantly increased plasma d_2-γ-tocopherol concentrations and decreased plasma d_2-γ-CEHC, as expected. In women, plasma d_2-γ-tocopherol and plasma d_2-γ-CEHC concentrations were unchanged by sesame oil consumption. Therefore, it seemed that sesame affects d_2-γ-tocopherol and d_2-γ-CEHC only in men. However, urinary d_2-γ-CEHC excretion in women were significantly decreased during the sesame oil trial compared with the corn oil trial (Figure 6 and 7). A similar trend was observed in men, but the difference did not reach significance ($p < 0.07$). In both men and women, urinary d_2-γ-CEHC excretion peaked prior to 24 hours and was significantly decreased by sesame oil consumption at 6-12 and 12-24 hours. A recent study has reported that sesamin metabolites excreted and disappeared in urine within 24 hours in a human (60). The present results are consistent the finding.

Therefore, these findings suggest d_2-γ-tocopherol metabolism is affected in both men and women by sesame oil, but gender differences exist in γ-tocopherol metabolism. No difference in plasma γ-tocopherol concentrations between the trials in women was observed, similar to a study in healthy women who consumed muffins prepared with

sesame oil, corn oil, or linola oil (88). The study by Lemcke-Norojärvi et al showed that plasma γ-tocopherol concentration increased significantly in both sesame and corn oil groups compared with baseline concentrations, but no significant differences in plasma γ-tocopherol concentrations between two treatment groups was observed. Both corn oil and sesame oil contain high amounts of γ-tocopherol (102), which probably increased the plasma γ-tocopherol concentrations.

The possible mechanisms to increase plasma γ-tocopherol concentration by sesame oil intakes are that sesame may 1) increase γ-tocopherol absorption, 2) increase γ-tocopherol secretion into plasma, 3) decrease γ-tocophetol excretion into bile, and 4) decrease γ-tocopherol metabolism to γ-CEHC. We verified two (the second and the fourth ones) feasible mechanisms by measuring deuterium labeled γ-tocopherol and its metabolite, d_2-γ-CEHC, in urine as well as plasma.

The fact that sesame oil increased plasma d_2-γ-tocopherol and decreased plasma d_2-γ-CEHC in men, but not in women, indicates that different CYPs may be involved or that activity differences may exist between genders. According to a recent review about gender difference in drug responses, CYP enzyme activities are affected by gender (103). The higher activity of CYP3A enzyme associated with vitamin E metabolism in human liver has been reported in women (103). Although gender differences in CYP system in uncertain, the present study suggests that gender differences may exist in vitamin E metabolism mediated by CYP enzymes.

We also observed that sesame oil consumption significantly decreased urinary d_2-γ-CEHC excretion in women, while plasma d_2-γ-tocopherol and plasma d_2-γ-CEHC

unchanged. These findings suggest that the kidney may partly account for gender differences in d_2-γ-tocopherol metabolism. Sontag and Parker reported tocopherol ω-hydroxylase activity in rat kidney homogenates and microsomes (24). Alternatively, as the present study was performed in a small number of human subjects, it is a possibility that the difference found between men and women's urinary d_2-γ-CEHC excretion would not have been significantly different if we had more subjects.

In the kinetics parameters for plasma d_2-γ-tocopherol, sesame oil intake increased men's AUCs, but there were no significant differences in fractional disappearance rate (FDR), C_{max}, and T_{max} between the two trials. This observation is not consistent with the idea that greater AUCs are accompanied with lower FDR, higher C_{max}, or prolonged T_{max} or vice versa, as we observed in d_2-γ-CEHC. However, it may be possible the plasma d_2-γ-tocopherol curve for men would be shifted slightly upward and shifted to the right by higher C_{max}, prolonged T_{max}, lower FDR, or lower fractional increase rate (FIR), in spite of no statistical significance.

In hypertension patients (75), sesame oil consumption with nifedipine, an antihypertensive drug, dramatically decreased blood pressure, improved the lipid profile than did nifedipine alone, sunflower oil with nifidipine, or peanut oil with nifidipine ($p < 0.05$). Plasma vitamin E, vitamin C, β-catotene, and reduced glutathione (GSH) also significantly increased with sesame oil intakes ($p < 0.05$). These findings may be a result from the sesame oil containing higher γ-tocopherol than the other oils (sesame oil: 6.6 mg/15 g, sunflower oil: 0.7, and peanuts: 3.2). sesame oil α-tocopherol content is least among the three different oils (sesame oil: 0.21 mg/15 g, sunflower oil: 6.6, and

peanuts : 3.2) (102). These previous studies demonstrated sesame's effect to increase plasma tocopherol concentrations in humans, but not the direct effect of sesame lignans on plasma tocopherol metabolism.

Our hypothesis is that sesame lignans from sesame seeds inhibit tocopherol ω-hydroxylase activity in humans, reducing γ-tocopherol metabolism to γ-CEHC. Unfortunately, it is not permissible to administer purified sesame lignans directly to human subjects in the US. Thus, in order to measure the effect of the only lignans detected in sesame oil (57) on tocopherol metabolism, we equalized concentrations of α-tocopherol (17.9 mg/100 g corn oil; 15.6 mg/100 g sesame oil) and γ- tocopherol (84.9 mg/100 g corn oil; 84.0 mg/100 g sesame oil) in the diets. Therefore, the present study suggested that consumption of sesame lignan may increase plasma γ-tocopherol concentration and inhibit γ-tocopherol metabolism to γ-CEHC.

CONCLUSIONS

γ-Tocopherol has some beneficial characteristics not shared by α-tocopherol in the antiinflamatory activity and the regulation of blood pressure. It has been shown to inhibit both cyclooxygenase 2 activity and the generation of prostaglandin E_2 (PGE_2) in macrophages and human epithelial cells to a greater extent than does α-tocopherol. However, compared with α-tocopherol, γ-tocopherol is maintained at much lower concentration in human plasma and tissue, which makes the physiological role of γ-tocopherol weak. It has been suggested that the structural feature of γ-tocopherols lower γ-tocopherol affinity for α-TTP, while higher affinity for metabolism enzyme.

In the present study, sesame oil consumption containing sesame lignans (94 mg sesamin, 42 mg sesamolin) decreases d_2-γ-tocopherol metabolism by inhibiting γ-tocopherol metabolism to CEHC in both men and women as we hypothesized. The data presented here also suggests that gender differences may exist in γ-tocopherol metabolism. Based on findings that sesame oil consumption significantly decreased γ-tocopherol metabolites in urine, but did not alter both of plasma γ-tocopherol and its metabolites in women, it can be concluded that the kidney may partly account for metabolism of d_2-γ-tocopherol and gender differences on it. This indicates that further research about sesame effect on CYP enzyme activity in kidney may be essential to understand vitamin E metabolism mediated ω-hydroxylase.

Therefore, sesame seed, oil or lignans consumption can increase plasma γ-tocopherol concentration and thereby improve the bioavailability of γ-tocopherol in humans. Increased γ-tocopherol levels would reduce oxidative damage by reactive nitrogen species and prevent atherosclerosis and coronary heart disease.

There were limits in our ability to investigate absorptive kinetics and biliary excretion of γ-tocopherol in humans. Thus, the investigation of the absorption and excretion pathways of vitamin E by measuring deuterium labeled tocopherols during the initial stages of absorption and excretion in humans, the study of the place where vitamin E can be metabolized, and of mechanism of a biliary excretion of vitamin E. can be suggested for the future studies.

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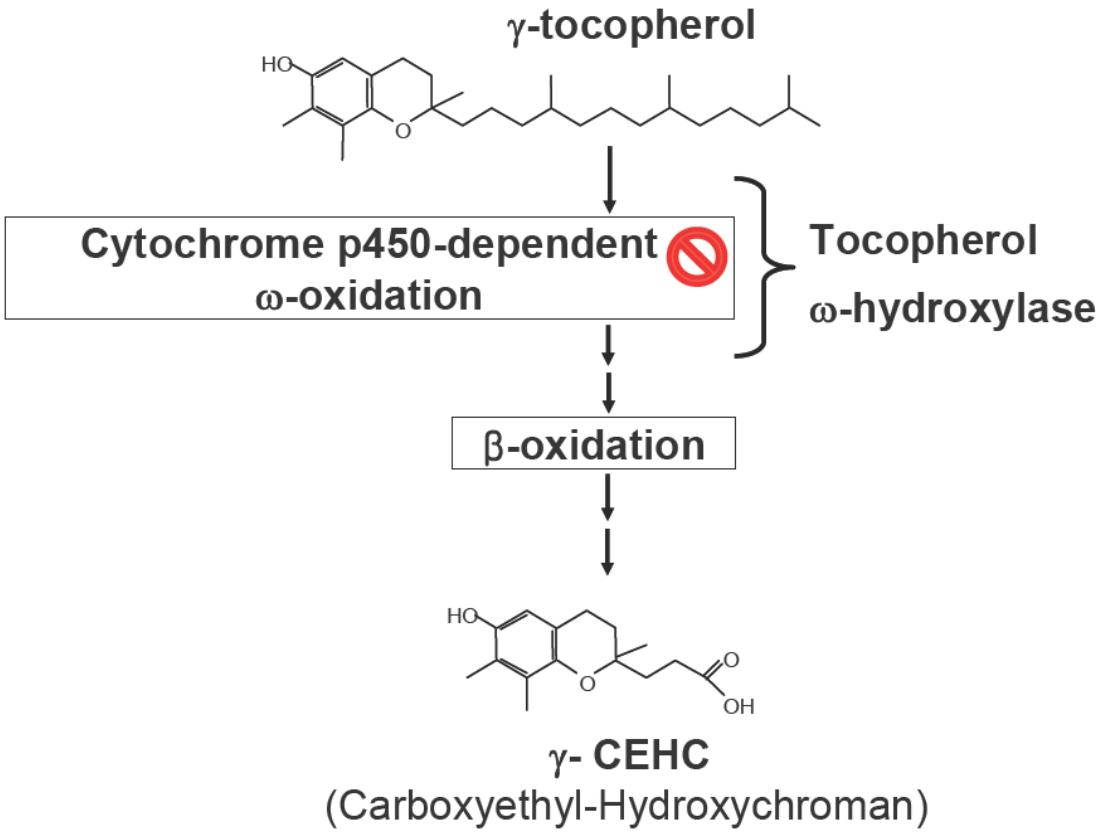
APPENDICES

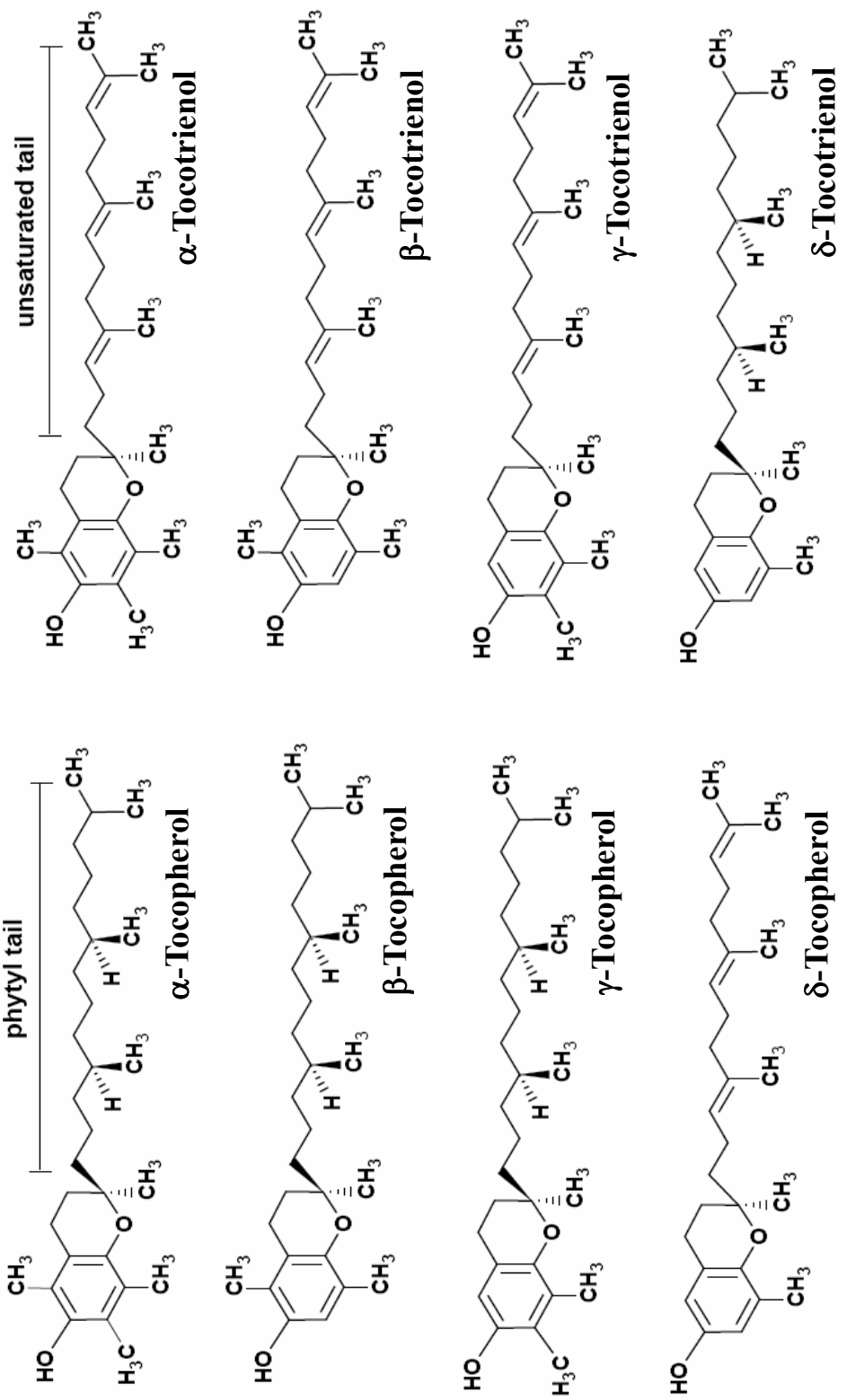
LIST OF APPENDICES

APPENDIX A. TOCOPHEROL ω-HYDROXYLASE ACTIVITY

Inhibition of Tocopherol ω-Hydroxylase Inhibits γ-Tocopherol Metabolism to γ-CEHC

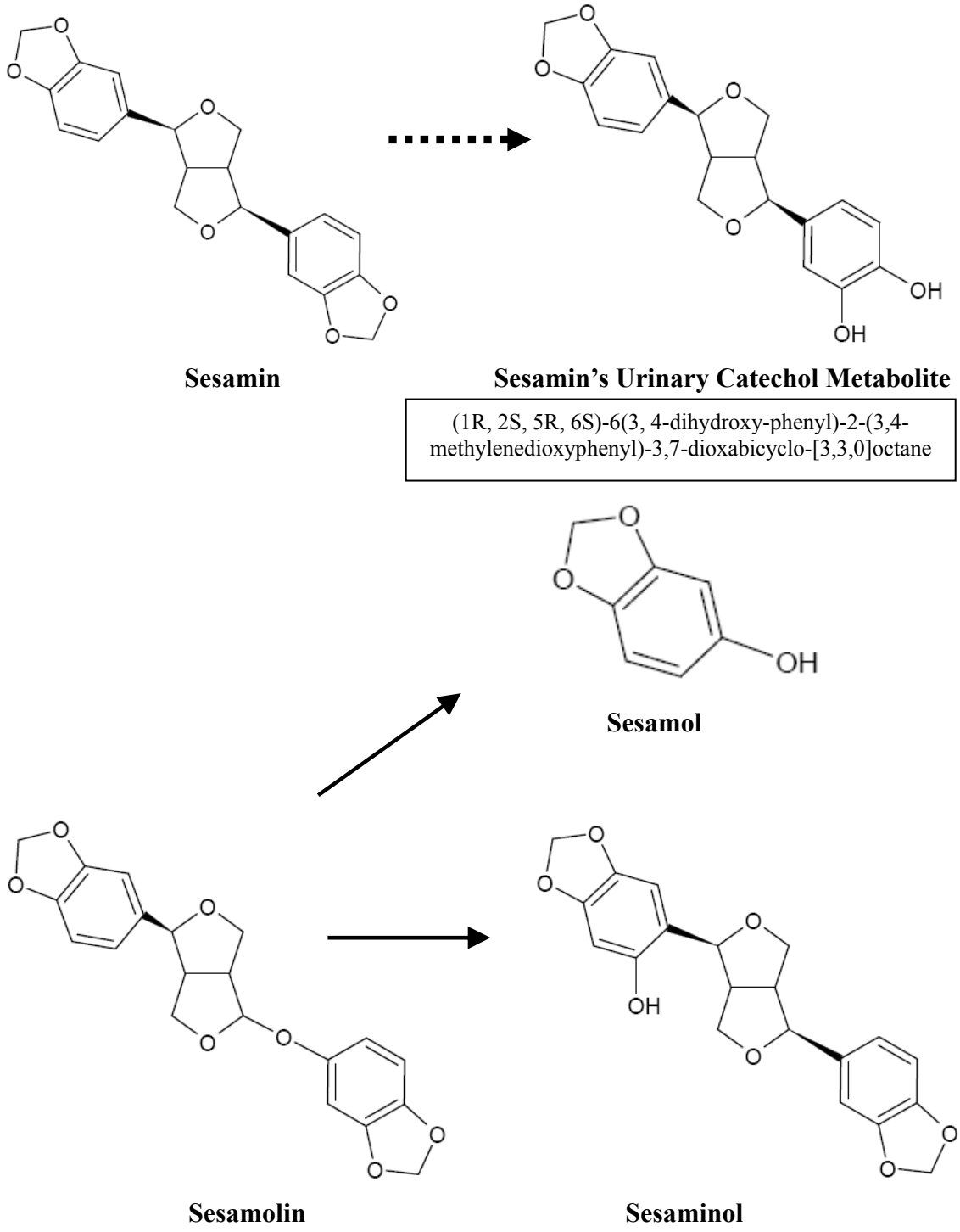


APPENDIX B. STRUCTURE OF TOCOPHEROLS



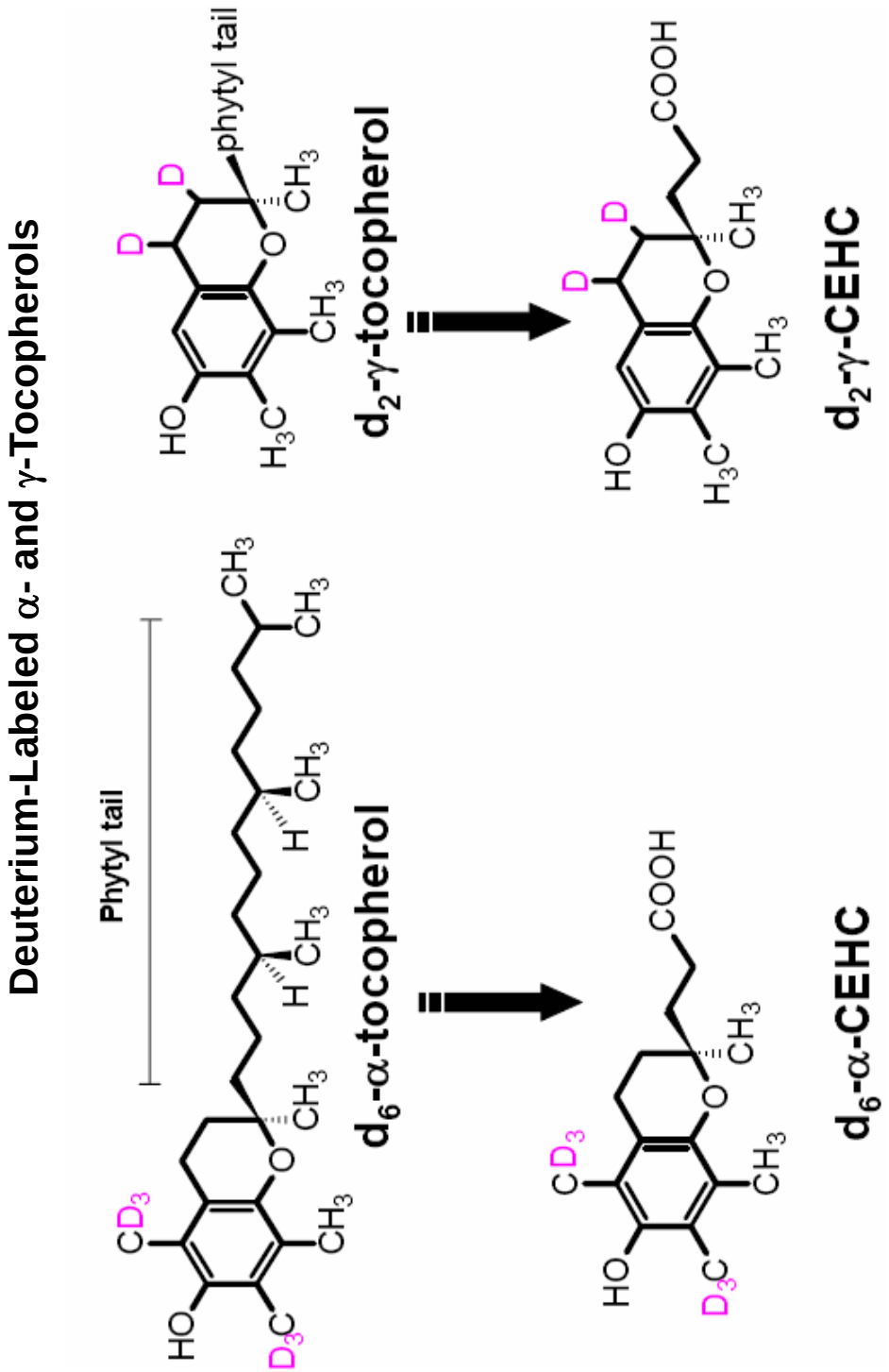
Appendix B. Structure of Toconherols

APPENDIX C. MAJOR SESAME LIGNANS



Adapted from J. Nutr 137 pp. 942 and JAOCS Vol. 83 pp.719

APPENDIX D. DEUTERIUM-LABELED TOCOPHEROLS



Appendix.D. Deuterium-Labeled Tocopherols

APPENDIX E. EXPERIMENTAL DESIGN