

The 4th Global Oils and Fats Business Forum USA

September 8-9th, 2005

San Diego, CA

Session 1: New Scientific and Nutrition Advances

Palm Minor Components and Health with Special Emphasis on

Palm Vitamin E and Carotenoids

Paul W. Sylvester, Ph.D.

Pfizer Endowed Professor of Pharmacology

College of Pharmacy, University of Louisiana at Monroe, Monroe, LA 71209-0470

Telephone: 318-342-1958, email: sylvester@ulm.edu

INTRODUCTION

Edible oils and fats contain variable amounts of natural antioxidants such as carotenoids (pro-vitamin A) and vitamin E. Antioxidants act not only to prevent lipid peroxidation and free-radical production, but also provide a wide range of health benefits that have been suggested to reduce the risk of cardiovascular disease and cancer. Crude palm oil has the highest content of natural carotenoids, ranging from 600 to 1000 parts per million (ppm), as well as, very high levels of vitamin E (500-800ppm) [1]. Conventional refining processes that produce refined, bleached, and deodorized palm olein (RBDPO), remove practically all of the natural carotenoids, but retain a substantial amount of vitamin E. In contrast, red palm oil, which is produced using a nonconventional refining process, retains the majority of carotenoids and vitamin E found in crude palm oil. It is the high level of carotenoids that gives red palm oil a deep reddish color, as compared to the straw-yellow color of RBDPO. The majority (85%) of the carotenoids found in red palm oil are α - and β -carotene, whereas the majority (75-80%) of the vitamin E found in palm oil are tocotrienols.

CAROTENOIDS

Carotenoids represent a family of lipid soluble naturally occurring pigments (usually red, orange or yellow in color) produced in plants and they are extensively used as safe, natural colorants for food, feed, and cosmetics. There are over 600 carotenoids found in nature, of which 40 are regularly consumed in the diet, and 6 can be routinely measured in the blood. They are essential for plant growth and photosynthesis, and are the main dietary source of pro-vitamin A in humans (Figure 1). As pro-vitamin A, carotenoids are converted to retinol (Vitamin A, Figure 1) in the intestinal mucosa of mammals, and then taken up and stored in the liver as vitamin A esters. Different carotenoids have different vitamin A potency, whereas other

carotenoids, such as lutein and lycopine are not converted to vitamin A, but still function as potent antioxidants in the body. The absorption of carotenoids from the gut is nonspecific in nature and depends upon the presence of bile and absorbable fat in the intestinal tract. Nevertheless, dietary consumption of β -carotene displays a direct correlation to the levels found in the blood.

Physiological Functions of Carotenoids and Retinoids: Carotenoids and Retinoids have a large number of important functions in the body. It has long been known that vitamin A deficiency interferes with vision in dim light (night blindness). Photoreception is accomplished by two types of specialized retinal cells called rods and cones. Rods are especially sensitive to light of low intensity, whereas cones act as receptors for high-intensity light and are responsible for color vision. When humans are fed diets deficient in carotenoids, their ability for dark adaptation gradually diminishes until the rods deteriorate and ultimately are destroyed. Vitamin A deficiency will also cause the ulceration of the cornea, which also contributes to the loss of vision and blindness. If vitamin A deficiency is short term, supplementation of carotenoids or retinoids will reverse these effects. However, if vitamin A deficiency persists for approximately ten months, there will irreversible destruction of the retina and cornea, and permanent blindness will occur.

It was found in a two-year study carried out by researchers from Canada and Burkino Faso that dietary supplementation with red palm oil resulted in a clear increase in vitamin A levels among mothers and children [2]. The number of women and children with a retinol levels below the recommended threshold ($0,70 \mu\text{mol/l}$) dropped from 62 to 30 per cent for women and from 84.5 to 67 per cent among children. Supplementation of red palm oil in the diet has subsequently been integrated in national programs aimed at preventing vitamin A deficiency in

several areas in north central and sub-Saharan Africa and has greatly enhanced vitamin A levels in the blood of rural school children in these areas of Africa [2].

In addition to preventing blindness, vitamin A is essential for maintaining the structural and functional integrity of epithelial cells throughout the body. Vitamin A deficiency will result in the atrophy of sweat and mucus glands, and cause the drying of the epidermis. These effects eventually lead to decreased elasticity, cracking, ulcerations of the skin, and greatly increase the risk of infection [3]. Carotenoids and retinoids are also important for normal bone formation and immune function [3].

Carotenoids and Breast cancer: Vitamin A has also been shown to reduce the growth of breast cancer cells grown in culture, but studies examining the effects of dietary carotenoid and retinoid intake in humans have shown a mixed response [4]. The majority of epidemiological studies have failed to find significant associations between retinol intake and a reduction in breast cancer risk [5-8]. Although some large studies have found that total vitamin A intake is inversely associated with the risk of breast cancer in women [9,10], other studies could not confirm these findings [11,12]. Presently, there is little evidence in humans that increased intake of carotenoids or retinoids reduce breast cancer risk in women.

Carotenoids and Lung Cancer: Cell culture and animal model studies have clearly shown that carotenoid treatment significantly reduces carcinogenesis in the skin, breast, liver, colon, prostate, and other sites [4]. However, the results of human studies examining the relationship between the consumption of preformed vitamin A and lung cancer are less clear. At least ten prospective studies have compared blood retinol levels at baseline among people who subsequently developed lung cancer and those who did not. Only one of those studies found a statistically significant inverse association between serum retinol and lung cancer risk [13]. The

results of the “Beta-Carotene and Retinol Efficacy Trial” (CARET) suggest that high-dose supplementation of vitamin A and/or β -carotene should be avoided in people at high risk of lung cancer [14]. About 9,000 people (smokers and people with asbestos exposure) were assigned a daily regimen of 25,000 IU of retinol and 30 milligrams of β -carotene, while a similar number of people were assigned a placebo. After four years of follow up, the incidence of lung cancer was 28% higher in the supplemented group. Presently, it seems unlikely that increased retinol intake decreases the risk of lung cancer, although the effects of retinol may be different for nonsmokers compared to smokers [13].

Vitamin A Toxicity: The condition caused by vitamin A toxicity is called hypervitaminosis A. It is caused by over consumption of retinoids, but not carotenoids. Retinoids are rapidly absorbed and slowly cleared from the body, so toxicity may result acutely from high-dose exposure over a short period of time, or chronically from much lower intake [4]. Vitamin A toxicity is relatively rare, but symptoms include nausea, headache, fatigue, loss of appetite, dizziness, dry itchy skin, and bone and joint pain. Severe cases of hypervitaminosis A may result in liver damage, hemorrhage, and coma. Generally, signs of toxicity are associated with long-term consumption of vitamin A in excess of 10 times the recommended daily allowance (RDA) or levels exceeding 8-10 mg/day or 25,000-33,000 IU/day.

Summary and Recommendations: The RDA for vitamin A (2,300 IU/day for women and 3,000 IU/day for men) is sufficient to support normal gene expression, epidermal health, immune function, and vision. However, because excessive vitamin A intake is associated with adverse effects, it is generally recommended that multivitamin supplements should provide no more than 2,500 IU of natural or synthetic retinoids (vitamin A) or provide no more than 5,000 IU of β -carotene (pro-vitamin A). High potency vitamin A supplements should not be used without

medical supervision due to the risk of toxicity. Taken together, it can be concluded that routine consumption of red palm oil will not only provide a safe means to maintain adequate levels of carotenoids in the diet and prevent vitamin A deficiency, but also eliminate the need to take vitamin A supplements and prevent the potential adverse effects and toxicity associated with retinoids overdose.

TOCOTRIENOLS

Vitamin E is another important antioxidant that regulates peroxidation reactions and controls free-radical production within the body [15]. However, it has become increasingly evident that many of the biological effects of vitamin E are mediated independently of its antioxidant action [15]. Vitamin E has been shown to modulate multiple intracellular signaling pathways involved in hormone-, growth factor- and cytokine-induced endothelial activation, platelet aggregation, monocyte-endothelial cell adhesion, vascular smooth muscle proliferation, as well as, mitogenic and apoptotic pathways in breast cancer cells [16-18].

Vitamin E represents a family of compounds that is further divided into two subclasses called tocopherols and tocotrienols. Tocopherols and tocotrienols have the same basic chemical structure characterized by a long phytyl tail attached to a chromane ring (Figure 2). However, tocopherols have a saturated, whereas tocotrienols have an unsaturated phytyl tail, and individual isoforms of tocopherols and tocotrienols differ from each other based on the degree of methylation (-CH₃ groups) on the chromane ring (Figure 2). Direct comparisons between the two vitamin E subclasses have shown that tocotrienols are significantly more potent than tocopherols in most biological actions, and display a consistent relationship corresponding to δ -tocotrienol \geq γ -tocotrienol > α -tocotrienol > δ -tocopherol > γ - and α -tocopherol [18,19].

Tocotrienols and the Prevention of Cardiovascular Disease: Atherosclerosis is a major cause of age-related disease and death in the United States and it is generally accepted that the cause of this disease can be explained by the “response-to-injury” hypothesis [20]. This hypothesis explains atherosclerosis as a process of chronic inflammatory response to injury of the endothelium or the layer of cells that line the lumen wall of blood vessels. Once the endothelium is injured, there results a series of molecular and cellular interactions between cells of the endothelium, smooth muscle and several blood cell components. The inflammatory response to injury triggers the release of cytokines, growth factors and the generation of free radicals that induce the migration, atherosclerotic plaque formation, and the proliferation of vascular smooth muscle cells, all of which leads to the subsequent narrowing of the vascular lumen [20]. Experimental evidence demonstrates that tocotrienols modulate multiple intracellular signaling pathways involved in many risk factors associated with the process of atherosclerosis, including cholesterol synthesis, endothelial activation, platelet aggregation, monocyte-endothelial cell adhesion, and vascular smooth muscle proliferation [16].

Cell culture and animal studies have shown that tocotrienols, in contrast to tocopherols, inhibit cholesterol synthesis in a similar manner as “statin drugs” by suppressing the activity of hydroxymethylglutaryl coenzyme A reductase (HMGCoAR), the rate-limiting enzyme in the cholesterol biosynthetic pathway [21,22]. A structure-activity relationship was also identified that showed that the unsaturated phytyl tail present on tocotrienols, in contrast to the saturated phytyl tail on tocopherols, is required for suppression of HMGCoAR activity [23]. Demethylation of the chromane ring was also found to significantly increase tocotrienol biopotency [23]. However, similar studies conducted in humans have shown mixed results and

have not firmly established whether or not dietary supplementation will significantly reduce blood cholesterol levels [24-28].

Oxidation of LDL and uptake by macrophages are also important first steps in atherosclerosis. Dietary supplementation with tocotrienols in humans was shown to be effective in reducing the rate of LDL oxidation as compared to the placebo control group [26]. Since tocotrienols have been shown to be a more potent antioxidant than α -tocopherol [29], tocotrienols may aid in preventing the risk of atherosclerosis by acting as a potent inhibitor of LDL oxidation. Likewise, monocyte adhesion to endothelial cells lining in lumen surface of blood vessels, and their subsequent differentiation into macrophages are important early events leading to the development of macrophage-derived foam cells in the atherosclerotic plaque. Studies have shown that tocotrienol is a potent inhibitor of monocytic-endothelial cell adherence and may prevent arterial plaque formation [30].

Enhanced growth factor and cytokine production in the surrounding tissues plays an important role in stimulating excessive smooth muscle cell proliferation and is associated with thickening of the atherosclerotic lesion. Cell culture studies have shown that tocotrienol treatment induces a dose-responsive inhibition of vascular smooth muscle cell proliferation [31]. Thrombus formation begins when platelets adhere and aggregate to the vascular endothelium forming a fibrous plaque that can dislodge and obstruct an artery [32]. Several epidemiological studies have shown that dietary supplementation of tocotrienols suppresses of platelet activation and aggregation and causes an antithrombotic effect [33].

In summary, numerous *in vitro* studies have demonstrated that tocotrienols are more potent than α -tocopherol as antioxidants, antithrombotic agents, suppressors of monocyte adhesion to the endothelium and inhibitors of vascular smooth muscle proliferation. These

findings strongly suggest that dietary supplementation of tocotrienols may have significant value as a therapeutic agent in the prevention and/or treatment of atherosclerosis. However, dietary supplementation with tocotrienols in animal and human studies have not produced consistent results or clearly demonstrated a protective effect against the development and progression of atherosclerosis. Evidence suggests that these discrepancies between *in vitro* and *in vivo* studies results from inefficient or sub-optimal delivery of tocotrienols to target tissues within the body. Further pharmaceuticals research is clearly needed, particularly in the areas of tocotrienol kinetics, formulation, and drug delivery, in order to subsequently clarify in clinical trials the potential value and use of these vitamin E isoforms as therapeutic agents in the prevention and/or treatment of atherosclerosis.

Tocotrienols and the Prevention of Breast Cancer: The anticancer effects of tocotrienols were first discovered in studies investigating the role of high dietary fat intake on carcinogen-induced mammary cancer development and growth in rats [34]. Dietary intake of crude palm oil, in contrast to other high fat diets, was to suppress carcinogen-induced mammary tumorigenesis in experimental animals [33,34]. Furthermore, if tocotrienols are removed from palm oil, the protective effects of high palm oil diets were no longer observed [33,35]. Since palm oil contains a mixture of α -tocopherol (20-25%) and α - γ -, and δ -tocotrienol (75-80%), subsequent studies were conducted to determine if one or all of these vitamin E isoforms were responsible for mediating the antitumor effects of palm oil. Direct comparisons between the two vitamin E subclasses showed that tocotrienols were significantly more potent in suppressing growth and inducing cell death than tocopherols, and these effects were observed using doses of tocotrienols that had no adverse effects on normal mammary epithelial cell growth or function [18,19]. These dose-response studies also showed that IC_{50} doses (dose that inhibited cell growth by 50%

as compared to untreated controls over a five day culture period) of individual tocotrienol isoforms were 5-6 times lower than their corresponding LD₅₀ doses (dose that induced 50% cell death after a 24 hr treatment exposure). Subsequent studies were then conducted to determine if the antiproliferative and cytotoxic effects of tocotrienol are mediated through similar or independent intracellular signaling mechanisms.

Recent experimental evidence indicates that tocotrienols inhibit cancer cell growth by suppressing specific hormone-dependent signaling pathways involved with mitogenesis. Specifically, tocotrienol treatment was found to inhibit hormone- and growth factor-dependent mitogenesis in breast cancer cells, whereas α -tocopherol does not [33,35]. The mitogenic actions of hormones and growth factors are mediated by specific membrane-bound receptors, but tocotrienols were not found to affect receptor level or function [36,37]. These findings indicate that the growth inhibitory effects of tocotrienols occur downstream of the receptor. Several signaling pathways have been shown to mediate hormone and growth factor receptor-dependent growth and survival, particularly the phosphatidylinositol 3-kinase (PI3K)/PI3K-dependent kinase (PDK)/Akt signaling pathways [38,39]. Elevated PI3K/PDK/Akt signaling shows a direct association with advanced tumor progression and poor prognosis in breast cancer patients [38,39]. PI3K is a lipid signaling enzyme that activates PDK-1, and activated PDK-1 then subsequently activates Akt. Activated Akt phosphorylates various proteins associated with cell proliferation and survival. Treatment with low doses (2-6 μ M) of γ -tocotrienol was found to significantly inhibit breast cancer cell growth while having no effect on cell viability, and was associated with a reduction in Akt activity within 2-3 days following treatment exposure. Previous studies have shown that tocotrienol serum levels ranged between 2-4 μ M and display a serum half-life of more than 4 h following a single oral dose of 300 mg mixed tocotrienols in

fasted volunteers, and indicate that treatment doses are within relevant physiological ranges [38,39].

In contrast to low doses (2-6 μ M), higher doses of tocotrienol (>15 μ M) induce breast cancer cells death within 24 hr after treatment exposure [40-42]. This cytotoxic effect results from the initiation of programmed cell death or apoptosis. Apoptosis is an important mechanism by which cancer cells are eliminated from the breast. Morphological and biochemical characteristics distinguish apoptosis from necrosis in terms of nuclear and cytoplasmic condensation, DNA fragmentation, and alterations in the cell membrane composition. The initiation of apoptosis involves the activation of specific enzymes known as caspases. Caspases are constitutively present in cells in an inactive precursor form that must then be cleaved and processed for activation. Recently, studies have shown that treatment with high, cytotoxic doses (20 μ M) of γ -tocotrienol, caused a relatively large increase in caspase-8 and caspase-3 activation, and combined treatment with a specific caspase-8 and/or caspase -3 inhibitors blocked tocotrienol-induced cancer cell death [40-42]. Furthermore, tocotrienol-induced caspase activation is associated with a reduction in FLIP levels. FLIP is an anti-apoptotic protein that acts as an intracellular inhibitor of caspase-8 activation [41]. Activation of various growth factors and cytokine receptors stimulate the PI3K/PDK-1/Akt mitogenic pathway, increase FLIP expression and promote cell proliferation and enhance cell survival. Studies have also shown that overexpression of FLIP is associated with tumor cell resistance to chemotherapeutic drug-induced apoptosis [40,41]. Although the exact mechanism by which Akt regulates FLIP expression is unknown, tocotrienol-induced suppression of PI3K/PDK-1/Akt mitogenic signaling leads to a decrease in FLIP levels and a corresponding increase in caspase-8 activity [40-41]. Taken together, these data strongly suggest that both the antiproliferative and apoptotic effects of

tocotrienol appear to be mediated by suppression in the PI3K/PDK-1/Akt signaling. A growth inhibiting, but not cytotoxic dose (4 μM) of γ -tocotrienol induced a slow and gradual decrease in Akt activity within 2-3 day following treatment exposure, whereas an apoptotic doses (20 μM) of γ -tocotrienol induce a rapid and large reduction in PI3K/PDK-1/Akt signaling and intracellular FLIP levels within 2-4 hrs following treatment exposure.

Conclusions and Recommendations: Since tocotrienols display significantly greater anticancer activity than tocopherols in a variety of *in vitro* experimental models, these finding suggest that dietary supplementation of tocotrienols may prevent the risk of breast cancer in women. Although cell culture studies have firmly established the anticancer effects of tocotrienols, and suggest that tocotrienols are mediating the anticancer effects observed in animals fed palm oil diets, this hypothesis has been difficult to prove. Further studies are needed to clarify the specific interactions between macronutrients (lipids) and micronutrients (antioxidants and vitamins) present in palm oil in providing protective effects against breast cancer development and growth. More extensive research is also needed in the areas related to tocotrienol pharmaceuticals and pharmacokinetics in order to optimized tocotrienol formulation, dosage and delivery to target tissues in the body. In addition, since tocotrienols induce cell death in multi-drug resistance breast cancer cells, further research is need to determine if tocotrienols can be utilized to provide additional therapeutic benefits when combined with traditional chemotherapeutic agents in the treatment of breast cancer.

REFERENCES

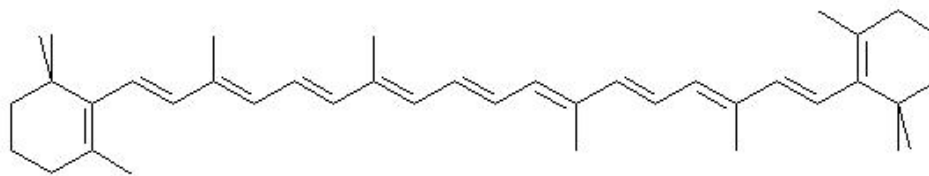
1. Choo YM. (1996) Antioxidants in red palm oil. *MOST* 5:15-16.
2. Solomons NW. (1998) Plant sources of vitamin A and human nutrition: red palm oil does the job. *Nutr Rev* 56:309-311.
3. Ross AC. Vitamin A and retinoids. In: Shils M, ed. *Nutrition in Health and Disease*. 9th ed. Baltimore: Williams & Wilkins; 1999:305-327.
4. Prakash P, Krinsky NI, Russell RM. (2000) Retinoids, carotenoids, and human breast cancer cell cultures: a review of differential effects. *Nutr Rev* 58:170-176.
5. Bohlke K, Spiegelman D, Trichopoulos A, Katsouyanni K, Trichopoulos D. (1999) Vitamins A, C and E and the risk of breast cancer: results from a case-control study in Greece. *Br J Cancer* 79:23-29.
6. Franceschi S. (1997) Micronutrients and breast cancer. *Eur J Cancer Prev* 6:535-539.
7. Longnecker MP, Newcomb PA, Mittendorf R, Greenberg ER, Willett WC. (1997) Intake of carrots, spinach, and supplements containing vitamin A in relation to risk of breast cancer. *Cancer Epidemiol Biomarkers Prev* 6:887-892.
8. Michels KB, Holmberg L, Bergkvist L, Ljung H, Bruce A, Wolk A. (2001) Dietary antioxidant vitamins, retinol, and breast cancer incidence in a cohort of Swedish women. *Int J Cancer* 91:563-567.
9. Zhang S, Hunter DJ, Forman MR, et al. (1999) Dietary carotenoids and vitamins A, C, and E and risk of breast cancer. *J Natl Cancer Inst* 91:547-556.
10. Ching S, Ingram D, Hahnel R, Beilby J, Rossi E. (2002) Serum levels of micronutrients, antioxidants and total antioxidant status predict risk of breast cancer in a case control study. *J Nutr* 132:303-306.

11. Dorgan JF, Sowell A, Swanson CA, et al. (1998) Relationships of serum carotenoids, retinol, alpha-tocopherol, and selenium with breast cancer risk: results from a prospective study in Columbia, Missouri (United States). *Cancer Causes Control* 9:89-97.
12. Hulten K, Van Kappel AL, Winkvist A, et al. (2001) Carotenoids, alpha-tocopherols, and retinol in plasma and breast cancer risk in northern Sweden. *Cancer Causes Control* 2:529-537.
13. Comstock GW, Helzlsouer KJ. Preventive nutrition and lung cancer. In: Bendich A, Decklebaum RJ, eds. *Preventive Nutrition: The Comprehensive Guide for Health Professionals*. 2nd ed. Totowa: Humana Press Inc; 2001:97-129.
14. Omenn GS, Goodman GE, Thornquist MD, et al. (1996) Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. *N Engl J Med* 334:1150-1155.
15. Packer L., Weber SU, Rimbach G. (2001) Molecular aspects of a-tocotrienol antioxidant action and cell signaling. *J Nutr* 131:369S-373S.
16. Keaney JF, Simon DI, Freedman JE. (1999) Vitamin E and vascular homeostasis: implications for atherosclerosis. *Fed Am Soc Exp Bio J* 13:965-976.
17. Ozer KK, Azzi A. (2000) Effect of vitamin E on the development of atherosclerosis. *Toxicology* 148:179-185.
18. McIntyre BS, Briski KP, Tirmenstein MA, Fariss MW, Gapor A, Sylvester PW. (2000) Antiproliferative and apoptotic effects of tocopherols and tocotrienols on normal mouse mammary epithelial cells. *Lipids* 35:171-180.

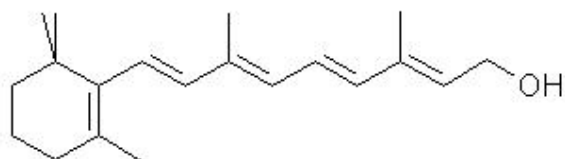
19. McIntyre BS, Briski KP, Gapor A, Sylvester PW. (2000) Antiproliferative and apoptotic effects of tocopherols and tocotrienols on preneoplastic and neoplastic mouse mammary epithelial cells. *Pro Soc Exp Biol Med* 224:292-301.
20. Ross R. (1995) Cell biology of atherosclerosis. *Annu Rev Physiol* 57:791-804.
21. Qureshi AA, Burger WC, Peterson DM, Elson CE. (1986) The structure of an inhibitor of cholesterol biosynthesis isolated from barley. *J Biol Chem* 261:10544-10550.
22. Parker RA, Pearce BC, Clark RW, Gordon DA, Wright JJ. (1993) Tocotrienols regulate cholesterol production in mammalian cells by post-transcriptional suppression of 3-hydroxy-3-methylglutaryl-coenzyme A reductase. *J Biol Chem* 268:11230-11238.
23. Pearce BC, Parker RA, Deason ME, Qureshi AA, Wright J J. (1992) Hypocholesterolemic activity of synthetic and natural tocotrienols. *J Medicinal Chem* 35:3595-3606.
24. Wahlqvist ML, Krivokuca-Bogetic Z, Lo CS, Hage B. (1992) Differential serum responses of tocopherols and tocotrienols during vitamin supplementation in hypercholesterolaemic individuals without change in coronary risk factors. *Nutr Res* 12:S181-S201.
25. Tomeo AC, Geller M, Watkins TR, Gapor A, Bierenbaum ML. (1995) Antioxidant effects of tocotrienols in patients with hyperlipidemia and carotid stenosis. *Lipids* 30:1179-1183.
26. O'Byrne D, Grundy S, Packer L, Devaraj S, Baldenius K, Hoppe PP, Kraemer K, Jialal I, Traber MG. (2000) Studies of LDL oxidation following alpha-, gamma-, or delta-tocotrienyl acetate supplementation of hypercholesterolemic humans. *Free Rad Biol Med* 29:834-845.
27. Mustad VA, Smith CA, Ruey PP, Edens NK, DeMichele SJ. (2002) Supplementation with 3 compositionally different tocotrienol supplements does not improve cardiovascular disease risk factors in men and women with hypercholesterolemia. *Am J Clin Nutr* 76:1237-1243.

28. Qureshi AA, Sami SA, Salser WA, Khan FA. (2002) Dose-dependent suppression of serum cholesterol by tocotrienol-rich fraction (TRF) of rice bran in hypercholesterolemic humans. *Atherosclerosis* 161:199-207.
29. Begum AN, Terao J. (2002) Protective effect of alpha-tocotrienol against free radical-induced impairment of erythrocyte deformability. *Biosci Biotech Biochem* 66:398-403.
30. Theriault A, Chao JT, Gapor A. (2002) Tocotrienol is the most effective vitamin E for reducing endothelial expression of adhesion molecules and adhesion to monocytes. *Atherosclerosis* 160:21-30.
31. Chatelain E, Boscoboinik DO, Bartoli GM, Kagan VE, Gey FK, Packer L, Azzi A. (1993) Inhibition of smooth muscle cell proliferation and protein kinase C activity by tocopherols and tocotrienols. *Biochim Biophys Acta* 1176:83-89.
32. Hirsh J. (1987) Hyperactive platelets and complications of coronary artery disease. *New Eng J Med* 316:1543-1544.
33. Sylvester PW, Theriault A. (2003) Role of tocotrienols in the prevention of cardiovascular disease and breast cancer. *Curr Top Nutraceutical Res* 1:121-136.
34. Sylvester PW, Russell M, Ip MM, Ip C. (1986) Comparative effects of different animal and vegetable fats fed before and during carcinogen administration on mammary tumorigenesis, sexual maturation, and endocrine function in rats. *Can Res* 46: 757-762.
35. Sylvester PW, Shah SJ. (2005) Mechanisms mediating the antiproliferative and apoptotic effects of vitamin E in mammary cancer cells. *Front Biosci* 10: 699-709
36. Sylvester PW, McIntyre BS, Gapor A, Briski KP. (2001) Vitamin E inhibition of normal mammary epithelial cell growth is associated with a reduction in protein kinase C(alpha) activation. *Cell Prolif* 34:347-57.

37. Sylvester PW, Nachnani A, Shah S, Briski KP. (2002) Role of GTP-binding proteins in reversing the antiproliferative effects of tocotrienols in preneoplastic mammary epithelial cells. *Asia Pacific J Clin Nutr* 11:S452-S459.
38. Shah S, Sylvester PW. (2005) Antiproliferative effects of γ -tocotrienol are associated with a reduction in Akt and NF κ B activity. *Exp Biol Med* 230:235-241.
39. Sylvester PW, Shah SJ, Samant GV. (2005) Intracellular signaling mechanisms mediating the antiproliferative and apoptotic effects of γ -tocotrienol in neoplastic mammary epithelial cells. *J Plant Phys* 162:803-810.
40. Shah S, Gapor A, Sylvester PW. (2003) Role of caspase-8 activation in mediating vitamin E-induced apoptosis in murine mammary cancer cells. *Nutr Cancer* 45: 236-246.
41. Shah S, Sylvester PW (2004) Tocotrienol-induced caspase-8 activation is unrelated to death receptor apoptotic signaling in neoplastic mammary epithelial cells. *Exp Biol Med* 229:745-755.
42. Shah S, Sylvester PW. (2005) Tocotrienol-induced cytotoxicity is unrelated to mitochondrial stress apoptotic signaling in neoplastic mammary epithelial cells. *Biochem Cell Biol* 83:86-95.

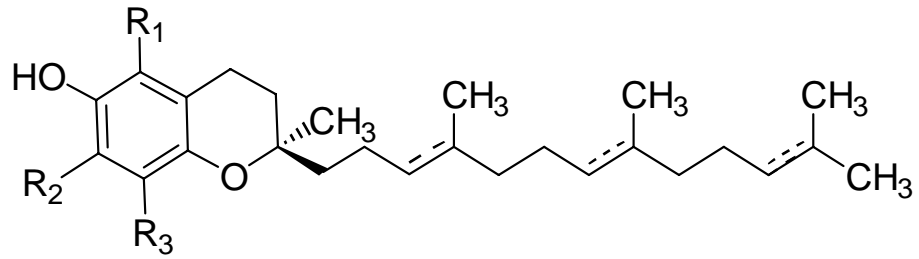


β -Carotene (Pro-Vitamin A)



Retinol (Vitamin A)

Figure 1. The General Chemical Structure of Carotenoids and Retinoids



Compound	R ₁	R ₂	R ₃	Phytyl Chain
α-tocopherol	CH ₃	CH ₃	CH ₃	Saturated
γ-tocopherol	H	CH ₃	CH ₃	Saturated
δ-tocopherol	H	H	CH ₃	Saturated
α-tocotrienol	CH ₃	CH ₃	CH ₃	Unsaturated
γ-tocotrienol	H	CH ₃	CH ₃	Unsaturated
δ-tocotrienol	H	H	CH ₃	Unsaturated

Figure 2: Generalized Structure of Vitamin E