Neurological Protection and Breast Cancer Prevention and Likely Treatment with Tocotrienols

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Vitamin E, in its natural form, comprises eight different compounds: alpha-, beta-, gamma-, and delta-**tocopherols** and alpha-, beta-, gamma-, and delta-**tocotrienols**. Both tocopherols and tocotrienols are important to human health. Known as the "master antioxidant," vitamin E has the ability to attenuate oxidative stress, and its antioxidant-related effects on various organs and systems have been the focus of vast research. More recently, non-antioxidant mechanisms have been proposed, such as those that affect cell signal transduction and gene expression. Though the vast majority of research has been on alpha-tocopherol, recent mechanistic studies indicate that other isomers of vitamin E, such as gamma- and deltatocopherols and tocotrienols, have superior antioxidant and cell signaling properties that offer greater health benefits

Studies have demonstrated that tocotrienols have superior antioxidant activity compared to tocopherols. Tocotrienols also exhibit biological activities related to neuroprotection, radioprotection, cell-life regulation, cytokine modulation, and lipid metabolism that are not shared by tocopherols

Metabolites 2022, 12, 608.

Antioxidant and Neuroprotective Activity of Vitamin E Homologues: In Vitro Study

Agnieszka Trela-Makowej , Monika Le´skiewicz, Jerzy Kruk, etal.

Abstract: Here we present comparative data on the inhibition of lipid peroxidation by a variety of tocochromanols in liposomes. We also show for the first time the potential neuroprotective role of all the vitamin E homologues investigated on the neuronally differentiated human neuroblastoma SH-SY5Y cell line. α-Tocopherol

had nearly no effect in the inhibition of lipid peroxidation, while β-, γ-, and δ-tocopherols inhibited the reaction completely when it was initiated in a lipid phase. Similar effects were observed for tocotrienol homologues. Moreover, in this respect plastochromanol-8 was as effective as β-, γ-, and δ-tocochromanols. When the prenyllipids were investigated in a 1,1-diphenyl-2-picrylhydrazyl (DPPH) test and incorporated into different lipid carriers, the rad-ical oxidation was most pronounced in liposomes, followed by mixed micelles and the micellar system. When the reaction of tocochromanols was examined in niosomes, the oxidation was most pronounced for α-tocopherol and plastochromanol-8, followed by α-tocotrienol. Next, using retinoic acid-differentiated SH-SY5Y cells, we tested the protective effects of the compounds investigated on hydrogen peroxide (H2O2)-induced cell damage. We showed that tocotrienols were more active than tocopherols in the oxidative stress model. Plastochromanol-8 had a strong inhibitory effect on H2O2-induced lactate dehydrogenase (LDH) release and H2O2-induced decrease in cell viability. The water-soluble α-tocopherol phosphate had neuroprotective effects at all the concentrations analyzed. **The results clearly indicate that structural differences between vitamin E homologues reflect their different biological activity and indicate their potential application in pharmacological treatments for neurodegenerative diseases. In this respect, the application of optimal tocochromanol-carrying might be critical.**

J Nutr Sci Vitaminol, 65, S185–S187, 2019

Koji Fukui

Neuroprotective and Anti-Obesity Effects of Tocotrienols

Summary Vitamin E is a natural lipophilic vitamin, and the most famous function of vitamin E is an antioxidant activity. Because we have a-tocopherol transfer protein, many vitamin E-related reports are about a-tocopherol. Recently, other vitamin E isoforms, tocotrienols are focusing. Because tocotrienols have unique biological functions such as induction of apoptosis, neuroprotective and anti-obesity effects. Tocotrienols contain in annatto, palm, whole wheat and rice bran. Rice is a typical food in the East Asian countries and Japan. Recently, intake of whole rice is a popular in young women of Japan. Previously, we demonstrated

that treatment with tocotrienols on the neuronal cells shows a strong antioxidant effect compared to the tocopherols. In this review, I introduce about neuroprotective and anti-obesity effects of tocotrienols. I would like to show daily intake of whole rice is very good for our health in this review.

Maznah Ismail, , Abdulsamad Alsalahi , Mustapha Umar Imam , Der Jiun Ooi, etal.

Nutrients 2020, 12, 521

Safety and Neuroprotective Efficacy of Palm Oil and Tocotrienol-Rich Fraction from Palm Oil: A Systematic Review

Abstract: Background: Several natural products have been reported to elicit beneficial effects against neurodegenerative disorders due to their vitamin E contents. However, the neuroprotective efficacy of palm oil or its tocotrienol-rich fraction (TRF) from the pre-clinical cell and animal studies have not been systematically reviewed. Methods: The protocol for this systematic review was registered in "PROSPERO" (CRD42019150408). This review followed the Preferred Reporting Items for SystematicReviews and Meta-Analysis (PRISMA) guidelines. The Medical Subject Heading (MeSH) descriptorsof PubMed with Boolean operators were used to construct keywords, including ("Palm Oil"[Mesh])AND "Nervous System"[Mesh], ("Palm Oil"[Mesh]) AND "Neurodegenerative Diseases"[Mesh],("Palm Oil"[Mesh]) AND "Brain"[Mesh], and ("Palm Oil"[Mesh]) AND "Cognition"[Mesh], to retrievethe pertinent records from PubMed, Scopus, Web of Science and ScienceDirect from 1990 to 2019, whilebibliographies, ProQuest and Google Scholar were searched to ensure a comprehensive identification of relevant articles. Two independent investigators were involved at every stage of the systematic review, while discrepancies were resolved through discussion with a third investigator. Results: All of the 18 included studies in this review (10 animal and eight cell studies) showed that palmoil and TRF enhanced the cognitive performance of healthy animals. In diabetes-induced rats, TRF and α-tocotrienol enhanced cognitive function and

exerted antioxidant, anti-apoptotic and anti-inflammatory activities, while in a transgenic Alzheimer's disease (AD) animal model, TRF enhanced the cognitive function and reduced the deposition of β-amyloid by altering the expression of several genes related to AD and neuroprotection. In cell studies, simultaneous treatment with α-tocotrienols and neurotoxins improved the redox status in neuronal cells better than γ- and δ-tocotrienols. Both pre-treatment and post-treatment with α-tocotrienol relative to oxidative insults were able to enhance the survival of neuronal cells via increased antioxidant responses.

Conclusions: Palm oil and its TRF enhanced the cognitive functions of healthy animals, while TRF and α-tocotrienol enhanced the cognitive performance with attenuation of oxidative stress, neuroinflammation and apoptosis in diabetes-induced or transgenic AD animal models. In cell studies, TRF and α-tocotrienol exerted prophylactic neuroprotective effects, while α-tocotrienol exerted therapeutic neuroprotective effects that were superior to those of γ- and δ-tocotrienol isomers.

Ageing Research Reviews
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Ahsan et al. Nutrition & Metabolism 2014, 11:52

Do tocotrienols have potential as neuroprotective dietary factors?

Abstract

Tocotrienols (T_3) belong to the family of vitamin E compounds (α-, β-, γ-, δ-tocopherols and -tocotrienols) and have unique biological properties that make them potential neuroprotective dietary factors. In addition to their antioxidant activity, T_3 at micromolar concentrations exert cholesterol-lowering activities in cells, animal models and some, but not all, human studies by means of inhibition of the activity of the rate-limiting enzyme in cholesterol biosynthesis, 3-hydroxy-3-methylglutaryl coenzyme A reductase. At lower concentrations (~10 nmol/L), T_3 modulate signalling pathways involved in neuronal cell death in cell culture experiments. Targets of T_3 include prenyl transferases, non-receptor tyrosine kinase, phospholipase A_2, 12-lipoxygenase, cyclooxygenase-2, and nuclear factor κB. The low

bioavailability and rapid excretion of T_3 represents a major hurdle in their preventive use. Fasting plasma concentrations, even after supplementation with high doses, are below 1 μmol/L. T_3 bioavailability may be enhanced by ingestion with a high-fat meal, self-emulsifying drug delivery systems, or phytochemicals that inhibit T_3 metabolism and excretion. T_3 have no known adverse effects when consumed as part of a normal diet and the studies reviewed here support the notion that they may have potential as neuroprotective agents. However, experiments in relevant animal models and randomised human intervention trials addressing the neuroprotection mediated by T_3 are scarce and, thus, highly warranted.

Highlights

► Tocotrienols (T_3) are natural vitamin E compounds with low bioavailability. ► Bioavailability is affected by dietary fat and ingestion of phytochemicals. ► T_3 are antioxidants, hypocholesterolemic agents, and alter cell signalling. ► Cellular targets include enzymes and transcription factors. ► T_3 are safe and have potential as neuroprotective dietary factors.

R EVI EW Open Access **Pharmacological potential of tocotrienols: a review**

Haseeb Ahsan1 , Amjid Ahad , Jahangir Iqbal and Waseem A Siddiqui

Abstract Tocotrienols, members of the vitamin E family, are natural compounds found in a number of vegetable oils, wheat germ, barley, and certain types of nuts and grains. Like tocopherols, tocotrienols are also of four types viz. alpha, beta, gamma and delta. Unlike tocopherols, tocotrienols are unsaturated and possess an isoprenoid side chain. Tocopherols are lipophilic in nature and are found in association with lipoproteins, fat deposits and cellular membranes and protect the polyunsaturated fatty acids from peroxidation reactions. The unsaturated chain of tocotrienol allows an efficient penetration into tissues that have saturated fatty layers such as the brain and liver. Recent mechanistic studies indicate that other forms of vitamin E, such as γ-tocopherol, δ-tocopherol, and γ-tocotrienol, have unique antioxidant and anti-inflammatory properties that are superior to those of α-tocopherol against chronic diseases. These forms scavenge reactive nitrogen

species, inhibit cyclooxygenase- and 5-lipoxygenase-catalyzed eicosanoids and suppress proinflammatory signalling, such as NF-κB and STAT. The animal and human studies show tocotrienols may be useful against inflammation-associated diseases. Many of the functions of tocotrienols are related to its antioxidant properties and its varied effects are due to it behaving as a signaling molecule. Tocotrienols exhibit biological activities that are also exhibited by tocopherols, such as neuroprotective, anti-cancer, anti-inflammatory and cholesterol lowering properties. Hence, effort has been made to compile the different functions and properties of tocotrienols in experimental model systems and humans. This article constitutes an in-depth review of the pharmacology, metabolism, toxicology and biosafety aspects of tocotrienols. Tocotrienols are detectable at appreciable levels in the plasma after supplementations. However, there is inadequate data on the plasma concentrations of tocotrienols that are sufficient to demonstrate significant physiological effect and biodistribution studies show their accumulation in vital organs of the body. Considering the wide range of benefits that tocotrienols possesses against some common human ailments and having a promising potential, the experimental analysis accounts for about a small fraction of all vitamin E research. The current state of knowledge deserves further investigation into this lesser known form of vitamin E.

Studies show women that exercised **three hours** per week had 30% less breast cancer, but those who exercised **four hours per week were 60% less** likely to get breast cancer.

The role of physical activity in breast cancer etiology.

Semin Oncol. 2010 Jun;37(3):297-302 . Friedenreich CM.

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Abstract

Considerable research interest has been given in the past 25 years to examining the role of physical activity in breast cancer prevention given the scarcity of modifiable risk factors for this major cause of cancer incidence and mortality in women. A review of the observational epidemiologic evidence and recent randomized exercise intervention trials on the association between physical activity and breast cancer risk is presented. As of March 2010, 73 separate studies out of 91 publications worldwide were identified as having sufficient data for this review. Across these 73 studies, the average reduction in breast cancer risk, when comparing the most to the least physically active women, was 25%. There also was evidence for a dose-response effect found in the majority of studies that examined this trend. The

strongest associations were found for recreational and household activities and for activity that was of at least moderate intensity and sustained over a lifetime. Within population subgroups, a stronger effect was seen in women who are normal weight, in women without a family history of breast cancer, and in women who are parous. Women of all races benefitted from physical activity; however, a particularly strong effect on breast cancer risk was observed in non-Caucasian women. Future research should focus on elucidating the exact type, dose, and timing of physical activity required to reduce breast cancer risk. Prospective observational epidemiologic studies of lifetime physical activity patterns and breast cancer risk would help in this regard, as well as randomized controlled exercise intervention trials employing hypothesized biomarkers of breast cancer risk as outcome measures. Additional consideration to the role of sedentary behavior and light-intensity activity also is needed, as well as improved physical activity assessment methods. These additional data will be useful in improving public health recommendations regarding physical activity for breast cancer risk reduction.

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Advancing the Role of Gamma-Tocotrienol as Proteasomes Inhibitor: A Quantitative Proteomic Analysis of MDA-MB-231 Human Breast Cancer Cells

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Abstract: Tocotrienol, an analogue of vitamin E has been known for its numerous health benefits and anti-cancer effects. Of the four isoforms of tocotrienols, gamma-tocotrienol (γT3) has been frequently reported for their superior anti-tumorigenic activity in both in vitro and in vivo studies, when compared to its counterparts. In this study, the effect of γT3 treatment in the cytoplasmic and nuclear fraction of MDA-MB-231 human breast cancer cells were assessed using the label-free quantitative proteomics analysis. The cytoplasmic proteome results revealed the ability of γT3 to inhibit a group of proteasome proteins such as PSMA, PSMB, PSMD, and PSME. The inhibition of proteasome proteins is known to induce apoptosis in cancer cells. As such, the findings from this study suggest γT3 as a potential proteasome inhibitor that can overcome deficiencies in growth-inhibitory or pro-apoptotic molecules in breast cancer cells. The nuclear proteome results revealed the involvement of important nuclear protein complexes which hardwire the anti-tumorigenesis mechanism in breast cancer following γT3 treatment. In conclusion, this study uncovered the advancing roles of γT3 as potential proteasomes inhibitor that can be used for the treatment of breast cancer.

Cancer Causes Control. 2010 Apr;21(4):577-86. Epub 2010 Jan 19

Moderate physical activity and breast cancer risk: the effect of menopausal status.

Angeles-Llerenas A, Ortega-Olvera C, Pérez-Rodríguez E, Esparza-Cano JP, Lazcano-Ponce E, Romieu I, Torres-Mejía G.

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Abstract

INTRODUCTION: It has been suggested that both moderate-and vigorous-intensity physical activity reduces the risk of breast cancer. However, the effect of moderate-intensity physical activity on breast cancer risk has not been consistently evaluated by menopausal status and has not been evaluated in Mexican women.

OBJECTIVE: To evaluate the effect of moderate-intensity physical activity (h/week and MET-h/week) on the risk of breast cancer by menopausal status in Mexican women.

METHODS: A population-based case-control study was conducted in Mexico. One thousand incident cases and 1,074 matched controls to cases by 5 years of age, site and health institution participated in the study. Women provided information on health, diet and physical activity by means of an in-person interview. Anthropometric measurements and blood samples were obtained from all women. A conditional logistic regression model was used to assess this association.

RESULTS: Participating in moderate-intensity physical activity decreased the risk of BC in both pre- and postmenopausal women (OR = 0.96; 95% CI 0.92.-0.99; OR = 0.90; 95% CI 0.86-0.93, respectively) for every 3 h per week of moderate-intensity physical activity. There was a statistically significant modification effect by menopausal status (p = 0.009).

CONCLUSIONS: Strategies need to be identified that will engage women in physical activity programs.

A study published in the Journal of Clinical Oncology reported that **exercise reduced mortality from all causes in breast cancer survivors by 50%,** when combined with a healthy diet. These results were true for lean and obese women, although obese women had more trouble sticking to a healthy diet.

Participation with the **Brain Back Body Exercise program** will satisfy the requirement of moderate exercise. It will provide aerobic and resistance training along with brain integration exercises. For an example of exercises go **to www.theneurotechnologies.com**

d1, inhibitor of differentiation, is a key protein mediating anti-tumor responses of gamma-tocotrienol in breast cancer cells.

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Gamma-tocotrienol has demonstrated anti-proliferative effect on breast cancer (BCa) cells, but mechanisms involved are largely unknown. This study aimed at deciphering the molecular pathways responsible for its activity. Our results showed that treatment of BCa cells with gamma-tocotrienol resulted in induction of apoptosis as evidenced by activation of pro-caspases, accumulation of sub-G1 cells and DNA fragmentations. Examination of the pro-survival

genes revealed that the gamma-tocotrienol-induced cell death was associated with suppression of Id1 and NF-kappaB through modulation of their upstream regulators (Src, Smad1/5/8, Fak and LOX). Meanwhile, gamma-tocotrienol treatment also resulted in the induction of JNK signaling pathway and inhibition of JNK activity by specific inhibitor partially blocked the effect of gamma-tocotrienol. Furthermore, synergistic effect was observed when cells were co-treated with gamma-tocotrienol and Docetaxel. Interestingly, in cells that treated with gamma-tocotrienol, alpha-tocopherol or beta-aminoproprionitrile were found to partially restore Id1 expression. Meanwhile, this restoration of Id1 was found to protect the cells from gamma-tocotrienol induced apoptosis. Consistent outcome was observed in cells ectopically transfected with the Id-1 gene. Our results suggested that the anti-proliferative and chemosensitization effect of gamma-tocotrienol on BCa cells may be mediated through downregulation of Id1 protein.

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A novel mechanism of natural vitamin E tocotrienol activity: involvement of ERβ signal transduction

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Vitamin E is a generic term used to indicate all tocopherol (TOC) and tocotrienol (TT) derivates. In the last few years, several papers have shown that a TT-rich fraction (TTRF) extracted from palm oil inhibits proliferation and induces apoptosis in a large number of cancer cells. However, the molecular mechanism(s) involved in TT action is still unclear. In the present study, we proposed for the first time a novel mechanism for TT activity that involves estrogen receptor (ER) signaling. In silico simulations and in vitro binding analyses indicated a high affinity of TTs for ERβ but not for ERα. In addition, in ERβ-containing MDA-MB-231 breast cancer cells, we demonstrated that TTs increase the ERβ translocation into the nucleus, which in turn activates estrogen-responsive genes (*MIC-1*, *EGR-1* and *cathepsin D*), asdemonstrated by cell preincubation with the ER inhibitor ICI-182,780. Finally, we observed that TT treatment is associated with alteration of cell morphology, DNA fragmentation, and caspase-3 activation. Altogether, these experiments elucidated the molecular mechanism underling γ- and δ-TT effects.

estrogen receptor-β; breast cancer; apoptosis; tocopherol; nuclear receptor

Suppression of cell proliferation and gene expression by combinatorial synergy of EGCG, resveratrol and gamma-tocotrienol in estrogen receptor-positive MCF-7 breast cancer cells.

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Numerous dietary phytochemicals have shown anti-breast carcinogenic activities when tested in vitro; however, in most cases, the demonstrated efficacy of individual phytochemicals requires doses not readily achievable in vivo. Therefore, whether diets might exert translational promises and benefits in clinical settings and prevention of breast cancer remain unclear. Since cancer cells are endowed with complex, redundant, converging and diverging pathways spanning both the genetic and metabolic networks that are not merely replicates of those in normal cells, it is of interest to test whether a multicomponent approach involving lower, physiologically relevant doses of natural dietary agents may be developed as a chemopreventive strategy for breast cancer. Herein, we investigated, using the estrogen receptor-positive MCF-7 breast cancer cells as a model, whether the combination of epigallocatechin gallate (EGCG), resveratrol and gamma-tocotrienol at suboptimal doses elicits synergism in suppressing cell proliferation, modulating gene expression, and increasing antioxidant activity, as compared to each of the three phytochemicals added alone. The results showed that there was a approximately 33, 50 and 58% inhibition of cell proliferation by > or =50 microM EGCG, > or =25 microM resveratrol and > or =10 microM gamma-tocotrienol, respectively, added as a single agent. When a suboptimal dose (10 microM) of each phytochemical was used, a significant additive effect in suppression of cell proliferation was observed with the combination of resveratrol and gamma-tocotrienol whereas the three phytochemicals added together did not produce more pronounced inhibition of cell proliferation. A significant additive effect in reducing cyclin D1 and bcl-2 expression was found when gamma-tocotrienol was added with either EGCG or resveratrol. Functional synergism among the three phytochemicals was only observed in the induction of quinone reductase NQO1. These results suggest that diet-based protection against breast cancer may partly derive from synergy amongst dietary phytochemicals directed against specific molecular targets in responsive breast cancer cells, and provide support for the feasibility of the development of a diet-based combinatorial approach in the prevention and treatment of breast cancer.

Tocotrienol levels in adipose tissue of benign and malignant breast lumps in patients in Malaysia.

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Data on dietary exposure to vitamin E by plasma or adipose tissue concentrations of alpha-tocopherol (alpha-T) in observational studies have failed to provide consistent support for the idea that alpha-T provides women with any protection from breast cancer. In contrast, studies indicate that alpha, gamma, and delta-tocotrienols but not alpha-T have potent anti-proliferative effects in human breast cancer cells. Our aim was to investigate whether there was a difference in tocopherol and tocotrienol concentrations in malignant and benign adipose tissue, in a Malaysian population consuming predominantly a palm oil diet. The study was undertaken using fatty acid levels in breast adipose tissue as a biomarker of qualitative dietary intake of fatty acids. The major fatty acids in breast adipose tissue of patients (benign and malignant) were oleic acid (45-46%), palmitic (28-29%) and linoleic (11-12%). No differences were evident in the fatty acid composition of the two groups. There was a significant difference (p=0.006) in the total tocotrienol levels between malignant (13.7 +/- 6.0 microg/g) and benign (20+/-6.0 microg/g) adipose tissue samples. However, no significant differences were seen in the total tocopherol levels (p=0.42) in the two groups. The study reveals that dietary intake influences adipose tissue fatty acid levels and that adipose tissue is a dynamic reservoir of

fat soluble nutrients. The higher adipose tissue concentrations of tocotrienols in benign patients provide support for the idea that tocotrienols may provide protection against breast cancer.

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Pro-apoptotic mechanisms of action of a novel vitamin E analog (alpha-TEA) and a naturally occurring form of vitamin E (delta-tocotrienol) in MDA-MB-435 human breast cancer cells.

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Vitamin E derivative, RRR-alpha-tocopheryl succinate (vitamin E succinate, VES), is a potent pro-apoptotic agent, inducing apoptosis by restoring both transforming growth factor-beta (TGF-beta) and Fas (CD95) apoptotic signaling pathways that contribute to the activation of c-Jun N-terminal kinase (JNK)-mediated apoptosis. Objectives of these studies were to characterize signaling events involved in the pro-apoptotic actions of a naturally occurring form of vitamin E, delta-tocotrienol, and a novel vitamin E analog, alpha-tocopherol ether acetic acid analog [alpha-TEA; 2,5,7,8-tetramethyl-2R-(4R,8R,12-trimethyltridecyl)chroman-6-yloxyacetic acid]. Like VES, alpha-TEA and delta-tocotrienol induced estrogen-nonresponsive MDA-MB-435 and estrogen-responsive MCF-7 human breast cancer cells to undergo high levels of apoptosis in a concentration- and time-dependent fashion. Like VES, the two compounds induced either no or lower levels of apoptosis in normal human mammary epithelial cells and immortalized but nontumorigenic human MCF-10A cells. The pro-apoptotic mechanisms triggered by the structurally distinct alpha-TEA and delta-tocotrienol were identical to those previously reported for VES, that is, alpha-TEA- and delta-tocotrienol-induced apoptosis involved up-regulation of TGF-beta receptor II expression and TGF-beta-, Fas- and JNK-signaling pathways. These data provide a better understanding of the anticancer actions of a dietary form of vitamin E (delta-tocotrienol) and a novel nonhydrolyzable vitamin E analog (alpha-TEA). Copyright 2004 Lawrence Erlbaum Associates, Inc.

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Disruption of mitochondria during tocotrienol-induced apoptosis in MDA-MB-231 human breast cancer cells.

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Tocotrienols, which are Vitamin E isoforms, are known to inhibit the growth of human breast cancer cells due partly to apoptosis. However, the characterization of tocotrienol-induced apoptosis is incomplete, particularly what happens during the initiation phase that precedes execution of the cells. The objective of this study was to clarify the apoptotic effects of tocotrienols, with special emphasis in determining if the mitochondria-mediated death pathway is activated when human breast cancer cells are incubated with a specific tocotrienol isomer. During incubation with gamma-tocotrienol, MDA-MB-231 human breast cancer cells showed membrane blebbing, and apoptotic bodies were present. Upon 4',6-diamidino-2-phenylindole staining of the cells, chromatin condensation and fragmentation were observed. Additionally, the annexin V-binding assay detected the translocation of membrane phospholipid during earlier analysis of the cells. Taken together, these results further establish that gamma-tocotrienol can induce apoptosis in human breast cancer cells. To help elucidate how gamma-tocotrienol induced the apoptosis, some important parameters related to the mitochondria-mediated death pathway were examined next. In gamma-tocotrienol-treated cells, the mitochondria were disrupted. Collapse of the mitochondrial membrane potential was detected, and cytochrome c was released later from mitochondria. However, expression of Bax and Bcl-2 (mRNA and protein) did not change. Furthermore, poly-(ADP-ribose)-polymerase cleavage was not detected, suggesting that

caspases were not involved in the gamma-tocotrienol-induced apoptosis. These results imply that cytochrome c is not the critical protein released from mitochondria that triggers gamma-tocotrienol-induced apoptosis in MDA-MB-231 cells.

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Does lack of tocopherols and tocotrienols put women at increased risk of breast cancer?

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Breast cancer is the leading site of new cancers in women and the second leading cause (after lung cancer) of cancer mortality in women. Observational studies that have collected data for dietary exposure to alpha-tocopherol with or without the other related tocopherols and tocotrienols have suggested that vitamin E from dietary sources may provide women with modest protection from breast cancer. However, there is no evidence that vitamin E supplements confer any protection whatever against breast cancer. Observational studies that have assessed exposure to vitamin E by plasma or adipose tissue concentrations of alpha-tocopherol have failed to provide consistent support for the idea that alpha-tocopherol provides any protection against breast cancer. In addition, evidence from studies in experimental animals suggest that alpha-tocopherol supplementation alone has little effect on mammary tumors. In contrast, studies in breast cancer cells indicate that alpha- gamma-, and delta-tocotrienol, and to a lesser extent delta-tocopherol, have potent antiproliferative and proapoptotic effects that would be expected to reduce risk of breast cancer. Many vegetable sources of alpha-tocopherol also contain other tocopherols or tocotrienols. Thus, it seems plausible that the modest protection from breast cancer associated with dietary vitamin E may be due to the effects of the other tocopherols and the tocotrienols in the diet. Additional studies will be required to determine whether this may be the case, and to identify the most active tocopherol/tocotrienol.

PMID: 11834215 [PubMed - as supplied by publisher]

Induction of apoptosis in human breast cancer cells by tocopherols and tocotrienols.

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The apoptosis-inducing properties of RRR-alpha-, beta-, gamma-, and delta-tocopherols, alpha-, gamma-, and delta-tocotrienols, RRR-alpha-tocopheryl acetate (vitamin E acetate), and RRR-alpha-tocopheryl succinate (vitamin E succinate) were investigated in estrogen-responsive MCF7 and estrogen-nonresponsive MDA-MB-435 human breast cancer cell lines in culture. Apoptosis was characterized by two criteria: 1) morphology of 4,6-diamidino-2-phenylindole-stained cells and oligonucleosomal DNA laddering. Vitamin E succinate, a known inducer of apoptosis in several cell lines, including human breast cancer cells, served as a positive control. The estrogen-responsive MCF7 cells were more susceptible than the estrogen-nonresponsive MDA-MB-435 cells, with concentrations for half-maximal response for tocotrienols (alpha, gamma, and delta) and RRR-delta-tocopherol of 14, 15, 7, and 97 micrograms/ml, respectively. The tocotrienols (alpha, gamma, and delta) and RRR-delta-tocopherol induced MDA-MB-435 cells to undergo apoptosis, with concentrations for half-maximal response of 176, 28, 13, and 145 micrograms/ml, respectively. With the exception of RRR-delta-tocopherol, the tocopherols (alpha, beta, and gamma) and the acetate derivative of RRR-alpha-tocopherol (RRR-alpha-tocopheryl acetate) were ineffective in induction of apoptosis in both cell lines when tested within the range of their solubility, i.e., 10-200 micrograms/ml. In summary, these studies demonstrate that naturally occurring tocotrienols and RRR-delta-tocopherol are effective apoptotic inducers for human breast cancer cells.

PMID: 10227040 [PubMed - indexed for MEDLINE]

Apoptosis and cell-cycle arrest in human and murine tumor cells are initiated by isoprenoids.

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Diverse classes of phytochemicals initiate biological responses that effectively lower cancer risk. One class of phytochemicals, broadly defined as pure and mixed isoprenoids, encompasses an estimated 22,000 individual components. A representative mixed isoprenoid, gamma-tocotrienol, suppresses the growth of murine B16(F10) melanoma cells, and with greater potency, the growth of human breast adenocarcinoma (MCF-7) and human leukemic (HL-60) cells. beta-Ionone, a pure isoprenoid, suppresses the growth of B16 cells and with greater potency, the growth of MCF-7, HL-60 and human colon adenocarcinoma (Caco-2) cells. Results obtained with diverse cell lines differing in ras and p53 status showed that the isoprenoid-mediated suppression of growth is independent of mutated ras and p53 functions. beta-Ionone suppressed the growth of human colon fibroblasts (CCD-18Co) but only when present at three-fold the concentration required to suppress the growth of Caco-2 cells. The isoprenoids initiated apoptosis and, concomitantly arrested cells in the G1 phase of the cell cycle. Both suppress 3-hydroxy-3-methylglutaryl CoA reductase activity. beta-Ionone and lovastatin interfered with the posttranslational processing of lamin B, an activity essential to assembly of daughter nuclei. This interference, we postulate, renders neosynthesized DNA available to the endonuclease activities leading to apoptotic cell death. Lovastatin-imposed mevalonate starvation suppressed the glycosylation and translocation of growth factor receptors to the cell surface. As a consequence, cells were arrested in the G1 phase of the cell cycle. This rationale may apply to the isoprenoid-mediated G1-phase arrest of tumor cells. The additive and potentially synergistic actions of these isoprenoids in the suppression of tumor cell proliferation and initiation of apoptosis coupled with the mass action of the diverse isoprenoid constituents of plant products may explain, in part, the impact of fruit, vegetable and grain consumption on cancer risk.

PMID: 10203554 [PubMed - indexed for MEDLINE]

Effect of tocotrienols on the growth of a human breast cancer cell line in culture.

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The tocotrienol-rich fraction (TRF) of palm oil consists of tocotrienols and some alpha-tocopherol (alpha-T). Tocotrienols are a form of vitamin E having an unsaturated side-chain, rather than the saturated side-chain of the more common tocopherols. Because palm oil has been shown not to promote chemically-induced mammary carcinogenesis, we tested effects of TRF and alpha-T on the proliferation, growth, and plating efficiency (PE) of the MDA-MB-435 estrogen-receptor-negative human breast cancer cells. TRF inhibited the proliferation of these cells with a concentration required to inhibit cell proliferation by 50% of 180 microgram/mL whereas alpha-T had no effect at concentrations up to 1000 microgram/mL as measured by incorporation of [3H]thymidine. The effects of TRF and alpha-T also were tested in longer-term growth experiments, using concentrations of 180 and 500 microgram/mL. We found that TRF inhibited the growth of these cells by 50%, whereas alpha-T did not. Their effect on the ability of these cells to form colonies also was studied, and it was found that TRF inhibited PE, whereas alpha T had no effect. These results suggest that the inhibition is due to the presence of tocotrienols in TRF rather than alpha T.

PMID: 8614304 [PubMed - indexed for MEDLINE]

Tocotrienols are members of the vitamin E family as well and are potent antioxidants against lipid peroxidation (the damaging of fats by oxidation). Human studies indicate that, in addition to their antioxidant activity, tocotrienols have other important functions, especially in maintaining a healthy cardiovascular system. In a double-blind study in patients with severe blocked arteries—the main artery supplying blood to the head—tocotrienol administration reduced the level of lipid peroxides in the blood, leading to reductions in clogged arteries. Recent clinical investigations have reported that tocotrienols can reduce total cholesterol levels significantly.

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Tocotrienol is the most effective vitamin E for reducing endothelial expression of adhesion molecules and adhesion to monocytes

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Atherosclerosis, Volume 164, Issue 2, October 2002, Page 389,
Andre Theriault, Jun-Tzu Chao, Abdul Gapor

Abstract

α-Tocopherol and its esterified derivatives have been shown to be effective in reducing monocytic-endothelial cell adhesion. However, the effect of α-tocotrienol (α-T3) has not been characterized. In the present study, using human umbilical vein endothelial cells (HUVEC) as the model system, we examined the relative inhibitory effects of α-T3 and other vitamin E derivatives on cell surface adhesion molecule expression under TNF-α stimulation. Using enzyme-

linked immunosorbent assay, we demonstrated that α-T3 markedly inhibited the surface expression of vascular cell adhesion molecule-1 in TNF-α activated HUVEC in a dose- and time-dependent manner. The optimal inhibition was observed at 25 μmol/l α-T3 within 24 h (77±5%) without cytotoxicity. In addition, the surface expression of intercellular adhesion molecule-1 and E-selectin were also reduced by 40±7 and 42±5%, respectively. In order to further evaluate the effects of α-T3 on the vascular endothelium, we investigated the ability of monocytes to adhere to endothelial cells. Interestingly, a 63±3% decrease in monocytic cell adherence was observed. Compared to α-tocopherol and α-tocopheryl succinate, α-T3 displayed a more profound inhibitory effect on adhesion molecule expression and monocytic cell adherence. This inhibitory action by α-T3 on TNF-α-induced monocyte adhesion was shown to be NF-κB dependent and was interestingly reversed with co-incubation with farnesol and geranylgeraniol, suggesting a role for prenylated proteins in the regulation of adhesion molecule expression. In summary, the above results suggest that α-T3 is a potent and effective agent in the reduction of cellular adhesion molecule expression and monocytic cell adherence.

Anti-atherosclerotic effects of vitamin E – myth or reality?

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KEYWORDS
vitamin E • atherosclerosis • non-antioxidant • gene expression • signalling • transcription factors • tocopherol binding proteins • clinical trials

ABSTRACT

Atherosclerosis and its complications such as coronary heart disease, myocardial infarction and stroke are the leading causes of death in the developed world. High blood pressure, diabetes, smoking and a diet high in cholesterol and lipids clearly increase the likelihood of premature atherosclerosis, albeit other factors, such as the individual genetic makeup, may play an additional role. Several epidemiological studies and intervention trials have been performed with vitamin E, and some of them showed that it prevents atherosclerosis. For a long time, vitamin E was assumed to act by decreasing the oxidation of LDL, a key step in atherosclerosis initiation. However, at the cellular level, vitamin E acts by inhibition of smooth muscle cell proliferation, platelet aggregation, monocyte adhesion, oxLDL uptake and cytokine production, all reactions implied in the progression of atherosclerosis. Recent research revealed that these effects are not the result of the antioxidant activity of vitamin E, but rather of precise molecular actions of this compound. It is assumed that specific interactions of vitamin E with enzymes and proteins are at the basis of its non-antioxidant effects. Vitamin E influences the activity of several enzymes (*e.g.* PKC, PP2A, COX-2, 5-lipooxygenase, nitric oxide synthase, NADPH oxidase, superoxide dismutase, phopholipase A2) and modulates the expression of genes that are involved in atherosclerosis (*e.g.* scavenger receptors, integrins, selectins, cytokines, cyclins). These interactions promise to reveal the biological properties of vitamin E and allow designing better strategies for the protection against atherosclerosis progression.

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Tomeo AC, Geller M, Watkins TR, et al. Antioxidant effects of tocotrienols in patients with hyperlipidemia and carotid stenosis. *Lipids*1995;30:1179–83.

In a double-blind trial in people with severe atherosclerosis of the carotid artery—the main artery supplying blood to the head—tocotrienol administration (200 mg per day) reduced the level of lipid peroxides in the blood. Moreover, people receiving tocotrienols for 12 months had significantly more protection against atherosclerosis progression, and in some cases reductions in the size of their atherosclerotic plaques, compared with those taking a placebo

Tocotrienols: Vitamin E beyond tocopherols.

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In nature, eight substances have been found to have vitamin E activity: alpha-, beta-, gamma- and delta-tocopherol; and alpha-, beta-, gamma- and delta-tocotrienol. Yet, of all papers on vitamin E listed in PubMed less than 1% relate to tocotrienols. The abundance of alpha-tocopherol in the human body and the comparable efficiency of all vitamin E molecules as antioxidants, led biologists to neglect the non-tocopherol vitamin E molecules as topics for basic and clinical research. Recent developments warrant a serious reconsideration of this conventional wisdom. Tocotrienols possess powerful neuroprotective, anti-cancer and cholesterol lowering properties that are often not exhibited by tocopherols. Current developments in vitamin E research clearly indicate that members of the vitamin E family are not redundant with respect to their biological functions. alpha-Tocotrienol, gamma-tocopherol, and delta-tocotrienol have emerged as vitamin E molecules with functions in health and disease that are clearly distinct from that of alpha-tocopherol. At nanomolar concentration, alpha-tocotrienol, not alpha-tocopherol, prevents neurodegeneration. On a concentration basis, this finding represents the most potent of all biological functions exhibited by any natural vitamin E molecule. An expanding body of evidence support that members of the vitamin E family are functionally unique. In recognition of this fact, title claims in manuscripts should be limited to the specific form of vitamin E studied. For example, evidence for toxicity of a specific form of tocopherol in excess may not be used to conclude that high-dosage "vitamin E" supplementation may increase all-cause mortality. Such conclusion incorrectly implies that tocotrienols are toxic as well under conditions where tocotrienols were not even considered. The current state of knowledge warrants strategic investment into the lesser known forms of vitamin E. This will enable prudent selection of the appropriate vitamin E molecule for studies addressing a specific need.

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D.K. Kooyenga, et al., "Palm Oil Antioxidants: Effects in Patients with Hyperlipidaemia and Carotid Stenosis-2 Year Experience," Asia Pacific J. Clin. Nutr. 6(1), 72-75 (1997).

A double-blind placebo-controlled human study conducted by the Kenneth Jordan Heart Foundation and University of Elmhurst showed that tocotrienols reverse atherosclerosis. In this study, patients withcarotid stenoses (narrowing of the main artery supplying the brain) were supplemented with 240 mg/ day of palm tocotrienol complex for 6 months. In the tocotrienol-supplemented group, 92% of the patients showed atherosclerotic plaque regression or remain unchanged. By contrast, none of the patients receiving the placebo showed atherosclerotic regression, whilst 40% showed progression of the disease. Tocotrienol is the first natural compound to reverse atherosclerosis in humans. Its role in preventing diseases such as heart attack and stroke deserve more intensive investigation.

Summary

Since the early 1990s, there were hints that antioxidants could have some effect in showing regression of arteriosclerosis (improvement of artery health and blood flow). Dr Anthony Verlangieri of the Atherosclerosis Laboratory at the University of Mississippi

published his research in reversing atherosclerosis in monkeys with antioxidant nutrients. A report in JAMA in 1995 showed that antioxidant vitamins slowed progression of coronary atherosclerosis. In a three-year, double-blind clinical study at the Kenneth Jordan Heart Foundation, New Jersey, on 50 patients with Carotid Stenosis (blockage of the carotid artery, the main artery that supplies blood to the brain), patients were given a supplementation of 240mg palm based tocotrienols per day. Within 6 months, 92% of the patients had an improvement in their blood flow through the carotid artery, indicating the ability of palm based tocotrienols to reverse artery blockage and improve artery health.

Palm based tocotrienols have been proven by numerous human and animal studies to have the ability to inhibit cholesterol production in the liver. Tocotrienols especially the delta-tocotrienol are potent and effective natural antioxidant nutrients to have the ability to inhibit the key enzyme that is responsible for cholesterol production in the body: HMG Co A Reductase. In human cholesterol lowering human studies, mild hypercholesterolemia patients were given a supplementation of 200mg palm based tocotrienols per day. Significant reduction of total serum cholesterol was observed within 6 to 8 weeks. The reduction of total serum cholesterol was between 15-33% whereas the HDL level was no affected. In another cross-over human study carried out at the Science University of Malaysia, randomly picked subjects with uncontrolled diet was given supplementation of 100mg palm tocotrienols per day. Within 8 to 10 weeks, there was a significant reduction of 10-12% of total cholesterol level in the group that received tocotrienols.

There are four reputable research centers that are currently furthering their research on the ability of palm tocotrienols to inhibit both the estrogen positive and negative human breast cancer cells. The research centers are University of Reading, UK, University of Louisiana, University of Western Ontario, and Palm Oil Research Institute of Malaysia (PORIM). At the moment, research is focused on elucidating the mechanism of inhibition of breast cancer cells by tocotrienols, In addition, the University of Wisconsin is currently carrying out studies on the inhibitory effect of tocotrienols on hepatocarcinogenesis.

Conclusions; Exercise five to six days per week, control weight, consume 240mg of tocotrienols daily. Enjoy your exercise and be happy.

Exercise Information: see the video and supportive documents at www.theneurotechnologies.com

Tocotrienols: Available at most health food stores or with vitamin distributors

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- Provides Mixed Tocopherols and Tocotrienols for Comprehensive Vitamin E Nutrition*

Xcellent E™ *features EVNol SupraBio™ full-spectrum palm tocopherol/tocotrienol complex. EVNol SupraBio's patented bio-enhancing technology has been shown to increase tocotrienol absorption rates in humans by an average of 250%. Tocotrienols confer unique health benefits not provided by tocopherols. This means Xcellent E not only enables superior absorption but also more comprehensive vitamin E benefits than tocopherol-only formulas.**

Discussion

Vitamin E, in its natural form, comprises eight different compounds: alpha-, beta-, gamma-, and delta-tocopherols and alpha-, beta-, gamma-, and delta-tocotrienols. Both tocopherols and tocotrienols are important to human health. Known as the "master antioxidant," vitamin E has the ability to attenuate oxidative stress, and its antioxidant-related effects on various organs and systems have been the focus of vast research. More recently, non-antioxidant mechanisms have been proposed, such as those that affect cell-signal transduction and gene expression.[1] Though the vast majority of research has been on alpha-tocopherol, recent mechanistic studies indicate that other isomers of vitamin E, such as gamma- and delta-tocopherols and tocotrienols, have superior antioxidant and cell-signaling properties that offer greater health benefits.*[2,3]

Tocotrienols

Studies have demonstrated that tocotrienols have superior antioxidant activity compared to tocopherols. Tocotrienols also exhibit biological activities related to neuroprotection, radioprotection, cell-life regulation, cytokine modulation, and lipid metabolism that are not shared by tocopherols.[3-5] Many of these benefits are thought to be mediated via their carboxychromanol metabolites.[2,6] Among other actions, tocotrienols have been shown to inhibit HMG-CoA reductase (3-hydroxy-3-methylglutaryl-coenzyme A reductase), attenuate transcription factor NF-κB activation, and inhibit COX-2.[7,8] Given these mechanisms, in addition to their antioxidant mechanisms, tocotrienols have a very broad range of applications. Due to the poor absorption and low bio-availability of tocotrienols, scientists developed EVNol SupraBio™.*

EVNol SupraBio: Bio-enhanced tocotrienol/tocopherol complex

EVNol SupraBio is a natural, full-spectrum tocopherol and tocotrienol complex extracted and concentrated from the red palm fruits (*Elaeis guineensis*) of sustainable plantations in Peninsular Malaysia. This vitamin E complex also contains minute amounts of other phytonutrients such as plant squalene, phytosterols, coenzyme Q10, and mixed carotenoids that are naturally extracted together with tocotrienols. This patented formula contains a precise mixture of oil and approved food emulsifiers at optimum ratio and processing that self-emulsifies in the gastrointestinal tract to facilitate and provide a rapid and consistent absorption of tocotrienols into the plasma, independent of dietary fat or food intake.*

EVNol SupraBio Human Absorption Studies

Kholsa et al were the first to establish that oral supplementation of EVNol SupraBio resulted in a peak plasma level 12- to 13-fold the level established for neuroprotection.[9] Later, in a two-period, two-sequence, crossover study performed in healthy human volunteers, researchers demonstrated that the SupraBio system increased the rate and extent of absorption of individual tocotrienols by an average of 250% compared to a regular tocotrienol oil extract.[10] Moreover, EVNol Suprabio is the only tocotrienol/tocopherol complex in the market that has been the subject of an actual human tissue distribution study. In that study, Patel et al demonstrated that orally supplemented tocotrienols from EVNol SupraBio are absorbed into plasma and delivered and accumulated in vital organs, including the brain.*[11]

EVNol SupraBio Human Clinical Studies

EVNol SupraBio is a heavily researched tocopherol/tocotrienol product that has been scientifically substantiated with human clinical studies on brain health, liver support, beauty, and cardiovascular health.[11-18] For example, in a randomized, placebo-controlled, two-year neuroprotection study (n = 121), supplementation with 200 mg/d EVNol SupraBio attenuated the progression of injury to brain white matter.[12] Three other studies demonstrated the positive effects of EVNol SupraBio on parameters of liver health[11,13,14], and studies related to cardiovascular health suggested that 50-200 mg/d EVNol SupraBio supports healthy lipid (cholesterol, low-density lipoprotein, triglyceride) metabolism and showed a trend toward improved arterial compliance (the ability to expand and contract).[15,16] Supplementation has also been shown to support the desired immune response to vaccine.[17] And in a randomized, double-blind, placebo-controlled trial (n = 38), volunteers

Continued on next page

Antioxidant Activity

Cardiovascular Support

Cytokine Balance

Liver Support

Neurological & Cognitive

Xcellent E™ Supplement Facts

Serving Size: 1 Softgel

	Amount Per Serving	%Daily Value
Vitamin E (as d-alpha tocopherol)	33.5 mg	223%
EVNol SupraBio™ Bio-Enhanced Natural Full Spectrum Tocotrienol/Tocopherol Complex	164.5 mg	**
Total Mixed Tocotrienols	25 mg	**
d-Gamma Tocotrienol	11.5 mg	**
d-Alpha Tocotrienol	7.4 mg	**
d-Delta Tocotrienol	4.1 mg	**
d-Beta Tocotrienol	822.5 mcg	**
Total Mixed Tocopherols	125 mg	**
Typical Composition:		
d-Gamma Tocopherol	75 mg	**
d-Delta Tocopherol	30 mg	**
d-Alpha Tocopherol	17.5 mg	**
d-Beta Tocopherol	2.5 mg	**

** Daily Value not established.

Other Ingredients: Sunflower oil, softgel (bovine gelatin, vegetable glycerin, and purified water), and polyglycerol esters of fatty acids.

DIRECTIONS: Take one softgel twice daily, or use as directed by your healthcare practitioner.

Consult a healthcare practitioner prior to use. Individuals taking medication should discuss potential interactions with their healthcare practitioner. Do not use if tamper seal is damaged.

STORAGE: Keep closed in a cool, dry place out of reach of children.

DOES NOT CONTAIN: Wheat, gluten, corn, yeast, soy protein, dairy products, fish, shellfish, peanuts, tree nuts, egg, ingredients derived from genetically modified organisms (GMOs), artificial colors, artificial sweeteners, or artificial preservatives.

with hair loss who were given 100 mg of EVNol SupraBio daily experienced a 34.5% increase in number of hairs at the end of eight months, compared to a 0.1% increase in the placebo group.[18] The higher activity of tocotrienols in certain organs may, in part, be explained by the fact that the unsaturated side-chain of tocotrienols allow more efficient penetration into tissues, such as brain and liver tissues, that have saturated fatty layers.*[3,13]

It is clear from the emerging data that tocopherols and tocotrienols have complementary, unique, and important functions.[3] Providing a formula that supplies the full spectrum of natural vitamin E isomers is an important option for practitioners and their patients.*

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Additional references available upon request

Note on Vitamin E Activity and International Units (IUs)

Only alpha-tocopherol contributes to IU of vitamin E activity: 1 mg d-alpha tocopherol equals 1.49 IU vitamin E activity. Other naturally occurring forms of vitamin E (beta-, gamma-, delta-tocopherol) and tocotrienols do not contribute toward meeting the vitamin E requirement. Hence, the IU is calculated based on alpha-tocopherol alone in all formulations. Other isomers of vitamin E are expressed as "mg." Each gram of EVNol SupraBio 20% contains approximately 152 mg d-mixed tocotrienols and 35-60 mg d-alpha-tocopherol. Hence, the minimum vitamin E activity in 1 gram of EVNol SupraBio 20% = 35 mg d-alpha-tocopherol x 1.49 = 52.15 IU.

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