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Tocotrienols: the other Half of Natural Vitamin E.

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ABSTRACT

Vitamin E, the most essential lipid-soluble antioxidant, are hydrophobic fat-soluble compounds found in a wide variety of food sources such as vegetable oils, fruits, corn oil, peanuts, nuts, and green leafy vegetables. Vitamin E occurs in nature in at least eight different isoforms: α, β, γ, and δ tocopherols and α, β, γ, and δ tocotrienols. The small structural differences between vitamin E isoforms have a significant influence on vitamin E metabolism. All forms of vitamin E possess antioxidant activity however, tocotrienols have been shown to have more powerful antioxidant potential than tocopherols. In addition to their potent antioxidant activity, tocotrienols have other significant functions, particularly in maintaining a healthy cardiovascular system and a possible role in protection against cancer and other diseases. In this review, the benefits and superior function of tocotrienols including their role and potential in cardiovascular disease and cancer are elaborated.

Keywords: Tocotrienols- tocopherols- vitamin E- tocochromanols- antioxidant.

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INTRODUCTION

Discovery of vitamin E in 1922 is credited to Evans HM et al. when they found that a substance in vegetable oils required for reproduction in female rats. This substance was named as vitamin E, because vitamins A, B, C and D was already discovered [1]. At that time, vitamin E scientifically named as "Tocopherol", a name obtained from the ancient Greek word "Phero", means "To bring" and the word "Tocos", means "child birth" The suffix 'ol' was added at the end to represent the phenolic nature of the substance [2].

In 1936, Evans et al. isolated and characterized two isoforms of tocopherol (TP) designated as α- and β-tocopherol [3]. In the subsequent years, two additional forms of tocopherols, γ- and δ- tocopherol as well as the tocotrienols (T3) were isolated from oil of edible plants, such as wheat germ oil, palm oil, soybean oil and annatto etc [4,5]. Thus, today a total of eight different isomers that belong to two classes α, β, γ, and δ tocopherols and α, β, γ, and δ tocotrienols are known to occur in nature.

It has long been demonstrated that vitamin E is associated with an antioxidant activity in 1966 reported by Epstein et al. Thereafter, the role of this vitamin as a radical chain breaking antioxidant was widely studied in humans and animals, and later in plants [6].

Tocotrienols Structure and Chemistry

Tocotrienols are novel part of the vitamin E family. The vitamin E contain two major homologous subgroups (Tocochromanols) based on the degree of saturation of the phytyl tail known as tocopherols (TP) and tocotrienols (T3) [7]. Both of tocopherols and tocotrienols have a chromanol nucleus, which is the site for their potent antioxidant activities, but they differ in the molecule's tail [8]. While tocopherols contain a saturated side chain in the chroman ring, tocotrienols contain an unsaturated phytyl side chain [9]. The C16 side chain of tocopherols is saturated, while in tocotrienols it contain three double bonds in transform. Tocopherols possess a 20-carbon phytyl tail involving the pyranol ring. Tocotrienols, in turn, have a 20-carbon geranyl geranyl tail with double bonds at the 3', 7' and 11' sites, linked to the benzene ring [10]. Tocopherols and tocotrienols are further classified into sub-groups for each α, β, γ and δ, distinguished by their methyl-groups at positions 5, 7 or 8 of the chroman ring. Each of these isomers share important aspects in that, all have a head or chromonal ring, all have a tail named the phytyl tail for tocopherols, and all have the hydroxyle group, which is the active group on the chroman ring of the molecule [11]. These 8 isoforms represent the natural form of this vitamin, however there are various forms of synthetic vitamin E consists of either a mix of naturally occurring tocopherols and tocotrienols; RRR-α-tocopherol (commonly referred to as d-α- tocopherol), synthetic α-tocopherol, which consists of the 8 stereo-isomers in similar values (all rac- α -tocopherol, commonly called dl- α -tocopherol); or their esters [12]. These eight isomeric forms (RRR, RRS, RSS, RSR, SSS, SRR, SSR, and SRS) are synthesized depending on the configuration at three chiralic centres found on the α-tocopherol molecule [13]. Studies comparing the bio-potency of the natural (RRR) versus synthetic (all-rac) α- tocopherol, and the estimated ratio was 1.36:1 (natural/ synthetic) [14]. Synthetic all-rac-α tocopherol increases in plasma to only about 50% of the level achieved by the natural RRR-α-tocopherol

and the degradation rate of all-rac-α-tocopherol is higher by three to four times. Tocotrienols have only the chiral stereocenter at C-2 and naturally occurring tocotrienols have the (2R, 3'E, 7'E) configuration. The chiral properties of these molecules should be considered in the evaluation of the activity of a compound in biological studies. Both of tocopherols and tocotrienols are amphipathic molecules, in which the lipophilic isoprenoic side chain is related to the lipids in the membrane and the polar chromanol ring is exposed to the membrane surface [15].

Tocotrienols Synthesis and Biosynthesis

Vitamin E are fundamental components of the human diet and are only produced by plants and other oxygenic, photosynthetic organisms [16]. In plants, there is a wide range of vitamin E contents, and the tissues of photosynthetic plant contain from 10 to 50 µg vitamin E contents per gram fresh weight [17]. Tocopherols are found in all photosynthetic organisms, whereas tocotrienols are present in certain groups of plant [18]. Tocopherols contain a chromanol ring and a 15-carbon tail, which is obtained from coupling of homogentisate (HGA) with phytyl diphosphate, followed by cyclization and methylation reaction. The condensation of homogentisate comes from the shikimate pathway. However, the condensation of phytyl pyrophosphate (phytyl-PP) is derived from the non-mevalonate pathway [19]. Condensation of both HGA and Phytyl diphosphate, the important step in tocopherol biosynthesis, is catalyzed by HGA phytyltransferase (HPT) to yield 2-methyl-6-phytyl-plastoquinol, the first reliable tocopherol intermediate and most common precursor of all tocopherols, which is first methylated to produce 2,3- dimethyl-5-phytyl-1,4-benzoquinol and then transformed by the enzyme tocopherol cyclase to γ- tocopherol. A further ring cyclization and methylation reactions result in production of α, β, and δ tocopherol derivatives [20].

Tocotrienols biosynthesized from an analogues series of reactions but with geranyl geranyl diphosphate as substrate in the condensation step. Tocotrienols are structurally differing from tocopherols in that they have double bonds in their hydrocarbon tail. Tocotrienols are the essential form of vitamin E in the seed endosperm of most monocots, including cereal grains i.e. rice, barley, and wheat. Moreover, tocotrienols are present in the seed endosperm of a few numbers of dicots, including *Apiaceae species* and certain *Solanaeceae* species, e.g. tobacco, which are found only in vegetative tissues of plants. Palm oil contains a large amounts of tocotrienol, with frank palm oil (also referred to as the ''tocotrienol rich fraction'') extracted from the fruits of *Elaeis guineensis* essentially contain a considerable quantity of tocotrienols (up to 800 mg/kg) mainly α-tocotrienol and γ tocotrienol [21]. Studies revealed the presence of tocotrienols in several non-photosynthetic and photo-synthetically active tissues [22]. Expression of the barley HGGT (homogentisic acid transferase, which catalyzes the first step of tocotrienol biosynthesis) into several plants such as *Arabidopsis thaliana* leaves by transgenic approaches results in sufficient production of tocotrienols, which were not found in the leaves of non-transformed plants. Over-expression of the barley HGGT in corn seeds raised tocotrienol and tocopherol content by six fold. These outcomes opens a field into the genetic basis for tocotrienol biosynthesis in plants and prove the capacity to enhance the antioxidant content of yields by introducing enzyme that helps in redirecting the metabolic flux [23]. Further, researchers have developed another way of tocotrienols biosynthesis involves genetic

engineering of metabolic pathways in plants. Both of p-hydroxyphenylpyruvate and homogentisate, the precursors of vitamin E, has been up-regulated by enhancing their synthesis directly at the level of prephenate which was accomplished by the expression of the prephenate dehydrogenase gene in tobacco plants that already over-express the Arabidopsis p-hydroxyphenylpyruvate dioxygenase coding sequence. Thus, the synthesis of p-hydroxyphenylpyruvate is an important step for the collection of vitamin E in plants [24].

Tocotrienols Availability in Nature

Since the discovery of α-tocotrienols as cholesterogenesis-inhibitory factor obtained from barley, reports have increased toward the unique importance of tocotrienols in health and disease status [25]. Tocotrienols were primarily isolated from the latex of the rubber plant *Havea brasiliensis* in 1964 [26]. The eight isomers of both tocopherols and tocotrienols are widely distributed in nature. While tocopherol present mainly in dicotyledoneous plants (e.g. olive, soybean, peanut), tocotrienols found in monocotyledoneous plants (e.g. rice bran, annatto, palm). Dicotyledoneous plants mainly contain γ-tocopherol as primary tocopherols, and σ-tocopherol and α-tocopherol as secondary form. However, monocotyledoneous plants primarily contain γ-tocotrienol, and secondarily contain σ-tocotrienol and α-tocotrienol [27]. The ratio of tocopherols: tocotrienols in rice bran, palm and annatto oils are 50:50; 25:75 and 0.1:99.9, respectively [28]. Tocotrienols are the predominant vitamin E and presented in high quantities in palm oil, rice bran and barley [29]. It is a round 70% of the vitamin E content in palm oil consists of tocotrienol isomers, while 30% are alpha tocopherol [30]. Other natural sources of tocotrienol are walnut, hazelnut, rye, amaranth, poppy, safflower, maize, and the seeds of grape, flax, and pumpkin. Furthermore, tocotrienols were also found in eggs and meat [31].

Tocotrienols Bioavailability

Absorption

Following oral administration, vitamin E are absorbed in the intestine by passive diffusion along with other non-polar lipids, e.g. triglycerides and cholesterol, and then transported to the systemic circulation through the lymphatic pathway [32]. All isoforms of vitamin E are absorbed by intestinal cells and liberated into the circulation with chylomicrons [12]. The absorption of vitamin E depends on lipid intake and the secretion of bile and esterases [33]. Bile emulsifies the tocopherols and packaged them into micelles together with other fat-soluble compounds, thereby facilitating absorption [34]. The excretion of bile, which produced by the liver, is generally depends on the level and type of dietary fat consumed, and reports have clearly identified that tocotrienol absorption is reduced in fasted individuals in compare to full fed individuals [35]. Oil palm tocotrienol identified to increase adequate bile excretion and micelle formation which enhance the absorption of tocotrienols, this is in part due to the high fatty acid composition present in palm. However, administration of isolated tocotrienols by oral gavage may lack adequate fat content to enhance sufficient bile excretion into the small intestine that necessary to promote tocotrienol absorption [34]. The liver contains a transfer protein that enriches VLDL with α-tocopherol [36]. Thus, α-tocopherol is secreted by the liver in a way that discriminates between tocopherols and tocotrienols. Moreover, the α-tocopherol transfer

protein (α-TTP) was identified as a product of gene for hereditary isolated vitamin E deficiency [37]. The existence of transfer protein that discriminatory selects α-tocopherol clarify why all other isoforms of vitamin E possess a lower biological properties in compared with α-tocopherol. Co-administration of α-tocopherol and α-tocotrienol at similar amounts resulted in a higher distribution of α-tocopherol than that of α-tocotrienol in various organs of rats due to the differences in their binding to α-TTP [38]. Studies revelled that giving equal doses of α, γ, and σ- tocotrienols (250 mg/day) result in different plasma concentration levels with α-tocotrienol identified to be higher plasma concentration than σ- and γ-tocotrienols. This identification supports the higher affinity of α-tocotrienol to α-TTP than those of other tocotrienols [39]. Thus, the physiological levels of vitamin E may depend on the differences in their affinities to α-TTP [40]. Deficiency of *α*-TTP results in decreased absorption of *α*-tocopherol as identified in *α*-TTP knockout mice fed synthetic all-rac-α-tocopherol. In compare, tocotrienols were well absorbed by *α*-TTP knockout mice and distributed to peripheral tissues even in the absence of *α*-TTP [38].

Distribution

Tocotrienols are distributed throughout the body via the bloodstream and accumulates in various tissues of rats, particularly adipose tissues, liver, heart, and skin after oral gavage, suggesting that tocotrienols are absorbed and distributed *in vivo* [41]. The estimated plasma levels of tocotrienols were reported to reach 1 mol/L in humans and between 3 and 20 mol/L in various animal species [42]. Khanna et al. studied the distribution of vitamin E in different tissue by gavaging rats with α-tocotrienol alone, α-tocopherol alone or in together. Various organs and tissues e.g. skin, adipose tissue, heart, lungs, brain, spinal cord, skeletal muscle, liver and blood were studied on five generations of rats over sixty weeks. Tocotrienol was delivered to all vital organs. However, in some tissues, the level of tocotrienol identified to be higher than that of tocopherols, this indicates the existence of an efficacious tocotrienol transport system *in vivo*. In the tocopherol-fed rats, which never given tocotrienols, the skin level of tocotrienol demonstrated to be negligible. Moreover, better uptake of α-tocopherol over α-tocotrienol have been demonstrated in rats treated with combination of both α-tocopherol and α-tocotrienol [38]. It has been concluded that the delivery of vitamin E to tissues could occur by three main routes; lipoprotein lipase mediated triglyceride-rich lipoprotein catabolism, low-density lipoprotein (LDL) uptake via the LDL receptor, and through high-density lipoprotein (HDL)-mediated delivery systems. Moreover, vitamin E rapidly transport between diverse lipoproteins and between lipoproteins and membranes, which may enrich cellular membranes with vitamin E [43,44].

Metabolism and excretion

Tocopherols and tocotrienols are metabolized and excreted via urine and faeces when they are not recognized by α-TTP [45]. Unlike other fat-soluble vitamins, vitamin E does not easily accumulate in the liver because metabolism and excretion are critical hepatic regulatory pathways to limit vitamin E concentrations [44]. Certain isoforms of vitamin E, e.g. γ-tocopherol, all rac-α-tocopherol, and tocotrienols, are metabolized to a higher extant than α-tocopherol [38]. All isomers of vitamin E metabolized similarly by omega oxidation followed by beta oxidation of the side chain resulting in the formation of 2-

carboxyethyl-6-hydroxychroman (CEHC) metabolites which accumulate mostly in the urine. Omega oxidation is mediated by xenobiotic metabolizing enzymes, cytochrome P450 (CYP450) enzymes, which are often regulated by their substrates themselves. However, vitamin E has been demonstrated to increase the activity of CYP enzymes, particularly CYP3A and CYP4F2 resulting in tight regulation of vitamin E concentrations [46]. Furthermore, tocotrienols were found to be degraded to a larger amount than tocopherols [47]. The mean apparent elimination half-life of α, γ, and δ-tocotrienols when given as a single dose of 300 mg of mixed tocotrienols is valued to be 4.4, 4.3, and 2.3 hours respectively, and between 4.5- to 8.7-fold shorter than that identified for α-tocopherol [48].

Tocotrienols Biological Activities

Antioxidant activity

Vitamin E is well known for its strong antioxidant activities and has been suggested as the most significant lipid soluble antioxidant in the human blood plasma and circulating lipoproteins [49]. Vitamin E exerts antioxidant effects by scavenging lipid peroxyl radicals *in vivo* as well as *in vitro* systems [50]. Researchers have suggested that tocotrienols possess superior antioxidants in compare to tocopherols at preventing cardiovascular diseases and cancer [51,52]. Results obviously indicated that d-α-tocotrienol have 40-60 times higher antioxidant potency than conventional d-α-tocopherol, although their absorption and distribution after oral intake are less than that of α-tocopherol [53]. The higher antioxidant efficiency of d-α-tocotrienol were shown to be due to the combined effects of three activities displayed by d-α-tocotrienol in contrast to d-α-tocopherol includes; its higher recycling efficiency from chromanoxyl radicals, its more constant distribution in the membrane lipid bilayer, and its more effective interaction of the chromanol ring with lipid radicals. These activities make the interaction of chromanols with lipids radicals more efficient [54].

The antioxidant activity of tocotrienols thought to be mediated through induction of phase II antioxidant enzymes such as; glutathione peroxidise (GPx) [55], NADPH:quinone oxidoreductase (NQO-1) [56], superoxide dismutase (SOD), etc [57]. Induction of phase II enzymes provide protection against free radical damage and reduce the incidence of the radical derived degenerative diseases such as cancer [31]. Further, researchers have linked the antioxidant activities of tocotrienols with nuclear factor-erythroid 2-related factor 2 (Nrf2), a member of the Cap 'n' collar (CNC) family of basic region-leucine zipper transcription factors [58]. Results shows that tocotrienol were able to affect activates of Nrf2 regulated enzymes such as UDP-Glucuronyltranferase (UDP-GT), γ-glutamyltransferase (GGT) and glutathione S- transferase (GST) [59].

Tocotrienols and cardiovascular diseases

Tocotrienols had been reported to possess superior antioxidants in compare to tocopherols at preventing cardiovascular diseases [51]. Most of cardiopreventive properties of tocotrienols are mediated through their ability to suppress 3-hydroxy-3-methyl glutaryl CoA reductase enzyme (HMG-CoA reductase), an important enzyme in cholesterol biosynthesis, resulting in less cholesterol being produced by liver cells [60]. Tocotrienols

significantly inhibit age related raises in systolic blood pressure of spontaneously hypertensive rats, and these effects were more pronounced than those of σ-tocopherol [62]. TRF from palm oil is known to decrease total cholesterol and LDL-cholesterol levels through down modulation of hepatic HMG-CoA reductase activity [59]. Reporters telling that TRF reduces concentrations of plasma cholesterol and apolipoprotein B, thromboxane B2 and platelet factor-4, suggesting its capability to protect against endothelial dysfunction and platelet aggregation. There are several mechanism identified to account for cholesterol lowering effects of tocotrienols. One possible mechanism by lecithin-cholesteryl ester transferase, which convert free cholesterol to cholesterol ester that increase HDL levels, or by convert LDL cholesterol, present in the arteries, to VLDL which in turn convert it into IDL then finally HDL. Other mechanisms by activate the HDL cholesterols, which destroy LDL by phagocytosis results in transform HDL within the HDL particle [63].

Nafeeza et al. demonstrate the effect of tocotrienol on the development of atherosclerosis and lipid peroxidation in the aortas of rabbits. They found that tocotrienol was able to reduce the occurrence of atherosclerosis [63]. Tocotrienol was able to reduce myocardial infarct size and improve post-ischemic ventricular function though down modulation of c-Src and up-regulation of phosphorylation of Akt, therefore it generates a survival signal [64]. Esterhuyse and co-workers demonstrated the effects of red palm oil on the myocardial nitric oxide–cGMP signalling pathway. They reported that red palm oil increased aortic output and levels of cGMP in rat hearts. They conclude that dietary intake of red palm oil could offer protection against myocardial ischemia by the nitric oxide–cGMP pathway [65]. Additionally, Newaz et al. studied the effects of γ-tocotrienol on lipid peroxidation and total antioxidant status of spontaneously hypertensive rats. They found that γ-tocotrienol (15 mg/kg diet) reduced blood and plasma levels of lipid peroxides and improved total antioxidant status and superoxide dismutase activity. This suggest that γ-tocotrienol may prevent development of increased blood pressure, reduce lipid peroxides in plasma and blood vessels and improve total antioxidant status, including superoxide dismutase activity [66].

Tocotrienols and cancer

A raising body of information regarding the anticancer effect of tocotrienols suggests that in addition to its antioxidative and pro-apoptotic activities, tocotrienols possesses a number of anticancer activities that make them supreme to tocopherols [67]. Probably the initial record about the therapeutic prospect of tocotrienols for cancer in animal models was by Kato et al. who demonstrated that tumour bearing rats treated with tocotrienols had an expanded life duration [68]. Early report compared the chemopreventive capability of tocopherols and tocotrienols in 7, 12-dimethylbenz (a) anthracene-induced rat mammary carcinoma models demonstrated that tocotrienols treated rats, in compared with the tocopherols group, is associated with considerably lower tumour incidence and prolonged tumour latency [69]. The molecular mechanisms behind the antitumor effects of tocotrienols are still scarcely understood. Evidence now suggests that tocotrienol affects diverse pathways linked with tumorigenesis and therefore has prospect in both the prevention and the treatment of cancer [28]. Tocotrienol exerted significant cell growth inhibition occurred concomitantly with G1 cell-cycle arrest and apoptosis [70]. There are several signaling pathways promoting cell growth arrest and apoptosis are activated by

tocotrienols these include; transforming growth factor-β(TGF-b) [71], Cyclin-dependent kinases inhibitors such as p27(Kip1), p53 [72,73], activation of caspase-8, which results to caspase-3 activation [74], up-regulation of Bax, cleavage of Bid [75], Apaf-1, Fas [71], caspases [76], DNA fragmentation [77], and release of cytochrome C [73]. Additionally, researchers suggested that the antitumor activities of tocotrienols seems to be mediated through their ability to inhibit angiogenesis [78]. Inhibition of angiogenesis is mediated through reduction in serum levels of VEGF and inhibition of the PI3K–AKT pathway. The inhibition of HMG-CoA reductase as well as the reduction in serum cholesterol level has been attributed with the tumour suppressive activity of tocotrienols [79]. When different isomers of tocotrienols were analyzed, δ-tocotrienol showed the highest activity [80].

Nesaretnam and his colleagues reported that tocotrienols from TRF inhibited the proliferation of human breast cancer cell lines. They found that the inhibition was independent of the estrogen receptor status of the cell lines [81]. Wada et al. studied the effect of oral tocotrienols (Dose of 0.05%) on spontaneous model of liver carcinogenesis in male mice, they found significant decrease in tumour number in treated animal versus untreated group. However in the same treated group, TRF was identified to suppress lung tumour formation induced by 4NQO and glycerol [82]. *In vitro* study, treatments of human breast cancer cell lines MCF-7, MDA-MB- 231 and the non-cancerous cell line MCF-10A resulted in a significantly higher growth inhibition in breast cancer cell lines [83]. Moreover, tocotrienols treated rats showed statistically significant increase in tumour latency in the DMBA-induced rat mammary tumour model. Inhibition of tumour progression by different palm oil tocotrienols was also reported by other studies [84]. There are also evolving research around tocotrienol and cancer prevention, Pierpaoli et al. studied the effect of annatto-tocotrienols supplementation on the development of mammary tumors in HER-2/neu transgenic mice. They found that tocotrienols derived from the annatto plant delayed mammary tumor growth and reduced the number and size of tumors and lung metastases. These effects thought to be mediated in part due to the ability of tocotrienols to create stress, cause death and growth arrest to the cancer cells [85]. In link of this, another study, examined the impacts of γ-tocotrienol in human colon cancer SW620 cells, showed that γ-tocotrienol induced a paraptosis like cell death in SW620 cells distinguished by a mass of cytoplasmic vacuoles formation, which may linked with the suppression of the Wnt signaling pathway [86]. Ultimately, all of these studies suggest that tocotrienols have prospect in both prevention and treatment of cancer.

CONCLUDING REMARKS

Vitamin E is an interesting group of compounds, able to exert multiple biological effects in plant, animal and human cell. A total of eight different isomers of vitamin E that belong to two classes α, β, γ, and δ tocopherols and α, β, γ, and δ tocotrienols are known to occur in nature. Tocotrienols make up a major portion of total vitamin E in several food sources predominantly palm oil, rice bran and barley. *In vitro*, they have been demonstrated to exhibit enhanced antioxidant activities compared with tocopherols. In addition, they have been shown to have cholesterol- lowering, anticarcinogenic and other biological properties, which may not be linked to their antioxidant effect. After oral administration, however, they are not recognized by the α-TTP and therefore only have a short half-life, which accounts for their low bioavailability. However, despite the body's poor absorption of tocotrienols,

human studies have established that tocotrienols are distributed in a large extent in tissues and plasma upon supplementation. Knowing that this part of vitamin E can cross membranes and enter tissues such as the brain combined with *in vitro* data suggesting strong antioxidant activity. Further, a more well-designed clinical studies are required to confirm the efficacy of these forms of vitamin E for specific health claims.

Conflicts of Interests

The authors declare that they have no conflict of interests.

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