

## Review

# Palm Oil-Derived Natural Vitamin E $\alpha$ -Tocotrienol in Brain Health and Disease

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A growing body of research supports that members of the vitamin E family are not redundant with respect to their biological function. Palm oil derived from *Elaeis guineensis* represents the richest source of the lesser characterized vitamin E,  $\alpha$ -tocotrienol. One of 8 naturally occurring and chemically distinct vitamin E analogs,  $\alpha$ -tocotrienol possesses unique biological activity that is independent of its potent antioxidant capacity. Current developments in  $\alpha$ -tocotrienol research demonstrate neuroprotective properties for the lipid-soluble vitamin in brain tissue rich in polyunsaturated fatty acids (PUFAs). Arachidonic acid (AA), one of the most abundant PUFAs of the central nervous system, is highly susceptible to oxidative metabolism under pathologic conditions. Cleaved from the membrane phospholipid bilayer by cytosolic phospholipase A<sub>2</sub>, AA is metabolized by both enzymatic and nonenzymatic pathways. A number of neurodegenerative conditions in the human brain are associated with disturbed PUFA metabolism of AA, including acute ischemic stroke. Palm oil-derived  $\alpha$ -tocotrienol at nanomolar concentrations has been shown to attenuate both enzymatic and nonenzymatic mediators of AA metabolism and neurodegeneration. On a concentration basis, this represents the most potent of all biological functions exhibited by any natural vitamin E molecule. Despite such therapeutic potential, the scientific literature on tocotrienols accounts for roughly 1% of the total literature on vitamin E, thus warranting further investment and investigation.

### Key teaching points:

- Palm oil is one of the most abundant natural sources of the lesser characterized vitamin E,  $\alpha$ -tocotrienol.
- $\alpha$ -Tocotrienol possesses unique neuroprotective properties not shared by other natural vitamin E family members.
- Oral supplementation of palm oil-derived  $\alpha$ -tocotrienol reaches the brain in sufficient quantity to attenuate stroke-mediated neuropathy.
- Antioxidant-independent mechanisms of  $\alpha$ -tocotrienol-mediated neuroprotection are discussed in detail.

## INTRODUCTION

The first evidence of palm oil consumption in human diets dates back as far as 3000 BC, with a long history of use in western Africa [1]. Derived from the fleshy orange-red mesocarp of the fruits of the oil palm tree *Elaeis guineensis*, oil palm trees were introduced to the West (Brazil, West Indies) in the 15th century by the Portuguese and to the East (Indonesia) by the Dutch in the 19th century [2]. Originally purposed as ornamental landscape, commercial oil palm farming and palm oil production is a relatively recent endeavor of the 20th century, with the first commercial oil palm estate established in Malaysia in 1917 [2]. Of the oil-bearing plants,

*E. guineensis* provides the highest yield of oil, with an average of between 4 and 10 tons of oil per hectare per annum [1,3]. Accounting for ~30% of the total world production of oils and fats, palm oil has dramatically increased its share of production over the past 10 years and overtaken soybean oil (~23%) as the most abundant plant-derived oil source worldwide [4]. Throughout the world, 90% of palm oil is purposed for dietary consumption, with the majority of palm oil production and consumption localized in the tropics of South-East Asia [5].

### Palm Oil Nutrition and Chemistry

Despite the surge in production and consumption of palm oil over the past decade, incorporation of palm oil into Western

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diets remains largely unpopular due in part to a higher saturated fatty acid (SFA) content when compared to most other commercially available vegetable oils including olive, canola, and soybean [6]. A positive and significant link between a diet rich in SFA and elevated low-density lipoprotein (LDL) cholesterol and increased risk for cardiovascular disease is widely recognized. However, the scientific evidence for palm oil–rich diets specifically contributing to elevated LDL cholesterol and cardiovascular disease is conflicting [7–10].

Palm oil use in the United States food industry is slowly gaining acceptance as a natural hydrogenated fat substitute [11]. Partial hydrogenation of vegetable oils converts them into semi-solid fats with greater shelf-life, stability during cooking, and palatability [12]. Hydrogenated fats contain high levels of *trans*-fatty acids, which are thought to have adverse health effects, including increased risk of coronary heart disease [13], increased systemic inflammation [14,15], and impaired endothelial cell function [16]. To promote awareness and discourage their use in food products, the U.S. Food and Drug Administration mandated *trans*-fatty acid content listing on Nutrition Facts panels in 2006 [17]. With a near 1:1 ratio of saturated to unsaturated fatty acids, palm oil remains in a semi-solid state at room temperature. In many instances, this distinctive property makes it a desirable *trans*-fatty acid–free alternative to hydrogenated oil for use in food products such as margarine, shortenings, wafers, and candies [11].

Beyond the unique lipid profile of palm oil, there is growing scientific interest in the lipophilic palm oil–associated tocol members of the vitamin E family that possess potent nutritional and therapeutic value. While lipid-soluble vitamin E accounts for less than 1% of total palm oil content, it plays an important role in preserving oil stability and shelf-life while contributing to the health benefit of dietary palm oil consumption. The vitamin E family members are widely recognized and characterized for their antioxidant capacity; a growing body of scientific literature now also recognizes their antioxidant-independent functions, with therapeutic potential in pathologic disorders ranging from cancer to cerebrovascular disease. This review examines the recent body of work on palm oil–derived  $\alpha$ -tocotrienol, a lesser characterized vitamin E isomer with therapeutic potential in neuropathology.

### The Natural Vitamin E Family

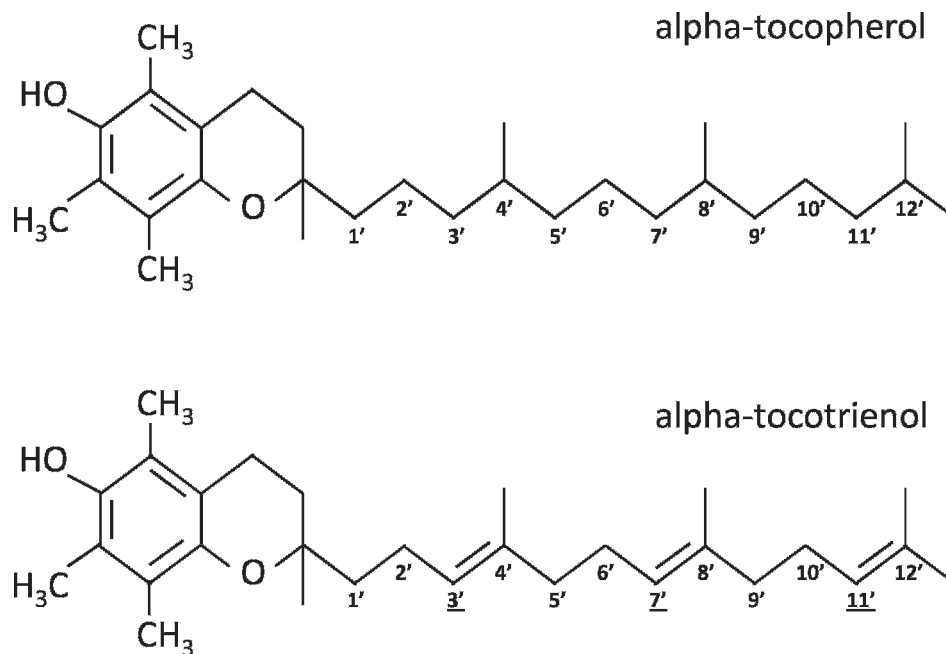
The discovery of vitamin E, as compared to the prior identified vitamins A–D, was uniquely different in that the effects of deficiency in animal models varied from species to species and a specific relevance to human deficiency was not realized for several decades. Herbert M. Evans is credited with the discovery of vitamin E in 1922, when he found that a particular “antisterility factor x” was necessary for reproduction in rats [1]. Specifically, pregnant rodents kept on a rancid

lard diet would resorb their embryos, but by enriching their diet with the chlorophyll-rich fraction of oil from lettuce leaves, Evans found that fertility was restored. Knowing that this factor was lipid soluble, he quickly resolved that his supplement was uniquely different from the other 2 known lipid-soluble vitamins of the time: vitamin A and vitamin D. Two years later, a chemistry professor from the University of Arkansas by the name of Barnett Sure named the fertility factor “vitamin E,” suggesting that Evans’ antisterility factor was also essential to humans despite no evidence yet to support such a claim. In 1936 Evans published the chemical formula of vitamin E in the *Journal of Biological Chemistry* [18]. Ascribing the chemical name of the compound was given to a colleague of Evan’s at The University of California at Berkeley, professor of Greek, George M. Calhoun. Evan’s personal account of the name’s origin from Dr. Calhoun gives humorous insight to what would otherwise be considered a prodigious effort:

“Most scientists, medical men especially,” said Calhoun, “have been guilty of coining Greek-Latin terms, bastards, of course, and we might have to do this.” “What does the substance do?” he asked. “It permits an animal to bear off-spring,” I replied. “Well, childbirth in Greek is *tocos*,” he said, “and if it confers or brings childbirth, we will next employ the Greek verb *phero*. You have also said that the term must have an ending consonant with its chemical — *ol*, it being an alcohol; your substance is ‘tocopherol,’ and the pleasant task assigned me quickly solved and not worth the delightful four-course dinner you have arranged.” [19]

The relevance of “tocopherols” to humans proved to be a difficult problem to solve until the late 1950s with the advent of the free-radical theory of aging and lipid peroxidation pioneered by Denham Harman [20,21]. According to this theory, membrane lipids are susceptible to oxidative damage, and the exact location of damage observed in different animal species depends on specific membrane lipid composition, vitamin E content, and cellular metabolic rate [1]. While it had already been known that vitamin E played an antioxidant role in protecting plant oils from oxidative damage and rancidity, this theory pioneered the concept of oxidative stress and the importance of antioxidants such as vitamin E as related to lipids in mammalian systems. Decades later, the human relevance of vitamin E deficiency became recognized in the medical field in cases of fat malabsorption or the disruption of low-density lipoprotein (LDL) processing, a lipid-rich transporter of vitamin E in the blood [22]. Progressive neuropathy and retinopathy leading to crippling ataxia and impaired vision were prevented by high-dose vitamin E supplementation in patients with abetalipoproteinemia [23,24].

Today, the naturally occurring vitamin E family is known to consist of both tocopherols and tocotrienols (Fig. 1). Tocopherols, as discovered by Evans, are more ubiquitous in



**Fig. 1.** Chemical structure of  $\alpha$ -tocopherol and  $\alpha$ -tocotrienol. Three unsaturations at carbon positions 3, 7, and 11 in the isoprenoid side chain account for the structural difference in  $\alpha$ -tocopherol and  $\alpha$ -tocotrienol chemical structure. This seemingly small difference has large implications for bioavailability and the specific biological functions of the vitamin E isomers.

plant tissues as compared to tocotrienols and are the principal vitamin E component in the leaves of plants and seeds of most dicots [25]. Tocopherols consist of a chromanol ring and a 15-carbon saturated tail derived from homogentisate (HGA) and phytyl diphosphate, respectively. Tocopherol biosynthesis in plants is catalyzed by homogentisate phytyltransferase which enacts the condensation reaction of HGA and phytyl diphosphate [26]. Unlike tocopherols, the 15-carbon phytyl tail of tocotrienols originates from geranylgeranyl diphosphate and contains 3 *trans* double bonds at the 3', 7', and 11' positions. Condensation of HGA and geranylgeranyl diphosphate is carried out by homogentisate geranylgeranyl transferase [27]. The position and degree of methylation on the chromanol head defines the  $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$  isoform designations within the tocopherol and tocotrienol families.

Compared to tocopherols, tocotrienols are considerably less widespread in the plant kingdom [25]. Tocotrienols are the major form of vitamin E in the seeds of most monocots (i.e., oil palm) and a limited number of dicots. A detailed analysis of tocotrienol accumulation revealed that the presence of this natural vitamin E is localized in nonphotosynthetic tissue [25]. Palm oil is one of the most abundant natural sources of tocotrienols, with crude palm oil (also referred to as the ‘tocotrienol-rich fraction’) containing up to 800 mg/kg weight of  $\alpha$ - and  $\gamma$ -tocotrienol isotypes. The distribution of vitamin E in palm oil is 30% tocopherols and 70% tocotrienols. In contrast, other commonly used dietary vegetable oils,

including corn, olive, peanut, sesame, soybean, and sunflower, contain tocopherols exclusively [2,28].

### Not All Vitamin E Is Created Equal

As the most bioavailable of all natural vitamin E isomers and with a well-characterized and selective transport mechanism in mammals, the vast majority of vitamin E research conducted to date has focused on  $\alpha$ -tocopherol ( $\alpha$ TOC). It is estimated that only 1% of all vitamin E literature and research during the last 30 years addresses tocotrienols [11]. Consequently, a striking asymmetry exists in characterizing the biological significance of non- $\alpha$ TOC family members to the degree that  $\alpha$ TOC and vitamin E are often used synonymously. Recent evidence supports unique biological functions of the lesser-characterized natural vitamin E homologues in mammalian tissue.  $\gamma$ -Tocopherol ( $\gamma$ TOC) is the most abundant form of vitamin E in the United States diet [29]. Desmethyl tocopherols, such as  $\gamma$ TOC, and specific tocopherol metabolites, such as the carboxyethyl-hydroxychroman products, exhibit functions that are not shared by  $\alpha$ TOC. The activities of these tocopherols are not directly related to their chemical antioxidant activity, but rather to anti-inflammatory, antineoplastic, and natriuretic function, possibly mediated through specific binding interactions [30].

Members of the tocotrienol family, known to be enriched in palm oil, also possess biological functions that are not shared

by  $\alpha$ TOC. Micromolar amounts of tocotrienol suppress the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the hepatic enzyme responsible for cholesterol synthesis [31,32]. HMG-CoA reductase is the same enzyme targeted by the statin class of drugs, one of the most widely prescribed group of drugs in the United States today [33]. Furthermore, tocotrienols, but not tocopherols, have been shown to suppress growth of human breast cancer cells [34]. Finally, dietary supplementation of the  $\alpha$ -tocotrienol ( $\alpha$ TCT) isoform uniquely protects against acute ischemic stroke injury *in vivo* [35]. At a nanomolar concentration range achievable by dietary supplementation,  $\alpha$ TCT derived from palm oil prevents inducible neurodegeneration by regulating specific mediators of cell death [35–40]. To date, the observed neuroprotective properties of palm oil-derived  $\alpha$ TCT are the most potent function of any natural form of vitamin E on a concentration basis.

### Bioavailability of Palm Oil-Derived $\alpha$ -Tocotrienol

While mechanisms of  $\alpha$ TOC tissue delivery and bioavailability are well characterized, the significance of dietary  $\alpha$ TCT bioavailability and tissue distribution has only recently been examined.  $\alpha$ -Tocopherol transfer protein (TTP) is a soluble 32-kDa protein expressed in liver that selectively binds  $\alpha$ TOC [41]. The TTP transporter is known to bind to  $\alpha$ TCT with 8.5-fold lower affinity than that for  $\alpha$ TOC [42], and subsequently contributed to the notion that availability of dietary tocotrienol to vital organs is negligible [43]. Recent *in vivo* evidence, however, suggests otherwise. Despite rodent chow enriched with  $\alpha$ TOC, it has been shown that TTP-deficient female mice are infertile due to vitamin E deficiency [44,45]. The placentas of pregnant TTP-deficient females are severely impaired with marked reduction of labyrinthine trophoblasts, and embryos die at midgestation even when fertilized eggs of TTP-sufficient wild-type mice are transferred into TTP-deficient recipients [44]. In 2005 it was reported that oral supplementation of palm oil-derived  $\alpha$ TCT in female TTP knockout mice restored fertility, suggesting that  $\alpha$ TCT is successfully delivered to relevant tissues in sufficient quantity to support reproductive function under conditions of  $\alpha$ TOC deficiency [46]. This observation was consistent with a second line of investigation in rats demonstrating that palm oil-derived  $\alpha$ TCT supplementation spared loss of fertility caused by long-term vitamin E deficiency in the diet [46]. Dietary palm oil-derived  $\alpha$ TCT supplementation was shown to enhance not only  $\alpha$ TCT bioavailability in reproductive tissues, but also in several vital organs, including the brain [46].

Over a decade ago in a study testing the ligand specificity of vitamin E isomers for TTP, it was concluded that the affinity of vitamin E analogues for TTP is one of the critical determinants of their biological activity [42]. This conclusion was based on the assumption that the biological function of

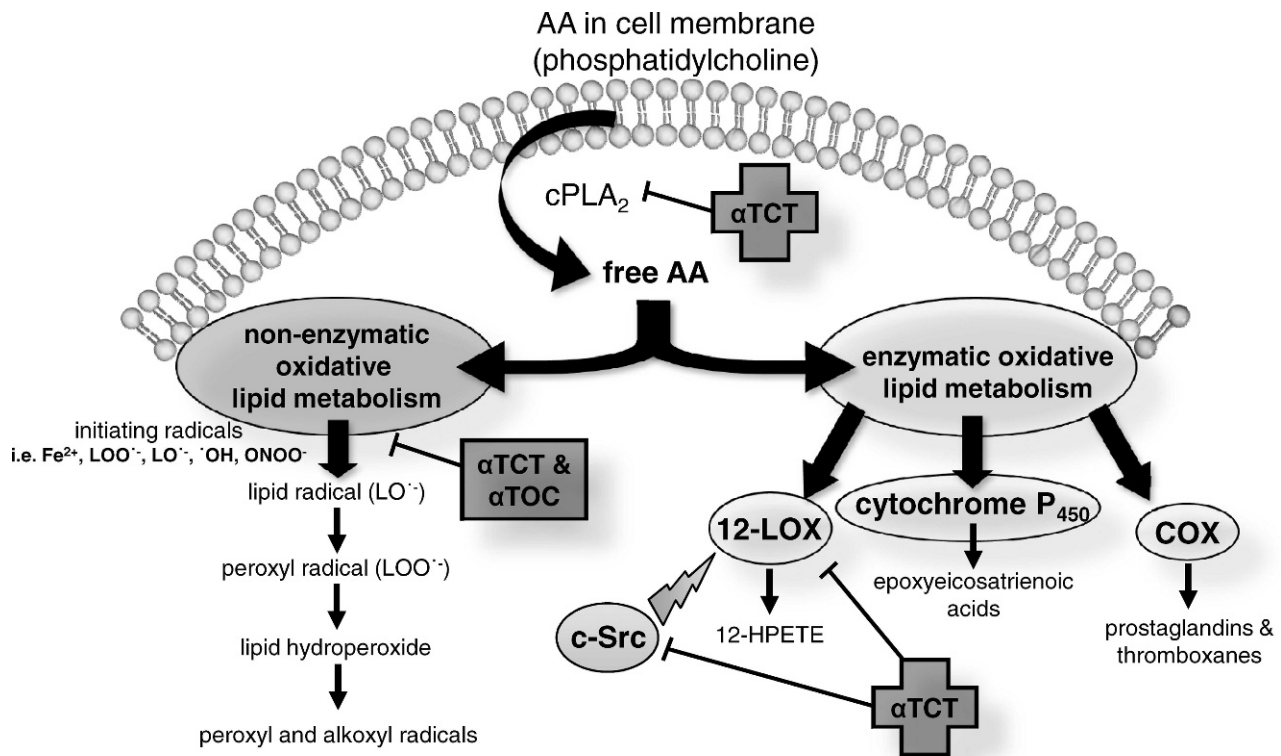
natural vitamin E family members is proportionate to their concentration in tissue and that vitamin E isotype function is redundant. Today, however, these assumptions have largely been disproven in light of new knowledge. It is now clear that oral supplementation of palm oil-derived  $\alpha$ TCT not only reaches the brain [46,47], but it does so in sufficient quantity to attenuate stroke-mediated neuropathy [35]. Furthermore,  $\alpha$ TCT supplementation in humans results in a peak blood plasma level that is over an order of magnitude higher than that required to protect neurons against a range of neurotoxic insults [35,37,46,48].

### The Arachidonic Acid Cascade of Oxidative Brain Injury and Neuroprotection by Palm Oil-Derived $\alpha$ -Tocotrienol

Brain tissue is highly susceptible to oxidative stress as it: (1) consumes an inordinate amount of oxygen to meet high metabolic demands, (2) contains high concentrations of polyunsaturated fatty acids (PUFAs) that are vulnerable to lipid peroxidation, and (3) has lower antioxidant capacity as compared to other organ systems. Neurons are particularly vulnerable to oxidative damage in pathologic brain tissue due to lower levels of endogenous antioxidant, glutathione, as compared to resident glial cells [27]. The consequence of increased generation of free radical species in injured brain tissue enriched with PUFAs is an accumulation of lipid peroxidation products that are proven to be culpable neuromodulators of the cell death cascade. Brain tissue is highly enriched with the n-6 PUFA arachidonic acid (AA, 20:4n-6) and the n-3 PUFA docosahexaenoic acid (DHA, 22:6n-3), which are major components of phospholipid membranes. Together, AA and DHA account for ~20% of all fatty acids in the mammalian brain [49]. Both AA and DHA are nutritionally essential to brain function and structure during early development and influence membrane fluidity, signal transduction, and gene transcription throughout life [50–54]. Neither AA nor DHA is synthesized *de novo*; rather, they are obtained from the diet and circulated to the brain directly in the plasma [54], or elongated from n-6 and n-3 PUFA linoleic (18:2n-6) and  $\alpha$ -linolenic acid (18:3n-3) precursors in the liver [55]. A number of neurodegenerative conditions in the human brain are associated with disturbed PUFA metabolism of AA [56,57]. Recent work supports palm oil-derived  $\alpha$ TCT as a potent natural neuroprotective agent in the AA cascade of neurodegeneration with the ability to uniquely target both nonenzymatic and enzymatic mechanisms of brain injury (Fig. 2).

### Phospholipase A<sub>2</sub>

Phospholipase A<sub>2</sub> (PLA<sub>2</sub>) isozymes encompass a diverse family of at least 15 different isozyme groups classified into 5



**Fig. 2.** Palm oil-derived natural vitamin E attenuates the arachidonic acid (AA) cascade in brain injury. Following release from the lipid membrane bilayer by phospholipase A<sub>2</sub> (PLA<sub>2</sub>), the polyunsaturated fatty acid AA undergoes oxidative metabolism in nonenzymatic and enzymatic pathways. Well known for their antioxidant function, natural vitamin E isomers α-tocotrienol (αTCT) and α-tocopherol (αTOC) inhibit nonenzymatic oxidative lipid metabolism of AA. Independent of antioxidant function common to all vitamin E isomers, αTCT is a specific and potent inhibitor of cytosolic phospholipase A<sub>2</sub> (cPLA<sub>2</sub>), c-Src kinase (c-Src), and 12-lipoxygenase (12-LOX) at nanomolar concentrations.

distinct categories: (1) secreted small molecular weight sPLA<sub>2</sub>, (2) larger cytosolic calcium-dependent cPLA<sub>2</sub>, (3) calcium-independent iPLA<sub>2</sub>, (4) platelet-activating factor acetylhydrolases (PAFA), and (5) lysosomal PLA<sub>2</sub> isozymes. The entire PLA<sub>2</sub> family is characterized by a common function—the enzymatic hydrolysis of the sn-2 ester bond of glycerophospholipids—thereby producing a free fatty acid (i.e., AA) and lysophospholipid (i.e., lysophosphatidylcholine, LPC). Currently, only sPLA<sub>2</sub> and cPLA<sub>2</sub> have well-defined roles in pathologic AA metabolism [58].

In a central nervous system (CNS) pathologic setting in which reactive oxygen and nitrogen species are abundant, there occurs a rapid accumulation of free fatty acids, due to increases in intracellular Ca<sup>2+</sup> and activation of PLA<sub>2</sub>s [59–62]. The sPLA<sub>2</sub>s are characterized by the requirement of histidine in their active site, Ca<sup>2+</sup> for catalysis, and the presence of 6 conserved disulfide bonds [63]. Under pathologic conditions of ischemic stroke, sPLA<sub>2</sub> mRNA and protein expression are significantly upregulated [64,65] and activity is induced by inflammatory cytokine tumor necrosis factor α (TNF-α) [66]. The cPLA<sub>2</sub>s are the only PLA<sub>2</sub> that demonstrate a preference for AA in the sn-2 position of phospholipids [67]. Localized

predominantly in grey matter, they lack the disulfide bonding network of sPLA<sub>2</sub>s and function through the action of a serine/aspartic acid dyad [68]. Under pathologic conditions, cPLA<sub>2</sub> subunit mRNA and protein expression are elevated [62]. Intracellular Ca<sup>2+</sup> accumulation mediates cPLA<sub>2</sub> subunit translocation to the membrane phospholipid bilayer [69] and activity is induced by phosphorylation of serine residue 505 by mitogen-activated protein kinase [70].

Once released, free AA has 3 potential fates: reincorporation into phospholipids, diffusion outside the cell, and metabolism. In a pathologic setting, free AA accumulates and undergoes uncontrolled oxidative metabolism by both nonenzymatic and enzymatic processes. This uncontrolled metabolism, referred to as the “arachidonic acid cascade,” includes the formation of harmful prostaglandins, leukotrienes, thromboxanes, isoprostanes, and nonenzymatic lipid peroxidation products [71]. The AA cascade amplifies the overall production of free radicals, both reactive oxygen and nitrogen species, and subsequently oxidative damage to lipids, proteins, and nucleic acids.

It has recently been shown that palm oil-derived αTCT attenuates cPLA<sub>2</sub> activity under conditions of glutamate-



mediated toxicity in neural cells [36]. Glutamate is the most abundant neurotransmitter of the CNS. Uncontrolled glutamate release at the synaptic cleft under pathologic conditions induces neurodegeneration in a process known as oxytosis. Glutamate activates cPLA<sub>2</sub> in neurons in a calcium-dependent manner, leading to the hydrolysis of AA from phospholipids [36]. Both phosphorylation and translocation of cPLA<sub>2</sub> are inhibited with nanomolar concentrations of  $\alpha$ TCT, a level previously demonstrated to be readily achievable by dietary supplementation [46,47].

### Nonenzymatic AA Oxidative Metabolism

Beyond the initial damage to lipid membranes, reaction of free radical species with double bonds of PUFAs produce alkyl radicals, which in turn react with molecular oxygen to form a peroxy radical (ROO<sup>•</sup>). Peroxy radical can abstract hydrogen from adjacent PUFAs to produce a lipid hydroperoxide (ROOH) and a second alkyl radical, thereby propagating a chain reaction of lipid oxidation [72]. Lipid peroxides degrade and give rise to  $\alpha,\beta$ -unsaturated aldehydes that include 4-hydroxynonenal, malondialdehyde, and acrolein [73–75]. These aldehydes covalently bind to proteins through reaction with thiol groups and alter their function. They also react with amino groups to form cyclic adducts. Vitamin E is widely known as the major chain-breaking antioxidant and the first line of defense in protecting lipid membranes from peroxidation [76]. The antioxidant properties of vitamin E are thought to be due to the fused heterocyclic ring. In this ring, the p-type lone electron pair of oxygen is kept nearly perpendicular to the aromatic plane. This p-type lone pair orbital overlaps with the semi-occupied molecular orbitals of the lipid radical, stabilizing the radical by conjugative electron delocalization [76].

Compared to tocopherols, the antioxidant capacity of the tocotrienol family is believed to be more potent [77]. Indeed, the tocotrienol-rich fraction of palm oil was found to be significantly more effective than  $\alpha$ TOC alone in inhibiting oxidative damage to lipids in isolated mitochondria from rats [78]. Due to the unsaturations found in their phytyl tail, tocotrienols assume a unique conformation in the lipid membrane bilayer with greater flexibility in the side chain that increases curvature stress on phospholipid membranes [43]. It is believed that the unsaturated side chain of tocotrienol also enables more efficient penetration into tissues that have saturated fatty layers, such as brain [79]. These phenomena may contribute to easier access of ascorbate to reduce the  $\alpha$ -tocotrienoxyl radical, thereby enhancing antioxidant regeneration of  $\alpha$ TCT more effectively than  $\alpha$ TOC in brain [80].

### Enzymatic AA Oxidative Metabolism

Cyclooxygenase, epoxygenase, and lipoxygenase enzymes are pivotal players in the generation of oxygenated derivatives

of AA in pathologic conditions affecting the brain. Cyclooxygenases convert AA to prostaglandins and thromboxanes, epoxygenase activity produces epoxyeicosatrienoic acids, and lipoxygenases catalyze the metabolism of AA into leukotrienes and lipoxins. The functional role of these enzymes and downstream lipid-derived products is largely dependent upon environment and cellular localization. In a pathologic setting of ischemic stroke, the lipoxygenase pathway and derivative products have been identified as key mediators of neurodegeneration and cell death in brain and are the focus of discussion here [35,39].

The first human lipoxygenase (LOX) activity was observed in platelets via the transformation of AA to a prostaglandin-like endoperoxide [81]. Today, the LOX enzyme family includes 4 members that are classified on the basis of the carbon position in which they oxidize AA: 5-, 8-, 12-, and 15-LOX. Despite variances in amino acid sequences and tissue distribution amongst family members, the active site of each isozyme is highly conserved [82]. The structure of LOX at the active site is composed of an N-terminal beta-barrel domain and a C-terminal domain containing a hydrophobic substrate-binding site [83]. A non-heme iron atom is coordinated by 3 histidine residues and the carboxy-terminal isoleucine. The oxidation of ferrous iron (Fe<sup>2+</sup>) to ferric iron (Fe<sup>3+</sup>) activates the enzyme, which is then capable of excising a hydrogen atom from a hydrocarbon at 1 of the 4 double bonds of AA. This abstraction generates a radical metabolite of AA that rapidly reorganizes its double bonds to take on a more stable conformation. Next, insertion of molecular oxygen generates a hydroperoxide radical that is reduced to the hydroperoxide anion by the simultaneous oxidation of iron to the ferric state [83]. A proton is then accepted to form a highly reactive fatty acid hydroperoxide; hydroperoxyeicosatetraenoic acid (HPETE). The hydroperoxide derivatives of AA are short-lived and readily metabolized into more stable compounds, including hydroxyeicosatetraenoic acids (HETEs) and leukotrienes.

While short-lived, HPETEs are potent neurotoxins. Highly reactive oxygen radicals are produced during the conversion of HPETEs to HETEs [84], contributing to the overall burden of oxidative stress following stroke. Under conditions of glutathione depletion, as in acute focal stroke, 12-LOX-derived 12-HPETE triggers nitric oxide (NO)-induced neural cell death [85]. Recent evidence supports that the hydroxy-(HETE) derivatives of LOX-mediated metabolism also possess potent biological activity. 12-HETE has been shown to increase mitochondrial NO production, induce cytochrome C release, and subsequently cause mitochondrial dysfunction [86].

The LOX-catalyzed dehydration reaction generates an epoxide intermediate, leukotriene A<sub>4</sub> (LTA<sub>4</sub>) [71]. LTA<sub>4</sub> is a highly unstable intermediate that is readily hydrolyzed to LTB<sub>4</sub> or conjugated with glutathione by cysteinyl leukotriene

C4 synthase to produce leukotriene C4 (LTC4), leukotriene D4 (LTD4), and leukotriene E4 (LTE4) [87]. Together, LTC4, LTD4, and LTE4 are collectively referred to as cysteinyl-leukotrienes (Cys-LTs). Total leukotriene levels accumulate in the mammalian brain and cerebral spinal fluid during and after cerebral ischemia [88,89]. LTB4 is characterized as a powerful chemotactic agent that induces adhesion of pro-inflammatory leukocytes to the endothelium; stimulates phagocytosis, and activates neutrophils and other leukocytes in a paracrine manner [90–92]. The Cys-LTs are potent vasoconstrictors of both venous and arterial smooth muscle [93,94] and are also reported to produce vascular leak and vasogenic edema [95].

While each LOX isoform carries out the same general reaction, namely hydrogen abstraction from AA, each has a unique gene structure, amino acid sequence, and tissue distribution profile. Only 3 forms of LOX are present in brain tissue, 5-, 12-, and 15-LOX [71]. Of these, 12-LOX is the most abundant isoform found in the brain [96], with significant mRNA expression in cortical neurons, astrocytes, and oligodendrocytes [97]. The LOX-mediated metabolites of AA serve as second messengers following stroke by modulating inflammation, apoptosis, and synaptic activity in brain tissue. Using immature cortical neurons and HT cells, it has been shown that a decrease in intracellular glutathione triggers the activation of neuronal 12-LOX, which leads to production of peroxides, influx of  $Ca^{2+}$ , and ultimately cell death [98].

Once cleaved from the sn-2 position of glycerophospholipids, AA may be metabolized by 12-LOX to pro-inflammatory HPETEs, HETEs, and leukotrienes. 12-LOX-deficient mice are highly resistant to ischemic stroke injury [35], and palm oil-derived  $\alpha$ TCT has been shown to be a potent 12-LOX inhibitor in neural cells subjected to glutamate-mediated neurotoxicity [39]. Furthermore, prophylactic supplementation of palm oil-derived  $\alpha$ TCT in stroke-prone spontaneously hypertensive rats significantly attenuated ischemic stroke lesion volume [35]. Studies addressing the effects of  $\alpha$ TCT on pure 12-LOX indicate that  $\alpha$ TCT directly interacts with the enzyme to suppress AA metabolism. *In silico* studies examining possible docking sites of  $\alpha$ TCT to 12-LOX support the presence of an  $\alpha$ TCT-binding solvent cavity close to the active site, potentially blocking AA access [39].

12-LOX catalytic function is believed to be regulated by tyrosine kinases. In response to glutamate-mediated neurodegeneration, 12-LOX is subject to rapid tyrosine phosphorylation in neuronal cells [35]. c-Src kinase is heavily expressed in CNS tissue, with rapid activation demonstrated to play a critical role in executing neurodegeneration [40,99]. Consistently, Src deficiency or blockade of Src activity in mice protects cortical brain tissue from stroke-induced neurodegeneration [100]. Findings from cell biology studies as well as from the study of c-Src kinase and 12-LOX in cell-free systems indicate that in response to glutamate challenge, c-Src is

rapidly activated and phosphorylates 12-LOX [35,38]. Importantly, palm oil-derived  $\alpha$ TCT prevents glutamate-induced cell death in active c-Src overexpressing cells [40]. Furthermore, glutamate-induced c-Src activity is completely blocked by nanomolar amounts of  $\alpha$ TCT. Taken together, the powerful effects of palm oil-derived  $\alpha$ TCT on c-Src and 12-LOX suggest that the lesser characterized vitamin E isomer is a potent inhibitor of 12-LOX-mediated AA metabolism and neurodegeneration.

## CONCLUSION

A growing body of literature has only just begun to delineate the unique and potent biological properties of the natural vitamin E  $\alpha$ TCT; as evidenced by more than two thirds of the entire PubMed literature on tocotrienols having only been published since 2000 [11]. To date, the neuroprotective qualities of  $\alpha$ TCT in neurodegenerative disorders of the CNS are well characterized, with specific molecular targets (cPLA<sub>2</sub>, 12-LOX, and c-Src) and mechanisms of action identified. Beyond the CNS,  $\alpha$ TCT has also demonstrated therapeutic promise in the treatment cancer and hypercholesterolemia. Originating from a dietary source with a long history of safe consumption in humans, the oil palm represents the richest source of  $\alpha$ TCT known today. Although tocotrienols are present in edible products such as palm oil, it remains questionable whether a dietary source alone could provide sufficient amounts of  $\alpha$ TCT to humans [11]. This is particularly relevant in Western diets that are typically devoid of palm oil and other natural sources of  $\alpha$ TCT. Enrichment of  $\alpha$ TCT from crude palm oil for dietary supplementation is achievable, and to date represents the most cost effective and readily available source of natural  $\alpha$ TCT.

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