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Vitamin E: necessary nutrient for neural development and cognitive function

Maret G. Traber

Linus Pauling Institute, College of Public Health and Human Sciences, Oregon State University, Corvallis, OR, USA

Vitamin E, discovered in 1922, is essential for pregnant rats to carry their babies to term. However, 100 years later, the molecular mechanisms for the vitamin E requirement during embryogenesis remain unknown. Vitamin E's role during pregnancy has been difficult to study and thus, a vitamin E-deficient (E–) zebrafish embryo model was developed. Vitamin E deficiency in zebrafish embryos initiates lipid peroxidation, depletes a specific phospholipid (DHA-phosphatidyl choline), causes secondary deficiencies of choline, betaine and critical thiols (such as glutathione), and dysregulates energy metabolism. Vitamin E deficiency not only distorts the carefully programmed development of the nervous system, but it leads to defects in several developing organs. Both the α-tocopherol transfer protein and vitamin E are necessary for embryonic development, neurogenesis and cognition in this model and likely in human embryos. Elucidation of the control mechanisms for the cellular and metabolic pathways involved in the molecular dysregulation caused by vitamin E deficiency will lead to important insights into abnormal neurogenesis and embryonic malformations.

α-Tocopherol: Neural tube defects: Neural crest cells

Vitamin E (α-tocopherol) was discovered about 100 years ago because it is required by pregnant rats to bring their fetuses to term[1]; it is still unknown as to whether vitamin E is needed for a specific function by the mother, the placenta or the developing embryo. It is well accepted that vitamin E functions as an antioxidant by scavenging lipid peroxyl radicals and preventing the propagation of lipid peroxidation (Fig. 1)[2], but it is unclear how the antioxidant function relates to the deficiency symptoms. Vitamin E deficiency in human subjects is well known to cause ataxia, which is a result of the dying back of the sensory neurons of the peripheral nervous system[3,4]. Further, long-term (decades) α-tocopherol supplementation can prevent progression of the degenerating nervous system caused by vitamin E deficiency[5].

Perhaps the most important vitamin E-related discovery in the past century has been the existence of the α-tocopherol transfer protein (α-TTP)[6–8]. α-TTP facilitates the hepatic secretion of α-tocopherol, but not other forms of vitamin E (β-, γ-, δ-tocopherols, or α-, β-, γ-, δ-tocotrienols, or synthetic-α-tocopherol (2*S*-α-tocopherols)) into the circulation[9,10]. Thus, the liver via α-TTP maintains not only the plasma but the entire body α-tocopherol supply[11]. In addition to the α-TTP gene initially being identified in human subjects, the National Center for Biotechnology Information lists 322 jawed vertebrates, including bony fishes, amphibians, birds and mammals, which have been reported to have the α-TTP gene (https://www.ncbi.nlm.nih.gov/gene/7274/ortholog/?scope=7776&term=TTPA).

Abbreviations: α-TTP, α-tocopherol transfer protein; DHA-PC, DHA-phosphatidyl choline; hpf, h post-fertilisation; lysoPL, lysophosphatidyl choline.

Corresponding author: Maret G. Traber, email maret.traber@oregonstate.edu

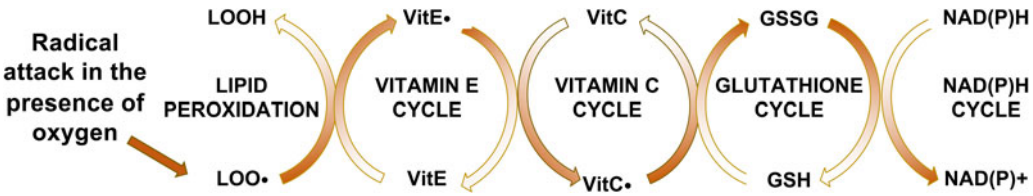

Fig. 1. Vitamin E interactions with lipid peroxidation and antioxidants. Vitamin E intercepts peroxyl radicals (LOO•), but becomes a radical itself (vitamin E•), which is reduced by VitC•, oxidizing it. Glutathione reduces the VitC. and becomes oxidised itself. The GSSG is then enzymatically reduced by glutathione reductase. Thus, the reversal of the entire oxidation process is energy (NADPH) dependent.

Apparently, the gene product, α-TTP, is critical for vertebrates.

Vitamin E background information

The vitamin E dietary reference intake for adult men and women (and individuals 14–18 years) was set in 2000 in the USA at a daily estimated average requirement of 12 mg α-tocopherol and an RDA of 15 mg[12]. Plants synthesise eight different forms of vitamin E[13], which all have antioxidant activity. The vitamin E forms are not interconvertible by animals or human subjects, only plants have the appropriate enzymes[13]. However, the human body preferentially retains α-tocopherol; α-tocopherol is the only form that has been shown to reverse clinical vitamin E deficiency symptoms. Therefore, only α-tocopherol meets human vitamin E requirements[12].

Human vitamin E deficiency is based on circulating α-tocopherol concentrations (<12 μmol/L serum or plasma). An increase in the prevalence of human vitamin E deficiency has been reported, based on low circulating α-tocopherol concentrations[14], which may be a result of increased consumption of vegetable oil that has become rancid through multiple frying uses[15] or other causes of rancidity (lipid peroxidation). Vitamin E food sources in addition to vegetable oils include nuts and seeds, and green/leafy vegetables[12].

α-Tocopherol absorption and transport have been studied using stable isotopes over the past 30 years. Recent studies show that intestinal absorption is about 55 %, α-tocopherol is then transported in chylomicrons from the intestine to the liver where it is preferentially secreted from the liver in newly synthesised lipoproteins into the plasma[16]. The α-tocopherol secretion from the liver mediated by α-TTP is the mechanism by which the plasma becomes α-tocopherol-enriched relative to the other forms of vitamin E[9,17]. Notably, the intestine does not require α-tocopherol absorption and secretion in chylomicrons[10]. Thus, the hepatic α-TTP is critical for plasma α-tocopherol enrichment.

Vitamin E and the nervous system

The clearest example of vitamin E deficiency in human subjects is caused by defective α-TTP and results in the disorder, ataxia with vitamin E deficiency (OMIM #277460). Ataxia with vitamin E deficiency is characterised by degeneration of sensory neurons, a progressive dying back of peripheral nerves, which causes a spinocerebellar ataxia with Purkinje cell death[18,19]. The defective α-TTP in ataxia with vitamin E deficiency causes low circulating α-tocopherol (<1 μM/l plasma) and low peripheral nerve and adipose tissue α-tocopherol concentrations[20]. In addition, the ataxia may also be a result of impaired α-tocopherol trafficking in the brain because α-TTP is located in the brain in Bergmann glial cells surrounding Purkinje cells[21,22], suggesting α-TTP in glial cells traffics α-tocopherol to neurons in the brain.

Although it is clear the human body needs α-tocopherol and that other forms of vitamin E do not fulfil this vitamin function because α-TTP does not maintain them, it remains unclear as to why the embryo specifically needs α-tocopherol and what is its molecular function. Critically, human subjects with deficient plasma α-tocopherol concentrations (<12 μM/l plasma) experience greater rates of miscarriage during early pregnancy[23], suggesting embryonic defects due to α-tocopherol deficiency. The Traber laboratory has been trying to address these questions by using the premier model of vertebrate embryogenesis, the zebrafish embryo.

Zebrafish embryo model system

Zebrafish embryos are widely used for investigating the molecular mechanisms of vertebrate development because the transcriptional networks, molecular responses and physiology are evolutionary conserved and similar to those in human subjects[24–26]. Zebrafish are also highly relevant for antioxidant research because they require the same dietary antioxidants as do human subjects, specifically both vitamins E and C[27,28]. Thus, the vitamin E-deficient zebrafish embryo model allows evaluation of developmental dysregulation in a highly relevant model to establish the mechanisms for the embryonic vitamin E requirement.

The Traber laboratory group has pioneered the use of vitamin E deficient or sufficient (E– or E+) diets fed to adult zebrafish that are spawned to obtain E– and E+ embryos[29,30]. These E– and E+ fish lay and fertilise eggs in similar numbers[31]. Biological variables, such as sex, age, weight and underlying health conditions (with the exception of vitamin E deficiency), are similar between the groups. Vitamin E deficiency causes >70 %

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of the E– embryos to die or be malformed by 72 h post-fertilisation (hpf)[32] with significant histologic abnormalities as early as 12 hpf[33], although the E+ and E– embryos appear phenotypically normal at 24 hpf[32]. Each embryo is a self-contained unit that does not require food until after 120 hpf. Thus, embryonic vitamin E deficiency can be studied as a progression of deleterious outcomes as the embryo progresses through the various developmental stages impacted by increasing lipid peroxidation.

Metabolic consequences of vitamin E deficiency in zebrafish embryos

Potentially, one reason cells in E– embryos die is due to increased lipid peroxidation because it is a self-propagating cycle that generates toxic compounds causing cell death[34,35], while vitamin E prevents the propagation of lipid peroxidation[36–38]. Thus, inadequate vitamin E in lipid peroxidation-susceptible cells, such as neural crest cells[39–42], could be a cause for cell death.

Both targeted and untargeted MS approaches (metabolomics and lipidomics) were used to determine why the E– embryo dies. McDougall *et al.*[32] discovered that vitamin E deficiency causes a fatal depletion of energy-producing nutrients (e.g. glucose for NADPH production via the pentose phosphate pathway) and that glucose repletion of the embryo by injection at an early stage could be used for rescue. Additionally, the E– embryos at 24 hpf were hypermetabolic, based on their oxygen consumption[32,43]. Thus, metabolic adaptation and compensation occur in the developing E– embryo to alleviate molecular, morphologic and biochemical phenotypes caused by the inadequate vitamin E supply. In support of this statement, E– embryos demonstrated a dysregulation of a complex, interwoven set of metabolic networks (Fig. 2)[32,43,44]. Further, quantitative measurements of glutathione, other thiols and methyl (one-carbon) donating molecules demonstrated that vitamin E deficiency leads to metabolic dysregulation, likely caused initially by lipid peroxidation of phosphatidyl choline-DHA (DHA-PC)[43,45,46], resulting in choline depletion and increased betaine production[46]. Vitamin E deficiency also dysregulates the methionine cycle[46]. These pathways are interconnected with the folate cycle, and it is well-appreciated that inadequate folate causes neural tube defects, in human subjects and in zebrafish[47,48]. During vitamin E deficiency, the depleted molecule is likely a thiol, probably glutathione, which then appears to dysregulate the balance of cysteine homostasis, both through generation from cystathionine and through the X_c^- antiporter[49]. Additionally, methyl group donors, such as S-adenosyl methionine, are dysregulated, possibly causing epigenetic dysregulation[45].

The critical role of vitamin E as an antioxidant and the relationship between lipid peroxidation, glutathione and other thiols, taken together, suggest that the abnormalities and lethality observed in the E– embryos are a result of lipid peroxidation-dependent death mechanisms, such as ferroptosis, as has been described for liver[36]. Vitamin E deficiency impairs the zebrafish embryo at a time *prior* to when a woman knows she is pregnant, very similar to the actions of folic acid deficiency on neural tube development. Although overt vitamin E deficiency is rare, the prevalence of vitamin E deficiency (serum α-tocopherol concentrations 12 μmol/L) in Bangladesh is estimated at 70 % of women[23]. Bangladeshi women with low α-tocopherol concentrations were about1·8 times more likely to miscarry[23]. Additionally, Balogun *et al.* in a Cochrane Database Systematic Review[50] reported that 'There was evidence of a *decrease* in the risk for stillbirth among women receiving multivitamins plus iron and folic acid compared to iron and folate only groups (RR 0·92, 95% CI 0·85 to 0·99, 10 trials, 79,851 women; high-quality evidence).' The beneficial results from supplementation with both multivitamins plus folate and iron support the idea that the stillbirth in human subjects induced by vitamin E inadequacy can be reversed by multivitamins containing vitamin E, but further research is needed. Additionally, vitamin E deficiency in zebrafish embryos induces secondary deficiencies of DHA, choline and glucose. Choline depletion may be the most important for human subjects because people do not consume sufficient choline[25]. Choline is a methyl donor that works in concert with folic acid and other B-vitamins and there is cross-talk between methylation status and energy homeostasis[51].

Vitamin E and neurogenesis

In early studies characterizing vitamin E deficiency in rodents, abnormalities were described to include exencephaly[52,53], dorsal root ganglia degeneration and defective blood–brain barrier formation[53]. Importantly, neural tube defects were also described in vitamin E-deficient mice[54–58]. Vitamin E protects zebrafish and rodent embryos at embryological states in which neural tube defects occur in human embryos 18–19 hpf in zebrafish[59], 9–12 d in rats[60] and 22–30 d in human subjects[61–63] (Table 1).

Neural crest cells are also important for the evaluation of the impact of vitamin E deficiency during embryonic development. Neural crest cells are stem cells that differentiate into precursors of the peripheral nervous system, as well as the cardiovascular system, craniofacial skeleton and pigment epithelia[64]. They migrate and differentiate into distinct populations along the embryo body axis during embryogenesis. Neural crest cells have a limited supply of nutrients for their migration through the embryo and are, thus, especially susceptible to oxidative damage[39]. Studies in E– embryos indicated these cells need more vitamin E antioxidant protection[33]. Specifically, neural crest cell formation is the result of a well-orchestrated gene regulatory network[65]. SRY-related HMG-box 10 protein (Sox10), a transcription factor, has a direct role in sensory neuron specification[66] and most peripheral nervous system neurons and glia are neural crest cell-derived and express Sox10[65]. These peripheral nervous system sites are also the most susceptible to damage in vitamin E-deficient human subjects[67].

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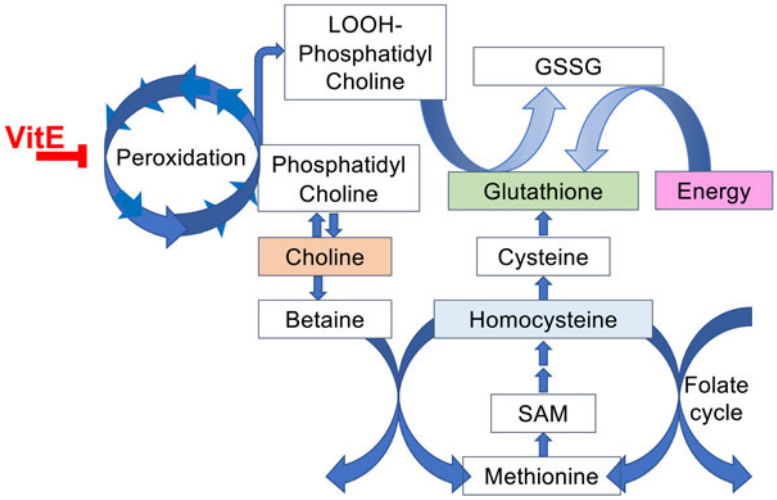

Fig. 2. Vitamin E interactions with lipid peroxidation and dysregulation of metabolism. In the absence of vitamin E, lipid peroxidation becomes a chain reaction and depletes critical phospholipids, such as phosphatidyl choline (oxidised phosphatidyl choline shown as a lipid hydroperoxide-phosphatidyl choline, LOOH-phosphatidyl choline). To replace these molecules, choline is needed, but choline is also needed via betaine for maintenance of the methionine cycle. Critically, lipid hydroperoxides (LOOH) also consume thiols, such as glutathione, which must be synthesised from the limited amino acid, cysteine. To maintain cysteine, the cell depends both on the methionine cycle as well as the X_c^- antiporter. Thus, with inadequate vitamin E, multiple overlapping pathways become depleted and dysregulated.

Importantly, E– zebrafish embryos demonstrated fewer cells expressing *Sox10*[33]. Collectively these data suggest that impaired neurodevelopment and degeneration are associated with neural crest cell abnormalities and cells derived from neural crest cells. Apparently, metabolic adaptation of the neural crest cells to vitamin E deficiency limits their migration, proliferation, differentiation and survival.

Requirement for α-TTP and α-tocopherol in embryonic development

α-TTP was reported in the developing human embryo at 5–12 weeks of gestation, specifically, α-TTP is expressed in the yolk sac[68]. Thus, zebrafish embryos with their yolk sac and ease of visualisation make a good model for these studies. The zebrafish embryo *Ttpa* mRNA increases 7-fold by 12 hpf and remains elevated at 24–36 hpf, while its knockdown causes 100 % embryonic mortality by 24 hpf[69]. Further, α-TTP is essential for normal neural plate and neural tube formation[69]. *Ttpa* is also found in developing brain, eyes and tail bud[69]. Thus, vitamin E uptake and trafficking occurs in the nervous system prior to the development of the liver or circulatory system, suggesting it is critical in the nervous system for delivery of vitamin E to specific regions. *Ttpa* is also highly expressed at the leading edges of the brain cavities during brain ventricle formation[33]. Importantly, in E– embryos, the development of the brain, the migration of neural stem cells and the formation of the spinal cord were impaired[33]. Taken together, these data show that both α-TTP and vitamin E are critical molecules during embryonic development, especially during neurulation (neural plate and tube formation)[69] and neural crest cell migration[33].

Neurodegeneration and cognition

Recent developments in neuroscience have shown that the human hippocampus, the site of memory and learning, undergoes neurogenesis in adults, but declines with ageing, which may be linked to cognitive impairments[70]. In 2015, 46·8 million people worldwide were living with dementia and this number will reach 131·5 million in 2050[71] Brain neurodegenerative disorders (e.g. Alzheimer's disease and -related diseases, and Down syndrome) are associated with (1) cognitive decline[72,73], (2) increased lipid peroxidation[74], (3) changes in metabolic function[75], and (4) mitochondrial dysfunctions and metabolic reprogramming[76,77]. The research community has focused on damaged proteins, but lipid peroxidation may be more dangerous in the brain because it is a self-propagating cycle that generates radicals and toxic lipid oxidation end-products (e.g. reactive aldehydes) that can damage proteins, DNA, etc. Our discoveries in adult zebrafish also show that low brain α-tocopherol is associated with a nearly 60 % depletion of 19 brain lysophosphatidyl cholines (lysoPL, combined $P = 0{\cdot}0003$), especially 3 lysoPL containing DHA: lysoP-choline, -ethanolamine, -serine[78]. The wide variety of lysoPL that are depleted suggests that

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Table 1. Comparisons of timing of developmental stages between zebrafish, rats and human subjects

Developmental stage	Zebrafish	Rat	Human subjects
Blastula/blastocyst	2–5 h	3–5 d	4–6 d
Implantation	n/a	6 d	8–10 d
Neural plate	10 h	9·5 d	17–19 d
Neural tube	18–19 h	9–12 d	22–30 d
First heartbeat	24 h	10·2 d	22 d
Birth/hatching	48–72 h	21 d	253 d

Embryological stages in zebrafish[59], 9–12 d in rats[60] and 22–30 d in human subjects[61–63].

the entire lysoPL substrate population is affected. LysoPL are needed for phospholipid remodelling during membrane synthesis, repair and replacement[79]. The brain acquires DHA as lysoPL-DHA[80]. A transporter from the major facilitator superfamily, MFSD2A, which is critical to maintain the blood–brain barrier[81], facilitates brain lysoPL-DHA uptake[82]. The MFSD2A transporter is a critical mechanism for lysoPL-DHA[80] delivery to the brain[82], resolving a long-standing mystery of how the brain acquires DHA[83]. Importantly, lysoPL-DHA depletion is linked to Alzheimer's disease[84].

Protection from lipid peroxidation is provided by a network of antioxidants, including vitamin E and glutathione[85], and is dependent on energy production (NADPH)[32]. The brain is particularly susceptible to lipid peroxidation due to its high concentration of polyunsaturated lipids (e.g. DHA-PC (18:0/22:6))[86,87]. DHA-PC is a significant membrane component in the brain[88] and a human serum biomarker of Alzheimer's disease[89]. To replace peroxidised DHA-PC requires (1) GSH to detoxify the oxidised lipids[85] and (2) choline[90], a one-carbon donor for DHA-PC synthesis[90,91]. The metabolic connection linking choline and one-carbon donors is through homocysteine.

Since homocysteine is an oxidation product and vitamin E is an antioxidant, oxidative damage has long been a focus in Alzheimer's disease research. Homocysteine elevation has been long recognised as a risk factor for dementia[92], is used as a biomarker to predict Alzheimer's disease pathology in human subjects[93], and homocysteinemia is decreased by increased B-vitamin intakes[94]. Nonetheless, clinical trials using B-vitamin supplements have shown no benefit for improving cognitive impairment or dementia[95], despite the slowing of brain shrinkage[96]. Could hyperhomocysteinemia be a result of inadequate brain vitamin E? Human clinical trials have shown that vitamin E supplements slowed the onset of dementia in patients with Alzheimer's disease[97,98]. However, meta-analyses of vitamin E supplements used in a number of Alzheimer's disease trials have shown no statistical benefit[99,100]. By contrast, low blood vitamin E levels are associated with high AD incidence[101]. Importantly, improved cognition and less brain shrinkage were associated with long-term dietary patterns that increase blood levels of both B-vitamins and vitamin E[101,102]. Based on the molecular interrelationships between vitamin E and B-vitamins in neurodegeneration, it is clear that chronic poor dietary choices such as diets low in vitamins B and E can promote neurodegeneration and cognitive decline.

Conclusions

Significant progress has been made in understanding the role of vitamin E in embryogenesis. The Traber laboratory has taken on these investigations because: (1) vitamin E is critical during neuro-embryogenesis; (2) the mechanisms by which vitamin E prevents defects during neural differentiation are heretofore unstudied; (3) the pathophysiological mechanisms of embryonic neurodegeneration are investigated in a highly relevant paradigm since women are increasingly consuming inadequate amounts of vitamin E[14,103]; and (4) the role of lipid peroxidation, as a mediator of embryonic neurodegeneration has largely been overlooked, although the embryonic environment is recognised to be under oxidative stress[104] and redox status is tightly regulated[105].

The Traber laboratory has focused on the unknown mechanism of an antioxidant vitamin in a vertebrate embryo to prevent nervous system abnormalities. Elucidation of the cellular and metabolic pathways involved in the molecular dysregulation caused by vitamin E deficiency will lead to important insights into abnormal neurogenesis and embryonic malformations. Identification of vitamin E-dependent pathways is necessary to provide critical knowledge necessary for effective progress in public policy concerning nutritional and therapeutic interventions to prevent malformations, such as neural tube defects during early embryonic development and potentially associated miscarriages[23].

Importantly, for human disease pathophysiology, the brain is particularly susceptible to lipid peroxidation due to its high concentration of polyunsaturated lipids, especially DHA-PC[86,87]. Based on the molecular interrelationships between vitamin E and B-vitamins in neurodegeneration, it is clear that chronic poor dietary choices may exacerbate deficiencies that can promote neurodegeneration and cognitive decline. What is less clear is whether any dietary changes can reverse damage or improve cognition.

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Conflict of Interest

None.

Authorship

The author had sole responsibility for all aspects of preparation of this paper.

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