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Commentary

Brain-E, Does It Equate to Brainy?

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The molecular mechanisms for the human vitamin E (α-tocopherol) requirement are unknown. In this issue of *The Journal of Nutrition*, Rhodes et al. (1) compared natural and synthetic vitamin E during pregnancy and lactation in mice for their studies on gene regulation in offspring brain vitamin E. These studies represent an important step forward because it is unknown how the brain acquires vitamin E, how it is trafficked within the brain, or how the brain compared with other tissues more effectively retains vitamin E during deficiency (2).

Vitamin E is a fat-soluble antioxidant that has gained new significance as an antiferroptotic agent, preventing programmed cell death caused by lipid peroxidation (3). Importantly, vitamin E deficiency enhances ferroptosis in the brain, especially the hippocampus (4). In 1922, vitamin E was identified in experiments in which it was found to be necessary for pregnant rats to carry their infants to term (5). Molecular mechanisms during embryogenesis and neurodevelopment are unknown, but low serum vitamin E concentrations early in human pregnancy are associated with increased miscarriage (6). Estimates of inadequate dietary vitamin E intakes exceed 80% of the global ≥14-y-old population (7) and 96% of American women (8). The 2015 Dietary Guidelines for Americans reported that vitamin E remains an under-consumed nutrient (9).

The vitamin E international unit (IU) was defined because natural and synthetic α-tocopherol had the same antioxidant activities, but different biological activities. Therefore, the IU was defined on the basis of the requirement for vitamin E during embryogenesis using the now obsolete rat "fetal resorption assay." The definition of the IU was deemed necessary because *1*) chemically synthesized α-tocopherol contains 8 different stereoisomers and *2*) plants make 8 different molecules with "vitamin E" antioxidant activity. This latter point was emphasized in the 1968 RDAs (10) because the American diet contains high amounts of γ-tocopherol, a form of vitamin E found in soybean, corn, and cottonseed oils (11). The expectation was that there was conversion between forms and that other vitamin E forms could substitute for α-tocopherol. However, more recent evidence shows that only plants can convert the 7 non-α-tocopherol forms to α-tocopherol.

The human body's preference for α-tocopherol was recognized in the 2000 DRIs (11). Both the α-tocopherol transfer protein (α-TTP) and vitamin E metabolism serve to ensure that α-tocopherol, but not naturally occurring non-α-tocopherols, is retained in the human body. Further, the FDA in 2016 limited the term "vitamin E" for labeling purposes to mean α-tocopherol, and stipulated that only half of the stereoisomers (*RRR*-, *RSR*-, *RRS*-, *RSS*-) of *all racemic* (*all rac*) α-tocopherol (of which *RRR* is the plant form) meet the human vitamin E requirement of 15 mg daily for adults (12). Importantly, α-TTP recognizes these 4 stereoisomers, generally termed 2*R*-α-tocopherol (11). Rhodes et al. (1) used the IU to normalize dietary natural and synthetic vitamin E in their studies on the effect of pregnancy and lactation on offspring brain vitamin E and discovered that the IU does not resolve the complexities of trying to match equivalencies. More confusing still, Kuchan et al. (13) showed that infant rhesus macaques fed synthetic vitamin E tend not to retain the synthetic 2-*R*-α-tocopherol in the brain, a phenomenon not related to brain α-TTP or RNA expression.

The role of vitamin E during pregnancy has been difficult to study. During human embryo development, α-TTP is expressed in the yolk sac (14). Vitamin E deficiency during embryogenesis causes neurologic defects in rats (15) and mice (16, 17). We study the zebrafish embryo because it develops from a fertilized egg to a free-swimming larvae in 5 d, it is transparent throughout development, and 70% of its genome is homologous to humans, making it a popular model for studies of vertebrate embryology (18). The zebrafish embryo expresses the α-TTP gene (*ttpa*) in yolk sac, developing brain, eyes, and tail bud, and *ttpa* is essential for neural plate and tube formation (19). *Ttpa* knockdown results in 100% lethality by 24 h postfertilization (hpf) (19). Vitamin E–deficient zebrafish embryos show that *ttpa* is highly expressed in the developing nervous system, but is not regulated by vitamin E (20). Importantly, *ttpa* is found at the leading edges of the brain cavities during brain ventricle formation at 24 hpf (20). Vitamin E is required for brain (fore-, mid-, and hindbrain) development at 12 hpf and by later stages (24 hpf) for formation of the dorsal root ganglia and notochord (20). Thus, both α-TTP and vitamin E are critical molecules during embryonic development, and vitamin E uptake and trafficking in the embryo occur in the nervous system before liver or circulatory system development (20). Critically, vitamin E deficiency in zebrafish embryos causes impaired behavior and cognition that is not repaired by subsequent vitamin E repletion (21, 22).

The hippocampus is important for learning and memory and contains similar vitamin E concentrations compared with other brain regions (23). Rhodes et al. (1) sought to evaluate whether

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Abbreviations used: *all rac*, *all racemic*; DEG, differentially expressed gene; hpf, hours post-fertilization; IU, vitamin E international unit; *ttpa*, α-tocopherol transfer protein gene; α-TTP, α-tocopherol transfer protein; 2*R*-α-tocopherol, *RRR*-, *RSR*-, *RRS*-, and *RSS*-α-tocopherol stereoisomers.

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gene expression in the hippocampus varies in mouse offspring from parents fed diets containing different α-tocopherol doses (37.5 or 75 IU/kg diet) or sources [natural (*RRR*) or synthetic (*all rac*) α-tocopherols] before pregnancy and through lactation. Interestingly, the brain α-tocopherol concentrations in offspring were highest in the 75 IU synthetic group and lowest in the low *RRR*-α-tocopherol group, but differences between the lowest and highest groups were <2 μmol/g, suggesting that doubling the vitamin E intake to the mothers had little impact on the offspring brain α-tocopherol concentrations. The authors emphasize, however, that in the synthetic vitamin E groups *RRR*-α-tocopherol was only ∼20% of the brain α-tocopherol, whereas 2*S*-forms (*SRR*-, *SSR*-, *SRS*-, *SSS*-α-tocopherol) were ∼10% of the total. Thus, the majority of the brain vitamin E was composed of 2*R*-stereoisomers. Unfortunately, no cognition or behavioral tests were performed (1). However, examination of the 16,755 hippocampal genes using DAVID analysis showed that 797 were differentially expressed genes (DEGs) and were in categories of transcription regulation, DNA binding, and synapse regulation (1). About 95% of these DEGs displayed a fold-change of <1.10. No genes showed a significant interaction between dose and source by the 20% false discovery rate criterion, suggesting that if vitamin E intake amounts were adequate, there was minimal impact on gene regulation.

Rhodes et al. (1) also defined "VIPs" or Vitamin E Interacting Proteins, based on literature reports, to construct a transcriptional regulatory network using the ASTRIX algorithm. They found that a large number of the DEGs resulted from synthetic α-tocopherol stereoisomer distribution. The authors raise the concern that human brain development for the first time in evolutionary history is being affected by foods and supplements with *all-rac* α-tocopherol, which contains 7 non-*RRR*-α-tocopherol forms. Importantly, genes in the protein kinase C family may be especially affected. Another protein, brain-derived neurotrophic factor, a member of the neurotrophin family of growth factors that promotes the proliferation and survival of neurons in the brain, was also decreased with increased *RRR*-α-tocopherol, suggesting decreased demand.

It is clear brain vitamin E plays a critical role in neurodevelopment at the earliest stages of brain formation (20). But, it remains unclear if the antioxidant function and postulated role of lipid peroxidation in ferroptosis cause the observed neurodegeneration of embryonic neurons and impaired proliferation during vitamin E deficiency. The importance of vitamin E roles in lipid fluidity and membrane repair is highlighted by the observed gene changes. Thus, the study of vitamin E in neurodevelopment remains an important new frontier in nutrition research.

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