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Review

Many tocopherols, one vitamin E

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ABSTRACT

Four tocopherols are available in nature and are absorbed with the diet, but only one RRR-α-tocopherol satisfies the criteria of being a vitamin. The biological activity of the different tocopherols studied in the rat by the resorption-gestation test has been inconsistently extrapolated to human beings where the tocopherols have no influence on a successful pregnancy. Diminution of RRR-α-tocopherol intake results in diseases characterized by ataxia, whose pathogenetic mechanism, despite vigorous claims, has not been clarified. The calculation of the Daily Reference Intake (DRI), necessary to prevent disease, is based on an obsolete test, the peroxide-induced erythrocyte hemolysis, called the gold standard, but of highly questioned validity. If many epidemiological studies have given positive results, showing prevention by high vitamin E containing diets of cardiovascular events, neurodegenerative disease, macular degeneration and cancer, the clinical confirmatory intervention studies were mostly negative. On the positive side, besides preventing vitamin E deficiency diseases, vitamin E has shown efficacy as anti-inflammatory and immune boosting compound. It has also shown some efficacy in protecting against nonalcoholic hepato-steatosis. At a molecular level, vitamin E and some of its metabolites have shown capacity of regulating cell signaling and modulating gene transcription.

1. Introduction

A literature search with ISI Web of Science conducted on February 19, 2017, using as keywords “vitamin_E or tocopherol” as topic, has given 65,247 papers. The story of vitamin E discoveries begins in 1922, when the first paper was published (Evans and Bishop, 1922). The entire information contained in all articles published afterwards, cannot be summarized in the following paragraphs; consequently, as a source of standard information on tocopherols and vitamin E, the reader can consult one or more of the 4 337 today available review articles.

In the next paragraphs, I will keep the classical information in the background to focus on some data and ideas that have been often omitted from most review articles, not being aligned with the general belief: “Vitamin E, is antioxidant and nothing more” (Traber and Atkinson, 2007) apparently expressed in contrast with Popper's philosophy of the scientific discovery (Popper, 1959). I have adopted herewith a critical attitude with respect to “established scientific facts” and I have rather concentrated on more novel data and ideas in part already summarized in few review articles (Azzi, 2007a; Azzi et al., 2016).

The notion that vitamin E, is an antioxidant and nothing more *in vivo* requires precise techniques to verify it. Some of these techniques will be critically reviewed. The action of vitamin E as free radical scavenger requires a mechanism for restoring it, after it

Abbreviations: ABCA, ATP binding cassette transporter A; AREDS, Age-Related Eye Disease Study; AMD, age dependent macular degeneration; AVED, ataxia with vitamin E deficiency; DRI, daily recommended intake; ERK, extracellular signal−regulated kinase; HOPE, Heart Outcomes Prevention Evaluation; IP3K, inositol 1,4,5-trisphosphate 3-kinase; NASH, nonalcoholic steatohepatitis; NIH3T3-L1, standard fibroblast cell line; NO, nitric oxide; PI3Kγ, gamma subunit of the inositol 1,4,5-trisphosphate 3-kinase; PKC, protein kinase C; PPARγ, peroxisome proliferator-activated receptor gamma; SELECT, Selenium and Vitamin E Cancer Prevention Trial; TAP, tocopherol associated protein; THP-1, human monocytic cell line; α-TP, α-tocopheryl phosphate; γ−CEHC, 2,7,8-trimethyl-2-(β-carboxyethyl)-6-hydroxychroman; α-CEHC, 2, 5, 7, 8-tetramethyl-2-(2'-carboxyethyl)-6-hydroxychroman; α-TTP, α-tocopherol transport protein; α-TTP−/−, α-tocopherol transport protein knock out mouse.

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has become itself a radical. Vitamin C and lipoic acid have been shown *in vitro* to have such a property. However, data will be presented by which the degree, if any, of vitamin E recycling in humans and by what antioxidants remains unconvincingly clarified. Current literature and companies' catalogues state that all four tocopherols available in nature can be called "vitamin": in fact, only one, RRR-α-Tocopherol, has been shown to protect the human body against the disease produced by its absence and can thus be called vitamin.

In the next paragraphs, it will be discussed why, although generally used, the measurements of the recommended daily intake are not reliable and the data on the need and type of tocopherol supplementation speculative. The notion that tocopherols and especially α-tocopherol are only antioxidants has been supported in such an extensive and assertive way that little space has been left for alternative concepts. I will discuss below controversial, somehow ignored aspects of this vitamin and its alleged mechanisms of action; I will also describe scientific data that hypothesize new and interesting functions of tocopherols and their molecular aspects.

Of the different tocopherols in the following paragraphs priority will be given to RRR-α-Tocopherol, a vitamin, whose diminution or absence is causing disease. The other tocopherols, although studied to a lesser extent, will be also considered.

2. RRR-α-tocopherol, vitamin E

Four tocopherols (α-, β-, γ−, and δ-Tocopherol) are synthesized and stored in plant leaves and seeds. Their chemical difference consists in the number and position of the methyl groups of the chromanol ring. Tocopherols' natural forms have RRR stereochemistry of the 3 asymmetric carbon atoms of the phytyl chain attached to the chromanol ring (Krinsky et al., 2000). Tocopherols are not *de novo* synthesized in animals, where also conversion of the different forms in each other by methylation or demethylation is not taking place. The major function of tocopherols in plants is that of antioxidants although non-antioxidant functions have been described (Hofius et al., 2004; Sakuragi et al., 2006).

A vitamin is an organic compound that is essential for the normal physiological functions of an organism; it is required in small quantities since it cannot be synthesized, or sufficiently synthesized by the organism. The insufficient availability of a vitamin causes disease.

Vitamin E deficiency in humans is avoided by administration of α-tocopherol. The other tocopherols have not been tested for their ability of preventing human avitaminosis and thus they cannot be considered "vitamers" or "other forms of vitamin E" for humans, as often reported. The attribution of vitamin function to β-, γ−, and δ-tocopherols although based on the protection against fetus resorption in rats, is illogically extended to the prevention of the neurological disease affecting humans.

The biological activity of the different tocopherols in the rat has been studied by the resorption-gestation test (Leth and Sondergaard, 1977). The following values have been obtained (with the chemically synthesized racemic α−tocopheryl acetate = 100%): RRR-α-tocopherol 80%; RRR-α-tocopheryl acetate 136%; RRR-β-tocopherol 45%; RRR-γ−tocopherol 13%; and RRR-δ-tocopherol less than 0.4%. This test is complicated by the fact that absorption and degradation of the various tocopherols is different among them and between rats and humans; consequently, the values of the efficacy of the different tocopherols in preventing fetus resorption do not necessarily correspond to their human effectiveness as vitamin. Additionally, it is not reasonable to extrapolate the information obtained in the rat to humans, where the lack of vitamin E does not cause fetus resorption and where the symptoms of avitaminosis are primarily represented by cerebellar ataxia.

In conclusion, only α-tocopherol has been successfully used in the prevention/treatment of the diseases associated with vitamin E deficiency and therefore only α-tocopherol can be *bona fide* called vitamin E. For the other tocopherols, the designation of vitamin E or vitamers is incorrect. Additionally, the frequent use of the attribute "isomers" is also incorrect since only the β− and γ form are isomers, with the chromanol ring methylated at two carbon atoms. The α- and δ− tocopherols have three and one methylated position, respectively, they cannot be called isomers and they are not isomer with the β-and γ forms.

3. The vitamin E deficiency diseases

An infrequent genetic disorder "Isolated Vitamin E Deficiency" or 'Ataxia with Isolated Vitamin E Deficiency" or AVED, is produced by mutations in the gene coding for the tocopherol transfer protein (α-TTP) (Arita et al., 1995; Catignani, 1975). These individuals have the capacity to absorb vitamin E in the intestine but have an extremely poor ability to retain it (Arita et al., 1995; Ouahchi et al., 1995; Sokol et al., 1988). The disease generally appears between ages five and 15 years and its symptoms include progressive ataxia, clumsiness of the hands, loss of proprioception, and areflexia (Schuelke, 1993). According to the pathological findings by Yokota et al. (2000) in a postmortem case with mutation of the tocopherol transfer protein (α-TTP) the major anomalies "were retinal atrophy; severe dying back-type degeneration of the posterior column; and massive accumulation of lipofuscin in neurons including dorsal root ganglion (DRG) cells, which were almost identical to those in vitamin E deficient animals and patients with fat malabsorption. Also, mild loss of Purkinje cells was noted. The mild Purkinje cell loss might be related to the mutant α-TTP in the cerebellum. By contrast, in the DRG, thought to be mainly responsible for ataxia, no expression of α-TTP was detected, and the tissue concentration of vitamin E increased to normal after supplementation. It is therefore considered that oral supplementation of vitamin E should effectively counteract the progression of ataxia" (Yokota et al., 2000). Vitamin E, after supplementation in α-TTP−/− mice, became normal in plasma but not in the cerebellum (Ulatowski et al., 2014). Regardless of all these incongruences, loss of cerebellar Purkinje cells has been considered to be at the molecular basis of the disease and studied in animal models (Ulatowski et al., 2014).

Due to its fat solubility, vitamin E requires lipid for its absorption in the gastrointestinal tract. Consequently, diminution of plasma vitamin E is associated with situations present in patients with fat malabsorption (premature, very low birth weight infants; cystic fibrosis; gastric bypass; Crohn's disease; liver disease or pancreatic insufficiency; abetalipoproteinemia) (Muller et al., 1983).

Although an attempt has been made to unify all the symptoms of the disease in terms of oxidative damage, which would occur in the absence or at low levels of vitamin E (Farrell et al., 1977), a closer analysis of the data indicates a large heterogeneity in the clinical presentation of the disease. For instance, Vitamin E deficiency symptoms have been found in patients with normal serum vitamin E (Sokol et al., 1984). Vitamin E deficiency in children with chronic cholestasis is associated with symptoms of disturbed hepatic function (Sokol et al., 1986); and in patients with cystic fibrosis and associated pancreatic insufficiency with a slight erythrocyte fragility (Farrell et al., 1977); in these erythrocytes, fragility may be related to the elevated plasma colic acids acting as a detergent (Hodate and Hamada, 1984). Although in cystic fibrosis and cholestasis vitamin E deficiency is frequently associated with erythrocyte fragility, no mention is made of erythrocyte fragility or

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