PERSPECTIVE

Vitamin E: A vitamin still awaiting the detection of its biological function

Regina Brigelius-Flohé[1] *and Francesco Galli*[2]

[1] Department of Biochemistry of Micronutrients, German Institute of Human Nutrition, Nuthetal, Germany
[2] Department of Internal Medicine, Faculty of Pharmacy, University of Perugia, Perugia, Italy

Introduction

Vitamin E was discovered in 1922 by Evans and Bishop [1] as a compound required for reproduction of rats. Since, innumerable attempts have been undertaken to finally find out why vitamin E is an essential micronutrient in humans. Even almost 90 years after its discovery, however, the real biological role of the vitamin remains enigmatic.

In 1937, the antioxidant function of vitamin E was described for the first time [2]. This detection started a decades-long attempt to prevent or cure diseases presumed to be associated with oxidative stress, such as cardiovascular diseases, cancer and neurodegenerative disorders by vitamin E supplementation. Unfortunately, the great efforts made by several groups to provide clinical evidence for a protective efficacy of vitamin E have so far failed. Largest secondary prevention trials on cardiovascular disease and cancer remained unsuccessful, and in some cases, they were prematurely stopped due to putative adverse effects of high-dose supplementation. Nor did these studies provide any novel insights to explain the two cardinal symptoms of vitamin E deficiency, female infertility and neuromuscular disorders.

To fill the intriguing gaps of knowledge, already two brainstorm meetings entirely devoted to vitamin E were organized in this millennium. The first one was held in Tremsbüttel, Germany, from July 30 to August 1, 2001 [3], the second in Boston, May 22–24, 2004 [4]. Both meetings focused on novel aspects of vitamin E research, such as its potential role in gene regulation and cellular signaling. Also the clinical trials undertaken to test the protective role of vitamin E in cardiovascular disease, cancer and neurological disorders were discussed. A real break-through was not achieved. To discuss the progress of the last 5 years, a satellite symposium on Vitamin E was held in Rome on August 26, 2009 prior to the annual meeting of the Society for Free Radical Research (SFRR)-Europe. The scientific program intended to learn more about the biological function of vitamin E. The breakthrough was not reached either. Again, however, some novel aspects arose which appear worth to be investigated in more detail, *e.g.* metabolism and metabolites of vitamin E, its transport, role in signaling, gene activity and brain function. Even topics related to plant metabolism were considered [5]. The most interesting topics were chosen for this special issue to update the frontiers of vitamin E research.

1 Metabolism and metabolites of vitamin E

A surprising milestone of vitamin E research was the finding that all forms of vitamin E are metabolized by ω-hydroxylation followed by side chain degradation while the chromanol structure is left intact [6]. These metabolic studies allowed two important conclusions: (i) the intact chromanol structure of the metabolites is hardly compatible with vitamin E having reacted as an antioxidant and (ii) forms of vitamin E not being α-tocopherol, as well as high dosages of α-tocopherol, are eliminated like xenobiotics [7, 8]. Nevertheless, tiny amounts of metabolites with an opened, *i.e.* oxidized, chroman structure such as tocopheronolactone and its conjugates are detectable in the urine [9]. If validated and standardized, such metabolites might indeed become suitable biomarkers for an antioxidative role of vitamin E that has been searched for so long. They might be more useful than the assays used to measure the antioxidant capacity since the relative antioxidant potential

Correspondence: Professor Regina Brigelius-Flohé, Department of Biochemistry of Micronutrients, German Institute of Human Nutrition, Potsdam-Rehbruecke, Arthur-Scheunert-Allee 114–116 D–14558 Nuthetal, Germany
E-mail: flohe@dife.de
Fax: +49-33200-88-407

Abbreviations: **α-TP,** α-tocopheryl phosphate; **α-TTP,** α-tocopherol transfer protein; **ApoE,** Apolipoprotein E; **TAP,** tocopherol-associated protein

of tocols depends on the assay and circumstances under which the assay is performed [10]. In this issue, the current methodology for the detection of metabolites and conjugates is compiled by Marc Birringer [11] and David Muller's group [9]. Further, tocopherol metabolites may have a pharmacodynamic profile of their own, such as interference, with the mitochondrial electron transport chain as discussed by Lars Gille [12]. Evidently, a better understanding of vitamin E metabolism is pivotal to predict the consequences of supplementation with α-tocopherol or, even more so, with other forms of vitamin E.

2 Transport of vitamin E

Uptake and distribution of vitamin E has mainly been investigated for α-tocopherol, while little is known about specific transport processes of other forms of vitamin E. Intestinal uptake of α-tocopherol occurs *via* the scavenger receptor SR-B1, which mediates selective uptake of HDL-associated lipids, or by the Niemann-Pick C1-like 1 (NPC1L1) receptor, which also transports cholesterol. Transfer into the circulation is achieved *via* incorporation into chylomicrons or *via* ABC transporters, which release vitamin E into HDL particles. In the circulation, chylomicrons are hydrolyzed by the lipoprotein lipase. Chylomicron remnants are taken up into the liver by LDL receptor-related protein. There the α-tocopherol transfer protein (α-TTP) is required for the selective resecretion of α-tocopherol by a still unknown mechanism. Incorporation into VLDL has for long been considered the only vehicle for α-tocopherol release from the liver, but recently also ABC transporters were shown to transfer α-tocopherol through membranes to the circulation where it is taken up by HDL. Peripheral tissue again takes up α-tocopherol by means of SR-B1. Transfer of α-tocopherol between circulating lipoproteins is made possible by the phospholipid transfer protein which together with tocopherol-associated proteins (TAP) also works intracellularly. Taken together, the transport and distribution of α-tocopherol between organs and within cells requires a remarkable number of transporters and transfer proteins and obviously is highly regulated. The increasing complexity of α-tocopherol uptake and distribution is here compiled by Lemaire-Ewing *et al.* [13] and Takada and Suzuki [14].

Expectedly, the fate of α-tocopherol in the body also depends on lipoproteins. Apolipoprotein E (ApoE) is the major protein in HDL particles and, thus, has a critical role in HDL-mediated α-tocopherol transport. Accordingly, ApoE$^{-/-}$ mice have increased levels of α-tocopherol in plasma and peripheral tissues but not in the brain where levels are decreased [15], indicating that the brain handles HDL-bound α-tocopherol different from peripheral tissue. In line with these findings, polymorphisms and mutations in the ApoE gene might influence vitamin E-associated diseases. As outlined by Huebbe *et al.* [16] in this issue, carriers of the ApoE4 variant have lower tissue α-tocopherol levels than ApoE3 carriers without significantly affected plasma levels. Furthermore, the apoE4 genotype is a significant risk factor for Alzheimer's disease. In view of the critical role ApoE has in the distribution of α-tocopherol, it appears worthy to reinvestigate the mechanism of α-tocopherol application in the atherosclerosis model, the ApoE$^{-/-}$ mouse.

Interestingly, excretion of excess α-tocopherol into the bile occurs *via* MDR transporters also known to mediate drug export and associated drug resistance. These transporters are upregulated by α-tocopherol which might point to a strict prevention of α-tocopherol accumulation in the body as outlined by Maret Traber [17], (see below).

3 Vitamin E in membrane processes

It appears reasonable that vitamin E as a hydrophobic vitamin exerts its physiological functions in a lipophilic environment, *i.e.* within membranes [18]. This does not necessarily imply that its sole function is to act as an unspecific lipophilic antioxidant. In fact, the rate constants for the reaction of vitamin E with most of the free radicals are too unfavorable to meet this task, reviewed in [19]. Only if concentrated in certain membrane domains, it can reach a concentration high enough to prevent oxidative modulation of proteins. Such membrane domains might be lipid rafts as suggested by Lemaire-Ewing *et al.* [13] or PUFA-containing phospholipid-rich domains as suggested by Atkinson *et al.* [20].

Lipid rafts are cholesterol and sphingolipid-enriched microdomains that serve as platform for signaling complexes. Intriguingly, α-tocopherol appears to preferentially affect proteins which are real membrane proteins or have to be recruited to membranes to form enzyme complexes, *e.g.* NADPH oxidase (NOX), enzymes involved in arachidonic acid metabolism (Lipoxygenase (LOX), Cyclooxygenases (COXs)) or proteins of the PI3K/AKT pathway as outlined by [19, 21] and by Zingg *et al.*, this issue [22]. It is not clear whether α-tocopherol is part of the rafts themselves, triggers recruitment of proteins/lipids to the membrane or modulates the formation of these microdomains. A first hint for the participation in the modulation of membranes processes comes from the here described finding that α- (and also β-) tocopherol increases vesicle-associated degranulation from mast cells [23].

A function within membranes and subcellular organelles requires proteins that transport vitamin E to intracellular destinations. Candidates might be TAPs from which three have been identified in humans belonging to the Sec14L family (hTAP1/2/3 or SEC14L2/3/4, respectively). TAPs bind lipid ligands, such as squalene, phosphatidylcholine and -ethanolamine, and different phosphatidylinositol phosphates. Their putative functions comprise intracellular transport as well as transport of lipids out of the cell by, *e.g.*

exocytosis. Apart from the SEC domain, TAPs harbor a GOLD domain which serves as protein–protein interaction module involved in Golgi function or trafficking [24]. Binding of vitamin E to TAPs has been discussed as a mechanism by which α-tocopherol is transported to intracellular targets where it modulates lipid-mediated signaling pathways, such as inhibition of the PI3K pathway or vesicular transports. Contributions relevant to these novel perspectives in this issue are [12, 13, 20, 22, 23].

4 Role of vitamin E in gene regulation and cellular signaling

A most attractive ''novel'' function of vitamin E is regulation of gene activity. However, despite an enormous number of vitamin E-affected genes found in microarrays performed with samples of differently fed rodents or α-TTP-deficient mice, a direct gene regulation by vitamin E could not be detected so far. Moreover, genes and/or pathways affected need months of vitamin E-feeding before a response could be detected. Also no vitamin E-specific transcription factor, as, *e.g.* known for vitamin A or D has so far been identified. Changes in gene clusters such as those for vesicular transport, neuronal or muscular functions might have been resulted from a permanent stimulation or depletion, respectively, of vitamin E. The only genes responding to high doses of α-tocopherol *in vivo* in a relatively short time were those involved in vitamin E elimination, *i.e.* hepatic cytochrome P450 and ABC transporters (work form the groups of Maret Traber and Regina Brigelius-Flohé). Induction is achieved by PXR activation, a transcription factor which responds to all kinds of lipophilic xenobiotics and drugs. Thus, regulation of xenobiotic metabolism (including its own) has been suggested to be the only signaling function of α-tocopherol to prevent accumulation of non-α-tocopherol forms and accumulation of excess α-tocopherol itself [17].

Also tocotrienols can regulate the activity of genes which, however, are different from those regulated by α-tocopherol [25]. The findings that tocotrienols but not tocopherols bind to ERβ (estrogen receptor β) and initiate the expression of estrogen-dependent genes, for example MIC-1, ECR-1 and cathepsin D, in ERβ-containing breast cancer cells resulted in the provocative question whether tocotrienols should still be grouped into the vitamin E family.

A recently detected form of α-tocopherol, α-tocopheryl phosphate (α-TP), has gained a lot of interest. α-TP is present only in small amounts of tissues, however, in doses as high as those commonly used for α-tocopherol and it was effective in a variety of test systems. Recent findings on α-TP are here discussed by Jean-Marc Zingg [22] with respect to uptake, intracellular transport and biological activities in comparison to α-tocopherol. Hypothetically, α-TP interacts with amphophilic compounds like phosphoinositides, which serve as intrinsic membrane signals that regulate intracellular membrane trafficking. Also hypothetically, α-TP is discussed to be the phosphorylated biologically active form, as it is common for other vitamins such as thiamine or pyridoxine. If this turns out to be true, the related kinase and phosphatase have to be identified as well as the site of phosphorylation. Thus, much work remains to be done before the question ''α-TP – an active lipid mediator?'' can be answered.

5 Vitamin E in neurological and neuromuscular functions

One of the major vitamin E-deficiency symptoms are neurological deficits. These affect neuronal and neuromuscular functions. Impairment of neuromuscular health by vitamin E-deficiency is here reviewed by Gohil *et al.* [26]. Very small amounts of α-tocopherol can prevent the onset of symptoms like ataxia and are sufficient to maintain normal muscle function. Also low levels of α-tocopherol are able to maintain retinal, motor and memory functions in α-TTP KO mice. Interestingly, TAPs and αTTP are expected to be saturated with minimal concentrations of α-tocopherol, taken as hint that vitamin E might have catalytic functions. In addition, it was concluded from exercise studies with normal and supplemented diets that supplementation with α-tocopherol does not improve gain of endurance. The authors provide a striking hypothesis that is supported by very old studies that oxidant products from vitamin deficient diets are responsible for neurological disorders in vitamin E deficiency. Effects of re-feeding vitamin E could have resulted from the absence of such toxic products. Therefore, instead of feeding an α-tocopherol-depleted diet, the use of animal-models with mutations in genes for absorption and distribution resulting in severe peripheral vitamin E-deficiency appears more promising. Such models are rodents harboring mutations in the microsomal triglyceride transfer protein and α-TTP genes. In these models, normal functions of the CNS and muscles are maintained for about 9 months before symptoms start to develop. Underlying mechanisms are to be characterized.

The second review written by David Muller [27] is a valuable synopsis of more than 30 years of investigations on the role of vitamin E in nerve functions. The central nervous system is more severely affected by vitamin E deficiency than the peripheral, and sensory axons are more involved than motor axons. Symptoms are caused by a damage of the neuron axons followed by demyelination. Morphological studies provided evidence for an abnormal axonal transport suggesting an abnormality of turnaround, which is the packaging of materials into lysosomes for their retrograde transport. A possible explanation for the long time needed before vitamin E-deficiency symptoms occur in neural tissues is provided. Two pools of vitamin E appear to exist, a rapidly mobilized pool of labile vitamin E and a second more

stable pool in which α-tocopherol is probably incorporated into membrane structures [28].

6 Miscellaneous aspects

Banks *et al.* [29] surprised with unexpected support for an anticancer effect of α-tocopherol which may be taken as a step from bench to bedside. Life-long supplementation with α-tocopherol indeed extended lifespan of mice maintained in the cold. From the affected genes investigated, p21 was upregulated by supplementation. The increase was independent of p53. P21 is mainly a tumor suppressor, but can also have pro-cancer functions. P21 stabilizes Nrf2, a transcription factor inducing protective, *i.e.* antioxidant enzymes. This may prevent carcinogenesis, and hence the interesting hypothesis was created that vitamin E extends lifespan due to inhibition of cancer development, the major cause of death in small rodents.

The outlook

Clearly, the known vitamin E deficiency syndromes, female infertility and neurological disorders, are not convincingly explained in the present special issue. Nevertheless, the numerous novel findings that are here compiled by experts of vitamin E research and related fields are expected to guide the way to further progress.

The frame to pinpoint the physiological action of vitamin E is set by its chemical nature: (i) It is a redox-active compound prone to undergo 1- and 2-electron transitions and (ii) it is highly lipophilic, although this property may be modulated by phosphorylation. Which of its chemical potentials are more relevant to its action(s) in biosystems has for too long been a matter of controversial debates. The antagonizing protagonists better try to synergize their energies by avidly absorbing novel insights from each other. In this context, only one restriction appears justified: tiny concentrations of a vitamin cannot likely fulfill a vital role as an unspecific free radical scavenger without being a catalyst.

The various novel aspects compiled in this issue point to specific roles of vitamin E, in particular of α-tocopherol, in regulating signaling cascades, gene activity and membrane processes. In none of the cases, however, a defined molecular partner of α-tocopherol or any other tocol has been elucidated. In search for a molecular mechanism of vitamin E action, we still are confronted with punctual insights, at best with the characterization of short reaction chains ending up in loose ends.

To solve this issue of vitamin E action is not just an academic challenge to explain why vitamin E-deficient rats fail to reproduce properly. The more recent studies on α-TTP knockout mice and even more convincingly the α-TTP-deficient AVED patients clearly teach how important vitamin E is for human health. Knowing this, it is indispensable to work out its precise role in order to define requirements, optimum form(s) and particular needs in clinical conditions. We accompany this issue with the hope that the mosaic stones collected therein will prove to be helpful tools in finally solving the problem.

References

[1] Evans, H. M., Bishop, K. S., On the existence of a hitherto unrecognized dietary factor essential for reproduction. *Science* 1922, *56*, 650–651.

[2] Olcott, H. S., Emerson, O. H., Antioxidants and the autoxidation of fats. IX. The antioxidant properties of the tocopherols. *J. Am. Chem. Soc.* 1937, *59*, 1008–1009.

[3] Brigelius-Flohé, R., Kelly, F. J., Salonen, J., Neuzil, J. *et al.*, The European perspective on vitamin E: current knowledge and future research. *Am. J. Clin. Nutr.* 2002, *76*, 703–716.

[4] Kelly, F., Meydani, M., Packer, L. (Eds.), *Vitamin E and Health*, The New York Academie of Sciences, New York 2004.

[5] Caretto, S., Nisi, R., Paradiso, A., De Gara, L., Tocopherol production in plant cell cultures. *Mol. Nutr. Food Res.* 2010, *54*, 726–730.

[6] Schultz, M., Leist, M., Petrzika, M., Gassmann, B. *et al.*, Novel urinary metabolite of α-tocopherol, 2,5,7,8-tetramethyl-2(2′-carboxyethyl)-6-hydroxychroman, as an indicator of an adequate vitamin E supply? *Am. J. Clin. Nutr.* 1995, *62*, 1527S–1534S.

[7] Brigelius-Flohé, R., Vitamin E and drug metabolism. *Biochem. Biophys. Res. Commun.* 2003, *305*, 737–740.

[8] Traber, M. G., Vitamin E, nuclear receptors and xenobiotic metabolism. *Arch. Biochem. Biophys.* 2004, *423*, 6–11.

[9] Sharma, G., Muller, D., O′Riordan, S., Bryan, S. *et al.*, A novel method for the direct measurement of urinary conjugated metabolites of α-tocopherol and its use in diabetes. *Mol. Nutr. Food Res.* 2010, *54*, 599–600.

[10] Müller, L., Theile, K., Böhm, V., *In vitro* antioxidant activity of tocopherols and tocotrienols and comparison of vitamin E concentration and lipophilic antioxidant capacity in human plasma. *Mol. Nutr. Food Res.* 2010, *54*, 731–742.

[11] Birringer, M., Analysis of vitamin E metabolites in biological specimen. *Mol. Nutr. Food Res.* 2010, *54*, 588–598.

[12] Gille, L., Staniek, K., Rosenau, T., Duvigneau, J. C. *et al.*, Tocopheryl quinones and mitochondria. *Mol. Nutr. Food Res.* 2010, *54*, 601–615.

[13] Lemaire-Ewing, S., Desrumeaux, C., Néel, D., Lagrost, D., Vitamin E transport, membrane incorporation and cell metabolism: is alpha-tocopherol in lipid rafts an oar in the lifeboat? *Mol. Nutr. Food Res.* 2010, *54*, 631–640.

[14] Takada, T., Suzuki, H., Molecular mechanisms of membrane transport of vitamin E. *Mol. Nutr. Food Res.* 2010, *54*, 616–622.

[15] Vatassery, G. T., Lam, C., Smith, W. E., Quach, H. T., Apolipoprotein E exerts selective and differential control over vitamin E concentrations in different areas of mammalian brain. *J. Neurosci. Res.* 2006, *84*, 1335–1342.

[16] Huebbe, P., Lodge, J. K., Rimbach, G., Implications of apo E genotype on inflammation and vitamin E status. *Mol. Nutr. Food Res.* 2010, *54*, 623–630.

[17] Traber, M. G., Regulation of xenobiotic metabolism, the only signaling function of α-tocopherol? *Mol. Nutr. Food Res.* 2010, *54*, 661–668.

[18] Quinn, P. J., Antioxidants and prophylactics. Is the distribution of α-tocopherol in membranes consistent with its putative functions? *Biochemistry (Moscow)* 2004, *69*, 58–66.

[19] Brigelius-Flohé, R., Vitamin E: the shrew waiting to be tamed. *Free Radic. Biol. Med.* 2009, *46*, 543–554.

[20] Atkinson, J., Harroun, T., Wassall, S. R., Stillwell, W., The location and behaviour of α-tocopherol in membranes. *Mol. Nutr. Food Res.* 2010, *54*, 641–651.

[21] Zingg, J.-M., Modulation of signal transduction by vitamin E. *Mol. Aspects Med.* 2007, *28*, 481–506.

[22] Zingg, J.-M., Meydani, M., Azzi, A., α-Tocopheryl phosphate – an active lipid mediator? *Mol. Nutr. Food Res.* 2010, *54*, 679–692.

[23] Hemmerling, J., Nell, S., Kipp, A., Schumann, S. *et al.*, Alpha-tocopherol enhances degranulation in RBL-2H3 mast cells. *Mol. Nutr. Food Res.* 2010, *54*, 652–660.

[24] Saito, K., Tautz, L., Mustelin, T., The lipid-binding SEC14 domain. *Biochim. Biophys. Acta* 2007, *1771*, 719–726.

[25] Comitato, R., Leoni, G., Canali, R., Ambra, R. *et al.*, Tocotrienols activity in MCF-7 breast cancer cells: involvement of ERβ signal transduction. *Mol. Nutr. Food Res.* 2010, *54*, 669–678.

[26] Gohil, K., Vasu, V. T., Cross, C. E., Dietary α tocopherol and neuromuscular health: search for optimal dose and molecular mechanisms continues! *Mol. Nutr. Food Res.* 2010, *54*, 693–709.

[27] Muller, D. R. P., Vitamin E and neurological function. *Mol. Nutr. Food Res.* 2010, *54*, 710–718.

[28] Bieri, J. G., Kinetics of tissue α-tocopherol depletion and repletion. *Ann. NY Acad. Sci.* 1972, *203*, 181–191.

[29] Banks, R., Speakman, J. R., Selman, C., Vitamin E supplementation and mammalian life span. *Mol. Nutr. Food Res.* 2010, *54*, 719–725.

