

# International Journal for Vitamin and Nutrition Research

# 2/13

[www.verlag-hanshuber.com/IJVNR](http://www.verlag-hanshuber.com/IJVNR)

Editor-in-Chief

R. F. Hurrell

Associate Editors

T. Bohn · M. Eggersdorfer · M. Reddy

HUBER



Contents

---

Original Communications	Noori N., Tabibi H., Hosseinpanah F., Hedayati M., Nafar M.: Effects of Combined Lipoic Acid and Pyridoxine on Albuminuria, Advanced Glycation End-Products, and Blood Pressure in Diabetic Nephropathy . . . . .	77
	Kajanachumpol S., Atamasirikul K., Tantibhedhyangkul P.: C677T Methylenetetrahydrofolate Reductase and Plasma Homocysteine Levels among Thai Vegans and Omnivores. . . . .	86
	Stöcklin E., Eggersdorfer M.: Vitamin D, an Essential Nutrient with Versatile Functions in Nearly all Organs . . . . .	92
	Eum S., Choi H.-D., Chang M.-J., Choi H.-C., Ko Y.-J., Ahn J.-S., Shin W.-G., Lee J.-Y.: Protective Effects of Vitamin E on Chemotherapy-Induced Peripheral Neuropathy: A Meta-Analysis of Randomized Controlled Trials . . . . .	101
	Büttner B. E., Öhrvik V. E., Köhler P., Witthöft C. M., Rychlik M.: Quantification of Isotope-Labeled and Unlabeled Folates and Folate Catabolites in Urine Samples by Stable Isotope Dilution Assay . . . . .	112
Renaud C., Berger J., Laillou A., Avallone S.: Quantification of Vitamin A in Fortified Rapeseed, Groundnut and Soya Oils Using a Simple Portable Device: Comparison to High Performance Liquid Chromatography . . . . .	122	
Workshop Conclusions	Péter S., Moser U., Pilz S., Eggersdorfer M., Weber P.: The Challenge of Setting Appropriate Intake Recommendations for Vitamin E: Considerations on Status and Functionality to Define Nutrient Requirements . . . . .	129
Research Note	Ebata Y., Takino J., Tsuchiya H., Sakabe T., Ikeda Y., Hama S., Kogure K., Takeuchi M., Shiota G.: Presence of Glyceraldehyde-Derived Advanced Glycation End-Products in the Liver of Insulin-Resistant Mice. . . . .	137

Workshop Conclusions

# The Challenge of Setting Appropriate Intake Recommendations for Vitamin E: Considerations on Status and Functionality to Define Nutrient Requirements

Szabolcs Péter<sup>1</sup>, Ulrich Moser<sup>2</sup>, Stefan Pilz<sup>3</sup>, Manfred Eggersdorfer<sup>1</sup>, and Peter Weber<sup>1</sup>

<sup>1</sup>DSM Nutritional Products Ltd., Kaiseraugst, Switzerland

<sup>2</sup>Basel, Switzerland

<sup>3</sup>Department of Internal Medicine, Division of Endocrinology and Metabolism, Medical University of Graz, Austria

Received: May 7, 2013; Accepted: August 14, 2013

**Abstract:** The main function of vitamin E is to protect against scavenging of reactive oxygen species; it is the primary protective agent against lipid peroxidation. Overt vitamin E deficiency is present only in patients with severe malnutrition and certain chronic diseases. The latest Recommended Dietary Allowance for vitamin E is based on the correlation between hydrogen peroxide-induced erythrocyte lysis and plasma  $\alpha$ -tocopherol concentrations (Institute of Medicine, United States), or the prevention of lipid peroxidation (National Nutrition Societies of Germany, Austria and Switzerland, D-A-CH). According to the current recommendations, the reference plasma concentration for vitamin E is 12–46  $\mu\text{mol/L}$  (daily intake of 15–30 mg  $\alpha$ -tocopherol equivalents). Epidemiological studies suggest a beneficial effect of vitamin E on cardiovascular health at a plasma concentration of 30  $\mu\text{mol/L}$  (a daily intake of ~50 IU). Vitamin E is also an important micronutrient for maintaining the immune system, especially in the elderly. A workshop was organized with the main objective to propose a concept for developing markers of status, functionality, and health in the field of nutritional research, in order to define desirable vitamin E requirements in healthy individuals.

**Key words:** vitamin E, markers, function, requirements, recommendations

## Introduction

The term vitamin E covers eight compounds found in nature ( $\alpha$ -,  $\beta$ -,  $\gamma$ -,  $\delta$ -tocopherol and  $\alpha$ -,  $\beta$ -,  $\gamma$ -,  $\delta$ -tocotrienol),  $\alpha$ -tocopherol being the most common and biologically most active form. Natural  $\alpha$ -tocopherol occurs in RRR-configuration only, whilst chemical synthesis results in a mixture of eight different stereoisomers, called all-rac- $\alpha$ -tocopherol. Vitamin E is a powerful antioxidant that can be regenerated by vitamin C after oxidation [1]. Due to its lipophilic nature, vitamin E localizes in lipid compartments, such as cell membranes, where it prevents both the peroxidation of lipids and oxidation of proteins. Incorporation of vitamin E into cell membranes may alter the activity of membrane-associated proteins. This in turn can change certain signal transduction pathways. The essential role of vitamin E as an antioxidant in the human body has been revisited by the recent approval of a 13.1 EFSA (European Food Safety Authority) health claim: "...contributes to the protection of cell constituents from oxidative damage" [2]. However, setting daily intake recommendations in humans based on the established antioxidative function of vitamin E remains challenging. A group of scientists addressed this issue in a workshop with the objective to propose a concept for using markers of status, functionality, and human health to define desirable vitamin E requirements. The main outcomes of this workshop are summarized below. The intention of the workshop was not only to take into account the findings from the "vitamin D case" but also to propose a more holistic approach in defining daily intake recommendations.

## What can we learn from vitamin D?

During the workshop it was discussed if the example of vitamin D could be a good benchmark for the definition of daily vitamin E requirements. In the case of vitamin D, established scientific evidence supports a key role of vitamin D in skeletal health. In providing a basis for determination of intake requirements it was important to relate vitamin D status, as measured by 25-hydroxyvitamin D (25[OH]D) in plasma, to markers of mineral metabolism (e. g., parathyroid hormone) and also to parameters of skeletal health [3]. Randomized controlled trials (RCTs) support the practice of vitamin D supplementation to benefit musculoskeletal health. A 25(OH)D plasma concentration of at least 20 ng/mL (50 nmol/L) is recommended for prevention of skeletal diseases, but there is an ongoing

debate as to whether concentrations of >30 ng/mL (75 nmol/L) are even more beneficial with regard to skeletal and probably some extraskelatal health outcomes, including mortality [4, 5]. A daily dietary intake of at least 700–800 IU vitamin D is required to exceed on average a serum concentration of 20 ng/mL (50 nmol/L). Based on these considerations, the vitamin D case has been targeted as a potential point of reference for the establishment of daily vitamin E requirements. However, more scientific data is required on clinical health benefits of vitamin E, possibly generated by RCTs. As with vitamin D, the plasma concentration of vitamin E has been suggested to form the basis of recommendations.

## Functionality as an indicator to define requirements of vitamin E in healthy people

### Integrity of cellular membranes

Redox signaling is an important cellular event that contributes to the regulation of cellular homeostasis. It is also known that excessive production of reactive oxygen species (ROS) may lead to oxidative stress, loss of cell function, and finally apoptosis or necrosis. According to Barry Halliwell, one of the researchers that originally promoted the concept of "oxidative stress," the key question remains: "does the oxidative stress that is likely to occur as a result of the tissue damage play any role at all in the disease pathology?" As discussed in a recent paper, his view is that the answer is still a firm "yes" for cancer and neurodegenerative diseases, a probable "yes" for inflammatory bowel disease, and a "maybe" for atherosclerosis [6]. A balance between oxidant and antioxidant intracellular systems is hence vital for cell function, regulation, and adaptation [7]. The ROS also mediate and enhance inflammation: Low plasma antioxidant concentrations have been reported in patients with rheumatoid arthritis and cystic fibrosis [8]. Being a potent antioxidant, the main function of vitamin E is protection against scavenging of ROS: It is the primary protective agent against lipid peroxidation. A number of biomarkers, including malondialdehyde, pentane, ethane, and the  $F_2$ -isoprostanes have been used to reflect the degree of lipid peroxidation *in vivo*. For example, a study by Roberts *et al.* investigated the dose-dependent effects of RRR- $\alpha$ -tocopherol in the suppression of plasma concentrations of  $F_2$ -isoprostanes in humans for 16

weeks. There was a linear trend between the dosage of vitamin E and percentage reduction in plasma F<sub>2</sub>-isoprostane concentrations which reached significance at doses of 1,600 IU [9]. However, since these markers are not certainly specific to vitamin E, estimates of lipid peroxidation products have not been used for establishing  $\alpha$ -tocopherol requirements [10]. Vitamin E minimizes free radical tissue damage by interrupting the chain reaction which produces fatty acid hydroperoxides. This is particularly relevant in the cell membrane, where vitamin E is vital to the prevention of polyunsaturated fatty acid (PUFA) oxidation. According to the German-Austrian-Swiss common recommendations (2008), the basic requirement to account for PUFA biosynthesis and enhancement of oxidation stability is 4 mg  $\alpha$ -tocopherol/day. Since the amount needed for protection increases with the number of double bonds in the fatty acid, additionally taking into consideration the approximate value of fat intake and proportional distribution of fatty acids in the diet, the daily intake requirement of  $\alpha$ -tocopherol increases to 12–14 mg [11]. By preserving intracellular and cellular membrane integrity and stability, vitamin E has an important role to play in the stability of erythrocytes and conductivity in central and peripheral nerves [12, 13]. Thus, vitamin E prevents hemolytic anaemia and neurological symptoms (ataxia, peripheral neuropathy, myopathy, pigmented retinopathy) occurring in vitamin E-deficient individuals. Vitamin E deficiency has been primarily reported in preterm infants, in patients with severe malnutrition, chronic gastrointestinal abnormalities, and genetic disturbances of lipid metabolism. Low vitamin E concentrations are also observed in certain hepatic and pancreatic diseases such as parenchymal liver cirrhosis, cholestasis, chronic pancreatitis, cystic fibrosis, and also in alcoholism. In addition to its role in the prevention of lipid peroxidation, vitamin E inhibits the oxidative low-density lipoprotein (LDL) formation and has a synergism with  $\beta$ -carotene, vitamin C, and selenium [14]. Suboptimal plasma tocopherol concentrations of 12–16  $\mu\text{mol/L}$  already cause hemolysis in >10 % of erythrocytes, along with creatinuria. Clinical symptoms such as impaired skeletal muscle function and accumulation of ceroid pigments in smooth muscle tissue have been reported at plasma tocopherol concentrations <12  $\mu\text{mol/L}$  and ataxia below 8  $\mu\text{mol/L}$  [15, 16]. In Austria, Germany and Switzerland, reported plasma concentrations for vitamin E range from 12–46  $\mu\text{mol/L}$ . In this context, it should be underlined that erythrocyte hemolysis occurs at tocopherol concentrations of 12–16  $\mu\text{mol/L}$ , which is well within this reference range [17]. On the other hand a minimum of 30  $\mu\text{mol/L}$  is suggested for

prevention of cardiovascular diseases (CVD) and cancer in individuals with normal plasma lipid concentrations; i. e. 5.7 mmol cholesterol/L and 1.3 mmol triacylglycerol/L [18]. The required dietary intake to reach the reference plasma concentration is 15–30 mg  $\alpha$ -tocopherol equivalents/day for persons without particular oxidative stress [11]. Current recommendations on vitamin E requirements vary considerably amongst countries, genders, and age groups. Generally, women, infants, children, and the elderly require a lower daily intake, while requirements for men, and for pregnant and lactating women, are higher. Of the eight naturally occurring forms of vitamin E only  $\alpha$ -tocopherol is maintained in human plasma, therefore the Recommended Dietary Allowance (RDA) is based solely on this form. Other naturally occurring forms ( $\beta$ -,  $\gamma$ -, and  $\delta$ -tocopherols and tocotrienols) do not contribute to vitamin E requirements because they are not converted to  $\alpha$ -tocopherol in humans. Based on the adverse effect of increased tendency to hemorrhage, the Tolerable Upper Intake Level (UL) for adults is set at 1,000 mg/day  $\alpha$ -tocopherol [10].

## Immune function

Vitamin E modulates immune functions, platelet aggregation and in addition, has an influence on eicosanoid synthesis: In healthy elderly people, vitamin E enhances certain clinically relevant *in vivo* indexes of T-cell-mediated function [19]. Furthermore, vitamin E plays an important role in the differentiation of immature T-cells in the thymus and restores age-associated decline in T-cell function. It is also known that vitamin E deficiency weakens the immune response in many model systems and in human studies is associated with increased risk of infectious diseases [20, 21]. A daily dose of 200 IU vitamin E was found to enhance immune function in the elderly, associated with a reduced risk of acquiring upper respiratory infections in nursing home residents [22]. Vitamin E also reverses the increase in prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) production associated with aging, thereby reducing its proinflammatory effects [23]. Therefore vitamin E may be an important micronutrient for maintaining the immune system, especially in the elderly. However, further studies are needed to investigate the effect of lower doses of vitamin E on immune functions and possible associations between vitamin E plasma concentrations and different immune parameters.

## Vitamin E status

The dietary requirement for vitamin E has been determined from intakes providing plasma  $\alpha$ -tocopherol concentrations that inhibit hydrogen peroxide-induced hemolysis. A number of studies have reported the determinants of plasma  $\alpha$ -tocopherol, measured by high-performance liquid chromatography methods, and provided kinetic models that aimed to correlate vitamin E intakes with plasma concentrations [24–26]. Such compartmental models might be useful for the development of improved RDA values for vitamin E. As defined by the Food and Nutrition Board, the lower limit of plasma  $\alpha$ -tocopherol is 12  $\mu\text{mol/L}$  for healthy, adult humans [10]. Plasma  $\alpha$ -tocopherol concentrations less than 8  $\mu\text{mol/L}$  are associated with neurologic disease, less than 12  $\mu\text{mol/L}$  with increased erythrocyte fragility [10], and over 32  $\mu\text{mol/L}$  with decreased risk of chronic diseases [16, 27]. Furthermore, calculation of effective plasma vitamin E concentrations needs to take into account possible elevated lipid concentrations, since in these patients “normal” vitamin E concentrations may not be sufficient to protect the tissues [28]. Low plasma vitamin E concentrations are indicative of vitamin E deficiency. According to National Health and Nutrition Examination Survey (NHANES) 2005–2006 data, the estimated total number of persons with low serum vitamin E concentration ( $<11.61 \mu\text{mol/L}$ ) in the US population aged 6 years and older is 1,835,000 [29]. Between 1988 and 1994, the dietary intake and the plasma concentrations of  $\alpha$ -tocopherol were examined in more than 16,000 adults in the NHANES III survey in the US: Thirty-two percent of participants had  $\alpha$ -tocopherol plasma concentrations  $<20 \mu\text{mol/L}$ . Human vitamin E requirements were assessed by Bruno *et al.* with the help of deuterium-labeled  $\alpha$ -tocopheryl acetate. Given an estimated 33 % absorption, the amount of dietary vitamin E needed daily to replace irreversible losses was  $\leq 15 \text{ mg}$  [30]. Data from NHANES 2003–2006 indicated that the average dietary intake of  $\alpha$ -tocopherol from food was 6.9 mg/day. This is well below the currently recommended intake of 15 mg/day. At this level of dietary intake, more than 90 % of Americans did not meet daily dietary recommendations for vitamin E [31]. In Germany, according to the report of the Nationale Verzehrsstudie (NVS) II study (2008), irrespective of the age group, 48 % of the men and 49 % of the women did not reach the recommended daily intake level of vitamin E [32]. Further analyses are proposed in order to globally characterize vitamin E status on the basis of intake surveys and plasma concentrations. These findings suggest that low intake of vitamin E

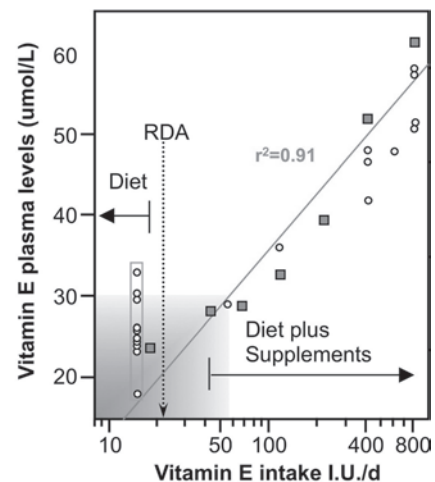


Figure 1: The relationship between vitamin E intake and serum concentrations (according to Weber *et al.* [33]). The filled squares represent data from Princen *et al.* [14]. The open circles stand for data from a number of former studies which employed different vitamin E concentrations in different populations [34–42].

is rather common in the US, which is also supported by the reported low vitamin E status and is actually consistent with the reported low intakes of vitamin E in the US population. Vitamin E intake correlates very well with plasma concentrations (Figure 1), therefore this could be used as a stable indicator for the basis for further recommendations, provided markers of functionality or health benefits can be related to it [33].

## Health benefits of vitamin E in human studies: Epidemiology versus randomized clinical trials

Interest in vitamin E as a possible protective micronutrient against atherosclerotic coronary artery disease has intensified with the recognition that oxidized LDL (oxLDL) may be involved in atherogenesis. Increased circulating oxLDL concentrations have been related to cardiovascular disease in some studies, although not always independently after adjustment of classical lipid markers [43, 44].

### Epidemiology

Evidence from various observational human studies consistently indicates that vitamin E has beneficial effects on the cardiovascular system. Outcomes from the



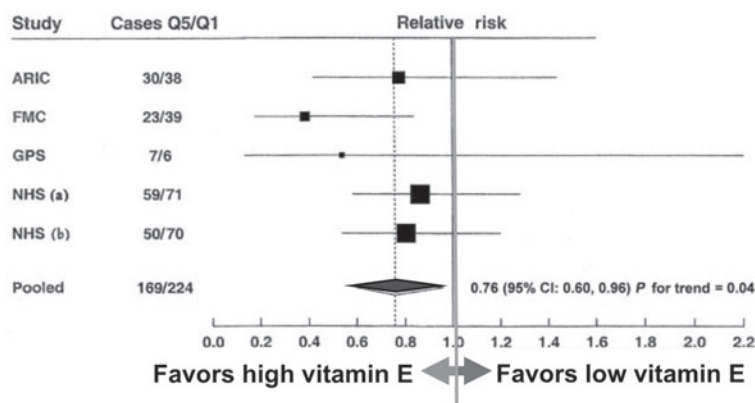


Figure 2: Major coronary heart disease risk in women (according to Knekt *et al.* [47]). Median dietary vitamin E intake (female/male, mg): ARIC, Atherosclerosis Risk in Communities Study (1987–1998): 4.69/5.61; FMC, Finnish Mobile Clinic Health Examination Survey (1966–1976): 4.47/5.46; GPS, Glostrup Population Study (1974–1985): 5.57/6.23; NHS (a), Nurses' Health Study (1980–1986): 3.82; NHS (b), Nurses' Health Study, (1986–1996): 5.78

main epidemiological studies show a risk reduction of 24 % for cardiovascular events, when comparing high versus low vitamin E concentrations. These results suggest a beneficial effect of vitamin E at a plasma concentrations of 30  $\mu\text{mol/L}$  [45–48]. Some of the key studies are listed in Figure 2. In contrast with the studies cited above, other epidemiological studies have not shown an association between vitamin E serum levels and cardiovascular mortality risk [49, 50]. Results from RCTs also did not detect a consistent benefit of vitamin E supplementation on cardiovascular health.

## Randomized clinical trials

Data from the Women's Health Study (WHS) and the Alpha-Tocopherol Beta Carotene Cancer Prevention Study (ATBC) suggest that protection against oxidative stress by vitamin E supplementation may reduce the risk of venous thromboembolism, and can increase the life expectancy of some population groups [51, 52]. However, most of the large-scale human intervention studies could not find a risk reduction in cardiovascular endpoints. For instance, discrepant results have been found by the Cambridge Heart Antioxidant Study (CHAOS), with a vitamin E supplementation of 400–800 IU/day: Alpha-tocopherol treatment significantly reduced the rate of non-fatal myocardial infarction (MI). However, there was also a non-significant excess of cardiovascular deaths in the alpha-tocopherol group [53]. The results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI) Prevenzione (300 mg/day) and Heart Outcomes Prevention Evaluation (HOPE) (400 IU/day) trials suggest the absence of relevant clinical effects of vitamin E in the risk of cardiovascular events [54]. Many reasons have been suggested to explain the neutral outcome of these RCTs, such as inadequate study design and methods of analysis, etc., as discussed elsewhere [55].

In addition, recent data show that the genotype of an individual appears to be an important factor in the effect of vitamin E supplements on cardiovascular endpoints – a fact which has not been considered in the studies reporting null results. In the Israel Cardiovascular Vitamin E (ICARE) study, comprising 1,434 diabetic individuals  $\geq 55$  years of age with the haptoglobin (Hp) 2–2 genotype, participants were randomized to vitamin E (400 IU/day) or placebo. The primary composite outcome was myocardial infarction, stroke, and cardiovascular death. At the first evaluation of events, 18 months after initiating the study, the primary outcome was significantly reduced in individuals receiving vitamin E (2.2 %) compared with placebo (4.7 %;  $p=0.01$ ) and led to early termination of the study [56]. These findings were confirmed in a *post-hoc* analysis of the HOPE study: Risk for cardiovascular events was significantly reduced only in the diabetics carrying the Hp 2–2 gene [57].

In conclusion, previously published RCTs on vitamin E might not be particularly useful in defining RDA, as not only do they have many limitations whilst looking at very narrowly defined cohorts, but also because they suffer from using intakes that far exceed the nutritional-physiological range. Based on epidemiological evidence, RCTs should have been conducted involving subjects with plasma concentrations in the lower 20  $\mu\text{mol/L}$  range or even lower, but there is no study with such a design. Moreover, the risk of bias by meta-analysis of RCTs is significant (e. g., publication bias, search bias, selection bias, etc.), therefore two different models of meta-analyses should show similar results in order to prove efficacy [58]. In that respect, the value of the epidemiological studies appears to be currently underestimated in setting RDAs. In particular, observational studies might be useful for identifying target plasma concentrations associated with health benefit(s), which then can be translated into respective intakes to achieve these plasma

concentrations. It should however be acknowledged that a great need still exists for well-designed RCTs to address potential health benefits of vitamin E on relevant clinical health outcomes.

## The way forward

Vitamin E is an essential micronutrient for humans; its main function is its powerful antioxidant activity, exerting its crucial effects particularly in the bilayer of the cell membrane. *Ex vivo* biomarkers of cellular and intracellular membrane damage are creatine-kinase in plasma and the *in vitro* hemolysis test. In order to preserve membrane integrity of erythrocytes and neurons and avoid hemolysis and deficiency symptoms occurring at <12  $\mu\text{mol/L}$  vitamin E plasma concentration, the recommended reference range is 12–46  $\mu\text{mol/L}$ . The lower part of this range can be questioned; the required dietary intake to reach it is 15–30 mg  $\alpha$ -tocopherol equivalents/day. Furthermore, 17 mg vitamin E supplementation per day decreases the susceptibility of LDL to oxidation. The essential daily intake requirement of  $\alpha$ -tocopherol to account for PUFA protection is 12–14 mg. Based on human studies, which measured vitamin E serum response, a daily intake of ~50 IU vitamin E would be required to achieve the desirable protective plasma concentration of 30  $\mu\text{mol/L}$ . Since vitamin E intake correlates very well with plasma concentration, the latter could be used as a stable indicator, and therefore form the basis of further recommendations. More studies are required on the vitamin E intake needed to exert an optimal protection of cell membrane constituents that are susceptible to oxidation, as this is a fundamental role of vitamin E which should be considered in defining daily vitamin E requirements, in addition to the desired plasma level of vitamin E, which will provide another important element to establish such recommendations.

## Conflicts of Interest

Szabolcs Péter, Manfred Eggersdorfer and Peter Weber are employees of DSM Nutritional Products Ltd.

## References

- Combs, G.F. (2012) *The Vitamins*, pp. 193–196, Academic Press
- <http://www.efsa.europa.eu/en/efsajournal/pub/1816.htm>
- Ross, A.C. et al. (2011) The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. *J. Clin. Endocrinol. Metab.* 96, 53–58.
- Pilz, S. et al. (2011) Vitamin D, cardiovascular disease and mortality. *Clin. Endocrinol. (Oxf)*. 75, 575–584.
- Hintzpeter, B. et al. (2008) Vitamin D status and health correlates among German adults. *Eur. J. Clin. Nutr.* 62, 1079–1089.
- Halliwell, B. (2012) Free radicals and antioxidants: updating a personal view. *Nutr. Rev.* 70, 257–265.
- Nordberg, J. and Arner, E.S. (2001) Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. *Free Radic. Biol. Med.* 31, 1287–1312.
- Heliövaara, M. et al. (1994) Serum antioxidants and risk of rheumatoid arthritis. *Ann. Rheum. Dis.* 53, 51–53.
- Roberts, L.J. et al. (2007) The relationship between dose of vitamin E and suppression of oxidative stress in humans. *Free Radic. Biol. Med.* 43, 1388–1393.
- Food and Nutrition Board, Institute of Medicine (2000) *Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids*, pp. 186–283, National Academy Press, Washington.
- Deutsche Gesellschaft für Ernährung, Ö.G.f.E., Schweizerische Gesellschaft für Ernährungsforschung, Schweizerische Vereinigung für Ernährung (2008) *Referenzwerte für die Nährstoffzufuhr*, pp 87–93, Neuer Umschau Buchverlag, Neustadt an der Weinstraße.
- Boda, V. et al. (1998) Monitoring erythrocyte free radical resistance in neonatal blood microsamples using a peroxyl radical-mediated haemolysis test. *Scand. J. Clin. Lab. Invest.* 58, 317–322.
- Sokol, R.J. et al. (1988) Isolated vitamin E deficiency in the absence of fat malabsorption – familial and sporadic cases: characterization and investigation of causes. *J. Lab. Clin. Med.* 111, 548–559.
- Princen, H.M. et al. (1995) Supplementation with low doses of vitamin E protects LDL from lipid peroxidation in men and women. *Arterioscler. Thromb. Vasc. Biol.* 15, 325–333.
- Stamp, G.W. et al. (1987) Accumulation of ceroid in smooth muscle indicates severe malabsorption and vitamin E deficiency. *J. Clin. Pathol.* 40, 798–802.
- Traber, M.G. and Kamal-Eldin, A. (2007) Oxidative stress and vitamin E in anemia. In: *Nutritional*



- anemia. (Kraemer, K. and Zimmermann, M.B., eds.) pp. 161–163, Slight and Life Press, Basel.
17. Farrell, P.M. et al. (1977) The occurrence and effects of human vitamin E deficiency. *J. Clin. Invest.* 60, 233–241.
  18. Gey, K.F. (1995) Cardiovascular disease and vitamins. Concurrent correction of 'suboptimal' plasma antioxidant levels may, as important part of 'optimal' nutrition, help to prevent early stages of cardiovascular disease and cancer, respectively. *Bibl. Nutr. Dieta.* 52, 75–91.
  19. Meydani, S.N. et al. (1997) Vitamin E supplementation and in vivo immune response in healthy elderly subjects. A randomized controlled trial. *JAMA* 277, 1380–1386.
  20. Beck, M.A. (1997) Increased virulence of coxsackievirus B3 in mice due to vitamin E or selenium deficiency. *J. Nutr.* 127, 966S–970S.
  21. Meydani, S.N. et al. (2004) Vitamin E and respiratory tract infections in elderly nursing home residents: a randomized controlled trial. *JAMA* 292, 828–836.
  22. Meydani, S.N., Han, S.N. and Wu, D. (2005) Vitamin E and immune response in the aged: molecular mechanisms and clinical implications. *Immunol. Rev.* 205, 269–284.
  23. Wu, D. and Meydani, S.N. (2008) Age-associated changes in immune and inflammatory responses: impact of vitamin E intervention. *J. Leukoc. Biol.* 84, 900–914.
  24. Kardinaal, A.F. et al. (1995) Relations between antioxidant vitamins in adipose tissue, plasma, and diet. *Am. J. Epidemiol.* 141, 440–450.
  25. Gascón-Vila, P. et al. (1997) Determinants of the nutritional status of vitamin E in a non-smoking Mediterranean population. Analysis of the effect of vitamin E intake, alcohol consumption and body mass index on the serum alpha-tocopherol concentration. *Eur. J. Clin. Nutr.* 51, 723–728.
  26. Novotny, J.A. et al. (2012) This kinetic, bioavailability, and metabolism study of RRR- $\alpha$ -tocopherol in healthy adults suggests lower intake requirements than previous estimates. *J. Nutr.* 142, 2105–2111.
  27. Wright, M.E. et al. (2006) Higher baseline serum vitamin E concentrations are associated with lower total and cause-specific mortality in the alpha-tocopherol, beta-carotene cancer prevention study. *Am. J. Clin. Nutr.* 84, 1200–1207.
  28. Traber, M.G. and Jialal, I. (2000) Measurement of lipid-soluble vitamins—further adjustment needed? *Lancet* 355, 2013–2014.
  29. National Center for Environmental Health (2012) Second National Report on Biochemical Indicators of Diet and Nutrition in the U.S. Population, p. 122, <http://www.cdc.gov/nutritionreport/>
  30. Bruno, R.S. et al. (2006) Human vitamin E requirements assessed with the use of apples fortified with deuterium-labeled alpha-tocopheryl acetate. *Am. J. Clin. Nutr.* 83, 299–304.
  31. Fulgoni, V.L. 3<sup>rd</sup>. et al. (2011) Foods, fortificants, and supplements: where do Americans get their nutrients? *J. Nutr.* 141, 1847–1854.
  32. Troesch, B. et al. (2012) Dietary surveys indicate vitamin intakes below recommendations are common in representative Western countries. *Br. J. Nutr.* 13, 1–7.
  33. Weber, P., Bendich, A. and Machlin, L.J. (1997) Vitamin E and human health: rationale for determining recommended intake levels. *Nutrition* 13, 450–460.
  34. Lehmann, J. et al. (1988) Vitamin E and relationships among tocopherols in human plasma, platelets, lymphocytes, and red blood cells. *Am. J. Clin. Nutr.* 47, 470–474.
  35. Greenberg, E.R. et al. (1994) A clinical trial of antioxidant vitamins to prevent colorectal adenoma. Polyp Prevention Study Group. *N. Engl. J. Med.* 331, 141–147.
  36. (1994) The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group. *N. Engl. J. Med.* 330, 1029–1035.
  37. Meydani, M. et al. (1993) Effect of long-term vitamin E supplementation on lipid peroxidation and immune response of young and old subjects. *FASEB J.* 7: A415.
  38. Meydani, S.N. et al. (1990) Vitamin E supplementation enhances cell-mediated immunity in healthy elderly subjects. *Am. J. Clin. Nutr.* 52, 557–563.
  39. Baker, H. et al. (1980) Plasma tocopherol in man at various times after ingesting free or acetylated tocopherol. *Nutr. Report. Int.* 21, 531.
  40. Willett, W.C. et al. (1983) Vitamins A, E, and carotene: effects of supplementation on their plasma levels. *Am. J. Clin. Nutr.* 38, 559–566.
  41. Yokota, K., Tamai, H. and Mino, M. (1990) Clinical evaluation of alpha-tocopherol in buccal mucosal cells of children. *J. Nutr. Sci. Vitaminol. (Tokyo)* 36, 365–375.

42. Brin, M.F. et al. (1989) Relationship between dose of vitamin E administered and blood levels. *Ann. N.Y. Acad. Sci.* 570, 421.
43. Wilson, R. et al. (2003) Do oxidized fatty acids activate coagulation factor VII during post-prandial lipemia in women? *Thromb. Haemost.* 89, 654–659.
44. Verhoye, E., Langlois, M.R. and Asklepios Investigators (2009) Circulating oxidized low-density lipoprotein: a biomarker of atherosclerosis and cardiovascular risk? *Clin. Chem. Lab. Med.* 47, 128–137.
45. Gey, K.F. et al. (1991) Inverse correlation between plasma vitamin E and mortality from ischemic heart disease in cross-cultural epidemiology. *Am. J. Clin. Nutr.* 53, 326S–334S.
46. Gey, K.F. (1993) Prospects for the prevention of free radical disease, regarding cancer and cardiovascular disease. *Brit. Med. Bull.* 49, 679–699.
47. Knekt, P. et al. (2004) Antioxidant vitamins and coronary heart disease risk: a pooled analysis of 9 cohorts. *Am. J. Clin. Nutr.* 80, 1508–1520.
48. Biesalski, H.K. et al. (1997) Antioxidant vitamins in prevention. *Clin. Nutr.* 16, 151–155.
49. Hense, H.W. et al. (1993) Lack of an association between serum vitamin E and myocardial infarction in a population with high vitamin E levels. *Atherosclerosis* 103, 21–28.
50. Eichholzer, M., Stähelin, H.B. and Gey, K.F. (1992) Inverse correlation between essential antioxidants in plasma and subsequent risk to develop cancer, ischemic heart disease and stroke respectively: 12-year follow-up of the Prospective Basel Study. *EXS* 62, 398–410.
51. Glynn, R.J. et al. (2007) Effects of random allocation to vitamin E supplementation on the occurrence of venous thromboembolism: report from the Women's Health Study. *Circulation* 116, 1497–1503.
52. Hemilä, H. and Kaprio, J. (2011) Vitamin E may affect the life expectancy of men, depending on dietary vitamin C intake and smoking. *Age & Ageing* 40, 215–220.
53. Stephens, N.G. et al. (1996) Randomised controlled trial of vitamin E in patients with coronary disease: Cambridge Heart Antioxidant Study (CHAOS) *Lancet* 347, 781–786
54. Marchioli, R. et al. (2001) Antioxidant vitamins and prevention of cardiovascular disease: epidemiological and clinical trial data. *Lipids* 36, S53–63.
55. Traber, M.G. et al. (2008) Vitamin E revisited: do new data validate benefits for chronic disease prevention? *Curr. Opin. Lipidol.* 19, 30–38.
56. Milman, U. et al. (2008) Vitamin E supplementation reduces cardiovascular events in a subgroup of middle-aged individuals with both type 2 diabetes mellitus and the haptoglobin 2–2 genotype: a prospective double-blinded clinical trial. *Arterioscler. Thromb. Vasc. Biol.* 28, 341–347.
57. Levy, A.P. et al. (2004) The effect of vitamin E supplementation on cardiovascular risk in diabetic individuals with different haptoglobin phenotypes. *Diabetes Care* 27, 2767.
58. Walker, E., Hernandez, A.V. and Kattan, M.W. (2008) Meta-analysis: Its strengths and limitations. *Cleve. Clin. J. Med.* 75, 431–439.

Szabolcs Péter MD, PhD

---

DSM Nutritional Products Ltd.  
P.O. Box 2676  
4002 Basel  
Switzerland  
Fax: +41 61 815 80 50  
szabolcs.peter@dsm.com