

Review

Relationship Between Vitamin E Requirement and Polyunsaturated Fatty Acid Intake in Man: a Review

Esther E.J. Valk and Gerard Hornstra*

Department of Human Biology, Maastricht University, Maastricht, the Netherlands

Received for publication: March 11, 1999

Abstract: Vitamin E is the general term for all tocopherols and tocotrienols, of which α -tocopherol is the natural and biologically most active form. Although γ -tocopherol makes a significant contribution to the vitamin E CONTENT in foods, it is less effective in animal and human tissues, where α -tocopherol is the most effective chain-breaking lipid-soluble antioxidant. The antioxidant function of vitamin E is critical for the prevention of oxidation of tissue PUFA.

Animal experiments have shown that increasing the degree of dietary fatty acid unsaturation increases the peroxidizability of the lipids and reduces the time required to develop symptoms of vitamin E deficiency. From these experiments, relative amounts of vitamin E required to protect the various fatty acids from being peroxidized, could be estimated.

Since systematic studies on the vitamin E requirement in relation to PUFA consumption have not been performed in man, recommendations for vitamin E intake are based on animal experiments and human food intake data. An intake of 0.6 mg α -tocopherol equivalents per gram linoleic acid is generally seen as adequate for human adults. The minimum vitamin E requirement at consumption of fatty acids with a higher degree of unsaturation can be calculated by a formula, which takes into account the peroxidizability of unsaturated fatty acids and is based on the results of animal experiments.

There are, however, no clear data on the vitamin E requirement of humans consuming the more unsaturated fatty acids as for instance EPA (20:5, n-3) and DHA (22:6, n-3). Studies investigating the effects of EPA and DHA supplementation have shown an increase in lipid peroxidation, although amounts of vitamin E were present that are considered adequate in relation to the calculated oxidative potential of these fatty acids. Furthermore, a calculation of the vitamin E requirement, using recent nutritional intake data, shows that a reduction in total fat intake with a concomitant increase in PUFA consumption, including EPA and DHA, will result in an increased amount of vitamin E required.

* Present affiliation: Novartis Nutrition Research Unit Maastricht, the Netherlands

In addition, the methods used in previous studies investigating vitamin E requirement and PUFA consumption (for instance erythrocyte hemolysis), and the techniques used to assess lipid peroxidation (e.g. MDA analysis), may be unsuitable to establish a quantitative relation between vitamin E intake and consumption of highly unsaturated fatty acids. Therefore, further studies are required to establish the vitamin E requirement when the intake of longer-chain, more-unsaturated fatty acids is increased. For this purpose it is necessary to use functional techniques based on the measurement of lipid peroxidation *in vivo*. Until these data are available, the widely used ratio of at least 0.6 mg α -TE/g PUFA is suggested. Higher levels may be necessary, however, for fats that are rich in fatty acids containing more than two double bonds.

Key words: Vitamin E, tocopherol, diet, polyunsaturated fatty acids, review

Introduction

In recent years increasing evidence is emerging that a decrease in total fat consumption, with an increase in the intake of mono- and polyunsaturated fatty acids at the expense of saturated fatty acids, has several health benefits, including a decrease in the incidence of cardiovascular disease.

Increasing the consumption of polyunsaturated fatty acids (PUFA), i.e. the essential fatty acids (EFA) linoleic and α -linolenic acid and their longer-chain, more unsaturated derivatives, the long-chain polyenes (LCP), may have consequences for the vitamin E requirement. Since more than 30 years it is known that, in animals as well as in man, vitamin E is needed to protect PUFA in membrane lipids from being peroxidized, and that the requirement of vitamin E is closely related to the dietary intake of PUFA. The dependency of the vitamin E status on the dietary consumption of PUFA has frequently been described in a qualitative way, and there is clear evidence from animal experiments as well as human studies that the vitamin E requirement increases with the amount and degree of unsaturation of the PUFA consumed [1–3]. However, the precise amount of vitamin E required to compensate for the increased demand caused by dietary PUFA has not been systematically investigated in man.

Currently, requirements of vitamin E depending upon the dietary intake of PUFA must be extrapolated from animal data. It is, however, not clear whether the present recommendations for the vitamin E intake at a higher consumption of PUFA with different degrees of unsaturation are sufficiently effective to prevent peroxidation of membrane lipids in humans.

Therefore, the aim of this review is to summarize the science base of these recommendations and to critically assess whether an increased PUFA consumption would require a higher vitamin E intake.

Vitamin E

Vitamin E is a fat-soluble vitamin with antioxidant activity and is the general term for all tocopherols and tocotrienols [4–7]. Both the tocopherols and tocotrienols consist of four homologues: the α -, β -, γ - and δ -tocopherols and the α -, β -, γ - and δ -tocotrienols. All tocopherols have 3 asymmetric C-atoms in their side chains; however, in nature only the RRR forms are occurring. The biologically most active form is α -tocopherol (formerly called d- α -tocopherol), also the most widely available form of vitamin E in food and the primary form found in animal tissues [4, 6]. α -Tocopherol is also synthesized commercially and this form should be designated as all-rac - α -tocopherol (formerly called dl- α -tocopherol) [8]. The acetate esters of α -tocopherol and all-rac- α -tocopherol are the two main sources of vitamin E available for commercial use [4]. For dietary purposes, the term α -tocopherol equivalent (α -TE) was introduced to express vitamin E activity. One α -TE is the activity of 1 mg of α -tocopherol or 1.36 mg of all-rac- α -tocopherol. The activity of 1 mg of the acetate form of all-rac- α -tocopherol has been defined as equivalent to 1 International Unit (IU) of vitamin E (or 0.67 α -TE), and 1 mg of α -tocopherol is equal to 1.49 IU [8].

The main biological functions of vitamin E are based on its antioxidant property. Vitamin E is the most effective chain-breaking lipid-soluble antioxidant [6]. This antioxidant property of vitamin E is believed to be based on its capability of scavenging lipid radicals in the course of initiation and propagation of lipid peroxidation [9]. Vitamin E especially protects PUFA in phospholipids of biological membranes and in plasma lipoproteins against peroxidation [5]. Steady-state concentrations of vitamin E in membranes are determined by the efficiency of its incorporation into membranes by transfer from blood lipoproteins, and its metabolism in these membranes [9].

In healthy humans vitamin E deficiency is rare, but it can occur in patients with lipid malabsorption syndromes

such as cholestatic liver disease or abetalipoproteinemia. Vitamin E deficiency is characterized by peripheral neuropathy and spinocerebellar ataxia. Also in children, lipid malabsorption (as in cystic fibrosis or biliary atresia) can produce severe neurological symptoms [6]. Although a normal, balanced diet contains sufficient vitamin E to prevent deficiency symptoms in humans, there are several epidemiological studies suggesting beneficial associations between dietary intake of vitamin E and coronary heart disease, cancer and the immune response [4, 6, 10], which are thought to result from its antioxidant effect.

Vitamin E in foods

When evaluating food products as adequate sources of vitamin E, a few points have to be considered. Firstly, there is large variation in the vitamin E content of foods, and in particular in the proportion of the different tocopherols and tocotrienols, depending on processing, storage and preparation procedures during which large losses can occur [8]. Secondly, the eight known homologues of vitamin E have different biological activities. Therefore, the conversion factors for the various active compounds of vitamin E, used to calculate total vitamin E activity, are different. Moreover, they are not always used in a consistent way [11]. Based on animal experiments it is generally assumed that the relative activities of γ -tocopherol, β -tocopherol and α -tocotrienol are 0.1, 0.5 and 0.3 respectively, compared to α -tocopherol [8]. Both α -tocopherol and γ -tocopherol make a significant contribution to the vitamin E activity in foods. However, as mentioned before, the antioxidant activity of vitamin E in human and animal tissues is most pronounced in the form of α -tocopherol [4, 6].

In this article, the vitamin E content of food products will be expressed as mg α -TE per 100 g food.

Vitamin E in vegetable products

Most fruits and vegetables contain rather low amounts of vitamin E (< 1 mg/100 g). Only green leafy vegetables supply considerable amounts of vitamin E.

In contrast, vegetable oils are among the richest sources of tocopherols, mainly α -tocopherol. Soybean oil and corn oil have relatively high concentrations of γ -tocopherol. Coconut oil is reported to have the lowest vitamin E activity, approximately 0.7 mg α -TE, while wheat germ oil has the highest at approximately 173 mg α -TE per 100 g of oil. Palm oil and rice bran oil are particularly rich in α - and γ -tocotrienol [12].

Food products containing large amounts of plant oils are margarines and salad dressings. The level of total vitamin E activity in these products varies with the kind of oil used, the extent of processing, and the storage time [13].

Vitamin E in animal products

In general, meat products do not contain large amounts of vitamin E (approximately 0.5 mg/100 g). This is to be expected, since animals derive vitamin E entirely from their diet. α -Tocopherol is the major form present, but animal tissues also contain small amounts of γ -tocopherol, approximately 0.03 mg/100 g [13, 14]. The lower γ -tocopherol content of animal tissues can be attributed to the specificity of a hepatic α -tocopherol binding protein that is less effective in binding γ -tocopherol. Although the intestinal absorption of α - and γ -tocopherol is similar, the liver, responsible for maintaining plasma vitamin E concentrations and for delivery of vitamin E to peripheral tissues, is able to discriminate between these two tocopherols. Consequently, the blood lipoproteins released by the liver will be particularly rich in α -tocopherol resulting in a higher delivery of α -tocopherol, compared to γ -tocopherol, to the membranes of peripheral tissues [5, 7].

The vitamin E content of fish varies with the species and apparently is related to the diet of the fish. As in meats, α -tocopherol is the principal form present. The α -tocopherol content in fish muscle is relatively low (< 2 mg/100 g) in comparison to fish liver in which the α -tocopherol content ranges from less than 1 mg/100 g to almost 30 mg/100 g [13, 14]. Furthermore, it has been observed that low-fat fish (e.g. cod) had lower levels of α -tocopherol than high-fat fish (e.g. salmon) [15].

Most common animal fats and oils contain less than 4 mg vitamin E per 100 g. There is large variation in the vitamin E content of fish oils among species. Moreover, body oil generally has a lower vitamin E content (1–75 mg/100 g) than liver oil, which can attain values as high as 200 mg/100 g [13, 14].

Methods used to relate vitamin E requirement to PUFA intake

As mentioned before, the antioxidant function of vitamin E is critical for the prevention of oxidation of tissue PUFA. Although there are a large number of studies reporting a relation between vitamin E consumption and PUFA intake, this discussion will be limited to the results of those studies upon which the current recommendations for vitamin E are based. In particular, the methods used to study vitamin E requirement in these experiments will be critically evaluated.

Myopathy in rats as a symptom of vitamin E deficiency

A rat study performed by Witting and Horwitt [2] is generally seen as the most thorough attempt to relate vitamin E requirement to PUFA intake [11, 16–18]. In this study it is suggested that there might be a relation between the

onset of α -tocopherol deficiency symptoms and a specific level of lipid peroxidation in animal tissues. Earlier studies demonstrated that animals fed a tocopherol deficient diet develop a variety of pathological conditions, including myopathy. Other animal experiments showed that deficiency symptoms could be prevented by supplementation with synthetic fat-soluble antioxidants [2].

Witting and Horwitt [2] postulated that in the case of a relation between tocopherol deficiency symptoms and lipid peroxidation, the rate at which deficiency develops should be related to the degree of unsaturation of the various fatty acids rather than to the total amount of unsaturation in the dietary fat. In the kinetics of autoxidation *in vitro*, the overall rate of oxidation is dependent on the rate of the slowest reaction, i.e. $\text{ROO}\cdot + \text{RH} \rightarrow \text{ROOH} + \text{R}\cdot$, where $\text{ROO}\cdot$ is a lipid peroxy radical, RH is a PUFA, ROOH is a lipid hydroperoxide and $\text{R}\cdot$ is a lipid radical. Indeed, this reaction is highly dependent on the nature of the R-group. Accordingly, from *in vitro* experiments it is known that the peroxidizability of unsaturated fatty acids increases almost linearly with the number of double bonds present in the molecule [19]. The relative maximal oxidation rates for mono-, di-, tri-, tetra-, penta- and hexaenoic acids appeared to be 0.025:1:2:4:6:8, respectively [19].

In their experiment, Witting and Horwitt [2] fed tocopherol deficient rats diets containing various specially prepared fats, which differed only in composition with respect to the PUFA but were, with one exception, of constant total unsaturation. The aim of this experiment was to investigate the interaction of dietary tocopherol and the degree of unsaturation of fatty acids. The rate of development of creatinuria, a symptom of myopathy, i.e. muscle cell membrane damage, was used as measure for the peroxidizability of the experimental diets [2].

The results showed that the fatty acid composition of the muscle phospholipids of the rats was affected by the different dietary lipids fed, and that there was a negative relation between the estimated peroxidizability of muscular fatty acids, which increases as the number of double bonds per molecule increases, and the rate at which creatinuria developed. Thus, the time required for the production of creatinuria in rats fed diets without added vitamin E is dependent on the nature of the dietary fatty acids. The higher the degree of unsaturation, i.e. the higher the number of double bonds in the fatty acid, the larger the amount of peroxidation products formed, and the earlier the onset of deficiency symptoms like myopathy.

The relative contributions of the various unsaturated fatty acids in inducing creatinuria were comparable to the relative oxidation rates of PUFA as shown in the *in vitro* experiments by Holman [19]. Accordingly, the relative amounts of tocopherol required to protect the various fatty acids from being peroxidized were estimated to be ap-

proximately in the ratios 0.3:2:3:4:5:6 for mono-, di-, tri-, tetra-, penta- and hexaenoic acids respectively [2]. However, since neither the tocopherol content of the muscle membranes nor the plasma tocopherol concentration were analyzed, it is not possible to directly relate the amount of tocopherol required to the amount of peroxidizable PUFA in phospholipids. In addition, it is not clear whether the same ratios as obtained and estimated from animal data are applicable to humans.

This study, as well as an experiment in ducklings by Jager [20], indicates that, when studying the vitamin E requirement, it is important to take into account not only the fatty acid composition and tocopherol content of the diet, but of the biomembranes as well. Furthermore, it should be evident that, although myopathy might be a valid indicator to study vitamin E requirement in animals, this method can not be used for human studies.

Hemolysis of human erythrocytes in relation to vitamin E requirement

A number of difficulties arise when studying the vitamin E requirement in man. Besides the ethical problems of feeding humans experimental diets, which provide minimal levels of α -tocopherol for prolonged periods of time, there are a number of variable conditions that determine vitamin E requirement. These conditions include the amount of peroxidizable compounds in tissues and diet, and the time required to deplete the stores of tocopherols which, in turn, depends on the level of peroxidizable compounds in the tissues [21].

The vitamin E status of erythrocytes has been evaluated by determining their resistance to hemolysis *in vitro* (erythrocyte hemolysis test). This test is based on the requirement for α -tocopherol to stabilize the erythrocyte membrane. Since vitamin E in the form of α -tocopherol is the major lipid-soluble antioxidant in red blood cell membranes, regulation of α -tocopherol concentrations in membranes is of critical importance in maintaining structure and function of these membranes. In the erythrocyte hemolysis test, the red blood cells are exposed to an oxidizing agent, e.g. hydrogen peroxide, under controlled conditions of time and temperature. Rose and György [22] were the first to demonstrate a protective effect of all four tocopherol isomers, both *in vivo* as *in vitro*, on erythrocyte hemolysis induced by dialuric acid in vitamin E deficient rats.

The erythrocyte hemolysis test was also used in the Elgin project, a long-term study (up to four years) to investigate the vitamin E requirements in relation to PUFA intake, mainly linoleic acid, in humans [1, 21, 23, 24]. Up till now, this is the only experimental study on vitamin E requirement of human adults. In this study, male subjects were divided in three groups. One group of subjects was

fed tocopherol-deficient diets (2–3 mg α -tocopherol/day) containing increasing amounts of linoleic acid (3.3 g of linoleic acid per day for 30 months, 16.5 g/day for 9 months and 33 g/d for 7 months). A second group of subjects received the same diets plus a supplement of 15 mg α -tocopherol acetate per day. A third group of males was fed a regular hospital diet, providing up to 10% linoleic acid in about 70 g of fat and approximately 10 mg α -tocopherol acetate [21]. Plasma tocopherol concentrations and the percentage hemolysis of erythrocytes induced by hydrogen peroxide were used as parameters for an adequate tocopherol status.

In the non-supplemented group, each increase in the ingestion of linoleic acid reduced the plasma tocopherol concentration and resulted in a higher percentage of peroxide-induced hemolysis. A period of almost two years was required before maximum susceptibility of erythrocytes to hydrogen peroxide occurred. Although in the supplemented group the plasma tocopherol levels also decreased with each increase in the intake of linoleic acid, the percentage of peroxide-induced hemolysis remained low, even at high intakes of linoleic acid. However, subjects did not appear to have any tocopherol stores, since erythrocyte hemolysis developed within one week after finishing the supplementation. Thus, these results show that erythrocyte hemolysis is only to a certain extent related to the plasma tocopherol content. Although the fatty acid composition of erythrocytes was analyzed, showing a higher linoleic acid content in subjects ingesting a diet high in corn oil than in volunteers consuming a regular hospital diet, these results were not directly related to the extent of erythrocyte hemolysis.

From these experiments Horwitt *et al* [1, 21, 23] concluded that the requirement of vitamin E varies as a function of the amount of PUFA, i.e. linoleic acid, in the diet. However, a direct quantitative relation between the amount of α -tocopherol required to prevent hemolysis (i.e. prevent peroxidation of unsaturated fatty acids in erythrocyte membrane phospholipids) and dietary fatty acid composition was not demonstrated. The α -tocopherol requirement was only estimated to range from a minimum of less than 5 mg/day to a maximum of about 30 mg/day, depending on the amount of unsaturated fatty acids present in the diet.

According to Horwitt [21] an allowance should be made for cellular interconversion and retention of PUFA when calculating the required dietary intake of vitamin E in relation to dietary PUFA consumption. Significant amounts of PUFA are found in tissues, predominantly in the phospholipids of biological membranes, which are susceptible to peroxidation. A calculation of the vitamin E requirement based on dietary PUFA intake does not take into account these PUFA. It is generally accepted that the fatty

acid composition of tissue lipids can be altered, within certain limits, by changing the dietary fatty acid composition. After reanalyzing the data of the Elgin project, Horwitt [21] introduced a formula for determining the α -tocopherol requirement, which includes both the percentage (% of dietary total lipids) and amount (g) of dietary PUFA, and also a baseline value (4 mg), allowing for tissue synthesis of peroxidizable compounds:

$$\text{mg } \alpha\text{-TE} = 0.25 (\% \text{ PUFA} + \text{g PUFA}) + 4 \text{ mg} \quad (\text{Formula 1})$$

In another evaluation of this study, a different formula was proposed to determine the α -tocopherol requirement [25]:

$$\text{mg } \alpha\text{-TE} = 5 + 0.5 * \text{g PUFA} \quad (\text{Formula 2})$$

Both formulas were derived from the same data of the Elgin project to establish the α -tocopherol requirement, and it is obvious that different amounts of α -tocopherol are obtained. Unfortunately, it is not adequately explained how both formula have been attained [21, 25].

Horwitt's analyses of the Elgin project demonstrate that it is very difficult to give exact information on vitamin E requirements in relation to PUFA intake, in particular when the erythrocyte hemolysis test is used. Horwitt [1] recognized that the hemolysis test is nothing more than an indication of the rate at which erythrocyte fatty acids can be oxidized. Furthermore, there is no evidence indicating that this *in vitro* assay closely reflects the stability of the erythrocyte *in vivo* [23, 26].

In conclusion, although the erythrocyte hemolysis test may be a valid indicator of vitamin E status, it has insufficiently been validated to allow assessment of the vitamin E requirement in relation to PUFA content and composition in diet and membranes.

A dietary vitamin E/PUFA ratio

Harris and Embree [3] proposed to use a vitamin E/PUFA ratio for comparison of various diets and foods with respect to mg α -tocopherol per g PUFA. According to Harris and Embree [3], the results of Horwitt's experiments showed that the minimum ratio of α -tocopherol/PUFA at which no deficiency symptoms developed, was between 0.5 and 0.8 mg/g for the experimental diets used. Analysis of the results of food consumption surveys in the United States revealed a ratio of 0.6 mg α -tocopherol per g PUFA, predominantly linoleic acid. They proposed to use the value of 0.6 as a reference ratio for an adequate supply of vitamin E, which seems to be well in agreement with ratios found in various animal experiments. However, it is difficult to compare the results of these studies because of species variation and differences in experimental techniques, including the deficiency symptoms that are under examination, time of depletion and interaction with

other nutrients. Moreover, this value is mainly based on linoleic acid as the major PUFA and may not be adequate when the intake of longer-chain, more-unsaturated fatty acids is increased.

The analysis of Harris and Embree has led to some criticism [20, 27]. In animals it was demonstrated that the vitamin E requirement increased only as long as an increase in the amount of dietary PUFA resulted in an increase in tissue [20, 27]. Consequently, the vitamin E/PUFA ratio for quantities of PUFA higher than those required to saturate tissue PUFA approaches zero. In addition, vitamin E requirement is not only increased during a high PUFA diet, but also for a certain period after returning to a normal mixed diet because tissue PUFA content is still high. Moreover, linoleic acid is not the only dietary unsaturated fatty acid to be considered in evaluating the vitamin E requirement [27]. According to Witting [27], the critical vitamin E/PUFA ratio is therefore not applicable to all dietary regimens.

Present recommendations for vitamin E intake

Despite the difficulties of establishing a relation between vitamin E and PUFA in a quantitative way, in recent years a number of recommendations for vitamin E intake have come forward.

In the 10th edition of the Recommended Dietary Allowances [8] of the United States Food and Nutrition Board, a ratio of approximately 0.4 mg α -tocopherol/g linoleic acid has been suggested as adequate for adult humans [8]. This RDA value is simply based on the observation that the amounts of vitamin E and PUFA consumed by healthy individuals ingesting balanced diets in the United States appeared to maintain normal physiological function and prevent deficiency symptoms. Therefore, the RDA for men and women was set to 10 and 8 mg α -TE (15 and 12 IU), respectively [8]. Indeed, based on the Second National Health and Nutrition Examination Survey (NHANES II) in the United States, mean dietary intakes of vitamin E, expressed in α -TE, were found to be 9.6 mg/day for men and 7.0 mg/day for women. Mean dietary intakes of PUFA were reported to be 16.3 g/day in men and 10.8 g/day in women [28]. However, it should be reiterated that the RDA value used is arbitrary, because it is not based on experimental studies and does not take into account potential differences in the amount of PUFA consumed.

Other recommendations for vitamin E intake as established by the FAO/WHO [29], the scientific Committee on Food (SCF) of the European Union [30], and the German Society of Nutrition [31], are related to the dietary PUFA intake.

The FAO/WHO recommended at high PUFA intakes a ratio of 0.6 mg α -TE/g PUFA [29], comparable to the value suggested by Harris and Embree [3]. Unfortunately, in this recommendation it is not explained what level of dietary PUFA consumption is considered as a high PUFA intake.

In contrast, the SCF [30] proposed a relative intake of 0.4 mg α -TE/g PUFA. In contrast, the SCF [30] proposed a relative intake of 0.4 mg α -TE/g PUFA. This recommendation is based on the α -TE/PUFA ratio found in a normal American diet. According to the SCF, there is no evidence that this level of α -tocopherol consumption is not sufficient, provided that the intake does not fall below 4 and 3 mg/day for male and female adults, respectively.

Consistent with Horwitt [21, 25], the German Society of Nutrition proposed in 1991 a minimum daily intake of 5 mg α -tocopherol to prevent lipid peroxidation in tissue lipids at moderate PUFA intakes (< 7 g linoleic acid per day) [31]. The German Society of Nutrition also suggested that an additional vitamin E requirement needs to be calculated on the basis of the maximal oxidation rates *in vitro* of PUFA with different degrees of unsaturation [2, 19], multiplied by a factor 0.5 (Formula 3). At the usual PUFA intake of about 21 g/day, it was thought unnecessary to include the minimum daily intake of 5 mg in the formula (see Formula 4). Accordingly, the following formulas could be used to calculate α -tocopherol requirement [11, 31]:

at linoleic acid intake $\leq$ 7 g/day:

$$\text{mg } \alpha\text{-TE} = 5 + 0.5 (0.025 G_1 + 1 G_2 + 2 G_3 + 4 G_4 + 6 G_5 + 8 G_6) \quad (\text{Formula 3})$$

at linoleic acid intake > 7 g/day:

$$\text{mg } \alpha\text{-TE} = 0.5 (0.025 G_1 + 1 G_2 + 2 G_3 + 4 G_4 + 6 G_5 + 8 G_6) \quad (\text{Formula 4})$$

where G_n = grams of unsaturated fatty acids with n double bonds.

When it is assumed that the average PUFA intake (21 g/d) consists of 18 g linoleic acid (2 double bonds) and 3 g α -linolenic acid (3 double bonds) per day, a dienoic + trienoic acid equivalent of 24 g will be obtained. Consequently, the daily α -tocopherol requirement to prevent lipid peroxidation at this level of PUFA intake will be $0.5 \times 24 = 12$ mg α -TE, which is in good agreement with the vitamin E intake recommended by the German Society of Nutrition [31]. This level is comparable to the mean dietary consumption of vitamin E in Germany, 14 and 11.5 mg α -TE per day for men and women, respectively [11].

After analyzing the results of animal and human studies, Muggli [16] concluded that a ratio of 0.6 mg α -tocopherol/g linoleic acid, as proposed by Harris and Embree

[3], could be used to determine human requirements. He introduced a formula in order to convert the relative vitamin E requirement for individual PUFA (0.3:2:3:4:5:6), as determined by Witting and Horwitt [2], into absolute amounts [16, 17]. A ratio of 0.6 mg α -tocopherol/g linoleic acid is used as a reference value. In order to obtain mg of α -TE required to protect grams of unsaturated fatty acids with different numbers of double bonds, Muggli's formula can be revised in the following way [11]:

$$\text{mg } \alpha\text{-TE} = 0.09 G_1 + 0.6 G_2 + 0.9 G_3 + 1.2 G_4 + 1.5 G_5 + 1.8 G_6 \quad (\text{Formula 5})$$

where G_n = grams of unsaturated fatty acids with n double bonds.

Revision of vitamin E intake and PUFA consumption

As is clear from the studies and recommendations described above, there is no agreement upon the exact amount of vitamin E required when consuming various unsaturated fatty acids. Critical points are that these recommendations are based on animal experiments and food intake data that are not adequately validated. Moreover, the methods used in the studies described have several limitations, which make it difficult to quantify the relation between vitamin E and PUFA.

In the following paragraphs, studies are summarized investigating the effects of the intake of the highly unsaturated n-3 fatty acids on vitamin E status and lipid peroxidation as measured with the MDA method. Furthermore, the applicability of the MDA method will be critically evaluated.

Effects of consumption of foods rich in PUFA on vitamin E status and lipid peroxidation

There is increasing evidence that consumption of fish and fish oils containing n-3 PUFA, especially eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), is beneficial in the prevention of cardiovascular disease [32] and possibly cancer [33]. Fish oils have been shown to be hypolipidemic by lowering total triglycerides in circulating blood and may also have anti-thrombotic effects [32].

However, the n-3 PUFA in fish and fish oils are also highly unsaturated and consequently, very susceptible to peroxidation. Since the requirement for vitamin E, as the major lipid-soluble antioxidant, is elevated when the intake of PUFA increases, an enhanced consumption of fish or fish oils, for instance by using supplements containing the major fish oil fatty acids EPA and DHA, may increase the need for vitamin E.

It is generally assumed that foods high in PUFA, e.g. EPA and DHA, often also contain considerable amounts of vitamin E, thereby providing an adequate vitamin E/PUFA ratio. However, some food products are rich in PUFA but contain relatively low levels of α -TE. Therefore, Bässler [18] stipulates that only the net vitamin E content should be considered when evaluating the vitamin E supply in food products that are high in PUFA. The net vitamin E content is the total vitamin E content minus vitamin E needed for PUFA protection, as calculated according to the formula of Muggli [16, 17]. Consequently, foods relatively high in PUFA and low in vitamin E may have a negative net vitamin E content.

With respect to the amounts of vitamin E and PUFA (primarily EPA and DHA) in fish and fish oils, it is evident that there is much variation between and within species. Furthermore, it is possible that there are seasonal variations in the vitamin E content of certain fish species [15].

Table I: Vitamin E, expressed in α -tocopherol equivalents (α -TE), and PUFA content of some fish species [13, 15, 34] and the amount of vitamin E required, according to Muggli [16, 17]

Fish type	Unsaturated fatty acids (g/100 g)						α -TE (mg/100 g)	α -TE required (mg/100 g)
	mono	di	tri	tetra	penta	hexa		
Cod	0.07	—	—	0.01	0.09	0.23	0.23	0.57
Haddock	0.07	—	—	0.02	0.08	0.14	0.39	0.40
Halibut	0.99	—	—	0.03	0.21	0.22	0.85	0.84
Herring	4.84	0.03	0.02	0.09	1.12	1.29	1.07	4.58
Mackerel	9.06	0.05	0.06	0.26	1.64	2.16	1.52	7.56
Rainbow trout	1.46	0.02	0.01	0.03	0.16	0.50	0.20	1.33
Salmon	2.65	0.04	0.02	0.07	0.39	0.73	2.02	2.26
Winter flounder	0.06	—	—	0.02	0.09	0.12	0.36	0.38
Wolffish	0.09	—	—	0.03	0.14	0.06	2.10	0.36
Crab	0.20	—	—	0.03	0.21	0.09	2.25	0.53
Lobster	0.44	0.01	—	0.03	0.24	0.12	1.47	0.66
Mussel	0.36	0.05	—	0.05	0.15	0.17	0.74	0.65
Oyster	0.17	—	—	0.02	0.11	0.09	0.85	0.37

Table I presents the vitamin E and unsaturated fatty acid contents of some fish species [13, 15, 34]. In addition, it shows the amount of vitamin E required, calculated according to the revised formula of Muggli (Formula 5). The unsaturated fatty acids with 1, 2, 3, 4, 5 and 6 double bonds are indicated by mono, di, tri, tetra, penta and hexa, respectively, and the amounts of vitamin E are given as the amounts of α -TE, since α -tocopherol is the major form of vitamin E present in fish [14].

As is clear from Table I, most of the fish species can not be regarded as containing adequate amounts of vitamin E. Indeed, more than half of the fish types presented, contain equivalent or lower levels of α -tocopherol than is required. In particular herring and mackerel have a large negative vitamin E content: -3.51 and -6.04 mg respectively. Only wolffish and crab have an additional α -tocopherol content of more than 1.5 mg/100 g. Thus, using Muggli's formula to evaluate the vitamin E status of fish food shows that the α -tocopherol content of fish is relatively low and that increased consumption of fish may compromise the vitamin E status in humans.

Results of an observational study showed that extreme fish consumption does not affect plasma antioxidant levels. Although subjects usually consuming a high-fish diet (103 g/day) had higher EPA and DHA intakes and higher levels of plasma n-3 fatty acids than subjects on an habitual low-fish diet (5 g/day), there were no differences in vitamin E intake and concentrations of α -tocopherol in plasma between the two groups [35]. When the formula of Muggli is applied to the intake data of unsaturated fatty acids in both groups, the α -tocopherol requirement will be 8.97 and 8.28 mg/day for the high-fish and low-fish group, respectively, values that are only slightly lower than the actual vitamin E intakes (9.0 and 10.5 mg/day). However, since in this study markers of lipid peroxidation were not measured, any possible adverse effects of the more oxidation-susceptible n-3 fatty acids in fish cannot be ruled out. Therefore, it is not clear whether the vitamin E intake of 10.5 mg/day in the high-fish group is sufficient to prevent lipid peroxidation.

Several studies have investigated the effect of increased intakes of the major fish fatty acids EPA and DHA, in the form of commercial fish oil supplements, on vitamin E status and lipid peroxidation. Most fish oil supplements contain amounts of vitamin E that are equal to or exceed the levels that are required according to Muggli's formula, taking into account the fatty acid composition of the supplements. Nevertheless, lipid peroxidation could not be prevented in subjects receiving fish oil supplements, suggesting that the vitamin E content of either the supplements or the total diet is not adequate [36-38]. Furthermore, additional vitamin E supplementation gave inconsistent results.

Brown and Wahle [37] found no difference in the plasma concentration of thiobarbituric acid responsive substances (TBARS) between volunteers only receiving fish oil supplements (15 g/day) and a group of subjects that was also supplemented with 268 mg additional vitamin E (α -TE), despite the fact that the extra vitamin E caused a significant increase in plasma vitamin E concentration. Haglund *et al* [39], found no effect of fish oil containing 1.0 mg α -TE/g oil on levels of plasma MDA and serum vitamin E, while fish oil containing only 0.2 mg α -TE/g significantly increased the concentration of plasma MDA and reduced serum vitamin E concentration. Allard *et al* [40] reported a significant increase in plasma MDA and lipid peroxide concentrations in subjects receiving supplements mainly containing EPA and DHA, irrespective of 604 mg additional vitamin E (α -TE). In contrast, the results of a cross-over trial with post-menopausal women receiving fish oil capsules (15 g/day) and different doses of vitamin E (0, 91, 182, 364 mg α -TE) showed that the concentration of urinary TBARS decreased linearly as the dose of vitamin E increased. However, no vitamin E dose-dependent response was found in the concentrations of the MDA-TBA adduct in both plasma and urine [41]. Wander *et al* [41] concluded that more studies are necessary to assess the oxidative potential of highly unsaturated fatty acids, i.e. EPA and DHA, and the amount of vitamin E that is required to prevent peroxidation. A study of Nair *et al* [38] also showed a protective effect of vitamin E on lipid peroxidation. Daily supplementation of 15 g fish oil, containing 4.53 g EPA and 1.96 g DHA, led to reduced plasma α -tocopherol concentrations and elevated MDA levels, but values returned to baseline levels after 147 mg α -TE was given in addition to the 15 g of fish oil.

Table II summarizes the results of studies investigating the effects of EPA and DHA (and vitamin E) supplementation on vitamin E status and lipid peroxidation in humans. The vitamin E content of the supplements is expressed in mg α -TE. From most fish oil supplements only the amounts of EPA and DHA present is exactly known. As a consequence, only these two fatty acids could be used to calculate the amount of α -TE that should have been present in the supplement. However, since in Muggli's formula EPA and DHA have the largest conversion factors and usually are the major PUFA in fish oil, other fatty acids will contribute only to a limited extent to the vitamin E requirement.

As is clear from Table II, different doses of EPA and DHA have been used, as is also true for the vitamin E content of the fish oil supplements. Furthermore, different doses of additional vitamin E have been supplemented. From the results presented it appears that in some studies even large doses of extra vitamin E could not prevent an

Table II: Summary of studies investigating the effects of n-3 PUFA and vitamin E on vitamin E status and lipid peroxidation in man. Vitamin E is expressed in α -tocopherol equivalents (α -TE)

Daily dose fatty acids in FO supplement (g)		Daily dose α -TE in FO supplement (mg)	Calculated requirement α -TE in FO supplement (mg)*	α -TE supplement (mg)	Lipid peroxidation	Plasma α -tocopherol	Duration (weeks)	Reference
EPA	DHA							
1.68	0.72	4.0	3.82		Increase	No effect	12	36
2.00	1.30	10.1	5.34		Increase	No effect	2	37
2.00	1.30	10.1	5.34		No effect	No effect	4	37
2.00	1.30	10.1	5.34	268	Increase	Increase	2	37
2.00	1.30	10.1	5.34	268	No effect	Increase	4	37
4.53	1.96	11.0	10.32		Increase	Decrease	10	38
4.53	1.96	11.0	10.32	147	Decrease	No effect	8	38
4.10	3.60	2.0	12.63		No effect	No effect	4	53
4.10	3.60	2.0	12.63		Increase	No effect	25	53
3.062	2.262		8.66		Increase	No effect	6	40
3.062	2.262		8.66	604	Increase	Increase	6	40
2.75	1.85	16.3	7.46		–	Increase	6	54
2.46	1.80		6.93	91	Decrease	Increase	5	41

* The requirement α -TE in the FO supplement is calculated according to Muggli's formula.

FO = Fish oil; EPA = Eicosapentaenoic acid (20:5 n-3); DHA = Docosahexaenoic acid (22:6 n-3).

increase in lipid peroxidation [37, 40], whereas in other studies a distinct protective effect is demonstrated.

The inconsistency in these results may be due to differences in the methodology that is used to measure lipid peroxidation. Furthermore, it is possible that nutritional co-factors play a role in the process of lipid peroxidation, for instance vitamin C. Vitamin C is the major line of antioxidative defense against radicals in the aqueous phase and lipid peroxidation in plasma, but it is also crucial for the recycling of vitamin E [42]. According to Gey [42], there is evidence that the vitamins C and E interact also *in vivo* in man, and that the functional coupling of these vitamins is a crucial part of the overall antioxidant potential. However, in the studies described above, the influence of vitamin C on lipid peroxidation and on the recycling process of vitamin E has not been taken into account.

Therefore, when investigating the amount of vitamin E that is needed to prevent lipid peroxidation when the consumption of fish fatty acids, e.g. EPA and DHA is increased, the influence of co-nutrients as vitamin C should also be taken into account.

Influence of a reduction in fat intake on vitamin E requirement

In previous chapters it is discussed that the vitamin E requirement is elevated when the consumption of PUFA is increased. Therefore, a decrease in total fat intake, with a shift in the ratio from saturated fatty acids (SAFA) to PUFA, may also have consequences for the vitamin E requirement.

In the following example it is pointed out that the vitamin E requirement, calculated according to the formula of Muggli as revised by Gassmann [11], is increased by 3 mg/day when the dietary fat content and composition is altered according to the recommendations of several public health organizations: a decrease in total fat intake to 30 energy percent (en%), and a decrease in SAFA and increase in PUFA and monounsaturated fatty acids (MUFA) at a ratio of 10:10:10 en% [6].

In the calculation it is assumed that the habitual daily fat intake is 40 en%. Recent nutritional intake data have shown that 40 en% of total daily fat intake consists of 16.7 en% SAFA, 15.6 en% MUFA and 7.7 en% PUFA and PUFA can be divided in 6.6 en% linoleic acid, 1.1 en% α -linolenic acid, 0.1 en% EPA and 0.1 en% DHA (R. P. Mensink, personal communication). Assuming a daily energy intake of 2250 kcal, the intake of the different fatty acids can be calculated in grams, i.e. 39 g MUFA, 16.6 g linoleic acid, 2.75 g α -linolenic acid, and 0.25 g of both EPA and DHA. Accordingly, vitamin E requirement will be 16.7 g/day.

In contrast, at a similar daily energy intake but a reduced total fat consumption of 30 en%, consisting of 10 en% of both SAFA, MUFA and PUFA, and PUFA consisting of 8 en% linoleic acid, 1.5 en% α -linolenic acid and 0.25 en% of both EPA and DHA, the calculated vitamin E requirement will be 19.7 mg/day.

The above calculation demonstrates that although total fat intake is reduced, vitamin E requirement, calculated according to the Muggli formula, is increased when the

SAFA/PUFA ratio is decreased. Thus, although a reduction in total fat intake with a concomitant increase in PUFA consumption is considered beneficial with respect to the risk of cardiovascular disease, the vitamin E status may be negatively influenced.

Evaluation of the MDA technique to measure lipid peroxidation

A method commonly used to assess lipid peroxidation is the determination of malondialdehyde (MDA), an end product of oxidative lipid degradation, or of a derivative of MDA generated by its reaction with thiobarbituric acid (TBA). At low pH and high temperature, MDA readily reacts with TBA to form a red, fluorescent 1:2 MDA:TBA adduct, which can be measured by fluorescence spectrophotometry [43, 44]. Several studies have demonstrated an increased production of MDA or TBA-reactive substances (TBARS) in subjects receiving dietary supplements with a high PUFA content, and close associations between vitamin E and PUFA have been observed [36–38, 45]. However, there are no studies in which this method was used to establish the amount of vitamin E required preventing MDA formation at different intake levels of PUFA. In addition, the determination of MDA as index of lipid peroxidation has several limitations that should be taken into account when attempting to quantify the relation between vitamin E and PUFA.

It is assumed that a quantitative relationship exists between lipid peroxidation and the formation of MDA and that the formation of TBARS during the TBA-test is specific for the presence and amount of lipid peroxides, i.e. lipid-derived MDA [43]. However, according to Janero [43], who extensively reviewed the available literature on MDA and TBA-reactivity, there are several important factors, which influence the relationship between MDA derived from lipid oxidation products and its reactivity in a TBA-test. Firstly, it is well documented that the TBA-test is not specific for MDA. Lipid peroxide-derived decomposition products other than MDA and substances not related to lipid peroxidation can form TBA complexes also. In addition, MDA is able to react with other molecules than TBA. Furthermore, MDA is not the only end product of lipid peroxidation nor is this substance exclusively generated through lipid peroxidation. An important point is that the formation of MDA as a result of lipid peroxidation varies with the nature of the PUFA peroxidized, especially with its degree of unsaturation. Therefore, the amount of MDA formed does not give reliable and unequivocal information on the precise nature of the MDA, precursors, nor can the TBA-test response be considered a quantitative measure of lipid peroxidation [43]. According to Gutteridge and Halliwell [46], the TBA-test may give misleading results when used on human mater-

ial, especially plasma, which contains many substances that react in the TBA-assay.

When assessing the vitamin E requirement in relation to PUFA consumption it is, however, necessary to determine the extent of lipid peroxidation as a quantitative marker of free radical generation.

Recommendations for future research

As discussed above, the vitamin E requirement in man has frequently been studied in relation to linoleic acid as the principal PUFA being consumed. However, the effects of consumption of the highly unsaturated LCP on vitamin E requirement have not been adequately investigated. In addition, the frequently used MDA-technique may not be suitable to study the extent of lipid peroxidation in relation to vitamin E requirement.

Therefore, to study the relation between vitamin E and PUFA, it is necessary to use functional techniques based on the measurement of lipid peroxidation *in vivo*. Recently, an immunoassay method for the measurement of isoprostanes in plasma or urine has been developed [47]. The F₂-isoprostane, 8-iso-PGF_{2α}, has been shown to be formed *in vivo* as a result of free radical-catalyzed lipid peroxidation of arachidonic acid. This reaction occurs independent of the cyclooxygenase pathway [48]. Studies have shown that the measurement of circulation amounts of 8-iso-PGF_{2α} is a reliable index for the determination of oxidative stress status in humans [49, 50], provided the tissue content of arachidonic acid is stable. Furthermore, assessment of oxidized low density lipoprotein (oxLDL) in plasma by using a monoclonal antibody raised against oxLDL may also provide an adequate marker of lipid peroxidation *in vivo* [51, 52].

Thus, a study should be considered to investigate vitamin E requirement in relation to the consumption of PUFA, especially the highly unsaturated LCP, using F₂-isoprostanes and oxLDL as markers of lipid peroxidation.

References

1. Horwitt, M.K. (1960) Vitamin E and lipid metabolism in man. *Am. J. Clin. Nutr.* 8, 451–461.
2. Witting, L.A. and Horwitt, M.K. (1964) Effect of degree of fatty acid unsaturation in tocopherol deficiency-induced creatinuria. *J. Nutr.* 82, 19–33.
3. Harris, P.L. and Embree N.D. (1963) Quantitative consideration of the effect of polyunsaturated fatty acid content of the diet upon the requirements for vitamin E. *Am. J. Clin. Nutr.* 13, 385–392.
4. Weber, P., Bendich, A. and Machlin, L.J. (1997) Vitamin E and human health: rationale for determining recommended intake levels. *Nutr.* 13, 450–460.

5. Traber, M. G. and Sies, H. (1995) Vitamin E in humans: demand and delivery. *Ann. Rev. Nutr.* 16, 321–347.
6. Meydani, M. (1995) Vitamin E. *Lancet* 345, 170–175.
7. Traber M. G., Cohn, W. and Muller D. P. R. (1993) Absorption, transport and delivery to tissues. In: *Vitamin E in health and disease* (Packer, L. and Fuchs, J., eds.), pp. 35–51, Marcel Dekker, Inc., New York.
8. NRC (National Research Council) (1989) Recommended dietary allowances, 10 th Edition ed. Food and Nutrition Board. National Academy Press, Washington D.C.
9. Packer, L. and Kagan, V.E. (1993) Vitamin E: the antioxidant harvesting center of membranes and lipoproteins. In: *Vitamin E in health and disease* (Packer, L. and Fuchs, J., eds.), pp. 179–192, Marcel Dekker, Inc., Wanshington D.C.
10. Diaz, M. N., Frei, B., Vita, J. A. and Keaney, J. F. (1997) Antioxidants and atherosclerotic heart disease. *N. Engl. J. Med.* 337, 408–416.
11. Gassmann, B. and Kübler, W. (1996) Ungesättigte Fettsäuren und Vitamin-E-Bedarf. *Ernährungs-Umschau* 43, 172–177.
12. Sheppard, A. J., Pennington J. A. T. and Weihrauch, J. L. (1993) Analysis and distribution of vitamin E in vegetable oils and foods. In: *Vitamin E in health and disease* (Packer, L. and Fuchs, J., eds.), pp. 9–31, Marcel Dekker, Inc., New York.
13. McLaughlin, P. J. and Weihrauch, J. L. (1979) Vitamin E content of foods. *J. Am. Diet. Assoc.* 75, 647–665.
14. Lehmann, J., Martin, H. L., Lashley, E. L., Marshall, M. W. and Judd, J. T. (1986) Vitamin E in foods from high and low linoleic acid diets. *J. Am. Diet. Assoc.* 86, 1208–1216.
15. Syväoja, E. L., Salminen, K., Piironen, V., Varo, P., Kerojoki, O. and Koivistoinen, P. (1985) Tocopherols and tocotrienols in Finnish foods: fish and fish products. *J. Am. Oil Chem. Soc.* 62, 1245–1248.
16. Muggli, R. (1994) Vitamin E-Bedarf bei Zufuhr von Polyenfettsäuren. *Fat. Sci. Technol.* 96, 17–19.
17. Muggli R. (1994) Physiological requirements of vitamin E as a function of the amount and type polyunsaturated fatty acid. In: *Fatty acids and lipids: Biological aspects* (Galli, C., Simopoulos, A. P. and Tremoli, E., eds.), pp. 166–168, Karger, Basel.
18. Bässler, K. H. (1991) On the problematic nature of vitamin E requirements: net vitamin E. *Z. Ernährungswiss.* 30, 174–180.
19. Holman, R. T. (1954) Autooxidation of fats and related substances. In: *Progress in the chemistry of fats and related substances* (Holman, R. T., Lundberg, W. O. and Malkin, T., eds.), pp. 51–98, Academic Press, New York.
20. Jager, F. C. (1973) Linoleic acid intake and vitamin E requirement. Unilever Research, Vlaardingen.
21. Horwitt, M. K. (1974) Status of human requirements for vitamin E. *Am. J. Clin Nutr.* 27, 1182–1193.
22. Rose, C. S. and György, P. (1952) Specificity of hemolytic reaction in vitamin E-deficient erythrocytes. *Am. J. Physiol.* 168, 414–420.
23. Horwitt, M. K., Harvey, C. C. and Harmon, E. M. (1968) Lipids, α -tocopherol, and erythrocyte hemolysis. *Vitam. Horm.* 26, 487–499.
24. Horwitt, M. K., Harvey, C. C., Duncan, G. D. and Wilson, W. C. (1956) Effects of limited tocopherol intake in man with relationships to erythrocyte hemolysis and lipid oxidations. *Am. J. Clin. Nutr.* 4, 408–419.
25. Horwitt, M. K. (1986) Interpretations of requirements for thiamin, riboflavin, niacin-tryptophan, and vitamin E plus comments on balance studies and vitamin B₆. *Am. J. Clin. Nutr.* 44, 973–985.
26. Bieri, J. G. and Poukka, R. K. H. (1970) In vitro hemolysis as related to rat erythrocyte content of α -tocopherol and polyunsaturated fatty acids. *J. Nutr.* 100, 557–564.
27. Witting, L. A. (1972) Recommended dietary allowance for vitamin E. *Am. J. Clin. Nutr.* 25, 257–261.
28. Murphy, S. P., Subar, A. F. and Block, G. (1990) Vitamin E intakes and sources in the United States. *Am. J. Clin. Nutr.* 52, 361–367.
29. FAO WHO (1994) Fats and oils in human nutrition. Report of a joint expert consultation. FAO Food and Nutrition Paper 57, Rome.
30. Europäische Kommission (1994) Nährstoff- und Energiezufuhr in der Europäischen Gemeinschaft (Stellungnahme vom 11. Dezember 1992). Berichte des wissenschaftlichen Lebensmittelausschusses, 31. Luxemburg.
31. Deutsche Gesellschaft für Ernährung (1991) Empfehlungen für die Nährstoffzufuhr. 5th ed. Umschau Verlag, Frankfurt.
32. Deckere, E. A. M. de, Korver, O., Verschuren, P. M. and Katan, M. B. (1998) Health aspects of fish and n-3 polyunsaturated fatty acids from plant and marine origin. *Eur. J. Clin. Nutr.* 52, 749–753.
33. Galli, C. and Butrum, R. (Dietary ω -3 fatty acids and cancer: an overview. In: *Health affects of ω -3 polyunsaturated fatty acids in seafoods* (Simopoulos, A. P. *et al*, eds.), pp. 446–461, Karger, Basel.
34. Ackman, R. G. (1989) Nutritional composition of fats in seafoods. *Prog. Food. Nutr. Sci.* 13, 161–241.
35. Anttolainen, M., Valsta, L. M., Alfthan, G., Kleemola, P., Salminen, I. and Tamminen, M. (1996) Effect of extreme fish consumption on dietary and plasma antioxidant levels and fatty acid composition. *Eur. J. Clin. Nutr.* 50, 741–746.
36. Meydani, M., Natiello, F., Goldin, B., Free, N., Woods, M., Schaefer, E., Blumberg, J. B. and Gorbach, S. L. (1991) Effect of long-term fish oil supplementation on vitamin E status and lipid peroxidation in women. *J. Nutr.* 121, 484–491.
37. Brown, J. E. and Wahle, K. W. J. (1990) Effect of fish oil and vitamin E supplementation on lipid peroxidation and whole-blood aggregation in man. *Clin. Chim. Acta* 193, 147–156.
38. Nair, P. P., Judd, J. T., Berlin, E., Taylor, P. R., Shami, S., Sainz, E. and Bhagavan, H. N. (1993) Dietary fish oil-induced changes in the distribution of α -tocopherol, retinol, β -carotene in plasma, red blood cells, and platelets: modulation by vitamin E. *Am. J. Clin. Nutr.* 58, 98–102.
39. Haglund, O., Luostarinen, R., Wallin, R., Wibell, L. and Saldeen, T. (1991) The effects of fish oil on triglycerides, cholesterol, fibrinogen and malondialdehyde in humans supplemented with vitamin E. *J. Nutr.* 121, 165–169.
40. Allard, J. P., Kurian, R., Aghdassi, E., Muggli, R. and Royall, D. (1997) Lipid peroxidation during n-3 fatty acid and vitamin E supplementation in humans. *Lipids* 32, 535–541.

41. Wander, R. C., Du, S., Ketchum, S. O., Rowe, K. E. (1996) α -Tocopherol influences in vivo indices of lipid peroxidation in postmenopausal women given fish oil. *J. Nutr.* 126, 643–652.
42. Gey, K. F. (1998) Vitamins E plus C and interacting constituents required for optimal health. *BioFactors* 7, 113–174.
43. Janero, D. R. (1990) Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Free Radic. Biol. Med.* 9, 515–540.
44. Esterbauer, H. and Cheeseman, K. H. (1990) Determination of aldehydic lipid peroxidation products: malondialdehyde and 4-hydroxynonenal. *Methods Enzymol.* 186, 407–421.
45. Brown, K. M., Morrice, P. C. and Duthie, G. G. (1998) Erythrocyte membrane fatty acid composition of smokers and non-smokers: effects of vitamin E supplementation. *Eur. J. Clin. Nutr.* 52, 145–150.
46. Gutteridge, J. M. C. and Halliwell, B. (1990) The measurement and mechanism of lipid peroxidation in biological systems. *Trends Biochem. Sci.* 15, 129–135.
47. Wang, Z., Ciabattini, G., Creminon, C., Lawson, J., Fitzgerald, G. A., Patrono, C. and MacLough, J. (1995) Immunological characterization of urinary 8-epi-prostaglandin $F_{2\alpha}$, excretion in man. *J. Pharmacol. Exp. Ther.* 275, 94–100.
48. Morrow, J. D., Hill, K. E., Burk, R. F., Nammour, T. M., Badr, K. F. and Roberts, L. J. (1990) A series of prostaglandin F_2 -like compounds are produced in vivo in humans by a non-cyclooxygenase, free radical-catalyzed mechanism. *Proc. Natl. Acad. Sci.* 87, 9383–9387.
49. Roberts, L. J. and Morrow, J. D. (1994) Isoprostanes. Novel markers of endogenous lipid peroxidation and potential mediators of oxidant injury. *Ann. N.Y. Acad. Sci.* 744, 237–242.
50. Awad, J. A., Il, L. J. R., Burk, R. F. and Morrow, J. D. (1996) Isoprostanes – prostaglandin-like compounds formed in vivo independently of cyclooxygenase. *Gastroenterol. Clin. North Am.* 25, 409–427.
51. Holvoet, P., Perez, G., Bernar, H., Bouwers, E., Vanloo, B., Rosseneu, M. and Collen, D. (1994) Stimulation with a monoclonal antibody (mAb4E4) of scavenger receptor-mediated uptake of chemically modified low density lipoproteins by THP-1-derived macrophages enhances foam cell generation. *J. Clin. Invest.* 93, 89–98.
52. Holvoet, P., Donck, J., Landeloos, M., Brouwers, E., Luijckens, K., Arnout, J., Lesaffre, E., Vanrenterghem, Y. and Collen, D. (1996) Correlation between oxidized low density lipoproteins and von Willebrand factor in chronic renal failure. *Thromb. Haemost.* 75, 663–669.
53. Palozza, P., Sgarlata, E., Luberto, C., Piccioni, E., Anti, M., Marra, G., Armelao, F., Franceschelli, P. and Bartoli, G. M. (1996) n-3 Fatty acids induce oxidative modifications in human erythrocytes depending on dose and duration of dietary supplementation. *Am. J. Clin. Nutr.* 64, 297–304.
54. Shapiro, A. C., Meydani, S. N., Meydani, M., Morrow, F., McNamara, J., Schaefer, E. J., Endres, S. and Dinarello, C. A. (1991) The effect of fish oil supplementation on plasma α -tocopherol, retinol, lipid and lipoprotein levels in normolipidemic subjects. *Nutr. Res.* 11, 539–548.

Prof. G. Hornstra, Ph.D. Med.

Dept. of Human Biology
Maastricht University
P.O. Box 616
6200 MD Maastricht, The Netherlands