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Review

Analysis of lipophilic antioxidants in human serum and tissues: tocopherols and carotenoids

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Abstract

Tocopherols and carotenoids are naturally occurring lipophilic micronutrients, suggested to play a role in the prevention of several degenerative diseases. Thus, methods for the quantification of these nutrients in human samples have been developed during recent years. Blood and tissue levels of tocopherols and carotenoids are influenced by a variety of parameters related to disease, age, diet and lifestyle. This review summarizes general aspects of chromatographic analysis of tocopherols and carotenoids in human samples and deals with information on the outcome of human studies, in which such measurements were applied.

Keywords: Reviews; Antioxidants; Tocopherols; Carotenoids; Vitamins

Contents

1. Introduction

Tocopherols and carotenoids are naturally occurring lipophilic compounds exhibiting a variety of biological activities in plants and animals. They are not synthesized de novo in humans and animals but ingested with the diet. Tocopherols (α, β, γ, δ) and tocotrienols (α, β, γ, δ) (Fig. 1) comprise eight compounds generically called ''vitamin E'', of which RRR-α-tocopherol is largely found in vegetable oils

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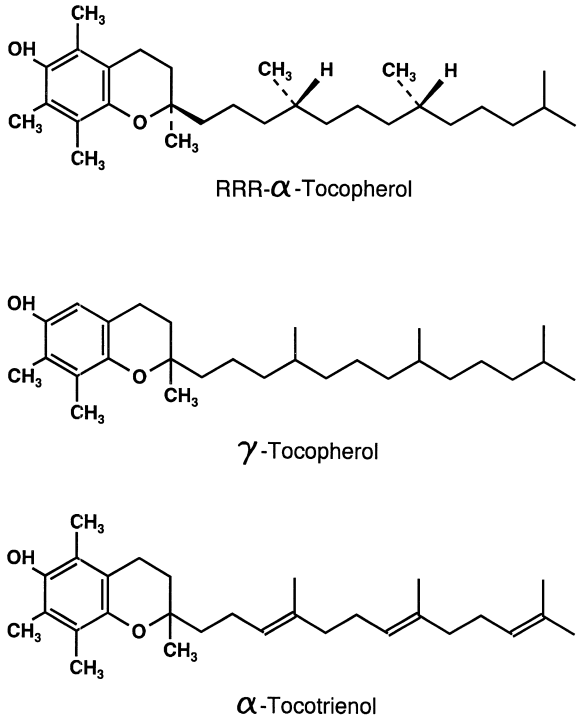

Fig. 1. Structures of selected tocopherols and tocotrienols.

and other plant-based food groups [1] and constitutes the predominant form circulating in human blood [2]. Vitamin E functions as a chain-breaking antioxidant, inhibiting the propagation of lipid peroxidation, and thus preventing membranes or lipoproteins from oxidative damage. This constitutes an important biological function of vitamin E, since the deterioration of cellular membranes is associated with cellular dysfunction and because oxidative modification of lipoproteins plays a role in the formation of the atherosclerotic plaque [3]. The elevation of plasma and tissue F_2-isoprostanes (a reliable index of in vivo oxidative lipid damage) in animals with vitamin E deficiency demonstrates the importance of vitamin E also in vivo [4]. Furthermore vitamin E was shown to protect against F_2-isoprostane generation and atherosclerosis in mice [5]. The chroman ring of vitamin E provides the redox system of the molecule which interacts with lipid radicals and forms relatively stable tocopheroxyl radicals. In biological systems, vitamin E is thought to be regenerated by other antioxidants such as ascorbate and glutathione. In addition to antioxidant and anti-atherosclerotic activities [6], vitamin E exhibits a number of other biological activities, including impact on cellular signaling and prevention of infertility in animals [7]. Physiology, biopotency and bioavailability are beyond the scope of this article and have been reviewed by Traber and Sies [8] and Hoppe and Krennrich [9]. Since vitamin E is transported by lipoproteins in human organisms, total cholesterol or total lipids concentrations in serum should be taken into account when measuring the vitamin E status. Vitamin E intake from supplements and body mass index (BMI) have been recently shown to constitute the major independent predictors of serum tocopherol levels in women; dietary factors are playing only a minor role [10].

Carotenoids are a group of pigments which are synthesized in plants and in several microorganisms; more than 700 carotenoids have been identified up to now. They exhibit a large number of different biological activities, including a role in light-harvesting and photoprotection in plants and microorganisms and, for selected carotenoids, a pro-vitamin A activity in humans and animals. Carotenoids are divided into two major classes: hydrocarbon carotenoids (carotenes) and oxygen-containing carotenoids (oxocarotenoids or xanthophylls); the xanthophylls which carry hydroxyl groups may be esterified with fatty acids to carotenol esters [11]. In human studies, numerous associations between a low carotenoid intake or status and increased risk for cancer, age-related macular degeneration, cataract, sunburn-induced skin damage or cardiovascular diseases have been observed [12–17]. Therefore, attention has been given to the uptake, distribution and metabolism of carotenoids after ingestion as supplements or as part of dietary yellow and green fruits and vegetables [18]. Whether carotenoids reveal the attributed beneficial health effects primarily by acting as antioxidants is still a matter of debate. They contain a system of conjugated double bonds which allow them to interact efficiently with reactive oxygen species [19]. Lutein, zeaxanthin, β-cryptoxanthin, lycopene, α- and β-carotene (Fig. 2) as well as several other dietary carotenoids are efficient quenchers of singlet molecular oxygen and scavengers of peroxyl radicals. Carotenoids are transported by

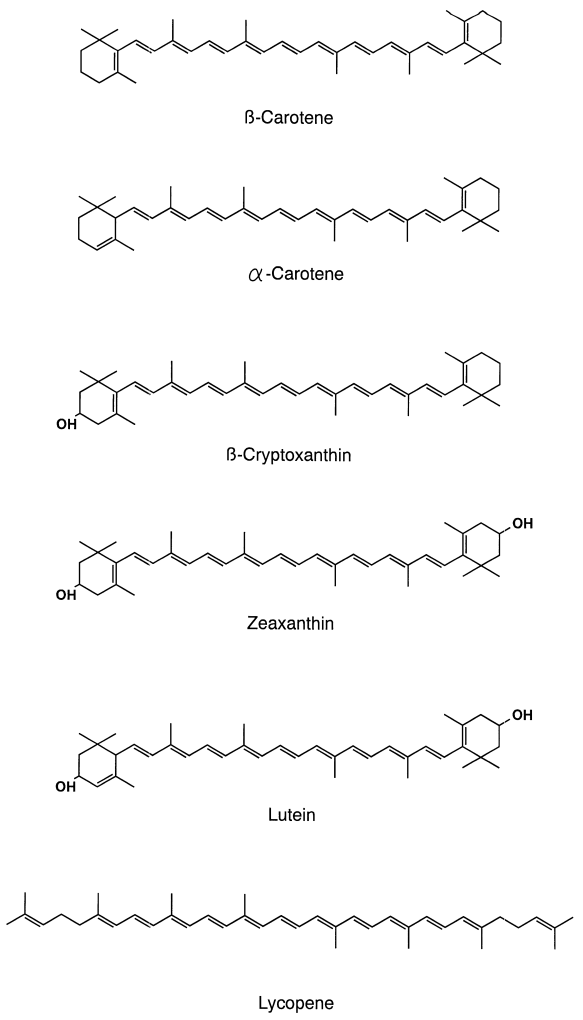

Fig. 2. Structures of major carotenoids in human serum and tissues.

lipoproteins in the body, and therefore the carotenoid status of the organism should be seen in context with the lipid profile. Detailed data regarding the bioavailability of carotenoids will be not discussed in this work (for reviews, see Refs. [20–22]).

Given the importance of assessing tocopherol and carotenoid status in human blood and tissues, this review will mainly focus on the major tocopherols (α- and γ-tocopherol) and the major carotenoids (lutein, zeaxanthin, β-cryptoxanthin, lycopene, α- and β-carotene) found in humans.

2. General aspects

Several methods for the analysis of tocopherols and carotenoids have already been described in detail and are currently widely used [23–43]. Rather than overlapping with previously published descriptions of commonly used methods, this section focuses on aspects of tocopherol and carotenoid analysis deserving particular attention, such as solubility and stability of the analytes and problems associated with sample purification and the application of high-performance liquid chromatography (HPLC).

2.1. Solubility

Tocopherols and tocotrienols are practically insoluble in water but can be readily dissolved in organic solvents like acetone, chloroform, diethyl ether, benzene, or different alcohols. Ethanol is a suitable solvent for the preparation of stock solutions of tocopherols and tocotrienols. The concentration of tocopherols in stock solutions can be measured by UV spectroscopy using extinction coefficients (Table 1). These metabolites and the synthetic tocopherol analog Trolox (6-hydroxy-2,5,7,7-tetramethylchroman-2-carboxylic acid) carry a carboxyl group and are soluble in water (buffer) or alcohols (e.g., ethanol) which can be used to prepare standard solutions. Tocopherols are stable to heat, alkali, and acids up to 100°C in the absence of oxygen but decompose readily in the presence of oxygen. Oxidation is accelerated by traces of copper or iron or exposure to light. Thus, it is recommended to use solvents of high purity and control the purity and the content of stock solutions and standard dilution before use.

Due to their structural features, carotenoids are even more lipophilic than tocopherols. They are insoluble in water and even their solubility in methanol or acetonitrile is limited. This has to be taken into account when worked-up samples (e.g., dry residues after extraction and evaporation) are dissolved in mobile phases for HPLC analysis. There are striking differences with respect to the solubility of carotenes and xanthophylls [44]; the solubility of the more polar lutein in acetonitrile is 10-fold higher (100 mg/l) than β-carotene (10 mg/l). In contrast, the hydrocarbon carotenoid β-carotene is more solu-

Table 1
Extinction coefficients of tocopherols and carotenoids in organic solvents

Compound	ϵ (l mol^{-1} cm^{-1})	Solvent	Ref.
α-Carotene	150 100 at 444 nm	Light petroleum (b.p. 40–60°C)	[62]
	145 300 at 445 nm	Hexane	[62]
β-Carotene	138 900 at 450 nm	Light petroleum (b.p. 40–60°C)	[62]
	140 400 at 450 nm	Light petroleum (b.p. 40–60°C)	[62]
Lycopene	184 900 at 470 nm	Light petroleum (b.p. 40–60°C)	[62]
β-Cryptoxanthin	131 900 at 449 nm	Light petroleum (b.p. 40–60°C)	[62]
	135 700 at 450 nm	Hexane	[62]
Lutein	144 800 at 445 nm	Ethanol	[62]
Zeaxanthin	140 900 at 450 nm	Ethanol	[62]
	144 300 at 450 nm	Ethanol	[62]
	133 400 at 449 nm	Light petroleum (b.p. 40–60°C)	[62]
α-Tocopherol	3102 at 290 nm	Ethanol	[41]
γ-Tocopherol	3868 at 298 nm	Ethanol	[111]
α-CEHC	3230 at 289 nm	Ethanol	[43]
γ-CEHC	3620 at 297 nm	Ethanol	[43]

ble in hexane (600 mg/l) than lutein (20 mg/l). Chloroform, dichloromethane, and tetrahydrofuran are good solvents for xanthophylls and carotenes, suitable to obtain solutions above 1000 mg/l. However, these solvents may be contaminated with traces of hydrochloride or hydroperoxides which chemically react with carotenoids, leading to decomposition. Among the carotenoids described here, lycopene is the most difficult carotenoid to handle, being very sensitive to decomposition and isomerization when dissolved. Mixtures of dichloromethane and hexane are suitable solvents for the dissolution of the carotenoids of interest in human serum and tissue. They can be used to prepare stock solutions which can be diluted with an appropriate solvent to determine the concentration by UV–Vis spectroscopy. Selected extinction coefficients for the major blood carotenoids are listed in Table 1. As for vitamin E, it is recommended to control for the purity and content before use.

Isomerization of carotenoids leading to the interconversion of *cis*/*trans* (*E*/*Z*)-isomers (geometrical isomers) is a general problem in carotenoid analyses and may occur during sample processing but also during injection of the sample on the HPLC column. Carotenoids in their natural environment, incorporated into lipoproteins or membranes, are relatively resistant towards this process. In homogenous solution (e.g., in stock solutions or analytes dissolved in the extraction solvent) isomerization is induced by light, acids or traces of metal ions. Therefore, special care has to be taken when the isomer pattern of carotenoids is to be analyzed.

2.2. Sample purification

As mentioned above, the current method of choice to measure tocopherols and carotenoids quantitatively in serum, plasma and tissues is HPLC. Before application of HPLC, there is need for sample purification. This includes in most cases an extraction step with a lipophilic organic solvent to remove water-soluble components which might interfere with the chromatographic system. Before extraction of the lipophilic analytes, the samples are deproteinized by the addition of ethanol or other alcohols like propanol [45]. Together with the deproteinizing reagent, an internal standard may be added to the sample [23–25,32,42,46]. Deproteinization may be also achieved with a mixture of perchloric acid and ethanol [47], although acids with strong oxidative properties may destroy the analytes and induce isomerization. Before extraction, plasma samples may be further treated using lipase and cholesteryl esterase in order to hydrolyze non-polar lipids [31]; but this is a cumbersome procedure and usually not indicated in routine analysis.

The extraction of the analytes (tocopherols and carotenoids) from the samples with organic solvents requires a complete separation of the aqueous and

the organic phases, and therefore solvents are used which are not miscible with water. Hexane is the most common solvent applied for extraction, being efficient in extracting hydrocarbon carotenoids from serum [24,35,36,40,42,48] but xanthophylls not always completely [34]. To improve extraction efficacy, mixtures of organic solvents such as hexane–dichloromethane have been utilized. Halogenated solvents were also used in mixtures with methanol for the analysis of lipophilic antioxidants in subcellular fractions such as mitochondria or microsomes [47,49–51].

Tissue samples must be homogenized before extraction. In most cases this can be achieved using an ultraturrax in combination with a tissue homogenizer (Potter). Working up samples at 0–5°C (on ice) improves the stability of the analytes. Another possibility is homogenization of the frozen samples with a pestle under liquid nitrogen; in this case the sample has to be cut into small pieces before homogenization. The homogenized samples can then be extracted with the same organic solvent following the procedure for serum analysis [52]. In order to obtain homogenous samples from fat or skin tissue, alkaline saponification or enzymatic digestion should be applied. However, in most cases losses and/or isomerization of carotenoids may occur, since the digestion step requires longer incubation times at elevated temperatures (25–37°C).The addition of synthetic antioxidants during sample work-up may help to prevent or limit degradation of tocopherols and carotenoids.

2.3. High-performance liquid chromatography

2.3.1. Stationary phases

As mentioned above, HPLC is the method of choice for the analysis of tocopherols and carotenoids. Reversed phases with C_{18} residues are used predominantly in applications for the determination of tocopherols and carotenoids in human samples [23,26,27,31,32,53]. A number of methods have been described in the literature which are suitable for the routine analysis of human serum or plasma and which have been appropriately validated. With some of these methods, the simultaneous analysis of tocopherols and carotenoids is feasible. Typical chromatograms are shown in Figs. 3 and 4.

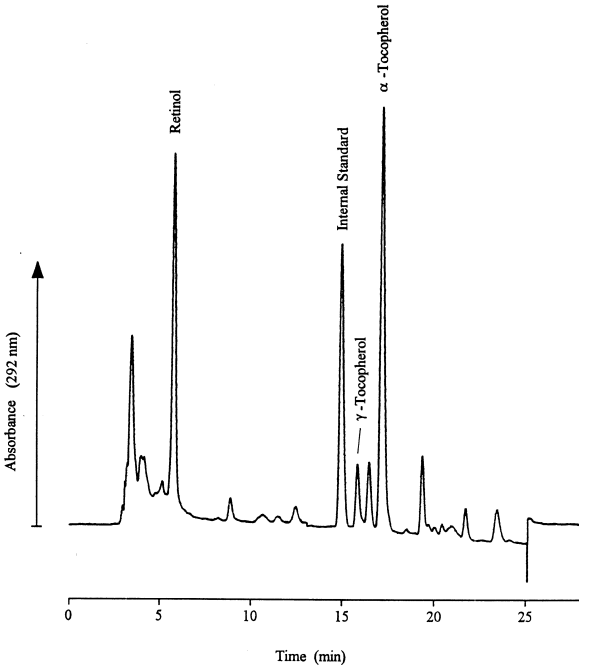

Fig. 3. Typical chromatogram of tocopherol analysis in human serum. Column: 25 cm×4.6 mm; stationary phase: Suplex pKb-100, 5 μm; ambient temperature; mobile phase: A, methanol–acetonitrile–2-propanol (54:44:2, v/v); B, methanol–acetonitrile–water–2-propanol (46:37:15:2, v/v); gradient (linear): 0–5 min A–B (64:36), 5–15 min 100% A, 15–27 min 100% A, 27–32 min A–B (64:36).

Other phases have been tested for specific applications, e.g., the analysis of geometrical isomers or oxidative metabolites of tocopherols and carotenoids [40,48]. The advantages of a nitrile-bound phase for the separation of geometric isomers and polar compounds in human breast milk were demonstrated by Khachik et al. [48]. It was pointed out that all forms of tocopherols and most carotenoids could be separated with a reversed phase, but the polar compounds especially the xanthophylls lutein and zeaxanthin and their geometrical isomers are better separated by a nitrile-bounded phase. These two separation systems were applied for the analysis of 34 carotenoids and the tocopherols [48].

Low resolution of lutein and zeaxanthin is in some cases observed with C_{18} reversed-phase (RP) HPLC [24,32,41,54]. Resolution may be improved using non end-capped material, as the non-derived silanol residues leave the stationary phase more polar [29].

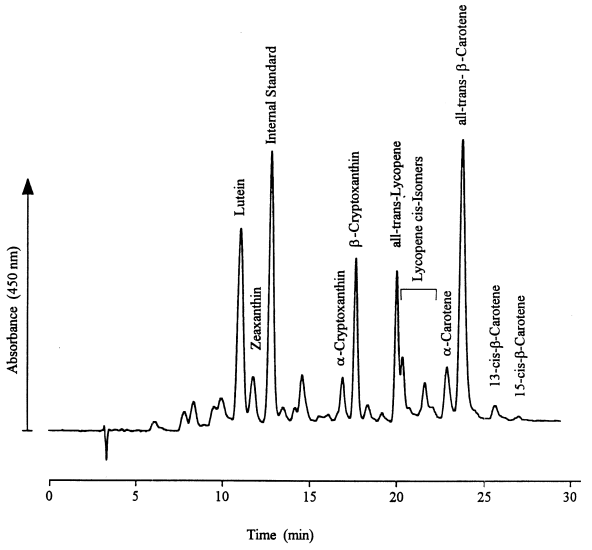

Fig. 4. Chromatogram of carotenoid analysis in human serum. Conditions: see Fig. 3.

On the other hand, end-capped materials provide better reproducibility of the assay and improve the separation of carotenoid geometrical isomers.

Sander and Wise [55] developed stationary phases with alkyl chain lengths between 20 and 30 carbon atoms. C_{30} columns were shown to be able to separate a number of geometrical carotenoid isomers [56]. A comparison between C_{30} and C_{18} phases for carotenoid separation has been previously described [56,57].

2.3.2. Mobile phases

Due to the polarity of carotenoids, the major constituents of the mobile phases are either acetonitrile, methanol or mixtures of these solvents. For the analysis of tocopherols on RP columns, mobile phases containing acetonitrile and/or methanol in combination with water are suitable. If electrochemical detection is applied, ionic compounds are added to the mobile phase [43]. A number of methods have been described using various mobile phases for the analysis of carotenoids [23,24,31,42,46,58,59]. Such variations refer mainly to the application of further solvents used as modifiers to optimize separation of specific carotenoids (e.g., lutein and zeaxanthin) or improve the separation of geometrical isomers, as for example *all-trans* and 9-*cis* β-carotene [60]. Water, hexane, chloroform, dichloromethane, tetrahydrofuran or various ethers are among these modifiers. A gradient may be applied to improve separation and analysis. For the analysis of the major carotenoids in human plasma, however, some isocratic methods are able to separate the compounds of interest within 30 min. Antioxidants are often added to mobile phases to stabilize solutions. A suitable antioxidant is 2,6-di-*tert*.-butyl-*p*-cresol (BHT) which is used at levels of 0.05 to 0.1%.

A careful preparation of the mobile phase is important. Since tocopherols and carotenoids are sensitive to oxidation, solvents should be degassed before use. In order to remove dissolved oxygen from the mobile phase, it is recommended to degas by bubbling nitrogen through the mixture.

2.3.3. Internal standard

Internal standards are usually applied in the analysis of tocopherols and carotenoids to improve reproducibility and accuracy in the quantitative assay. Complex purification steps may lead to losses or decomposition of analytes during extraction, aliquoting and separation. With the internal standard method, such interferences can be corrected for. Usually, the ratio between peak height or area of the analyte and that of the internal standard is used for quantitation [61]. Based on a regression curve calculated from calibration samples, the concentration of the analytes in the samples is calculated. An internal standard should be a definite, stable compound of high purity. It must exhibit similar properties with respect to extraction, chromatographic separation and detection and it should not interfere with the analytes or other signals from the sample matrix. It should be commercially available or otherwise easily to synthesize. A list of standards used in vitamin E and carotenoid analysis is given in Table 2.

2.3.4. Peak identification and detection

Tocopherols are usually detected either with UV or electrochemical detectors. Gaziano et al. [51] reported a method for detection of both tocopherols and carotenoids with electrochemical detection (HPLC–ED). Electrochemical detection is more sensitive but less suitable than UV detection for the simultaneous determination of the analytes. Carotenoids are commonly quantified with UV–Vis de-

Table 2
Internal standards used in analysis of tocopherols and carotenoids

Compound	Analysis of		Ref.
	Carotenoids	Tocopherols	
Tocopheryl acetate	x	x	[46]
	x	x	[28]
	x		[100]
	x	x	[101]
	x	x	[41]
	x	x	[36]
	x	x	[42]
Tocopheryl nicotinate	x	x	[35]
δ-Tocopherol		x	[102]
		x	[43]
Trolox		x	[96]
Echinenone	x		[24]
	x	x	[39]
	x		[103]
Retinyl acetate	x	x	[25]
	x	x	[104]
β-Apo-carotenal			[31]
O-Alkyl-oximes	x		
(3*R*)-8′-Apo-β-carotene-3,8-diol	x		[64]
Ethyl-β-apo-8′-carotenoate	x		[48]
Alkyl β-apo-8′-carotenoates	x		[105]
Decapreno-β-carotene (C-50)	x		[106]
	x		[107]
Dimethyl-β-carotene	x		[108]
β-Apo-8′-carotinol	x		[109]
Canthaxanthin-2,4-dinitrophenylhydrazone	x		[45]

tection, but it should be taken into account that they have different spectral absorption characteristics, including differences in the maxima ranging normally from 400 to 500 nm. Compounds of interest in serum and tissues can be normally detected at one wavelength, e.g., 450 nm [62]. Several specific carotenoids like phytofluene and phytoene, which also occur in human plasma and tissue, exhibit maximal absorption at considerably shorter wavelengths. Phytofluene and phytoene as tomato components are detected at 346 and 286 nm, respectively [63]. Retention times constitute the usual criteria of identification, compared to retention times of reference compounds, if available. Isolation or semi/total synthesis is otherwise required to obtain reference compounds [64]. Addition of the analyte of interest to a sample (''spiking'') is a useful technique to prove peak identity. Sometimes it is additionally useful to record online the UV–Vis spectrum of signals by means of photodiode array detection (DAD), which is of sufficient sensitivity [30]. In some cases, spectra of carotenoids (e.g., β-carotene and zeaxanthin or lutein and α-carotene) do not differ very much, so this technique is limited.

More specific methods may be applied for further identification, such as mass spectrometry (MS) or nuclear magnetic resonance spectroscopy (NMR) of isolated peaks [65,66]. A number of carotenoids, especially xanthophyll esters, have been identified by means of mass spectrometry after separation with HPLC [11]. NMR offers information on definite structures of stereoisomers, as applied for the assignment lutein and zeaxanthin isomers in bovine retina by Glaser et al. [67]. Advanced techniques like LC–MS are of increasing interest. The most widely applied coupling techniques are the electrospray (ESI) and the atmospheric pressure chemical ionization (APCI) techniques [68]. The separation and identification of a wide range of xanthophylls and of β-carotene isomers has been achieved by Lacker et

al. [69] using LC–MS in the APCI mode. An interesting method has been described by Careri et al. [70], who coupled high-resolution HPLC (two narrow-bore columns in series) with ESI-MS.

Labeled compounds like C^{13} or deuterated substances are preferentially used for metabolism studies. A novel double-tracer (hexadeuterated [$^2H_6$]β-carotene and [$^2H_6$]retinyl acetate) approach has been found to be adaptable for identifying efficient converters of carotenoid to retinoid [71] Mass spectrometry also provides a good tool to detect stable isotope labeled compounds as described by Duecker et al. [72], Liang et al. [73], and Yao et al. [74].

3. Plasma and tissue levels

The determination of lipophilic antioxidants in human samples contributes information to the antioxidant status of an individual and maybe useful for the evaluation of the nutritional status and the risk for degenerative diseases. Individual antioxidant profiles may also help to identify deficiencies and consequently initiate supply for prevention. The outcome of selected studies which made use of the determination of lipophilic antioxidants in context with the evaluation of human health and disease risk are discussed in this section.

With respect to compounds exhibiting antioxidant properties, epidemiological surveys have provided a large body of evidence that diets rich in specific antioxidants (especially from vegetables, fruits and vegetable oils) lower the relative risk of premature death from cardiovascular disease and cancer. The relative epidemiological risk seems to be lower at ''optimal'' antioxidant plasma levels: 30 μ*M* lipid-standardized vitamin E or more (α-tocopherol/cholesterol ratio≥5.1), 2.2 μ*M* vitamin A or more, 0.4 μ*M* β-carotene or more, and 0.4–0.5 μ*M* α- plus β-carotene or more. Antioxidant levels which are 25 to 35% below these levels are predictive for an at least twofold higher risk of developing disease. Low levels of any single antioxidant may increase the relative risk independently, and concomitant ''suboptimal'' levels of various antioxidants may act synergistically in further increasing the risk for a disease (for review, see Ref. [75]).

The profiles of lipophilic antioxidants in plasma from healthy subjects are usually comparable between studies, allowing the determination of a concentration range in which plasma levels of tocopherols and carotenoids might be considered ''normal'' (Table 3). Despite this, levels of tocopherols and carotenoids in plasma might show considerable inter-individual variation, likely due to geographical, seasonal, lifestyle and dietary factors. Thus the range might be relatively broad; 90% of the β-carotene levels determined in the USA are between 0.09 and 0.9 μmol/l. Smoking for example is a condition associated with free radical-induced damage to tissues and organs [76], and might significantly influence the lipophilic antioxidant status in healthy subjects [22].

For vitamin E, the estimated average requirement for adults has been established at 13 mg/day, and the current recommended dietary allowance in the USA is 15 mg/day [77]. Much higher intakes of vitamin E, however, have been discussed to be needed for disease prevention (for review, see Ref. [76]).

Disease states have also been shown to be associated with specific patterns of plasma antioxidants. A pronounced decrease of β-carotene plasma levels in comparison to α-tocopherol has been reported in patients with cystic fibrosis [78].

In patients with certain types of cancer, selected plasma antioxidants have been shown to be lowered more than others. Plasma levels of ascorbic acid, α- and β-carotene, cryptoxanthin, lutein/zeaxanthin, lycopene, α-tocopherol and selenium were measured in patients with lung cancer as compared to controls. Significant differences between groups were found for cryptoxanthin, lutein/zeaxanthin and β-carotene levels. Non-significant differences between patients and controls were found for α-carotene, whereas

Table 3
Lipophilic antioxidants in human plasma (from Sies et al. [110])

Antioxidant	Plasma content (μmol/l)
α-Tocopherol	15–40
γ-Tocopherol	3–5
α-Carotene	0.05–0.1
β-Carotene	0.3–0.6
Lycopene	0.5–1.0
Lutein	0.1–0.3
Zeaxanthin	0.1–0.2

lycopene, α-tocopherol and selenium were similar between lung cancer patients and controls [79]. Plasma contents of α-tocopherol and β-carotene were evaluated in women with uterine cervix dysplasia and cancer, and were both found to be significantly lower in patients as compared to matched controls [80]. In a case-control study of serum and tissue carotenoids in prostate cancer patients, significantly lower serum and tissue lycopene levels (44% and 78% less, respectively) were found in patients as compared to controls, whereas the other major carotenoids were not different between groups both in serum and prostate tissue [81].

Kontush et al. [82] have shown that among a variety of lipophilic antioxidants – α-tocopherol, γ-tocopherol, α-carotene, β-carotene and ubiquinol-10 – only α-carotene and γ-tocopherol were significantly lower in plasma from patients suffering from coronary heart disease as compared to controls. It should be noted that the determination of the antioxidant pattern in plasma is only one aspect in the evaluation of nutritional deficiencies or disease risk [82].

Disease- and/or nutrition-related antioxidant depletion may coexist, with a separation of the two being impossible without further information [22].

An important question regards the correlation between antioxidant plasma and tissue levels in order to estimate the antioxidant level at the target site from plasma analysis. For instance, positive relationships between plasma and skin carotenoid levels as well as between dietary carotenoid intake and plasma/tissue carotenoid levels exist and both are related to the prevention of erythema by carotenoids after UV-light exposure [16]. Freeman et al. [83] evaluated how prostatic levels of a number of antioxidants relate to plasma levels and to self-reported dietary intake. They observed that prostate levels of tocopherols and carotenoids, but not of retinol, are significantly correlated with plasma levels; the strongest association between tissue and plasma was reported for lycopene, β-carotene and γ-tocopherol. In this context it should be mentioned that a high intake of lycopene is associated with a diminished risk for prostate cancer [84].

A positive relationship was found in patients with age-related macular degeneration (AMD) between serum and macula lutea (yellow spot in the eye) concentrations of several carotenoids [85]. Increasing serum levels of lutein, the major carotenoid in the human macula lutea, were found to be positively correlated with optical density of the increasing macula lutea after carotenoid supplementation [86]. It has been suggested that lutein and zeaxanthin may play a role in the prevention of AMD via mechanisms related to their antioxidant properties [87].

4. Metabolites

Metabolites of specific antioxidants exhibit important biological activities. The most prominent example is retinol, which can be synthesized in vivo from its precursor β-carotene by 15,15′-dioxygenase [88]. There is increasing evidence that metabolites of other carotenoids and other antioxidants also exhibit biological activity which may be relevant in context with disease prevention. As mentioned below, decomposition products of carotenoids are capable of stimulating gap junctional communication and a major metabolite of γ-tocopherol shows natriuretic properties. Therefore, the determination of these metabolites is important to further discover and evaluate biological activities in comparison to the parent compound. Regarding active metabolites of carotenoids, among plasma carotenoids identified, only β-carotene has been extensively studied in different conditions [89–92], and there are data suggesting that further investigation on lutein and lycopene metabolites is strongly needed [93].

Several studies have been conducted on vitamin E metabolism, but knowledge in this field is still limited. These studies have led to important findings also object of debate, such as the fact that there is no difference between SRR-α-toc and RRR-α-toc in metabolic pathways [9,94]. A number of compounds including 2,5,7,8-tetramethyl-2(2′-carboxyethyl)-6-hydroxychroman (α-CEHC) and 2,7,8-trimethyl-2-(β-carboxyethyl)-6-hydroxychroman (γ-CEHC) have been detected in human plasma [43] and urine [95–97], which are metabolites of α- and γ-tocopherol, respectively. γ-CEHC has been described to exhibit natriuretic activity, apparently mediated by the inhibition of a potassium channel in the apical membrane of the thick ascending limb of the kidney, and has

been named LLU-α [98]. Furthermore, γ-CEHC, along with γ-tocopherol, inhibits cyclooxygenase activity in macrophages and epithelial cells, and it may therefore possess anti-inflammatory activity [99].

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