Dietary Phenolic Compounds and Vitamin E Bioavailability

Model studies in rats and humans

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An expert is a man who has made all the mistakes, which can be made, in a narrow field.

Niels Bohr (1885-1962)

If we knew what we were doing, it wouldn't be called research, would it?

Albert Einstein (1879-1955)
Abstract


The human diet contains a vast number of dietary phenolic compounds of which vitamin E represents only one class. Vitamin E is a generic name for all substances exerting the biological functions of β-tocopherol. The two quantitatively most important E vitamers are α- and β-tocopherol (α-T and β-T). The fat soluble vitamin E is absorbed and transported in the circulation to the liver where α-T is preferentially re-secreted into the bloodstream while the other vitamers are degraded by cytochrome P450 enzymes to the water-soluble carboxyethyl hydroxychroman (CEHC) metabolites excreted in the urine. Thus, α-T blood concentrations are usually 4-10 times higher than those of β-T. Vitamin E is mainly recognized to protect cell components from oxidative damage, but has also been reported to inter alia control gene expression and cellular signalling pathways.

This thesis aimed at investigating the effects of dietary phenolic compounds on the bioavailability of vitamin E in model studies. To this purpose, polyphenols were incorporated into standardized, semi-synthetic diets and fed to male Sprague-Dawley rats for 4 weeks. Blood plasma, liver and lung tissue concentrations of α-T and β-T were determined. The sesame lignan sesamin and cereal alkylresorcinols greatly increased the bioavailability of β-T, but not α-T, in all tissues. In contrast, the flaxseed lignan secoisolariciresinol diglucoside reduced the bioavailability of both tocopherols. The flavanols (+)-catechin and (-)-epicatechin and the preservative butylated hydroxytoluene (BHT) markedly enhanced the bioavailability of α-T in all analysed tissues. Curcumin and the tested anthocyanins and phenolic acids exerted only minor, inconsistent effects in different tissues in the rat model.

In order to study the impact of selected polyphenols on the enzymatic degradation of vitamin E, HepG2 cells were incubated together with phenolic compounds in the presence of tocopherols and the formation of metabolites was determined. Sesamin almost completely inhibited tocopherol side-chain degradation and cereal alkylresorcinols inhibited it, dose-dependently, by 20-80%. BHT and (+)-catechin had no effect on tocopherol-β-hydroxylase activity in HepG2 cells.

To verify the inhibition of β-T metabolism by sesame lignans in humans, sesame oil or corn oil muffins together with deuterated d6-β-T and d2-β-T were given to volunteers. Blood and urine samples were collected for 72 hours and analysed for deuterated and non-deuterated tocopherols and their metabolites. Consumption of sesame oil muffins significantly reduced the urinary excretion of d2-β-CEHC.

Overall, the findings from this thesis show that dietary phenolic compounds alter vitamin E bioavailability in humans and animals through various mechanisms.

Keywords: Bioavailability, blood, carboxyethyl hydroxychromans, CEHC, cells, cytochrome P450, CYP, HepG2, humans, livers, lungs, rats, tocopherols, tocopherol-β-hydroxylase, vitamin E.

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Sammanfattning


Konkreta rekommendationer angående intag av en bestämd mängd och form av vitamin E som kan skydda mot insjuknande kan inte ges i nuläget. Det grundar sig bl.a. på att E-vitaminets biotillgänglighet, dvs. mängden av en viss dos E vitamin som efter intag är tillgänglig för fysiologiska processer i kroppen påverkas av ett stort antal faktorer. Som exempel kan nämnas typen och mängden av samtidigt konsumerade fenoliska substanser (antioxidanter) och metabolismen av E-vitaminet till vattenlösiga metaboliter och deras utsöndring i urinen. Vår kost innehåller utöver E-vitamin ett stort antal fenoliska ämnen, framför allt växtsubstanser, som kan utöva en rad biologiska effekter i kroppen.

I denna avhandling har effekter av fenoliska substanser på E-vitaminets biotillgänglighet undersökts med hjälp av olika modellstudier. Rättor har matats med fenoliska ämnen och blodplasma, lever och lungor har analyserats för halten av de två viktigaste formerna av E-vitamin, α- och γ-tokoferol. Jag har identifierat ämnen som betydligt förbättrar E-vitaminets biotillgänglighet, t.ex. sesamin, en väsentlig beståndsdel av sesamfrö och sesamolja, alkylresorcinoler som förekommer i fullkorns-cerealier och flavanolerna (+)-catechin och (-)-epicatechin som finns i te, choklad och många frukter. I motsats till dessa substanser försämrade secoisolariciresinol diglukosid, en viktig beståndsdel av linfrö och linfröolja, biotillgängligheten av både α- och γ-tokoferol.


Resultaten av undersökningarna i denna doktorsavhandling visar att fenoliska substanser i maten kan påverka E-vitaminets biotillgänglighet med hjälp av ett antal olika mekanismer.
Zusammenfassung

Vitamin E ist ein Sammelbegriff für alle chemischen Verbindungen mit der biologischen Wirkung von $\alpha$-Tokopherol. Die acht natürlich vorkommenden Vitamin E-Verbindungen, $\alpha$-, $\beta$-, $\delta$- und $\gamma$-Tokopherol und $\alpha$-, $\beta$-, $\delta$- und $\gamma$-Tokotrienol, werden ausschließlich von Pflanzen gebildet und müssen daher dem Körper über die Nahrung zugeführt werden. Die Hauptfunktion von Vitamin E ist es, als Antioxidans andere Moleküle vor freien Radikalen zu schützen. Freie Radikale sind extrem reaktive Verbindungen, die im Körper als Nebenprodukte physiologischer Prozesse entstehen und Kettenreaktionen auslösen, die zur Zerstörung wichtiger Zellstrukturen, wie z.B. der Membranlipide, Proteine und DNS, führen. Aus diesem Grund wird dem Vitamin E eine schützende Rolle bei der Vorbeugung verschiedener chronischer Erkrankungen, wie z.B. Herzinfarkt, Schlaganfall und Krebs, zugesprochen, bei deren Entstehung freie Radikale vermutlich eine zentrale Rolle spielen. Darüber hinaus stützen epidemiologische Studien einen Zusammenhang zwischen einer hohen Vitamin E-Zufuhr sowie hohen Vitamin E-Blutspiegeln und einem verminderten Auftreten chronischer Erkrankungen.

Konkrete Empfehlungen bezüglich der Zufuhr einer bestimmten Menge und Form von Vitamin E die vor Erkrankung schützen kann, sind nach dem heutigen Stand der Forschung nicht verfügbar. Dies gründet sich u.a. darauf dass die Bioverfügbarkeit von Vitamin E, also diejenige Menge einer bestimmten Dosis die nach dem Verzehr dem Körper für biologische Prozesse zur Verfügung steht, von einer Vielzahl von Faktoren beeinflusst wird. Als Beispiele können hier u.a. gleichzeitig aufgenommene phenolische Verbindungen (Antioxidantien) oder der Abbau von Vitamin E zu wasserlöslichen Endprodukten und deren Ausscheidung mit dem Urin genannt werden. Die menschliche Nahrung enthält neben Vitamin E noch eine Vielzahl weiterer phenolischer Verbindungen, überwiegend pflanzlicher Herkunft, die im Körper eine Reihe biologischer Wirkungen entfalten können.

Die vorliegende Doktorarbeit beschäftigt sich mit den Auswirkungen von über die Nahrung zugeführten phenolischen Verbindungen auf die biologische Verfügbarkeit von Vitamin E unter Zuhilfenahme verschiedener Modellstudien. Hierzu wurden phenolische Verbindungen an Ratten verfüttert und anschließend die Gehalte der zwei quantitativ wichtigsten Vitamin E-Formen, nämlich $\alpha$- und $\gamma$-Tokopherol, im Blutplasma, Leber- und Lungengewebe bestimmt. Dabei wurden Verbindungen identifiziert die die biologische Verfügbarkeit von Vitamin E deutlich erhöhen, wie z.B. Sesamin, ein wesentlicher Bestandteil von Sesamsamen und -öl, Alkylresorcinole, die besonders in Vollkorngetreide enthalten sind, sowie die in Tee, Schokolade und vielen Früchten enthaltenen Flavanole (+)-Catechin und (−)-Epicatechin. Im Gegensatz zu diesen Stoffen verschlechterte Secoisolariciresinoldiglukosid, ein quantitativ bedeutender Bestandteil von Leinsamen und -öl, die Bioverfügbarkeit von $\alpha$- und $\gamma$-Tokopherol.

Weiterhin wurden die Auswirkungen von ausgesuchten Polyphenolen auf den enzymatischen Abbau von Vitamin E zu seinen wasserlöslichen Metaboliten in einem Zellmodell untersucht. Sesamin verhinderte hier die Umwandlung von $\gamma$-Tokopherol zu seinen Metaboliten fast vollständig, während die Alkylresorcinolcetine dies in Abhängigkeit von der verabreichten Dosis taten.

Die Hemmung des Metabolismus von $\gamma$-Tokopherol durch Sesamlignane wurde auch am Menschen untersucht. Der Verzehr von mit Sesamöl gebackenen Muffins führte zu einer deutlich geringeren Ausscheidung von $\gamma$-Tokopherol-Metaboliten im Urin.

Die Untersuchungsergebnisse dieser Doktorarbeit zeigen, dass phenolische Nahrungsbestandteile die biologische Verfügbarkeit von Vitamin E mittels einer Anzahl verschiedener Mechanismen beeinflussen können.
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Appendix

Papers I-VII
The present thesis is based on the following papers, which will be referred to by their Roman numerals.


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List of abbreviations

TAP  [a]-tocopherol-associated protein
[a]-TTP  [a]-tocopherol transfer protein
all rac  all racemic
AUC  area under the curve
BHT  butylated hydroxytoluene
CEHC  carboxyethyl hydroxychroman (vitamin E metabolite)
C<sub>max</sub>  peak concentration
CVD  cardiovascular disease
CYP  cytochrome P<sub>450</sub>
DNA  deoxyribonucleic acid
HDL  high density lipoprotein
HepG2  human hepatoblastoma cells
HMG-CoA  3-hydroxy-3-methylglutaryl coenzyme A
IDL  intermediate density lipoprotein
IU  international unit
LDL  low density lipoprotein
mRNA  messenger ribonucleic acid
oxLDL  oxidised LDL
PKC  protein kinase C
PXR  pregnane X receptor
RNA  ribonucleic acid
RNS  reactive nitrogen species
ROS  reactive oxygen species
SDG  secoisolariciresinol diglucoside
SPF  supernatant protein factor
SR-BI  scavenger receptor class B type I
T  tocopherol
T<sub>3</sub>  tocotrienol
t<sub>1/2</sub>  half life
t<sub>max</sub>  time to reach peak concentration
T[a]H  tocopherol-[a]-hydroxylase
VLDL  very low density lipoprotein
Vitamin E

In 1922, Herbert Evans and Katherine Bishop discovered what they called ‘factor X’, an essential factor for successful reproduction in rats (Evans & Bishop, 1922). Three years later, this ‘factor X’ was assigned its vitamin status and the letter E, being the next serial alphabetical designation after the preceding discovery of the vitamins A-D (Mason, 1977). A decade later, Evans and co-workers isolated an alcohol with the biological activity of vitamin E from wheat germ oil and proposed the name \( \alpha \)-tocopherol (Greek: tokos = child birth; phero = to bear; and -ol, indicating an alcohol) (Evans, Emerson & Emerson, 1936).

Structures and stereochemistry

Vitamin E is a generic name for all substances exerting the biological activity of \( \alpha \)-tocopherol (\( \alpha \)-T). The eight recognized natural vitamin E compounds (subsequently referred to as ‘vitamers’) consist of a chroman head substituted with a 16-carbon side-chain and are classified into tocopherols, with a saturated phytol side-chain, and tocotrienols, with an unsaturated isoprenoid side-chain with three isolated double bonds. The Greek letters \( \alpha \)-, \( \beta \)-, \( \gamma \)-, and \( \delta \)- are added as prefixes to denote the number and positions of methyl groups linked to the chroman head (Figure 1). The phytol side chain of the tocopherols has three chiral centers at positions 2, 4’, and 8’, which can be either in the R- or S-conformation, giving rise to eight different stereoisomers (RRR, RSR, RRS, RSS, SRR, SSR, SRS, and SSS) for each tocopherol. The tocotrienols have only one chiral center at position 2 and can therefore only be in R- or S-configuration. However, the double bonds at the 3’ and 7’ positions of the tocotrienol side-chain give rise to four cis/trans geometrical isomers. Hence, at least in theory, eight isomers are possible for each tocotrienol (Kamal-Eldin & Appelqvist, 1996).

Occurrence and dietary intake

Vitamin E is exclusively synthesised by photosynthetic organisms. Plants accumulate \( \alpha \)-T in their green tissues, while \( \beta \)-T and \( \gamma \)-T are mainly present in seeds, and tocotrienols are predominant in cereal grains and palm oil (Lampi, Kamal-Eldin & Piironen, 2002; Munne-Bosch & Alegre, 2002). The richest sources of vitamin E are vegetable oils, with wheat germ, safflower, and sunflower oils being particularly rich in \( \alpha \)-T, and soybean, corn, and sesame oils in \( \gamma \)-T. Other good sources of vitamin E include lipid-rich plant parts such as nuts, seeds and grains. The dietary intake of vitamin E in Western diets is mainly from fats and oils used in margarine, mayonnaise, salad dressings, and also from fortified foods such as breakfast cereals and fruit juices. In contrary to other Western populations where \( \alpha \)-T is the predominant form in the diet, \( \gamma \)-T is the major dietary form of vitamin E in the USA due to the widespread use of soybean and corn oils (Packer & Obermüller-Jevic, 2002). The naturally occurring tocopherols exist solely as RRR-stereoisomers. Synthetic tocopherols, on the other hand, are composed of an equimolar mixture of all eight stereoisomers, a so-called all...
racemic (all rac) mixture. The vitamer most frequently used in supplements and fortified foods is [-T (mostly all rac-[-]-T, but also RRR-[-]-T); often in the form of esters with acetate, succinate or nicotinate to improve its storage stability (Packer & Obermüller-Jevic, 2002).

![Chemical structures and methyl positions of the eight naturally occurring forms of vitamin E and their biological activities.](image)

<table>
<thead>
<tr>
<th>Common Name</th>
<th>R1</th>
<th>R2</th>
<th>Human plasma concentrations</th>
<th>Activity based on rat assay IU/mg</th>
<th>Relative to [-]-T</th>
</tr>
</thead>
<tbody>
<tr>
<td>[-]-Tocopherol</td>
<td>CH₃</td>
<td>CH₃</td>
<td>~25-32 [M]²</td>
<td>1.49</td>
<td>100</td>
</tr>
<tr>
<td>[-]-Tocopherol</td>
<td>CH₃</td>
<td>H</td>
<td>~-0.4 [M]³</td>
<td>0.75</td>
<td>50</td>
</tr>
<tr>
<td>[-]-Tocopherol</td>
<td>H</td>
<td>CH₃</td>
<td>~1.4-4.3 [M]²</td>
<td>0.15</td>
<td>10</td>
</tr>
<tr>
<td>[-]-Tocopherol</td>
<td>H</td>
<td>H</td>
<td>~-0.3 [M]²</td>
<td>0.05</td>
<td>3</td>
</tr>
<tr>
<td>[-]-Tocotrienol</td>
<td>CH₃</td>
<td>CH₃</td>
<td>n.d.⁴</td>
<td>0.75</td>
<td>50</td>
</tr>
<tr>
<td>[-]-Tocotrienol</td>
<td>CH₃</td>
<td>H</td>
<td>n.d.⁴</td>
<td>0.08</td>
<td>5</td>
</tr>
<tr>
<td>[-]-Tocotrienol</td>
<td>H</td>
<td>CH₃</td>
<td>n.d.⁴</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>[-]-Tocotrienol</td>
<td>H</td>
<td>H</td>
<td>n.d.⁴</td>
<td>?</td>
<td>?</td>
</tr>
</tbody>
</table>

Figure 1. Chemical structures and methyl positions of the eight naturally occurring forms of vitamin E and their biological activities.

¹One IU (international unit) is defined as the biological activity of 1 mg all rac-[-]-tocopheryl acetate (Hoppe & Krennrich, 2000).

²(Hensley et al., 2004).

³(Cooney et al., 2001).

⁴Tocotrienols are usually not detectable in plasma. Supplementation with 250 mg tocotrienols/day for 8 weeks did not raise their plasma concentrations above 1 [M] (O’Byrne et al., 2000).
Absorption, transport, and metabolism

On intake, the esterified vitamin is rapidly hydrolysed in the gut, thus releasing the free form. The intestinal absorption of vitamin E generally parallels the absorption of dietary fat (Figure 2). In humans, only a fraction, most likely ~15-45%, of the ingested vitamin E is absorbed and the remainder excreted with the faeces (Traber & Sies, 1996; Traber, 2000). The liver secretes bile acids into the small intestine to aid the digestion of lipids and the formation of mixed micelles. Although dietary fat is needed to aid the absorption of vitamin E, the amount of dietary fat is of minor importance and even low-fat diets grant a sufficient uptake of the vitamin (Parks & Traber, 2000). Integrated in micelles, vitamin E is taken up into the enterocytes by passive diffusion. Unlike other lipid soluble vitamins, vitamin E has no specific plasma transport protein. In order to be transported in the aqueous environment of the circulation, vitamin E is incorporated into a type of lipoprotein, the chylomicrons, which are secreted into the lymphatic system by the intestinal cells. The chylomicrons pass through the thoracic duct into the systemic circulation where they come in contact with lipoprotein lipase, an enzyme located on the surface of the vascular endothelium. Endothelial lipoprotein lipase decomposes the chylomicrons and transfers a fraction of the transported vitamin E to tissues. Vitamin E is also transferred from chylomicrons to high density lipoproteins (HDL) from where it can easily be distributed to all circulating lipoproteins. Chylomicron degradation ultimately results in the chylomicron remnants, which are taken up into the liver by a receptor-mediated process. Right to this point, the extent of vitamin E absorption and transport to the liver appears to be similar for all vitamers (Figure 2, Kayden & Traber, 1993; Traber & Sies, 1996; Traber, 2000).

Once vitamin E enters the liver, RRR-α-T is preferentially secreted into very low density lipoproteins (VLDL), facilitated by the action of a cytosolic α-tocopherol transfer protein (α-TTP) with pronounced selectivity towards the 2R-isomers (R-configuration at carbon 2; Figure 2). Hosomi and co-workers (1997) determined the affinities of α-TTP for some E-vitamers relative to that for RRR-α-T and found the following lower values: RRR-β-T, 38%; RRR-γ-T, 9%; RRR-δ-T, 2%; SRR-α-T, 11%; and α-tocotrienol, 12%. Interestingly, the affinity values for the tocopherols are comparable to their biological activities (Figure 1). Consequently, α-TTP was proposed as the determinant of the biological activity of the vitamers (Hosomi et al., 1997). The selective secretion of RRR-α-T into the blood stream does, at least partly, explain why α-T concentrations are usually 4-10 times higher than those of γ-T. Circulating VLDL, carrying relatively high amounts of RRR-α-T and significantly lower amounts of the other vitamers (including the non-RRR isomers of α-T), may transfer vitamin E to HDL, undergo conversion to low density lipoproteins (LDL), and/or return to the liver as VLDL remnants and do, thus, increase the RRR-α-T concentrations of all lipoproteins. Tissues with an LDL-receptor internalise LDL actively by a receptor-mediated process, which represents a major route of vitamin E delivery to peripheral tissues where vitamin E is mainly located in the lipid layer of biological membranes (Wang & Quinn, 2000). The mechanisms for vitamin E
Figure 2. Absorption, transport, and metabolism of \( \alpha \)- and \( \gamma \)-tocopherol in the body. Abbreviations used: CEHC, carboxyethyl hydroxychroman metabolites; \( \alpha \)-TTP, \( \alpha \)-tocopherol transfer protein; T, tocopherol; TH, tocopherol-[\( \gamma \)]-hydroxylase; VLDL, IDL, LDL, and HDL; very low-, intermediate-, low-, and high-density lipoproteins, respectively. Enzymes, transfer proteins, and membrane receptors are shown in italics, quantitatively major forms of tocopherols and their metabolites are shown in bold letters.
release from peripheral tissues are as yet unknown. Normal blood concentrations of \( \alpha\)-T and \( \gamma\)-T in humans have been reported to be in the range of 25-30 \( \mu \)M and 1.3-4.3 \( \mu \)M, respectively (Figure 1). In discarded human surgical tissues, the proportions of \( \alpha\)-T (as % of \( \gamma\)-T), 31% in adipose tissue, 33% in vein, 38% in muscle, and 53% in skin, were found to be appreciably higher than in blood (Burton et al., 1998). The non-\( \gamma\)-T vitamers that are retained in the liver are metabolised and excreted (Traber, 2000; Packer & Ostermüller-Jevec, 2002; Hensley et al., 2004).

The lipid-soluble vitamin E is degraded to water-soluble carboxyethyl hydroxychroman (CEHC) metabolites by side-chain degradation (Figure 3) without modification of the chromanol head (Sontag & Parker, 2002). CEHC’s are conjugated with glucuronic acid or sulphate to increase their solubility and excreted in the urine. Although the exact location of vitamin E-metabolism has not been determined yet, hepatocytes are likely to play a central role. It has been shown that human hepatoblastoma cells (HepG2) and rat primary hepatocytes as well as human and rat liver microsomes convert vitamin E to CEHC’s (Parker, Sontag & Swanson, 2000; Birringer, Droger & Brigelius-Flohe, 2001; Birringer et al., 2002; Brigelius-Flohe et al., 2002b; Sontag & Parker, 2002). The first step in the metabolism of tocopherols and tocotrienols consists of a terminal \( \beta\)-hydroxylation of the side-chain by cytochrome P_{450} (CYP) isozymes (most likely CYP4F2, but a role for CYP3A has also been proposed) followed by a stepwise shortening of the tail by \( \beta\)-oxidation (Figure 3, Parker, Sontag & Swanson, 2000; Birringer et al., 2002; Sontag & Parker, 2002) similar to that of saturated and unsaturated fatty acids (see biochemistry textbooks for details). In vitro, tocopherol-\( \beta\)-hydroxylase, the enzyme that initiates vitamin E metabolism, showed similar binding affinities for \( \gamma\)-T and \( \alpha\)-T, but exhibited much higher catalytic activity towards \( \gamma\)-T, suggesting a central role for this enzyme in the selective retention of \( \gamma\)-T in the body and the regulation of \( \gamma\)-T plasma concentrations (Sontag & Parker, 2002). This notion is further supported by findings in human subjects showing that up to ~50% of the ingested \( \gamma\)-T is excreted in urine as the corresponding \( \gamma\)-CEHC metabolite (Swanson et al., 1999), while only 1-3% of the consumed \( \gamma\)-T dose is converted to urinary \( \gamma\)-CEHC (Schuelke et al., 2000). Furthermore, when Traber and co-workers compared the urinary excretion of deuterated \( \gamma\)-CEHC derived from RRR-\( \gamma\)-T and all rac-\( \gamma\)-T, they found 2-4 times more “all rac”-metabolites (Traber, Elsner & Brigelius-Flohe, 1998). Previously, secretion into the bile was proposed to be the major route of vitamin E elimination (Kayden & Traber, 1993), but evidence from a rat study, together with the recent discovery of the degradation and urinary excretion of the vitamin suggests that biliary excretion only plays a minor role (Yamashita, Takeda & Ikeda, 2000; Sontag & Parker, 2002). Likewise, only a small fraction of the vitamin E secreted in bile is reabsorbed during enterohepatic circulation while the remainder is excreted in faeces (Lee-Kim et al., 1988).
Figure 3. Side-chain degradation of tocopherols to their 3'- and 5'-carboxychromanol metabolites.
Functions of vitamin E

When Evans and Bishop studied the duration of the oestrous cycle in response to dietary changes in laboratory rats, they discovered that the absence of the then unknown ‘factor X’, later designated vitamin E, resulted in foetal death and resorption (Evans & Bishop, 1922). During the following years, a multitude of vitamin E deficiency syndromes were described in various species (Mason, 1977), but no specific function could be ascribed to the vitamin. Decades later, the antioxidant activity of $\alpha$-T was discovered and assumed to be its major function in vivo (Kamal-Eldin & Appelqvist, 1996; Brigelius-Flohé & Traber, 1999). Recently, other biological functions of vitamin E, unrelated to its antioxidant properties, have been discovered. These include roles in cellular signalling, gene expression, immune response, and apoptosis, and are now considered to be of importance (Azzi, Ricciarelli & Zingg, 2002; Brigelius-Flohé et al., 2002a).

Protection against free radicals as part of the antioxidant network

According to the definition by Barry Halliwell (1996), “A free radical is any species capable of independent existence…that contains one or more unpaired electrons, that is, one that is alone in an orbital.” Free radicals (reactive oxygen species (ROS) like superoxide, hydroperoxide, peroxyl and hydroxyl radicals, and reactive nitrogen species (RNS) like nitric oxide, etc.) are constantly produced in the body as a result of physiological processes, such as xenobiotic metabolism, aerobic respiration in mitochondria or disposal of infected cells by phagocytes (Ames, Shigenaga & Hagen, 1993). Once formed, radicals rapidly react with macromolecules (e.g. poly-unsaturated fatty acids, lipoproteins, proteins, carbohydrates, RNA, DNA, etc.), thus starting self-propagating radical chain reactions, which alter or destroy the structure and function of important cell components. Alternatively, reactive species may react with other free radicals to form stable products or be scavenged by antioxidants, thus being transformed into non-radical species, while the antioxidants become ‘antioxidant-radicals’, which are much less reactive and do not efficiently attack adjacent macromolecules (Halliwell, 1996).

The excess formation of free radicals, caused by an imbalance of oxidative and antioxidative processes, leads to oxidative stress (Sies, 1997), which is believed to be at the basis of many degenerative diseases, such as atherosclerosis, cardiovascular disease (CVD), stroke, cancer, arthritis, and Alzheimer’s disease (Davies, 1995). It has also been suggested that oxidative stress may not only be a result but also a cause of diabetes mellitus type II and CVD (Ceriello & Motz, 2004). The body defends itself against oxidative damage through an antioxidant network in which vitamin E plays a central role (Packer & Obermüller-Jevic, 2002). $\alpha$-T is the major lipid-soluble, chain-breaking antioxidant in human plasma (Burton, Joyce & Ingold, 1982) preventing the progression of free radical reactions and lipid peroxidation, thereby protecting lipoproteins and biological membranes (Kamal-Eldin & Appelqvist, 1996; Brigelius-Flohé & Traber, 1999; Packer & Obermüller-Jevic, 2002). The excellent antioxidant properties of vitamin E, which vary in degree for its different vitamers, are due to the rapid abstraction
of a phenolic hydrogen from the hydroxyl group at the chromanol head during the reaction with free radicals (Kamal-Eldin & Appelqvist, 1994; Kamal-Eldin & Appelqvist, 1996; Packer & Oermüller-Jevic, 2002). The membrane-bound tocopheroxyl radical (at the surface of the lipid-water interface) is then reduced back to tocopherol by ascorbate (vitamin C) in the aqueous phase (Packer, Slater & Willson, 1979). The ascorbyl radical is, in turn, regenerated to ascorbate by thiol (e.g. glutathione, dihydrolipoic acid, or thioredoxin) or polyphenol (e.g. flavonoids) antioxidants. The thiol antioxidants, eventually, are recycled by the conversion of NAD(P)H+H\(^+\) to NAD(P)\(^+\). This concept is likely to apply to the tocotrienols as well, although the relevance will be limited, due to the low concentrations observed in tissues (Packer & Oermüller-Jevic, 2002).

The differences in reactivity of the vitamers in vitro can be explained by two main factors, namely inductive effects caused by electron-releasing substituents in ortho- and/or para-positions to the phenolic hydrogen and stereo-electronic effects due to the orientation of these substituents towards the aromatic plane. The presence of more methyl groups, especially in ortho- and para-positions, enhances the antioxidant activity. Hence, \(\alpha\)-T with its two ortho-methyl groups is expected to be a better hydrogen donor than \(\gamma\) and \(\delta\)-T with only one ortho-methyl group each, which are expected to be better antioxidants than \(\beta\)-T with no ortho-methyl substituent (Kamal-Eldin & Appelqvist, 1996). This concept of relative reactivity becomes even more complicated when applied to the situation in vivo. In the body, antioxidant activity is not only determined by chemical reactivity, but also by compartmentalisation and the kinetics of absorption, transport, metabolism, and excretion. As a result of the chemical and biological characteristics described above, the two basic requirements for a good in vivo chain-breaking E-vitamer are a fully methylated phenolic ring and stereochemistry with a 2R-configuration (Kamal-Eldin & Appelqvist, 1996).

Reactive nitrogen species, in particular nitric oxide, are formed endogenously by inter alia macrophages and endothelial cells and occur in large amounts in cigarette smoke (Cooney et al., 1993). Cooney’s group demonstrated that \(\gamma\)-T is much more efficient than \(\alpha\)-T in the detoxification of nitrogen dioxide (Cooney et al., 1993). The superiority of \(\gamma\)-T to \(\alpha\)-T in the disposal of RNS has been confirmed in subsequent experiments (Cooney et al., 1995; Christen et al., 1997). In contrast to the structural requirements for the scavenging of ROS where the unsubstituted 5-position of \(\gamma\)-T is a disadvantage, this structural feature promotes the nitration of \(\gamma\)-T to form 5-nitro-\(\gamma\)-T. The nitration of \(\alpha\)-T is not possible because of the methyl substituent at carbon 5 (Hensley et al., 2004). RNS are known contributors to carcinogenesis (Hofseth et al., 2003), therefore \(\gamma\)-T may play a specific role in cancer prevention that cannot be assumed by \(\alpha\)-T (see below).
Non-antioxidant functions

In addition to its important role as a free radical scavenger, vitamin E has recently been recognised to affect cellular signalling, gene transcription, and enzyme activity, in a manner independent of its antioxidant properties.

α-T, specifically, inhibits smooth muscle cell proliferation and platelet aggregation via the inhibition of protein kinase C (PKC). PKC inhibition by α-T has been observed in a number of different cell types (monocytes, macrophages, neutrophils, fibroblasts, and mesangial cells) and is mediated by dephosphorylation of the enzyme via an activation of protein phosphatase 2A. α-T also decreases the release of the pro-inflammatory cytokine interleukin-1β via the inhibition of the 5-lipoxygenase pathway (Azzi et al., 2000; Azzi & Stocker, 2000; Ricciarelli, Zingg & Azzi, 2002; Rimbach et al., 2002).

The regulation of gene transcription by α-T has been reported for several proteins. α-T up-regulates the expression of α-tropomyosin and inhibits liver collagen α1 gene expression. In rat liver cells, the expression of α-TTP and its mRNA are modulated as a result of vitamin E deficiency. α-T down-regulates the expression of the scavenger receptors class A and CD36 in macrophages and smooth muscle cells at a transcriptional level (Azzi et al., 2000; Azzi & Stocker, 2000; Ricciarelli, Zingg & Azzi, 2002; Rimbach et al., 2002).

In animal and human studies, dietary tocotrienols (T3) lowered blood concentrations of lipids, particularly cholesterol. Experiments in cell cultures revealed that α-T3 reduces the endogenous synthesis of cholesterol by inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, a rate-limiting enzyme in the biosynthesis of cholesterol. The reduction in enzyme activity and the hypcholesterolemic effect of α-T3 was attenuated if α-T was co-administered (Qureshi et al., 1997; Khor & Ng, 2000; Packer, Weber & Rimbach, 2001). Recently, supernatant protein factor (SPF), a protein stimulating cholesterol biosynthesis, was shown to be identical with α-tocopherol-associated protein (TAP). SPF/TAP binds α-T, translocates to the nucleus and activates gene expression. Thus, α-T was suggested to affect cholesterol homeostasis via SPF/TAP and the down-regulation of scavenger receptors (Porter, 2003).

Vitamin E in health and disease

A multiplicity of disorders, such as atherosclerosis, stroke, heart disease, cancer, rheumatoid arthritis, Alzheimer’s disease, Parkinson’s disease, diabetes mellitus type I and II, and even obesity, to name a few, have been proposed to result from or to result in an excess formation of free radicals (Davies, 1995; Keaney et al., 2003; Maritim, Sanders & Watkins, 2003; Ceriello & Motz, 2004). Therefore, it is a common belief that antioxidants, which are capable of detoxifying free radical species, may be helpful in the prevention and/or treatment of these conditions. Also, the non-antioxidant functions of vitamin E give rise to a wide array of potential health effects. Discussing the role of vitamin E with respect to the pathophysiology of all of the aforementioned disorders would by far exceed the
scope of this introduction. For this reason, only two major diseases causing death and morbidity that are frequently discussed in connection with vitamin E, namely atherosclerosis and cancer, are described below.

**Vitamin E and atherosclerosis**

Atherosclerosis is a disorder affecting the arteries by thickening of the arterial wall, thus causing narrowing and loss of elasticity of the blood vessels and ultimately leading to thrombus formation and obstruction, thereby causing stroke, CHD and the like. In their ‘oxidation hypothesis of atherosclerosis’, Steinberg and colleagues suggest a central role for oxidised LDL (oxLDL) in the aetiology of atherosclerosis (Steinberg et al., 1989). Some key events in the development of atherosclerosis are (i) entrapment and oxidative modification of LDL (initiated by free radicals and macrophages) in the endothelial intima, (ii) uptake of oxLDL by macrophages via scavenger receptors, subsequently transforming them into foam cells, (iii) smooth muscle cell proliferation (induced by oxLDL), and (iv) platelet adhesion and aggregation (leading to obstructive thrombus formation) (Steinberg et al., 1989; Berliner & Heinecke, 1996). 

α-T has been shown (1) to protect LDL particles from oxidation, (2) to down-regulate the expression of macrophage scavenger receptors, thus reducing the uptake of oxLDL, (3) to inhibit smooth muscle cell proliferation and (4) to reduce platelet adhesion and aggregation; (3) and (4) are facilitated by inhibition of PKC activity (Azzi, 2002).

There is increasing evidence that γ-T may play a special role in the prevention of atherosclerosis. A diet rich in γ-T (containing minor amounts of other E vitamers) was more effective than α-T alone in the prevention of iron-induced lipid peroxidation and occlusive thrombus formation in a rat model (Saldeen, Li & Mehta, 1999). Hensley and co-workers confirmed these results and proposed the superiority of γ-T in the detoxification of RNS to be at the basis of this effect, supported by a clear association of thrombus formation and the appearance of 5-nitro-γ-T in the circulation (Hensley et al., 2004). In line with these findings, reduced blood concentrations of γ-T, but not α-T, have been found in patients suffering from coronary heart disease and myocardial infarction (Öhrvall, Sundlöf & Vessby, 1996; Kristenson et al., 1997; Kontush et al., 1999; Ruiz Rejón et al., 2002). However, high α-T concentrations were associated with a lower risk of ischemic heart disease (Gey et al., 1991).

The outcome of prospective studies assessing the effects of dietary supplementation with γ-T on cardiovascular events are inconclusive and have been reviewed elsewhere (Jialal & Devaraj, 2002; Stocker et al., 2002). It may suffice to say that, despite initial positive reports associating a high intake of vitamin E with a reduction in CHD risk (Rimm et al., 1993), the results from clinical supplementation trials, reporting positive, negative, or no effects, have been disappointingly inconsistent and do not allow for recommendations regarding the supplementation of vitamin E with regard to atherosclerosis prevention (Jialal & Devaraj, 2002; Stocker et al., 2002).
Vitamin E and cancer

Although conclusive evidence from human intervention trials with vitamin E is still lacking, some promising findings suggest a protective role of supplemental \( \alpha \)-T on the incidence of and death from prostate cancer in smokers (Heinonen et al., 1998; Chan et al., 1999). In support of these data, observational studies found an association between high vitamin E blood concentrations, especially of \( \gamma \)-T, and a reduced incidence of prostate cancer (Giovannucci, 2000; Helzlsouer et al., 2000). Similarly, patients suffering from cancer of the upper aero-digestive tract had significantly lower \( \gamma \)-T plasma levels than comparable controls (Nomura et al., 1997). Gysin and colleagues (2002) found that \( \gamma \)-T, more so than \( \alpha \)-T or \( \beta \)-T, inhibited the growth of prostate and colon cancer cells. In confirmation of these findings, \( \gamma \)-T and also its metabolite \( \gamma \)-CEHC inhibited prostate cancer cell proliferation by \( \geq 75\% \), while the respective \( \alpha \)-forms only reduced cell growth by \(< 50\% \) (Galli et al., 2004). In contrast to the multitude of in vitro and in vivo data supporting a protective role for vitamin E, especially \( \gamma \)-T, on prostate cancer in smokers, Schwenke, in an extensive review of the breast cancer risk and its relation to vitamin E, concluded that the scientific literature provides only modest evidence for a protective effect (Schwenke, 2002). A variety of mechanisms have been proposed to explain how vitamin E might exert its beneficial effects on cancer. These concepts include: protection of DNA from oxidative modification by free radicals, detoxification of RNS, inhibition of tumour cell growth through cell cycle arrest and apoptosis, and enhanced elimination of cancer cells by stimulation of the immune system (Jiang et al., 2001; Kline et al., 2003).

Interactions of vitamin E with xenobiotic metabolism

In HepG2 cells, all tocopherols and tocotrienols (T) activate the nuclear receptor PXR (pregnane X receptor). PXR regulates the expression of a variety of drug metabolising enzymes, including cytochrome \( \text{P}_{\text{450}} \) isozymes. The activation of PXR by vitamin E analogues followed the order \( \gamma \)-T1>\( \gamma \)-T3>\( \gamma \)-T2>\( \alpha \)-T1 in vitro. \( \gamma \)-T3 up-regulated CYP3A mRNA to a similar degree as rifampicin, a known inducer of PXR and CYP3A (Landes et al., 2003). It was mentioned earlier that initial \( \omega \)-hydroxylation is a key step in vitamin E metabolism and that the CYP isozymes 3A and 4F2 were proposed to potentially catalyze this reaction (Parker, Sontag & Swanson, 2000; Sontag & Parker, 2002). CYP3A metabolises more than 50% of the drugs currently used for therapy (Cholerton, Daly & Idle, 1992; Kliewer, Goodwin & Willson, 2002). Hence, by means of induction of drug metabolising enzymes, vitamin E may enhance its own elimination, but also the clearance of therapeutic drugs and other xenobiotics, posing a possible explanation for unexpected negative outcomes in clinical trials where drugs were co-administered with vitamin E (Traber, 2004).
Dietary phenolic compounds – contribution to human health

Occurrence and dietary intake

Vitamin E is only one of the many classes of phenolic compounds in the diet. The vast majority of dietary phenolic compounds, often referred to as polyphenols, originate from plant foods (Scalbert & Williamson, 2000). In addition, synthetic phenols are frequently added as preservatives to lipid rich foodstuffs (Leclercq, Arcella & Turrini, 2000). In plants, phenolic compounds fulfil essential physiological purposes, such as protecting from ultraviolet radiation, pathogens and predators, contributing to their colour and flavour, and facilitating growth and reproduction (Bravo, 1998; Harborne & Williams, 2000; Heim, Tagliaferro & Bobilya, 2002). Several thousand of these natural compounds have been identified in plants, with a large diversity in their structural features (Harborne & Williams, 2000). These may be grouped into classes according to the shared structural characteristics of their carbon skeletons. The main classes of natural polyphenols comprise phenolic acids and derivatives, flavonoids, lignans, and stilbenes (Figure 4), as well as tannins and lignins (Shahidi & Naczk, 2003).

Figure 4. Basic chemical structures of important subclasses of polyphenols.
Phytochemicals are synthesized in the secondary metabolism of plants, therefore sometimes called ‘secondary plant metabolites’, and stem from two major synthetic pathways: the shikimate and the acetate pathway (Bravo, 1998). All plant phenolic compounds share one common feature, namely an aromatic ring with at least one hydroxyl substituent, but may vary greatly in their complexity from simple phenols to the highly polymerized tannins and lignins. They occur predominantly as conjugates with sugars (mono-, di-, or oligosaccharides), with glucuronic or galacturonic acids, or even with other phenols that are linked to hydroxyl groups or, less frequently, aromatic carbon atoms. The principal sugar residue is glucose while others, e.g. galactose, rhamnose, xylose or arabinose residues, are also encountered (Bravo, 1998). The structural diversity of phenolic compounds results in a plethora of phytochemicals ingested by man. It would be almost impossible to describe them all, hence, only those classes of phenolic substances that are abundant in the human diet and/or may exert important effects on human health are discussed here.

**Phenolic acids**
The hydroxycinnamic acids and their derivatives are the most important subclass of phenolic acids, but benzoic acid derivatives and hydrolysable tannins (polymers of gallic and ellagic acids) are also present in foods. Some common hydroxycinnamates are $p$-coumaric, ferulic, sinapic, and caffeic acids; the latter is thought to be the most abundant in the diet (Clifford, 2000). Phenolic acids exist primarily as conjugates of e.g. sugars, polysaccharides, or organic acids, whereas the free forms are less frequently observed in nature. The quantitatively most important conjugate of caffeic acid is its ester with quinic acid, 5-caffeoylquinic acid (also known as chlorogenic acid). Phenolic acid conjugates are ubiquitously distributed in the plant kingdom, e.g. in fruits and vegetables. Especially high concentrations are found in coffee, apples, citrus fruits and juices, and the bran of cereal grains. Excessive coffee drinkers, may achieve a daily consumption of phenolic acids in excess of 1 g (Clifford, 2000). The intake of caffeic acid alone was reported to be up to 983 mg per day in a southern German population, but also as low as 5 mg per day in some individuals. However, the mean intake of phenolic acids in this population was 222 mg/d (Radtke, Linseisen & Wolfram, 1998).

**Flavonoids**
In 1937, the group of Szent-Györgyi observed that certain flavonoids increased the biological activity of ascorbic acid and could even heal scorbutic pigs and, therefore, introduced the term ‘vitamin P’ for flavonoids (Bentsath, Rusznyak & Szent-Györgi, 1937). However, the essentiality of flavonoids for humans or animals has never been proven and, therefore, the classification as a vitamin was never warranted (Kühnau, 1976). Nevertheless, flavonoids exert a number of health effects that may justify a semi-essential status for these compounds (Lampe, 1999), as will be discussed below.
The flavonoids are the most abundant class of dietary phenolic substances and, as of 1999, more than 6400 different flavonoids have been identified (Harborne & Williams, 2000). The basic structural feature of all flavonoids is the flavane (2-phenyl-benzo[2][pyrane]) nucleus, a system of two benzene rings (A and B) linked by an oxygen-containing pyrane ring (C; Figures 4 & 5) (Kühnau, 1976). According to the degree of oxidation of the C-ring, the hydroxylation pattern of the nucleus, and the substituent at carbon 3, the flavonoids can be categorized into the subclasses flavones, isoflavones, flavanols (catechins), flavonols, flavanones, anthocyanins, and proanthocyanidins (Figure 5) (Scalbert & Williamson, 2000). Some flavonoids, for example the flavonol quercetin, are widely spread in edible plants, while others, e.g. the soy isoflavones genistein and daidzein, are restricted to certain foodstuffs. Flavonols, flavanols, and anthocyanins are abundant in the human diet, while flavones and isoflavones are less common (Scalbert & Williamson, 2000). In the Netherlands, Hollman & Katan (1999) found an average consumption of flavonols and flavones of 23 mg/d and Arts and co-workers (2001) estimated the average daily intake of catechins to be 50 mg. In a Japanese city, the mean intake of isoflavones was 39 mg/d (Kimira et al., 1998).

Lignans

Plant lignans are a large class of phytochemicals that are formed by fusion of two coniferyl alcohol residues and are structurally related to the lignins present in plant cell walls. Lignans can be found throughout the plant kingdom, predominantly in foodstuffs such as cereals, nuts, and seeds, where they occur as glycosides or in free form (Mazur, 2000). Flaxseeds, with a content of 1-4% by weight, are one of the richest dietary sources of the plant lignan secoisolariciresinol (Johnsson et al., 2000; Eliasson et al., 2003). The average daily consumption of total plant lignans in Finland was reported as 434 mg/d, where 396 mg/d was secoisolariciresinol and 38 mg/d was matairesinol (Valsta et al., 2003). Sesame seeds and oils contain significant amounts of the lignans sesamin (up to 1.1% by weight in oil) and sesamolin (up to 0.6% by weight in oil) (Kamal-Eldin & Appelqvist, 1994). During the refining of sesame oils, sesamin is partially transformed to episesamin while sesamolin is transformed to sesaminol and episesaminol (Fukuda et al., 1994).
Absorption and metabolism

The exact fate of ingested polyphenols in the digestive tract, including their absorption and metabolism, remains largely unknown. Research in this area has produced conflicting results, which are subject to debate. The current knowledge in this regard may be briefly summarized as follows. Ingested polyphenols enter the digestive system primarily in form of glycosides, although some aglycones may be present. The glycosides may then be de-conjugated by the action of non-specific \(\beta\)-glucosidases, present in the food itself or on the surface of or inside of mucosal cells (Day et al., 2000; Aherne & O'Brien, 2002). Both aglycones and glycosides have been reported to be absorbed. The conjugates are more hydrophilic than the aglycones and the removal of the hydrophilic moiety appears to be a requirement for the passive diffusion across the intestinal mucosa (Scalbert & Williamson, 2000; Aherne & O'Brien, 2002). It was also suggested that the intestinal sodium-glucose transporter might carry phenolic glucosides through the intestinal cell.
wall. This has, however, not been proven in vivo (Aherne & O’Brien, 2002). The polyphenols undergo extensive metabolism, mainly conjugation reactions, during their passage through the enterocytes, e.g. O-methylation and/or conjugation with glucuronides and/or sulphates. Certain transporter proteins (e.g. multidrug resistance-associated protein-2) in the enterocytes may actively transfer the glycosides back into the intestinal lumen. Absorbed polyphenols are transported in the circulation and reach the liver via the portal vein. In the liver, they are metabolised or secreted into the bile (Aherne & O’Brien, 2002). Un-absorbed or re-excreted polyphenols reach the large intestine where they may undergo metabolism to more simple compounds by the colonic microflora and the degradation products (e.g. phenolic acids in the case of flavonoid metabolism) may be absorbed by passive diffusion. The polar, and therefore water-soluble, polyphenol glucuronides and sulphates that escaped biliary excretion and enterohepatic circulation are eliminated from the body by urinary excretion (Scalbert & Williamson, 2000; Aherne & O’Brien, 2002; Murota & Terao, 2003; Spencer, 2003).

Following the ingestion of plant phenols, either as pure compound or as part of a test meal, substantial amounts of conjugated metabolites, and sometimes of the unconjugated compound, have been detected in human blood (Scalbert & Williamson, 2000). Hollman et al. (1997) reported the sum of quercetin aglycones and metabolites in blood after the consumption of onions (containing 68 mg quercetin) to reach a maximum of 0.74 μM. For a detailed overview over trials reporting blood concentrations of flavonoids, the interested reader is referred to the review article by Scalbert & Williamson (2000). Nardini and colleagues (2002) detected 91 ng/mL (~0.5 μM) caffeic acid (sum of conjugates and free form) in blood plasma of volunteers 1 hour after they drank 200 mL of coffee.

**Biological activities and health implications**

Epidemiological studies provide evidence for a protective role of a diet rich in vegetables, fruits, and wholegrain cereals against a range of degenerative diseases including certain cancers, cardiovascular diseases, and diabetes mellitus (Lampe, 1999; Segasothy & Phillips, 1999). Because of the proposed involvement of free radical species in the aetiology of degenerative disorders (see above), the major focus of research in the past has been on the effects of plant foods and compounds isolated from them on the antioxidant defence system (Lampe, 1999). The structural requirements for efficient antioxidant function of flavonoids and phenolic acids have been reviewed (Rice-Evans, Miller & Paganga, 1996). It was also suggested that, owing to their one-electron reduction potentials, polyphenols may spare endogenous antioxidants similar to the recycling of vitamin E by ascorbic acid (Buettner, 1993). Phenolic acids, for example, have been reported to efficiently scavenge free radicals in various model systems (Laranjinha, Almeida & Madeira, 1994; Chen & Ho, 1997), to delay lipid oxidation, spare vitamin E, and to regenerate tocopherol from its tocopheroxyl radical in human LDL, erythrocyte membrane ghosts, and monocytic cells (Laranjinha et al., 1995; Nardini et al., 1995; Nardini et al., 1998; Laranjinha & Cadenas, 1999; Liao & Yin, 2000). In a rat model, caffeic acid spared vitamin E and enhanced the resistance of LDL
towards oxidative stress (Nardini et al., 1997). A recent publication reported the antioxidant potential of polyphenols from apples (quercetin, (+)-catechin, (−)-epicatechin, chlorogenic acid, and others) in vitro and in vivo (Lotito & Frei, 2004). The authors found in vitro that flavonoids and phenolic acids from apples delayed the oxidation of ascorbic acid and [·]-T in blood plasma. However, no increased resistance to oxidation of endogenous antioxidants was found in blood plasma collected from volunteers up to 4 hours after the consumption of five apples (Lotito & Frei, 2004). This illustrates one of the weak spots in our current knowledge about the antioxidant (and other biological) functions of polyphenols, namely the sparse information on the metabolites that are present in vivo. It is these metabolites that may exert biological effects, rather than the parent compounds, which are conventionally employed in scientific experiments. Hence, effects observed by the parent compounds in vitro may not readily translate into similar effects in vivo. This should be kept in mind when interpreting results, especially from in vitro studies with pure phenolic substances. For example, it was shown that the flavonoid glycosides have lower antioxidant potentials than their parent aglycones (Ross & Kasum, 2002) and similar results may be expected for the conjugated metabolites and might explain the above findings.

Considering the relatively low blood concentrations of dietary phenolic compounds and/or their metabolites compared to the much higher levels of endogenous antioxidants, doubts have been raised regarding their contribution to the antioxidant defence in vivo (Williams, Spencer & Rice-Evans, 2004). Alternatively, it was suggested that the modulation of cell signalling pathways might be important for their positive effects on certain disorders. Polyphenols, including flavonoids and their metabolites, were reported to modulate a range of protein kinases (e.g. PKC) and transcription factors (e.g. nuclear factor-[κB]), thereby affecting cell proliferation and apoptosis (Orzechowski et al., 2002; Williams, Spencer & Rice-Evans, 2004).

Phase I enzymes, such as cytochrome P₄₅₀ isozymes, catalyse oxidation, hydroxylation, and reduction reactions by which they convert xenobiotics into electrophiles in a preparatory step for their subsequent conjugation with water-soluble moieties by phase II enzymes (e.g. sulphotransferases, glutathione transferases, and UDP-glucuronosyltransferases) to enhance their excretion. Most chemical carcinogens become carcinogenic only after activation by phase I reactions. Consequently, reactions catalysed by CYP enzymes may not only activate some carcinogens, they may also result in the production of free radical species. Plant polyphenols, on the other hand, have been shown to modulate the activity of certain phase I and II enzymes (Lampe, 1999; Orzechowski et al., 2002). For example, curcumin, the colouring principle in turmeric and mustard, was found to dose-dependently inhibit the activities of CYP1A1, CYP1A2, and CYP2B1 in vitro and ex vivo in cells isolated from rats previously fed turmeric (Thapliyal & Maru, 2001). The sesame lignan sesamin inhibited CYP3A and CYP4F2 activity in vitro in human and rat liver cells (Parker, Sontag & Swanson, 2000; Sontag & Parker, 2002). CYP3A is a major CYP in humans and known to metabolise more than 50% of the commonly prescribed drugs (Cholerton, Daly & Idle, 1992; Kliewer, Goodwin & Willson, 2002). Dietary phenolic compounds
have also been reported to inhibit a wide range of other enzymes, such as lipoxygenase, cyclooxygenase, and phospholipase A2, to name but a few (Raj Narayana et al., 2001). Again, sesamin, as one example, was reported to reduce endogenous cholesterol biosynthesis by inhibition of HMG-CoA reductase activity (Hirose et al., 1991) and episesamin was shown to increase cholesterol excretion via induction of 7α-hydroxylase (Ogawa et al., 1995).

The dietary lignans secoisolariciresinol and matairesinol have received much attention from medical researchers because of their conversion by intestinal bacteria into the mammalian lignans enterolactone and enterodiol. Mammalian lignans have been identified in various bodily fluids (Axelson et al., 1982). Mammalian lignans and other phyto-oestrogens (e.g. isoflavones) bind to oestrogen receptors and exert weak oestrogenic activity, thereby altering the concentrations of endogenous sex hormones. Hence, phyto-oestrogens, such as lignans and isoflavones, may have important effects on hormone dependent tumours (Lampe, 1999; Ross & Kasum, 2002). Indirect support of this notion can be derived from epidemiological studies reporting a lower incidence of the hormone-related breast, testicular, and prostate cancers in countries with a higher intake of phyto-oestrogens (Ross & Kasum, 2002).

**Biopotency and bioavailability**

The biopotency, or simply potency, of a substance is a measure of its biological effects (Hoppe & Krennrich, 2000). For example, the biopotency of vitamin E, often referred to as its biological activity (Figure 1), has traditionally been assessed in fertility-restoration assays in rodents, such as the rat foetal resorption-gestation test (Leth & Sondergaard, 1977). In these animal models, RRR-α-T has a higher biological potency than all rac-α-T and the other E vitamers (Bieri & Evarts, 1974; Leth & Sondergaard, 1977; Leth & Sondergaard, 1983). Fertility-restoration tests have been utilised because of the original discovery of vitamin E as an essential factor for reproduction in rats (Evans & Bishop, 1922) and the absence of appropriate quantifiable parameters in humans. Two fundamental prerequisites for the reliable determination of the biopotency of a substance in a certain species are (1) the exact knowledge of its biological effects and (2) the ability to measure these effects in the species of interest. In the case of vitamin E, these basic requirements have been ignored for decades. Instead, the efficacy of vitamin E to reverse infertility in model animals has been determined and was assumed to correlate with its biological functions in humans (Hensley et al., 2004). Considering the dramatic increase in our knowledge about the diverse biological functions of the different E vitamers (see earlier), the established biological potencies and their relevance for humans appear questionable. Consequently, properly designed vitamin E potency studies in humans using adequate functional endpoints are highly warranted. However, despite all scientific progress since the original discovery of vitamin E, appropriate methods to measure vitamin E biopotency in humans are still lacking (Hoppe & Kraemer, 2002). Instead, its bioavailability, which relies on the assumption that the magnitude of
its biological effect(s) depends on its concentration at the site of action, is commonly determined as a surrogate measure for its biopotency (Hoppe & Krennrich, 2000).

The term ‘bioavailability’ and the underlying concept were initially introduced in the field of pharmacology, where bioavailability was defined as “the rate and extent to which a drug reaches its site of action”. Because of the problems in the quantification of a compound at its ‘site of action’, this concept was modified to account for the fraction of an oral dose of a substance or its metabolite(s) that reaches the systemic circulation (Stahl et al., 2002). Thus, bioavailability can be determined in a single-dose experiment by measuring the peak blood concentration ($C_{\text{max}}$), the time to reach the peak concentration ($t_{\text{max}}$), and the area under the blood concentration time curve (AUC). The latter is the most reliable measure because it takes into account the entire response over time, whereas $C_{\text{max}}$ measures only one point in time. In a multiple-dose study, the compound of interest is given for at least five times its half-life ($t_{1/2}$) in the tissue intended for analysis (e.g. blood) to establish the steady state concentration, which is the most important parameter in this type of bioavailability study. A fundamental assumption of the bioavailability concept is that the blood concentration and the biological activity of a compound are proportional (Hoppe & Krennrich, 2000). However, this may not always be the case. In scavenger receptor class B type I (SR-BI) knockout mice, for example, $\alpha$-T blood concentrations were increased while tissue levels remained normal or even decreased (Mardones et al., 2002) and SR-BI knockout mice are reportedly prone to female infertility and accelerated atherogenesis (Trigatti et al., 1999).

Bioavailability is influenced by a multitude of factors, including absorption, distribution, metabolism, excretion, and bioactivity, which in turn are governed by a large number of parameters themselves (Stahl et al., 2002). In the specific case of vitamin E, bioavailability may be affected by its release from the food matrix, the secretion of bile acids and pancreatic enzymes, the amount and type of concurrently ingested fat, the dose and composition of vitamers consumed, the action of $\alpha$-TTP, the quantity of circulating lipoproteins, vitamin E degradation and excretion, the exposure to cigarette smoke, oxidative stress or other processes consuming vitamin E, the presence of vitamin E-regenerating antioxidants (e.g. ascorbate), simultaneously consumed phenolic compounds, and many other parameters (Stahl et al., 2002). The influence that other dietary phenolic compounds may exert on vitamin E bioavailability is in turn determined by their own bioavailability. Eventually, this brings us to the fundamental question addressed in this thesis.
How do dietary phenolic compounds affect the bioavailability of vitamin E?

To answer the central question of this thesis, first of all one needs to identify the characteristics of the effects of dietary phenolic compounds on vitamin E bioavailability (increasing, decreasing, or not altering it). Then, one may seek for and investigate the mechanism(s) by which these effects are facilitated. In this thesis, these issues have been addressed using the following approach. In a series of model experiments, phenolic compounds that occur frequently in the human diet were selected and screened for their potential effects on the bioavailability of \( \alpha \) - and \( \gamma \) -tocopherol in blood plasma, liver and lung tissues of Sprague-Dawley rats (Papers I-VI). Next, substances with pronounced effects on vitamin E bioavailability in the rat model were selected and their impact on the enzymatic degradation of tocopherols to their water-soluble metabolites was tested in vitro, using a human liver cell line and rat liver microsomes as models (Papers IV & V). Finally, one group of compounds that had interesting effects in the animal model was tested in humans (Paper VII).

Animal model of vitamin E bioavailability

This section briefly describes the animal model employed to investigate the effects of dietary polyphenols on the bioavailability of vitamin E. For a more detailed description, the reader is referred to Papers I-VI. A semi-synthetic diet, containing low but adequate amounts of vitamin E and 0.2% cholesterol, was prepared and used as control diet and as basis for the experimental feeds. The test substances were incorporated into this basic diet at concentrations ranging from 0.1% to 0.4% (by weight) and these or control diets were fed to 21-23 days old male Sprague-Dawley rats for 4 weeks. The low but adequate amounts of vitamin E in the diets were utilized to avoid saturation of absorptive and metabolic processes, which could mask potential effects on vitamin E bioavailability. The high doses of polyphenols, on the other hand, were used in order to provoke potential physiological reactions. The 4-weeks duration of the feeding period was chosen to establish steady-state concentrations of vitamin E in the analysed tissues (Bjorneboe, Bjorneboe & Drevon, 1987; Ingold et al., 1987). A secondary aim of this project was to measure potential effects of dietary phenolic compounds on cholesterol concentrations in the animals and therefore cholesterol was added to the diets (see Papers I-VI).

Perhaps one of the first questions to cross one’s mind in regard to the approach described above is: “Why study something in the rat that is expected to occur in humans?” The answer is simple: Although the human diet contains a large variety of phenolic compounds in significant quantities (see above), many of these have not yet been approved for human consumption as isolated substances by the authorities concerned. Furthermore, human studies require a tremendous input of resources, such as money, labour, and time, and experimental conditions are much more difficult to control than during animal experiments. For these reasons,
human studies are not suitable for screening experiments and animal models are generally preferred. The rat was chosen as model animal because its physiology and metabolism have been comprehensively studied and documented (Krinke, 2000) and organ samples are readily accessible. Also, rat studies are easy to repeat and, thus, the results obtained can be reproduced without much effort. What is more, the physiological processes involved in nutrient digestion, absorption, transport, metabolism, and excretion in the rat are for the most part comparable to those in humans. DeSesso and Jacobson (2001) discussed the factors affecting gastrointestinal absorption in rats and humans in a comprehensive review. The alimentary tracts of both species consist, on a macroscopic level, of the same morphological sections (oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon, and rectum) that fulfil similar functions. In humans and rats, the relative lengths (in percent of the total length of the intestinal tract) of the small and large intestines (human, 81% and 19%; rat, 83% and 17%, respectively) are similar, although the relative lengths of the subdivisions may deviate. For example, the jejunum stretches over ca. 38% of the human small intestine, while the respective figure in rats is 90%. Another important difference between the two species is that the relative surface area (normalised for body surface) of the human small intestine, where the majority of absorptive processes takes place, is ~4 times larger than that of the rat. Consequently, dietary compounds that are poorly absorbed in both species will be taken up to a greater extent in humans and substances that are equally well absorbed will be taken up much faster by humans (DeSesso & Jacobson, 2001). The uptake of alimentary substances is not only determined by the absorptive surface, but also by the transit time of the intestinal contents (chyme). The chyme transit time in the small intestine of both rats and humans is 3-4 hours. However, the transit times for the large intestine differ and are 2-4 days in humans and approximately 15 hours in rats. At the microscopic level, the alimentary tracts of humans and rats, e.g. in morphology and cell types, is very similar (DeSesso & Jacobson, 2001). In accordance with these general similarities of the alimentary tracts of rats and humans, the substances tested in the present work (Figure 6) were reported to be absorbed, metabolised, and excreted in both species (see Papers I-VI for details and respective references). More support for the validity of the animal model can be derived from the fact that the findings are largely in agreement with results from other in vitro and in vivo models and, where available, from human studies (see Papers I-VII for discussion and literature).

Effects of dietary phenolic compounds on vitamin E bioavailability in the rat model

During the initial phase of the current work, a variety of common dietary polyphenols was selected for the screening experiments (Figure 6), namely the phenolic acids ferulic and caffeic acid and the phenolic acid conjugate 5-caffeoylquinic acid (Papers I & III), the anthocyanin cyanidin-3-O-glucoside and anthocyanin concentrates from blackcurrant and elderberry (Paper II), the flavanols (+)-catechin and (−)-epicatechin (Paper IV and unpublished results), the lignans sesamin, secoisolariciresinol diglucoside (SDG) and SDG-oligomer (Papers I &
VI), the curcuminoid curcumin, the synthetic antioxidant butylated hydroxytoluene (BHT; Papers I & IV), and the cereal phenolic lipids alkylresorcinols (Paper V). In order to be able to compare the findings from the different experiments despite of the variations in reference (control) values, the results in Figure 7 are given as percent increase or decrease in the bioavailability of \( \alpha \) - and \( \gamma \) - tocopherol.

Dietary plant lignans markedly affect vitamin E bioavailability in rats

The most striking result in Figure 7 is the dramatic increase in \( \gamma \) -T concentrations in blood plasma (900%), liver (1350%) and lung tissues (1556%) in response to supplementation with sesamin, which is in agreement with previous findings (see Paper I and references therein). Sesamin was later shown to effectively inhibit tocopherol-\( \gamma \)-hydroxylase activity in human and rat liver cell models (Parker, Sontag & Swanson, 2000; Sontag & Parker, 2002). The pronounced increase of \( \gamma \) -T bioavailability in response to dietary sesamin was proposed to result from a reduced degradation and urinary excretion of the vitamer (Parker, Sontag & Swanson, 2000; Sontag & Parker, 2002), which was later confirmed in male Wistar rats (Ikeda, Tohyama & Yamashita, 2002).

In contrast to the findings for the sesame lignan sesamin, the flaxseed lignan SDG, in monomeric and oligomeric form, reduced the bioavailability of both tocopherols equally by ca. 50% (Paper VI). Consumption of a diet containing 10-40% flaxseed previously resulted in a dose dependent reduction of \( \alpha \)-T and \( \gamma \)-T in liver and other tissues of rats, while lipid peroxidation, measured by the urinary excretion of thiobarbituric acid reactive substances, increased (Ratnayake et al., 1992). Although flaxseed lignans may undergo oxidative modification catalysed by CYP enzymes, the major part of an ingested dose appears to be converted to mammalian lignans prior to absorption and no appreciable amounts of plant lignans have been found in urine from flaxseed lignan-supplemented rats (Niemeyer et al., 2003). The vitamin E-metabolising enzymes as well as the hepatic \( \gamma \)-TTP are unlikely to be involved in the effect brought about by dietary flaxseed lignans because both discriminate between \( \alpha \)-T and \( \gamma \)-T (Hosomi et al., 1997; Sontag & Parker, 2002) and would therefore result in differential effects on the two tocopherols.

The turmeric constituent curcumin has little effect on vitamin E bioavailability in rats

Curcumin is regularly consumed in some populations and the daily intake from curry powder in India may be in the range of 0.4-1.5 mg/kg bodyweight (Srinivasan & Satyanarayana, 1988). Curcumin is absorbed, metabolised, and excreted in the form of glucuronidated metabolites in rats (Holder, Plummer & Ryan, 1978). When tested in the rat model, curcumin only changed \( \alpha \)-T concentrations in lungs but had no statistical effect on vitamin E in the liver and blood plasma (Paper I).
Figure 6. Substances tested in this thesis. All compounds except BHT are natural plant constituents. See Paper VI for the oligomeric structure of SDG.

*R=15-27 odd-numbered hydrocarbon saturated or unsaturated side-chain.
Figure 7. Changes (in percent) in the concentrations of \( \alpha \)-tocopherol (grey bars) and \( \gamma \)-tocopherol (white bars) in blood plasma, liver, and lungs of rats in response to 4 weeks supplementation with phenolic compounds. Statistically significant changes are given in bold; n.a. = not analysed. All compounds and extracts were fed at the 0.2% dietary level, except alkylresorcinols, BHT, curcumin, and sesamin, fed at 0.4%, and SDG and its oligomer fed at 0.1% in the diet. Due to the extreme values for sesamin, the broken bars do not reflect the actual proportions.
Anthocyanins have little impact on vitamin E bioavailability in rats

Anthocyanins were previously reported to possess antioxidant activity and to counteract DNA damage and lipid peroxidation in rats (Tsuda, Horio & Osawa, 1998; Ramirez-Tortosa et al., 2001). Therefore, cyanidin-3-O-glucoside and anthocyanin-rich extracts of blackcurrant and elderberry were tested in the rat model (Paper II). Neither the isolated compound nor the extracts exerted any major effects on vitamin E bioavailability. However, slight increases in a-T concentrations in the livers and of g-T in the lungs of cyanidin-3-O-glucoside-fed rats were observed. The generally negligible outcome of this experiment may be in part due to the small amounts of experimental substances given (as a consequence of the impurity of the polyphenolic raw material) compared to the other experiments. The concentrations of anthocyanins fed in this study were comparable to the average intake in humans (see Paper II for discussion and literature).

Dietary phenolic acids slightly improve vitamin E bioavailability in rats

In the animal model, dietary caffeic acid increased g-T in liver while its derivative, 5-caffeoylquinic acid, increased a-T in lung tissue. This is similar to previous reports of an a-T sparing action of and the recycling of tocopheroxyl-radicals by caffeic acid. 5-Caffeoylquinic acid appears to be cleaved into caffeic and quinic acids prior to absorption. In response to dietary 5-caffeoylquinic acid, an increase in blood concentrations of caffeic acid but not 5-caffeoylquinic acid was observed in rats and humans. Consequently, its biological effects may be mediated by and be similar to those of caffeic acid (see Paper III for discussion and references). Ferulic acid, on the other hand, consistently exerted no effects on vitamin E bioavailability in the rat model despite being a potent antioxidant in vitro (see Papers I & III). A study on the partitioning of ferulic acid in plasma and LDL showed that the major fraction appears to be associated with albumin and only a minor part with the lipid fraction (Castelluccio et al., 1996). Therefore, ferulic acid and tocopherols may exist in separate compartments and their interactions may be limited in vivo.

Dietary catechins enhance a-tocopherol bioavailability in rats

The flavanols (+)-catechin and (−)-epicatechin are stereoisomers that differ only in the spatial configuration of the hydroxyl group on ring C (Figure 6) and were reported to have comparable antioxidant capacities (Pedrielli & Skibsted, 2002). Both isomers markedly increased the bioavailability of a-T in blood plasma and liver tissue in the present study. Interestingly, the 2R,3R-isomer (−)-epicatechin also enhanced a-T bioavailability in both tissues whereas the 2R,3S-isomer (+)-catechin was without effect on this tocopherol (Paper IV and unpublished results). The effect on a-T is possibly due to antioxidant interactions between the flavanols and the vitamin (see Paper IV for discussion and references). The increase in a-T facilitated only by (−)-epicatechin may be due to differential effects on some cytochrome P450 enzymes. Tea catechins were reported to affect a range of CYPs including CYP1A1, CYP1A2, CYP2B1, and CYP3A4 (Muto et al., 2001; Huynh & Teel, 2002; Vaclavikova et al., 2003). CYP3A was previously proposed to participate in vitamin E metabolism (Parker, Sontag & Swanson, 2000).
although the same group later suggested a role for CYP4F2 rather than CYP3A (Sontag & Parker, 2002).

The synthetic antioxidant BHT enhances α-tocopherol bioavailability
Butylated hydroxytoluene (BHT) is a synthetic preservative frequently used in e.g. convenience foods to preserve the oxidative stability of oils and fats. In the rat model, BHT markedly enhanced α-T bioavailability when fed at 0.2% (by weight; Paper IV) and 0.4% (Paper I) dietary levels. Interestingly, BHT reduced the bioavailability of α-T to less than half the control values when given at 0.2% but not at 0.4% in the diet. This discrepancy cannot be explained at present. Contrary to our results, Simán & Eriksson (1996) found a dose dependent decrease in liver concentrations of α-T in BHT-fed female Sprague-Dawley rats. They attributed this effect to the formation of pro-oxidative metabolites arising from the catabolism of BHT by CYP enzymes, which were suggested to consume vitamin E (Simán & Eriksson, 1996). In fact, BHT is metabolised by CYP and excreted in urine in form of water-soluble conjugates (Daniel & Gage, 1965; Bolton & Thompson, 1991). Following oral administration of BHT, female rats had higher liver concentrations of BHT and excreted larger quantities of urinary BHT-metabolites at a faster rate than male rats treated in the same manner (Daniel & Gage, 1965). The expression of CYP isozymes in an organism is far from static and is known to vary depending on gender and age (Rich & Boobis, 1997). Therefore, it appears possible that the contrasting results obtained during the present work and by Simán & Eriksson (1996) result from gender and/or age differences in BHT metabolism by CYP enzymes as seen in our young male and their older female rats.

Cereal alkylresorcinols increase α-tocopherol bioavailability in rats
Alkylresorcinols are a class of cereal phenolic lipids predominantly present in the outer layers of wholegrain wheat and rye and are absorbed and metabolised in humans and rats (Ross, Kamal-Eldin & Åman, 2004). In the rat model, dietary supplementation with cereal alkylresorcinols dose dependently increased α-T concentrations in liver and lung tissues without altering α-T (Paper V) similar to sesamin, although to a lesser extent. Because of the structural similarities between tocopherols and alkylresorcinols it was hypothesised that they may share common metabolic pathways and competitively inhibit enzymatic degradation of tocopherols (see Paper V and below).
In vitro model of vitamin E metabolism

The discovery that sesamin inhibits the initial \( \alpha \)-hydroxylation of tocopherols and thereby reduces their degradation to carboxychroman metabolites (Parker, Sontag & Swanson, 2000; Sontag & Parker, 2002) not only offered a ready explanation for the observed increase in \( \alpha \)-T bioavailability in the sesamin-fed rats (Paper I), but also opened new opportunities for the investigation of some of the mechanisms underlying the effects observed in the screening experiments. In order to determine if the tested phenolic compounds interfere with the side-chain degradation of vitamin E, human HepG2 cells and rat liver microsomes were incubated for 48 hours with the respective polyphenol in the presence of \( \alpha \)-T or \( \beta \)-T and the conversion to their respective 3'- and 5'-carboxychromanol metabolites (Figure 3) was determined by GC-MS (Parker, Sontag & Swanson, 2000). The advantage of the in vitro model lies in the general ability of HepG2 cells to metabolise polyphenols (Parker, Sontag & Swanson, 2000; Parker & Swanson, 2000; Sontag & Parker, 2002; O'Leary et al., 2003). Consequently, the effects observed in the hepatoblastoma cell model may be mediated by the parent compound and/or its metabolites in resemblance of the situation in vivo.

Alkylresorcinols and sesamin inhibit tocopherol metabolism in vitro

To clarify whether alkylresorcinols affect vitamin E metabolism or not, HepG2 cells were incubated with 5 \( \mu \)M or 20 \( \mu \)M of mixed alkylresorcinols from rye or synthetic pentadecylresorcinol (C15:0 alkylresorcinol) in the presence of \( \alpha \)-T. The alkylresorcinol mixture and the pure compound both dose-dependently inhibited the formation of 3'- and 5'-carboxychromanol. The pure compound was a more potent inhibitor of tocopherol-\( \alpha \)-hydroxylase activity than the mixed alkylresorcinols (Paper V). With regard to the similar side-chains of tocopherols and alkylresorcinols, it seems likely that both classes of compounds share a common catabolic pathway and compete for the same metabolic enzymes. This is supported by the recent discovery of urinary metabolites of alkylresorcinols in humans, apparently resulting from \( \beta \)-oxidation of the side-chain (Ross, Kamal-Eldin & Åman, 2004). Consistent with previous reports (Parker, Sontag & Swanson, 2000; Sontag & Parker, 2002), sesamin, at a concentration of only 2 \( \mu \)M, almost completely inhibited tocopherol-\( \alpha \)-hydroxylase activity and the degradation of \( \alpha \)-T (Paper V). The inhibition of tocopherol side-chain degradation by alkylresorcinols and sesamin gives a ready explanation for the increase in \( \alpha \)-T bioavailability observed in the rat model. Sesamin was a much more potent inhibitor of tocopherol-\( \alpha \)-hydroxylase activity in the cell model than the alkylresorcinols and, accordingly, had a much more pronounced elevating effect on \( \alpha \)-T concentrations in the rats (Papers I & V). Chemicals with a methylenedioxyphenyl function, such as sesamin, are known to form complexes with CYP’s, thereby irreversibly inactivating the enzymes (Murray, 2000). The dose-dependent effect of alkylresorcinols on tocopherol-\( \alpha \)-hydroxylase activity in the present work, on the other hand, suggests a (reversible) competitive inhibition of CYP’s to be at the basis of the underlying mechanism (Paper V).
BHT and (+)-catechin do not affect tocopherol metabolism in vitro

As mentioned before, \( \Delta^T \) is a much better substrate for the tocopherol-[\( \Delta^\text{-} \)]-hydroxylase pathway than [\( \Delta^\text{T} \)] (Sontag & Parker, 2002). Hence, substances that increase the bioavailability of [\( \Delta^\text{T} \)] without affecting that of [\( \Delta^\text{T} \)] are unlikely to exert this effect through modulation of the enzymes involved in tocopherol side-chain degradation. However, the involvement of distinct CYP isozymes in the metabolism of the different E vitamers cannot be ruled out (Sontag & Parker, 2002). To investigate whether the tocopherol-[\( \Delta^\text{-} \)]-hydroxylase pathway was involved in the increase in [\( \Delta^\text{T} \)] bioavailability observed in the rats fed BHT and (+)-catechin, these compounds were also tested in the in vitro model. In accordance with the above reasoning, neither BHT nor (+)-catechin inhibited tocopherol-[\( \Delta^\text{-} \)]-hydroxylase activity (Paper IV).

Dietary sesame oil lignans decrease the urinary excretion of [\( \Delta^\text{-} \)] T metabolites in humans

In the final stage of this project, the knowledge obtained in the animal and in vitro models was applied to humans. Based on the drastic increase in [\( \Delta^\text{T} \)] bioavailability in rats and the apparent inhibition of [\( \Delta^\text{T} \)] metabolism in liver cells in response to sesamin, it was hypothesised that, in humans, oral application of sesame lignans would result in a reduced excretion of urinary metabolites of co-administered [\( \Delta^\text{T} \)]. In order to test this, ten volunteers (5 females, 5 males) were given two muffins prepared from unrefined sesame oil (equalling a dose of 94 mg sesamin and 42 mg sesamolin) or corn oil (control) together with a capsule containing 100 mg [\( \Delta^\text{a} \text{-} \)] and [\( \Delta^\text{g} \text{-} \)]tocopherol (50 mg of each vitamer). The administered two sesame oil muffins contained a dose of 2.4 mg [\( \Delta^\text{a} \text{-} \)]-T and 13.0 mg [\( \Delta^\text{g} \text{-} \)]-T and the two corn oil muffins of 2.8 mg [\( \Delta^\text{a} \text{-} \)]-T and 13.2 mg [\( \Delta^\text{g} \text{-} \)]-T. The tocopherols in the pills were stably labelled with different amounts of deuterium (d6-[\( \Delta^\text{a} \text{-} \)]-T and d2-[\( \Delta^\text{g} \text{-} \)]-T) in order to be able to differentiate between the newly ingested and the endogenous tocopherols and their resulting metabolites in the blood and urine samples that were collected for 72 hours.

The single dose of sesame lignans did not alter the bioavailability (measured as \( C_{\text{max}} \), \( t_{\text{max}} \), or absolute concentrations at the given time points) of [\( \Delta^\text{T} \)] or [\( \Delta^\text{a} \text{-} \)]-T (Paper VII). Comparing these results to those of Cooney et al. (2001), who found a 19% increase in blood plasma concentrations of [\( \Delta^\text{T} \)] after only 3 days of supplementation with sesame seed enriched muffins (equalling a dose of 35 mg sesamin and 13 mg sesamolin per day), suggests that a repeated intake of sesame lignans is a prerequisite to enhance [\( \Delta^\text{T} \)] bioavailability. This seems even more likely when compared to the 42% increase in [\( \Delta^\text{T} \)] blood concentrations in women adding unrefined sesame oil to their habitual diets for 4 weeks (Lemcke-Norojärvi et al., 2001).

Serum concentrations of tocopherol metabolites were slightly lower in subjects eating the sesame instead of the corn oil muffins, but the differences were not statistically significant. The responses in d0-[\( \Delta^\text{a} \text{-} \)]CEHC and d0-[\( \Delta^\text{g} \text{-} \)]CEHC were rather weak compared to the steep rise and steady decline in d2-[\( \Delta^\text{g} \text{-} \)]CEHC (Paper VII).
CEHC was previously reported to possess specific biological functions, e.g. as natriuretic factor (Wechter et al., 1996) and anti-inflammatory agent (Jiang et al., 2000), and its blood concentrations may therefore be subject to regulation (Galli et al., 2003).

The urinary excretion of tocopherol metabolites was generally lower in subjects receiving the sesame oil muffin treatment compared to those consuming corn oil muffins. The urinary excretion of the newly ingested d2-\(\gamma\)-T in form of d2-\(\gamma\)-CEHC was significantly reduced from 6-12 hours (P<0.01) and at peak excretion from 12-24 hours (P<0.05) following the treatment with sesame oil muffins (Paper VII). In consequence, the total amount of ingested d2-\(\gamma\)-T recovered in urine in form of d2-\(\gamma\)-CEHC was significantly (P<0.05) lower after consumption of sesame oil muffins (7.8 ± 3.7%) than corn oil muffins (12.3 ± 5.6%; Paper VII). Also, the total urinary excretion of \(\gamma\)-CEHC (sum of d2- and d0-\(\gamma\)-CEHC) was lower in subjects consuming sesame oil muffins than those consuming corn oil muffins, but this effect was statistically significant only at 12 hours after treatment (P<0.02; Figure 8).

It may be concluded that the one-time ingestion of sesame oil muffins containing 136 mg sesame lignans significantly reduced the metabolism and urinary excretion of newly ingested \(\gamma\)-T in humans (Paper VII). Future studies should also address the effects of a repeated intake of sesame lignans on vitamin E bioavailability, metabolism, and excretion.
Some general remarks on vitamin E bioavailability

Changes in tocopherol concentrations could result from an altered availability of vitamin E transport vehicles, the lipoproteins, in the blood. If that would be the case, one would also expect similar changes in the cholesterol content of the lipoprotein fractions, because tocopherol and cholesterol concentrations are closely correlated (Kayden & Traber, 1993; Perugini et al., 2000). However, the decreases and increases in vitamin E bioavailability in the rat model were not accompanied by corresponding changes in cholesterol levels (Papers I-VI). Hence, it appears unlikely that the observed effects on vitamin E bioavailability in this project were due to altered lipoprotein concentrations.

The bioavailability of vitamin E might also be influenced by the ratio of $\alpha$-T/$\gamma$-T in the diet. In earlier studies, $\gamma$-T supplements increased blood concentrations of both $\gamma$-T and $\alpha$-T in humans and rats (Handelman et al., 1985; Clément & Bourre, 1997), while $\gamma$-T supplements increased $\gamma$-T but reduced $\alpha$-T blood concentrations in humans (Melchert & Pabel, 1998; Huang & Appel, 2003). Recently, the activation of PXR by all major forms of vitamin E was reported (Landes et al., 2003). PXR is a nuclear receptor controlling the expression of drug metabolising enzymes (Kliwer, Goodwin & Willson, 2002). In this manner, vitamin E may induce the enzymes necessary for its own metabolic breakdown (Landes et al., 2003). Sontag & Parker (2002) reported a higher catalytic activity of these enzymes towards $\gamma$-T than $\alpha$-T. Taken together, these studies suggest that $\gamma$-T supplementation activates PXR and, thus, induces the expression of tocopherol-$\gamma$-hydroxylase, which then might preferentially degrade $\gamma$-T. However, a conclusive explanation for the $\gamma$-T elevating effect of $\gamma$-T supplements, other than perhaps a simple protection of $\alpha$-T from oxidative decay by $\gamma$-T, is still lacking.

In theory, at least one explanation for the increase in $\gamma$-T concentrations due to $\gamma$-T supplementation seems possible, even though it has never been conclusively proven to play a role in mammals. The only structural difference between $\gamma$-T and $\alpha$-T lies in a methyl substituent in position 5 on the chromanol head (see Figure 1). While plants can methylate $\gamma$-T to $\alpha$-T, catalysed by a methyltransferase (Munne-Bosch & Alegre, 2002), no such enzyme system has been reported in mammals. Nevertheless, a few research groups have tried to shed light on this issue and gathered some evidence for a methylation of the desmethyl-forms of vitamin E to the fully methylated $\gamma$-forms in vivo (Emmel & Celle, 1959; Elmadfa et al., 1989; Goh et al., 1992; Qureshi et al., 2001). However, all of these studies have methodological limitations that render the data interpretation difficult. Hence, the conversion of $\gamma$-T to $\alpha$-T or the lack of conversion has yet to be conclusively proven in vivo.
Conclusions and future research

The findings of the present thesis can be summarised as follows:

- Certain simultaneously ingested dietary phenolic compounds alter the bioavailability of \( \alpha \)- and \( \gamma \)-tocopherol in rats:

  - The tested anthocyanins, phenolic acids and derivatives, and curcumin have only minor and varying effects on vitamin E bioavailability.
  - Butylated hydroxytoluene, (+)-catechin, and (\( \beta \))-epicatechin markedly increase \( \alpha \)-tocopherol bioavailability.
  - Secoisolariciresinol diglucoside and its oligomer considerably decrease the bioavailability of \( \gamma \)- and \( \beta \)-tocopherols.
  - Cereal alkylresorcinols and sesamin enhance \( \gamma \)-T bioavailability significantly but do not alter that of \( \alpha \)-T.

- The bioavailability of \( \alpha \)- and \( \gamma \)-tocopherol in rats can be affected to a different extent in different tissues.

- Butylated hydroxytoluene and (+)-catechin do not affect the side-chain degradation of tocopherols in vitro.

- Sesamin almost completely inhibits the side-chain degradation of tocopherols in vitro.

- Cereal alkylresorcinols dose-dependently reduce the side-chain degradation of tocopherols in vitro.

- Singular consumption of sesame oil muffins reduces the urinary excretion as \( \gamma \)-CEHC of concurrently ingested \( \alpha \)-tocopherol in humans.

As is so often the case in science, one starts research with a single question. As one attempts to find the answer to that one question, more and more questions arise along the way. Eventually, one may end up a tiny step closer to the answer to the original question, but far away from the answers to a plethora of new ones.

One such subject for future research relates to the still unknown mechanisms by which BHT and the catechins increased the concentrations of \( \gamma \)-T in rats. Since these compounds did not inhibit vitamin E metabolism, other mechanisms are likely to be involved and deserve investigation. The mechanisms by which polyphenolic chemicals might spare \( \gamma \)-T may involve (1) scavenging of free radical species that consume \( \alpha \)-T; (2) inhibition of enzyme systems that produce free radicals (e.g. lipoxygenase, cyclooxygenase, and CYP’s); (3) induction of antioxidant enzyme systems (e.g. catalase and glutathione peroxidase); (4) enhancement of vitamin E absorption; (5) increased expression of \( \alpha \)-TTP through induction of (i) gene expression and/or (ii) translational processes; and (6) improvement of the binding capacity of \( \alpha \)-TTP. Appropriate experiments to
investigate these mechanisms are highly warranted. Furthermore, future research
should not only try to substantiate and characterise the interactions of vitamin E
with co-administered phenolic compounds, it should also consider the metabolic
interactions between different simultaneously ingested E vitamers.

The animal models employed in this thesis largely made use of pharmacological
doses of the tested compounds, in order to identify interactions with vitamin E
bioavailability. In continuation of the work initiated with the human study
reported in Paper VII, it would be of particular interest to study in the future the
interactions with vitamin E bioavailability of the effective compounds at common
dietary levels, in order to assess the importance of the observed metabolic
interactions for human nutrition.

Among the broader questions that frequently came up during the course of this
work has been one that is as yet unanswered: “What makes vitamin E a vitamin?”
For decades, the antioxidant activity of vitamin E has been the sole focus of
vitamin E research. But can we really replace vitamin E with any other potent
lipid-soluble antioxidant? This seems unlikely given that in recent years the non-
antioxidant functions of vitamin E have become evident and unique properties of
specific vitamers and their metabolites have been revealed (see earlier). A related
question of particular interest is: Which of the E vitamers is/are indispensable?
The most recent dietary reference intakes for vitamin E, given out by the Food and
Nutrition Board of the Institute of Medicine (2000), consider \( \alpha \)-tocopherol to be
the only essential vitamer. However, until the exact physiological tasks of all
vitamers and their metabolites have been identified and characterised, it appears
premature to disregard any form of vitamin E.

It was pointed out before, that plants are capable of converting \( \delta \)-tocopherol to
\( \alpha \)-tocopherol by a single methylation-reaction and that no such \( \delta \)-tocopherol
methyltransferase has been reported in animals. The existence of such an enzyme
in mammals would give some credence to the sole essentiality of \( \alpha \)-tocopherol,
while its absence may hint toward physiological roles of the desmethyl vitamers.
However, sound experiments substantiating or disproving the in vivo conversion
of the desmethyl- to the \( \alpha \)-vitamers are highly warranted.

Finally, it should be borne in mind that we only measure vitamin E
bioavailability as a marker of its biopotency. High blood concentrations of
vitamin E do not necessarily reflect high levels in tissues and its presence in the
blood alone does not guarantee its biological activity. Hence, vitamin E research
would greatly benefit from the development of a reliable in vivo-assay for vitamin
E-biopotency. However, for the time being the bioavailability concept is the most
accurate tool to our disposal.
References


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