Expert Meeting of the Research Training Programme on “Natural Antioxidants- Effects in Plants, Foods, Animals and Humans” (GRK 820)

„Occurrence, Metabolism and Function of Vitamin E in Plants, Man and Animals“

Salzau
Germany
March 9-13, 2005
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Salzau is one of the manors that are being named in Kiel’s oldest chronicle from 1264 until 1289. The "Geheime Rath" and "Kammerherr" Wolf Blome purchased Salzau manor in 1758 after it had been in possession of several different owners. Salzau manor being situated close to Lake Selent encompassed 4,000 hectares with all the neighboring villages included. It remained in possession of Earl Blome for almost 200 years. In 1881 Otto Blome let the castle built up again after a fire had destroyed much of it. The rebuilding was managed by the Silesian architect J.E. Mose, and it took only a year to restore the castle. In 1945 Salzau by succession passed over to the Earls Thun and Hohenstein. By distribution of the estate, the castle had been sold to a merchant from Hamburg in 1973. Later the federal state Schleswig-Holstein purchased the manor-house with its adjacent buildings and its park. The gate lodge and the barn had been purchased in 1988.
Programme

March 9th

18.00  Dinner

20.00  Welcome

Krupinska, Rimbach
Presentation of the Research Training Programme GRK820
Information concerning the meeting

Opening Lecture

20.30  Hannelore Daniel, University of Weihenstephan, Freising, Germany
Systems biology in nutrition research

March 10th

Session 1  Tocochromanol biosynthesis in plants
Chairperson: Achim Trebst

9.15  Dean DellaPenna, Michigan State University, USA
Progress in understanding vitamin E synthesis in plants

10.15  Peter Dörmann, MPI Potsdam, Golm, Germany
The role of tocopherol cyclase (VTE1) in Arabidopsis in the regulation of
tocopherol synthesis

10.45  Coffee break

11.15  Bernhard Grimm, Humboldt University, Berlin, Germany
Effects of reduced and lacking content of tocopherol and phytlated
chlorophyll on photosynthesis in transgenic tobacco plants and Synechocystis
mutants

11.45  Jon Falk, CAU Kiel, Germany
Tocotrienols: Occurrence in plants and genetic engineering approaches

12.30  Lunch break and walk around Salzau

15.30  Coffee

Session 2  Functions of tocochromanols in plants
Chairperson: Dean DellaPenna

16.00  Achim Trebst, University of Bochum, Germany
Alpha-tocopherol as singlet oxygen quencher in photosystem II

16.30  Rüdiger Schulz-Friedrich, CAU Kiel, Germany
Influences on tocopherol biosynthesis in the cyanobacterium
Synechocystis sp. PCC 6803
17.00 Susanne Römer, University of Konstanz, Germany
Impact and interaction of lipophilic antioxidants in transgenic tobacco

17.30 Christine Desel, CAU Kiel, Germany
The impact of tocochromanols on germination and NO release

18.15 Poster presentation and discussion I

19.30 *Dinner (Italian buffet) and Jazz*

**March 11th**

**Session 3**  **Vitamin E status and intervention studies**  
*Chairperson: John Lodge*

8.30 Achim Stocker, Swiss Federal Institute of Technology, Zürich, Switzerland
Molecular determinants of vitamin E retention in man

9.30 Jan Frank, Swedish University of Agricultural Sciences, Uppsala, Sweden
Beyond vitamin E supplementation: alternative strategies to improve the vitamin E status

10.00 *Coffee break*

10.30 Brigitte M. Winklhofer-Roob, Karl-Franzens-University, Graz, Austria
Effects of vitamin E on oxidative stress in health and disease: evidence obtained from human intervention studies

11.45 *Lunch*

**Session 4**  **Vitamin E supplementation**  
*Chairperson: Gerald Rimbach*

13.00 Edgar Miller, Johns Hopkins Medical University, Baltimore, USA
High dose vitamin E supplementation is associated with increased all-cause mortality: results of a meta-analysis of randomized controlled clinical trials

14.00 John Hathcock, Council for Responsible Nutrition, Washington, USA
Vitamin E - comparison of risk assessments

14.45 Discussion on Vitamin E supplementation

15.30 *Coffee and tour to “Hessenstein” followed by the Congress Dinner at the “Genueser Schiff” in Hohwacht*
March 12th

**Session 5**  **Vitamin E metabolism and in vivo effects**  
*Chairperson: Achim Stocker*

9.15  Johannes von Lintig, University of Freiburg, Germany  
Towards a better understanding of carotenoid metabolism in animals

9.45  Regina Brigelius-Flohé, German Institute of Nutrition, Potsdam, Germany  
Vitamin E and drug metabolism

10.45  *Coffee break*

11.15  Peter D. Weinberg, Imperial College, London, UK  
Cardiovascular effects of vitamin E *in vivo*

12.00  *Lunch*

13.30  Poster Presentations and Discussion II

**Session 6**  **Vitamin E bioavailability, function and signalling**  
*Chairperson: Regina Brigelius-Flohé*

15.30  John Lodge, School of Biomedical and Molecular Sciences, Surrey, UK  
Vitamin E bioavailability in humans

16.30  *Coffee break*

17.00  Luca Barella, DSM, Basel, Switzerland  
Vitamin E and gene expression profiling

17.30  P.W. Sylvester, University of Monroe, Lousiana, USA  
Intracellular signalling mechanisms mediating the antiproliferative and apoptotic effects of γ-tocotrienol in neoplastic mammary epithelial cells

18.30  *Dinner*

20.00  *Wine and Music*

March 13th

DEPARTURE
Session 1

Tocochromanol biosynthesis in plants

Chairperson: Achim Trebst
Progress in understanding vitamin E synthesis in plants

Dean DellaPenna

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The tocopherols are a group of lipid-soluble, amphipathic compounds that collectively constitute vitamin E, an essential nutrient for both humans and other animals. Tocopherols are synthesized only by oxygenic photosynthetic organisms, including all plants and some cyanobacteria, and the vitamin E content of a given plant tissue is a consequence of both the levels and types of tocopherols produced. During the past seven years my laboratory has pursued a genomic and molecular approach to dissect and engineer the tocopherol biosynthetic pathway using the complementary model systems Arabidopsis and Synechocystis PCC6803. Cloning of a tocopherol pathway enzyme from one organism has in general facilitated isolation of the respective ortholog from the other such that the full suite of pathway genes has now been isolated and used to manipulate tocopherol synthesis and study tocopherol functions in photosynthetic tissues. My presentation will provide 1) an overview of our current understanding of the pathway and its manipulation in photosynthetic organisms, 2) the use of quantitative genetics to understand the genetic basis for natural variation in tocopherol levels in plant tissues and, 3) recent insights into tocopherol functions obtained from mutant studies of tocopherol deficient mutants in Arabidopsis and Synechocystis PCC6803.
Tocopherol cyclase (VTE1) catalyzes the penultimate step of tocopherol synthesis in plants, the closure of the second ring of the chromanol head group. Based on an Arabidopsis mutant deficient in tocopherol synthesis, the gene encoding tocopherol cyclase was isolated via map-based cloning. Functional activity of the enzyme was confirmed after expression in E. coli. Overexpression of VTE1 in Arabidopsis resulted in an increase in total tocopherol of seven fold in leaves, and a dramatic shift from α-tocopherol to γ-tocopherol. Expression of tocopherol cyclase (VTE1) was found to be induced at early time points during high light stress in Arabidopsis leaves. Furthermore, VTE1 was induced in two antioxidant mutants, vtc1 (ascorbate deficient) and cad2 (glutathione deficient). Tocopherol deficiency in the Arabidopsis vte1 mutant resulted in an increase in ascorbate and glutathione. Deficiency in one antioxidant in vte1, vtc1 or cad2 led to increased oxidative stress and to the concomitant increase in alternative antioxidants. Double mutants of vte1 were generated with vtc1 and cad2 and analyzed with regard to growth, chlorophyll content and photosynthetic quantum yield.
Effects of reduced and lacking content of tocopherol and phytylated chlorophyll on photosynthesis in transgenic tobacco plants and *Synechocystis* mutants

Bernhard Grimm

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Vitamin E is the collective term for a group of amphiphatic compounds, the tocopherols and tocotrienols, which are exclusively synthesised by photosynthetic organisms. Vitamin E has long been accepted to be essential in protection of animal cells from oxidative reaction. Although tocopherols may be important in plants to similar extent, precise functions in antioxidative defence of plants could not be assigned. We analysed the phototolerance of transgenic tobacco plants expressing antisense *CHLP* RNA and of a *Synechocystis* PCC 6803 ∆*CHLM* knock out mutant. *CHLP* codes for the geranylgeranyl reductase, which catalyses the reduction of geranylgeranyl pyrophosphate to phytyl pyrophosphate required for synthesis of tocopherol, chlorophyll and phylloquinone. While the cyanobacterial ∆*CHLP* mutant accumulated only little α tocotrienol and geranylgeranylated chlorophyll, the transgenic tobacco plants showed only reduced levels of tocopherol and accumulation of geranylgeranylated chlorophyll. The photosynthetic performance was determined under high and low light conditions and low temperature to test susceptibility to photo-oxidative stress. The correlation will be presented between tocopherol contents in tobacco and cyanobacterial mutants and the effects of photooxidation on the stoichiometry, composition and activity of photosystem I and II.

The presented data are based on collaboration between Dr. Michel Havaux, Dr. Peter Jahns and Dr. Vladislav Zinchenko, respectively, and my group at the IPK, Gatersleben/HU, Berlin.
Tocotrienols are structurally related to tocopherols, share the same biosynthetic pathway and are known to have vitamin E activity, too. Tocopherols are found in all plants and almost all plant parts, while tocotrienols are not synthesized by all plants and predominately found in seeds. The capability of cereals to produce tocotrienols could be shown to be a result of an additional tocotrienol specific prenyltransferase. On the other hand plants without such a tocotrienol specific prenyltransferase, which normally are not accumulating tocotrienols, could be shown to produce tocotrienols after transgenic manipulation of other steps of the biosynthetic pathway of vitamin E. These results indicate that additional factors might be responsible for the presence or absence of tocotrienols in plants and might explain the appearance of tocotrienols in diverse groups of the plant kingdom. Despite the importance as lipophilic antioxidants it is still unknown what makes tocopherols and tocotrienols a vitamin. Therefore, also non-antioxidant functions of vitamin E became a main focus of research. Especially tocotrienols have gained much interest since it has a very high therapeutic potential to prevent and reduce cardiovascular disease and cancer. The function of tocotrienols in plants is not clear so far and is discussed in the context of their tissue specific distribution in seeds.
Session 2

Function of tocochromanols in plants

Chairperson: Dean DellaPenna
Singlet oxygen is formed in the photosystem II reaction center in the quench of P680 triplets. The yield is depending on light intensity and a reduced state of plastoquinone. Singlet oxygen in PS II triggers the degradation of the D1 protein.

We probed for the participation of tocopherol in this system in its property as singlet oxygen scavenger. For that we inhibited tocopherol biosynthesis at the level of the HPP-dioxygenase in the alga Chlamydomonas reinhardtii at conditions that plastoquinone did not limit the photosynthesis rate. In the presence of the inhibitor and in high light for 2 h photosynthesis and photosystem II get inactivated, the D1 protein is degraded, the tocopherol pool is depleted and falls below its turnover rate/h. The inhibited system can be fully rescued on addition of a chemical singlet oxygen quencher (diphenylamine), partly by synthetic, cell wall permeable shortchain tocopheryl derivatives.

We conclude that under conditions of photoinhibition and D1 protein turnover tocopherol has a protective function as singlet oxygen scavenger.
Influences on tocopherol biosynthesis in the cyanobacterium *Synechocystis sp. PCC 6803*

Rüdiger Schulz-Friedrich

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To elucidate the function of tocopherol in cyanobacteria and tocopherol biosynthesis, wild-type and methyltransferase mutant cells of *Synechocystis sp. PCC 6803* were grown under different conditions. The vitamin E content of cells grown under different light regimes, photomixotrophic or photoautotrophic conditions and varying carbon dioxide supplies were compared by HPLC measurements. The tocopherol levels in wildtype cells increased under higher light conditions and low carbon dioxide supply. Photomixotrophic growth led to lower vitamin E amounts in the cells compared to those grown photoautotrophically. We were able to segregate a homozygous ∆*sll0418* mutant under photoautotrophic conditions. In contrast to former suggestions the deletion of this gene is not lethal under photomixotrophic conditions and the influence on tocopherol and plastoquinone amounts is diminutive. Our results indicate that tocopherol plays a major role in protection against photooxidative damage and that its biosynthesis is correlated to the redox-state of the cyanobacterial cell. The methyltransferase encoded by the gene *sll0418* is not essential neither for tocopherol nor plastoquinone synthesis.
Impact and interaction of lipophilic antioxidants in transgenic tobacco

Sonja Woitsch, Susanne Römer

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We have analyzed the interaction of lipophilic and soluble antioxidants in transgenic plants with genetically modified carotenoid biosynthesis. In the case of β-carotene hydroxylase antisense plants short-term high light treatment did not result in a drastically altered stress sensitivity, despite a clear reduction in β-carotene hydroxylase mRNA levels and strongly reduced zeaxanthin contents. Determination of the expression of genes encoding ROS (reactive oxygen species) detoxifying enzymes and measurements of the corresponding enzyme activities gave similar results for wild type and transgenic plants. However, a drastic change in the amount of α-tocopherol was observed leading to a 2.5fold increase of this lipophilic antioxidant in the transformants. Our findings indicate a close functional interaction between carotenoid and tocopherols revealing a putative compensatory mechanism.
The impact of tocochromanols on germination and NO release

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Plant seeds and fruits are the main source for tocochromanols (tocopherols and tocotrienols) collectively known as Vitamin E in human nutrition. Tocochromanols are the most important lipophilic antioxidants in plants and animals known to date and most likely essential for the viability of plant seeds. Seeds are particularly rich in \( \gamma \)-tocopherol. The reason for the abundance of \( \gamma \)-tocopherol in seeds is not yet clear. However, different tocopherol variants differ in their individual biochemical properties. Thus, \( \gamma \)-tocopherol is a better nucleophil and scavenges electrophiles such as nitric oxides (NO\(_x\)) more efficiently than \( \alpha \)-tocopherol. Since nitric oxides stimulate seed germination and root development it is conceivable that \( \gamma \)-tocopherol by specifically controlling the NO content in seedlings may have an indirect impact on the time course of germination. To test this hypothesis we compared the influence of \( \gamma \)- and \( \alpha \)-tocopherols on the development of barley roots during the first stages of germination. Our observations suggest that endogenous \( \gamma \)-tocopherol in barley seeds modulates germination as evident by an inhibition of root elongation.
Session 3

Vitamin E status and supplementation

Chairperson: John Lodge
Molecular determinants of vitamin E retention in man

Achim Stocker

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Vitamin E is the major lipid-soluble radical quencher in humans. Specific tocopherol binding proteins are involved in the uptake and the regulation of the most potent vitamin E homologue, RRR-α-tocopherol (RRR-α-T) in man. α-Tocopherol transfer protein (α-TTP), a member of the SEC14-like protein family, is the key regulator of vitamin E homeostasis and a specific binder for RRR-α-T. α-TTP is presumed to function by transporting the hydrophobic RRR-α-T between cellular compartments.

We have solved the crystal structures of both, the ligand-charged and the apo-forms of human α-tocopherol transfer protein (α-TTP) and of human supernatant protein factor (SPF). The renewed interest in the biological function of tocopherol binders is based on the discovery of AVED, a characteristic neurological disorder, that is caused by genetic defects of the α-TTP gene and/or vitamin E deficiency. The analysis of the crystal structure of α-TTP provides insight into the molecular mechanisms of RRR-α-T specificity and transfer activity.

SPF has been reported to enhance cholesterol biosynthesis by facilitating the conversion of squalene to lanosterol. Nevertheless, the physiological role of SPF as well as its ligand specificity is not known. Investigations on the substrate specificity of SPF have uncovered binding of RRR-α-tocopherylquinone (RRR-α-TQ), the major physiological oxidation product of RRR-α-T. The ligand-protein interactions of RRR-α-TQ in the crystal structure of human SPF point towards a link between oxidized Vitamin E and cholesterol biosynthesis.
Beyond vitamin E supplementation: Alternative strategies to improve vitamin E status

Jan Frank

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Vitamin E is a generic name for all substances (tocopherols and tocotrienols) exerting the biological functions of \(\alpha\)-tocopherol. The two quantitatively most important E vitamers are \(\alpha\)- and \(\gamma\)-tocopherol (\(\alpha\)-T and \(\gamma\)-T). The fat soluble vitamin E is absorbed and transported in the circulation to the liver where \(\alpha\)-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 which are excreted in the urine. Thus, \(\alpha\)-T blood concentrations are usually 4-10 times higher than those of \(\gamma\)-T. Vitamin E has many reported health effects and is recognized as the most important lipid-soluble, chain-breaking antioxidant in the body. Vitamin E has also been reported to play a regulatory role in cell signaling and gene expression. Epidemiological studies show that high blood concentrations of vitamin E are associated with a decreased risk of cardiovascular diseases and certain cancers. Yet, high doses of supplemental vitamin E have been associated with an elevated risk in all-cause mortality. Therefore, establishing alternative strategies to improve vitamin E status without potentially increasing mortality risk is highly warranted.

To identify dietary phenolic compounds capable of increasing blood and tissue concentrations of vitamin E, selected 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 \(\alpha\)-T and \(\gamma\)-T were determined. The sesame lignan sesamin and cereal alkylresorcinols substantially increased the concentrations of \(\gamma\)-T, but not \(\alpha\)-T, in all tissues. In contrast, the flaxseed lignan secoisolariciresinol diglucoside reduced the levels of both tocopherols. The flavanols (+)-catechin and (-)-epicatechin and the preservative butylated hydroxytoluene (BHT) markedly elevated the amount of \(\alpha\)-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, at concentrations as low as 2 \(\mu\)M, almost completely inhibited tocopherol side-chain degradation and cereal alkylre-
sorcinols inhibited it, dose-dependently (5-20 µM), by 20-80%. BHT and (+)-catechin had no effect on tocopherol-ω-hydroxylase activity in HepG2 cells.

To verify the inhibition of γ-T metabolism by sesame lignans in humans, sesame oil or corn oil muffins together with deuterium-labelled d6-α-T and d2-γ-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-γ-CEHC.

Overall, the findings from these studies show that some dietary phenolic compounds alter vitamin E bioavailability in humans and animals through various mechanisms and, thus, have the potential to improve vitamin E status.
Vitamin E acts as an antioxidant both in vitro and in vivo. In this overview, studies conducted in healthy subjects and in patients who exhibit either acute or chronic oxidative stress will be presented.

In the EU-funded VITAGE project (QLK1-CT-1999-00830) (Rock et al., Nutr Metabol Cardiovasc Dis 2001, 11, suppl 4, 70-73) we investigated the status and effects of vitamin E on different biomarkers of oxidative stress in 300 healthy volunteers. They had to pass strict inclusion/exclusion criteria to be classified as healthy. Ages were equally distributed from 20 to 75 years to allow for study of possible age effects. While there was a significant increase in plasma \( \alpha \)-tocopherol and cholesterol concentrations with age, ratios of \( \alpha \)-tocopherol to cholesterol did not show any changes, neither did buccal mucosal cell \( \alpha \)-tocopherol. Bio-markers of oxidative stress including plasma malondialdehyde (by HPLC), \( \text{F}_2 \)-isoprostane, and plasma total peroxide concentrations increased significantly with age. A depletion/repletion study limiting dietary vitamin E intake to \( \sim 25\% \) of RDA, followed by unrestricted dietary intake and supplementation with a relatively high dose of vitamin E (800 IU/d RRR-\( \alpha \)-tocopherol) showed significant changes in ex vivo LDL oxidizability, total plasma peroxide concentrations and urinary 8-oxo-7,8-dihydro-2'-deoxyguanosine excretion (by HPLC with EC detection). Taken together, these data demonstrate that healthy ageing is associated with increased oxidative stress and that biomarkers of oxidative stress and resistance to oxidation in healthy individuals can be modulated by changes in vitamin E status.

Patients with chronic renal failure on hemodialysis present with increased oxidative stress in the presence of normal vitamin E but impaired vitamin C status. Parenteral iron supplements are frequently required for treating anemia in these patients. We studied the effect of a high single oral dose of vitamin E (1200 IU all-rac-\( \alpha \)-tocopheryl acetate) taken 6 hours prior to a single dose of intravenous iron (Roob et al., J Am Soc Nephrol 2000;11:539-549). Vitamin E significantly reduced and in combination with a vitamin C supplement (500 mg) it completely protected against the acute oxidative stress induced by excess iron. The iron-binding capacity of transferrin was exceeded, as demonstrated by bleomycin-detectable iron. In this setting, vitamin C in combination with vitamin E did not act as a pro-oxidant. An antioxidant sup-
plement taken prior to the intravenous application of iron might prove beneficial also in other conditions with severe anemia. Patients with cystic fibrosis experience chronic oxidative stress due to an over-production of reactive oxygen species as a result of neutrophil-dominated lung inflammation and impaired glutathione and fat-soluble vitamin status. Vitamin E status of these patients can successfully be corrected with intermediate doses of vitamin E (400 IU/d) even in the presence of persistent fat malabsorption, using either RRR-\(\alpha\)-tocopherol or all-rac-\(\alpha\)-tocopheryl-acetate. Responses to supplementation did not differ between RRR- and all-rac and between the usual fat-soluble and water-miscible preparations (Winklhofer-Roob et al., Am J Clin Nutr 1996;63:722-728). Long-term supplementation is associated with correction to normal of increased ex vivo LDL oxidizability and in vivo lipid peroxidation (Winklhofer-Roob et al., Free Radic Biol Med 1995; 19:725-733). It remains to be demonstrated whether early initiation of antioxidant supplementation and continuation for a life time will improve quality of life and life expectancy of patients with cystic fibrosis.
Session 4

Vitamin E supplementation

Chairperson: Gerald Rimbach
High dose vitamin E supplementation is associated with increased all-cause mortality: results of a meta-analysis of randomized controlled clinical trials

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Introduction: There is strong evidence from observational studies of benefit of Vitamin E supplementation for the prevention of cardiovascular disease and cancer, yet evidence from clinical trials is inconsistent, due in part to differences in patient populations, heterogeneity in study design, and differences in primary outcomes.

Methods: We performed a meta-analysis of randomized trials that tested the effects of vitamin E supplementation on all-cause mortality. Nineteen trials meeting the inclusion criteria were identified. Random-effects models and meta-regression methods were used to pool study results and to determine the dose-response relationship of vitamin E and total mortality.

Results: The 19 studies included 9 of vitamin E alone and 10 of vitamin E contained in a multivitamin. The doses of vitamin E ranged from 15 to 2000 IU/day (median 400 IU/d). The pooled all-cause mortality risk difference in high dose (>400 IU/day) was 39 per 10,000 persons (95% CI, 3 to 74 per 10,000 persons, P=0.035). The dose-response analysis showed a statistically significant relationship between Vitamin E dose and all-cause mortality, with increased risk of dosages above 150 IU/day. The possibility of a small mortality benefit with low-dose vitamin E supplementation was apparent in secondary analyses.

Conclusions: Our meta-analysis detected a dose dependent increase in total mortality with increasing vitamin E dose above 150IU/day. Significantly higher risk for mortality were seen with high-dose trials >400 IU/day. These results suggest that high-dose vitamin E supplementation may be harmful and should be avoided.
Risk assessment is the most appropriate method for safety evaluation of nutrients, including vitamin E. The Tolerable Upper Intake Level (UL) method widely is accepted. The UL is derived by (1) evaluation of the content and quality total relevant data set, (2) restrict selection of the human data if sufficiently robust, (3) identification of the critical effect, (4) dose-response evaluation to identify a No Observed Adverse Effect Level (NOAEL), (5) quantitative assessment of uncertainty, and calculation of the UL. Risk assessments of vitamin E have been performed by the US Food and Nutrition Board (UL = 1,000 mg), the EC Scientific Committee on Food (UL = 300 mg), the UK Expert Group on Vitamins and Minerals (guidance level = 540 mg), the Council for Responsible Nutrition, Washington, DC, 2004 (UL = 1,000 mg); Azzi et al., 2004 (UL = 540-800 mg) and Hathcock et al., 2005 (UL = 16,00 IU = 1,073 mg). Vitamin E can diminish platelet aggregation but the hemorrhagic stroke increase in the ATBC study has not been observed in later long-term studies at higher doses. No adverse effects in healthy adults have been established after substantial clinical trials. The meta-analysis finding of risk as dose increases has not persuaded investigators and monitoring boards that there is a clinical risk, and therefore several high-dose clinical trials are being continued. The absence of adverse effects at 3,200 IU supports good confidence that the data for intakes of 1,600 IU warrant selection of this intake as the NOAEL. The absence of adverse effect at 3,200 IU or any other dose warrants selection of an Uncertainty Factor (UF) of 1 for calculation of the UL from the NOAEL of 1,600 IU (1,073 mg). The ULS (UL for supplementation) is identical because intakes from the diet are a trivial fraction of the UL.
Session 5

Vitamin E metabolism and *in vivo* effects

*Chairperson: Achim Stocker*
Towards a better understanding of carotenoid metabolism in animals

Johannes von Lintig, Susanne Hessel, Andrea Isken, Cornelia Kiefer, Johanna M. Lampert, Olaf Voolstra, Vitus Oberhauser, Klaus Vogt

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In recent years it has become increasingly clear that nutrition has an important influence on our health. Particularly, dietary lipids or metabolites thereof are recognized as controlling a complex framework of gene activities. Carotenoids are C\textsubscript{40} isoprenoids with characteristic molecular structures and properties responsible for light absorption as well as for the inactivation of aggressive radicals. In animals, these plant-derived compounds are currently intensely investigated regarding their potential to lower the risk of chronic disease and vitamin A deficiency. To become biologically active, dietary carotenoids must first be absorbed, then delivered to the site of action in the body, and in the case of the provitamin A function metabolically converted. We identified molecular players in this pathway by the analysis of vitamin A-deficient Drosophila mutants. Similar genome sequences were found in vertebrates. Subsequently, these genes were cloned and their gene products were biochemically characterized. By loss-of-function studies in zebrafish and in knock out mouse models we studied the physiological roles of carotenoids in functional detail. This presentation will summarize the advanced state of knowledge about animal carotenoid metabolism and will discuss biochemical, physiological, developmental and medical aspects of carotenoids and their numerous derivatives.
Vitamin E is an essential micronutrient involved in various processes relevant to human health and disease. Whereas it has for long been considered just as an antioxidant, it now becomes clear that vitamin E has functions by far exceeding an antioxidative one. These include regulation of cellular signaling processes and gene expression. We learnt about these functions from the findings that vitamin E is not inert but is metabolized and degraded. All forms of vitamin E are metabolized by side chain degradation initiated by an ω-hydroxylation catalyzed by a cytochrome P450 enzyme (CYP). This mechanism is identical for all forms of vitamin E, the degree by which individual forms are degraded, however, varies dramatically, which will definitely influence their biological activity.

CYPs degrade various endogenous and exogenous compounds and many of them are induced by their substrates via the activation of the pregnane-X-receptor (PXR). Also vitamin E identified as substrate of CYPs induced a reporter gene driven by PXR. The induction was highest with α- and γ-tocotrienol (T3) and low but significant with α-tocopherol. This roughly correlated with the in vitro binding of vitamin E to PXR. In addition, γ-T3 increased the mRNA of endogenous CYP3A4 in HepG2 cells (1). This shows that vitamin E is able to directly influence gene activity in principle, which may explain many of the novel functions described for vitamin E in the last decade. Since, however, these findings may also imply an interference of vitamin E with drug metabolism it was deemed necessary to investigate their in vivo relevance. Therefore, mice were grown for 3 months with α-tocopherol-deficient, -adequate, and -supranutritional diet, i.e. 2, 20 and 200 mg RRR-α-tocopheryl acetate/kg diet, respectively. Half of them received 250µg γ-tocotrienol/day for the last 7 days. Cyp3a11 mRNA, the murine homolog to human CYP3A4, increased about 2.5-fold with 20 and 200 mg α-tocopherol. In contrast, γ-tocotrienol did not induce Cyp3a11 mRNA. This could be explained by its high metabolism as demonstrated by the 20-25-fold increase in the urinary excretion of γ-CEHC, the final metabolite of γ-tocotrienol degradation (2). Thus, α-tocopherol maintains an adequate level of xenobiotic-metabolizing enzymes. If fed in supranutritional dosages, α-tocopherol induces Cyp3a11 to levels which might interfere with drug metabolism.
In vitro studies of the biochemical and cellular effects of vitamin E are consistent with the view that it could have beneficial cardiovascular effects in vivo, and in particular that through a number of putative mechanisms it will protect against endothelial dysfunction and cardiovascular disease. However, in vivo trials involving supplementation of animal or human diets with vitamin E have given confusing and contradictory results that do not unequivocally support this hypothesis. This lecture will summarise current ideas concerning the processes involved in endothelial dysfunction and the development of atherosclerotic disease, will examine why in vitro studies suggest potentially beneficial effects of vitamin E, and will review in detail the data from animal and human trials, discussing possible reasons for their disappointing outcomes.
Session 6

Vitamin E bioavailability, function and signalling

Chairperson: Regina Brigelius-Flohé
Vitamin E bioavailability in humans

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Vitamin E appears to have a cardioprotective role therefore it is important understand factors that can influence its bioavailability, and especially in populations with increased risk of CHD such as cigarette smokers. There is also a need to clarify the bioavailability of natural and synthetic vitamin E, which is currently a subject of some controversy. Previous studies using a competitive uptake approach how found bioavailability ratios of natural:synthetic vitamin E close to 2:1, different from the accepted biopotency ratio of 1.36:1. We have used a non-competitive uptake approach to compare the plasma biokinetics of deuterated natural (RRR) and synthetic (all-rac) α-tocopheryl acetate in smokers and non-smokers. The study comprised two 4-week treatments with 400 mg/d (either RRR-α-tocopheryl or all-rac-α-tocopheryl acetates) with a 12-week wash-out period between. Prior to and after each treatment subjects underwent a 48 h biokinetic protocol with 150 mg deuterated α-tocopherol acetate in either the RRR or all-rac form. Smokers had a lower deuterated α-tocopherol AUC than non-smokers following administration of RRR, but there was no difference following all rac. The ratio RRR:all-rac from AUCs and Cmax was 1.3:1 in non-smokers and 0.9:1 in smokers. Following vitamin E supplementation deuterated tocopherol AUCs were lower in both groups. These data suggest that non-smokers and smokers differ in their handling of vitamin E, that the relative bioavailability of natural and synthetic vitamin E is close to the currently accepted biopotency ratio of 1.36:1, and that following supplementation the ability of the plasma to take up newly absorbed vitamin E is decreased.
Vitamin E and gene expression profiling

Luca Barella

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The abstract will be available at the meeting.
Intracellular signaling mechanisms mediating the antiproliferative and apoptotic effects of γ-tocotrienol in neoplastic mammary epithelial cells

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Tocotrienols, a subgroup within the vitamin E family of compounds, display potent antiproliferative and apoptotic activity against neoplastic mammary epithelial cells at treatment doses that have little or no effect on normal cell growth and function. Recent studies have shown that treatment with a growth inhibitory, but non-cytotoxic dose (4 µM) of γ-tocotrienol inhibits PI3K/PDK1/Akt mitogenic signaling over a 2-3 day period following treatment exposure, and these effects were not found to be associated with an increased in either PTEN or PP2A phosphatase activity. In addition, this treatment caused a large decrease in NFκB transcriptional activity, apparently by suppressing IκB-kinase (IKK)-α/β activation, an enzyme associated with inducing NFκB activation. Since Akt and NFκB are intimately involved in mammary tumor cell proliferation and survival, these findings strongly suggest that the antiproliferative effects of γ-tocotrienol result, at least in part, from a reduction in Akt and NFκB activity. In contrast, treatment with 20 µM γ-tocotrienol (cytotoxic dose) resulted in caspase-8 and –3 activation and apoptosis. It was also shown that this same treatment caused a rapid and large decrease in PI3K/PDK/Akt signaling within 2-4 hr following treatment exposure, and a corresponding decrease in intracellular levels of FLIP, an anti-apoptotic protein that inhibits caspase-8 activation. In summary, both the antiproliferative and apoptotic effects of γ-tocotrienol appear to be mediated by a reduction in the PI3K/PDK-1/Akt signaling, an important pathway associated with cell proliferation and survival in neoplastic mammary epithelial cells.
A. **Vitamine E (VE)**

**Overexpression of the barley *hpd* gene in tobacco plants: Effects on photosynthesis and plastid gene transcription**

Gaby Andersen, Falk Jon, Krupinska Karin  
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**Influence of vitamin C and E supplementation on hyperbaric oxygen-induced (HBO) oxidative stress in healthy men**

Nicole Bader, A. Bosy-Westphal, A. Koch, M.J. Müller  
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**The role of tocopherol in preventing oxidative damage at the cyanobacterial photosystem II of *Synechocystis sp.* PCC 6803**

Ninja Bakasch, Jens Appel, Mathias Schultzé, Rüdiger Schulz-Friedrich  
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**The influence of supplemental tocopherol on stress-induced ROS generation in suspension cells of *Arabidopsis thaliana***

Christine Desel, Wiebke Häusgen, Karin Krupinska  
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**Techniques for quantifying effects of dietary antioxidants on nitric oxide production and transcription factor nuclear translocation in cultured cells**

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²Institute of Human Nutrition and Food Science, Christian Albrechts University, Kiel, Germany; ³Department of Bioengineering, Imperial College London, London, UK  
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**Characterization of transplastomic tobacco plants with a plastid localized barley 4-hydroxyphenylpyruvate dioxygenase**

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**Improving α-tocopherol production in plant cell cultures**

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Tocopherol content and activities of tyrosine aminotransferase and cystine lyase in Arabidopsis under stress conditions
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Effects of Vitamin E and polyphenols on ochratoxin induced cytotoxicity in liver (HepG2) cells
Christoph Hundhausen, Christine Bösch-Saadatmandi, Kay Augustin, Ralf Blank, Siegfried Wollfram, Gerald Rimbach
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Characterization of the tocochromanol content and composition of different species of the parasitic plant genus Cuscuta
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Isolation and functional analysis of genes involved in the synthesis of tocopherols in rapeseed (Brassica napus L.)
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Tocopherols are important for long-term low temperature adaptation in Arabidopsis thaliana
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Tocopherols protect Synechocystis sp. PCC 6803 from PUFA-induced lipid peroxidation in concert with carotenoids
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The more - the less? α-tocopherol as inhibitor for lipidoxidation
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The role of tocopherols in germination and seedling development in Arabidopsis
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HPLC screening of natural Vitamin E from mediterranean plant biofactories-a novel tool for pilot-scale bioreactor technology
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Regulation and function of human TAP1 in epithelial cells (HeLa)
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Effects of polymorphisms of apolipoprotein A5 (apo5) and microsomal triglyceride transfer protein (MTP) genes on Vitamin E status in healthy male volunteers
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B. **GRK820**

**NOD2-dependent signal transduction and gene expression**
Susanne Billmann, Philip Rosenstiel and Stefan Schreiber
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**Different levels of gene expression in amyloplasts of potato tubers**
Mario Brosch, Jon Falk, Karin Krupinska
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**Identification of cell-wall bound phenolic compounds in Pak-Choi (Brassica campestris L. ssp. chinensis var. communis)**
Britta Harbaum, Heiko Stöckmann, Karin Schwarz,
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**Differential gene expression in Arabidopsis thaliana induced by light stress and nutrition**
Anne Hoffmann, Sabine Milde, Mirva Piippo, Arto Soitamo, Anja Hümpel,
Christine Desel, Burkhard Sattelmacher, Eva-Mari Aro, Ulf-Peter Hansen
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**Comparative gene expression studies in leaves of Arabidopsis thaliana exposed to different abiotic stressors**
Karena Hoffmann-Wuelfing, Sabine Milde, Anne Hoffmann, Arto Soitamo,
Mirva Piippo, Eva-Mari Aro, Wolfgang Bilger
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**Epidermal screening by anthocyanin pigments in leaves**
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**The bioavailability of quercetin in pigs is influenced by the dietary fat content**
Stephanie Lesser, Rainer Cermak, Siegfried Wolffram,
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**Do spice ingredients act as antioxidants in the formation of mutagenic/carcinogenic heterocyclic amines ?**
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**Functional Analysis of a pathophysiologically relevant Polymorphism (Met196 \(\rightarrow\) Arg) in human TNF\(_{\alpha}\) - Receptor 2**
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Stefan Schreiber
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Overexpression of the barley hpd gene in tobacco plants: effects on photosynthesis and plastid gene transcription

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With the aim to enhance plant vitamin E content, the barley gene encoding 4-hydroxy-phenylpyruvate-dioxygenase (HPPD), which is a key enzyme of tocochromanol and plastoquinone biosynthesis, was overexpressed in tobacco plants (Falk et al. 2003). Under normal growth conditions the tocochromanol and the plastoquinone content of the leaves are not affected, but under conditions of low temperature in combination with enhanced irradiance the tocochromanol content as well as the plastoquinone content is increased in leaves of the transgenic lines compared to wild-type plants (Andersen et al., in preparation). Also, under these conditions the transgenic lines show a higher efficiency of photosystem II, and an altered redox state of QA as revealed by measuring the photochemical quenching. Since the redox state of the plastoquinone pool is known to affect the expression of photosynthesis related genes in the plastid (Pfannschmidt 2002) it could be speculated that such abiotic stress conditions may lead to an altered expression of plastid genes encoding relevant photosynthetic proteins in the transgenic lines. Investigations of plastid gene transcription are underway. First results will be presented.

References:
Influence of vitamin C and E supplementation on hyperbaric oxygen-induced (HBO) oxidative stress in healthy men

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Objective comparison of the degree of HBO-induced oxidative stress before and after 4 weeks of vitamin C and E supplementation.

Design:
19 healthy men (age: 29.3 ±5.0 y; BMI: 24.4 ±3.1 kg/m²) were exposed to HBO (100%O₂, 2.4 bar, 131 min) before (T1) and after 4-weeks of supplementation with 500mg/d vitamin C and 200 mg/d vitamin E (T2). A normoatmospheric protocol (21%O₂, 1.0bar, 131 min) served as control treatment (Control). Blood samples were taken before (B) and immediately after treatment (A). Plasma levels of vitamin A, C, E, β-Carotene, reduced glutathione and malondialdehyde were measured by HPLC and UV-detection. Antioxidative capacity and lipidperoxides in plasma were analysed by ELISA.

Results:
When comparing values before and after treatment vitamin C (74.8 ±18.9 vs. 70.6 ±18.3 µmol/l), β-Carotene (423.2 ±317.7 vs. 393.7 ±274.8 ng/ml) and antioxidative capacity (263.3 ±38.4 vs. 245.3 ±33.9 µmol/l H₂O₂ equivalents) decreased at T1. At T1 delta A-B of vitamin C (-5.6 vs. -0.7%), antioxidative capacity (-7.3 vs. 4.4%) and lipidperoxides (6.2 vs. -13.3%). were different from the control. Vitamin supplementation increased plasma levels of vitamin C and E by 26 % and 47%, respectively. When comparing before treatment values of vitamin C (94.2 ±19.3 vs. 88.1 ±19.4 µmol/l) and reduced glutathione (314.4 ±58.7 vs. 278.8 ±50.6 µmol/l) decreased at T2. At T2 delta A-B of vitamin C, antioxidative capacity and lipidperoxides were significantly different from the control (-6.9 vs. -0.7%; -2.9 vs. 4.4%, 23.1 vs. -13.3%). Vitamin supplementation led to decreased concentrations of lipidperoxides, reduced glutathione and β-Carotene at T2-before.

Conclusion:
Oxidative stress, induced by HBO, decreased levels of vitamin C and antioxidative capacity and increased plasma lipidperoxides. Supplementation with vitamin C and E before HBO does not prevent these effects.
The role of tocopherol in preventing oxidative damage at the cyanobacterial photosystem II of *Synechocystis* sp. PCC 6803

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In contrast to the situation in higher plants the deletion of the gene encoding 4-hydroxyphenylpyruvate dioxygenase (*hpd*) in *Synechocystis* leads to a tocopherol lacking mutant without affecting the plastoquinone biosynthesis (Dähnhardt et al. 2002).

In the wildtype of *Synechocystis* light, glucose and carbon dioxide can influence the tocopherol content. Under higher light intensities, increasing the oxidative stress at Photosystem II (PS II), more tocopherol is measurable. Cultures growing photomixotrophically, shifting the ratio of PSII/PSI towards PSI compared to cultures growing photoautotrophically, contain less tocopherol than those grown photoautotrophically. Carbon dioxide deficiency, slowing down the dark reaction of photosynthesis, which causes oxidative stress at PS II, leads to higher amounts of tocopherol in *Synechocystis*. Additionally a PS II free mutant of *Synechocystis* contains almost no tocopherol at all.

Fluorescence experiments, which quantify the portion of functional PS II, showed, that PS II is degraded successively in the *hpd*-mutant under high light conditions. In the wildtype the number of intact PS II remains constant over the time.

All the data support the hypothesis, that tocopherol prevents oxidative damage at PS II. In contrast to experiments in *Chlamydomonas reinhardtii* (Trebst et al. 2002), our model allows a distinct differentiation of tocopherol from plastoquinone effects and the results indicate that the tocopherol content is correlated with the amount of oxidative stress at PS II.

References


Reduction of stress-induced ROS generation in a suspension cell culture of Arabidopsis thaliana by pre-treatment of the cells with tocopherols

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The main membrane antioxidant vitamin E exists in two major forms, α-tocopherol and γ-tocopherol. α-tocopherol has the highest vitamin efficacy, while γ-tocopherol is known as a strong antioxidant preventing oxidation of fatty acids in vegetable oil products (Pongracz et al., 1995; Kamal-Eldin and Appelqvist, 1996). Since most leaves are particularly rich in α-tocopherol, in plants the effects induced by other tocochromanols than α-tocopherol are difficult to analyze. We used instead a cell culture system of Arabidopsis thaliana to investigate the influence of supplementary tocopherol on stress-induced ROS generation. After addition of tocopherols to the medium of cultured cells, the paraquat-induced increase of ROS was monitored by quantification of DCF (5-(and-6-carboxy-2','7'-dichlorofluorescein) – fluorescence intensity. The ROS production was analysed in cells which had been fed with different concentrations and several forms of tocopherols (α- and γ- tocopherole, and tocopherol-phosphate).

The determination of the tocopherol content in the cell pellet by HPLC indicated an increase of the intracellular tocopherol concentration after supplementation. In addition, the growth rate of cell cultures preincubated with tocopherol was analysed by measuring the cell density and protein content of the cell culture.

References:
Techniques for quantifying effects of dietary antioxidants on nitric oxide production and transcription factor nuclear translocation in cultured cells

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There is increasing interest in effects of dietary antioxidants on cellular processes relevant to disease, particularly the chronic inflammatory diseases such as atherosclerosis that account for much of the morbidity and mortality in industrialised countries. We have developed techniques for quantifying (a) constitutive and induced nitric oxide production and (b) nuclear translocation of the transcription factor NF-κB, both of which are important in cardiovascular and other diseases and may be modifiable by dietary antioxidants. Nitric oxide was quantified by electrochemical methods, after reduction of its oxidation products in cell culture supernatants. NF-κB nuclear translocation was quantified using laser scanning immuno-confocal microscopy and ratiometric image analysis. We employed these techniques to study effects of isoflavones on endothelial cells and macrophages. Data were in agreement with results obtained using the Griess reaction and electromobility shift assays, but the new techniques were more sensitive, gave more detailed information, were more convenient and avoided the use of radioisotopes. The methods we have developed could be applicable in many other studies of the effects of dietary antioxidants, including vitamin E, on key processes in inflammatory diseases.

Acknowledgements: This work was funded by the Gen Foundation, Unilever plc and the Research Endowment Trust Fund of the University of Reading
Does the barley 4-hydroxyphenolpyruvate dioxygenase gene when inserted into the plastid genome of tobacco affect the tocopherol level?

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The 4-hydroxyphenylpyruvate dioxygenase (HPD) is the only enzyme of the biosynthetic pathway of tocopherols and tocotrienols localised outside of the plastid. In order to investigate whether a plastid localized HPD could give rise to an increase in tocopherol levels of tobacco leaves and seeds, in the present study the HPD enzyme was transferred to the plastid by introducing a cDNA specific for the barley hpd gene into the plastome of tobacco via homologous recombination. Expression of the hpd gene expression cassette was demonstrated by accumulation of the hpd gene specific transcript and by a higher resistance towards the HPD specific inhibitor sulcotrione. The α-tocopherol content was only slightly increased in leaves of the transplastomic plants, whereas the transplastomic seeds contained a significantly increased γ-tocochromanol level. Overexpression of the hpd gene in plastids did not proof advantageous over an increase in the HPD level within the cytoplasm by genetic engineering to yield an higher tocopherol level in plants. It is hypothesized that homogentisate synthesized in plastids will have to pass the envelope membrane in order to be accessible to the following enzymes of the tocopherol biosynthetic pathway.
Improving $\alpha$-tocopherol production in plant cell cultures

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Plant cell cultures have been widely used for the production of specific metabolites such as natural food ingredients. On the other hand, various strategies using plant in vitro systems have been successfully exploited not only for improving the production of valuable plant compounds, but also for studying their biosynthesis and metabolism. By investigating the production of tocopherols in sunflower ($Helianthus annuus$ L., cv. Gloriasol) cell cultures, we established a suitable in vitro production system of natural $\alpha$-tocopherol, which has the strongest vitamin E activity in the tocopherol isomers. It was also the main tocopherol form produced by sunflower cell cultures. The tocopherol production was studied in various culture media with or without the addition of casaminoacids, myo-inositol and biosynthetic precursors (homogentisic acid and phytol). Significant improvement of $\alpha$-tocopherol production was obtained by the addition of both casaminoacids and myo-inositol or homogentisic acid. In order to better understand the regulation of tocopherol production in plant cell cultures, we also analysed the $\alpha$-tocopherol levels of Arabidopsis thaliana (ecotype Landsberg) suspension cell cultures subjected to various growth conditions. In these cell cultures, we observed the highest value of tocopherol production twenty days after the subculture, during the stationary phase. Such value was doubled after the addition of casaminoacids and myo-inositol to the culture medium. The administration of homogentisic acid resulted in a 25% increase. Furthermore, we tested the effects of jasmonic acid and methyl-jasmonate on the tocopherol production of Arabidopsis cell cultures. A 50% enhancement of tocopherol levels was observed only when 5 $\mu$M jasmonic acid was added to the medium, while the addition of methyl-jasmonate had no effect. In these cell culture conditions analyses of gene expression are also in progress in order to investigate the regulation of tocopherol production.
Tocopherol content and activities of tyrosine aminotransferase and cystine lyase in Arabidopsis under stress conditions

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Tocopherols are supposed to be important antioxidants and scavengers of lipid radicals and reactive oxygen species in plants. In leaves of aging plants of Arabidopsis thaliana, a condition when oxidative stress increases, the content of α-tocopherol as well as of γ-tocopherol increase drastically. The activity of tyrosine aminotransferase, supplying the biosynthetic pathway with 4-hydroxyphenylpyruvate, is enhanced as well. Coronatine, a phytotoxin mimicking octadecanoids and leading to symptoms of senescence, on the other hand causes a moderate increase in α-tocopherol as well as some enhancement in γ-tocopherol.
Effect of vitamin E and polyphenols on ochratoxin induced cytotoxicity in liver (HepG2) cells

Christoph Hundhausen¹, Christine Bösch-Saadatmandi¹, Kay Augustin¹, Ralf Blank², Siegfried Wolffram², Gerald Rimbach¹

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Ochratoxin A (OTA) is a hepato- and nephrotoxic mycotoxin frequently present in food and feedstuffs. It has been suggested that oxidative damage contributes to the cytotoxicity of OTA. Therefore we examined the effect of vitamin E and different polyphenols (epigallocatechin gallate, quercetin and genistein) on OTA-induced cytotoxicity in HepG2 liver cells. Incubation of HepG2 with increasing concentrations of OTA resulted in a dose- and time-dependent cytotoxicity as measured by the neutral red assay. Half lethal concentrations (LC50) of OTA were 35 and 10 µM after 48 and 72 h incubation. Supplementation of HepG2 with alpha-tocopherol as well as different polyphenols (exhibiting different antioxidant potency as determined by the TEAC and DPPH assay) did not counteract OTA-induced cytotoxicity. Current data indicate that hepatic antioxidant defence mechanism, other than those directly affected by alpha tocopherol or polyphenols (e.g. cellular GSH), may be impaired by the OTA treatment.
Characterization of the tocochromanol content and composition of different species of the parasitic flowering plant genus Cuscuta

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The holoparasitic plant genus Cuscuta comprises plant species with different degrees of plastid functionality, ranging from intact photosynthetically active chloroplasts, via plastids with impaired protein production and gene expression to plastids with reduced plastome gene content (van der Kooij et al. 2000, Berg et al. 2003). While some species are photosynthetically active and have a chlorophyll and carotenoid composition similar to non-parasitic plants, other species are non-photosynthetic and do not contain any chlorophyll. To investigate whether the tocochromanol content and composition is related to the photosynthetic capacity of the species, the overall content of tocochromanols and the levels of the individual tocochromanols were determined in stem sections of eight Cuscuta species. All species including the non-photosynthetic ones contain plastoquinone and tocochromanols at variable levels. In addition unusual tocochromanol species were detected in C. japonica and C. grandiflora.

References:
Rapeseed is the most important oilseed crop in Germany. The oil contains three derivatives of vitamin E namely α-, γ- and small amounts of δ–tocopherol. According to the nutritional value of vitamin E oil-mills are interested in rapeseed varieties with higher contents of tocopherol. The pathway leading to the different tocopherol species has been investigated during the last years. Three of the enzymes involved in the tocopherol biosynthesis are HPPD (hydroxyphenylpyruvate dioxygenase), γ-TMT (γ-tocopherol methyltransferase) and α-cyclase. From *A. thaliana* the corresponding genes *hpd*, *vte1* and *vte4* have been cloned and sequenced.

This work aims at the identification and functional analysis of the orthologous genes in rapeseed as well as in the determination of allelic variation within these genes. Primers were designed with homology to the *A. thaliana* sequences and three fragments with homology to *hpd*, *vte1* and *vte4* were amplified and sequenced from the *B. napus* genome with a size range between 750 and 1000 bp. The overall similarity between the *B. napus* and the *A. thaliana* sequences ranged between 78 and 91%. Presently, full length clones are selected by RT-PCR and by 3’ and 5’ RACE using the *B. napus* cultivar “Express”. To demonstrate their function in the tocopherol-synthesis pathway, the full length clones will be transferred to *A. thaliana* mutants lacking the respective vitamin E genes for *hpd*, *vte1* or *vte4* using the floral-dip method and the vitamin E content of the transgenic plants will be determined by HPLC.

In a second experiment allelic variation within the rapeseed genes for tocopherol biosynthesis will be investigated. First, the number of gene copies within the allotetraploid rapeseed genome will be determined. Second, single nucleotide polymorphisms will be uncovered by sequencing the alleles of 60 rapeseed accessions and comparing them with the consensus sequence. The vitamin E content of the rapeseed accessions will be determined to find associations between sequence variation and phenotypic variation.
Tocopherols are important for long-term low temperature adaptation in *Arabidopsis thaliana*

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Tocopherols (vitamin E) are lipid-soluble antioxidants produced only by photosynthetic organisms. In contrast to the well-studied functions of tocopherols in animals, assessing the functions of tocopherols in photosynthetic organisms has become approachable only recently due to molecular dissection of the tocopherol biosynthetic pathway and isolation of mutants affecting tocopherol accumulation and composition. Recent studies using the tocopherol-deficient mutants in both *Arabidopsis thaliana* and *Synechocystis* sp. PCC 6803 demonstrated that tocopherols are essential in protecting photosynthetic organisms from lipid peroxidation during development and stress [Sattler et. al., (2004) Plant Cell 16, 1419; Maeda et. al., accompanying poster].

To further examine the physiological roles of tocopherols in mature plants, we assessed the susceptibility of tocopherol-deficient mutants of *Arabidopsis thaliana* to various abiotic stresses. These mutants were found to be sensitive to long-term low temperature treatments. The growth of specific tocopherol mutants was reduced relative to wild-type and their mature leaves accumulated anthocyanins and starch. A series of phenotypic and biochemical analyses during a time course of low temperature treatment suggested that the low temperature sensitive phenotype is not related to severe oxidative stress but rather to altered mobilization or utilization of sugars during low temperature treatment of tocopherol-deficient mutants. These results demonstrate that tocopherols play important role(s) in long-term low temperature adaptation of *Arabidopsis thaliana* by mechanisms other than acting as bulk-antioxidants.
Tocopherols protect *Synechocystis* sp. PCC 6803 from PUFA-induced lipid peroxidation in concert with carotenoids

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Tocopherols (vitamin E) are lipid-soluble antioxidants only synthesized by photosynthetic eukaryotes and some cyanobacteria, and have been assumed to play important roles in protecting photosynthetic membranes from oxidative stress. To test this hypothesis, tocopherol-deficient mutants of *Synechocystis* sp. strain PCC 6803 were challenged with a series of reactive oxygen species-generating and lipid peroxidation-inducing chemicals in combination with high-light (HL) intensity stress. The tocopherol-deficient mutants and wild-type were indistinguishable in their growth responses to HL in the presence and absence of superoxide and singlet oxygen-generating chemicals. However, the mutants showed enhanced sensitivity to linoleic or linolenic acid treatments in combination with HL. The tocopherol-deficient mutants were also more susceptible to HL treatment in the presence of sub-lethal levels of norflurazon, an inhibitor of carotenoid synthesis. These results are consistent with tocopherols playing an essential role in protecting *Synechocystis* sp. strain PCC 6803 cells from lipid peroxidation and also suggest that carotenoids and tocopherols functionally interact or have complementary roles *in vivo*. 
The more - the less? α-Tocopherol as inhibitor for lipidoxidation

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PUFA (Poly unsaturated fatty acids) display quite low stability since they are susceptible to lipidoxidation. Antioxidants, such as tocopherols, contribute to an improved shelf-life of fats and are therefore added to many foods. In addition, tocopherols are naturally found in bulk oils. Rapeseed oil for example, which is rich in unsaturated fatty acids, contains α- and γ-tocopherol in concentrations of more than 500 mg/kg. However, the efficiency of tocopherols in inhibiting lipid oxidation decrease with the degree of unsaturation of an oil (Witting, 1969).

Moreover, α-tocopherol is assumed to cause prooxidative effects in higher concentrations (Kamal-Eldin et al., 2001). Thiyam et al. (2004) found that 50 μmol/kg of a natural combination of α- and γ-tocopherols (1:1.5) protect the unsaturated fatty acids in rapeseed oil triglycerides better from oxidation than 500 μmol/kg.

The aim of this study was to systematically determine the concentration dependent antioxidant activity of α- tocopherol in rapeseed oil triglycerides and to investigate the effect of the oxidative status of the oil. Therefore the oil was purified by column chromatography to remove all naturally occurring antioxidants in the oil before different concentrations of α-tocopherol were added. To investigate the influence of the oxidative status, purified rapeseed oil was mixed with oxidated oil before adding α-tocopherol.

In order to follow lipid oxidation primary oxidation products were determined by using the conjugated dienes- and the thiocyanat-test. Secondary oxidation products were determined by gaschromatographic headspace analysis.

References:
The role of tocopherols in germination and seedling development in Arabidopsis

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In animals and in vitro systems, tocopherols have been shown to scavenge a variety of reactive oxygen species (ROS) and prevent the propagation of lipid peroxidation within polyunsaturated fatty acid (PUFA)-rich membranes by inactivating lipid peroxyl radicals. Hence, tocopherols have been presumed to protect PUFA-enriched plastids membranes from ROS and lipid peroxyl radicals generated as by-products of photosynthesis and metabolism. Two loci (VITAMIN E 1 and 2) were identified in Arabidopsis that result in tocopherol deficiency when mutated. VTE1 encodes the tocopherol cyclase and is required for forming the chromanol ring. VTE2 encodes homogentisate phytyl transferase, the committed step in the tocopherol biosynthetic pathway. vte1 mutants lack tocopherols but accumulate a tocopherol pathway intermediate DMPBQ, while vte2 mutants lack tocopherols and all tocopherol pathway intermediates. Visible defects were only observed in germinating vte2 seedlings, which displayed a range of cotyledon defects that were correlated with massive increases in lipid peroxidation. However, subsequent vegetative development in vte2 was morphologically indistinguishable from wild type. Neither cotyledon defects nor increased lipid peroxidation were observed in vte1 suggesting DMPBQ is able to functionally replace tocopherols in this regard. During germination, seedlings catabolize their storage lipids through β-oxidation, while simultaneously becoming photosynthetically competent. Because both processes are known to generate ROS, seedling development represents an oxidative hurdle in the life cycle of plants. We are using biochemical analysis, genetics and gene expression profiling to address the role of tocopherols in seedling development and to understand the impact of tocopherol deficiency during this critical stage in the lifecycle of plants.
The study was performed in order to investigate a simple, efficient, reliable and rapid method of extracting and quantifying natural vitamin E for Accelerated Solvent Extraction (ASE) as well as High-performance liquid chromatography (HPLC) analysis. Lyophilized Corylus avellana L. nut samples were powdered by high-speed milling with Waring blender for 40 s. α-Tocopherol was extracted from the nut tissue powder using dehydrated hexane fortified with 0.01% butylated hydroxytoluene (BHT). The rate of α-tocopherol accumulation showed differences among nut samples collected in different areas of Italy. Sarda piccola nut biofactory contained higher amount (81.17 µg/g d.w) of α-tocopherol than other-local eleven Italian genotype nuts. These results provide insight into the biofactory basis for α-tocopherol accumulation in hazelnuts and give the suitable Italian genotype tissues to establish pilot-scale bioreactors production of natural bioactive vitamin E.
This project investigates the antioxidative effects of vitamin E in the cell culture.

In the standard cell culture neither the medium nor the FCS contain vitamin E. According to this HeLa cells are vitamin E deficient. Incubation with tocopherols in the culture medium increases the tocopherol-content of the cells.

To analyze if vitamin E has protective effects Hela cells were exposed to oxidative stress and cell-viability, intracellular ROS and lipidperoxidation were measured. The oxidative stress was induced by 250 μM H₂O₂ or 80 μM Cuminperoxid + 80 nM Hemin.

The cell-viability was measured using MTS-Assays. Oxidative stress (250 μM H₂O₂, 48 h) reduced the cell-viability. α-tocopherol revokes this effect while the resolvent ethanol had no influence.

The level of intracellular ROS was detected by DCFH-fluorescence. The fluorescence increased after stimulation with H₂O₂. Incubation with α-tocopherol could decrease the intracellular ROS in a dose-dependent manner.

The ratio-dye Bodipy incorporates into the membrane and indicates the redox-state of the surrounding lipids. Lipidperoxidation was induced by 80 μM Cuminperoxid + 80 nM Hemin. Incubation with α-tocopherol reduced the lipidoxidation.

Conclusion: In non-stressed cells α-tocopherol had no effects. In cells exposed to oxidative stress α-tocopherol increases cell-viability und reduces intracellular ROS and lipidperoxidation.
Effects of polymorphisms of apolipoprotein A5 (apo A5) and microsomal triglyceride transfer protein (MTP) genes on vitamin E status in healthy male volunteers.


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Background: Single nucleotide polymorphisms (SNPs) of the apo A5 gene have been shown to increase plasma triglyceride levels, while SNPs in the microsomal triglyceride transfer protein (MTP) gene lower total cholesterol and low density lipoprotein concentrations (LDL) in humans. However, both polymorphisms are associated with an increased risk of coronary heart disease (CHD).

Study aim: The aim of this study was to investigate the effects of the polymorphisms apo A5 -1131T>C and MTP -493G>T on vitamin E status in 300 healthy subjects.

Subjects and Methods: Concentrations of α- and γ-tocopherol in plasma, buccal mucosal cell (BMC) vitamin E and in LDL as well as the lipid profile were determined along with the 2 polymorphisms apo A5 -1131T>C and MTP -493G>T in 300 healthy male non-smoking subjects 20-75 years of age recruited in the VITAGE project (FP5, QLK1-CT-1999-00830). Effects of the polymorphisms on vitamin E status and total cholesterol, triglycerides, LDL, high density lipoproteins (HDL) were studied.

Results: Plasma α-tocopherol concentrations were significantly increased in carriers of the apo A5 C allele (P = 0.015), which disappeared after standardization for cholesterol or triglycerides. The apo A5 polymorphism had no effect on plasma γ-tocopherol and LDL α- and γ-tocopherol and BMC total vitamin E concentrations. There were small but statistically not significant increases in total cholesterol, triglycerides and LDL. The MTP polymorphism did not show any effects on α- and γ-tocopherol concentrations in plasma and LDL, while there was a small but statistically not significant decrease in concentrations of α-tocopherol standardized for triglycerides in the homozygote T carriers due to increased triglyceride concentrations in this genotype. In contrast, BMC vitamin E was significantly increased in the heterozygote genotype (P = 0.013).

Conclusions: Differences in vitamin E concentrations in carriers of the apo A5 and MTP polymorphisms are related to increased total cholesterol and triglyceride concentrations.
The Schreiber group is interested in the role of a group of tocopherol-binding proteins (tocopherol-associated proteins, TAPs) in the signal transduction of chronic inflammatory processes of the intestine. The barrier function of the gut is maintained by a combination of mechanical, biochemical and immunological mechanisms of the intestinal epithelium, which detects pathogenic organisms via PAMP (pathogen associated molecular pattern)-receptors such as Toll-like receptors (TLR) and NOD proteins. Intestinal barrier dysfunction, e.g. by deficient NOD receptor signalling, plays a pivotal role in the etiopathogenesis of chronic inflammatory bowel disease (Hampe et al. 2001, 2002, Rosenstiel et al. 2003) or septic infections of the immunocompromised host.

To clarify the role of NOD2 in the inflammatory response to pathogens, this PhD project investigates the functional differences of NOD2wt and the loss of function mutation NOD21007fs. Differential gene expression is assessed using the microarray technology of Affymetrix.

Recently, a regulatory role of VE on signal cascades downstream of TLR4 has been demonstrated. Analysis of NOD2–dependent signal transduction and gene expression will reveal similar effects of antioxidants on NOD2 mediated inflammation.

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Hampe J. et.al.: Association between insertion mutation in NOD2 gene and Crohn's disease in German and British populations. Lancet 2001
Different levels of gene expression in amyloplasts of potato tubers

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While chloroplast gene expression has been analysed intensively in the past, almost nothing is known about its regulation in amyloplasts. Here we report on plastid gene expression in amyloplasts of potato tubers.

Transcription in amyloplasts analysed by run-on transcription assay changes significantly during tuber development. While in amyloplasts isolated from young tubers transcription of housekeeping genes are dominant, in amyloplasts derived from elder tubers only the \textit{rrn} operon and a still not identified gene is transcribed. On the other hand composition of accumulated plastid encoded transcripts is rather similar between the two different development stages of tubers as analysed by hybridization of 3-prime labelled RNA. When the composition of amyloplast RNA was compared to RNA derived from potato chloroplasts by hybridization with plastid DNA fragments one fragment including three genes shows a stronger signal in amyloplasts than in chloroplasts. These results provide evidence for differential stabilization of certain RNAs in amyloplasts compared to chloroplasts. Hybridization of 16S rDNA to ribosomal fractions showed the formation of polysomes in amyloplasts, which are indicative for active translation. Present studies are now focussing on the identification of the actively translated transcripts in amyloplasts.
Identification of cell wall bound phenolic compounds in Pak-Choi

(Brassica campestris L. ssp. chinensis var. communis)

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Phenolic compounds are found in significant quantities in plant cell walls. These compounds are esterified with the cell wall polysaccharides. Phenolic esters particularly hydroxycinnamic acids and aldehydes may cross-link plant cell wall carbohydrates due to dimer formation and determine the mechanical properties and biodegradability. Furthermore, the presence of esterified phenolic compounds may protect against pathogen infestation of plants and may be an important factor for the digestability of cell wall in animal intestine (Waldron et al., 1996; Weber et al., 1995; Buchanan et al., 1996).

The ester-linked phenolic acids in Chinese cabbage (Brassica campestris L. ssp. chinensis var. communis) were released from isolated cell wall material of fresh plants by hydrolysis under alkaline conditions as described by Beveridge et al. (2000) and Rodriguez-Arcos et al. (2002). Characterization was carried out using RP-HPLC (NUCLEODUR C18 column) with detection at 280 and 330nm. The identification of different components particularly monomeric phenolic acids and aldehydes relies on the comparison of their retention times and UV-spectra with standard compounds.

The main esterified phenolic compounds found in the cell wall of Chinese cabbage were vanillin and trans-ferulic acid. Furthermore, cis-ferulic acid, sinapic acid, p-hydroxybenzoic acid, p-hydroxybenzoic aldehyde and p-coumaric acid were found in significant amounts in the plant material. The concentrations were in the range of µg/g cell wall material. Unidentified peaks in the chromatograms are supposed to be dimers of the monomeric compounds and remain to be identified.

References:
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Buchanan CJ et al. Journal of the Science of Food and Agriculture
Differential gene expression in *Arabidopsis thaliana* induced by light
stress and nutrition

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*Arabidopsis thaliana* was grown with nitrate or ammonium as the sole nitrogen source for 14
days and exposed to light stress for 2 h. cDNA Arrays and Northern Blots were employed for
the gene expression analysis. The form of N-nutrition was found to influence photosynthesis
and the interaction of the chloroplasts with other organelles of the cell. As expected the
genes of amino acid metabolism were exclusively upregulated in nitrate grown plants. The
enhanced amino acid synthesis seems to be accompanied by additional effects on gene
expression in the related metabolic pathways:
Nitrate grown plants displayed higher expression of the enzymes of the Calvin cycle and of
photorespiration under light stress than ammonium grown ones. Amino acid synthesis
requires $\alpha$-ketoglutarat for the GS-GOGAT cycle which has to be supplemented by the TCA
cycle. Gene expression of some TCA enzyme seems to be adapted to this function.
More complex is the picture in the case of radical scavenging systems.
Plants are exposed to a plethora of unfavourable environmental conditions during their life span. Since they cannot move away, they have to acclimate to them in order to survive. One example is the epidermal accumulation of UV-absorbing phenylpropanoids such as flavonoids and hydroxy cinnamic acid esters (HCAs) for screening UV radiation. However, many plants such as *Arabidopsis thaliana* also accumulate these compounds under several other adverse environmental situations such as low temperature or high irradiance. We observed epidermal flavonoid accumulation at low temperature, high light or supplemental UV-B radiation. Therefore, we assumed merging signal transduction pathways under these conditions.

In order to investigate this assumption, gene expression studies were performed on InCyte micro-array chips using the Cy3/Cy5 labelling system. The chips displayed about 25% of the *Arabidopsis* genome, i.e. about 6500 genes. A sampling time-point of four hours after transfer to new conditions (9 °C; 360 µmol/m²s white light; 143 mW/m² UV-B radiation) was chosen.

In comparison to control plants, which were grown at 22 °C and 120 µmol/m²s white light, only 23 and 8 genes were up-regulated or down-regulated more than 1.5 times, respectively, in all separate replicates and in all treatments. 10 of these up-regulated genes were known to be stress-induced. 6 genes were involved in signal transduction as well as translational and transcriptional processes. Genes involved in vitamin E biosynthesis such as VTE1 and VTE3 were not regulated under the conditions investigated.

Surprisingly, no enzymes of the biosynthetic pathway leading to flavonoids and HCAs were markedly up-regulated in the cold or high light treatment, although these conditions led to flavonoid accumulation within few days. Only UV-B treated plants displayed a strong up-regulation of the key enzymes of flavonoid biosynthesis, phenylalanin ammonia-lyase (PAL) and chalcone synthase (CHS). Only low temperature induced several O-methyltransferases, whereas 4 hours of high-light had virtually no effect on enzymes of the flavonoid biosynthetic pathway suggesting either slow induction of flavonoid pathway gene expression or a post-transcriptional regulation and enzyme activation.
Epidermal screening by anthocyanin pigments in leaves

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Anthocyanins are leaf pigments which are supposed to possess a photoprotective function. This would result from their ability to screen UV-B and blue-green photosynthetically active radiation. In addition they are able to act as antioxidants against reactive oxygen species (ROS) induced by light (Gould et al., 2002). To investigate the photoprotective function of anthocyanins it is necessary to quantify their screening function. In principle it is possible to calculate epidermal absorbance from a methanolic leaf extract. However, the absorbance of an anthocyanin containing extract will be different from the absorbance in vivo, since anthocyanin spectral properties are strongly dependent on the solvent, the pH of the solution and are further influenced by the presence of copigmenting factors such as flavonoids.

This study involves several red-leafed plant species such as Fagus sylvatica purpurea, Rhoeo discolor and Tradescantia spec. Epidermal screening of blue-green light is measured in vivo by a chlorophyll fluorescence technique and in epidermal strips by microspectrometry (Bilger et al., 2001). In vivo absorbance is also estimated from reflectance spectra of the leaves. These results shall be compared to absorption characteristics of leaf extracts. Characterization of anthocyanin species and their potential flavonoid copigments will be performed by HPLC-MS. This information combined with direct determination of vacuolar pH shall help to reconstitute the in vivo absorbance from in vitro measurements.

Preliminary fluorescence measurements in leaves sampled along a light gradient in the crown of a copper beech tree (F. sylvatica purpurea) showed strong adjustment of blue light screening to light conditions. This was highly correlated to the specific leaf weight, which is an indicator for anatomical light acclimation of the leaves. In shade and half shade leaves, there was a good correlation between blue light screening and screening of UV-A radiation, which suggests a function of flavonoids as copigments in these leaves.

References:
The bioavailability of quercetin in pigs is influenced by the dietary fat content

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The flavonoid quercetin is one of the most prevalent flavonoids found in edible plants. Several effects of quercetin have been demonstrated in vitro which could be beneficial for human health, e.g. antiproliferative, antioxidative and anticarcinogenic effects. It is assumed that a high dietary intake of quercetin correlates with a lower risk for cancer and atherosclerosis in humans. Bioavailability after oral consumption is a prerequisite for health effects. Investigations by Azuma et al. suggest that lipids and emulsifiers as an administration vehicle for quercetin influence its bioavailability in rats (Azuma, K.; Ippoushi, K.; Ito, H.; Higashio, H. & Terao, J. (2002): Combination of lipids and emulsifiers enhances the absorption of orally administered quercetin in rats. J Agric Food Chem, 50, 1706-12). In this study we investigated the effect of dietary fat content on the oral bioavailability of quercetin applied in two chemical forms in the animal model pig. Quercetin (30 µmol kg⁻¹ body weight) was administered either as the lipophilic aglycone or as the more hydrophilic quercetin-3-O-glucoside in test meals of different fat content (standard swine diet and swine diet enriched with 15% or 30% of lard). Blood samples were repeatedly drawn over a 24-h period and analysed by HPLC. The main metabolite found in plasma was always conjugated quercetin (78 ± 0,8%). In each diet, quercetin bioavailability from the glucoside was always higher than from the aglycone. Dietary fat enhanced the total bioavailability of quercetin (P < 0.05). This applied to the administration of both chemical forms. An increase of the fat content from 15% to 30% did not further enhance quercetin bioavailability. The addition of fat accelerated uptake of quercetin from both sources. Thus, besides the chemical form of quercetin the composition of the diet seems to play a pivotal role in the overall bioavailability from the diet.

Reference:
Do spice ingredients act as antioxidants in the formation of mutagenic/carcinogenic heterocyclic amines?

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Heterocyclic amines (HCA) are formed during grilling and frying of meat and fish products at high temperatures. Related to a high consumption of well done meat products in the Western diet, HCA are discussed to be potent mutagens and/or carcinogens to humans. Since free intermediate radicals have been reported to be formed during the formation of some HCA, the use of antioxidants and antioxidative spice ingredients has been investigated in various studies to inhibit HCA formation in meat products. Rosemary extracts are commonly used in commercial meat production as a potent antioxidant to inhibit lipid oxidation during heat treatment and storage. However, contradictory results were obtained when investigating the effect of rosemary extracts on the formation of HCA in different meat and model systems. Therefore it was of interest to clarify whether the principal antioxidants of rosemary and related compounds function as antioxidants during HCA formation.

In the present study different benzoic acid and cinnamic acid derivatives, cinnamic aldehyde and Trolox have been investigated regarding their influence on the formation of IQx, MeIQx and DiMeIQx in chemical model systems during heating at 130 °C for 120 min. Apart from benzoic acid, which increased the formation of MeIQx and DiMeIQx, all additives decreased the contents of IQx-compounds between 13 and 67 % compared to the control. The investigations clearly indicate that the inhibiting efficiency of additives did not necessarily depend on an antioxidant potential, since cinnamic acid and cinnamic aldehyde e.g., do not possess hydroxyl groups that can reduce free radicals. Therefore other mechanisms may be also involved in the inhibition of HCA formation by benzoic acid and cinnamic acid derivatives.

Studies on heating stability of rosmarinic acid at 130 °C and at 180 °C resulted in a loss of rosmarinic acid between 10 and 30 %, respectively, and degradation products have been identified by LC-MS-analysis. During heating of rosmarinic acid in water/diethylene glycol (60/40) and in presence of glycine, two novel compounds have been detected: a degradation product of rosmarinic acid esterified with diethylene glycol (267 m/z) and a reaction product of rosmarinic acid and glycine (236 m/z). An inhibiting mechanism is therefore suggested which is based on the removal e.g. of free glycine as a precursor of HCA formation.
Functional analysis of a pathophysiologically relevant polymorphism (Met196 → Arg) in human TNFα - Receptor 2

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Tumor necrosis factor α (TNFα) is a pleiotropic cytokine which plays an important role in coordination of immune responses and in pathophysiological processes. TNFα displays its biological functions by binding to two distinct cell surface receptors (TNFR1 and TNFR2). Several studies show a genetic association of the biallelic polymorphism T676G in human TNFR2 with chronic inflammatory and non-inflammatory diseases (i.e. rheumatoid arthritis, colitis ulcerosa, periodontitis, alzheimer´s disease). The nucleotide substitution leads to exchange of aminoacid 196 in the extracellular domain of the receptor protein (methionine196 → arginine). The aim of this study was to evaluate differences in the activation of signal transduction pathways, ROS production during TNFR1-mediated apoptosis and expression of proinflammatory, antioxidative and antioxidative TNFα-target genes. Stable and transient overexpression of TNFR2 variants in HeLa cells and in immortalized TNFR1/TNFR2-deficient mouse fibroblasts was used for radioactive binding studies, reportergene assays and gene expression analyses using recombinant TNFα and TNFR2-specific TNFα - mutants as stimulus. Signaltransduction events triggered by both TNFR2 variants were characterized and TNFR1-induced apoptotis was analyzed under varying conditions.

The results indicate an impaired ability of the 196Arg variant of TNFR2 to (i) form a signaling complex with the cytosolic adaptor protein TRAF2, (ii) activate the antiapoptotic NFκB- signaling pathway, (iii) induce NFκB-dependent expression of antiapoptotic, antioxidative and proinflammatory genes, and (iv) counteract TNFR1-mediated proapoptotic signaling. The deficiency of the 196Arg variant in NFκB-signaling finally results in enhanced sensitivity to TNF-induced apoptosis coinciding with excess of oxidative stress.

Regarding the in vivo- functions of human TNFR2, the mutation could lead to (i) defective cytokine release by immune-modulatory cells resulting in impaired proliferation of pathogen-specific immune cells, and (ii) diminished apoptotis of activated T-effector cells in the context of adaptive immune response.
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