

## Dietary vitamin E, brain redox status and expression of Alzheimer's disease-relevant genes in rats

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Oxidative stress is one of the major pathological features of Alzheimer's disease (AD). Here, we investigated whether dietary vitamin E (VE) depletion may induce adverse effects and supplementation with  $\alpha$ -tocopherol ( $\alpha$ T) may result in beneficial effects on redox status and the regulation of genes relevant in the pathogenesis of AD in healthy rats. Three groups of eight male rats each were fed diets with deficient (<1 mg  $\alpha$ T equivalents/kg diet), marginal (9 mg  $\alpha$ T equivalents/kg diet) or sufficient (18 mg  $\alpha$ T equivalents/kg diet) concentrations of natural-source VE for 6 months; a fourth group was fed the VE-sufficient diet fortified with  $\alpha$ T (total VE, 146 mg  $\alpha$ T equivalents/kg diet). Feeding of the experimental diets dose dependently altered  $\alpha$ T concentrations in the cortex and plasma. No significant changes in F<sub>2</sub>-isoprostane concentrations, activities of antioxidative enzymes (total superoxide dismutase, Se-dependent glutathione peroxidase) and concentrations of glutathione or the expression of AD-relevant genes were observed. In this non-AD model, depletion of VE did not induce adverse effects and supplementation of  $\alpha$ T did not induce positive effects on the parameters related to the progression of AD.

### Vitamin E: Brain: Rat: Gene expression: Redox status: Alzheimer's disease

Oxidative stress appears to be centrally involved in the pathogenesis of Alzheimer's disease (AD) and many of the pathological processes involved in its development and progression are either caused by or lead to an augmented generation of reactive oxygen and nitrogen species<sup>(1)</sup>. Therefore, the importance of vitamin E (VE) for the maintenance of neuronal integrity and brain function is becoming increasingly recognised.

Epidemiological studies indicate that a high intake of VE from food sources, but not from dietary supplements, may contribute to the prevention of age-related neurodegenerative disorders such as AD<sup>(2–4)</sup>. Morris *et al.* evaluated the contribution of the different VE congeners towards the prevention of AD. While a higher dietary intake of both  $\alpha$ -tocopherol ( $\alpha$ T) and  $\gamma$ -tocopherol ( $\gamma$ T) was associated with a decreased risk of developing AD, their data suggested that the combination of the different VE isoforms may be more effective in preventing AD than the intake of  $\alpha$ T alone<sup>(4)</sup>. In line with this, in some intervention studies, supplementation of high doses of  $\alpha$ T, the major lipid-soluble antioxidant in human plasma and the VE congener with the highest biological potency<sup>(5)</sup>, failed to delay

the onset of dementia<sup>(6)</sup> and to slow down the progression of AD<sup>(7)</sup>. Other clinical intervention studies employing high dosage  $\alpha$ T supplements, on the other hand, found a significant reduction in AD progression<sup>(8,9)</sup>.

Little is known regarding the long-term effects of dietary VE on the expression of genes involved in the development or prevention of AD and on the antioxidant status in the healthy brain. The aim of the present study was therefore to investigate (i) whether suboptimal supply of dietary VE may cause unfavourable physiological changes with regard to the parameters of oxidative stress and the expression of genes relevant in the pathogenesis of AD in the healthy brain, and (ii) whether supplementation of a high dose of  $\alpha$ T on top of an already sufficient dietary supply of VE may induce AD-preventive effects.

### Materials and methods

#### Experimental diets

The tocopherol-rich rapeseed variety S-NAP 944 (a kind gift from Norddeutsche Pflanzenzucht Hans-Georg Lembke KG,

**Abbreviations:** AD, Alzheimer's disease; APP, amyloid  $\beta$ -(A4) precursor protein; Bax, Bcl-2-associated X protein; Bcl-2, B-cell leukaemia/lymphoma 2; BHT, butylated hydroxytoluene; dVE, vitamin E deficient; fVE, vitamin E fortified; HO-1, haeme oxygenase 1; mVE, vitamin E marginal; sVE, vitamin E sufficient;  $\alpha$ T,  $\alpha$ -tocopherol;  $\gamma$ T,  $\gamma$ -tocopherol; Tris, 2-amino-2-hydroxymethyl-propane-1,3-diol;  $\alpha$ TTP,  $\alpha$ T transfer protein; VE, vitamin E.

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Holtsee, Germany) was cold pressed under a nitrogen atmosphere. The resulting oil was vacuum filtered and contained 341 mg  $\alpha$ T, 386 mg  $\gamma$ T and 11 mg  $\delta$ -tocopherol per kg oil. A part of the native rapeseed oil was stripped of tocopherols by column chromatography as described by Lampi *et al.*<sup>(10)</sup>. The fatty acid composition of the oil (oleic acid, 49.1 % of total fatty acids; linoleic acid, 19.7 %; linolenic acid, 10.8 %; erucic acid, 7.2 %; gadoleic acid, 4.9 %; palmitic acid, 4.5 %) was not altered by the removal of antioxidants (data not shown). The tocopherol-stripped oil was used as follows: (i) unmodified or (ii) blended at a ratio of 1:1 with the native oil, (iii) the native oil was used unaltered or (iv) fortified with 4 g natural RRR- $\alpha$ -tocopherol (a kind gift of Cognis, Monheim am Rhein, Germany) per kg oil. Butylated hydroxytoluene (BHT 200 mg/kg; Carl Roth GmbH, Karlsruhe, Germany) was added to all oils as a preservative to protect the tocopherols from oxidative decay.

These oils were used to prepare diets (ssniff Spezialdiäten GmbH, Soest, Germany) with VE concentrations that, according to the *Nutrient Requirements of Laboratory Rats*<sup>(11)</sup>, can be classified as VE deficient (dVE), marginal (mVE), sufficient (sVE) and fortified (fVE).

The composition of the four semi-synthetic experimental diets was as follows (g/kg diet): casein, 240; maize starch, modified, 480; glucose, 110; cellulose, 50; VE-free vitamin premix (E15313-2), 10; mineral premix (E15000), 60; rapeseed oil, 50. All VE in the experimental diets originated from the rapeseed oil and were as follows (as analysed by HPLC and given in mg/kg diet). dVE:  $\alpha$ T, <1;  $\gamma$ T, <1; mVE:  $\alpha$ T, 6;  $\gamma$ T, 11; sVE:  $\alpha$ T, 12;  $\gamma$ T, 24; fVE:  $\alpha$ T, 140;  $\gamma$ T, 24.

#### Experimental animals and study design

The animal experiment was conducted in accordance with the German regulations on animal care and with the permission of the responsible authority. Thirty-two male Fisher 344 rats (Charles River Laboratories, Sulzfeld, Germany) with a mean initial body weight of 51 (SD 5) g were randomly divided into four groups of eight animals each and fed the experimental diets for 6 months. The rats were housed in Makrolon III cages in a conditioned room (22  $\pm$  2°C, 55 % relative humidity, 12 h light–dark cycle). The animals had free access to tap water and the experimental diets throughout the experiment. Food consumption and animal weight were recorded daily and weekly, respectively. At the end of the experiment, the rats were fasted for 12 h before CO<sub>2</sub>–anaesthesia and decapitation. Blood samples were collected in tubes containing heparin as anticoagulant and plasma was obtained by centrifugation (4°C, 3000 g, 10 min) and stored at –80°C until analysed. The brain was excised and dissected; one part of the cortex and the hippocampus were stored in RNA-later (Qiagen, Hilden, Germany). The remaining brain was snap frozen in liquid nitrogen and stored at –80°C.

#### Quantification of tocopherols

Tissues (100 mg) were weighed into test tubes with screw caps on ice. Ethanol (2 ml) containing 1 % (by weight) ascorbic acid, 700  $\mu$ l H<sub>2</sub>O and 300  $\mu$ l saturated aqueous KOH were

added. The samples were saponified at 70°C in a shaking water-bath for 30 min and then cooled on ice. Fifty microlitres of BHT (1 mg/ml) and 2 ml of *n*-hexane were added, and the samples were centrifuged (167 g, 4°C, 5 min) to aid phase separation. One millilitre of the supernatant was transferred to a clean tube and dried under a stream of nitrogen gas. The residue was dissolved in 250  $\mu$ l mobile phase (methanol–water (98:2, v/v)) and injected into the HPLC system. Plasma samples (100  $\mu$ l) were processed as described previously, but without saponification. Tocopherols were analysed using a Jasco (Gross-Umstadt, Germany) HPLC system (pump PU2080Plus, autosampler AS2057Plus, detector FP2020Plus). The separation of tocopherols was performed on a Waters Spherisorb ODS-2 column (100 mm  $\times$  4.6 mm, 3  $\mu$ m). The fluorescence detector was set to an excitation wavelength of 296 nm and emission wavelength of 325 nm. The concentrations of  $\alpha$ T and  $\gamma$ T were quantified by the use of authentic tocopherols (Calbiochem, Schwalbach, Germany) as external standards (limit of quantification,  $\approx$  50 pg). Analyses were performed in duplicate. Porcine plasma with known concentrations of  $\alpha$ T and  $\gamma$ T was analysed in parallel as quality control. Plasma VE concentrations were normalised for plasma cholesterol concentration, which was determined spectrophotometrically using a commercial kit (Fluitest<sup>®</sup> CHOL 4241, Biocon Diagnostik, Voehl/Marienhagen, Germany), according to the manufacturer's protocol.

#### Determination of rat cortex concentrations of F<sub>2</sub>-isoprostanes

Cortex samples were homogenised using Dounce homogeniser to yield 10 % homogenates in 180 mM-KCl, 10 mM-EDTA and 0.1 mM-BHT (pH 7.4). To hydrolyse esterified F<sub>2</sub>-isoprostanes, the cortex homogenates (100  $\mu$ l) were treated with 4 M-KOH at 45°C for 30 min and neutralised by the addition of 4 M-HCl (pH adjusted to 2.0 with 0.1 M-HCl). 9 $\alpha$ ,11 $\alpha$ -PGF<sub>2 $\alpha$</sub> -d<sub>4</sub> (0.5 ng; Cayman Chem. Co., Ann Arbor, MI, USA) was used as an internal standard. Fatty acids, including isoprostanes, were extracted two times by ethyl acetate. The samples were centrifuged for phase separation and the upper phases were applied onto NH<sub>2</sub> cartridges. Solid phase extraction and derivatisation steps were performed as described previously<sup>(12)</sup>. F<sub>2</sub>-isoprostanes were separated and measured by GC/negative-ion chemical ionisation MS (DSQ/Trace GC Ultra; Thermo Fisher Scientific, Dreieich, Germany) with ammonia as reagent gas using selected ion monitoring of the carboxylate anion [M-181] at *m/z* 569 and 573 for F<sub>2</sub>-isoprostanes and the deuterated internal standard. The F<sub>2</sub>-isoprostane peak co-eluted with authentic 8-*iso*-PGF<sub>2 $\alpha$</sub> , but it is known that other F<sub>2</sub>-isoprostane isomers occur at the same retention time<sup>(13)</sup>. All analyses were performed in triplicate for each tissue sample. The protein content was determined using the method of Bradford<sup>(14)</sup>.

#### Antioxidant enzyme activities and glutathione concentration in the cortex

Cortex tissue (100 mg) was diluted 1:10 (by weight) in ice-cold PBS and homogenised. The homogenate was centrifuged (3000 g, 4°C, 10 min), the supernatant collected and used

for the measurements after appropriate dilution in PBS. Superoxide dismutase enzyme activity was measured according to the method of Marklund & Marklund<sup>(15)</sup>. Se-dependent glutathione peroxidase activity was measured according to the method of Lawrence & Burk<sup>(16)</sup> using bovine GPx (Sigma Aldrich, Darmstadt, Germany) to generate the external standard curve. GSH concentrations were measured in homogenates according to Tietze<sup>(17)</sup> with modifications<sup>(18)</sup>. All measurements were carried out on a DU 800 spectrophotometer (Beckman Coulter, Krefeld, Germany). Results for antioxidant enzyme activities and GSH concentrations were adjusted for protein content as assessed using a commercial kit (BCA Protein Assay; Pierce, Rockford, IL, USA).

#### RNA isolation and real-time qRT-PCR

Total RNA was extracted according to the RNeasy<sup>®</sup> Lipid Tissue Protocol (Qiagen). DNA digestion was performed with the RNase-Free DNase Set (Qiagen). The concentration of isolated RNA was spectrophotometrically determined (Beckmann Instruments, Munich, Germany) by measuring the absorbance at 260 nm; the purity was determined by the ratio of 260 and 280 nm and a ratio of 1.6–1.9 was considered as acceptable. RNA aliquots were stored at  $-80^{\circ}\text{C}$  until PCR analysis. Primer sequences for real-time RT-PCR experiments were designed with Primer3 software (version 0.4.0; [http://frodo.wi.mit.edu/cgi-bin/primer3/primer3\\_www.cgi](http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi)). Primer pairs (Table 1) were obtained from MWG Biotech AG (Ebersberg, Germany). The Bcl-2 primer was obtained from Qiagen. One-step quantitative RT PCR was carried out with the QuantiTect<sup>™</sup> SYBR<sup>®</sup> Green RT-PCR kit (Qiagen). Real-time PCR amplification was performed in a Rotor-Gene 3000 thermocycler (Corbett Research, Sydney, Australia). Relative mRNA concentrations of genes were quantified by the use of a standard curve. Target gene mRNA concentration was normalised to the mRNA concentration of the housekeeping gene  $\beta$ -actin.

#### Western blot experiments in the cortex homogenates

Cortex tissue (100 mg) was homogenised in RIPA buffer (NaCl, 150 mM; sodium deoxycholate, 0.5 %; SDS, 0.1 %;

NP-40, 1 %; EDTA, 20 mM; 2-amino-2-hydroxymethyl-propane-1,3-diol (Tris)-HCl, 50 mM (pH 7.4); dithiothreitol, 1 mM; protease inhibitor cocktail). Lysates were purified by centrifugation (14 000 g,  $4^{\circ}\text{C}$ , 30 min). Total protein concentrations in each lysate were quantified using a BCA Protein Assay kit (Pierce). Total proteins of the lysate (40  $\mu\text{g}$  per lane) were separated by SDS gel electrophoresis followed by transferring the proteins to a PVDF membrane, which was then blocked for 1.5 h in blocking buffer (5 % non-fat milk in Tris-buffered saline). Primary antibodies were diluted in 5 % non-fat milk (Bax, Bcl-2, 1:200;  $\beta$ -actin, 1:1000) and the blots were incubated overnight at  $4^{\circ}\text{C}$ . The blots were washed and incubated with secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA; anti-mouse horseradish peroxidase) in blocking buffer (Bax, Bcl-2, 1:5000;  $\beta$ -actin, 1:1000) with gentle agitation for 1 h at room temperature. The blots were washed and exposed to Immun-Star Western Chemiluminescent kit (Bio-Rad Laboratories, Hercules, CA, USA) and scanned with a ChemiDoc (Bio-Rad). Digital images were captured and quantified using the Quantity-One system (Bio-Rad). Relative concentration of the proteins were quantified as the ratio between the amount of target protein and the amount of the housekeeping protein  $\beta$ -actin.

#### Statistical analysis

Statistical calculations were conducted with SPSS Version 13.0 (SPSS, Munich, Germany). For the comparison of group means, one-way ANOVA was performed with the Scheffé test (normal distribution, homogeneity of variances) or the Games–Howell test (normal distribution, heterogeneity of variances) as *post hoc* tests. When data were not normally distributed, the Mann–Whitney *U* test was performed. Significance was accepted at  $P < 0.05$ .

## Results

#### Feed intake and body weight gain

Total feed intake (data not shown) and final body weights (means dVE, 362.8 (SD 11.5) g; mVE, 366.6 (SD 18.3) g; sVE, 372.1 (SD 21.2) g; fVE, 349.9 (SD 21.0) g) did not differ between the dietary groups.

**Table 1.** Nucleotide sequences of primers used for the real-time qRT-PCR experiments

Gene	Primer sequence forward (5'–3')	Primer sequence reverse (5'–3')
$\beta$ -Actin	GGG GTG TTG AAG GTC TCA AA	TGT CAC CAA CTG GGA CGA TA
APP	CAG CGT GTC ATC CCA AAG T	GCG TGC TCG TTC TCA TCT C
Apba1	AGC AGT AAG CCG GAT CAA GA	GAT CCT CTG GGT GGA GAT GA
ADAM-10	GGG CAA TGT GCA GGT TCT AT	TGA ACT GCT TGT TCC ACT GC
BACE-1	AAT CAG TCC TTC CGC ATC AC	GGC TCG ATC AAA GAC CAC AT
ECE	CTA TGT CAG TGC CGA CTC CA	CCC AGC TGG ACC ATG TAG TT
IDE	TCA AAG GGC TGG GTA AAC AC	CCT TGC ACT CTT GGA AAA CC
NEP	ACC TAG CAG GTG GAC AGC AT	ACA CCT GGG CAA AGT TCA AG
Bax	AGC TGC AGA GGA TGA TTG CT	GAT CAG CTC GGG CAC TTT AG
AChE	CAG CAA TAC GTG AGC CTG AA	TTC CAG TGC ACC ATG TAG GA
$\alpha$ TTP	GCT TTT CAA ATT ACC CCA TC	GAT CCC ACG AAC TTT CAA TG
HO-1	GGG TGA CAG AAG AGG CTA AG	GCT GAT CTG GGA TTT TCC TC

APP, amyloid  $\beta$ -(A4) precursor protein; Apba1, APP-binding family member 1; ADAM-10, a disintegrin and metalloprotease domain 10; BACE-1,  $\beta$ -site amyloid  $\beta$ -(A4) precursor protein-cleaving enzyme 1; ECE, endothelin-converting enzyme; IDE, insulin-degrading enzyme; NEP, neprilysin; AChE, acetylcholinesterase;  $\alpha$ TTP,  $\alpha$ -tocopherol transfer protein; HO-1, haeme oxygenase 1.

### Plasma and tissue concentrations of $\alpha$ - and $\gamma$ -tocopherols

Plasma concentrations of total cholesterol were significantly lower in the dVE group than in the mVE ( $P < 0.05$ ) and fVE ( $P < 0.001$ ) groups (means dVE, 2.23 (SD 0.22) mmol/l; mVE, 2.6 (SD 0.24) mmol/l; sVE, 2.51 (SD 0.17) mmol/l; fVE, 2.80 (SD 0.24) mmol/l; one-way ANOVA with Scheffé *post hoc* test) and were used to normalise plasma tocopherol concentrations. Consumption of the experimental diets with graded concentrations of VE for 6 months significantly and dose dependently affected  $\alpha$ T concentrations in the plasma, lung, muscle, cortex and hippocampus of male Fisher 344 rats (Table 2).

### F<sub>2</sub>-isoprostanes and antioxidant enzymes in the cortex

The concentrations of F<sub>2</sub>-isoprostanes in the cortex homogenates were not significantly different between the dietary groups (Fig. 1).

Superoxide dismutase and glutathione peroxidase activities as well as glutathione concentrations in the cortices of the rats were similar in all groups (Table 3).

### Gene expression in cortex and hippocampus

In the cortex and hippocampus, the differences in dietary supply with VE did not result in significant changes in relative mRNA concentrations of amyloid  $\beta$ -(A4) precursor protein (APP), APP-binding family member 1, a disintegrin and metalloprotease domain 10,  $\beta$ -site APP-cleaving enzyme 1, endothelin-converting enzyme, insulin-degrading enzyme, neprilysin, Bax (Bcl-2-associated X protein), Bcl-2 (B-cell leukaemia/lymphoma 2), acetylcholinesterase,  $\alpha$ TTP ( $\alpha$ -tocopherol transfer protein) and HO-1 (haeme oxygenase 1) (Table 4).

### Protein concentrations of apoptosis markers in the cortex homogenates

Protein concentrations of Bcl-2 and Bax in the cortex did not differ between the groups (Fig. 2). Protein concentrations of activated caspases 3 and 8 were undetectable (data not shown).

### Discussion

Epidemiological as well as clinical intervention studies indicate that a high intake of VE may be important for the prevention of age-related neurodegenerative disorders such as AD<sup>(19)</sup>. Of the different tocopherols present in foods, RRR- $\alpha$ -tocopherol is thought to be the most important to human health and has the highest VE activity in human and animal systems<sup>(5)</sup>. Plant oils are the major dietary source of tocopherols and rapeseed oil, due to its high concentrations of tocopherols and *n*-3 fatty acids<sup>(20)</sup>, is considered of high value for human nutrition. Therefore, in the present study, we investigated the effects of graded dietary concentrations of VE from rapeseed oil on the antioxidant status and the regulation of genes relevant in the pathogenesis of AD in the brains of Fisher 344 rats during growth and maturation. During the 6-month feeding period of the present experiment, no differences in feed intake and life weight gain were observed between the dietary groups, which is in accordance with our previous findings<sup>(21)</sup>.

### Vitamin E in plasma and tissues

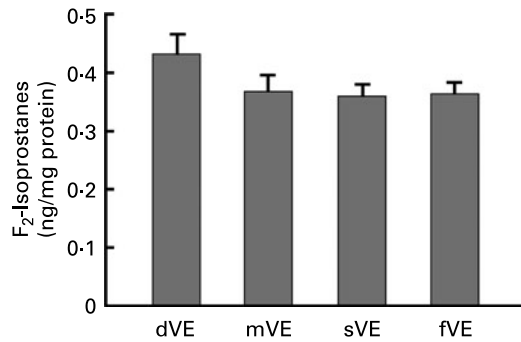
In correspondence with the intake of  $\alpha$ T, we observed dose-dependent changes in the concentrations of  $\alpha$ T in the brain (cortex and hippocampus), plasma and other tissues in our rats (Fig. 1). In accordance with the data from the previously

**Table 2.** Tocopherol concentrations in the plasma, lung, muscle, cortex and hippocampus of rats fed diets deficient (dVE), marginal (mVE) or sufficient (sVE) in vitamin E or fortified (fVE) with  $\alpha$ -tocopherol ( $\alpha$ T) for 6 months (Mean values with their standard errors, *n* 8 except where indicated differently)

Tissue	Diet								<i>P</i>
	dVE		mVE		sVE		fVE		
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	
Plasma	<i>n</i> 8		<i>n</i> 8		<i>n</i> 8		<i>n</i> 8		
$\alpha$ T (nmol/ml)	0.34 <sup>a</sup>	0.01	10.8 <sup>b</sup>	0.49	17.7 <sup>c</sup>	0.43	38.1 <sup>d</sup>	1.26	<0.001
$\gamma$ T (nmol/ml)	0.01 <sup>a</sup>	0.001	0.57 <sup>b</sup>	0.01	0.55 <sup>b</sup>	0.02	0.07 <sup>c</sup>	0.01	<0.001
$\alpha$ T (nmol/mol cholesterol)	0.15 <sup>a</sup>	0.002	4.15 <sup>a</sup>	0.09	7.05 <sup>c</sup>	0.15	13.6 <sup>d</sup>	0.18	<0.001
$\gamma$ T (nmol/mol cholesterol)	<0.01 <sup>a</sup>		0.22 <sup>b</sup>	0.01	0.22 <sup>b</sup>	0.01	0.02 <sup>c</sup>	0.002	<0.001
Lung (nmol/g)									
$\alpha$ T	0.40 <sup>a</sup>	0.04	12.1 <sup>b</sup>	0.39	21.2 <sup>c</sup>	1.24	43.1 <sup>d</sup>	1.21	<0.001
$\gamma$ T	0.04 <sup>a</sup>	0.02	0.54 <sup>b</sup>	0.02	0.65 <sup>c</sup>	0.03	0.09 <sup>a</sup>	0.02	<0.05
Muscle (nmol/g)									
$\alpha$ T	0.39 <sup>a</sup>	0.04	7.37 <sup>b</sup>	0.28	11.4 <sup>c</sup>	0.64	20.0 <sup>d</sup>	0.92	<0.001
$\gamma$ T	0.03 <sup>a</sup>	0.004	0.21 <sup>b</sup>	0.01	0.36 <sup>c</sup>	0.03	0.06 <sup>d</sup>	0.005	<0.05
Cortex (nmol/g)	<i>n</i> 8		<i>n</i> 8		<i>n</i> 6		<i>n</i> 8		
$\alpha$ T	3.78 <sup>a</sup>	0.44	16.7 <sup>b</sup>	0.97	21.2 <sup>b</sup>	1.27	30.0 <sup>c</sup>	1.59	<0.001
$\gamma$ T	0.01 <sup>a</sup>	0.001	0.13 <sup>b</sup>	0.01	0.16 <sup>b</sup>	0.01	0.05 <sup>c</sup>	0.005	<0.001
Hippocampus (nmol/g)	<i>n</i> 7		<i>n</i> 6		<i>n</i> 5		<i>n</i> 6		
$\alpha$ T	2.00 <sup>a</sup>	0.39	7.86 <sup>a,b</sup>	1.60	9.64 <sup>b,c</sup>	1.30	15.6 <sup>c</sup>	1.52	<0.05
$\gamma$ T	ND		ND		ND		ND		

$\gamma$ T,  $\gamma$  tocopherol.

<sup>a,b,c</sup> Mean values within a row with unlike superscript letters are statistically different at *P*.



**Fig. 1.** F<sub>2</sub>-isoprostane concentrations (ng/mg protein) in the cortex of Fisher 344 rats fed diets deficient (dVE), marginal (mVE) or sufficient (sVE) in vitamin E (VE) or fortified (fVE) with  $\alpha$ -tocopherol for 6 months. Values are represented as means with their standard errors (*n* 8). No statistical differences were observed.

published rat experiments<sup>(22,23)</sup>, the changes in  $\alpha$ T concentrations in the brain, however, were less pronounced than the changes observed in all other tissues. Similarly, in Parkinson's disease patients who, over a period of 5 months, consumed high amounts of  $\alpha$ T (from 400 IU/d (364 mg all-rac- $\alpha$ -tocopherol/d) during the first month to 4000 IU/d (3636 mg all-rac- $\alpha$ -tocopherol/d) during the last month of supplementation), Pappert *et al.*<sup>(24)</sup> observed large increases in  $\alpha$ T concentrations in plasma (from 19 to 111  $\mu$ mol/l), while its concentrations in ventricular cerebrospinal fluid remained unchanged (in a range of 0.114–0.164  $\mu$ mol/l).

In our rats, a deficient supply of  $\alpha$ T (< 1 mg/kg diet) resulted in an almost complete depletion of  $\alpha$ T in the plasma (0.15 (SD 0.002) mmol/mol cholesterol), while again changes in the brain were less pronounced (Table 2). In the cortex and hippocampus, 3.7 (SD 0.4) and 2.0 (SD 0.4) nmol  $\alpha$ T/g tissue, respectively, were retained even after 6 months of deficient VE intake. These data are in agreement with the results of Clement *et al.*<sup>(25)</sup>, who fed rats from VE-deficient mothers a VE-deficient diet for 8 weeks, and observed a marked reduction in the concentrations of  $\alpha$ T in the serum, liver and muscle, while significant concentrations of  $\alpha$ T were preserved in all analysed nervous tissues. Vatassery *et al.*<sup>(22)</sup> observed that even though other tissues could be almost completely depleted of  $\alpha$ T by feeding of a VE-deficient diet for 4 months, in the brain low levels of  $\alpha$ T were retained. Together, these and the present results suggest that the brain is especially efficient at

maintaining  $\alpha$ T within a certain physiological range, preventing both excessive  $\alpha$ T accumulation and its depletion.

In the present study, the dVE, mVE and sVE diets provided increasing concentrations of  $\alpha$ T and  $\gamma$ T. The fVE diet, on the other hand, was prepared by the addition of  $\alpha$ T to the natural rapeseed oil and thus provided the same amount of  $\gamma$ T as the sVE diet but about 10 $\times$  more  $\alpha$ T. With decreasing  $\gamma$ T concentrations in the dVE, mVE and sVE diets, we observed dose-dependent decreases in the concentrations of  $\gamma$ T in the lung, muscle and cortex tissue, but not in the plasma. Interestingly, the fVE-fed rats consuming additional  $\alpha$ T also had significantly reduced plasma and tissue  $\gamma$ T concentrations compared with the sVE-fed rats despite a similar  $\gamma$ T intake (Table 2). In the human system, the phenomenon of reduced  $\gamma$ T concentrations caused by a high intake of  $\alpha$ T was first described by Handelman and co-workers. In eighty-six subjects (> 50 years of age, mean age 71.8 (SD 6.4)), they observed an inverse relationship between  $\alpha$ T and  $\gamma$ T concentrations in the plasma. Supplementation of eight volunteers (30–60 years of age) with 1200 IU (1091 mg) all-rac- $\alpha$ T per day for 8 weeks resulted in a significant increase in plasma  $\alpha$ T and a significant decrease in  $\gamma$ T<sup>(26)</sup>. These findings were later confirmed in human subjects by other researchers<sup>(27,28)</sup> and are thought to be the result of an induction of cytochrome P<sub>450</sub> enzymes, which are involved in VE metabolism and preferentially degrade the desmethyl vitamins<sup>(29)</sup>.

#### F<sub>2</sub>-isoprostanes

With increasing age, lipid peroxidation of membrane fatty acids in the brain appears to augment<sup>(30)</sup> and oxidative stress in the brain is thought to be an important factor in the pathology of AD<sup>(19)</sup>. Accordingly, the concentrations of F<sub>2</sub>-isoprostanes, which are non-enzymatic free-radical-induced peroxidation products of arachidonic acid<sup>(31)</sup>, have been found to be significantly higher in the cerebrospinal fluid or brains of AD patients than those of matched healthy controls<sup>(32)</sup>. We investigated the effects of increasing doses of dietary VE on F<sub>2</sub>-isoprostane concentrations in the cortex of male Fisher 344 rats and found a non-significant trend towards higher F<sub>2</sub>-isoprostane concentrations in the group fed the dVE diet, but overall no significant differences were observed between the dietary groups (Fig. 1). Similar to our findings, Cuddihy *et al.* did not observe significant differences between the F<sub>2</sub>-isoprostane

**Table 3.** Antioxidant enzyme activities and glutathione concentrations in the cortex of rats fed diets deficient (dVE), marginal (mVE) or sufficient (sVE) in vitamin E or fortified (fVE) with  $\alpha$ -tocopherol for 6 months\*

(Mean values with their standard errors, *n* 8)

Enzyme	Diet							
	dVE		mVE		sVE		fVE	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
SOD (U/mg protein)	13.8	0.77	13.0	0.79	13.5	0.51	14.2	0.55
SeGPx (mU/mg protein)	88.0	4.67	95.6	5.20	90.4	4.35	98.0	6.61
GSH (nmol/mg protein)	24.9	1.40	25.1	0.76	23.9	0.76	26.0	0.49

SOD, superoxide dismutase; SeGPx, Se-dependent glutathione peroxidase.

\*No statistical differences were observed.

**Table 4.** Relative mRNA expression (corrected for the housekeeping gene  $\beta$ -actin) of Alzheimer's disease-relevant genes in the cortex and hippocampus of rats fed diets deficient (dVE), marginal (mVE) or sufficient (sVE) in vitamin E or fortified (fVE) with  $\alpha$ -tocopherol for 6 months\* (Mean values with their standard errors,  $n$  8)

Gene	Cortex								Hippocampus							
	dVE		mVE		sVE		fVE		dVE		mVE		sVE		fVE	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
APP	1.21	0.06	1.13	0.08	1.13	0.10	1.21	0.04	0.89	0.08	0.88	0.04	1.01	0.08	0.95	0.07
Apba1	1.06	0.11	1.13	0.11	1.35	0.14	1.16	0.09	0.94	0.06	0.92	0.06	0.95	0.08	0.95	0.06
ADAM-10	0.94	0.08	0.91	0.08	1.06	0.06	0.97	0.08	0.90	0.06	0.83	0.03	1.00	0.04	0.83	0.05
BACE-1	0.96	0.07	0.88	0.09	1.06	0.08	0.98	0.08	0.79	0.06	0.79	0.03	0.76	0.04	0.73	0.02
Bax	0.94	0.04	0.89	0.07	1.08	0.07	0.96	0.03	1.02	0.08	0.84	0.03	0.90	0.02	0.90	0.04
Bcl-2	1.09	0.05	1.04	0.05	1.16	0.07	1.14	0.05	1.15	0.08	1.01	0.04	1.15	0.05	1.08	0.05
ECE	1.03	0.06	0.87	0.05	1.10	0.09	0.96	0.04	0.95	0.06	0.90	0.04	0.95	0.04	0.89	0.05
IDE	0.92	0.04	0.86	0.07	1.07	0.06	0.97	0.08	0.93	0.12	0.88	0.07	0.99	0.08	0.86	0.09
Neprilysin	1.58	0.29	1.18	0.30	1.25	0.17	1.12	0.06	0.88	0.07	0.98	0.06	0.94	0.05	0.89	0.05
$\alpha$ TTP	1.06	0.06	1.08	0.09	1.17	0.11	1.10	0.06	0.99	0.07	0.91	0.04	1.01	0.09	1.02	0.05
HO-1	1.35	0.09	1.39	0.10	1.45	0.13	1.31	0.11	0.85	0.10	0.68	0.06	0.81	0.05	0.63	0.04
AChE	1.35	0.23	1.04	0.09	1.11	0.06	1.27	0.10	0.96	0.06	1.02	0.10	1.05	0.06	0.94	0.07

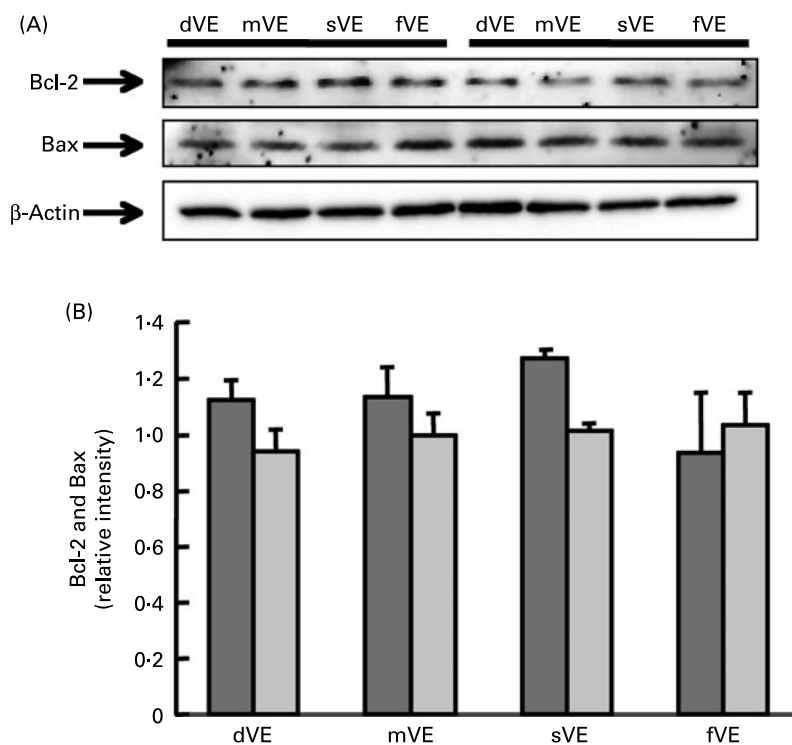
APP, amyloid  $\beta$ -(A4) precursor protein; Apba1, APP-binding family member 1; ADAM-10, a disintegrin and metalloprotease domain 10; BACE-1,  $\beta$ -site amyloid  $\beta$ -(A4) precursor protein-cleaving enzyme 1; ECE, endothelin-converting enzyme; IDE, insulin-degrading enzyme;  $\alpha$ TTP,  $\alpha$ -tocopherol transfer protein; HO-1, haeme oxygenase 1; AChE, acetylcholinesterase.

\* No statistical differences were observed.

concentrations in the cortices of mice fed dVE or sVE diets for 40 weeks. Surprisingly, cortex concentrations of F<sub>4</sub>-neuroprostanes, which are oxidation products of DHA (22:6), an abundant fatty acid in the brain, were positively correlated with  $\alpha$ T concentrations<sup>(33)</sup>.

The present results indicate that the low concentrations of  $\alpha$ T retained in the brain may have been sufficient to protect the cortex from lipid peroxidation.

Another aim of the present study was to investigate whether or not the intake of different amounts of VE may influence the activity of antioxidative enzymes and modulate cellular glutathione levels. Activities of total superoxide dismutase and Se-dependent glutathione peroxidase as well as concentrations of total GSH in the cortices of our rats did not differ between the experimental groups. It appears likely that the lack of oxidative stress in our animals may be responsible for the absence



**Fig. 2.** (A) B-cell leukaemia/lymphoma 2 (Bcl-2), Bcl-2-associated X protein (Bax) and  $\beta$ -actin protein concentrations in the cortex homogenates and (B) relative concentration of Bcl-2 (■) and Bax (□) of rats fed diets deficient (dVE), marginal (mVE) or sufficient (sVE) in vitamin E (VE) or fortified (fVE) with  $\alpha$ -tocopherol for 6 months.  $\beta$ -Actin served as endogenous (loading) control. Values are represented as means with their standard errors ( $n$  6).

of the differences in the activities and concentrations, respectively, of antioxidant enzymes. Supplementation with  $\alpha$ T (FVE group) did not result in enhanced enzyme activities or concentrations, either. However, there is no consistent opinion regarding this in the literature: while some groups report no changes in antioxidative enzyme activities or concentrations<sup>(34)</sup>, others report increases in the activities or concentrations of antioxidant enzymes caused by VE depletion<sup>(35)</sup>, as well as supplementation<sup>(36)</sup>.

### Gene expression

The accumulation and aggregation of misfolded proteins (particularly amyloid- $\beta$ ), the loss of neurons due to programmed cell death (e.g. apoptosis) and impaired neuronal signal transduction in specific parts of the brain are major pathological features of AD<sup>(1)</sup>. Therefore, we measured mRNA concentrations of genes involved in the formation (APP-binding family member 1, a disintegrin and metalloprotease domain 10,  $\beta$ -site APP-cleaving enzyme 1) or degradation (neprilysin, insulin-degrading enzyme, endothelin-converting enzyme) of amyloid- $\beta$ , programmed cell death (Bcl-2, Bax) and neurotransmitter degradation (acetylcholinesterase) in the brains of our rats.

In the cortex and hippocampus of our rats, dietary VE did not affect mRNA concentrations of the measured genes (Table 4). Some of the analysed genes (e.g. acetylcholinesterase,  $\beta$ -site APP-cleaving enzyme 1)<sup>(37,38)</sup> are thought to be regulated by oxidative stress, others experience age-related loss of activity and/or protein concentration (neprilysin, insulin-degrading enzyme)<sup>(39,40)</sup>. As an additional biomarker for oxidative stress, we determined haeme oxygenase type 1 (HO-1) mRNA concentrations in the brains of our rats. HO-1 is up-regulated in the situations of oxidative and nitrosative stress, presenting a cytoprotective effect against various forms of stress by releasing the vasoactive molecule carbon monoxide and the potent antioxidant bilirubin<sup>(41)</sup>. A significantly higher expression of HO-1 was observed in the neurons and astrocytes of AD hippocampus and cerebral cortex samples relative to age-matched controls<sup>(42)</sup>. Consistent with our F<sub>2</sub>-isoprostane data, mRNA concentrations of HO-1 were not affected by VE depletion or supplementation, indicating the absence of oxidative stress in the rat brains in the present study.

$\alpha$ TTP mRNA has been found in the human and rat brains, although at lower concentrations than in the liver<sup>(43,44)</sup>. The exact function of  $\alpha$ TTP in the brain is not known, but it was suggested to function in maintaining  $\alpha$ T concentrations in the brain<sup>(45)</sup>. In the present study,  $\alpha$ TTP mRNA concentrations were not changed by VE depletion or by  $\alpha$ T supplementation. Other groups, however, observed that VE deficiency led to transcriptional alterations in the cortex<sup>(46)</sup> and hippocampus<sup>(47)</sup> of rats, as well as in  $\alpha$ TTP<sup>-/-</sup> mice<sup>(48)</sup>. Rota *et al.*<sup>(47)</sup> have shown that certain genes related to the pathophysiology of neurodegenerative diseases were regulated in rat hippocampi by dietary VE. One main difference between these two studies is that we fed our rats for 6 months, while Rota *et al.* fed theirs for 9 months. In both studies, however, animals fed a VE-deficient diet had significantly reduced VE concentrations in the plasma and hippocampus, although the VE depletion of the brains remained incomplete, suggesting that the brain has a strong capability

to retain VE. Additionally, the diets in our trial contained the synthetic antioxidant BHT to protect the diets from lipid peroxidation during storage and BHT might have exerted additional antioxidant effects in our rats by direct scavenging of reactive species and/or regenerating  $\alpha$ T from its radical form<sup>(49)</sup>. However, with 10 mg/kg, the concentration of BHT in our diets was low compared with 2000–4000 mg/kg diet employed in the rat studies where antioxidant effects of BHT were observed<sup>(50,51)</sup>. BHT is extensively metabolised in the liver, and both BHT and its metabolites are excreted with the bile, faeces and urine, which in turn results in a low rate of tissue accumulation of BHT<sup>(52–54)</sup>. Hence, potential antioxidant effects of BHT are likely to be more potent in the liver than in the extra-hepatic tissues. However, hepatic HO-1 mRNA concentrations were significantly higher in the dVE than in the sVE animals in conjunction with reduced micro-RNA expression<sup>(55)</sup>. Therefore, the absence of oxidative stress, as assessed by the concentrations of F<sub>2</sub>-isoprostanes and relative mRNA levels of HO-1, in the brains of our VE-depleted as well as  $\alpha$ T-supplemented rats compared with control (sVE) is unlikely to be a result of the BHT in the diet.

Apoptosis seems to be an important process in AD, as suggested by increased levels of apoptosis markers in the brains of AD patients<sup>(56)</sup>. Bcl-2 and Bax, anti- and pro-apoptotic members, respectively, of the Bcl-2 family of signalling molecules, play a central role in apoptotic cell death. Both proteins function independently to regulate cell death, but can also form heterodimers. Therefore, the ratio of Bcl-2 to Bax is indicative of a pro- or anti-apoptotic cell status<sup>(57)</sup>. In the present study, neither Bcl-2 nor Bax mRNA (Table 4) and protein (Fig. 2) concentrations were different depending on the diet.

### Conclusions

The present data suggest that stepwise reduction in dietary  $\alpha$ T and  $\gamma$ T for 6 months does not impair antioxidant status, induce apoptosis or alter the expression of selected genes in the brains of rats. Similarly,  $\alpha$ T supplementation of already sVE animals does not improve antioxidant status, affect apoptosis or alter the expression of AD-relevant genes. In conclusion, VE does not appear to change the parameters involved in the aetiology and progression of AD in healthy animals. Furthermore, investigations into the impact of VE on these parameters in an AD disease model are warranted.

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G. R. designed the study. S. G. prepared the oils, conducted the feeding trial and determined antioxidant enzymes. S. G. and J. F. analysed tocopherols. X. Z. performed the western blot experiments. S. G., C. B.-S. and P. H. performed PCR analyses. I. W. and A. G. determined F<sub>2</sub>-isoprostanes. Y. L. and G. R. provided funding. S. G., J. F. and G. R. performed statistical calculations, and prepared and edited

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## References

- Christen Y (2000) Oxidative stress and Alzheimer disease. *Am J Clin Nutr* **71**, 621S–629S.
- Engelhart MJ, Geerlings MI, Ruitenberg A, *et al.* (2002) Dietary intake of antioxidants and risk of Alzheimer disease. *J Am Med Assoc* **287**, 3223–3229.
- Morris MC, Beckett LA, Scherr PA, *et al.* (1998) Vitamin E and vitamin C supplement use and risk of incident Alzheimer disease. *Alzheimer Dis Assoc Disord* **12**, 121–126.
- Morris MC, Evans DA, Tangney CC, *et al.* (2005) Relation of the tocopherol forms to incident Alzheimer disease and to cognitive change. *Am J Clin Nutr* **81**, 508–514.
- Food and Nutrition Board of the Institute of Medicine (2000) *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids*. Washington, DC: National Academy Press.
- Fillenbaum GG, Kuchibhatla MN, Hanlon JT, *et al.* (2005) Dementia and Alzheimer's disease in community-dwelling elders taking vitamin C and/or vitamin E. *Ann Pharmacother* **39**, 2009–2014.
- Petersen RC, Thomas RG, Grundman M, *et al.* (2005) Vitamin E and donepezil for the treatment of mild cognitive impairment. *N Engl J Med* **352**, 2379–2388.
- Sano M, Ernesto C, Thomas RG, *et al.* (1997) A controlled trial of selegiline, alpha-tocopherol, or both as treatment for Alzheimer's disease. The Alzheimer's Disease Cooperative Study. *N Engl J Med* **336**, 1216–1222.
- Onofrij M, Thomas A, Luciano AL, *et al.* (2002) Donepezil versus vitamin E in Alzheimer's disease: Part 2: mild versus moderate-severe Alzheimer's disease. *Clin Neuropharmacol* **25**, 207–215.
- Lampi AM, Hopia A, Ekholm P, *et al.* (1992) Method for the preparation of triacylglycerol fractions from rapeseed and other oils for autoxidation studies. *Food Sci Technol* **25**, 386–388.
- National Research Council Nutrient Requirements of the Laboratory Rat (1995) *Nutrient Requirements of Laboratory Animals*, pp. 11–79. Washington, DC: National Academic Press.
- Wiswedel I, Hirsch D, Kropf S, *et al.* (2004) Flavanol-rich cocoa drink lowers plasma F(2)-isoprostane concentrations in humans. *Free Radic Biol Med* **37**, 411–421.
- Morrow DM, Zackert WE, Van der Ende DS, *et al.* (2002) Quantification of isoprostanes as indicators of oxidant stress *in vivo*. In *Handbook of Antioxidants*, 2nd ed., pp. 57–74 [E Cadenas and L Packer, editors]. New York: Marcel Dekker.
- Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**, 248–254.
- Marklund S & Marklund G (1974) Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem* **47**, 469–474.
- Lawrence RA & Burk RF (1976) Glutathione peroxidase activity in selenium-deficient rat liver. *Biochem Biophys Res Commun* **71**, 952–958.
- Tietze F (1969) Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. *Anal Biochem* **27**, 502–522.
- Dringen R & Hamprecht B (1996) Glutathione content as an indicator for the presence of metabolic pathways of amino acids in astroglial cultures. *J Neurochem* **67**, 1375–1382.
- Grundman M, Grundman M & Delaney P (2002) Antioxidant strategies for Alzheimer's disease. *Proc Nutr Soc* **61**, 191–202.
- Grusak MA & DellaPenna D (1999) Improving the nutrient composition of plants to enhance human nutrition and health. *Annu Rev Plant Physiol Plant Mol Biol* **50**, 133–161.
- Barella L, Muller PY, Schlachter M, *et al.* (2004) Identification of hepatic molecular mechanisms of action of alpha-tocopherol using global gene expression profile analysis in rats. *Biochim Biophys Acta Mol Basis Dis* **1689**, 66–74.
- Vatassery GT, Brin MF, Fahn S, *et al.* (1988) Effect of high doses of dietary vitamin E on the concentrations of vitamin E in several brain regions, plasma, liver, and adipose tissue of rats. *J Neurochem* **51**, 621–623.
- Martin A, Janigian D, Shukitt-Hale B, *et al.* (1999) Effect of vitamin E intake on levels of vitamins E and C in the central nervous system and peripheral tissues: implications for health recommendations. *Brain Res* **845**, 50–59.
- Pappert EJ, Tangney CC, Goetz CG, *et al.* (1996) Alpha-tocopherol in the ventricular cerebrospinal fluid of Parkinson's disease patients: dose-response study and correlations with plasma levels. *Neurology* **47**, 1037–1042.
- Clement M, Dinh L & Bourre JM (1995) Uptake of dietary RRR-alpha- and RRR-gamma-tocopherol by nervous tissues, liver and muscle in vitamin-E-deficient rats. *Biochim Biophys Acta* **1256**, 175–180.
- Handelman GJ, Machlin LJ, Fitch K, *et al.* (1985) Oral alpha-tocopherol supplements decrease plasma gamma-tocopherol levels in humans. *J Nutr* **115**, 807–813.
- Melchert H-U & Pabel E (1998) The tocopherol pattern in human serum is markedly influenced by intake of vitamin E drugs – results of the German national health surveys. *J Am Oil Chem Soc* **75**, 213–216.
- Huang HY & Appel LJ (2003) Supplementation of diets with alpha-tocopherol reduces serum concentrations of gamma- and delta-tocopherol in humans. *J Nutr* **133**, 3137–3140.
- Sontag TJ & Parker RS (2002) Cytochrome P450 omega-hydroxylase pathway of tocopherol catabolism. Novel mechanism of regulation of vitamin E status. *J Biol Chem* **277**, 25290–25296.
- Floyd RA & Hensley K (2002) Oxidative stress in brain aging. Implications for therapeutics of neurodegenerative diseases. *Neurobiol Aging* **23**, 795–807.
- Morrow JD, Hill KE, Burk RF, *et al.* (1990) A series of prostaglandin F2-like compounds are produced *in vivo* in humans by a non-cyclooxygenase, free radical-catalyzed mechanism. *Proc Natl Acad Sci U S A* **87**, 9383–9387.
- Montine KS, Quinn JF, Zhang J, *et al.* (2004) Isoprostanes and related products of lipid peroxidation in neurodegenerative diseases. *Chem Phys Lipids* **128**, 117–124.
- Cuddihy SL, Ali SS, Musiek ES, *et al.* (2008) Prolonged alpha-tocopherol deficiency decreases oxidative stress and unmasks alpha-tocopherol-dependent regulation of mitochondrial function in brain. *J Biol Chem* **283**, 6915–6924.
- Kolosova NG, Shcheglova TV, Sergeeva SV, *et al.* (2006) Long-term antioxidant supplementation attenuates oxidative stress markers and cognitive deficits in senescent-accelerated OXYS rats. *Neurobiol Aging* **27**, 1289–1297.
- De AK & Darad R (1988) Physiological antioxidants and anti-oxidative enzymes in vitamin E-deficient rats. *Toxicol Lett* **44**, 47–54.
- Devi SA & Kiran TR (2004) Regional responses in antioxidant system to exercise training and dietary vitamin E in aging rat brain. *Neurobiol Aging* **25**, 501–508.
- Tong Y, Zhou W, Fung V, *et al.* (2005) Oxidative stress potentiates BACE1 gene expression and Abeta generation. *J Neural Transm* **112**, 455–469.



38. Melo JB, Agostinho P & Oliveira CR (2003) Involvement of oxidative stress in the enhancement of acetylcholinesterase activity induced by amyloid beta-peptide. *Neurosci Res* **45**, 117–127.
39. Caccamo A, Oddo S, Sugarman MC, *et al.* (2005) Age- and region-dependent alterations in Abeta-degrading enzymes: implications for Abeta-induced disorders. *Neurobiol Aging* **26**, 645–654.
40. Fukami S, Watanabe K, Iwata N, *et al.* (2002) Abeta-degrading endopeptidase, neprilysin, in mouse brain: synaptic and axonal localization inversely correlating with Abeta pathology. *Neurosci Res* **43**, 39–56.
41. Calabrese V, Scapagnini G, Colombrita C, *et al.* (2003) Redox regulation of heat shock protein expression in aging and neurodegenerative disorders associated with oxidative stress: a nutritional approach. *Amino Acids* **25**, 437–444.
42. Schipper HM, Cisse S & Stopa EG (1995) Expression of heme oxygenase-1 in the senescent and Alzheimer-diseased brain. *Ann Neurol* **37**, 758–768.
43. Hosomi A, Goto K, Kondo H, *et al.* (1998) Localization of alpha-tocopherol transfer protein in rat brain. *Neurosci Lett* **256**, 159–162.
44. Copp RP, Wisniewski T, Hentati F, *et al.* (1999) Localization of alpha-tocopherol transfer protein in the brains of patients with ataxia with vitamin E deficiency and other oxidative stress related neurodegenerative disorders. *Brain Res* **822**, 80–87.
45. Leonard SW, Terasawa Y, Farese RV Jr, *et al.* (2002) Incorporation of deuterated RRR- or all-rac-alpha-tocopherol in plasma and tissues of alpha-tocopherol transfer protein-null mice. *Am J Clin Nutr* **75**, 555–560.
46. Hyland S, Muller D, Hayton S, *et al.* (2006) Cortical gene expression in the vitamin E-deficient rat: possible mechanisms for the electrophysiological abnormalities of visual and neural function. *Ann Nutr Metab* **50**, 433–441.
47. Rota C, Rimbach G, Minihane AM, *et al.* (2005) Dietary vitamin E modulates differential gene expression in the rat hippocampus: potential implications for its neuroprotective properties. *Nutr Neurosci* **8**, 21–29.
48. Gohil K, Schock BC, Chakraborty AA, *et al.* (2003) Gene expression profile of oxidant stress and neurodegeneration in transgenic mice deficient in alpha-tocopherol transfer protein. *Free Radic Biol Med* **35**, 1343–1354.
49. Frank J, Lundh T, Parker RS, *et al.* (2003) Dietary (+)-catechin and BHT markedly increase alpha-tocopherol concentrations in rats by a tocopherol-omega-hydroxylase-independent mechanism. *J Nutr* **133**, 3195–3199.
50. Frank J, Lundh T, Parker RS, *et al.* (2003) Dietary (+)-catechin and BHT markedly increase alpha-tocopherol concentrations in rats by a tocopherol-omega-hydroxylase-independent mechanism. *J Nutr* **133**, 3195–3199.
51. Kamal-Eldin A, Frank J, Razdan A, *et al.* (2000) Effects of dietary phenolic compounds on tocopherol, cholesterol, and fatty acids in rats. *Lipids* **35**, 427–435.
52. Daniel JW & Gage JC (1965) The absorption and excretion of butylated hydroxytoluene (BHT) in the rat. *Food Cosmet Toxicol* **3**, 405–415.
53. Thompson JA, Bolton JL & Malkinson AM (1991) Relationship between the metabolism of butylated hydroxytoluene (BHT) and lung tumor promotion in mice. *Exp Lung Res* **17**, 439–453.
54. Conacher HB, Iverson F, Lau PY, *et al.* (1986) Levels of BHA and BHT in human and animal adipose tissue: interspecies extrapolation. *Food Chem Toxicol* **24**, 1159–1162.
55. Gaedicke S, Zhang X, Schmelzer C, *et al.* (2008) Vitamin E dependent microRNA regulation in rat liver. *FEBS Lett* **582**, 3542–3546.
56. Honig LS & Rosenberg RN (2000) Apoptosis and neurologic disease. *Am J Med* **108**, 317–330.
57. Lu J, Moomhala S, Kaur C, *et al.* (2000) Changes in apoptosis-related protein (p53, Bax, Bcl-2 and Fos) expression with DNA fragmentation in the central nervous system in rats after closed head injury. *Neurosci Lett* **290**, 89–92.