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Effect of Vitamin E on Cytochrome P450 mRNA Levels in Cultured Hepatocytes (HepG2) and in Rat Liver

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Abstract. *Vitamin E has been described in the literature as a regulator of gene expression. The gene-regulatory activity of vitamin E with regard to genes encoding cytochrome P450 (CYP) enzymes, which play a pivotal role both in the metabolism of xenobiotics and vitamin E, has not been conclusively characterised. The objective of the current study was, therefore, to elucidate the short- and long-term effects of natural and synthetic vitamin E on CYP gene expression using Affymetrix GeneChip® technology. To this end, HepG2 cells were incubated with 0, 10, 30, 80 and 300 μM RRR-α-tocopheryl acetate (natural vitamin E) or all rac-α-tocopheryl acetate (synthetic vitamin E) for 7 days and the mRNA of CYP genes was quantified. The expression of only one (CYP20A1) of 14 CYP genes with detectable mRNA levels was dose-dependently up-regulated. No differences in gene-regulatory activity were observed between RRR- and all rac-α-tocopheryl acetate. To study the role of vitamin E in CYP gene expression in vivo, Fisher 344 rats were randomly assigned to either a vitamin E-enriched (60 mg/kg RRR-α-tocopheryl acetate) or -deficient (1.7 mg/kg RRR-α-tocopheryl acetate) diet for 290 days. Neither in the vitamin E-enriched, nor in the vitamin E-deficient rats, were significant changes in the liver CYP, mRNA levels observed. In conclusion, our data indicated that vitamin E does not appear to modulate cytochrome P450 mRNA expression in HepG2 cells or in rats.*

α-Tocopherol, the predominant form of vitamin E *in vivo*, is the most important lipid-soluble, chain-breaking anti-oxidant in human plasma (1). In the past, studies have focussed on the ability of vitamin E to prevent diseases that are believed to have an oxidative stress component, such as atherosclerosis, Alzheimer's disease and cancer (2-4). However, more recent findings have indicated that vitamin E also has other important functions that are independent of its anti-oxidant activity, such as the regulation of gene expression (5-8).

Vitamin E is not one particular substance, but a generic term for a group of lipid-soluble, chain-breaking anti-oxidants. The eight recognized natural vitamin E compounds consist of a chromanol head substituted with a 16-carbon side chain. They can be subdivided into tocopherols, with a saturated side chain and tocotrienols, with an unsaturated side chain with three isolated double bonds. The lower case greek letters α, β, γ, δ are assigned as prefixes according to the number and position of methyl groups attached to the chromanol head. Because the side chain of the tocopherols features three chiral centres at positions 2, 4', and 8', that can be in either R or S configuration, eight stereoisomers for each tocopherol are possible. In nature, tocopherols exist exclusively as *RRR*-stereoisomers, whereas synthetic vitamin E is an equimolar mixture of all eight stereoisomers, a so-called *all racemic (all rac)* mixture (9).

Upon ingestion, vitamin E generally follows the absorption and transport pathways of dietary lipids in the body. The metabolic degradation of vitamin E seems to occur in the liver, involving cytochrome P450 (CYP) enzymes (10, 11).

CYP enzymes constitute a large family of membrane-bound oxidative proteins. In liver cells, CYPs are located either in the inner membrane of the mitochondria or in the endoplasmic reticulum, where they metabolize a wide variety of endogenous compounds and xenobiotics. They are, however, also present in other tissues of the body including the mucosa of the gastrointestinal tract and kidney cells. In mammals, CYP enzymes are the most important enzymes of phase I metabolism. Additionally, they play an

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important role in the biosynthesis of steroids, fatty acids and bile acids. In humans, CYP3A4 and its closely related form CYP3A5 are the most abundant forms in the liver (from 30 to 60% of total CYP, depending on genetic background, food *etc.*). CYP3A4 metabolises more than 50% of the drugs currently used for therapy (12).

The initial step in the metabolism of tocopherols and tocotrienols is the hydroxylation of the terminal methyl group of the side-chain, followed by a step-wise shortening by β-oxidation. The resulting water-soluble carboxye-thylhydroxychromans (CEHCs) are excreted in the urine. Both CYP4F2 and CYP3A4 were suggested to facilitate this initial ω-hydroxylation and their inhibition resulted in a reduced production of CEHCs in various *in vitro* systems (13, 14).

High-throughput techniques for the analysis of changes in mRNA levels are powerful tools to study the gene-regulatory activity of vitamin E. These state-of-the-art techniques have been successfully used to identify genes that are differentially expressed in response to dietary vitamin E in various tissues including liver (5), testes (6) , adrenal glands (15), cortex (16) and hippocampus (7). Although some studies indicate an induction of CYP mRNA by certain vitamin E isoforms (11, 17), the role of the vitamin on the expression of CYPs has not been studied in sufficient detail.

The aim of this trial was to perform a large-scale gene-chip experiment to determine the role of vitamin E on the gene expression of CYP enzymes both *in vitro* and *in vivo*.

Materials and Methods

Cell culture experiments. HepG2 cells (ATCC HB-8065) were cultured in 6 cm dishes in DMEM medium (GIBCO-Invitrogen, Basel, Switzerland) with 10% NU serum™ (Becton Dickinson, Basel, Switzerland) containing 1% penicillin/streptomycin and no detectable amounts of vitamin E (detection limit 20 nM). *RRR*- and all *rac*-α-tocopheryl acetate (purity: 99.0-99.5 and 98.0-99.5 weight%, respectively, DSM Nutritional Products Ltd, Kaiseraugst, Switzerland) were dissolved in 100% ethanol to prepare stock solutions. Treatment media were prepared by the addition of RRR- or all rac-α-tocopheryl acetate (*RRR*-α-Tac or all *rac*-α-Tac, respectively) to the basic medium at the following final concentrations: 0, 10, 30, 80 and 300 μM. The maximal ethanol concentration in the medium was 1‰. Treatment media were aliquoted and stored at –20˚C. The vitamin E treatment was performed for seven days during the logarithmic growth phase of the cells. All treatment media were exchanged for fresh media every 24 h. All treatments were performed in quadruplicate dishes.

Animals and diets. Sixty recently-weaned male Fisher 344 rats (Charles River, Les Oncins, France) with an initial average weight of 50-60 g were randomly assigned to either a VE-containing diet (VE+) or to a control diet deficient in vitamin E (VE) for 290 days. The VE+ diet contained 60 mg/kg *RRR*-α-tocopheryl acetate (DSM Nutritional Products Ltd), which corresponds to 80 IU per kg diet. The VE-diet contained 60 mg/kg of the vehicle (16% fish gelatine, 8% micro gel E, 1% Sipernat 50) resulting in a final dietary concentration of 1.7 mg/kg *RRR*-α-tocopheryl acetate. The VE- and VE+ rats consumed the diets *ad libitum* and had free access to water. The rats were maintained under standard conditions at 22±1˚C with 12:12 h dark:light cycles. The Laboratory Animal Care Committees of F. Hoffmann-La Roche Ltd, Basel and the Veterinary Office of Basel-Stadt, Switzerland approved all animal protocols.

Beginning from day 17 of feeding, five animals per group were euthanised every 3 months under isoflurane anaesthesia by withdrawing blood from the *vena cava*. Livers were removed, rinsed in ice-cold phosphate-buffered saline (pH 7.4) and snap-frozen in liquid nitrogen. Samples were stored at –80˚C prior to RNA extraction.

Vitamin E concentrations in HepG2 cells and in rat liver and plasma. Adherent HepG2 cells were trypsinised, collected and washed three times with PBS containing 1% bovine serum albumin. The HepG2 cells or liver tissue, respectively, was saponified in a methanolic potassium hydroxide solution. The solution was diluted in 35% ethanol and extracted with hexane/toluol. α-Tocopherol was quantified by isocratic HPLC analysis using a Lichrosorb™ Si 60.5 mm, 20x4 mm pre-column coupled to a Lichrosorb™ Si 60.5 mm, 125x4 mm column (Stagroma, Reinach, Switzerland) and 3% 1.4-dioxane in n-hexane as the mobile phase (flow rate ~1.6 ml/min, pressure 35-55 bar). Fluorescence was measured at 330 nm after excitation at 295 nm.

The plasma samples were analysed for α-tocopherol using a routine HPLC method. Briefly, the plasma proteins were precipitated using ethanol and tocopherol was extracted by liquid/liquid extraction with hexane. After centrifugation, an aliquot of the organic phase was chromatographed isocratically on a normal phase HPLC system. α-Tocopherol was quantified by fluorimetry (excitation at 298 nm, emission at 326 nm).

Total RNA extraction, cRNA preparation and Affymetrix GeneChip® hybridization. The cells were washed three times with PBS and lysed with RTL buffer (Qiagen, Basel, Switzerland). Total RNA isolation was performed using RNeasy mini spin columns (Qiagen) and DNase digested on the columns (RNase-Free DNase Set, Qiagen) according to the manufacturer's description. cRNA preparation and Affymetrix GeneChip® (U133A) hybridisation were performed as described (5).

GeneChip® microarray expression and data analysis. Data processing was carried out using the RACE-A analysis tool (Roche Bioinformatics, Basel, Switzerland) as previously described (5). Briefly, the arrays were normalised against the mean of the total sum of Average Difference (AvgDiff) values across all arrays used. Mean average difference values (MeanAvgDiff) were calculated as the means of one experiment performed in quadruplicate. Possible outliers were identified using the procedure of Nalimov with a 95% confidence interval. Subsequently, mean change factors (Chgf) for each individual gene were calculated among the different treatment groups and control using pairwise comparisons and statistical significance was assessed by the Student's *t*-test with prior testing for the normal distribution of the data. The analysis of the experimental data obtained upon stimulation of the HepG2 cells with *RRR*-α-Tac or all *rac*-α-Tac was performed independently from each other. Confirmation of the gene expression data by RT-PCR was omitted because, in previously published

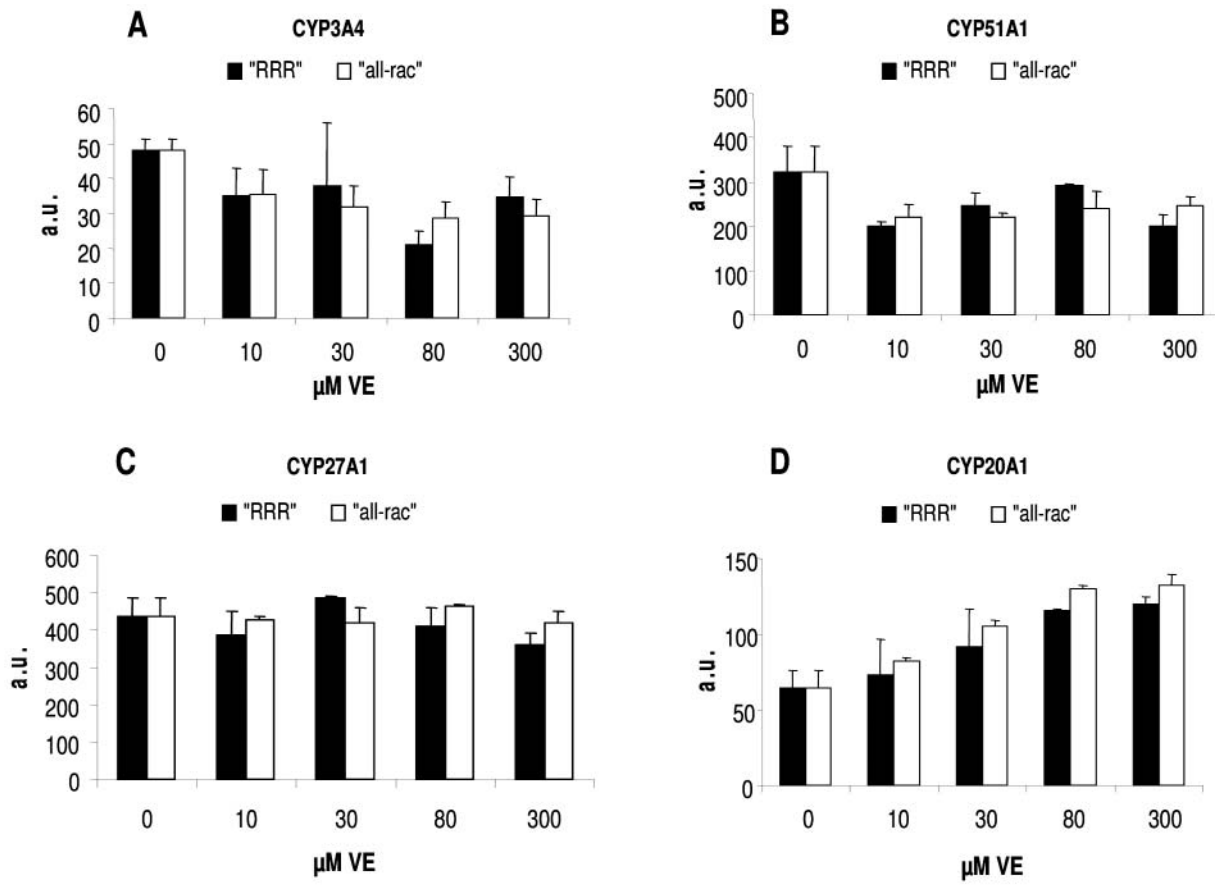

Figure 1. *Cytochrome P450 mRNA levels in HepG2 cells – given in arbitrary units (a.u.) – after treatment with RRR-α-tocopheryl acetate ("RRR") or all rac-α-tocopheryl acetate ("all rac") at the concentrations given for 7 days. The data represent means±SD.*

experiments performed on the same experimental animals, the GeneChip® data were always consistent with data confirmed by independent methods (5-7). A differential expression profile analysis between the two treatment groups was performed at four different time-points (T1=day 17, T2=day 91, T3=day 191 and T4=day 269 of feeding) over the 9-month study period in five individual liver samples.

Results

α-Tocopherol concentrations in HepG2 cells. The incubation of HepG2 cells with media containing 0, 10, 30, 80 or 300 μM *RRR*- or *all rac*-α-Tac for 7 days dose-dependently increased the intracellular content of free vitamin E. A plateau was reached at supplementation with 80 μM vitamin E and a further increase in vitamin E did not cause higher intracellular vitamin E concentrations. There was no significant difference between the intracellular concentrations of *RRR*- and *all rac*-α-Tac (data not shown).

CYP mRNA expression in vitro. In the HepG2 cells, 14 out of the 44 CYP enzymes represented on the RNA microarray were expressed. The highest expression levels of these 14 CYPs were observed for CYP27A1 and CYP51A1 (Figure 1). Four members of the CYP3A subfamily, namely CYP3A4 (Figure 1), CYP3A5, CYP3A7 and CYP3A43, as well as CYP4F3 and CYP4F12, showed low to moderate expression levels. CYP4F2 was only marginally expressed.

In response to vitamin E supplementation, only the mRNA levels of CYP20A1 dose-dependently increased (Figure 1). All other 13 CYPs with detectable mRNA levels showed no dose-dependency. Moreover, there was no substantial difference in CYP mRNA expression between treatment with *RRR*-α-Tac and *all rac*-α-Tac. A detailed list of the CYP mRNA levels for each vitamin E concentration is given in Table I.

Body weight, plasma and liver vitamin E concentrations in rats. No differences in weight gain and final body weight were observed between the rats fed VE+ and VE– diets. Rats fed the VE+ diet had significantly higher plasma levels (27.40±3.17 μmol/l, day 269) compared to the controls receiving the VE–

Table I. *Cytochrome P450 mRNA levels in arbitrary units (a.u.) in HepG2 cells after treatment with RRR-α-tocopheryl acetate ("RRR") and all rac-α-tocopheryl acetate ("all rac") at the concentrations indicated for 7 days. Data represent means±SD.*

CYP450 enzyme, HepG2			mRNA expression (a.u.±SD)				
	Function	VE form	0	10	30	80	300 μM VE
CYP1A2	metabolism of xenobiotics, drugs	*"all rac"*	45 (15)	67 (10)	56 (5)	74 (5)	63 (1)
		"RRR"	45 (15)	62 (21)	64 (12)	67 (1)	48 (9)
CYP2A6	metabolism of xenobiotics, drugs	*"all rac"*	72 (18)	61 (16)	54 (13)	44 (2)	43 (6)
		"RRR"	72 (18)	59 (11)	75 (3)	45 (13)	53 (2)
CYP2C9	metabolism of xenobiotics, drugs	*"all rac"*	66 (10)	36 (8)	34 (6)	42 (3)	35 (1)
		"RRR"	66 (10)	45 (24)	65 (6)	36 (4)	37 (1)
CYP3A4	metabolism of xenobiotics, drugs	*"all rac"*	48 (3)	35 (7)	31 (6)	28 (4)	29 (4)
		"RRR"	48 (3)	35 (7)	38 (17)	20 (4)	34 (5)
CYP3A5	metabolism of xenobiotics, drugs	*"all rac"*	36 (9)	23 (4)	23 (2)	38 (9)	24 (1)
		"RRR"	36 (9)	28 (6)	29 (6)	37 (3)	25 (4)
CYP3A7	metabolism of xenobiotics, drugs	*"all rac"*	38 (13)	25 (12)	27 (8)	26 (5)	23 (1)
		"RRR"	38 (13)	29 (7)	34 (10)	25 (1)	22 (5)
CYP3A43	metabolism of xenobiotics, drugs	*"all rac"*	45 (12)	45 (2)	40 (9)	46 (5)	34 (3)
		"RRR"	45 (12)	31 (11)	39 (2)	38 (6)	41 (2)
CYP4F3	arachidonic acid or fatty acid metabolism	*"all rac"*	37 (7)	18 (12)	23 (3)	26 (3)	20 (2)
		"RRR"	37 (7)	26 (9)	24 (4)	24 (8)	20 (5)
CYP4F12	arachidonic acid or fatty acid metabolism	*"all rac"*	77 (21)	39 (10)	36 (2)	61 (6)	59 (8)
		"RRR"	77 (21)	55 (12)	61 (7)	47 (7)	51 (12)
CYP11B1	steroid biosynthesis	*"all rac"*	35 (13)	13 (1)	21 (3)	21 (3)	15 (2)
		"RRR"	35 (13)	25 (4)	29 (5)	18 (6)	17 (4)
CYP20A1	unknown	*"all rac"*	64 (10)	82 (2)	105 (3)	130 (2)	132 (6)
		"RRR"	64 (10)	72 (24)	91 (25)	115 (1)	119 (5)
CYP24A1	vitamin D degradation	*"all rac"*	40 (8)	50 (1)	72 (10)	44 (8)	40 (7)
		"RRR"	40 (8)	43 (4)	52 (13)	56 (10)	45 (6)
CYP27A1	bile acid biosynthesis	*"all rac"*	435 (51)	430 (8)	420 (41)	463 (4)	418 (33)
		"RRR"	435 (51)	386 (63)	488 (1)	410 (49)	359 (33)
CYP51A1	cholesterol biosynthesis	*"all rac"*	323 (57)	219 (32)	220 (9)	239 (38)	246 (20)
		"RRR"	323 (57)	201 (9)	245 (31)	294 (1)	200 (24)

diet (0.20±0.09 μmol/l, day 269). Differences in dietary α-tocopherol were also reflected by significant differences in its hepatic concentrations after 269 days (VE+: 83.91±15.86 nmol/g tissue, VE–: 0.35±0.20 nmol/g tissue) (5).

CYP mRNA expression in vivo. In rat liver, 34 out of the 44 CYP enzymes represented on the RNA microarray were expressed. The highest mRNA expression levels were observed for CYP2C7 and CYP2D3. CYP3A4 and CYP4F2 also showed high expression levels (Figure 2). CYP1A1, CYP2A3, CYP2D18, CYP3A2, CYP3A9, CYP4A8, CYP11B3, CYP17 and CYP51 were only marginally expressed (data not shown). A detailed list of all the CYPs with moderate or high mRNA expression levels is given in Table II.

No significant differences in CYP mRNA concentrations were found at any time between rats deprived of or supplemented with vitamin E. Furthermore, no significant changes in mRNA levels were observed over time.

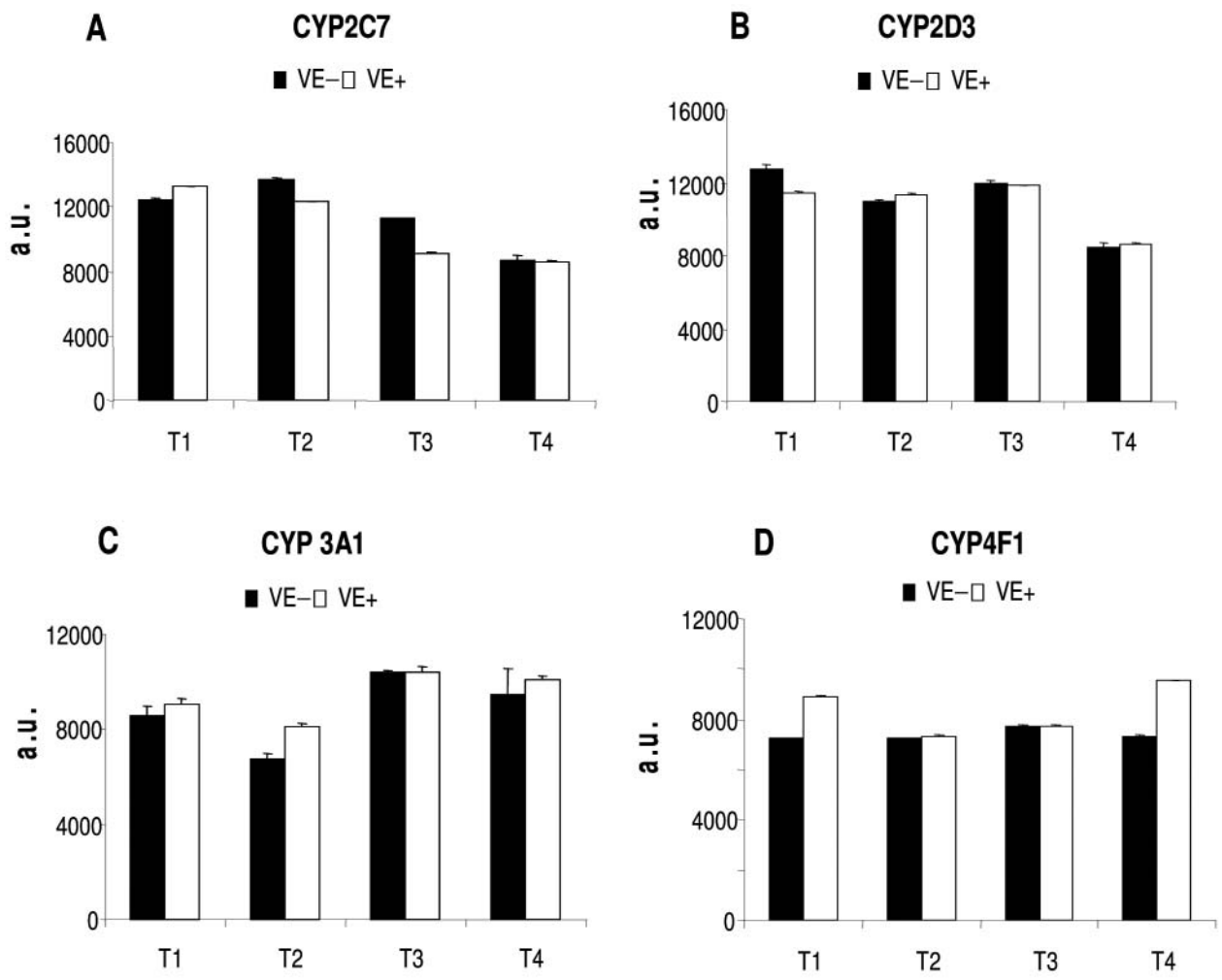

Figure 2. *Cytochrome P450 mRNA levels in rat hepatocytes – given in arbitrary units (a.u.) – after treatment with a vitamin E-enriched diet (60 mg/kg RRR-α-tocopheryl acetate, VE+) or a vitamin E-deficient diet (1.7 mg/kg RRR-α-tocopheryl acetate, VE–) for 17 d (T1), 91 d (T2), 191 d (T3) or 269 d (T4). The data represent means±SD.*

Discussion

The objective of this study was to investigate the short- and long-term effects of vitamin E on CYP mRNA expression. To this end, HepG2 cells were supplemented with RRR- or all rac-α-tocopheryl acetate at concentrations of 0, 10, 30, 80 and 300 μM. Only CYP20A1 mRNA was dose-dependently up-regulated by vitamin E *in vitro*. The function of CYP20A1 in humans is currently unknown. Its amino acid sequence has a 23% similarity to that of rat CYP17A1, which is known to be involved in sexual development during foetal life and at puberty (18, 19). CYP20A1 may play a role in the metabolism of human sex hormones. This is consistent with the discovery of vitamin E as an essential factor for successful reproduction in rats (20).

The remaining 13 CYP enzymes expressed in HepG2 cells showed no dose-dependent regulation by vitamin E. Previously, incubation of human HepG2 cells with a single dose of 50 μM γ-tocotrienol, a different form of vitamin E, for 48 hours, resulted in a significant up-regulation of CYP3A4 and CYP3A5 mRNA (21). However, γ-tocotrienol is usually not detectable in human plasma. Supplementation with 250 mg tocotrienols/d for 8 weeks did not raise the plasma tocotrienol concentrations above 1 mM (22). Thus, the γ-tocotrienol concentrations used by Landes and co-workers are not physiologically achievable and the significance of their findings await confirmation or refutation in properly designed *in vivo* experiments. Moreover, in our study, the HepG2 cells were supplemented with vitamin E for 7 days, because we thought it important to keep the vitamin E concentrations

Table II. *Cytochrome P450 mRNA levels in arbitrary units (a.u.) in rat hepatocytes after treatment with a vitamin E-enriched (60 mg/kg RRR-α-tocopheryl acetate, VE+) or a vitamin E-deficient diet (1.7mg/kg RRR-α- tocopheryl acetate, VE–) for 17 d (T1), 91 d (T2), 191 d (T3) or 269 d (T4)). The data represent means±SD.*

CYP450 enzyme, rat	Function	mRNA expression (a.u.±SD) VE form	T1	T2	T3	T4
CYP1A2	may play a role in xenobiotic metabolism	VE–	2910 (14)	2202 (10)	2098 (50)	4583 (9)
		VE+	2420 (16)	1826 (39)	1524 (14)	2389 (40)
CYP2A1	steroid hormones 7-alpha-hydroxylase	VE–	8842 (552)	7758 (742)	7268 (225)	6883 (1543)
		VE+	7169 (735)	6717 (48)	7858 (431)	7324 (596)
CYP2A2	testosterone 15-alpha-hydroxylase	VE–	6583 (59)	5464 (19)	5688 (39)	7353 (46)
		VE+	6307 (46)	4816 (45)	5367 (12)	7470 (6)
CYP2B2	unknown	VE–	3943 (37)	887 (32)	1529 (49)	2007 (57)
		VE+	3598 (40)	1095 (35)	1896 (20)	2694 (40)
CYP2B3	oxidises a variety of compounds, including steroids, fatty acids, xenobiotics	VE–	6958 (6)	6868 (55)	5258 (20)	6260 (41)
		VE+	6973 (46)	7713 (38)	8621 (4)	6866 (38)
CYP2C6	may play a role in drug metabolism	VE–	9635 (208)	5583 (489)	5374 (197)	6885 (298)
		VE+	8584 (384)	4252 (258)	7225 (441)	7068 (431)
CYP2C7	a retinoic acid and testosterone hydroxylase	VE–	12482 (41)	13689 (94)	11292 (27)	8682 (327)
		VE+	13257 (53)	12374 (11)	9144 (41)	8596 (103)
CYP2C11	unknown	VE–	6673 (363)	10021 (872)	7935 (461)	4656 (1376)
		VE+	7126 (356)	10949 (79)	8516 (299)	5578 (723)
CYP2C13	metabolizes arachidonic acid	VE–	7322 (9)	5767 (6)	6166 (3)	4649 (60)
		VE+	8921 (7)	6074 (13)	5509 (16)	6034 (63)
CYP2C22	unknown	VE–	6596 (48)	3190 (60)	4494 (57)	2914 (63)
		VE+	9940 (97)	3823 (35)	4627 (29)	5757 (9)
CYP2C23	arachidonic acid epoxygenase	VE–	9266 (41)	10034 (18)	11794 (65)	12426 (82)
		VE+	10562 (34)	10174 (53)	12254 (19)	11044 (50)
CYP2D3	debrisoquine 4-hydroxylase	VE–	12763 (277)	10947 (166)	11975 (167)	8467 (215)
		VE+	11433 (80)	11349 (64)	11863 (29)	8617 (93)
CYP2D4	debrisoquine 4-hydroxylase	VE–	1323 (163)	1364 (81)	1332 (50)	754 (380)
		VE+	1139 (336)	1305 (243)	1153 (46)	705 (89)
CYP2D5	debrisoquine 4-hydroxylase	VE–	6495 (80)	6068 (57)	6690 (36)	7071 (60)
		VE+	7849 (57)	5884 (5)	6652 (15)	8954 (32)
CYP2F4	unknown	VE–	588 (16)	720 (27)	732 (15)	1117 (12)
		VE+	534 (16)	636 (7)	836 (18)	996 (25)
CYP2J3	unknown	VE–	1505 (10)	1963 (19)	1316 (6)	1051 (100)
		VE+	1900 (21)	1602 (4)	1201 (6)	1494 (52)
CYP3A1	unknown	VE–	8549 (433)	6776 (220)	10376 (111)	9472 (1082)
		VE+	9025 (276)	8143 (139)	10392 (227)	10079 (187)
CYP3A18	catalyses 16 beta- and 6-alpha hydroxylations of testosterone	VE–	3965 (34)	4531 (55)	3875 (43)	4086 (27)
		VE+	3980 (29)	3808 (68)	5410 (36)	3839 (18)

continued

Table II. *continued.*

CYP450 enzyme, rat	Function	mRNA expression (a.u.±SD)				
		VE form	T1	T2	T3	T4
CYP4A1	lauric acid omega-hydroxylase	VE–	1484 (65)	1121 (65)	934 (50)	2860 (56)
		VE+	1400 (35)	1096 (108)	767 (65)	3005 (62)
CYP4F1	plays a role in metabolism of arachidonic acid	VE–	7250 (13)	7244 (7)	7694 (52)	7305 (86)
		VE+	8933 (51)	7330 (55)	7743 (9)	9531 (45)
CYP4F4	plays a role in metabolism of arachidonic acid	VE–	1699 (27)	1381 (7)	1439 (6)	2018 (39)
		VE+	1728 (12)	1347 (3)	1186 (11)	2016 11)
CYP4F6	catalyses the conversion of leukotriene B(4) into 19-hydoxy- and 18-hydroxy-leukotriene B(4); plays a role in inflammation	VE–	1586 (462)	1658 (133)	1371 (437)	1683 (217)
		VE+	1770 (833)	1794 (264)	2219 (216)	2220 (26)
CYP7A1	cholesterol 7-alpha-monooxygenase	VE–	664 (15)	854 (23)	1746 (36)	780 (58)
		VE+	675 (16)	803 (20)	2063 (12)	237 (23)

stable over time and to establish intracellular steady state concentrations of vitamin E (23), whereas in most studies reported in the literature, the incubation periods were only 24 or 48 h (14, 21). Thus, the previously reported changes of CYP expression may be transient effects.

In our *in vitro* model, no significant differences were found in CYP gene expression between the natural and synthetic forms of vitamin E. This is in agreement with our previously published data showing that *RRR*- and all *rac*-α-tocopherol share identical transcriptional activity in HepG2 cells (23).

Feeding male rats over a period of 9 months with diets deficient in (< 2 mg *RRR*-α-tocopheryl acetate/kg diet) or supplemented with vitamin E (60 mg *RRR*-α-tocopheryl acetate/kg diet; approximately twice the recommended α- tocopherol content of diets for growing rats (24)), resulted in an almost complete depletion or a significant accumulation of α-tocopherol in the plasma and liver, respectively (5). Despite these pronounced differences in the plasma and liver α-tocopherol concentrations, no changes in gene expression of CYP enzymes were observed (Table II). In previous experiments, the employed rat model was successfully used to identify vitamin E-sensitive genes (5, 6).

Kluth *et al.* (17) fed mice for 3 months with diets containing 2, 20, or 200 mg *RRR*-α-tocopheryl acetate/kg. The hepatic levels of Cyp3a11 (the murine homolog of human CYP3A4 and rat CYP3A1 (25)) mRNA were about 2.5-fold higher in the 20- and 200-mg α-tocopherol groups compared to the 2-mg group. Furthermore, after feeding 200 mg α-tocopherol for 9 months, Cyp3a11 mRNA was 1.7-fold higher than after 3 months. The differences between our results and those of Kluth *et al.* (17) may be related to species-specific differences in the regulation of CYP gene expression. It has been reported that the induction of CYP3As by rifampicin is much more pronounced in humans than in rodents (26), and that rat CYP3A1 is not affected by rifampicin at all (27).

In conclusion, it was shown that vitamin E does not affect the CYP mRNA levels of the majority of CYPs in HepG2 cells or in rat liver *in vivo*. Moreover, no differences between natural and synthetic vitamin E on CYP gene expression were observed. Since the current study measured the effect of vitamin E on CYP gene expression at the level of mRNA, future experiments are warranted to study these effects at the level of CYP protein expression and enzyme activity.

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