

Comparative ^2H -labelled α -tocopherol biokinetics in plasma, lipoproteins, erythrocytes, platelets and lymphocytes in normolipidaemic males

Yvonne M. Jeanes, Wendy L. Hall and John K. Lodge*

Centre for Nutrition and Food Safety, School of Biomedical and Molecular Sciences, University of Surrey, Guildford, Surrey GU2 7XH, UK

(Received 17 May 2004 – Revised 19 January 2005 – Accepted 20 January 2005)

The biokinetics of newly absorbed vitamin E in blood components was investigated in normolipidaemic males. Subjects (n 12) ingested encapsulated 150 mg ^2H -labelled *RRR*- α -tocopheryl acetate with a standard meal. Blood was collected at 3, 6, 9, 12, 24 and 48 h post-ingestion. ^2H -labelled and pre-existing unlabelled α -tocopherol (α -T) was determined in plasma, lipoproteins, erythrocytes, platelets and lymphocytes by LC–MS. In all blood components, labelled α -T concentration significantly increased while unlabelled decreased following ingestion ($P < 0.0001$). Significant differences in labelled α -T biokinetic parameters were found between lipoproteins. Time of maximum concentration was significantly lower in chylomicrons, while VLDL had a significantly greater maximum α -T concentration and area under the curve (AUC) ($P < 0.05$). The largest variability occurred in chylomicron α -T transport. Erythrocyte labelled α -T concentrations increased gradually up to 24 h while α -T enrichment of platelets and lymphocytes was slower, plateauing at 48 h. Platelet enrichment with labelled α -T was biphasic, the initial peak coinciding with the labelled α -T peak in chylomicrons. Erythrocyte and HDL AUC were significantly correlated ($P < 0.005$), as was platelet and HDL AUC ($P < 0.05$). There was a lower turnover of pre-existing α -T in platelets and lymphocytes (maximum 25 % labelled α -T) compared to plasma and erythrocytes (maximum 45 % labelled α -T). These data indicate that different processes exist in the uptake and turnover of α -T by blood components and that chylomicron α -T transport is a major determinant of inter-individual variation in vitamin E response. This is important for the understanding of α -T transport and uptake into tissues.

Deuterated: Tocopherol: Plasma: Erythrocytes: Platelets: Lymphocytes

Vitamin E, in particular α -tocopherol (α -T) which constitutes over 90 % of vitamin E in the body (Burton & Traber, 1990; Brigelius-Flohe & Traber, 1999), has been widely researched owing to its antioxidant and non-antioxidant functions, and its putative cardioprotective role. Vitamin E biokinetics within the systemic circulation is governed by plasma lipoprotein metabolism. α -T is absorbed by the intestinal cells at the same rate as the other forms of vitamin E (γ -, β - and δ -tocopherols and α -, β -, δ - and γ -tocotrienols). Chylomicrons (CM) transport newly absorbed vitamin E, and during circulation they transfer lipids and vitamin E to peripheral tissues by way of lipid hydrolysis (Traber *et al.* 1985). Excess CM surface area is consequently produced and along with vitamin E it is transferred to HDL (Traber & Kayden, 1989). The resultant CM remnants are taken up into the liver and *RRR*- α -T is incorporated into VLDL by the α -tocopherol transport protein in preference to the other forms of vitamin E, and then secreted into the blood for systemic distribution (Traber *et al.* 1993). VLDL is catabolised to LDL and during this process vitamin E is transferred to HDL (Kayden & Traber, 1993). Vitamin E is then taken up into peripheral tissues via LDL receptors (Traber & Kayden, 1984). There is a constant flux of vitamin E between circulating lipoproteins (Traber *et al.* 1992), and presumably, also between the vascular endothelium and blood components.

To monitor directly the specific form of vitamin E administered, stable isotope-labelled (deuterated) vitamin E is commonly used. These so-called biokinetic studies involve sampling over a period of time following administration of the label. Using this approach the uptake and distribution of newly absorbed vitamin E has been reported in plasma (Acuff *et al.* 1994; Traber *et al.* 1994, 1998), lipoproteins (Traber *et al.* 1988, 1998) and erythrocytes (Cheeseman *et al.* 1995; Roxborough *et al.* 2000), but not together within the same individuals. In plasma it has been demonstrated that newly absorbed α -T rapidly displaces pre-existing 'old' α -T in the circulation (Traber *et al.* 1994), reaching a maximum concentration between 9 and 12 h (Traber *et al.* 1998) consistent with the known pathways of lipoprotein transport. There is also transfer of vitamin E between lipoproteins during lipoprotein metabolism (Traber *et al.* 1988). Vitamin E uptake into erythrocytes is more gradual, with previous studies showing a maximum concentration after 24 h (Roxborough *et al.* 2000). Erythrocytes contain vitamin E within their membranes, which is obtained via tocopherol-binding proteins (Kitabchi & Wimalasena, 1982). To date, there have been no studies to investigate the biokinetics of vitamin E uptake into platelets and lymphocytes. It is currently unknown how lymphocytes and platelets obtain their vitamin E. A variety of cell lines have been shown to receive α -T preferably via HDL (Kolleck *et al.* 1999; Goti *et al.*

Abbreviations: CM, chylomicrons; Cmax, maximum concentration of deuterated α -T; α -T, α -tocopherol; Tmax, time of Cmax.

* **Corresponding author:** Dr John K. Lodge, fax +44 (0)1483 876 416, email j.lodge@surrey.ac.uk

2000), and *in vitro* evidence has implicated the scavenger receptor class B type I (SR-BI) as being important for cellular α -T uptake (Goti *et al.* 2001; Mardones *et al.* 2002). Both platelets and lymphocytes are functionally affected by vitamin E, and play key roles in thrombo- and atherogenesis. α -T inhibits platelet aggregation *in vitro* and *in vivo* (Freedman *et al.* 1996), and also influences lymphocyte proliferation *in vitro* (Roy *et al.* 1991). Therefore it is of interest to study the uptake of vitamin E into these blood components. This study describes the comparative biokinetics of newly absorbed vitamin E into plasma and lipoproteins, erythrocytes, platelets and lymphocytes in healthy normolipidaemic males.

Materials and methods

Tocopherols

RRR- α -5,7-(CD₃)₂-tocopheryl acetate and *all rac*- α -5 (CD₃)-tocopheryl acetate were kind gifts from Cognis Nutrition and Health (Düsseldorf, Germany). Purity of the acetates was 98.8% for both species. Isotopic purity was >99.9% as determined by LC-MS. Hexadeuterated *RRR*- α -tocopheryl acetate was encapsulated (150 mg) for human consumption. Trideuterated *all rac*- α -tocopheryl acetate was used as an internal standard.

Reagents and solvents

LC-MS grade methanol (LC-MS Chromasolv), butylated hydroxytoluene, ascorbic acid and lithium perchlorate were purchased from Sigma-Aldrich Chemical Co. (Poole, UK). SDS and potassium hydroxide were from BDH (Poole, UK), hexane (HPLC grade) was from Fisher (Loughborough, UK), absolute ethanol was from Hayman Ltd (Witham, UK) and PBS tablets were from Oxoid Ltd (Basingstoke, UK).

Study protocol

Twelve healthy males were recruited from the general population by advertisements in local newspapers. The study was limited to male volunteers to reduce inter-individual variation. Selection criteria stated subjects must be non-smoking, not taking dietary supplements and with no gastrointestinal or blood lipid disorders. Subjects with lipid abnormalities were also excluded; the selection criteria being total plasma cholesterol <6 mmol/l and plasma triacylglycerol <1.8 mmol/l. Subjects were determined healthy if they reported no gastrointestinal and no other medical condition via a questionnaire and their blood lipids (as measured by study coordinators) were within the healthy range. Habitual dietary vitamin E intake was not measured.

Volunteers were given 150 mg encapsulated hexadeuterated *RRR*- α -tocopheryl acetate with a standard breakfast that contained 40 g fat and approximately 0.2 mg vitamin E. Volunteers consumed a standard lunch 4.5 h after the standard breakfast, and after 8 h food and beverage consumption was no longer controlled. Blood samples were taken 3, 6, 9, 12, 24 and 48 h after ingestion of the capsule. Blood components were immediately isolated from the whole blood.

The study was approved by the University of Surrey Advisory Committee on Ethics.

Sample isolation and analysis

Platelets were isolated from whole blood by firstly centrifuging whole blood at 280 g for 14 min at 2°C to obtain platelet-rich plasma. The platelet-rich plasma was then centrifuged at 1120 g for 15 min at 2°C to obtain a pellet of platelets. After removal of the platelet-poor plasma the platelets were washed with Tris-HCl buffer (pH 7.4) before reconstituting in 1 ml Tris-HCl buffer. Samples were aliquoted and snap frozen in liquid nitrogen and stored at -80°C prior to analysis.

Lymphocytes were isolated from whole blood using Histopaque-1077 (Sigma-Aldrich) according to the manufacturer's protocol. After washing the lymphocytes three times with PBS, pH 7.4, the lymphocytes were reconstituted into 0.5 ml PBS, aliquoted into cryo-tubes containing 10 μ l of 1 mg/ml butylated hydroxytoluene (Sigma-Aldrich), and snap frozen in liquid nitrogen and stored at -80°C prior to analysis.

Erythrocytes were carefully removed from whole blood after centrifugation at 1550 g for 10 min and washed three times with saline. Haematocrit was then measured and the cells aliquoted with 10 μ l of 1 mg/ml desferrioxamine (Sigma-Aldrich).

Plasma was collected following platelet removal (platelet-poor plasma). Plasma was then aliquoted into cryo-tubes containing 10 μ l of 1 mg/ml butylated hydroxytoluene, snap frozen in liquid nitrogen, and stored at -80°C prior to analysis.

CM were isolated from previously frozen plasma by overlaying an equi-volume of saline and centrifuging at 110 000 g for 15 min as described by Weintraub *et al.* (1987). VLDL, LDL and HDL were extracted from CM-free plasma by sequential ultracentrifugation through a discontinuous KBr gradient and collected by aspiration (Havel *et al.* 1955). A Beckman Coulter 70-1 Ti rotor and Beckman Optima XL-100 ultracentrifuge were used for all lipoprotein isolation. Aliquots of isolated lipoproteins were immediately analysed for cholesterol, triacylglycerol, protein and α -T using the methods described below.

Vitamin E extraction and analysis

Prior to extraction, plasma and erythrocyte aliquots (100 μ l) were diluted 1:5 and 1:10 with PBS, respectively (Hall *et al.* 2003). Platelet and lymphocyte aliquots (100 μ l) were not diluted and were sonicated for 3 min prior to extraction to obtain a homogenous solution. Total vitamin E (mainly α - and γ -tocopherol) was extracted from all samples using a combination of SDS, ethanol and hexane as described (Burton *et al.* 1985). α -T was analysed by LC-MS using a method we have recently developed (Hall *et al.* 2003). The system used was a Micromass LCT™ (Waters Ltd, Elstree, UK) which combines a Waters Alliance System comprising a solvent delivery system, online degasser, peltier-cooled autosampler (set at 4°C), controller and column oven (set at 25°C) in conjunction with a Time-of-Flight Mass Spectrometer. Tocopherols were separated on a Waters Symmetry™ Column (2.1 \times 50 mm, C18, 3.5 μ m) with a mobile phase consisting of 100% methanol (LC-MS Chromasolv, Sigma-Aldrich). Trideuterated *all rac*- α -tocopherol (final concentration 0.5 μ mol/l) was used as an internal standard, and added to the blood component aliquots prior to extraction.

Biochemical analysis

Total plasma cholesterol and triacylglycerol were determined using enzymatic kits supplied by Randox (County Antrim, UK),

and analysed automatically using a SPACE biochemical analyser (Alfa-Wasserman, The Netherlands). Total protein content of lipoproteins, platelets and lymphocytes were determined using a colorimetric protein kit based on the Bradford assay (BioRad).

Data analysis and statistics

Non-compartmental kinetic parameters associated with ²H-labelled α-T concentration *v.* time profiles in individual blood components were determined using the PK Solutions Version 2.0 software (Summit Research Services). Data in the figures are expressed as means with their standard error of the mean, and in the tables as means with their standard deviation. Coefficients of variation (SD/mean × 100) were used as a measure of inter-individual variation. The change in labelled and unlabelled α-T with time was analysed using repeated measures ANOVA. Differences between kinetic parameters was also analysed by ANOVA. Tukey's HSD *post hoc* test was used when significance was found. Pearson correlations were used to correlate area under the curve (AUC) between components. Results were considered significant at the 95 % confidence level ($P < 0.05$). The software package Statistica for Windows (Statsoft Inc., Tulsa, OK, USA) was used for all statistical analysis.

Results

Subject characteristics

Table 1 shows the characteristics of the subjects in the study. Subjects were all asymptomatic normolipidaemic males with average age of 48 years and average BMI of 25.6 kg/m². The average BMI was slightly higher than the ideal recommended range of 20–25 kg/m².

Uptake of labelled α-tocopherol into plasma and lipoproteins

Fig. 1 shows the biokinetic profile of labelled, unlabelled and total α-T in the plasma following ingestion of a capsule containing 150 mg *RRR*-α-tocopheryl acetate. Labelled α-T concentration significantly increased over time ($P < 0.0001$) up to a maximum after about 12 h following which the concentration slowly decreased. Unlabelled α-T concentration significantly decreased over time ($P < 0.001$), with a maximum decrease after 12 h, following which there was no further change in concentration. Total α-T concentration also showed a sharp increase in concentration up to 12 h, followed by a gradual decrease in concentration. Statistical differences in labelled α-T concentration

Table 1. Subject information

Variable	Mean	SD	Range
Age (years)	47.8	10.2	31–58
BMI (kg/m ²)	25.6	2.5	23–32
Labelled α-tocopherol/kg body weight (mg/kg)	1.9	0.2	1.7–2.2
Plasma cholesterol (mmol/l)	5.0	0.6	3.88–5.84
LDL cholesterol (mmol/l)	3.3	0.5	2.24–3.7
HDL cholesterol (mmol/l)	1.1	0.2	0.75–1.54
Plasma triacylglycerols (mmol/l)	1.2	0.3	0.76–1.72

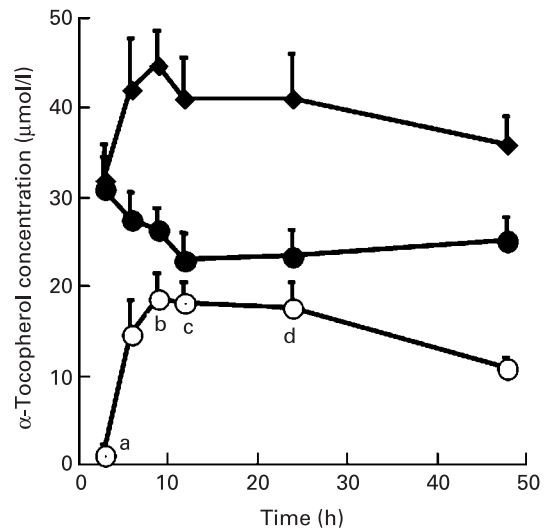


Fig. 1. Unlabelled (●), labelled (○) and total (◆) α-tocopherol concentration in plasma over time following ingestion of a capsule containing 150 mg deuterated *RRR*-α-tocopheryl acetate. Values are means with their standard errors shown by vertical bars. There was a significant increase in labelled α-tocopherol over time ($P < 0.0001$), and a significant decrease in unlabelled α-tocopherol over time. Statistical differences between time points are also shown: ^a3 *v.* 6, 9, 12, 24 and 48 h, $P < 0.001$; ^b9 *v.* 48 h, $P < 0.01$; ^c12 *v.* 48 h, $P < 0.01$; ^d24 *v.* 48 h, $P < 0.05$.

were found between time points 3 and 6, 9, 12, 24, 48 h ($P < 0.001$), 9 and 48 h ($P < 0.01$), 12 and 48 h ($P < 0.01$), and 24 and 48 h ($P < 0.05$).

Profiles of labelled α-T in CM, VLDL, LDL and HDL following ingestion of a capsule containing 150 mg *RRR*-α-tocopheryl acetate are shown in Fig. 2. In all lipoproteins there was a significant increase in labelled α-T over time ($P < 0.0001$). Labelled α-T uptake into CM increased sharply up to 6 h, then decreased rapidly. The uptake of labelled α-T into the other lipoproteins followed similar time courses to each other, with a

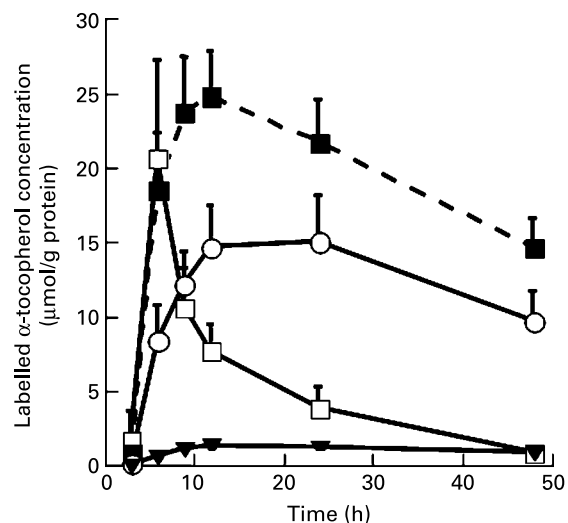


Fig. 2. Labelled α-tocopherol concentration in individual lipoproteins (□, chylomicrons; ■, VLDL; ○, LDL; ▼, HDL) over time following ingestion of a capsule containing 150 mg deuterated *RRR*-α-tocopheryl acetate. Values are means with their standard errors shown by vertical bars. There was a significant increase in labelled α-tocopherol over time ($P < 0.0001$). For reasons of clarity significant differences between time points are explained in the text.

gradual increase in labelled α -T up to a maximum at about 12 h in VLDL and HDL and between 12 and 24 h in LDL. As with plasma, in all lipoproteins there was a significant decrease in unlabelled α -T over time ($P < 0.005$, data not shown) up to 12 h. Statistical differences in labelled α -T concentration were found between time points 3 and 6, 9, 12, 24, 48 h in VLDL, LDL and HDL ($P < 0.001$), 3 and 6 h in CM ($P < 0.001$), 6 and 12 ($P < 0.05$), 24, 48 h ($P < 0.001$) in CM, 6 and 24 h in LDL ($P < 0.05$), 6 and 9, 12, 24 in HDL ($P < 0.001$), 9 and 48 h in VLDL and HDL ($P < 0.05$), 12 and 48 h in VLDL ($P < 0.01$) and HDL ($P < 0.001$), and 24 and 48 h in HDL ($P < 0.01$).

Uptake of labelled α -tocopherol into erythrocytes, platelets and lymphocytes

Biokinetic profiles of labelled, unlabelled and total α -T in erythrocytes, platelets and lymphocytes following ingestion of a capsule containing 150 mg *RRR*- α -tocopheryl acetate are shown in Fig. 3(A)–(C). In all components, labelled α -T significantly increased over time ($P < 0.0001$). In erythrocytes (Fig. 3(A)), labelled α -T concentration showed a slow increase up to a maximum after about 24 h followed by a gradual decrease. Unlabelled α -T showed a gradual decrease in concentration until 24 h. In platelets (Fig. 3(B)) there appeared to be a biphasic response characterised by an initial peak in labelled α -T concentration at 6 h, followed by a slower increase which appeared to plateau at 48 h. This biphasic response occurred in four out of twelve subjects. The other eight subjects showed a gradual increase in labelled α -T concentration from 3 to 24 h. By 9 h all subjects had a similar labelled α -T concentration. Unlabelled α -T decreased in concentration after 9 h. In lymphocytes (Fig. 3(C)) the increase in labelled α -T concentration after 6 h was slower,

and appeared to have not reached a maximum at the last sampling point (48 h). Unlabelled α -T appeared to increase from 3 to 6 h, followed by a return to basal levels at 12 h and no change following this. Statistical differences in labelled α -T concentration were found between time points 3 v. 9, 12, 24, 48 h in erythrocytes ($P < 0.001$), 3 v. 6, 9, 12, 24, 48 h in platelets ($P < 0.001$ except for 3 v. 9 h, $P < 0.01$), 3 v. 12, 24, 48 h in lymphocytes ($P < 0.001$ except 3 v. 12 h, $P < 0.01$), 6 v. 9 ($P < 0.01$), 12 h ($P < 0.001$) in erythrocytes, 6 v. 12 h in lymphocytes ($P < 0.01$), 6 v. 24, 48 h in all components ($P < 0.001$), 9 v. 12 h in erythrocytes ($P < 0.01$), 9 v. 24, 48 h in all components ($P < 0.001$), and 12 v. 24, 48 h in all components ($P < 0.001$, except 12 v. 24 h in platelets, $P < 0.01$; and 24 v. 48 h in erythrocytes, $P < 0.05$).

Comparisons of labelled α -tocopherol biokinetics in blood components

Biokinetic measurements calculated from deuterated α -T concentration with time profiles in blood components are shown in Table 2. Within lipoproteins, VLDL had a significantly higher maximum concentration of deuterated α -T (C_{max}) and AUC than other lipoproteins ($P < 0.05$), while CM had a significantly lower time of C_{max} (T_{max}) at 6 h ($P < 0.05$) and a significantly shorter half-life ($P < 0.05$). Large variation ($> 70\%$ CV) was observed in CM C_{max} , AUC, elimination rate and half-life. In blood components, T_{max} was significantly lower in plasma ($P = 0.001$). Variation was relatively similar between the blood components. Pearson correlations were performed on AUC for all components. There was a positive correlation between plasma and CM ($r = 0.715$, $P = 0.013$) but not between plasma and other lipoproteins or in AUC between lipoproteins. Positive correlations were found between AUC in HDL and

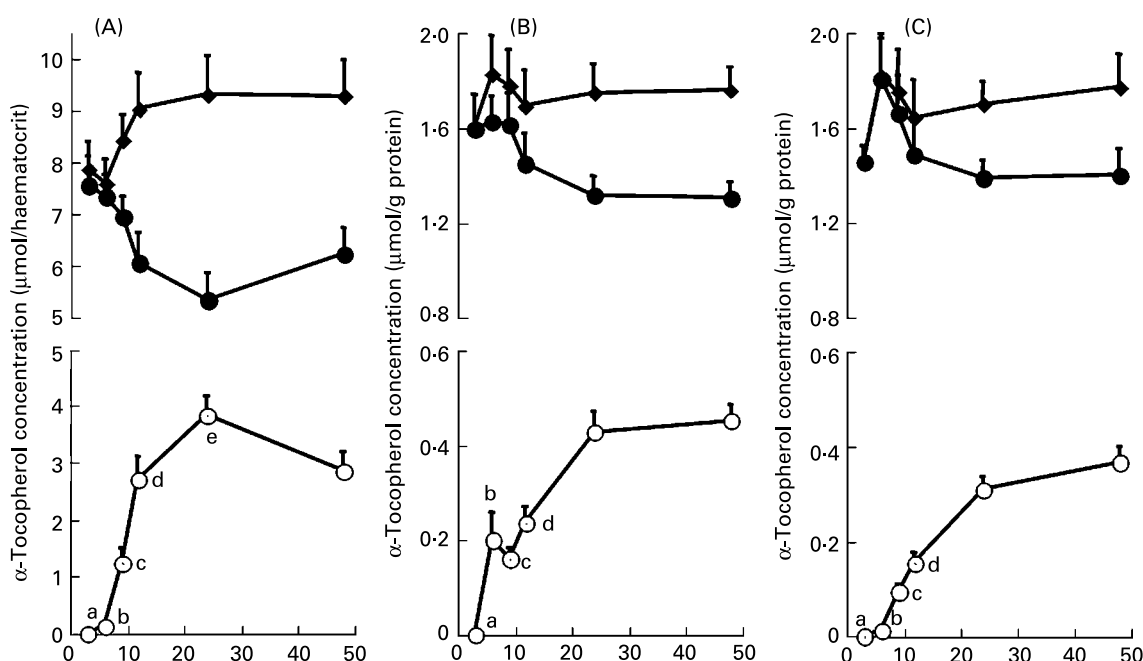


Fig. 3. Unlabelled (●), labelled (○) and total (◆) α -tocopherol concentration in erythrocytes (A), platelets (B) and lymphocytes (C) over time following ingestion of a capsule containing 150 mg of deuterated *RRR*- α -tocopheryl acetate. Values are means with their standard errors shown by vertical bars. There was a significant increase in labelled α -tocopherol over time ($P < 0.0001$). Statistical differences between time points are also shown: (A), ^a3 v. 9, 12, 24 and 48 h, $P < 0.001$; ^b6 v. 9 h, $P < 0.01$, 6 v. 12, 24 and 48 h, $P < 0.001$; ^c9 v. 12 h, $P < 0.01$, 9 v. 24 and 48 h, $P < 0.001$; ^d12 v. 24 h, $P < 0.001$; ^e24 v. 48 h, $P < 0.001$; (B), ^a3 v. 6, 12, 24 and 48 h, $P < 0.001$, 3 v. 9 h, $P < 0.01$; ^b6 v. 24 and 48 h, $P < 0.001$; ^c9 v. 24 and 48 h, $P < 0.001$; ^d12 v. 24, $P < 0.01$ and 48 h, $P < 0.001$; (C), ^a3 v. 24 and 48 h, $P < 0.001$, 3 v. 12 h, $P < 0.01$; ^b6 v. 24 and 48 h, $P < 0.001$, 6 v. 12 h, $P < 0.01$; ^c9 v. 24, and 48 h, $P < 0.001$; ^d12 v. 24 and 48 h, $P < 0.001$.

Table 2. ^2H -Labelled α -tocopherol kinetic measurements for all blood components up to 48 h following ingestion of 150 mg deuterated *RRR*- α -tocopheryl acetate

	Cmax			Tmax			AUC			Rate of absorption			Rate of elimination			Half-life (h)		
	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV
Chylomicrons	22.5	17.6	78	6.6 ^b	1.3	19	223 ^c	154	69	1.41	0.79	56	0.190	0.19	100	11 ^d	9	84
VLDL	28.1 ^a	10.2	36	12.6	6.3	50	868 ^c	286	33	0.37	0.25	69	0.019	0.01	57	54 ^d	43	80
LDL	16.7	8.1	48	16.2 ^b	8.4	52	503 ^c	255	51	0.26	0.20	77	0.024	0.01	52	36	16	45
HDL	1.43	0.4 ^a	28	13.5	5.7	42	47 ^c	13	29	0.24	0.08	37	0.021	0.01	31	37	15	41
Plasma	20.6	6.6	32	12.8 ^e	5.8	46	930	328	35	0.40	0.24	60	0.018	0.004	27	43	21	48
Erythrocytes	3.8	1.1	28	25.5	10	39	119	36	30	0.24	0.04	19	0.014	0.003	24	51	14	27
Platelets	0.48	0.14	29	30.0	16.9	56	16	5	30	0.28	0.10	37	ND	ND	ND	ND	ND	ND
Lymphocytes	0.37	0.10	27	43.6 ^e	9.7	22	12	3	27	0.43	0.18	42	ND	ND	ND	ND	ND	ND

AUC, area under the deuterated α -tocopherol concentration with time curve ($\mu\text{mol}\cdot\text{h}/\text{g}$ protein in lipoproteins, platelets and lymphocytes; $\mu\text{mol}\cdot\text{h}/\text{l}$ in plasma; $\mu\text{mol}\cdot\text{h}/\text{haematocrit}$ in erythrocytes); Cmax, maximum concentration of deuterated α -tocopherol ($\mu\text{mol}/\text{g}$ protein in lipoproteins, platelets and lymphocytes; $\mu\text{mol}/\text{l}$ in plasma; $\mu\text{mol}/\text{haematocrit}$ in erythrocytes); ND, not determined; rate of absorption and elimination ($\mu\text{mol}/\text{g}$ protein/h in lipoproteins, platelets and lymphocytes; $\mu\text{mol}/\text{l}$ per h in plasma; $\mu\text{mol}\cdot\text{haematocrit}/\text{h}$ in erythrocytes); Tmax, time of Cmax (h).

^{abcde}Significant difference in: ^aCmax between VLDL and HDL, $P=0.003$; ^bTmax between CM and LDL, $P=0.017$; ^cAUC between VLDL and HDL, $P<0.0001$, CM and VLDL, $P=0.001$, and LDL and HDL, $P=0.025$; ^dhalf-life between CM and VLDL, $P=0.032$; ^eTmax between plasma and lymphocytes, $P=0.001$.

erythrocytes ($r\ 0.804$, $P=0.003$) and HDL and platelets ($r\ 0.612$, $P=0.046$). Positive correlations were also found between AUC in plasma and erythrocytes ($r\ 0.659$, $P=0.025$), plasma and platelets ($r\ 0.666$, $P=0.025$), and platelets and lymphocytes ($r\ 0.768$, $P=0.006$).

Fig. 4 shows the percentage of total α -T in the labelled form in the lipoproteins (Fig. 4(A)), and in each blood component (Fig. 4(B)). In plasma lipoproteins, VLDL, LDL and HDL show a very similar pattern, all reaching maximum labelling at about 12 h of approximately 36%. CM, however, show a very different pattern with a maximum at 6 h at 55% labelled α -T, followed by a rapid decrease. In blood components, labelled α -T firstly enters the plasma, with up to 45% of the α -T being in the labelled form at the maximum time of 12 h. Within erythrocytes the maximum labelled α -T occurs at 24 h, with approximately 45% enrichment. Platelets and lymphocytes, at the time Cmax of 48 h, contain 25 and 20% of the total α -T in the labelled form, respectively.

Discussion

This study aimed to determine and compare the uptake and distribution profiles of labelled α -T into plasma, lipoproteins,

erythrocytes, platelets and lymphocytes in healthy males following a single dose (150 mg) of ^2H -labelled *RRR*- α -tocopheryl acetate. This is the first report of vitamin E biokinetics in platelets and lymphocytes and the first time the uptake and distribution of newly absorbed vitamin E has been compared in plasma lipoproteins, erythrocytes, platelets and lymphocytes within the same individual.

Plasma α -T concentrations represent the combined total of α -T within circulating lipoproteins. Plasma labelled α -T concentration with time profiles were similar to those published previously (Traber *et al.* 1994, 1998; Cheeseman *et al.* 1995; Roxborough *et al.* 2000). The initial rise in plasma labelled α -T corresponded to the profile of labelled α -T in CM, which peaked at 6 h. The rate of α -T appearance and disappearance in CM was between 4- and 10-fold greater than that for the other lipoproteins, consistent with the secretion of CM following a meal and rapid removal from the circulation. VLDL enrichment with labelled α -T peaked at 12 h. VLDL was the major carrier of labelled α -T as demonstrated by the significantly higher labelled α -T per g protein Cmax and AUC, and this is consistent with previous studies (Goulinet & Chapman, 1997; Perugini *et al.* 2000). Labelled α -T peaked between 12 and 24 h in LDL and HDL, and kinetic parameters

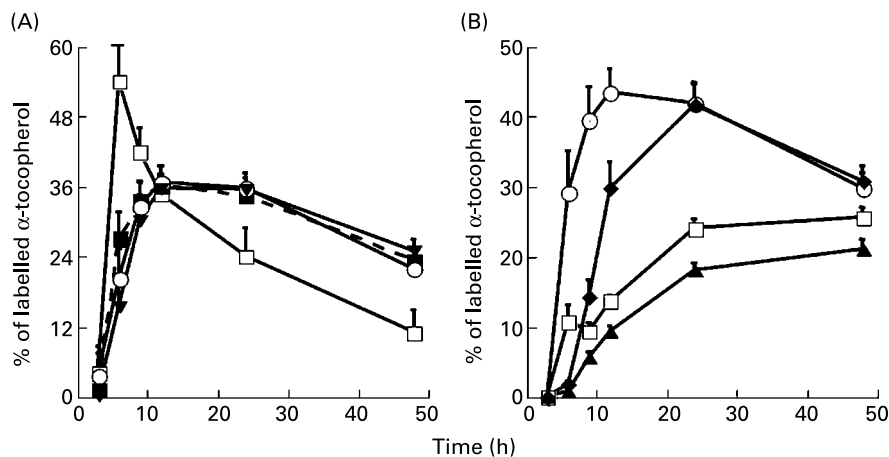


Fig. 4. The percentage of total α -tocopherol in the labelled form in blood components over time following ingestion of a capsule containing 150 mg of deuterated *RRR*- α -tocopheryl acetate. (A), Individual lipoproteins (\square , chylomicrons; \blacksquare , VLDL; \circ , LDL, \blacktriangledown , HDL); (B), blood components (\circ , plasma; \square , platelets; \blacklozenge , erythrocytes; \blacktriangle , lymphocytes). Values are means with their standard errors shown by vertical bars.

were also very similar between these. HDL contained significantly lower concentrations of labelled α -T compared to the other lipoproteins, and this reflects the preference of α -T to more hydrophobic environments. The labelled α -T profiles in the lipoproteins are consistent with the known routes of vitamin E intestinal absorption and packaging into CM, hepatic secretion in VLDL and plasma transport (Traber *et al.* 1988, 1998).

During lipoprotein transport α -T can also be transferred between lipoproteins (Traber *et al.* 1992) and, presumably, tissues. *In vitro* studies have shown that during hydrolysis of triacylglycerol-rich lipoproteins by lipoprotein lipase there is transfer of α -T to cells (Traber *et al.* 1985). The transfer of α -T among lipoproteins and to cells can also be catalysed by the phospholipid transfer protein (Kostner *et al.* 1995). These various processes result in an equilibration of α -T between the lipoproteins, which was demonstrated in the present study by the similar profiles of percentage labelled α -T in VLDL, LDL and HDL (Fig. 3(A)).

There was large inter-individual variation in the plasma response to newly absorbed labelled α -T, as shown by %CV (Table 2). It is likely that many cumulative factors influence variation. To reduce variation, only males with a similar age and BMI were used in the current study. Also, the dose of administered labelled α -T per kg body weight was similar between subjects, which could reduce variation caused by differences in the volume of α -T distribution. To date there have been no studies addressing potential differences in vitamin E biokinetics between males and females. However, it is likely that the current data are applicable to women as the processes involved in vitamin E absorption and transport should not be markedly influenced by gender. The largest variation occurred in all biokinetic parameters of CM α -T transport. Therefore processes involved in the packaging of lipid and vitamin E into CM appears to be a major determinant of α -T variation. It is likely that genetic polymorphisms in genes associated with lipoprotein metabolism are responsible for a large proportion of inter-individual variation in vitamin E biokinetics, as we have recently hypothesised (Doring *et al.* 2004). It is also likely that vitamin E status itself will influence the kinetics of newly absorbed α -T as we have recently shown that following a period of vitamin E supplementation, the uptake of newly absorbed α -T is reduced (Lodge *et al.* 2004). One limitation of the current study is the lack of baseline α -T concentrations and measures of habitual vitamin E intake, therefore inter-individual differences in vitamin E status were not assessed.

Ultimately the amount of α -T in plasma will influence the extent of uptake into blood components and tissues. Labelled α -T enters the erythrocytes following the plasma. There was no increase in labelled α -T until 9 h, subsequent to peak CM concentrations. It therefore appears that CM do not readily transfer α -T to erythrocytes. Erythrocyte labelled α -T concentrations peaked at 24 h, whilst VLDL, LDL and HDL were all at their maximum α -T labelling at 12 h, which would support the theory that erythrocytes obtain their α -T via transfer from these lipoproteins. It has been shown that transfer of α -T from HDL to erythrocytes is more efficient compared with other lipoproteins (Kayden & Bjornson, 1972; Kostner *et al.* 1995). In the present study, a significant positive correlation was found between erythrocyte and HDL labelled α -T AUC, but not with other lipoproteins. There was also a significant correlation between platelet and HDL labelled α -T AUC, also reflecting the role of HDL as an important donor of vitamin E, which has been shown in various cell lines (Kolleck *et al.*

1999; Goti *et al.* 2000, 2001). The mechanism of α -T transfer to erythrocytes may involve more than just spontaneous transfer, as erythrocytes are thought to obtain vitamin E via tocopherol binding proteins (Kitabchi & Wimalasena, 1982), although the role of these binding proteins in α -T uptake has not been elucidated.

The results show a more gradual and later uptake of labelled α -T into platelets and lymphocytes compared to plasma and erythrocytes. The uptake of labelled α -T into platelets was particularly interesting. A gradual increase in labelled α -T concentration was observed in eight subjects; however, a distinct biphasic uptake at 6 and 12 h was observed in the other four subjects. Presently it is not known how platelets acquire vitamin E. The initial peak in labelled α -T concentration in subjects with a biphasic response coincided with the α -T peak in CM at 6 h and therefore may reflect uncontrolled diffusion from CM, perhaps during CM hydrolysis. These interesting findings deserve further investigation into how platelets obtain their vitamin E and the cause of this biphasic response. We have observed that this phenomenon is more noticeable in hyperlipidaemic subjects (Hall *et al.* 2005), and it would be of interest to establish if there is a threshold of labelled α -T in plasma before platelets rapidly take up newly absorbed α -T. The post-9 h uptake profile in platelets was similar to that in lymphocytes. The gradual uptake of labelled α -T into platelets and lymphocytes in all subjects suggests controlled uptake by unknown mechanisms. A number of mechanisms are thought to play a role in cellular α -T uptake (Mardones & Rigotti, 2004). Cells containing LDL receptors can take up α -T by the LDL receptor pathway (Kaempf *et al.* 1994), and recent evidence also suggests an important role for the scavenger receptor class B type I in α -T uptake in certain cell lines (Goti *et al.* 2001; Mardones *et al.* 2002), using HDL as a donor (Goti *et al.* 2001). Lymphocytes contain LDL receptors (Kaempf *et al.* 1994), and so may obtain α -T in this way. Neither tocopherol binding proteins (Kitabchi & Wimalasena, 1982) or LDL receptors (Kaempf *et al.* 1994) have been found in platelets to date. However, more recent studies have suggested that the apoB moiety of LDL does interact with an unidentified receptor on the platelet membrane which allows for the transfer of lipids (Relou *et al.* 2003). Another limitation of the current study is the sampling period of 48 h, as at this time labelled α -T in lymphocytes and platelets had only reached plateau, and so rates of elimination and half-lives could not be calculated.

It has been previously shown that newly absorbed α -T displaces pre-existing α -T within plasma (Traber *et al.* 1994, 1998). In the present study, there was an increase in plasma labelled α -T and total α -T, and a concomitant decrease in unlabelled α -T. At T_{max}, 45 % of the total α -T was in the labelled form, indicative of rapid displacement of pre-existing α -T. It has been estimated that approximately 1.4 pools (about 74 μ mol) of α -T are replaced daily (Traber *et al.* 1994), so even though the plasma α -T half-life is in the order of 45 h there is rapid turnover. This phenomenon was thought to be responsible for the limitation in plasma α -T levels following supplementation (Traber *et al.* 1998), such that plasma levels are never increased by more than 2–3-fold following any dose amount. A similar situation was observed in erythrocytes, with a dramatic decrease in unlabelled α -T in response to newly absorbed labelled α -T, with almost 45 % of total α -T in the labelled form at T_{max}.

In contrast to this situation in plasma and erythrocytes, unlabelled α -T decreased only marginally in platelets in response to newly absorbed labelled α -T, and did not change in lymphocytes. In both platelets and lymphocytes the percentage of labelled α -T reached only 25%. Therefore, turnover of α -T is slower in platelets and lymphocytes compared to plasma and erythrocytes. It is possible that controlled uptake mechanisms restrict the amount of α -T that can be incorporated, at least acutely, into platelets and lymphocytes. Further research is required to investigate whether this reflects the case in other tissues, e.g. vascular and adipose tissue.

In summary, this study is the first to compare the biokinetics of labelled α -T into plasma, lipoproteins, erythrocytes, platelets and lymphocytes. We have previously suggested that both platelets and lymphocytes are useful functional biomarkers of vitamin E status (Jeanes *et al.* 2004), and they have been shown to be functionally responsive to vitamin E (Roy *et al.* 1991; Freedman *et al.* 1996). The results presented from this study elucidate the simultaneous uptake of newly absorbed α -T into blood cells and lipoproteins, and provide a useful insight into the possible mechanisms of tocopherol uptake into tissues.

Acknowledgements

We are grateful to the British Heart Foundation and The Royal Society for funding, and to the Medical Research Council for supporting Y. M. J. We are also grateful to Dr Christine Gärtner and Dr James Clark of Cognis Nutrition and Health (Düsseldorf, Germany and LaGrange, IL, USA, respectively) for the gift of the deuterated tocopherols. We thank our subjects who participated in the study.

References

- Acuff RV, Thedford SS, Hidiogrou NN, Papas AM & Odom TA Jr (1994) Relative bioavailability of RRR- and all-rac-alpha-tocopheryl acetate in humans: studies using deuterated compounds. *Am J Clin Nutr* **60**, 397–402.
- Brigelius-Flohe R & Traber MG (1999) Vitamin E: function and metabolism. *FASEB J* **13**, 1145–1155.
- Burton GW & Traber MG (1990) Vitamin E: antioxidant activity, biokinetics and bioavailability. *Annu Rev Nutr* **10**, 357–382.
- Burton GW, Webb A & Ingold KU (1985) A mild, rapid, and efficient method of lipid extraction for use in determining vitamin E/lipid ratios. *Lipids* **20**, 29–39.
- Cheeseman KH, Holley AE, Kelly FJ, Wasil M, Hughes L & Burton G (1995) Biokinetics in humans of RRR- α -tocopherol: the free phenol, acetate ester, and succinate ester forms of vitamin E. *Free Radic Biol Med* **19**, 591–598.
- Doring F, Rimbach G & Lodge JK (2004) *In silico* search for single nucleotide polymorphisms in genes important in vitamin E homeostasis. *IUBMB Life* 615–620.
- Freedman JE, Farhat JH, Loscalzo J & Keaney JF (1996) α -Tocopherol inhibits aggregation of human platelets by a protein kinase C-dependent mechanism. *Circulation* **94**, 2434–2440.
- Goti D, Hammer A, Galla HJ, Malle E & Sattler W (2000) Uptake of lipoprotein-associated alpha-tocopherol by primary porcine brain capillary endothelial cells. *J Neurochem* **74**, 1374–1383.
- Goti D, Hrzencak A, Levak-Frank S, Frank S, van der Westhuyzen DR, Malle E & Sattler W (2001) Scavenger receptor class B, type I is expressed in porcine brain capillary endothelial cells and contributes to selective uptake of HDL-associated vitamin E. *J Neurochem* **76**, 498–508.
- Goulinet S & Chapman MJ (1997) Plasma LDL and HDL subspecies are heterogeneous in particle content of tocopherols and oxygenated and hydrocarbon carotenoids. Relevance to oxidative resistance and atherogenesis. *Arterioscler Thromb Vasc Biol* **17**, 786–796.
- Hall WL, Jeanes YM & Lodge JK (2005) Hyperlipidemic subjects have reduced uptake of newly absorbed vitamin E into their plasma lipoproteins, erythrocytes, platelets, and lymphocytes, as studied by deuterium-labeled {alpha}-tocopherol biokinetics. *J Nutr* **135**, 58–63.
- Hall WL, Jeanes YM, Pugh J & Lodge JK (2003) Development of a liquid chromatographic time-of-flight mass spectrometric method for the determination of unlabelled and deuterium-labelled alpha-tocopherol in blood components. *Rapid Commun Mass Spectrom* **17**, 2797–2803.
- Havel RJ, Eder HA & Bragdon JH (1955) The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J Clin Invest* **34**, 1345–1353.
- Jeanes YM, Hall WL, Proteggente AR & Lodge JK (2004) Cigarette smokers have decreased lymphocyte and platelet α -tocopherol levels and increased excretion of the γ -tocopherol metabolite γ -carboxyethylhydroxychroman (γ -CEHC). *Free Radic Res* **38**, 861–868.
- Kaempf DE, Miki M, Ogihara T, Okamoto R, Konishi K & Mino M (1994) Assessment of vitamin E nutritional status in neonates, infants and children – on the basis of alpha-tocopherol levels in blood components and buccal mucosal cells. *Int J Vitam Nutr Res* **64**, 185–191.
- Kayden HJ & Bjornson L (1972) The dynamics of vitamin E transport in the human erythrocyte. *Ann N Y Acad Sci* **203**, 127–140.
- Kayden HJ & Traber MG (1993) Absorption, lipoprotein transport, and regulation of plasma concentrations of vitamin E in humans. *J Lipid Res* **34**, 343–358.
- Kitabchi AE & Wimalasena J (1982) Specific binding sites for D-alpha-tocopherol on human erythrocytes. *Biochim Biophys Acta* **684**, 200–206.
- Kolleck I, Schlame M, Fechner H, Looman AC, Wissel H & Rustow B (1999) HDL is the major source of vitamin E for type II pneumocytes. *Free Radic Biol Med* **27**, 882–890.
- Kostner GM, Oetl K, Jauhiainen M, Ehnholm C, Esterbauer H & Dieplinger H (1995) Human plasma phospholipid transfer protein accelerates exchange/transfer of alpha-tocopherol between lipoproteins and cells. *Biochem J* **305**, Pt 2, 659–667.
- Lodge JK, Hall WL, Jeanes YM & Proteggente AR (2004) Physiological factors influencing vitamin E biokinetics. *Ann N Y Acad Sci* **1031**, 60–73.
- Mardones P & Rigotti A (2004) Cellular mechanisms of vitamin E uptake: relevance in alpha-tocopherol metabolism and potential implications for disease. *J Nutr Biochem* **15**, 252–260.
- Mardones P, Strobel P, Miranda S, Leighton F, Quinones V, Amigo L, Rozowski J, Krieger M & Rigotti A (2002) Alpha-tocopherol metabolism is abnormal in scavenger receptor class B type I (SR-BI)-deficient mice. *J Nutr* **132**, 443–449.
- Perugini C, Bagnati M, Cau C, Bordone R, Paffoni P, Re R, Zoppis E, Albano E & Bellomo G (2000) Distribution of lipid-soluble antioxidants in lipoproteins from healthy subjects. II. Effects of in vivo supplementation with alpha-tocopherol. *Pharmacol Res* **41**, 65–72.
- Relou IA, Hackeng CM, Akkerman JW & Malle E (2003) Low-density lipoprotein and its effect on human blood platelets. *Cell Mol Life Sci* **60**, 961–971.
- Roxborough HE, Burton GW & Kelly FJ (2000) Inter- and intra-individual variation in plasma and red blood cell vitamin E after supplementation. *Free Radic Res* **33**, 437–445.
- Roy RM, Petrella M & Ross WM (1991) Modification of mitogen-induced proliferation of murine splenic lymphocytes by in vitro tocopherol. *Immunopharmacol Immunotoxicol* **13**, 531–550.

- Traber MG, Cohn W & Muller DPR (1993) Absorption, transport and distribution to tissues. In *Vitamin E in Health and Disease*, pp. 35–52 [L Packer and J Fuchs, editors]. New York: Marcel Dekker.
- Traber MG, Ingold KU, Burton GW & Kayden HJ (1988) Absorption and transport of deuterium-substituted 2R,4'R,8'R- α -tocopherol in human lipoproteins. *Lipids* **23**, 791–797.
- Traber MG & Kayden HJ (1984) Vitamin E is delivered to cells via the high affinity receptor for low density lipoprotein. *Am J Clin Nutr* **40**, 747–751.
- Traber MG & Kayden HJ (1989) α -Tocopherol as compared with γ -tocopherol is preferentially secreted in human lipoproteins. *Ann N Y Acad Sci* **570**, 95–108.
- Traber MG, Lane JC, Lagmay N & Kayden HJ (1992) Studies on the transfer of tocopherol between lipoproteins. *Lipids* **27**, 657–663.
- Traber MG, Olivecrona T & Kayden HJ (1985) Bovine milk lipoprotein lipase transfers tocopherol to human fibroblasts during triglyceride hydrolysis in vitro. *J Clin Invest* **75**, 1729–1734.
- Traber MG, Rader D, Acuff RV, Ramakrishnan R, Brewer HB & Kayden HJ (1998) Vitamin E dose–response studies in humans with use of deuterated RRR- α -tocopherol. *Am J Clin Nutr* **68**, 847–853.
- Traber MG, Ramakrishnan R & Kayden KJ (1994) Human plasma vitamin E kinetics demonstrate rapid recycling of plasma RRR- α -tocopherol. *Proc Natl Acad Sci U S A* **91**, 10005–10008.
- Weintraub MS, Eisenberg S & Breslow JL (1987) Different patterns of postprandial lipoprotein metabolism in normal, type IIa, type III, and type IV hyperlipoproteinemic individuals. Effects of treatment with cholestyramine and gemfibrozil. *J Clin Invest* **79**, 1110–1119.