Comparative $^{2}H$-labelled $\alpha$-tocopherol biokinetics in plasma, lipoproteins, erythrocytes, platelets and lymphocytes in normolipidaemic males

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

Deuterated: Tocopherol: Plasma: Erythrocytes: Platelets: Lymphocytes

Vitamin E, in particular $\alpha$-tocopherol ($\alpha$-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, $\alpha$-T is absorbed by the intestinal cells at the same rate as the other forms of vitamin E ($\gamma$-, $\beta$- and $\delta$-tocopherols and $\alpha$-, $\beta$-, $\delta$- and $\gamma$-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-$\alpha$-T is incorporated into VLDL by the $\alpha$-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 $\alpha$-T rapidly displaces pre-existing ‘old’ $\alpha$-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 $\alpha$-T preferably via HDL (Kolleck et al. 1999; Goti et al.

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

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Materials and methods

**Tocopherols**

\( \text{RRR-\(\alpha\)-5,7-(CD}_3\rangle \text{\text{\(\alpha\)}-tocopheryl acetate and all rac-\(\alpha\)-5 (CD3)\text{\(\alpha\)}-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 \( \text{RRR-\(\alpha\)-tocopheryl acetate was encapsulated (150 mg) for human consumption. Trideuterated all rac-\(\alpha\)-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 \( \text{RRR-\(\alpha\)-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 µl 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 µl 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 µl 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 Weintrab 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-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 \( \text{\(\alpha\)}-T\) using the methods described below.

**Vitamin E extraction and analysis**

Prior to extraction, plasma and erythrocyte aliquots (100 µl) were diluted 1:5 and 1:10 with PBS, respectively (Hall et al. 2003). Platelet and lymphocyte aliquots (100 µl) were not diluted and were sonicated for 3 min prior to extraction to obtain a homogenous solution. Total vitamin E (mainly \( \text{\(\alpha\)}-\text{and} \text{\(\gamma\)}\)-tocopherol) was extracted from all samples using a combination of SDS, ethanol and hexane as described (Burton et al. 1985). \( \text{\(\alpha\)}-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 × 50 mm, C18, 3.5 µm) with a mobile phase consisting of 100% methanol (LC–MS Chromasolv, Sigma-Aldrich). Trideuterated all rac-\(\alpha\)-tocopherol (final concentration 0.5 µmol/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 $^{3}$H-labelled $\alpha$-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 $\times$ 100) were used as a measure of inter-individual variation. The change in labelled and unlabelled $\alpha$-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 (Systat 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 $\alpha$-tocopherol into plasma and lipoproteins**

Fig. 1 shows the biokinetic profile of labelled, unlabelled and total $\alpha$-T in the plasma following ingestion of a capsule containing 150 mg deuterated RRR-$\alpha$-tocopheryl acetate. Values are means with their standard errors shown by vertical bars. There was a significant increase in labelled $\alpha$-tocopherol over time ($P<0.0001$), and a significant decrease in unlabelled $\alpha$-tocopherol over time. Statistical differences between time points are also shown: $^{a}v.6,9,12,24$ and 48 h, $P<0.01$; $^{b}v.48$ h, $P<0.01$; $^{c}12$ v. 48 h, $P<0.01$; $^{d}24$ v. 48 h, $P<0.05$.

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Profiles of labelled $\alpha$-T in CM, VLDL, LDL and HDL following ingestion of a capsule containing 150 mg RRR-$\alpha$-tocopheryl acetate are shown in Fig. 2. In all lipoproteins there was a significant increase in labelled $\alpha$-tocopherol over time ($P<0.0001$), and a significant decrease in unlabelled $\alpha$-tocopherol over time. Statistical differences between time points are also shown: $^{a}v.6,9,12,24$ and 48 h, $P<0.01$; $^{b}v.48$ h, $P<0.01$; $^{c}12$ v. 48 h, $P<0.01$; $^{d}24$ v. 48 h, $P<0.05$.

Table 1. Subject information

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>47.8</td>
<td>10.2</td>
<td>31–58</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.6</td>
<td>2.5</td>
<td>23–32</td>
</tr>
<tr>
<td>Labelled $\alpha$-tocopherol/kg body weight (mg/kg)</td>
<td>0.94</td>
<td>0.2</td>
<td>1.7–2.2</td>
</tr>
<tr>
<td>Plasma cholesterol (mmol/l)</td>
<td>5.0</td>
<td>0.6</td>
<td>3.88–5.64</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>3.3</td>
<td>0.5</td>
<td>2.24–3.7</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.1</td>
<td>0.2</td>
<td>0.75–1.54</td>
</tr>
<tr>
<td>Plasma triacylglycerols (mmol/l)</td>
<td>1.2</td>
<td>0.3</td>
<td>0.76–1.72</td>
</tr>
</tbody>
</table>
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

Biokinetie 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 (Cmax) and AUC than other lipoproteins (P<0.05), while CM had a significantly lower time of Cmax (Tmax) at 6 h (P<0.05) and a significantly shorter half-life (P<0.05). Large variation (>70 %) was observed in CM Cmax, AUC, elimination rate and half-life. In blood components, Tmax was significantly lower (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), *3 v. 9, 12, 24 and 48 h, P<0.001; †6 v. 5, h, P<0.01, 6 v. 12, 24 and 48 h, P<0.001; ‡9 v. 12 h, P=0.01, 9 v. 24 and 48 h, P<0.001; §12 v. 24, h, P=0.01; ¶24 v. 48 h, P<0.001; (B), *3 v. 6, 12, 24 and 48 h, P<0.001, 3 v. 9 h, P<0.01; †6 v. 24 and 48 h, P<0.001; ‡9 v. 24 and 48 h, P<0.001; §12 v. 24, h, P=0.01 and 48 h, P=0.001; (C), *3 v. 24 and 48 h, P<0.001, 3 v. 12 h, P<0.01; †6 v. 24 and 48 h, P<0.001, 6 v. 12 h, P<0.01; ‡9 v. 24, and 48 h, P<0.001; §12 v. 24 and 48 h, P<0.001.](image-url)
Patterns with a maximum at 6 h at 55 % labelled at 12 h of approximately 36 %. CM, however, show a very different pattern, all reaching maximum labelling at about (Fig. 4(B)). In plasma lipoproteins, VLDL, LDL and HDL show a rapid decrease. In blood components, labelled \( \alpha \)-tocopherol in the labelled form occurs at 24 h, with approximately 45 % enrichment.

**Discussion**

This study aimed to determine and compare the uptake and distribution profiles of labelled \( \alpha \)-T into plasma, lipoproteins, erythrocytes, platelets and lymphocytes in healthy males following a single dose (150 mg) of \( ^{2} \text{H} \)-labelled \( RRR \)-\( \alpha \)-tocopheryl acetate. This is the first report of vitamin E bio kinetics 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 \( \alpha \)-T concentrations represent the combined total of \( \alpha \)-T within circulating lipoproteins. Plasma labelled \( \alpha \)-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 \( \alpha \)-T corresponded to the profile of labelled \( \alpha \)-T in CM, which peaked at 6 h. The rate of \( \alpha \)-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 \( \alpha \)-T peaked at 12 h.

**Table 2.** \( ^{2} \text{H} \)-Labelled \( \alpha \)-tocopherol kinetic measurements for all blood components up to 48 h following ingestion of 150 mg deuterated \( RRR \)-\( \alpha \)-tocopheryl acetate.

<table>
<thead>
<tr>
<th>Component</th>
<th>Cmax (Mean SD)</th>
<th>Tmax (Mean SD)</th>
<th>AUC (Mean SD)</th>
<th>Rate of absorption (Mean SD)</th>
<th>Rate of elimination (Mean SD)</th>
<th>Half-life (h) (Mean SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chylomicrons</td>
<td>22.5 17.6 78</td>
<td>6.6 1.3 19</td>
<td>223 154 69</td>
<td>1.41 0.79 56</td>
<td>0.190 0.19 100</td>
<td>11 9 84</td>
</tr>
<tr>
<td>VLDL</td>
<td>28 10 36</td>
<td>12.6 6.3 50</td>
<td>286 33 0.37</td>
<td>0.25 0.69</td>
<td>0.019 0.01 57</td>
<td>54 43 80</td>
</tr>
<tr>
<td>LDL</td>
<td>16.7 6.1 48</td>
<td>16.2 8.4 52</td>
<td>503 255 51</td>
<td>0.26 0.20 77</td>
<td>0.024 0.01 52</td>
<td>36 16 45</td>
</tr>
<tr>
<td>HDL</td>
<td>1.43 0.4 28</td>
<td>1.3 5.7 42</td>
<td>47 13 29</td>
<td>0.24 0.08 37</td>
<td>0.021 0.01 31</td>
<td>37 15 41</td>
</tr>
<tr>
<td>Plasma</td>
<td>20.6 6.6 32</td>
<td>12.8 5.8 46</td>
<td>930 328 35</td>
<td>0.40 0.24 60</td>
<td>0.018 0.004 27</td>
<td>43 21 48</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>3.8 1.1 28</td>
<td>25.5 10 39</td>
<td>119 36 30</td>
<td>0.24 0.04 19</td>
<td>0.014 0.003 24</td>
<td>51 14 27</td>
</tr>
<tr>
<td>Platelets</td>
<td>0.48 0.14 29</td>
<td>30 16.9 56</td>
<td>16 5 30</td>
<td>0.28 0.10 37</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>0.37 0.10 27</td>
<td>43.8 9.7 22</td>
<td>12 3 27</td>
<td>0.43 0.18 42</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

AUC, area under the deuterated \( \alpha \)-tocopherol concentration with time curve (\( \mu \text{mol.L}^{-1} \text{protein} \)), platelets and lymphocytes; \( \mu \text{mol.L}^{-1} \text{plasma} \), \( \mu \text{mol.L}^{-1} \text{haematocrit} \) in erythrocytes; Cmax, maximum concentration of deuterated \( \alpha \)-tocopherol (\( \mu \text{mol.g}^{-1} \text{protein} \) in lipoproteins, platelets and lymphocytes; \( \mu \text{mol.L}^{-1} \text{plasma} \), \( \mu \text{mol.L}^{-1} \text{haematocrit} \) in erythrocytes); ND, not determined; rate of absorption and elimination (\( \mu \text{mol.g}^{-1} \text{protein.h}^{-1} \) in lipoproteins, platelets and lymphocytes; \( \mu \text{mol.L}^{-1} \text{plasma} \), \( \mu \text{mol.L}^{-1} \text{haematocrit} \) in erythrocytes); Tmax, time of Cmax (h).
were also very similar between these. HDL contained significantly lower concentrations of labelled \( \alpha\)-T compared to the other lipoproteins, and this reflects the preference of \( \alpha\)-T to more hydrophobic environments. The labelled \( \alpha\)-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 \( \alpha\)-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 \( \alpha\)-T to cells (Traber et al. 1985). The transfer of \( \alpha\)-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 \( \alpha\)-T between the lipoproteins, which was demonstrated in the present study by the similar profiles of percentage labelled \( \alpha\)-T in VLDL, LDL and HDL (Fig. 3(A)).

There was large inter-individual variation in the plasma response to newly absorbed labelled \( \alpha\)-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 \( \alpha\)-T per kg body weight was similar between subjects, which could reduce variation caused by differences in the volume of \( \alpha\)-T distribution. To date there have been no studies addressing potential differences in vitamin E biochemistry 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 \( \alpha\)-T transport. Therefore processes involved in the packaging of lipid and vitamin E into CM appears to be a major determinant of \( \alpha\)-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 biochemistry, 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 \( \alpha\)-T as we have recently shown that following a period of vitamin E supplementation, the uptake of newly absorbed \( \alpha\)-T is reduced (Lodge et al. 2004). One limitation of the current study is the lack of baseline \( \alpha\)-T concentrations and measures of habitual vitamin E intake, therefore inter-individual differences in vitamin E status were not assessed.

Ultimately the amount of \( \alpha\)-T in plasma will influence the extent of uptake into blood components and tissues. Labelled \( \alpha\)-T enters the erythrocytes following the plasma. There was no increase in labelled \( \alpha\)-T until 9 h, subsequent to peak CM concentrations. It therefore appears that CM do not readily transfer \( \alpha\)-T to erythrocytes. Erythrocyte labelled \( \alpha\)-T concentrations peaked at 24 h, whilst VLDL, LDL and HDL were all at their maximum \( \alpha\)-T labelling at 12 h, which would support the theory that erythrocytes obtain their \( \alpha\)-T via transfer from these lipoproteins. It has been shown that transfer of \( \alpha\)-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 \( \alpha\)-T AUC, but not with other lipoproteins. There was also a significant correlation between platelet and HDL labelled \( \alpha\)-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 \( \alpha\)-T transfer to erythrocytes may involve more than just spontaneous transfer, as erythrocytes are thought to obtain vitamin E via tocopherol binding protein (Kitabchi & Wimalasena, 1982), although the role of these binding proteins in \( \alpha\)-T uptake has not been elucidated.

The results show a more gradual and later uptake of labelled \( \alpha\)-T into platelets and lymphocytes compared to plasma and erythrocytes. The uptake of labelled \( \alpha\)-T into platelets was particularly interesting. A gradual increase in labelled \( \alpha\)-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 \( \alpha\)-T concentration in subjects with a biphasic response coincided with the \( \alpha\)-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 \( \alpha\)-T in plasma before platelets rapidly take up newly absorbed \( \alpha\)-T. The post-9 h uptake profile in platelets was similar to that in lymphocytes. The gradual uptake of labelled \( \alpha\)-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 \( \alpha\)-T uptake (Mardones & Rigotti, 2004). Cells containing LDL receptors can take up \( \alpha\)-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 \( \alpha\)-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 \( \alpha\)-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 \( \alpha\)-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 \( \alpha\)-T displaces pre-existing \( \alpha\)-T within plasma (Traber et al. 1994, 1998). In the present study, there was an increase in plasma labelled \( \alpha\)-T and total \( \alpha\)-T, and a concomitant decrease in unlabelled \( \alpha\)-T. At Tmax, 45% of the total \( \alpha\)-T was in the labelled form, indicative of rapid displacement of pre-existing \( \alpha\)-T. It has been estimated that approximately 1-4 pools (about 74 µmol) of \( \alpha\)-T are replaced daily (Traber et al. 1994), and even though the plasma \( \alpha\)-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 \( \alpha\)-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 \( \alpha\)-T in response to newly absorbed labelled \( \alpha\)-T, with almost 45% of total \( \alpha\)-T in the labelled form at Tmax.
In contrast to this situation in plasma and erythrocytes, unlabelled \( \alpha \)-T decreased only marginally in platelets in response to newly absorbed labelled \( \alpha \)-T, and did not change in lymphocytes. In both platelets and lymphocytes the percentage of labelled \( \alpha \)-T reached only 25%. Therefore, turnover of \( \alpha \)-T is slower in platelets and lymphocytes compared to plasma and erythrocytes. It is possible that controlled uptake mechanisms restrict the amount of \( \alpha \)-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 \( \alpha \)-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 (Jeane 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 \( \alpha \)-T into blood cells and lipoproteins, and provide a useful insight into the possible mechanisms of tocopherol uptake into tissues.

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References


