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Short communication

The absorption of vitamin E is influenced by the amount of fat in a meal and the food matrix

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Vitamin E absorption requires the presence of fat; however, limited information exists on the influence of fat quantity on optimal absorption. In the present study we compared the absorption of stable-isotope-labelled vitamin E following meals of varying fat content and source. In a randomised four-way cross-over study, eight healthy individuals consumed a capsule containing 150 mg ^{2}H-labelled *RRR*-α-tocopheryl acetate with a test meal of toast with butter (17·5 g fat), cereal with full-fat milk (17·5 g fat), cereal with semi-skimmed milk (2·7 g fat) and water (0 g fat). Blood was taken at 0, 0·5, 1, 1·5, 2, 3, 6 and 9 h following ingestion, chylomicrons were isolated, and ^{2}H-labelled α-tocopherol was analysed in the chylomicron and plasma samples. There was a significant time ($P<0·001$) and treatment effect ($P<0·001$) in ^{2}H-labelled α-tocopherol concentration in both chylomicrons and plasma between the test meals. ^{2}H-labelled α-tocopherol concentration was significantly greater with the higher-fat toast and butter meal compared with the low-fat cereal meal or water ($P<0·001$), and a trend towards greater concentration compared with the high-fat cereal meal ($P=0·065$). There was significantly greater ^{2}H-labelled α-tocopherol concentration with the high-fat cereal meal compared with the low-fat cereal meal ($P<0·05$). The ^{2}H-labelled α-tocopherol concentration following either the low-fat cereal meal or water was low. These results demonstrate that both the amount of fat and the food matrix influence vitamin E absorption. These factors should be considered by consumers and for future vitamin E intervention studies.

Tocopherol: Fat: Plasma: Chylomicrons

Vitamin E has been the subject of a number of clinical trials investigating its potential cardioprotective effects, even though there is limited information on factors that influence its bioavailability. It is widely accepted that dietary fat is required for the absorption of vitamin E; however, the amount of fat required for maximal absorption is unknown. The process is similar for all the fat-soluble vitamins and other dietary fats in that there is a prerequisite for the formation of mixed micelles containing dietary lipids and products of lipid hydrolysis, emulsified in the presence of bile salts (Cohn *et al.* 1992; Cohn, 1997). The importance of bile salts and pancreatic secretions is demonstrated in subjects with either cholestatic liver disease or cystic fibrosis who malabsorb vitamin E and become vitamin E deficient (Sokol *et al.* 1989). The amount of fat necessary for maximal vitamin E absorption in man in currently undetermined (Cohn, 1997; Leonard *et al.* 2004). There are only a few human studies that have investigated the influence of dietary fat on vitamin E absorption. Dimitrov *et al.* (1991) reported significantly greater plasma α-tocopherol (the major form of vitamin E in tissues) levels in human subjects after 5 d when given 800 mg synthetic vitamin E with a high-fat diet compared with a low-fat diet. However, no difference in plasma vitamin E levels was found following a 50 mg supplement taken with either 3 or 36 g fat for 7 d (Roodenburg *et al.* 2000). Supplemental vitamin E is usually encapsulated in the esterified form, which requires de-esterification before absorption. This, however, does not appear to be rate limiting, as the biokinetics of α-tocopherol, α-tocopheryl acetate and α-tocopheryl succinate have been found to be all similar (Cheeseman *et al.* 1995). A recent study compared vitamin E bioavailability from a fortified breakfast cereal to that from a capsule (Leonard *et al.* 2004) and found increased bioavailability from the fortified meal; both forms were taken with 'fat-free' milk.

Vitamin E capsules represent an important source of vitamin E in terms of consumer use and in clinical studies. Such studies rarely include any indication of relative bioavailability. As there are limited controlled human data on the

Abbreviation: TAG, triacylglycerol.

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influence of dietary fat on vitamin E absorption we investigated the influence of both the amount of fat and the food matrix on plasma α-tocopherol uptake as assessed by stable-isotope (^{2}H)-labelled α-tocopherol kinetics. We chose to study this using common breakfast meals as sources of fat for our test meals, as it is common in clinical studies for subjects to take vitamin capsules in the morning at breakfast.

Materials and methods

Materials

RRR-α-5, 7-(C^2H_3)$_2$-tocopheryl acetate and *all rac*-α-5-(C^2H_3)-tocopheryl acetate were kind gifts from Cognis Nutrition and Health (Düsseldorf, Germany and LaGrange, IL, USA). The purity of the acetates was 98·8 % for both species. Isotopic purity was determined to be > 99·9 % by LC-MS. The ^{2}H-labelled *RRR*-α-tocopheryl acetate was encapsulated (150 mg) into hard gelatine capsules, with no other ingredients, for human consumption. The ^{2}H-labelled *all rac*-α-tocopheryl acetate was used as an internal standard. All reagents for vitamin E extraction and analysis were obtained from Sigma-Aldrich Chemical Co. (Poole, Dorset, UK).

Subjects

To estimate the sample size required to demonstrate a significant difference between treatments with 0·05 probability and 80 % power, we used a within-group variation of 20 %, based on previous observations (Roxborough *et al.* 2000), and a difference to detect between treatments of 25 %. This was based on the relatively large difference between amounts of fat in our test meals. A sample size of ten was calculated. In total eight healthy volunteers were recruited from within the University of Surrey. Selection criteria stated that subjects must be non-smoking, not taking dietary supplements and with no gastrointestinal disorders as determined from a written questionnaire. Subjects with blood lipid abnormalities were also excluded (criteria of total cholesterol < 6 mmol/l and triacylglycerol (TAG) < 1·5 mmol/l).

Study design

A within-subject, repeated-measures design was used, with each subject serving as their own control. Each subject consumed a capsule containing 150 mg ^{2}H-labelled *RRR*-α-tocopheryl acetate with a different test meal on four separate occasions in a randomised order, each study day separated by 7 d. On study days each subject consumed the capsule with one of four test meals. These were: (1) two slices of white toast with 20 g butter (containing 17·5 g fat, 6·8 g protein, 41·2 g carbohydrate, 1356 kJ); (2) 40 g cornflakes with 75 g full-fat milk plus 75 g single cream (containing 17·5 g fat, 7·5 g protein, 41·0 g carbohydrate, 1436 kJ); (3) 40 g cornflakes with 75 g semi-skimmed milk (containing 2·7 g fat, 8·1 g protein, 41·9 g carbohydrate, 894 kJ); (4) a glass of water (containing 0 g fat, protein, carbohydrate and 0 kJ). Subjects (fasted for 12 h) were cannulated at 07.30 hours and immediately a blood sample was taken (10 ml). Subjects then consumed the capsule with the test meal at 08.00 hours, and further blood samples were taken at 08.30, 09.00, 09.30, 10.00, 11.00 and 14.00 hours. Subjects then consumed a standard lunch comprising a sandwich and low-fat yoghurt (containing 2·5 g fat, 23 g protein, 59 g carbohydrate, 1428 kJ). A further blood sample was taken at 17.00 hours. Blood sampling therefore corresponded to times 0, 0·5, 1, 1·5, 2, 3, 6 and 9 h following ingestion of the vitamin E capsule with the test meal. This time period was chosen, as we were primarily interested in chylomicron vitamin E transport. Subjects were only allowed water during the study period.

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

Isolation of blood components

At each time point, plasma was harvested from whole blood following centrifugation at 3500 rpm for 10 min at 10°C. A sample of plasma (4 ml) was used for chylomicron isolation, while the remainder was snap-frozen in liquid N_2 and stored at − 80°C ready for analysis. To isolate chylomicrons, plasma (4 ml) was overlaid with an equal volume of saline solution (density 1·006 g/ml) in a Beckman ultracentrifuge tube. The samples were spun at 110 000 ***g*** for 15 min at 16°C using a 70.1 Ti Beckman Coulter rotor and a Beckman Optima XL-100 ultracentrifuge (Weintraub *et al.* 1987). The top 1 ml fraction, which comprises the chylomicrons, was harvested using a syringe and needle and subsequently snap-frozen in liquid N_2 and stored at − 80°C ready for analysis.

Vitamin E extraction and analysis

Total vitamin E was extracted from plasma and chylomicron samples by a combination of sodium dodecyl sulfate, ethanol and hexane as described previously (Burton *et al.* 1985). Tocopherols were 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, Herts., 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).

Occasionally the presence of a small amount (< 0·2 μmol/l) of ^{2}H-labelled α-tocopherol was found in baseline plasma samples as a result of carry-over from the previous intervention (only when the meal followed the toast with butter meal; six occasions in total). In these circumstances, the labelled α-tocopherol concentrations were corrected for baseline by subtracting this baseline value from all time points over that study period.

Biochemical analysis

Total plasma cholesterol and TAG were determined using enzymic kits supplied by Randox (Crumlin, County

Antrim, UK), and analysed automatically using a SPACE biochemical analyser (Alfa-Wasserman, Woerden, Holland).

Statistical analysis

Data were analysed using two-way repeated-measures ANOVA (Statistica version 5.1; StatSoft Inc., Tulsa, OK, USA), with meal and time as within-subject factors. *Post hoc* analysis of effects was carried out using Tukey's honestly significant difference test. Statistical significance was assigned at $P<0{\cdot}05$, and a trend towards significance if $P<0{\cdot}1$ and $>0{\cdot}05$. Values shown are means and standard deviations.

Results

Subject characteristics

Eight healthy normolipidaemic volunteers (five female, three male) participated in the study. Their mean age was 28 (SD 6) years and mean BMI was 23 (SD 4) kg/m^2. Fasting plasma cholesterol and TAG levels were 4·2 (SD 0·7) and 0·95 (SD 0·2) mmol/l respectively. Fasting plasma α-tocopherol was 23·6 (SD 2·2) μmol/l, and their labelled α-tocopherol/kg body weight following dosing was 2·3 (SD 0·1) mg/kg.

Influence of the test meals on plasma and chylomicron lipids

The concentration of plasma cholesterol did not differ significantly during the study period (data not shown). Baseline (pre-meal) plasma TAG concentrations were 0·9 mmol/l. Following the cereal with full-fat milk meal, plasma TAG increased to a maximum of 1·19 mmol/l after 1 h then gradually returned to baseline between 3 and 6 h. Plasma TAG did not increase above baseline following the other meals (data not shown). Chylomicron TAG concentration showed a significant difference over time ($P=0{\cdot}025$), and a trend towards a difference between test meals ($P=0{\cdot}051$). Increases in chylomicron TAG concentration only occurred following the higher-fat meals. Following the cereal with full-fat milk meal, chylomicron TAG increased from 0·4 mmol/l to a maximum of 0·58 mmol/l following 1 h, then decreased back to baseline by 3 h. Following the toast with butter meal, chylomicron TAG increased from 0·28 mmol/l to a maximum of 0·37 mmol/l after 1 h, then decreased back to baseline also by 3 h.

Influence of the test meals on ^{2}H-labelled α-tocopherol uptake into plasma and chylomicrons

Fig. 1 shows ^{2}H-labelled α-tocopherol concentration in chylomicrons and plasma following the ingestion of a capsule containing 150 mg ^{2}H-labelled *RRR*-α-tocopheryl acetate with the test meals. There was a significant difference in ^{2}H-labelled α-tocopherol concentration in both chylomicrons and plasma over time ($P<0{\cdot}001$), and a significant difference in ^{2}H-labelled α-tocopherol concentrations between the test meals ($P<0{\cdot}001$). The test meal of toast and butter (17·5 g fat) resulted in the earliest response, and the largest ^{2}H-labelled α-tocopherol concentration, in both plasma and chylomicrons over the study period. There was a significantly greater ^{2}H-labelled α-tocopherol concentration when the capsule was ingested with the high-fat cereal meal (17·5 g) compared with the low-fat cereal meal (2·7 g fat) ($P<0{\cdot}05$). There was a trend towards a greater ^{2}H-labelled α-tocopherol concentration when the capsule was ingested with the toast and butter meal compared with the cereal with full-fat milk meal, both containing 17·5 g fat ($P=0{\cdot}065$). There was no difference in either chylomicron or plasma ^{2}H-labelled α-tocopherol concentration following either the low-fat cereal meal or water, which remained negligible over the study period.

There was considerable inter-individual variation in labelled α-tocopherol responses in plasma and chylomicrons (data not shown). Subjects varied not only in the labelled α-tocopherol concentration (for example, toast

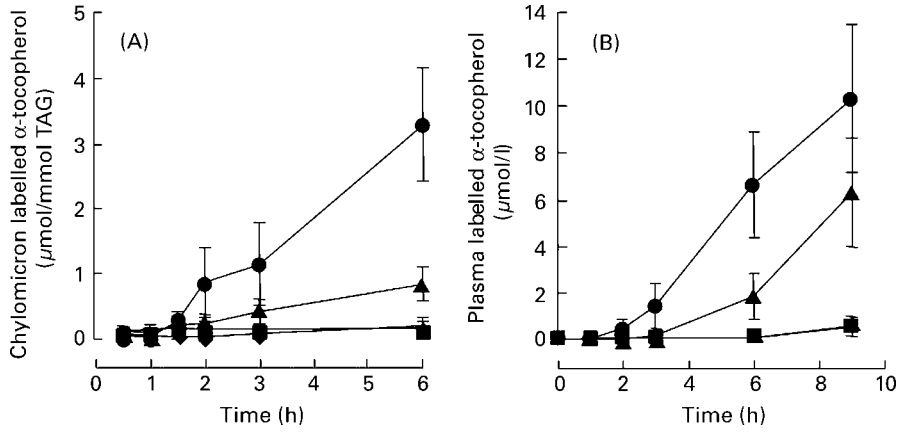

Fig. 1. ^{2}H-labelled α-tocopherol concentration in (A) chylomicrons and (B) plasma following ingestion of a capsule containing 150 mg ^{2}H-labelled *RRR*-α-tocopheryl acetate with various test meals. There was a significant difference over time ($P<0{\cdot}001$) and between test meals ($P<0{\cdot}001$) in ^{2}H-labelled α-tocopherol concentration in both chylomicrons and plasma. Values are means for eight subjects, with standard deviations represented by vertical bars. (–●–), Toast with butter; (–▲–), cereal with full-fat milk; (–◆–), cereal with semi-skimmed milk; (–■–), water; TAG, triacylglycerol.

with butter meal, plasma range 0·7 to 26·1 μmol/l at 9 h) but also in the time that labelled α-tocopherol began to increase in concentration (toast with butter meal, plasma range 2 to 6 h). However, the variation was consistent intra-individually, subjects showing the same responsiveness between meals. For example, the least responsive subject following the toast and butter meal was also the least responsive in the other meals. Variation was also consistent between the chylomicron and plasma data for each individual subject.

Discussion

One of the major determinants of bioavailability is the extent of absorption (Jackson, 1997). Thus it is expected that factors that increase absorption will increase bioavailability. In the present study we have shown that both the amount of dietary fat in a meal and the food matrix of the meal influence the absorption, and hence bioavailability, of vitamin E. There was little or no increase in plasma and chylomicron labelled α-tocopherol when a capsule containing labelled vitamin E was consumed with a low-fat cereal meal (2·5 g fat) or with water (0 g fat). Indeed the presence of 2·5 g fat did not appear to influence vitamin E absorption over the study period (9 h). However, the meals comprising 17·5 g fat resulted in significantly higher ^{2}H-labelled α-tocopherol concentrations in plasma and chylomicrons. The plasma and chylomicron concentration of ^{2}H-labelled α-tocopherol was also greater when the vitamin E capsule was consumed with a toast and butter meal compared with a cereal with full-fat milk meal, even though both meals contained 17·5 g fat. Thus, both the amount of fat in a meal and also the physical properties of a meal influence vitamin E absorption and bioavailability.

These data increase the current understanding of the role of fat in vitamin E absorption. Although a few studies have looked at vitamin E levels following diets of differing fat content, this is the first controlled study directly comparing the influence of varying dietary fat amounts on vitamin E absorption and bioavailability. Previously, a 3-week diet rich in unsaturated fat was found to increase serum concentrations of α-tocopherol by 7 %, whereas a diet rich in saturated fat decreased α-tocopherol concentration by a similar amount (Ohrvall *et al.* 2001). Plasma α-tocopherol levels were found to be similar following 7 d of supplementation with 50 mg vitamin E consumed with either a low-fat (< 6·5 g fat/d) or a high-fat (about 45 g fat/d) meal (Roodenburg *et al.* 2000). However, significantly greater plasma uptake of α-tocopherol was found in human subjects fed a high-fat diet (about 115 g fat/d) compared with a low-fat diet (about 51 g fat/d) over 5 d of supplementation with 800 mg synthetic vitamin E (Dimitrov *et al.* 1991). These studies have attempted to assess relative bioavailability by measuring steady-state plasma levels; however, this is impractical due to the regulation of plasma concentrations and the unknown relationship between intake and plasma concentrations (Cohn, 1997). We have used stable isotopes so that the ingested and newly absorbed vitamin E can be directly monitored, which eliminates complications due to endogenous vitamin E.

We have also shown in the present study that the matrix of the meal is important for vitamin E absorption, as differences were observed between test meals of different composition but the same fat content. A few studies have investigated the influence of the physical properties of the meal on vitamin E bioavailability. Hayes *et al.* (2001) reported that milk enhanced vitamin E uptake, irrespective of fat content. Their conclusions were based on a similar percentage increase in plasma α-tocopherol after a 4-week supplementation with a fat-soluble version of vitamin E dispersed in 1 % fat milk, and a water-soluble vitamin E in skimmed milk (0·1 % fat). Greater vitamin E bioavailability was found when a capsule was consumed with cereal and fat-free milk, rather than with fat-free milk alone (Leonard *et al.* 2004). Even though the micro-dispersion of vitamin E in milk appears to increase vitamin E bioavailability in previous studies (Hayes *et al.* 2001), in the present study the high-fat meal containing milk produced a lesser response to that of the isoenergetic toast with butter meal. Thus the physical properties of toast and butter appear to provide a better medium for vitamin E absorption. The physical properties of food are known to affect gastric emptying; foods that are high in fibre, viscosity and protein slow gastric emptying (Low, 1990), thus providing a potential explanation for the differences between meals.

The present study was aimed at vitamin E uptake during the absorptive period, measurements were taken up to 9 h, which approximates to the peak in chylomicron vitamin E transport (Traber *et al.* 1998). In the circulation, chylomicrons undergo extensive hydrolysis by endothelial bound lipoprotein lipase, during which time vitamin E can be transferred to peripheral tissues and circulating lipoproteins (Traber *et al.* 1985). Excess chylomicron surface area is produced and along with vitamin E is transferred to HDL. HDL can donate its vitamin E, therefore there is a constant flux of vitamin E among circulating lipoproteins (Traber *et al.* 1992). Also, we have previously shown that vitamin E metabolism is rapid, as the specific vitamin E metabolites, the carboxyethyl-hydroxychromans, are found in the urine 6 h following vitamin E ingestion and peak at 9 h (Lodge *et al.* 2001). Therefore in the present study we can assume that in this 9 h study period there is multi-compartmentalisation of labelled α-tocopherol with α-tocopherol pools in lipoproteins (plasma), the liver and peripheral tissues.

The large inter-individual variation in response to labelled vitamin E observed in the present study is consistent with previous observations (Roxborough *et al.* 2000). The fact that this intra-individual variation was consistent between meals does suggest that individuals do differ in their response to vitamin E supplementation. As the variation was large within the chylomicrons, it is probable that the mucosal handling of vitamin E is an important source of variability. This could reflect either the transport of α-tocopherol into the enterocyte, or in the packaging of chylomicrons themselves. Further work is required in this area.

In summary, the present study demonstrates that both the amount of fat and the physical properties of a meal influence the absorption of supplemental vitamin E. As vitamin

E supplements are frequently used by consumers and in clinical studies, these findings are of relevance and need to be considered.

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