DEPENDENCE OF PLASMA $\alpha$-TOCOPHEROL FLUX ON VERY LOW-DENSITY TRIGLYCERIDE CLEARANCE IN HUMANS

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Abstract—To evaluate the effect of dietary fat-induced alterations in triglyceride (TG) metabolism on plasma and very low-density lipoprotein (VLDL)-$\alpha$-tocopherol, nine healthy males (mean ± SEM, age: 36 ± 3 years, BMI: 24.7 ± 1.1) consumed a 35%-fat diet (control) for one week followed by a 15% low-fat, high-carbohydrate diet for 5 weeks. After each dietary phase, the subjects ingested an evening meal along with a 50 mg capsule of $^2$H$_6$-RRR-$\alpha$-tocopheryl acetate; blood samples were drawn over a 24 h period while the subjects remained fasted. Low-fat feeding increased fasting plasma TG concentrations by 53% (116 ± 27 to 178 ± 32, mg/dl, $p < 0.0001$) primarily by reducing VLDL-TG clearance. Total plasma $\alpha$-tocopherol concentrations (labeled + unlabeled) were unchanged (25.8 ± 2.3 vs. 26.4 ± 3.0 nmol/ml plasma) and no differences between the diets were observed for plasma $^2$H$_6$-$\alpha$-tocopherol concentration (4.8 ± 0.6 nmol/ml, for both diets) or enrichments (18.1 ± 1.8% average for both diets). However, low-fat feeding significantly increased the amount of $\alpha$-tocopherol in the VLDL fraction (43%, $p = 0.04$) in concert with elevations in VLDL-apoB and TG. The $\alpha$-tocopherol and TG content of VLDL varied in parallel in individual subjects and fractional replacement rates and clearance of $\alpha$-tocopherol and TG in VLDL were closely correlated. Kinetic parameters were decreased by 32–39% from high-fat to low-fat. These data suggest that vitamin E bioavailability is similar between a 15 and 35% fat diet, with a redistribution of $\alpha$-tocopherol in lipoproteins occurring during low-fat feeding (increased in the VLDL fraction, reduced in the other lipoproteins), and transfer of $\alpha$-tocopherol from VLDL depends upon TG removal from the particle, consistent with previous observations in vitro and in animal studies. © 2000 Elsevier Science Inc.

Keywords—$\alpha$-Tocopherol, Stable isotopes, Lipoprotein, Triglyceride, Kinetics, Free radicals

INTRODUCTION

In humans, elevated concentrations of serum lipids (triglycerides, phospholipids, and cholesterol), are associated with elevated vitamin E levels, as reviewed elsewhere [1–3]. Because it is fat soluble, vitamin E absorption is aided by both fat in a meal and lipase activity present in the intestine. Once in the blood, vitamin E is transported in plasma lipoproteins and distributed among the lipoproteins depending upon the subject’s lipoprotein pattern. For this reason, it had been thought that vitamin E was absorbed and distributed into lipoproteins in a passive manner. However, the description of patients who were vitamin E deficient with no obvious cause provided evidence that plasma vitamin E transport was not a passive process [4–6]. Studies in healthy subjects using deuterated tocopherols indicated that vitamin E was absorbed and secreted in chylomicrons, and that some portion of the vitamin E was distributed into circulating lipoproteins during chylomicron catabolism [2,7]. Subsequent to chylomicron clearance by the liver, the vitamin E is resecreted into the blood in VLDL.

The hypothesis that the liver was responsible for secreting $\alpha$-tocopherol in nascent lipoproteins has been confirmed by liver perfusion studies [8]. The specificity of the process is supported by studies carried out in patients with ataxia with vitamin E deficiency, who are now known to have a genetic defect in an $\alpha$-tocopherol transfer protein [4]. These patients had normal vitamin E absorption, but defective secretion of $\alpha$-tocopherol in

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VLDL [5,6]. The purpose of the current study was to determine how the secretion of α-tocopherol from the liver, or its clearance from the plasma, would be affected by diet-induced changes in lipoprotein metabolism in healthy subjects. Our objective here was to compare the content and kinetics of VLDL-triglyceride and vitamin E in humans in a diet with minimal fat content to understand how triglyceride and vitamin E metabolism may be co-regulated.

**METHODS**

**Study design**

Volunteers were recruited by advertisement and gave informed consent before enrolling in the study. Protocols were approved both by the University of California-Berkeley, and University of California-San Francisco Committees on Human Research. The present study was part of a larger project designed to investigate the effects of a low-fat diet on lipoprotein triglyceride (TG) production in healthy subjects; the specifics of the study design and the lipid kinetic data of the larger project have been presented separately [9]. Due to lack of sufficient sample volume, the plasma vitamin E data presented here represent that from 9 of the 11 subjects, while VLDL vitamin E data are from seven of the subjects. The TG metabolism data from the nine subjects included here are representative of that from the 11 subjects described in the previous publication. Briefly, all subjects were non-smoking, healthy males who consumed two isoenergetic, whole food diets: a control diet (35% fat, 49% carbohydrate, 16% protein) for 1 week, followed by a low-fat (15% fat, 69% carbohydrate, 16% protein) diet for 5 weeks (Fig. 1). Foods making up the control diet were chosen to be similar to those consumed by the subjects ad libitum during screening (e.g., stir-fry, casseroles, sandwiches, cereal, and some commercially available entrees), as determined by 3 d food records, food frequency questionnaires, and diet history interviews. The diets used in the present study were prepared by the staff of the Clinical Research Center at the University of California-San Francisco. As described in detail previously, subjects consumed the main meal in the research unit and took home the rest of the day’s food to be consumed on an outpatient basis [9]. The control diet contained 31 g/d of fiber, 278 mg/d cholesterol, and 16.7 mg/d α-tocopherol equivalents. The low-fat diet was designed to correspond to dietary patterns of free living
individuals by replacing dietary fat with carbohydrate, while limiting the consumption of monosaccharides and sucrose. It contained 46 g/d fiber, 149 mg/d cholesterol, and 8.5 mg/d α-tocopherol equivalents.

Administration of stable isotopes

At the end of each dietary phase the subjects were admitted to the Clinical Research Center for stable isotope studies (Fig. 1). A capsule containing 2R 4R 8R-α-(5,7,8-(C2H3)3)-tocopheryl acetate (dα-RRR-α-tocopheryl acetate) was administered with the evening meal prior to the study. The composition of the meal reflected the feeding phase; that is, a 35%-fat meal was fed the night before isotope study #1 and a 15%-fat meal was fed the night before isotope study #2. At midnight, an infusion was begun which contained [5,5,5-2H3]-leucine; then at 4 AM, a second infusion of [1,2,3,4-13C4]-palmitate was started. The leucine label was used to track VLDL apolipoprotein B (apoB) synthesis, while palmitate was used to label VLDL-TG production and clearance. Subjects remained fasted and rested, watched TV, or read until the end of the isotope study (5 PM on day 2). Non-energy containing, non-caffeinated drinks (e.g., herbal tea, diet soda) were available upon request. All blood was drawn into vacutainers chilled to 4°C containing one mg/ml EDTA. A cocktail was added to plasma, which brought each ml of plasma to 0.03% w/v benzamidine (to prevent scission of apoB); 0.02% Na azide (preservative), and 10 μM trolox (antioxidant). Trolox was kept at this concentration in all salt solutions used throughout lipoprotein isolation. The particulars of plasma and lipoprotein isolation and lipid analysis have been described in detail previously [9].

VLDL isolation and subfractionation

Within 24 h of each infusion study, plasma samples were subjected to ultracentrifugation at 35,000 rpm for 25 min in a 50.3 rotor at 15°C (1.6 × 109 g) to separate chylomicrons [10], then VLDL (d < 1.006 g/ml) were isolated by ultracentrifugation for 20 h in a 50.3 Beckman rotor (1.3 × 108 g at 12°C). Subsequently, the total VLDL fraction was subjected to an additional ultracentrifugation step in order to isolate large, buoyant VLDL particles [11]. This strategy was employed to isolate VLDL that most closely represented particles newly secreted by the liver [12]. VLDL-apoB content was measured by maximal radial immunodiffusion.

Isolation of metabolites and gas chromatography/mass spectrometric analysis

3H9-RRR-α-tocopherol acetate was a gift from Dr. James Clark (Cognis Nutrition, Lagrange, IL, USA); 3H13-RRR-α-tocopherol, for use as an internal standard, was the generous gift of Dr. Graham Burton of the National Research Council of Canada. Other stable isotope-labeled substrates were purchased from Cambridge Isotope Laboratory (Andover, MA, USA) and/or Isotec (Miamisburg, OH, USA). The measurement of palmitate and leucine enrichment by gas chromatography/mass spectrometry (GC/MS) using standard curves has been described in detail previously [9]. The percentage of dα-α-tocopherol, as well as the absolute concentrations of dα- and dγ-α-tocopherols in plasma and buoyant VLDL were determined using a method of Burton and Daroszewska [13] with some modifications. An internal standard (2R 4R 8R-α-(5,7,8-(C2H3)3)-α-tocopherol (dα-RRR-α-tocopherol) was added to a VLDL sample (250 μl density solution containing VLDL from an original 6 ml plasma volume) from each timepoint and the total volume brought up to 1 ml with water. The sample was mixed before and after the subsequent addition of 100% ethanol (1 ml) and heptane (1 ml). Following centrifugation at 1500 rpm for 5 min, the organic layer was transferred to a vial and dried under nitrogen.

Alpha-tocopherols were converted to trimethylsilyl ethers by the addition of 100 μl pyridine and 50 μl BSTFA and heat (65°C for 20 min). The dα, dγ, and dδ enrichments were assessed by GC/MS (HP-5971 instrumentation, Hewlett-Packard, Palo Alto, CA, USA) using a 15m × 0.25 DB-17HT column (J&W Scientific, Folsom, CA, USA) and helium as a carrier gas at a flow rate of 1 ml/min. The temperature program was as follows: initial temperature was 85°C, then increasing at a rate of 40°C/min to 310°C, then an increase of 3°C/min to 317°C, which was held for 8 min. The injector temperature was 300°C and the detector 300°C.

Calculations

Measurement of biosynthetic rates of VLDL apoB and TG were according to the precursor-product relationship [14]. VLDL-TG synthetic rate was calculated from the incorporation of [1,2,3,4-13C4]-palmitate into TG as described [15]. The absolute concentrations of unlabeled (dα-) and labeled (dα-) α-tocopherols in plasma and VLDL were calculated using two standard curves. A dα/dδ-α-tocopherol standard curve was used to calculate the percentage of dα-α-tocopherol to total (dα + δdδ) α-tocopherol, and a dα/dγ-α-tocopherol standard curve was used to calculate the concentrations of dα- and dγ-α-tocopherol in the samples by comparison to a known amount of dδ internal standard. Changes in the composition of VLDL particles were measured as follows.

Since each VLDL is known to contain one apoB (molecular weight of apoB taken as 530,000 daltons), the concentration of this apolipoprotein in the VLDL frac-
tion can be converted to the number of molecules present and used to determine particle number. Similarly, the molecular weights of TG (885 daltons) and α-tocopherol (430 daltons) were used to calculate the number of moles of these constituents in the VLDL fraction. These numbers were then divided by the moles of apoB in that fraction to determine the average number of molecules of TG and α-tocopherol per VLDL particle (mol/mol apoB).

The kinetic data were fit to the equation $y = A_x(1 - e^{-k(t-c)})$, where $y = VLDL$-α-tocopherol enrichment, $A_x = \text{the plateau or asymptote value of } \alpha$-tocopherol enrichment, $t = \text{time in hours}$, and $c = \text{lag period}$ before isotope incorporation into secreted VLDL, and $k_x$ is the fractional replacement rate constant for VLDL-α-tocopherol. The transport rate of VLDL-α-tocopherol was then determined as follows: $VLDL-\alpha$-tocopherol transport rate $(\text{nmol} \cdot \text{kg} \cdot \text{FFM}^{-1} \cdot \text{h}^{-1}) = VLDL-\alpha$-tocopherol concentration $(\text{nmol/ml}) \times \text{Plasma volume (ml)} \times k_x (\text{h}^{-1})/\text{FFM (kg)}$. The clearance rate of a substance is equivalent to the amount of plasma completely cleared of the substance per min and reflects its efficiency of removal [16]. Steady-state clearance was calculated as the ratio of the substance’s transport or turnover rate to its concentration in plasma or VLDL. For example, to calculate the clearance rate of VLDL α-tocopherol (ml/min), the transport rate (nmol/min) was divided by the VLDL concentration (nmol/ml).

**Statistics**

Data are presented as mean ± SEM, unless otherwise noted. Statistical analyses were performed using SPSS statistical software [17]. Variables whose values were not normally distributed (e.g., plasma TG concentration) were log transformed before statistical analysis. A $p$ value of < 0.05 was considered statistically significant.

**RESULTS**

**Research subjects and study diets**

Nine healthy, nonsmoking males participated in the study. The average (± SEM) age of the subjects was 36 ± 3 years, body weight was 79 ± 11 kg, body fat: 20.8 ± 1.6%, and body mass index: 24.7 ± 1.1 kg/m². All subjects tolerated the diets well and body weights remained constant throughout the study period. The study diets differed in their percentage of energy from fat, as well as other characteristics [9]. The dietary content of vitamin E in the control diet (16.7 ± 4.9 mg α-tocopherol equivalents/d) was significantly higher than that in the low-fat diet (8.5 ± 1.4 mg α-tocopherol equivalents/d, $p < 0.04$). The foods that provided that bulk of vitamin E in both diets were prepared food items such as frozen lasagna, bran cereals, etc.

**Plasma metabolite concentrations**

Plasma lipid responses to dietary fat reduction are shown in Table 1. All subjects experienced significant elevations in fasting TG concentrations on the low-fat diet. Although no differences in the concentrations of total cholesterol, apoB, or apoA1 were observed, the distribution of cholesterol in plasma lipoproteins
changed. Significant elevations in VLDLc and reductions in LDLc were observed, as well as a trend toward lower HDLc.

Concentrations and enrichments of total plasma α-tocopherol were measured at various time points during the stable isotope study and are reported for the steady-state period (between 10 AM and 5 PM on day 2). No differences were observed between the diets in plasma concentration of d0-, d6-α-tocopherol, or the total α-tocopherol (d0 + d6) concentrations (Table 1). Following both diets, the plasma total α-tocopherol concentrations were within normal ranges (25.8 and 26.4 nmol/ml, for the control and low-fat diets, respectively). However, at the end of the low-fat regimen, the ratio of α-tocopherol to total plasma cholesterol and TG was significantly reduced by 16% (p = 0.05), which reflected the elevation in total plasma TG concentration induced by the low-fat diet.

The fraction of the labeled vitamin E dose in the plasma at steady state can provide evidence for variations in delivery of the labeled vitamin E to the plasma. To calculate the percentage of the 50 mg dose of d6-α-tocopherol (taken at 5 PM on day 1) in the plasma at steady state on day 2, the d6-α-tocopherol concentration (nmol/ml plasma) was multiplied by the estimated total plasma volume [18], then converted to mg and divided by the dose. This calculation revealed that 1 d after taking the capsule similar amounts of the original dose (14.8 ± 1.5% and 15.1 ± 1.6%) were present in the subjects’ plasma after the control and low-fat diets.

**VLDL composition**

The concentrations of apoB, TG, and α-tocopherol were measured in the buoyant VLDL fraction (Table 2). Following low-fat feeding, this fraction contained significantly more apoB (62%) and tended to have more TG (69%), indicating that the increase in plasma TG observed on the low-fat diet resulted primarily from more apoB-containing lipoprotein particles in the VLDL fraction. An increase in the concentrations of d6-α-tocopherol (by 50%, p = 0.01) and total α-tocopherol (by 43%, p = 0.04) were found at steady state in this fraction.
following the low-fat diet. To determine their average concentration per VLDL particle, the data were expressed on a molar basis with respect to VLDL-apoB. No differences between the diets were observed in the average amount of TG or α-tocopherol (labeled or unlabeled) per particle. Further, the average d₆-α-tocopherol enrichment within the buoyant VLDL fraction (d₆/sum d₆ + d₀ in VLDL) did not vary (21.7 ± 0.4% and 22.6 ± 0.4% for the control and low-fat diets, respectively, p = 0.12).

Although the average amount of TG or α-tocopherol per VLDL particle was unchanged for the group as a whole, neither the transport rate, the fractional turnover-rate (kₛ), nor the clearance of plasma d₆-α-tocopherol changed on the low-fat diet (Table 3). By contrast, the kinetics of d₀-α-tocopherol in the large VLDL fraction

Table 3. Kinetics of Plasma α-tocopherol and of Components of VLDL: ApoB, Triglyceride, and α-Tocopherol

<table>
<thead>
<tr>
<th></th>
<th>Control diet</th>
<th>Low-fat diet</th>
<th>p-Value</th>
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<tbody>
<tr>
<td><strong>Plasma d₆-α-tocopherol</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Transport rate (nmol/kg FFM⁻¹h⁻¹)</td>
<td>39.1 ± 4.2</td>
<td>35.4 ± 5.4</td>
<td>0.25</td>
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<tr>
<td>kₛ (h⁻¹)</td>
<td>0.16 ± 0.01</td>
<td>0.14 ± 0.01</td>
<td>0.18</td>
</tr>
<tr>
<td>Clearance rate (ml/min)</td>
<td>209 ± 52</td>
<td>201 ± 57</td>
<td>0.45</td>
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<tr>
<td><strong>VLDL-d₆-α-tocopherol</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Transport rate (nmol/kg FFM⁻¹h⁻¹)</td>
<td>13.5 ± 3.9</td>
<td>9.8 ± 1.9</td>
<td>0.38</td>
</tr>
<tr>
<td>kₛ (h⁻¹)</td>
<td>0.19 ± 0.02</td>
<td>0.13 ± 0.02</td>
<td>0.05</td>
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<tr>
<td>Clearance rate (ml/min)</td>
<td>11.3 ± 1.0</td>
<td>6.9 ± 0.9</td>
<td>0.02</td>
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<tr>
<td><strong>VLDL-apoB</strong></td>
<td></td>
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<tr>
<td>Transport rate (nmol/kg TBW⁻¹h⁻¹)</td>
<td>0.16 ± 0.04</td>
<td>0.20 ± 0.05</td>
<td>0.66</td>
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<tr>
<td>kₛ (h⁻¹)</td>
<td>0.26 ± 0.04</td>
<td>0.20 ± 0.02</td>
<td>0.35</td>
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<tr>
<td>Clearance rate (ml/min)</td>
<td>16.1 ± 2.1</td>
<td>11.9 ± 1.3</td>
<td>0.34</td>
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<tr>
<td><strong>VLDL-triglyceride</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Transport rate (μmol/kg FFM⁻¹h⁻¹)</td>
<td>18.9 ± 6.7</td>
<td>19.7 ± 7.0</td>
<td>0.69</td>
</tr>
<tr>
<td>kₛ (h⁻¹)</td>
<td>0.41 ± 0.03</td>
<td>0.28 ± 0.05</td>
<td>0.04</td>
</tr>
<tr>
<td>Clearance rate (ml/min)</td>
<td>23 ± 3</td>
<td>15 ± 3</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Data are mean ± SEM. Abbreviations used; FFM = fat-free mass; TBW = total body weight; kₛ = fractional replacement rate.

α Data signify measurement made on the large VLDL fraction.

β Measurements made on the total VLDL fraction.

Plasma and lipoprotein vitamin E and VLDL kinetics

The kinetics of plasma and large VLDL α-tocopherol were assessed by following the changes in enrichment of d₀-α-tocopherol from 4 AM to 5 PM on day 2 of the study. The incorporation curves for d₀-α-tocopherol into the total plasma vitamin E pool were not different between the diets (Table 3), as exemplified by representative data from one subject (Fig. 3A). For the group as a whole, neither the transport rate, the fractional turnover-rate (kₛ), nor the clearance of plasma d₆-α-tocopherol changed on the low-fat diet (Table 3). By contrast, the kinetics of d₀-α-tocopherol in the large VLDL fraction

Fig. 3. Incorporation of labeled vitamin E (d₆) into the total plasma vitamin E pool and into large VLDL vitamin E.
revealed that the transport rate tended to be reduced (but not significantly so), while the fractional turnover rate was significantly reduced 32% (from 0.19 ± 0.02 to 0.13 ± 0.02 pools/h, p = 0.05), and the clearance rate slowed 39%, from 11.0 ± 1.3 to 6.9 ± 0.9 ml/min (p = 0.02, Table 3 and Fig. 3B for a representative subject).

No changes were observed in the production, turnover, or clearance rate of new VLDL particles, as calculated by the buoyant VLDL-apoB kinetics (Table 3). Neither did total VLDL-TG transport rate change on the low-fat diet. Similar to the results for vitamin E, the fractional replacement rate of TG was significantly reduced by 32% (p = 0.04) and the TG clearance rate was also significantly lower following the low-fat diet (by 35%, p = 0.04).

LDL and HDL fractions were isolated in addition to the VLDL fraction for one subject (Fig. 4). The incorporation of d$_{4}$-α-tocopherol into LDL and HDL lags behind that of VLDL, consistent with a precursor-product relationship.

**DISCUSSION**

The very low-fat, high-carbohydrate diet used in the present study significantly increased VLDL particle number, VLDL-TG concentration, and the proportion of the plasma vitamin E found in the VLDL fraction. The metabolic basis for the carbohydrate-induced elevation in VLDL-TG was reduced TG clearance, and VLDL α-tocopherol clearance was also significantly reduced in parallel. Among the subjects, changes in the quantity of both TG and α-tocopherol per VLDL particle were significantly associated. These findings have several implications for vitamin E transport in humans.

On a per particle basis, no changes were observed in the mean composition of particles in the buoyant VLDL fraction in response to diet (lower portion, Table 2). Thus, the average number of TG molecules per VLDL particle and the number of labeled or unlabeled α-tocopherol molecules per VLDL (~65 molecules of α-tocopherol per particle) remained constant; there were simply more particles in this fraction. The total number of molecules of α-tocopherol per VLDL is of interest given the lipoprotein’s role in vitamin E transport. Although these data are known for LDL (8–12 molecules of vitamin E per LDL apoB), the quantity within VLDL has not been reported previously. This larger number of molecules of vitamin E per VLDL particle compared to LDL may result from the greater volume of the VLDL particle. It should be noted that the VLDL particles under study here were obtained using an ultracentrifugation procedure that isolated the largest, most buoyant particles within the VLDL fraction. This strategy optimized our ability to follow the kinetics of particles that most closely represented VLDL newly secreted by the liver. These particles turn over more quickly than smaller particles that can be found within the whole VLDL fraction [11]. Thus, the average amount of vitamin E per particle in the total VLDL fraction may be slightly less than observed here.

The present study is the first report to compare TG and α-tocopherol kinetics in humans. As a result of low-fat feeding, a greater percentage of the total plasma vitamin E was found in the buoyant VLDL fraction. Biologic mechanisms for this observation include: (i) increased hepatic secretion of vitamin E in nascent VLDL, (ii) decreased VLDL clearance and retention of vitamin E, and/or (iii) an increased vitamin E transfer from plasma lipoproteins into circulating VLDL. If the first hypothesis were correct the total content of vitamin E in the plasma should have increased with the increasing TG content. This was not observed. In fact, the total plasma concentration of labeled and unlabeled vitamin E was the same on both diets and yet we found a 43% increase in the vitamin E content of buoyant VLDL (Table 1). This redistribution of α-tocopherol among the plasma lipoproteins can be explained by changes in VLDL kinetics.

The present data, and that from both in vitro and in vivo studies, support the second mechanism above, and highlight the strong role TG clearance has on transfer of vitamin E from lipoproteins into membranes. Lipoprotein lipase-mediated TG clearance has been shown to be required for the transfer of vitamin E from a lipid emulsion (Intralipid) into fibroblasts in culture [19]. In transgenic animals an increase in muscle-specific lipoprotein lipase activity, due to overexpression, has been shown to result in increased α-tocopherol concentrations in skeletal muscle cells [20]. Lastly, a patient with a lipoprotein lipase deficiency, studied with labeled tocopherols had the majority of her plasma tocopherol in TG-rich lipoproteins [6]. In the present study, an impairment of TG
lipolysis was documented by significantly slower TG fractional turnover rates and clearance rates, accompanied by a parallel reduction in VLDL-\(d_{60}\)-\(\alpha\)-tocopherol fractional turnover rate and clearance. An impairment of lipoprotein lipase activity, reducing transfer of \(\alpha\)-tocopherol out of the VLDL particle, may serve as a compensatory mechanism. The resulting relocation of vitamin E (similar to the redistribution of cholesterol and TG out of LDL and HDL and into VLDL) may provide benefit to increase the antioxidant protection for the increased lipid in VLDL. These changes were not enough, however, to maintain the vitamin E to lipid ratio in the total plasma (Table 1).

The increased vitamin E found in the VLDL fraction could have resulted from an increased transfer of vitamin E into VLDL from other lipoproteins (the third mechanism above). This mechanism is supported by in vitro studies documenting movement of vitamin E from HDL into apoB-containing lipoproteins [21], but not by our analysis of the labeled vitamin E enrichment in all lipoprotein fractions from one subject, which revealed an earlier incorporation into VLDL than into LDL and HDL (Fig. 4). Furthermore, previous labeling studies in humans have shown that most, but not all, vitamin E secreted from the intestine in a chylomicron remains with that particle until the chylomicron is cleared to the liver, and our results indicate that vitamin E is resecreted with VLDL and then moves from this lipoprotein into LDL and HDL. Indeed, this scenario is also supported by the observation in the present study that the enrichment of total plasma vitamin E began to fall when that in the buoyant VLDL fraction was still rising (data not shown). Note that patients with a genetic form of vitamin E deficiency, who lack the liver \(\alpha\)-tocopherol transfer protein and have impaired secretion of VLDL vitamin E, also have normal plasma vitamin E concentrations and lipoprotein distribution when they are supplemented with 1000 mg vitamin E per day. Such data suggest that direct vitamin E transfer from chylomicrons to all circulating lipoproteins can be enhanced by large increases in vitamin E intake [22].

When our subjects were switched to a very low-fat diet containing significantly less vitamin E (16.7 and 8.5 mg/d \(\alpha\)-tocopherol equivalents on the higher-fat and low-fat diet, respectively), total plasma vitamin E concentrations were not decreased. Similarly, Sarkkinen et al. [23] and Velthuis-te Wierik et al. [24] fed low-fat diets containing significantly less vitamin E and reported unchanged blood vitamin E concentrations. In contrast, Swinburn et al. reported constant dietary vitamin E intakes when 110 adults were switched from a 35% fat to a 26% fat diet; no reduction in serum vitamin E levels were observed [25]. In seminal studies of vitamin E’s antioxidant function, dietary fatty acid composition was shown to effect the course of vitamin E deficiency [26]. Polyunsaturated fatty acids exacerbated deficiency symptoms. However, the potential for dietary polyunsaturates to impact antioxidant needs, or reduce blood vitamin E concentrations may only hold when dietary fat provides a significant proportion of energy.

With regard to calculating dietary vitamin E content, some caution should be used when interpreting these data as determined by nutrient analysis software because its content is missing from some foods in these databases. Such foods used in the present study were analyzed directly and the highest sources of vitamin E in the low-fat diet were the commercially prepared foods. Even though the low-fat diet was calculated to have significantly less vitamin E in it, similar concentrations of labeled vitamin E plasma concentrations support comparable absorption kinetics of \(d_{60}\)-\(\alpha\)-tocopherol when the high- or low-fat meals were administered preceding each metabolic study. Clearly, a 15% compared with a 35% fat meal provides sufficient fat in the intestine and/or stimulus for bile acid secretion to support similar vitamin E absorption.

In summary, compared to a high-fat diet, the chronic consumption of a very low-fat, high-carbohydrate diet increased plasma TG without changing plasma vitamin E concentrations, but did alter the distribution of vitamin E among plasma lipoproteins resulting in an increase in the percentage of plasma vitamin E carried in large VLDL. Both the content and kinetics of VLDL-vitamin E correlated with the TG in that fraction. These findings suggest that vitamin E transport from the liver via VLDL and out of VLDL during lipolysis are controlled by the metabolism of TG. The potential significance of the co-secretion of vitamin E and TG, with respect to VLDL assembly, should be investigated further.

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