Transport of deuterium-labeled tocopherols during pregnancy

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**ABSTRACT**  With use of deuterium-labeled isotopes of RRR- and all-rac-α-tocopheryl acetate, the transport of vitamin E in pregnancy was evaluated to determine whether the placenta discriminates between these compounds. Fifteen pregnant subjects were recruited 5 d before delivery to receive 15, 30, 75, 150, or 300 mg vitamin E/d in capsules containing 3R-RRR-α-tocopheryl acetate and 6R-all-rac-α-tocopheryl acetate (1:1, by wt). Maternal blood was obtained before dosing, at hospital admission, and at parturition. Cord blood samples were obtained at parturition. Deuterium-labeled and unlabeled tocopherol contents were determined by gas chromatography–mass spectrometry in plasma and lipoproteins (chylomicrons, VLDL, LDL, and HDL). Maternal plasma and lipoproteins obtained at delivery had higher concentrations of 3R-RRR-α-tocopherol than 6R-all-rac-α-tocopherol regardless of the vitamin E dose administered (P < 0.05). Cord plasma at delivery also had higher concentrations of 3R-RRR-α-tocopherol than 6R-all-rac-α-tocopherol in plasma irrespective of the dose administered (P < 0.05). In lipoproteins isolated from cord blood, tocopherol concentrations were greatest in the HDL fraction (P < 0.05), whereas in maternal blood they were greatest in the LDL fraction (P < 0.05). We conclude that the placental-fetal unit, the fetal liver, or both further discriminate between RRR- and all-rac-α-tocopherol.

**KEY WORDS** Pregnancy, vitamin E, bioavailability, tocopheryl acetates, stable isotopes, isomers, humans, women, α-tocopherol

**INTRODUCTION**

Before this report, the assessment of fetal vitamin E status relied on the relation between tocopherol concentrations in cord and maternal blood, with an inability to document actual transport of supplemented tocopherols consumed by the mother. Plasma vitamin E concentrations in newborn infants are low compared with those in older infants or adults (1, 2). Very-low-birth-weight, low-birth-weight, and premature infants are often vitamin E deficient (3). This finding has been associated with an increased susceptibility to pulmonary oxygen toxicity (4) and a deficient antioxidant status in premature infants and their mothers (5, 6).

As an antioxidant, vitamin E may have potential benefits in counteracting hemolysis (7), retrolental fibroplasia (8), bronchopulmonary dysplasia (9, 10), and periventricular hemorrhage (11) in premature infants. It is in this patient population that supplemental vitamin E has been suggested to lessen the consequences of oxidative stress (12, 13).

In the United States, prenatal vitamins are commonly prescribed during pregnancy (14, 15). These preparations contain predominantly all-rac-α-tocopheryl acetate (16). The difference in bioavailability of all-rac-α-tocopheryl acetate and RRR-α-tocopheryl acetate in normal healthy males and females (15) has been an ongoing interest of our group and others (17–21).

The placenta is intimately involved in the transport of nutrients and other compounds from mother to fetus. We investigated the transport of deuterium-labeled tocopherols after administration of these compounds before delivery to evaluate whether the placenta further discriminates between natural (RRR-) and synthetic (all-rac-) tocopherols.

**SUBJECTS AND METHODS**

**Subjects**

Fifteen pregnant women were recruited for the study. The subjects were in their last trimester of pregnancy and were within 5–9 d of delivery. Subjects were healthy with no history of alcohol or tobacco use and were between 25 and 35 y of age. Baseline blood cholesterol concentrations were 6.55 ± 0.23 mmol/L and baseline triacylglycerol concentrations were 2.43 ± 0.13 mmol/L (X ± SE). All subjects had been prescribed prenatal vitamins containing 30 mg (30 IU) all-rac-α-tocopheryl acetate for the duration of their pregnancy. No other medications were prescribed. Prior review and approval of this protocol were obtained from the Institutional Review Board of East Tennessee State University and all subjects signed an informed consent form.

**Protocol**

Deuterated forms of vitamin E were synthesized by Eastman Chemical Company (Kingsport, TN) and were obtained through distribution from the Natural Source Vitamin E Association (Washington, DC). Baseline blood samples (20 mL) were obtained from each subject 5–9 d before delivery. Subjects were...
given soft gelatin capsules containing 15, 30, 75, 150, or 300 mg of a 1:1 (by wt) mixture of \textit{d}3-\textit{RRR}-\textit{a}-tocopheryl acetate and \textit{d}6-\textit{all-rac-\textit{a}-tocopheryl acetate daily (Figure 1). Assignment of subjects into each dose group \((n = 3)\) was performed randomly. Each group was instructed to consume one capsule daily with breakfast until they reported to the hospital for delivery. All subjects received deuterium-labeled tocopherols for \(\geq 5\) d before delivery; two subjects were supplemented for 9 d.

### Methods

Maternal blood samples (20 mL) were obtained during the 5–9-d dosing period (8–12 h after the last deuterium-labeled vitamin E dose) as well as with cord blood samples at parturition. Samples were collected in evacuated tubes containing EDTA and E dose) as well as with cord blood samples at parturition. Samples were collected in evacuated tubes containing EDTA and immediately separated by centrifugation (504 \(\times\) g for 10 min at 4 \(^\circ\)C). Cord blood was obtained after parturition by clamping the cord proximal and distal to the infant, cutting the cord proximally, and draining the cord blood into EDTA-containing tubes. For extracting tocopherol, plasma (0.1 mL) and lipoproteins isolated from 1.0 mL plasma were pipetted into screw-top glass vials (28 \(\times\) 61 mm), followed by the addition of 0.01 mL \(\text{d}9\)-\textit{all-rac-\textit{a}-tocopherol} (100 ng) in ethanol as an internal standard. Doubly distilled water (0.9 mL) and 2.0 mL ethanolic ascorbic acid (1%) were added to the vials, and the contents were mixed by vortexing the sample. After the addition of 0.3 mL of 11.9 mol KOH/L, the samples were mixed and placed in a 70 \(^\circ\)C water bath for 30 min to achieve saponification. The vials were then allowed to cool to room temperature and 1 mL of the 1% ascorbic acid solution was added to each vial, followed by mixing. HPLC grade \(\eta\)-heptane (4 mL) was added to each vial and after they were mixed by vortexing the vials were allowed to stand until the phases separated. The upper heptane layer (3 mL) was removed and evaporated to dryness with a clean stream of nitrogen at 60 \(^\circ\)C.

Trimethylsilyl ethers were prepared by adding 0.05 mL pyridine and 0.025 mL \(\text{N,O-bis-(trimethylsilyl)trifluoroacetamide}^\dagger\) containing 1% trimethylchlorosilane (Pierce, Rockford, IL), followed by heating for 15 min at 65 \(^\circ\)C (22). After they were cooled to room temperature, the samples were transferred to crimp-top glass vials (12 \(\times\) 32 mm) containing 0.1-mL glass inserts. The relative proportions of \(\text{d}0\)- (unlabeled), \(\text{d}3\)-, \(\text{d}6\)-, and \(\text{d}9\)-\textit{a}-tocopherols were determined by gas chromatography–mass spectrometry. A 1-\(\mu\)L sample was injected with a split ratio of 20:1 onto a fused-silica capillary column (12 m \(\times\) 0.2 mm \(\times\) 0.33 \(\mu\)m) with a polymethoxysilane-bonded liquid phase (Ultra 1; Hewlett-Packard, Palo Alto, CA). A Hewlett-Packard 5890 Series II gas chromatograph was programmed with an initial oven temperature of 40 \(^\circ\)C for 1 min, followed by a temperature increase of 50 \(^\circ\)C/min until a final temperature of 285 \(^\circ\)C was reached, which was maintained for 7 min. The gas chromatograph was connected to a Hewlett-Packard 5970B mass selective detector. The mass selective detector was programmed to continuously monitor \(m/z\) 502 (\(d\)0), 505 (\(d\)3), 508 (\(d\)6), and 511 (\(d\)9) ions for \(\alpha\)-tocopherol.

Plasma lipoproteins were isolated by using a modification of the method of Havel et al (23) with an ultracentrifuge (model L8–80M; Beckman Instruments, Inc, Palo Alto, CA). One milliliter of plasma was divided into G-Max Quick-Set polycarbonate tubes (16 \(\times\) 38 mm; Beckman Instruments, Inc) filled with potassium bromide solution (density: 1006 g/L). The tubes were sealed and centrifuged in an L8–80M ultracentrifuge at 14 \(^\circ\)C for 28 min at 59000 \(\times\) g with an SW41 Ti Rotor (Beckman Instruments, Inc). Chylomicrons were collected by slicing the tube with a tube slicer (Beckman Instruments, Inc). The bottom portion of the remaining isolate was placed in another tube, which was filled with potassium bromide solution (density: 1006 g/L), sealed, and centrifuged at 14 \(^\circ\)C for 100 min at 602000 \(\times\) g with an 80 Ti Rotor (Beckman Instruments, Inc). The VLDL fraction was collected by slicing the tube. Again, the bottom fraction remaining in the tube was adjusted to a density of 1063 g/L by adding 0.3 g solid potassium bromide. The tube was filled with potassium bromide solution (density: 1063 g/L), sealed, and centrifuged at 14 \(^\circ\)C for 110 min at 602000 \(\times\) g with an 80 Ti Rotor to separate the LDL (upper) and HDL (lower) layers. These two fractions were recovered by slicing the tube with a tube slicer after centrifugation. After separation of chylomicrons, VLDL, LDL, and HDL, these fractions were extracted and evaluated for \(\alpha\)-tocopherol content in the same manner as described for plasma samples.

Lipoproteins were identified by electrophoretic separation in a buffered agarose system (Ciba Corning Diagnostics Corp, Alameda, CA). After electrophoresis, the lipoproteins were detected with lipoprotein stain (Fat Red FB Stain; Sigma Chemical Co, St Louis). Visual comparisons were made by using a lipoprotein control sample for reference lipoprotein location.

### Statistics

Plasma and lipoprotein \(\alpha\)-tocopherol concentrations were subject to analysis of variance (ANOVA) along with Tukey’s stu-
RESULTS

Maternal deuterium-labeled vitamin E concentrations in plasma (Table 1) after the administration of d3-RRR- and d6-all-rac-α-tocopherol acetate at various doses indicated a greater concentration of the RRR-form at delivery irrespective of the dose administered (P < 0.05). The amount of d3-RRR-α-tocopherol in maternal plasma at delivery was not significantly different when the 15-, 30-, and 75-mg doses were compared; however, a difference did exist when the three lower doses (15, 30, and 75 mg) were compared with the two higher (150 and 300 mg) doses (P < 0.05). Although there were no differences in maternal d6-all-rac-α-tocopherol concentrations when the 15- and 30-mg doses or the 150- and 300-mg doses were compared, there was a difference when the 75-mg dose and the low doses were compared, as well as when the 75-mg dose and the higher doses were compared (P < 0.05).

Cord blood collected at delivery from mothers receiving various doses of deuterated vitamin E during the last 5–9 d of pregnancy showed higher concentrations of d3-RRR- than d6-all-rac-α-tocopherol across all doses (P < 0.05). Deuterium-labeled d3-RRR-α-tocopherol increased in cord blood as the dose increased, but was not different when the 15-, 30-, and 75-mg doses were compared. The d3-RRR-α-tocopherol content of cord blood evaluated after the higher doses (150 and 300 mg) was different from that evaluated after the lowest administered dose (15 mg) (P < 0.05). d6-all-rac-α-Tocopherol concentrations were not different in cord blood at any dose.

The ratio of d3-RRR-α-tocopherol to d6-all-rac-α-tocopherol (d3:d6) in maternal blood (Figure 2) varied from 1.77 to 2.02 with an average ratio of 1.86 (±0.10) in maternal blood and 3.42 (±0.03) in cord blood. When d3:d6 was evaluated, the ratio in cord blood was greater than that in maternal blood (P < 0.05). This difference existed across all doses.

Concentrations of d3-RRR-α-tocopherol were greater than concentrations of d6-all-rac-α-tocopherol in each lipoprotein fraction (P < 0.05) from maternal (Figure 3) and cord (Figure 4). The LDL and HDL fractions obtained from maternal plasma contained the highest deuterium-labeled tocopherol concentrations, with the LDL fraction containing significantly more than the other lipoprotein fractions (P < 0.05). No chylomicrons were present in cord blood as determined by electrophoretic separation; the cord blood HDL fraction had a greater concentration of deuterium-labeled tocopherol than did the other lipoprotein fractions (P < 0.05).

DISCUSSION

The purpose of this study was to investigate whether the form of supplemental vitamin E (natural versus synthetic) administered during pregnancy affected the human placenta’s ability to deliver the vitamin to the fetus. We administered various biocompetitive doses of deuterium-labeled d3-RRR- and d6-all-rac-α-tocopherol acetate (1:1, by wt) in the third trimester of pregnancy during the last 5–9 d before delivery. The use of deuterium-labeled vitamin E made it possible to distinguish between the forms administered as supplements and transport of the supplemented dose to the fetal compartment (cord blood).

It was determined previously that the bioavailability of the natural compared with the synthetic form of vitamin E is in the order of 2 to 1, in favor of the RRR-form (17), which is different from the accepted ratio of 1.36 (24). This difference was determined in healthy males and nonpregnant females. Our justification for examining the possibility of further discrimination by the placenta is based on the fact that most prenatal vitamins contain all-rac-α-tocopherol acetate as the vitamin E source (14, 15). In addition, other investigators found that the human placenta is stereospecific for the uptake of certain nutrients (25, 26).

The differences observed in d3-RRR- and d6-all-rac-α-tocopherol concentrations in maternal and cord blood (Table 1) show that the placental-fetal unit, the fetal liver, or both discriminate beyond what the maternal organism already does during first-pass metabolism of the stereoisomers of vitamin E. Several investigators (27–29) suggested that a tocopherol-binding protein (31 kDa) is present in liver and is responsible for the differences observed (30) between RRR- and all-rac-α-tocopherol concentrations. Traber et al (31) showed that humans discriminate between the naturally occurring RRR- and the SRR-α-tocopherol form, pre-

### Table 1

Comparison of total, labeled, (d3 and d6) and unlabeled (d0) α-tocopherol in plasma from maternal blood before and in maternal and cord blood at delivery after supplementation with d3-RRR and d6-all-rac-α-tocopheryl acetate.

<table>
<thead>
<tr>
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<th>Maternal</th>
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<tr>
<td></td>
<td>d0</td>
<td>d3</td>
<td>d6</td>
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<td>µmol/L</td>
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<tr>
<td>(n = 12)</td>
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<td>0.00</td>
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<td>37.63 ± 1.90</td>
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<tr>
<td>Dose (n = 3) (mg)</td>
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<tr>
<td>15</td>
<td>29.44 ± 3.28</td>
<td>6.50 ± 0.72</td>
<td>3.39 ± 0.32</td>
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<tr>
<td>30</td>
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<td>3.16 ± 0.99</td>
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<td>75</td>
<td>23.74 ± 2.35</td>
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<td>5.02 ± 0.60</td>
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<tr>
<td>150</td>
<td>32.89 ± 5.64</td>
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<td>8.82 ± 0.29</td>
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</tr>
<tr>
<td>300</td>
<td>34.14 ± 0.95</td>
<td>16.02 ± 0.95</td>
<td>8.87 ± 0.28</td>
<td>59.03 ± 0.73</td>
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* x ± SEM. dT is the sum of d0 + d3 + d6 extracted from plasma. NA, no sample available. Means in a row or column with different superscript letters are significantly different, P < 0.05.
umbilical cord blood compared with maternal blood and indicated
that HDL appears to transport a greater proportion of cholesterol in
the HDL₃ subfraction. In addition, the HDL umbilical cord plasma
was particularly enriched in lipids, and LDL cholesterol was signifi-
cantly reduced in the fetoplacental circulation. Sufficient information
is not available to explain the role of HDL-mediated tissue uptake of
vitamin E. The preferential transport of 3-RRR-α-tocopherol by
HDL in cord blood may indicate that this lipoprotein serves an
important role for vitamin E transport in the fetus. Whether this is in
response to the delivery of tocopherol to nervous tissue (36) or other
fetal tissues (37, 38) remains to be elucidated.

The significant increase of 3-RRR-α-tocopherol as opposed

to $\delta$-$\text{all-rac-}$-$\alpha$-tocopherol in HDL, LDL, and VLDL derived from cord blood (Figure 2) further underscores the placenta’s preference for 2R isomers. The uptake of lipoproteins by the placenta is enhanced by trophoblastic secretion of apolipoprotein E (39). However, maternal lipoproteins are not transported to the fetal side of the placenta (40–42) and it seems unlikely that the placental unit synthesizes lipoproteins, whereas fetal liver is capable of lipoprotein synthesis (35, 43). In view of vitamin E transport and metabolism, we propose that vitamin E is delivered to the placenta and transported to the fetal side with the placental tocopherol-binding protein selectively shutting the 2R isomers to the umbilical vein for transport to the fetal liver, returning others (eg, 2S isomers) to the maternal circulation. Because $\delta$-$\text{all-rac-}$-$\alpha$-tocopherol concentrations in cord blood did not significantly increase as the dose administered to the mother increased, it is likely that the predominant isomers transported to the fetal side are of the 2R configuration. If 100% of the $d3$-$\text{RRR}$-isomer was transported in the maternal plasma along with the 2R ($\text{RRR}^+$ $\text{RRS}^-$ $\text{RSS}^+ + \text{SRS}^-$) isomers (50% of all-$\alpha$-tocopherol is 2R and the remaining 50% is 2S), then $d3$:$d6$ would approach 2:1, close to the ratio found in this investigation and that reported for males and nonpregnant females (17). By the same reasoning, the $d3$:$d6$ in cord blood, which transports only the $\text{RRR}$-isomer of all-$\alpha$-tocopherol, would yield a ratio of 8:1. Transport of the $\text{RRR}$-isomer plus an additional 2R isomer would produce a $d3$:$d6$ equal to 4:1, close to the 3.42:1 reported here. Transport of the $\text{RRR}$-isomer and two additional 2R isomers would yield a ratio of 2.67:1.

In conclusion, our results show that the human placental-fetal unit, the fetal liver, or both further discriminate between 2R and all-$\alpha$-tocopherol acetate. In addition, we speculate that the placental tocopherol-binding protein is more selective than the liver tocopherol-binding protein in transporting the 2R isomers of all-$\alpha$-tocopherol. These findings merit further investigation because the maternal-fetal unit has a preference for natural $\alpha$-tocopherol over synthetic $\alpha$-tocopherol.

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REFERENCES


