Transport of deuterium-labeled tocopherols during pregnancy\textsuperscript{1–3}

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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 d3-RRR-α-tocopheryl acetate and d6-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 d3-RRR-α-tocopherol than d6-all-rac-α-tocopherol regardless of the vitamin E dose administered ($P < 0.05$). Cord plasma at delivery also had higher concentrations of d3-RRR-α-tocopherol than d6-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.


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 d before 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.

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-tocopherol acetate (16). The difference in bioavailability of all-rac-α-tocopherol acetate and RRR-α-tocopherol 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.
given soft gelatin capsules containing 15, 30, 75, 150, or 300 mg of a 1:1 (by wt) mixture of \( \text{d}3-\text{RRR}-\alpha \)-tocopheryl acetate and \( \text{d}6-\text{all-rac}-\alpha \)-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 tocophers 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 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 immediately separated by centrifugation (504 \( \times g \) for 10 min at 4 °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 11.9 mol KOH/L, 504 \( \times g \) for 10 min at 4 °C. The vials were then allowed to cool 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 \( \text{d}0-\text{(unlabeled)}, \text{d}3-, \text{d}6-, \) and \( \text{d}9-\alpha \)-tocopherols were determined by gas chromatography–mass spectrometry. A 1-μL sample was injected with a split ratio of 20:1 onto a fused-silica capillary column (12 m \( \times \) 0.2 mm \( \times \) 33 μm) with a polydimethoxysilane-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 °C for 1 min, followed by a temperature increase of 50 °C/min until a final temperature of 285 °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 (\( \text{d}0 \)), 505 (\( \text{d}3 \)), 508 (\( \text{d}6 \)), and 511 (\( \text{d}9 \)) 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-Seal 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 °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 °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 °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 \( d_3 \)-RRR- and \( d_6 \)-all-rac-\( \alpha \)-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 \( d_3 \)-RRR-\( \alpha \)-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 \( d_6 \)-all-rac-\( \alpha \)-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 \( d_3 \)-RRR- than \( d_6 \)-all-rac-\( \alpha \)-tocopherol across all doses (\( P < 0.05 \)). Deuterium-labeled \( d_3 \)-RRR-\( \alpha \)-tocopherol increased in cord blood as the dose increased, but was not different when the 15-, 30-, and 75-mg doses were compared. The \( d_3 \)-RRR-\( \alpha \)-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) (\( P < 0.05 \)). \( d_6 \)-all-rac-\( \alpha \)-Tocopherol concentrations were not different in cord blood at any dose.

The ratio of \( d_3 \)-RRR-\( \alpha \)-tocopherol to \( d_6 \)-all-rac-\( \alpha \)-tocopherol (\( d_3/d_6 \)) 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 \( d_3/d_6 \) 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 \( d_3 \)-RRR-\( \alpha \)-tocopherol were greater than concentrations of \( d_6 \)-all-rac-\( \alpha \)-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 bio-competitive doses of deuterium-labeled \( d_3 \)-RRR- and \( d_6 \)-all-rac-\( \alpha \)-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-\( \alpha \)-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 \( d_3 \)-RRR- and \( d_6 \)-all-rac-\( \alpha \)-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-\( \alpha \)-tocopherol concentrations. Traber et al (31) showed that humans discriminate between the naturally occurring RRR- and the SRR-\( \alpha \)-tocopherol form,pre-

### Table 1

<table>
<thead>
<tr>
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<th>Maternal</th>
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<td><strong>Baseline</strong></td>
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<td><strong>Dose (n = 3) (mg)</strong></td>
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<td>15</td>
<td>29.44 ± 3.28</td>
<td>6.05 ± 0.72</td>
<td>3.39 ± 0.32</td>
<td>39.35 ± 2.86</td>
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<td>30</td>
<td>34.64 ± 1.96</td>
<td>6.39 ± 1.49</td>
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<td>75</td>
<td>23.74 ± 2.35</td>
<td>9.03 ± 1.35</td>
<td>5.02 ± 0.60</td>
<td>37.80 ± 2.45</td>
<td>6.31 ± 0.10</td>
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<tr>
<td>150</td>
<td>32.89 ± 5.64</td>
<td>15.60 ± 0.58</td>
<td>8.82 ± 0.29</td>
<td>57.32 ± 6.22</td>
<td>6.86 ± 0.33</td>
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<tr>
<td>300</td>
<td>34.14 ± 0.95</td>
<td>16.02 ± 0.93</td>
<td>8.87 ± 0.28</td>
<td>59.03 ± 0.73</td>
<td>7.42 ± 0.50</td>
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\( \bar{x} \) ± SEM. \( d_7 \) is the sum of \( d_3 + d_6 + d_7 \) 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
VLDL moiety. Neary et al (35) evaluated the lipoprotein content of
tocopherol-binding protein) and vitamin E is secreted as a part of the
taken up by the liver (preferentially repackaging the 2

catabolism of chylomicrons, after which the resulting remnants are
by the intestine (20). This event is followed by lipoprotein lipase
lipoproteins occurs first by secretion of vitamin E into chylomicrons
administration of
ferred into lipoproteins of subjects after a multidose
protein or proteins are part of the placental makeup, they may transport
other 2R isomers (eg, RRS-, RSR-, and SRR-) in addition to the
R- form. We base this speculation on the fact that the d3sd6 observed in cord compared with maternal blood (3.42 compared with 1.86) suggests that the placental tocopherol-binding protein may be more selective than the liver tocopherol-binding protein.

The elevation of maternal plasma tocopherol concentrations throughout gestation has been attributed to the elevation of \( \beta \)-lipoprotein concentrations (34). Indeed, our data (Figure 2) support the theory that LDL is the major carrier for maternal \( \alpha \)-tocopherol in the third trimester of pregnancy, as reflected in the vitamin E content of lipoproteins at parturition. The increased d3-RRR-\( \alpha \)-tocopherol content of maternal VLDL, LDL, and HDL can be explained by a previous study in which the investigators showed that the absorption and transport of deuterium-labeled RRR-\( \alpha \)-tocopherol in human lipoproteins occurs first by secretion of vitamin E into chylomicrons by the intestine (20). This event is followed by lipoprotein lipase catabolism of chylomicrons, after which the resulting remnants are taken up by the liver (preferentially repackaging the 2R isomers by tocopherol-binding protein) and vitamin E is secreted as a part of the VLDL moiety. Neary et al (35) evaluated the lipoprotein content of umbilical cord blood compared with maternal blood and indicated

that HDL appears to transport a greater proportion of cholesterol in the HDL\(_1\) subfraction. In addition, the HDL umbilical cord plasma was particularly enriched in lipids, and LDL cholesterol was significantly 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 d3-RRR-\( \alpha \)-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 d3-RRR-\( \alpha \)-tocopherol as opposed

FIGURE 2. Ratios of d3-RRR-\( \alpha \)-tocopherol to d6-all-rac-\( \alpha \)-tocopherol (d3:d6) in cord and maternal blood obtained from subjects and from cord blood samples at delivery after the administration of deuterium-labeled tocopherols (1:1, by wt) in various doses. Plasma deuterium-labeled d3-RRR- and d6-all-rac-\( \alpha \)-tocopherols were measured by gas chromatography–mass spectrometry as described in the Methods. Shown are the mean (± SEM) ratios (n = 3 for each dose) for cord and maternal plasma after each dose. Maternal d3:d6 for each dose remained constant. d3:d6 was significantly higher in cord blood than in maternal blood (P < 0.05).

FIGURE 3. Maternal blood lipoprotein deuterium-labeled vitamin E concentrations at delivery after doses of deuterated vitamin E. Lipoprotein concentrations of d3-RRR-\( \alpha \)-tocopherol were greater than concentrations of d6-all-rac-\( \alpha \)-tocopherol in each fraction (P < 0.05). There was significantly more deuterium-labeled tocopherol in the LDL fraction than in the other lipoprotein fractions (P < 0.05). CHYLO, chylomicron. \( \bar{x} \) ± SEM; n = 3.

FIGURE 4. Cord blood lipoprotein deuterium-labeled vitamin E concentrations at delivery after doses of deuterated vitamin E. Lipoprotein concentrations of d3-RRR-\( \alpha \)-tocopherol were greater than concentrations of d6-all-rac-\( \alpha \)-tocopherol in each fraction (P < 0.05). There was significantly more deuterium-labeled tocopherol in the HDL fraction than in the other lipoprotein fractions (P < 0.05). \( \bar{x} \) ± SEM; n = 3.
to \( \text{d6-all-rac-} \alpha \text{-tocopherol in HDL, LDL, and VLDL derived from cord blood (Figure 2)} \) further underscores the placenta’s preference for 2\( \alpha \) 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 shuttling the 2\( \alpha \) isomers to the umbilical vein for transport to the fetal liver, returning others (eg, 2\( \delta \) isomers) to the maternal circulation. Because \( \text{d6-all-rac-} \alpha \text{-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 2\( \alpha \) configuration. If 100% of the \( \delta3\text{-RRR} \)-isomer was transported in the maternal plasma along with the 2\( \alpha \) (\( \text{RRR} + \text{RKS} + \text{RSS} + \text{RSR} \)-) isomers (50% of \( \text{all-rac-} \alpha \text{-tocopherol is 2\( \alpha \) and the remaining 50% is 2\( \delta \)\)}, then \( \delta3: \delta6 \) 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 \( \delta3: \delta6 \) in cord blood, which transports only the \( \text{RRR}- \) isomer of \( \text{all-rac-} \alpha \text{-tocopherol}, \) would yield a ratio of 8:1. Transport of the \( \text{RRR-} \) isomer plus an additional 2\( \alpha \) isomer would produce a \( \delta3: \delta6 \) equal to 4:1, close to the 3.42:1 reported here. Transport of the \( \text{RRR-} \) isomer and two additional 2\( \alpha \) 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 \( \text{RRR-} \) and \( \text{all-rac-} \alpha \text{-tocopherol acetate. In addition, we speculate that the placental tocopherol-binding protein in transporting the 2\( \alpha \) isomers to the maternal circulation. Because} \) the maternal-fetal unit has a preference for natural \( \alpha \)-tocopherol over synthetic \( \alpha \)-tocopherol.

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