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Infant Rhesus Macaque Brain α-Tocopherol Stereoisomer Profile Is Differentially Impacted by the Source of α-Tocopherol in Infant Formula

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ABSTRACT

Background: α-Tocopherol (αT) in its natural form [2′*R*, 4′*R*, 8′*R* αT (*RRR*-αT)] is more bioactive than synthetic α-tocopherol (*all rac*-αT). *All rac*-αT is widely used in infant formulas, but its accretion in formula-fed infant brain is unknown.

Objective: We sought to compare αT and stereoisomer status in infant rhesus macaques (*Macaca mulatta*) fed infant formula *(RRR*-αT or *all rac*-αT) with a reference group fed a mixed diet of breast milk and maternal diet.

Methods: From 1 d after birth until 6 mo of age, infants ($n = 23$) were either nursery reared and exclusively fed 1 of 2 formulas by staff personnel or were community housed with their mothers and consumed a mixed reference diet of breast milk (69 mL/d at 6 mo) transitioning to monkey diet at ∼2 mo (MF; $n = 8$). Formulas contained either 21 μmol *RRR*-αT/L (NAT-F; $n = 8$) or 30 μmol *all rac*-αT/L (SYN-F; $n = 7$). Total αT and αT stereoisomers were analyzed in breast milk at 2, 4, and 6 mo and in monkey plasma and liver and 6 brain regions at 6 mo of age. α-Tocopherol transfer protein (α-TTP), lipoprotein αT, and urinary α-carboxyethyl-hydroxychroman (α-CEHC) were measured. One-way ANOVA with Tukey's post-hoc test was used for analysis.

Results: At study termination, plasma, liver, lipoprotein, and brain total αT did not differ between groups. However, the NAT-F–fed group had higher *RRR*-αT than the SYN-F–fed group ($P < 0.01$) and the MF group ($P < 0.0001$) in plasma (1.7- and 2.7-fold) and brain (1.5- and 2.5-fold). Synthetic αT 2*R* stereoisomers (SYNTH-2*R*) were generally 3- and 7-fold lower in brain regions of the NAT-F group compared with those of the SYN-F and MF groups ($P < 0.05$). SYNTH-2*R* stereoisomers were 2-fold higher in MF than SYN-F ($P < 0.0001$). The plasma percentage of SYNTH-2*R* was negatively correlated with the brain percentage of *RRR*-αT ($r = -0.99$, $P < 0.0001$). Brain αT profiles were not explained by α-TTP mRNA or protein expression. Urine α-CEHC was 3 times higher in the NAT-F than in the MF group ($P < 0.01$).

Conclusions: Consumption of infant formulas with natural (NAT-F) compared with synthetic (SYN-F) αT differentially impacted brain αT stereoisomer profiles in infant rhesus macaques. Future studies should assess the functional implications of αT stereoisomer profiles on brain health. *J Nutr* 2020;150:2305–2313.

Keywords: infant, rhesus macaque, *Macaca mulatta*, breast milk, lactation, vitamin E, α-tocopherol, stereoisomer, *RRR*-α-tocopherol, *all rac*-α-tocopherol

Introduction

Vitamin E is an essential nutrient for vertebrates and therefore must be acquired through the diet. Although the term vitamin E describes 4 tocopherols (α-, β-, γ- and δ-) and 4 corresponding tocotrienols, only α-tocopherol (αT) meets vitamin E requirements (1). Plants synthesize only "natural" or 2′*R*, 4′*R*, 8′*R* αT (*RRR*-αT), whereas dietary supplements and fortified foods typically contain a "synthetic" all-racemic mixture of 8 stereoisomers, *RRR*, *RRS*, *RSR*, *RSS*, *SSS*, *SRR*, *SRS*, and *SSR* (*all rac*-αT). Only the 4 stereoisomers with the 2*R* configuration are recognized by the hepatic α-tocopherol transfer protein (α-TTP), are bioavailable, and contribute to dietary vitamin E requirements (2). For these reasons, *RRR*-αT is thought to have between 1.36 and 2 times more vitamin E activity than an equal mass of *all rac*-αT (2,3), perhaps depending on dose (4–6).

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Vitamin E deficiency in humans is rare, but its complications of ataxia and myopathy highlight the functional importance of this vitamin in the nervous system (7–9). After birth, infants acquire αT from human milk or infant formula, which commonly contains *all rac*-αT. Human milk αT concentrations vary widely between women, commonly ranging from 2 to 50 μM, with an average of about 10 μM after 1 mo of lactation (10), and also decrease with stage of lactation (11, 12). Infant formula manufacturers generally provide at least 7 μM *RRR*-αT or at least 12 μM *all rac*-αT in order to meet prevailing regulations. Autopsied human infant brain samples from 36 decedents for which no dietary history was available contained predominantly *RRR*-αT; synthetic 2R stereoisomers (SYNTH-2*R*; i.e., *RSS*, *RSR*, and *RSR*) were also detected (13). The minimum percentage of total αT found as *RRR* was 58%, but more than half of the decedents had at least 30% of total αT as SYNTH-2*R* stereoisomers. In a rodent fetal resorption assay of vitamin E activity, the SYNTH-2*R* stereoisomers did not have preventative activity equivalent to that of *RRR*-αT (14). In addition, dietary SYNTH-2*R* stereoisomers result in the displacement of tissue *RRR*-αT when dietary ratios of *all rac*-αT to *RRR*-αT are high (4–6). More recent reports suggest that *RRR*-αT and the synthetic αT stereoisomers may differentially affect gene expression in lymphocytes (15) and brain (16). Because infant formula is routinely supplemented with *all rac*-αT, it is important to determine if *RRR*-αT and *all rac*-αT differentially affect the brain αT status and stereoisomer profile. Because this question cannot be addressed in human infants, we studied infant rhesus macaques fed formulas supplemented with either *RRR*-αT or *all rac*-αT. We compared these animals to a reference group of infant macaques fed breast milk with the addition of a commercially made monkey diet beginning at 2 mo.

Methods

Animals and diets

Other results from this cohort of infant monkeys have previously been reported, and additional details are available (17). All procedures were approved by the Institutional Animal Care and Use Committee of Oregon Health and Science University and carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. On the day after birth, rhesus macaques (*Macaca mulatta*) of the Indian-origin subspecies were randomized to groups that received 1 of 3 diets: infant formula containing natural *RRR*-α−tocopherol [Similac Advance with OptiGRO (NAT-F group, $n = 8$)]; infant formula with synthetic *all rac*-α−tocopherol [Similac Advance base formulation (SYN-F group, $n = 7$)]; and a combination of breast milk and Fiber-Balanced Monkey Diet 5000 (LabDiet) (MF group, $n = 8$). Formula-fed infants were nursery reared from 1 d after birth and were fed by bottle. MF infants were housed with their dam and breastfed. Formulas were labeled with a numerical code by Abbott Nutrition, to which investigators were blinded. All staff were blinded until sample analyses were complete. Randomization was stratified by gender and birth weight as previously described (17). The health of all infant monkeys was continuously monitored by veterinary staff. Formula intake, weight gain, rate of weight gain, and final body and tissue weights and age on the day they were killed were previously reported (17). Briefly, there were 4 females and 3–4 males per group. There were no differences in days of age at sacrifice (MF: 179 ± 4 d; SYN-F: 178 ± 3 d; and NAT-F: 178 ± 3 d), final body weight (MF: 1.41 ± 0.12 kg; SYN-F: 1.47 ± 0.10 kg; and NAT-F: 1.40 ± 0.10 kg), or rate of weight gain among the 3 dietary groups. Formula intake volume did not differ between the NAT-F and SYN-F groups, while breast milk intake volume was not measured. There were no differences in organ weights except for liver (NAT-F group > MF group) and adrenal glands (SYN-F group > MF group). The dietary intervention period ended when the infants ($n = 23$) were 6 mo of age, at which time the infants were humanely killed under pentobarbital anesthesia by a veterinarian as recommended by the Panel on Euthanasia of the American Veterinary Medical Association.

Breast milk samples were collected at 8, 16, and 24 wk of lactation. Blood samples were collected on day 1 after birth, and at 4, 8, 12, 16, 20, and 24 wk as previously reported (17), but only the 24-wk samples were analyzed for the current study. At the time of death, liver, brain, and urine from the bladder were also collected.

MF infants were housed with their dam, which was fed Fiber-Balanced Monkey Diet 5000 plus a variety of fresh fruits and vegetables. MF infants had access to their mother's diet. As required, study infants were not restricted from eating the maternal diet, as it provides an increasingly important source of calories as they mature. The infants generally began ingesting small amounts of monkey diet at approximately 2 mo of age, then progressively increased their intake to approximately 75–90 g per d at 6 mo of age. In contrast, formula-fed infants were nursery reared starting at 1 d after birth, according to an established protocol (15), and received only their assigned infant formula for the 6-mo study period. The volume of formula consumed during each feeding period was recorded from day 1 through 12–13 wk of age and then for 5 consecutive d every 4 wk through week 24 (17). We calculated αT intakes using volumes from the last 5 d of week 24 to correspond with samples collected at study termination.

Details of the αT composition of the diets are presented in **Table 1**. Dam breast milk (31.4 μmol αT/L) and SYN-F (29.7 μmol αT/L) contained similar concentrations of αT, and NAT-F contained 20.9 μmol αT/L. Formula concentrations of αT were normalized on an international unit (IU) per liter basis; 1 IU of vitamin E equals 1 mg all *rac*-αT, or 0.74 mg *RRR*-αT. The αT in the formulas was added as the acetate derivative. The concentration of αT in SYN-F formula was approximately 1.4 times that of NAT-F, consistent with documented differences in bioactivity between natural and synthetic sources of αT (3). *RRR*-αT constituted 100% of the αT in NAT-F, but was only 12.5% in SYN-F. The remaining αT composition of SYN-F was synthetic 2*R* stereoisomers (37.5%) and 2*S* stereoisomers (50%). Although dam breast milk contained all stereoisomers, *RRR* and SYNTH-2*R* stereoisomers comprised ~92% of the total αT. Like SYN-F, the Fiber-Balanced Monkey Diet 5000 contained the acetate derivative of *all-rac*-αT (44 μmol αT/100 g diet).

A more complete composition of each diet is provided in **Supplemental Table 1**. Briefly, NAT-F was supplemented with a carotenoid blend (lutein, 0.135; zeaxanthin, 0.011; β-carotene, 0.0397, and lycopene, 0.182 μg/mL). In contrast, SYN-F was not supplemented with the carotenoid blend but contained inherent concentrations of lutein (0.022 μg/mL), zeaxanthin (0.001 μg/mL), and β-carotene (0.012 μg/mL). Lycopene was not detected in SYN-F. Concentrations of the other nutrients and compounds, including docosahexaenoic acid/L and arachidonic acid, were consistent between NAT-F and SYN-F, as they came from the same formula batch.

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Supplemental Tables 1 and 2 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.
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Abbreviations used: *ACTG1*, actin γ 1; *all rac*-αT, synthetic α-tocopherol (all-racemic mixture stereoisomers *RRR*, *RRS*, *RSR*, *RSS*, *SSS*, *SRR*, *SRS*, and *SSR*); α-CEHC, α-carboxyethyl-hydroxychroman; αT, α-tocopherol; α-TTP, α-tocopherol transfer protein; ECD, electrochemical chemical detection; MF, mixed diet of breast milk and monkey diet; IU, international unit; NAT-F, infant formula with natural *RRR*-α−tocopherol; OC, occipital cortex; *RRR*-αT, 2′*R*, 4′*R*, 8′*R* α-tocopherol (natural); SYN-F, infant formula with synthetic *all rac*-α−tocopherol; SYNTH-2*R*; synthetic α-tocopherol 2*R* stereoisomers.

TABLE 1 Total α-Tocopherol and α-tocopherol stereoisomer profiles of monkey breast milk, monkey diet, and infant formulas[1]

	Formulas				Mixed diet			
	NAT-F		SYN-F		Breast milk[2]		Monkey diet[2]	
	μmol/L	%	μmol/L	%	μmol/L	%	μmol/100 g	%
Total αT	20.9	100	29.7	100	31.4 ± 5.22	100	44.0	100
RRR	20.9	100	3.70	12.5	10.7 ± 2.00	32.4	5.50	12.5
RRS	0	0	3.70	12.5	7.84 ± 1.57	23.1	5.50	12.5
RSR	0	0	3.70	12.5	3.76 ± 0.37	13.8	5.50	12.5
RSS	0	0	3.70	12.5	7.09 ± 1.32	22.5	5.50	12.5
2S	0	0	14.9	50.0	2.03 ± 0.27	8.20	22.0	50.0

[1] Values are presented as means ± SEMs, $n = 6$ dams (means of 2–4 samples/dam collected at 2, 4, and 6 mo for a total of 18 samples). Individual values ranged from 12 to 74 μmol/L. Total αT values are presented as means of triplicate analyses. Stereoisomers were calculated based on known composition of the respective αT source. *all rac*-αT, synthetic α-tocopherol (all-racemic mixture stereoisomers *RRR, RRS, RSR, RSS, SSS, SRR, SRS,* and *SSR*); αT, α-tocopherol; MF, mixed diet of breast milk and monkey diet (LabDiet 5000); NAT-F, formula supplemented with *RRR*-αT; SYN-F, formula supplemented with all *rac*-αT.
[2] LabDiet Fiber-balanced Monkey Diet 5000. The total αT value is from the manufacturer.

Breast milk, plasma, urine, and tissue collection

Breast milk samples were collected while the animals were under ketamine sedation (5–10 mg/kg intramuscular) from 5 of the 8 dams, at 4 and 6 mo for 1 of the dams, but milk collection was not possible for 2 of the dams. Samples were collected directly into cryotubes, frozen on dry ice, and stored at –80°C until analysis. Fasting blood samples (1 mL) were collected in EDTA-containing blood collection tubes and were processed for plasma, placed in cryotubes, frozen in liquid nitrogen, and then stored at –80°C until analysis. Urine samples collected from the bladder were frozen in liquid nitrogen and stored at –80°C until analysis. Liver and brain samples (~0.5–1 g each) were flash frozen in liquid nitrogen and then stored at –80°C until analysis. Brain samples were dissected from the dorsolateral prefrontal cortex, occipital cortex, superior temporal cortex, striatum, cerebellum, and motor cortex.

Analysis of αT and its stereoisomers

αT and its stereoisomers were assessed as we described previously (16). In brief, samples were extracted with hexane following saponification as described (18). A portion of the hexane was dried and reconstituted to measure αT by HPLC with electrochemical chemical detection (ECD) as described (16). αT was quantified at the dominant oxidation potential relative to external αT standard (Sigma) that was validated against certified reference material (NIST SRM 968f). To assess αT stereoisomers, the remaining portion of the aforementioned hexane extract was used to measure the percentage distribution of αT stereoisomers as we described (16). In brief, the hexane extract was dried under nitrogen gas and resolubilized, and the reconstituted sample was methylated under basic conditions prior to extracting with hexane. Samples were then separated and detected by HPLC with fluorescence detection using a chiral separation column and excitation/emission settings of ($290_{nm}/330_{nm}$). Under these conditions, each specific *2R* stereoisomer of αT (*RRR*-, *RRS*-, *RSR*-, and *RSS*-αT) was determined along with a single peak for total *2S* stereoisomers. The peak area of each stereoisomer was calculated to determine the percentage distribution, and their molar concentrations were determined based on the concentration of total α-T obtained by HPLC-ECD.

Urine α-CEHC analysis

Urinary α-CEHC was extracted and analyzed as described previously (19), with minor modifications. Urine diluted with water was mixed with ascorbic acid (2%, wt/vol) and 6N hydrochloric acid, incubated in a shaking water bath (1 h, 60°C), extracted with diethyl ether, dried under nitrogen, and reconstituted in 30% acetonitrile/0.05% acetic acid. Samples were analyzed as described previously (19), except that nebulizing and drying gases were supplied at 1.5 L/min and 15 L/min, respectively, and heating block and desolvation temperatures were set to 400°C and 200°C, respectively. Urinary α-CEHC concentrations were normalized to urinary creatinine measured using a clinical assay (Pointe Scientific).

Lipoprotein fractions

Serum apoA-I, apoB-48, and apoB-100 levels were assessed with commercial ELISA kits (MyBioSource) according to the manufacturer's instructions.

Western blotting

Procedures were conducted as previously described (20). Briefly, protein was extracted from frozen brain tissues, separated on SDS-PAGE, and transferred onto a membrane. Membranes were blocked (5% fat-free milk) and probed with polyclonal rabbit anti-α-TTP antibody (1:1000) overnight at 4°C. (LifeSpan BioSciences, Inc). The secondary antibody was horseradish peroxidase–linked anti-rabbit IgG, 1:50,000 (Cell Signaling). GAPDH monoclonal antibody (1:3000) was used as a loading control (Cell Signaling). Liver was used as a positive control for α-TTP. Immunoblots were visualized with an ImageQuant LAS 4000 (GE Healthcare) and quantified via Quantity One (Bio-Rad). Samples from each of the 3 diet groups and 2 brain regions were evenly distributed between immunoblots to allow for direct comparisons between groups and to prevent bias between batches. One cerebellum sample was included in every batch to normalize band intensity values between blots.

Total RNA isolation and RT-qPCR analysis

Procedures were conducted as reported previously for brain tissues (20). After isolating total RNA from each sample, RNA purities and concentrations were determined and cDNA was synthesized. RT-qPCR was performed on a QuantStudio™ 7 Flex Real-Time PCR System (Applied Biosystems) following the manufacturer's protocol for SYBR® Green ROX qPCR Mastermix (Qiagen). Actin γ 1 (*ACTG1*) was chosen as the reference gene due to its low variability between the occipital cortex and the cerebellum samples. Relative quantitation of gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method. The following primer sequences were used: *TTPA* (designation of gene for α-TTP) forward: 5′-CAGAATCGCACACTGGGACC-3′; *TTPA* reverse: 5′-GCCAGCCTTCCAGATCAAAG-3′; *ACTG1* forward: 5′-GCTCC TGAACCAGTTTCTGC-3′; *ACTG1* reverse: 5′-AGTAACAGCCCAC GGTGTTC-3′.

Statistical analysis

All data, except Western blot and RT-qPCR data, were analyzed with GraphPad Prism version 7.01 for Windows, GraphPad Software, www.graphpad.com. Dietary effects on total αT and αT stereoisomers (*RRR*, SYNTH-*2R*, and *2S*) were analyzed using 1-way ANOVA followed by Tukey's Multiple Comparison Tests. Pearson's correlation was used to test for significant correlations between study variables. Unless indicated otherwise, data are expressed as mean ± standard deviation of the mean. Western blot and RT-qPCR data were analyzed using SPSS Statistics for Windows version 25 (IBM Corp). Shapiro-Wilk and Levene's tests were used to evaluate normality and homogeneity of variance, respectively. When necessary, data were transformed to account for unequal variances. Two-way ANOVA was used to assess the

TABLE 2 Estimated food and α-tocopherol intake at 6 mo of age in infant rhesus macaques that were fed infant formulas supplemented with either *RRR*-α-tocopherol or *all rac*-α-tocopherol or fed a mixed diet[1]

	Dietary group		
	NAT-F	SYN-F	MF
Formula intake			
mL/d	418 ± 36.8[2]	408 ± 27.5[2]	—
kcal/d	297 ± 26.2[2]	291 ± 19.6[2]	—
Estimated kcal/d	—	—	294
Monkey diet intake			
Estimated g/d	—		82.0[3]
Estimated kcal//d		—	235[3]
Breast milk intake			
Estimated kcal/d	—	—	59.0[4]
Estimated mL/d			69.0[5]
αT intake, μmol/d			
Formula	8.65 ± 0.76[6,b]	12.1 ± 0.80[6,a]	—
Monkey diet	—	—	36.1[6]
Breast milk	—	—	2.17[6]
αT stereoisomer intake, μmol/d			
RRR	8.65 ± 0.762[a]	1.52 ± 0.102[b]	5.22
SYNTH-2*R*	0[b]	4.55 ± 0.306[a]	14.8
2*S*	0[b]	6.06 ± 0.409[a]	18.2

[1] Values are means ± SDs. SYN-F: $n = 7$, received infant formula with 29.7 μmol *all rac*-αT/L; NAT-F: $n = 8$, received infant formula with 20.7 μmol *RRR*-αT/L. Formula αT values are means of triplicate analyses. Formula intake values previously reported (17). Values for group calculated based on caloric intake by SYN-F and NAT-F groups. Values within row with unlike superscripts differ, $P < 0.0001$ by 1-way ANOVA. *all rac*-αT, synthetic α-tocopherol (all-racemic mixture stereoisomers *RRR*, *RRS*, *RSR*, *RSS*, *SSS*, *SRR*, *SRS*, and *SSR*); αT, α-tocopherol; MF, mixed diet of breast milk and monkey diet (LabDiet 5000); NAT-F, infant formula with natural *RRR*-α-tocopherol; *RRR*-αT, 2′*R*, 4′*R*, 8′*R* α-tocopherol (natural); SYN-F, infant formula with synthetic all rac-α-tocopherol.

[2] From Jeon et al (17), intake per day calculated from caloric density of the infant formulas, 0.712 kcal/mL. Calculated daily calorie intake for the formula groups combined was 294 kcal/d at 6 mo of age. Assumed MF infants consumed the same number of calories per day as the formula fed infants as they grew at the same rate and finished at the same body weight (15).

[3] Estimated daily intake of monkey diet by the MF infants at 6 mo of age. Monkey diet (LabDiets, Inc) had a caloric density of 2.87 kcal/g.

[4] Estimated caloric intake from breast milk by subtracting monkey diet caloric intake from the estimated daily calorie intake. Rhesus macaque breast milk has a caloric density of approximately 0.854 kcal/mL (21).

[5] Monkey diet contained 0.44 μmol αT/g (provided by the manufacturer); breast milk contained an average of 31.4 μmol αT/L. Breast milk values were calculated by determining a mean for each dam, then calculating a mean for the dams together.

[6] Values were calculated by multiplying daily αT intake by the percentage composition information presented in Table 1 divided by 100.

effects of diet and brain region on TTPA protein and mRNA expression. No post hoc tests were conducted because only the brain region main effect was significant (there were only 2 brain regions tested). Hepatic TTPA protein expression was analyzed by 1-way ANOVA.

Results

Breast milk, monkey diet, and αT intake

We estimated the daily intake of αT for each dietary group at 6 mo of age based on calorie intake calculated from week 24 formula intake data previously reported for these monkeys (15) (**Table 2**). Daily formula intake was very similar between NAT-F and SYN-F, resulting in a combined average intake of 294 kcal/d during the last week of the study. Breast milk intake values were not available for the MF infants, but from 2 mo of age MF infants consumed increasing amounts of monkey diet, culminating in an estimated intake of approximately 235 kcal/d. We assumed that MF infants had the same daily energy intake as the formula-fed infants since there were no differences between the dietary groups for either growth velocity or final body weight (15). Based on this assumption, monkey diet provided most of the energy for the MF group at 6 mo of age. accompanied by a modest intake of breast milk of approximately 69 mL per d. The estimated intakes of *RRR*-αT and SYNTH-2*R* in the MF group were directionally higher than in the formula groups. Estimated intake of αT in the SYN-F group was 33% higher than that of the NAT-F group; the difference was driven by the higher αT fortification rate in SYN-F, as noted above. Table 2 also shows the estimated daily 2*S*-αT stereoisomer intake.

TABLE 3 Plasma total α-tocopherol and α-tocopherol stereoisomer distribution in 6-mo old infant rhesus macaques that were fed infant formulas with different sources of α-tocopherol or a mixed diet[1]

	NAT-F	SYN-F	MF
Plasma, μmol αT/L			
Total	32.0 ± 4.99	25.7 ± 7.86	32.5 ± 7.53
RRR	30.9 ± 4.87^{aA}	18.2 ± 6.15^{bA}	11.4 ± 2.92^{cB}
Synthetic 2*R*	0.713 ± 0.163^{cB}	6.76 ± 1.95^{bB}	20.7 ± 4.80^{aA}
2*S*	0.354 ± 0.129^{B}	0.687 ± 0.410^{B}	0.453 ± 0.163^{C}
Liver, μmol αT/g			
Total	41.2 ± 10.5	46.7 ± 16.7	38.9 ± 13.0
RRR	39.6 ± 10.2^{aA}	28.7 ± 9.67^{aA}	13.9 ± 5.02^{bB}
Synthetic 2*R*	1.51 ± 0.380^{cB}	12.1 ± 4.24^{bB}	23.5 ± 7.43^{aA}
2*S*	0.331 ± 0.688 ^{cB}	5.79 ± 2.87 ^{aB}	2.45 ± 0.891 ^{bcC}

[1] Values are means ± SDs. $n = 23$; MF, $n = 8$; SYN-F, and $n = 7$; and NAT-F, $n = 8$. Labeled means in a row without a common lowercase superscript letter differ, ^{ab,ac} $P < 0.001$; ^{bc} $P < 0.05$; by 1-way ANOVA and Tukey's post hoc test. Labeled means in a column without a common uppercase superscript (plasma or liver) $^{A-C}$ differ $P < 0.001$ by 1-way ANOVA and Tukey's post hoc test. *all rac*-αT, synthetic α-tocopherol (all-racemic mixture stereoisomers *RRR*, *RRS*, *RSR*, *RSS*, *SSS*, *SRR*, *SRS*, and *SSR*); αT, α-tocopherol; MF, received breast milk (31.4 μmol αT/L) and Monkey Diet 5000 (44 μmol αT/100 g); NAT-F, formula with 20.9 μmol *RRR*-αT/L; *RRR*-αT, 2′*R*, 4′*R*, 8′*R* α-tocopherol (natural); SYN-F: formula with 29.7 μmol all *rac*-αT/L.

Plasma and liver αT and αT stereoisomer profiles

There were no differences in total plasma αT concentration between the 3 dietary groups (**Table 3**). However, the proportions of plasma αT stereoisomers differed among the groups. Plasma *RRR*-αT concentrations were significantly higher in the NAT-F group than both the SYN-F group ($P < 0.0001$) and the MF group ($P < 0.0001$), and the SYN-F group was significantly higher than the MF group ($P < 0.05$). In contrast, plasma SYNTH-2*R* concentrations were significantly lower in the NAT-F group compared with those in both the SYN-F group ($P < 0.001$) and the MF group ($P < 0.0001$) and were significantly lower in the SYN-F group compared with the MF group ($P < 0.001$). The sum of the 2*S* αT stereoisomers in plasma was not different between groups.

The αT accumulation pattern in liver was similar, but not identical, to that in plasma. There were no differences among groups for total αT. Liver *RRR*-αT in the NAT-F group was not different from that in the SYN-F group ($P = 0.064$) but was significantly higher than in the MF group ($P < 0.0001$). The SYN-F group had significantly higher *RRR*-αT than the MF group ($P < 0.001$). The pattern of liver SYNTH-2*R* stereoisomers was reversed compared with that of *RRR*-αT.

TABLE 4 Concentration of α-CEHC in urine samples from 6-mo old infant rhesus macaques that were fed infant formulas with different sources of α-tocopherol or a mixed diet[1]

	Urine creatinine (g/L)	Urine α-CEHC (μmol/L)	Urine α-CEHC/creatinine (μmol/g)
NAT-F	0.382 ± 0.339	2.21 ± 1.36[a]	6.66 ± 3.62[b]
SYN-F	0.428 ± 0.523	1.32 ± 1.04[ab]	4.15 ± 2.31[bc]
MF	0.384 ± 0.179	0.551 ± 0.410[b]	1.74 ± 1.46[c]

[1] Values are means ± SDs. $n = 23$; MF, $n = 8$; SYN-F, $n = 7$; NAT-F, $n = 8$. Labeled means in a column without a common lowercase superscript letter differ: [a, b] $P < 0.05$; [b, c] $P < 0.01$ by 1-way ANOVA and Tukey's post hoc test. α-CEHC, α-carboxyethyl-hydroxychroman; *all rac*-αT, synthetic α-tocopherol (all-racemic mixture stereoisomers *RRR*, *RRS*, *RSR*, *RSS*, *SSS*, *SRR*, *SRS*, and *SSR*); αT, α-tocopherol; MF: received breast milk (31.4 μmol αT/L) and Monkey Diet 5000 (44 μmol αT/100 g); NAT-F: formula with 20.9 μmol *RRR*-αT/L; *RRR*-αT, 2′*R*, 4′*R*, 8′*R* α-tocopherol (natural); SYN-F: formula with 29.7 μmol *all rac*-αT/L.

Specifically, the concentration of SYNTH-2*R* in the NAT-F group was significantly lower than the concentrations in both the SYN-F group ($P < 0.002$) and the MF group ($P < 0.0001$). The SYN-F group had significantly lower SYNTH-2*R* than the MF group ($P < 0.001$). Mean liver 2*S* stereoisomer concentrations were significantly lower in the NAT-F group than in the SYN-F group ($P < 0.0001$), but not the MF group ($P = 0.07$). The 2*S* stereoisomer liver concentrations were significantly lower in the SYN-F group than the MF group ($P < 0.004$).

Urinary α-CEHC concentrations

Concentrations of α-CEHC in urine samples at the time of death are presented in Table 4. The concentration of urinary creatinine was not different between the dietary groups. Infants in the NAT-F group had a significantly higher concentration of α-CEHC than MF infants ($P < 0.05$). This difference persisted ($P = 0.004$) when α-CEHC concentration was normalized to creatinine concentration. The α-CEHC concentrations and α-CEHC normalized to creatinine concentrations in the SYN-F group did not differ from those in the NAT-F or MF groups.

Lipoprotein fraction αT concentrations

Approximately two-thirds of plasma αT was found in the HDL apoA-I fraction after a 6-h fast (Table 5). The remaining one-third of plasma αT was found in the apoB fraction (apoB-48 + apoB-100). The mode of feeding did not influence either the concentration of αT in these fractions, or the mol/mol ratio of αT to apo B.

TABLE 5 Lipoprotein fraction α-tocopherol concentrations from 6-mo old infant rhesus macaques that were fed infant formulas with different sources of α-tocopherol or a mixed diet[1]

	NAT-F	SYN-F	MF
αT, μmol/L[2]	28.9 ± 3.28	29.3 ± 7.69	33.1 ± 8.46
HDL fraction αT, μmol/L	18.7 ± 7.24	20.5 ± 5.86	20.7 ± 5.83
ApoB fraction αT, μmol/L	10.2 ± 3.86	8.80 ± 2.62	12.3 ± 3.41
αT/apoB, mol/mol	10.1 ± 6.19	9.80 ± 4.94	7.34 ± 3.17

[1] Values are means ± SDs. $n = 23$; MF, $n = 8$; SYN-F, $n = 7$; NAT-F, $n = 8$. Values were compared using 1-way ANOVA. *all rac*-αT, synthetic α-tocopherol (all-racemic mixture stereoisomers *RRR*, *RRS*, *RSR*, *RSS*, *SSS*, *SRR*, *SRS*, and *SSR*); αT, α-tocopherol; MF, received breast milk (31.4 μmol αT/L) and Monkey Diet 5000 (44 μmol αT/100 g); NAT-F, formula with 20.9 μmol *RRR*-αT/L; *RRR*-αT, 2′*R*, 4′*R*, 8′*R* α-tocopherol (natural); SYN-F, formula with 29.7 μmol all *rac*-αT/L.
[2] Total αT in HDL fraction (apoA-I) plus that in the apoB (apoB-48 + apoB-100) fraction.

TABLE 6 α-Tocopherol stereoisomer distribution by brain region in infant rhesus macaques that were fed infant formulas with different sources of α-tocopherol or fed a mixed diet[1]

	Dietary group (nmol αT/g)		
Brain Region	NAT-F	SYN-F	MF
Occipital cortex			
Total αT	28.9 ± 5.48[A]	23.9 ± 4.89[A]	27.8 ± 8.81[A]
RRR-αT	27.3 ± 5.12[aA]	17.2 ± 3.45[cA]	10.6 ± 3.27[dA]
Synthetic 2*R* αT	1.51 ± 0.529[aB]	6.42 ± 1.51[bB]	17.0 ± 5.52[dB]
Σ 2*S* αT	0.113 ± 0.165	0.315 ± 0.084	0.242 ± 0.063
Cerebellum			
Total αT	16.9 ± 5.73[B]	13.4 ± 3.32[B]	15.0 ± 5.16[B]
RRR-αT	15.3 ± 5.05[aA]	9.54 ± 2.41[cd]	6.12 ± 1.78[d]
Synthetic 2*R* αT	1.44 ± 0.75[abB]	3.66 ± 0.86[b]	8.71 ± 2.95[d]
Σ 2*S* αT	0.173 ± 0.39	0.218 ± 0.14	0.172 ± 0.06
Temporal cortex			
Total αT	21.2 ± 3.04[A]	18.9 ± 3.92[B]	20.1 ± 5.67[A]
RRR-αT	19.5 ± 2.52[aA]	13.2 ± 2.63[cA]	7.88 ± 2.24[d]
Synthetic 2*R* αT	1.57 ± 0.513[aB]	5.42 ± 1.29[cB]	12.1 ± 3.44[d]
Σ 2*S* αT	0.126 ± 0.252	0.276 ± 0.117	0.114 ± 0.061
Striatum			
Total αT	27.8 ± 3.06[C]	23.5 ± 3.25[A]	26.4 ± 7.33[A]
RRR-αT	25.3 ± 2.90[aA]	16.5 ± 2.09[cA]	10.1 ± 2.76[dA]
Synthetic 2*R* αT	2.41 ± 0.194[aB]	6.74 ± 1.15[cB]	16.1 ± 4.65[dB]
Σ 2*S* αT	0.088 ± 0.171	0.264 ± 0.129	0.213 ± 0.127
Motor cortex			
Total αT	20.2 ± 3.88[A]	17.8 ± 3.08[A]	18.3 ± 6.27[A]
RRR-αT	18.4 ± 3.50[aA]	12.5 ± 1.89[cA]	7.01 ± 2.37[d]
Synthetic 2*R* αT	1.66 ± 0.427[aB]	5.11 ± 1.17[cB]	11.16 ± 3.90[d]
Σ 2*S* αT	0.141 ± 0.290	0.189 ± 0.067	0.130 ± 0.055
Prefrontal cortex			
Total αT	19.6 ± 4.62[A]	17.6 ± 3.21[B]	19.1 ± 5.85[A]
RRR-αT	17.9 ± 4.30[aA]	12.3 ± 2.23[cA]	7.43 ± 2.33[d]
Synthetic 2*R* αT	1.52 ± 0.444[aB]	5.01 ± 1.06[cB]	11.62 ± 3.58[d]
Σ 2*S* αT	0.185 ± 0.291	0.291 ± 0.122	0.084 ± 0.078

[1] Values are means ± SDs. $n = 23$; MF, $n = 8$; SYN-F, $n = 7$; NAT-F, $n = 8$. Labeled means in a row without a common lowercase superscript letter differ: [a, b] $P < 0.05$; [a, c] $P < 0.01$; [ad, bd, cd] $P < 0.0001$ by 1-way ANOVA and Tukey's post hoc test. Labeled *RRR* and synthetic 2*R* or Total αT means in a column without a common uppercase superscript letter differ $P < 0.01$ by 1-way ANOVA and Tukey's post hoc test. *all rac*-αT, synthetic α-tocopherol (all-racemic mixture stereoisomers *RRR*, *RRS*, *RSR*, *RSS*, *SSS*, *SRR*, *SRS*, and *SSR*); αT, α-tocopherol; MF: received breast milk (31.4 μmol αT/L) and Monkey Diet 5000 (44 nmol αT/100 g); NAT-F: formula with 20.9 μmol *RRR*-αT/L; *RRR*-αT, 2′*R*, 4′*R*, 8′*R* α-tocopherol (natural); SYN-F: formula with 29.7 μmol all *rac*-αT/L.

Brain αT

αT concentrations in 6 brain regions (occipital cortex, cerebellum, superior temporal cortex, striatum, motor cortex, and prefrontal cortex) are presented in Table 6. Total αT was not significantly different between the dietary groups for any of the brain regions tested, and generally, αT concentrations did not differ among brain regions. However, within each dietary group, αT concentrations were significantly lower in cerebellum than in occipital, superior temporal, motor, and cortices and striatum ($P < 0.01$ for each), with the exception that total αT concentrations were not different between the cerebellum and temporal cortex in the SYN-F group.

Brain αT stereoisomer concentrations

αT stereoisomer concentrations are presented by dietary group and brain region in Table 6. In each brain region, infants

fed NAT-F had a significantly greater concentration of *RRR*-α-T than both the SYN-F group (occipital, temporal, motor, and prefrontal cortices: $P = 0.01$; striatum: $P < 0.0001$) and the MF group ($P < 0.0001$ all cortices). The SYN-F group had a significantly higher concentrations of *RRR*-α-T than the MF group ($P < 0.0001$, all regions except cerebellum). The concentrations of *RRR*-α-T in the cerebellum were not different between the SYN-F and MF groups. Generally, the opposite pattern was found for SYNTH-2*R*. The NAT-F group had significantly lower concentrations of SYN-2*R* than the SYN-F group in the temporal cortex, striatum, motor cortex, prefrontal cortex (all $P < 0.01$), and the occipital cortex ($P < 0.05$), but not the cerebellum (not significant). Additionally, both the NAT-F and SYN-F groups had significantly lower SYN-2*R* than the MF group ($P < 0.0001$ all cortices). The concentrations of the individual SYNTH-2*R* stereoisomers by brain region and dietary group are presented in **Supplemental Table 2**. The sum of the 2*S* stereoisomers was low in all 3 groups for all brain regions tested (1–2% of total α-T). In the NAT-F group, *RRR*-α-T was significantly higher ($P < 0.01$) than the sum of the SYNTH-2*R* stereoisomers in all cortices. The same was true for SYN-F, except for the cerebellum, where *RRR*-α-T and SYNTH-2*R* were not different. In contrast, in the MF group *RRR*-α-T values were not different from the SYNTH-2*R* group values, except that SYNTH-2*R* was significantly higher than *RRR*-α-T in the cerebellum ($P < 0.01$) and striatum ($P < 0.01$). With the 3 diet groups combined, the plasma percentage of *RRR*-α-T positively correlated ($r = 0.99$, $P < 0.0001$) with the percentage of *RRR*-α-T in the occipital cortex (**Figure 1**A) but the plasma percentage of SYNTH-2*R* was negatively correlated with the percentage of *RRR*-α-T in the occipital cortex ($r = -0.99$, $P < 0.0001$) (Figure 1B). Results for the other brain regions were similar.

α-TTP expression in cerebellum and occipital cortex

α-TTP protein expression was compared between the 3 diet groups and between 2 brain regions of interest (cerebellum and occipital cortex). These brain regions were chosen because of their relatively low and high total α-T concentrations, respectively; it was hypothesized that α-TTP expression levels may be associated with tissue α-T levels. α-TTP protein expression did not significantly differ by diet group in the brain ($P = 0.363$) but was ~2-fold higher in the cerebellum than the occipital cortex ($P < 0.001$) (**Figure 2**A and B). Analysis of α-TTP mRNA expression corroborated the protein expression findings, as levels were ~10-fold higher in the cerebellum than the occipital cortex (Figure 2C). Hepatic α-TTP protein levels were also determined, but they did not significantly differ between diet groups ($P = 0.319$, data not shown).

Comparison of diet stereoisomer profile with that of plasma and brain

The α-T stereoisomer profiles in infant diet, liver, infant plasma, and occipital cortex (as a representative brain region) are presented by dietary group in **Figure 3**. *RRR*-α-T constituted 100% of the α-T in the NAT-F formula, as well as the majority of α-T in the liver (96%), plasma (97%), and occipital cortex (94%) in the NAT-F group. In contrast, *RRR*-α-T constituted only 12.5% of the SYN-F diet, and synthetic α-T stereoisomers made up 87.5% (37.5% SYNTH-2*R*) of the SYN-F diet. SYN-F tissues were appreciably enriched in *RRR*-α-T; liver (61%), plasma (70%), and the occipital cortex (72%), but also each contained about 27% SYNTH-2*R*. Likewise, MF tissues were enriched in *RRR*-α-T (36–38%) and SYNTH-2R (35–38%) relative to their diet.

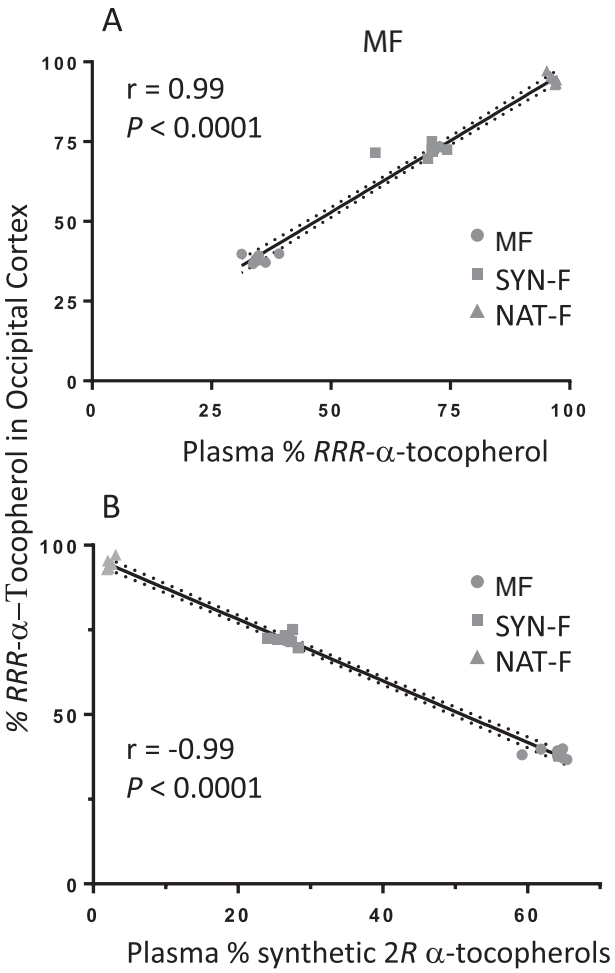

FIGURE 1 Relation between occipital cortex and plasma percentages of *RRR*-α-tocopherol (A) and SYNTH-2*R* (B) from 6-mo-old infant rhesus macaques. MF infants consumed breast milk and monkey diet (LabDiet 5000), $n = 8$, SYN-F–fed infants received formula supplemented with 29.7 μmol all rac-α-T/L ($n = 7$), and NAT-F infants received formula supplemented with 20.7 μmol *RRR*-α-T/L ($n = 8$). Synthetic 2*R* stereoisomers: *RRS* + *RSR* + *RSS*. Panel A, $r = 0.99$, $P < 0.0001$; panel B, $r = -0.99$, $P < 0.0001$. Lines indicate linear regression line with 95% CI indicated by dashed lines. α-T, α-tocopherol; MF, mixed diet of breast milk and monkey diet (LabDiet 5000); NAT-F, infant formula with natural *RRR*-α–tocopherol; *RRR*-α-T, 2′*R*, 4′*R*, 8′*R* α-tocopherol (natural); SYN-F, infant formula with synthetic *all rac*-α–tocopherol; SYNTH-2*R*; synthetic α-tocopherol 2*R* stereoisomers.

Discussion

Contrary to our hypothesis, neither the infant formula α-T source (*RRR*-α-T compared with *all rac*-α-T), nor the mode of feeding impacted total α-T concentration in the infant primate brain, plasma, or liver. However, the α-T stereoisomer profile in each group was substantially impacted by both the infant formula α-T source and by the mode of feeding. Our data reveal for what is to our knowledge the first time that infant formula supplemented with *RRR*-α-T results in higher accumulation of brain *RRR*-α-T and lower accumulation of SYNTH-2*R* compared with a formula supplemented with *all rac*-α-T. This is the first direct comparison of α-T sources in infant formula together with analysis of brain α-T. The results clearly indicate that synthetic α-T stereoisomers accumulate in the brain despite the selective activity of hepatic α-TTP and

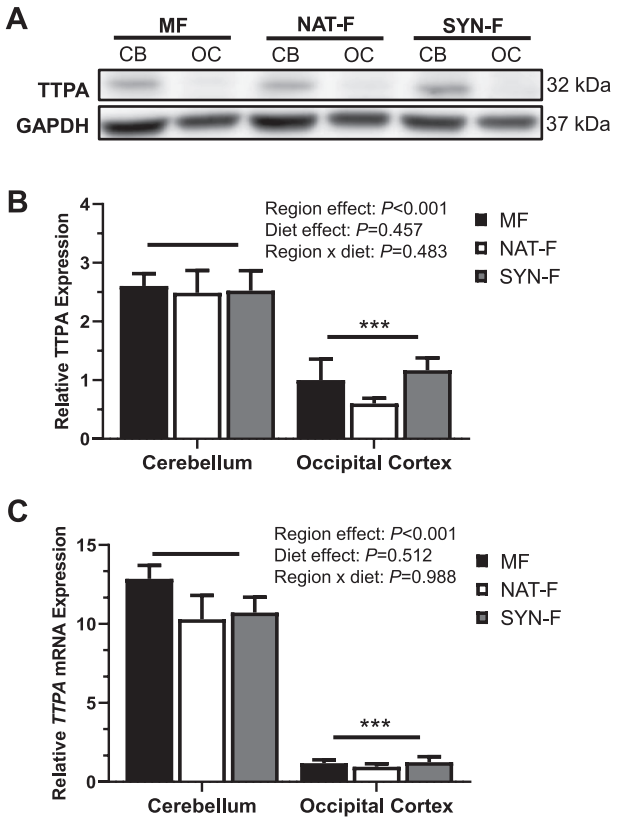

FIGURE 2 α-TTP protein and mRNA expression in cerebellum (CB) and occipital cortex (OC) of infant rhesus monkeys that were fed MF, NAT-F, or SYN-F for 6 mo. Representative Western blot images (A); relative α-TTP (TTPA is the gene designation for α-TTP) protein expression (B), with GAPDH used as a loading control. Relative *α-TTP* mRNA expression (C); *ACTG1* was used as the reference gene. All values are expressed as mean ± SEM (n = 7–8/diet group) and are relative to the MF group's occipital cortex mean value (set at 1.0). Diet and brain region effects were determined by 2-way ANOVA. ***$P < 0.001$. *ACTG1*, actin γ 1; αT, α-tocopherol; MF, mixed diet of breast milk and monkey diet (LabDiet 5000); NAT-F, infant formula with natural RRR-α-tocopherol; RRR-αT, 2′R, 4′R, 8′R α-tocopherol (natural); SYN-F, infant formula with synthetic all *rac*-α-tocopherol; SYNTH-2R; synthetic α-tocopherol 2R stereoisomers.

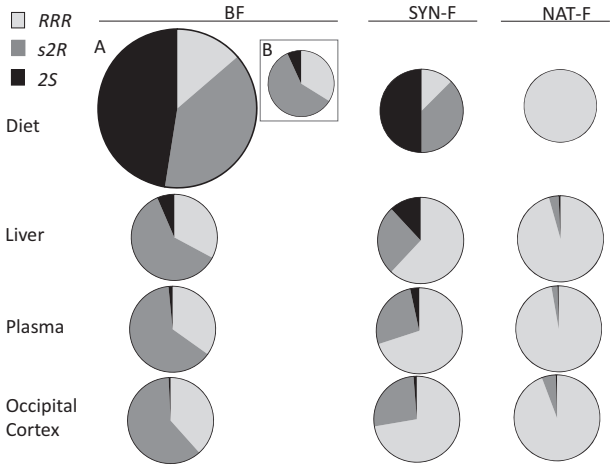

FIGURE 3 Mean αT stereoisomer proportions in diet, liver, plasma, and occipital cortex of 6-mo-old infant rhesus macaques. The area of each diet pie chart is proportional to the estimated average daily αT intake for each individual diet group. For the MF group, 2 pie charts are presented, Combined intake of αT from monkey diet (LabDiet 5000) and breast milk (A); and expanded view of the breast milk αT stereoisomer composition, which comprises 5% of the total intake of the MF group (B). SYNTH-2*R*: *RRS* + *RSR* + *RSS;* 2*S*: *SSR* + *SRS* + *RSS* + *SSS*. MF infants were fed a mixed diet of breast milk and monkey diet, n = 8, or fed infant formulas differing in the source of αT. Infant formulas were SYN-F, formula supplemented with 29.7 μmol *all rac*-αT/L, n = 7, and NAT-F, formula supplemented with 20.7 μmol *RRR*-αT/L, n = 8. *all rac*-αT, synthetic α-tocopherol (all-racemic mixture stereoisomers *RRR*, *RRS*, *RSR*, *RSS*, *SSS*, *SRR*, *SRS*, and *SSR*); αT, α-tocopherol.

reveal an inverse relation between plasma SYNTH-2*R* and brain *RRR* concentration.

Surprisingly, our data revealed that MF infant monkeys accreted less brain *RRR*-αT and more SYNTH-2*R* stereoisomers than SYN-F infants, possibly due to the consumption of a substantially higher quantity of SYNTH-2*R* and αT from the dam's diet. Note that the MF group was a reference group that consumed both breast milk and the maternal monkey diet and therefore inferences from comparisons with this group should be made with caution. This observation is, however, consistent with previous findings in other animals. Increasing doses of *all rac*-αT in rats and lambs led to decreased *RRR*-αT and increased SYNTH-2*R* in most nonbrain tissues analyzed (5, 6, 22). Mink fed *all rac*-αT had decreased brain *RRR*-αT and increased SYNTH-2*R* compared with those fed *RRR*-αT (4). Stereoisomer differences between the NAT-F and SYN-F groups are also generally consistent with previous reports. Previously, tissues, including brains of piglets (23, 24) and rodents (25, 26), preferentially accumulated *RRR*-αT over *all rac*-αT stereoisomers by ~1.8:1 to 2:1 when equimolar labeled quantities of both αT forms were fed. Notably, individual α stereoisomers were not measured in those reports. Thus, our findings indicate that brain from infant monkeys fed infant formula with *all rac*-αT or an MF diet had lower concentrations of *RRR*-αT and higher concentrations of SYNTH-2*R* compared with those fed infant formula containing *RRR*-αT.

Jeon et al. (17) previously reported higher concentrations of blood apoB and apoAI in the reference MF group in this cohort of infant primates. We sought to determine if differential distribution of αT in the lipoprotein fractions helped to explain our observations. We observed higher apoB fraction αT and lower αT/apoB molar ratios in the MF-fed infants compared with formula-fed infants, but neither was significantly different. Therefore, these findings did not offer mechanistic insight into our observations.

We also measured protein and mRNA concentrations of α–TTP in the brain regions with the highest (occipital cortex) and lowest (cerebellum) αT concentrations to determine if the selective activities of α–TTP toward specific αT stereoisomers could explain our observations. Contrary to our hypothesis, however, we found that α–TTP protein and mRNA expressions were higher in cerebellum than in occipital cortex. Clearly there was not a direct relation between αT concentration in these brain regions and α–TTP expression. This finding is particularly interesting in view of the proposal by Ulatowski and Manor (27) that cerebellar α–TTP is essential for the coordination of the various brain pools of αT.

It is noteworthy that brain *RRR*-αT concentrations in the MF group were significantly lower than those of the SYN-F group. This finding was surprising, as *RRR*-αT intake was estimated to be higher in the MF infants (5.2 μmol/L/d) than in

the SYN-F infants (1.5 μmol/L/d), with similar *RRR*/SYNTH-2*R* ratios (1:2.8 compared with 1:3, respectively). Consistent with this finding, MF SYNTH-2*R* concentrations in liver were 2-fold higher than those in SYN-F liver and were accompanied by MF brain and plasma SYNTH-2*R* concentrations that equaled those of *RRR* in most cases and even exceeded those of *RRR* in some cases. Taken together, these findings indicate that SYN-F infants were able to select for *RRR*-αT and to markedly increase the *RRR* to SYNTH-2*R* ratio in tissues and plasma, but MF infants were not.

Mechanistic studies are needed to explain the inability of MF infants to select for *RRR* to the same extent as SYN-F infants, despite the similar dietary αT stereoisomer profile consumed by the 2 groups. As discussed earlier, a possible explanation is that the elevated intake of *all rac*-αT by MF infants, which was obtained from Monkey Diet 5000, saturated stereoisomer-selective mechanisms including hepatic α-TTP. This explanation is consistent with the elevated hepatic SYNTH-2*R* and depressed *RRR* that we observed in the MF liver.

With respect to the high accumulation of SYNTH-2*R* in the MF brain, it is interesting that the αT stereoisomer profiles of the MF tissues were remarkably similar to those of the dam breast milk. Daily breast milk consumption was estimated to be approximately 69 mL per d at 6 mo of age; therefore, it would be surprising if breast milk αT, which comprised 5% of total αT intake, directly impacted tissue profiles. Instead, we speculate that enhanced chylomicron production driven by breast milk consumption might be consistent with this observation. Human breastfed infants have higher amounts of plasma chylomicron sooner after a feeding than formula-fed infants (28) and higher fasting plasma concentrations of apoB (apoB B-48 + apoB-100) and HDL (29, 30). Similarly, this cohort of MF infant macaques had higher fasting plasma concentrations of apoB and apoA-I compared with the formula groups at 6 mo of age (17). Like retinol, dietary αT is incorporated into chylomicrons (31). Chylomicrons directly deliver fatty acids to neonatal brain in hepatectomized neonatal rats (32) and have been proposed to deliver an important amount of retinol to neonatal rodent brain (33), suggesting that brain and other tissues have access to chylomicron-associated nutrients prior to their delivery to the liver.

When considered together, the current hepatic αT stereoisomer and urinary α-CEHC observations further indicate that αT was handled differently in the MF group. Despite having the highest hepatic concentration of SYNTH-2*R*, the MF group had lower urine α-CEHC concentrations than the NAT-F group and trended toward lower concentrations than the SYN-F group. These findings are consistent with the possibility of chylomicron-dependent delivery of αT that bypasses hepatic catabolism of synthetic stereoisomers in the MF infant monkey.

Total brain αT concentrations reported here are higher than those previously reported for autopsied human infant brain (13), and are similar to those reported for an autopsied older adult human (34, 35), rat (36), and piglet (23) brain, but are lower than autopsied human centenarian brain (37). Our results confirmed those of previous reports revealing that cerebellum has lower concentrations of αT than those measured in other brain regions (38, 39).

One confounding factor of this investigation was that the MF-group infants were housed with their biologic mothers. Thus, rearing conditions for this group differed from those in the nursery-reared formula groups, including differences in feeding patterns; breastfeeding typically involves ingestion of small but frequent volumes of breast milk, while the formula-fed groups were bottle-fed at regular intervals by humans on a predetermined schedule, which generally resulted in larger and less frequent feedings. Findings derived from the MF group should be further tested in controlled studies since the MF group was different from the formula-fed groups in potentially important ways.

In summary, the current data reveal that infant formulas containing *RRR*-αT or *all rac*-αT lead to significant differences in brain αT stereoisomer profiles. It is unknown whether an increased proportion of *RRR*-αT is advantageous to the developing brain, or if the accumulation of SYNTH-2*R* stereoisomers has unfavorable outcomes. However, it is known that *RSR*- and *RSS*-αT have lower vitamin E activity than *RRR*-αT (14). Further, αT may modulate gene expression in the brain (40–42), and the αT stereoisomers may also differentially affect gene expression (15, 16). Thus, future studies should consider whether synthetic αT stereoisomers differentially affect brain development compared with *RRR*-αT. Our data indicate that a mixed diet of breast milk and solid foods containing synthetic αT stereoisomers leads to higher brain SYNTH-2*R* αT and lower brain *RRR*-αT. These findings raise key questions for human translation, as women are routinely provided supplements containing *all rac*-αT, and they accumulate SYNTH-2*R* αT in their milk. Importantly, human infants are also commonly transitioned to solid foods that are supplemented with *all rac*-αT. Whether human infants have brain αT stereoisomer profiles like those of the infant monkeys in this study is an important question raised by the current finding that may yield interesing findings in future research.

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