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M Neuringer, … , C Van Petten, L Barstad

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Research Article

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Rapid Publication

Dietary Omega-3 Fatty Acid Deficiency and Visual Loss in Infant Rhesus Monkeys

Martha Neuringer, William E. Connor, Cyma Van Petten, and Louise Barstad

Section of Clinical Nutrition and Lipid Metabolism, Department of Medicine, The Oregon Health Sciences University, Portland, Oregon 97201; Oregon Regional Primate Research Center, Beaverton, Oregon 97006

Abstract. Linolenic acid (18:3ω3) is a dietary precursor of docosahexaenoic acid (22:6ω3), the major fatty acid in the photoreceptor membranes of the retina. We hypothesized that rhesus monkeys deprived of dietary ω-3 fatty acids during prenatal and postnatal development would show plasma depletion of these fatty acids and visual impairment. Semipurified diets low in ω-3 fatty acids were fed to one group of adult female rhesus monkeys throughout pregnancy and to their infants from birth. A control group of mothers and infants received similar diets but supplying ample linolenic acid. In the plasma phospholipids of deficient infants, linolenic acid was generally undetectable and 22:6ω3 levels became progressively depleted, falling from 42% of control values at birth to 21% at 4 wk, 9% at 8 wk, and 6% at 12 wk of age. In the other plasma lipid classes, 22:6ω3 was undetectable by 12 wk. The visual acuity of the deprived infants, as measured by the preferential looking method, was reduced by one-fourth at 4 wk ($P < 0.05$) and by one-half at 8 and 12 wk ($P < 0.0005$) compared with control infants. These results suggest that ω-3 fatty acids may be an essential nutrient, and that 22:6ω3 may have a specific function in the photoreceptor membranes of the retina.

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Introduction

Essential fatty acids include two families distinguished by the position of the double bond closest to the methyl terminal group of the fatty acid chain: ω-6 fatty acids, including linoleic acid (18:2ω6)[1,2] and its longer-chain derivatives, and ω-3 fatty acids, comprising α-linolenic acid (18:3ω3) and its derivatives. Neither series of fatty acids can be synthesized by animals. Studies in rats, monkeys, and humans have established a nutritional requirement for ω-6 fatty acids (1). The effects of dietary deficiency include reduced growth and feed efficiency, reproductive failure, skin and hair changes, and liver pathology. The existence of a comparable deficiency syndrome resulting from ω-3 fatty acid deprivation has been demonstrated for fish (2), but not for mammals. Rats, even after two generations of deprivation, maintained normal growth and reproduction (3).

The tissue distribution of ω-3 fatty acids suggests that more specific tests may be necessary to detect the effects of deficiency. These fatty acids are present in high concentrations in the photoreceptor membranes of the retina (4) and in cerebral gray matter (5), primarily as docosahexaenoic acid (22:6ω3). The high content, specific incorporation, and strong retention of 22:6ω3 in these tissues suggests a specific functional role. Tests of retina and brain function in rats deprived of ω-3 fatty acids have revealed two deficits: reduced amplitude of the electroretinogram (6) and impaired ability to learn a visual discrimination (7).

Using a specific test of visual function, we have tested the effects of ω-3 fatty acid deprivation in rhesus monkeys. Deprivation is likely to have maximum impact during prenatal and

1. *Abbreviations used in this paper:* 20:4ω6, arachidonic acid; 22:6ω3, docosahexaenoic acid; 18:2ω6, linoleic acid; 18:3ω3, linolenic acid.
2. Fatty acid nomenclature: first number indicates length of carbon chain; second number, following colon, specifies number of double bonds; third number, after ω, gives number of carbons before first double bond, counting from methyl end.

early postnatal development, when these fatty acids are initially incorporated into tissue lipids. Therefore, we fed a diet low in ω-3 fatty acids to female rhesus monkeys before conception and throughout pregnancy. Their infants then received a similarly deficient diet from birth.

Methods

Adult female rhesus monkeys in the experimental group were fed a semipurified diet deficient in ω-3 fatty acids (Tables IA and B). Safflower oil was used as the sole fat source because it has a very low content of linolenic acid and a very high ratio of ω-6 to ω-3 fatty acids. Because ω-6 and ω-3 fatty acids compete for the same desaturating enzyme system, a high level of linoleic acid suppresses the conversion of linolenic acid to the longer-chain forms (primarily 22:6ω3) found in tissue phospholipids. A control group of female monkeys, matched for body weight, age, and parity, received an identical diet except that the source of fat was soybean oil, rich in linolenic acid (Table IB). Neither diet contained detectable amounts of 22:6ω3. All females received these diets for a minimum of 2 mo before conception and throughout pregnancy. To prevent ingestion of maternal breast milk, the infants were delivered by caesarian section at 160 d of gestation, 5 d earlier than average full-term gestation for rhesus monkeys. They were housed in a primate nursery. From the day of birth each infant received an artificial infant formula with the same fat source as its mother's diet (Table IA).

Blood samples were taken from adult females and infants at monthly intervals for analysis of plasma lipid fatty acid composition. At the time of caesarian section, samples were taken of both maternal blood and fetal (umbilical cord) blood. Lipids were extracted from plasma by the

Table IA. Composition of Semipurified Diets

For adult female rhesus monkeys			For infant rhesus monkeys*		
	g/100 g	*% of kcal*		*g/100 g*	*% of kcal*
Sucrose	44.8	72.3	Lactose	30.0	54.9
Corn starch	15.0		Dextrose	30.0	
Fat-free casein	12.0	14.3	Fat-free casein	17.0	15.1
Oil (safflower or soybean)	5.0	13.4	Oil (safflower or soybean)	15.0	30.0
Salt mix‡	3.2	—	Salt mix‡	4.0	—
Vitamin mix§	2.0	—	Vitamin mix§	2.5	—
Alphacel	8.0	—	Taurine	0.065	—
Water	12.0	—	Carrageenan	1.5	—

* Fed in liquid form at a concentration of 140 g/liter.

‡ Hegsted IV salt mix. Each gram contained 299.74 mg $CaCO_3$, 74.93 mg $CaHPO_4$, 0.299 mg $CuSO_4 \cdot 5H_2O$, 27.47 mg $FeC_6H_5O_7 \cdot 5H_2O$, 101.91 mg $MgSO_4 \cdot 7H_2O$, 4.99 mg $MnSO_4 \cdot 4H_2O$, 0.799 mg KI, 322.22 mg K_2HPO_4, 167.35 mg NaCl, and 0.249 mg $ZnCl_2$.

§ Each gram of vitamin mix contained 0.625 mg retinyl acetate, 5 mg α-tocopherol, 25 mg ascorbic acid, 50 mg myo-inositol, 250 mg choline chloride, 2 mg menaquinone, 2.45 mg niacin, 0.5 mg riboflavin, 0.5 mg thiamine, 0.5 mg pyridoxine, 1.5 mg calcium pantothenate, 10 μg biotin, 50 μg folic acid, 1 μg vitamin B-12, 2.5 μg crystalline cholecalciferol, and 661.86 mg dextrose (8).

Table IB. Fatty Acid Composition of Diets (Percent of Total Fatty Acids)

	Control diet (soybean oil)	ω-3 fatty acid deficient diet (safflower oil)
16:0	10.7	7.1
18:0	4.2	2.5
18:1ω9	23.7	13.3
18:2ω6	53.1	76.0
18:3ω6	0	0.3
20:3ω6	0.3	0.2
18:3ω3	7.7	0.3
ω-6/ω-3	7	255

procedure of Bligh and Dyer (9). Lipid classes were separated by thin-layer chromatography (10), and fatty acid methyl esters were analyzed by capillary column gas-liquid chromatography as previously described (11).

Visual acuity thresholds were determined at 3–4, 7–8, and 10–12 wk of age with the forced-choice preferential looking method (12), a behavioral method for assessing visual function in young human and monkey infants. The method uses the strong tendency of young infants to gaze at patterned stimuli. A 10 × 10-cm stimulus card covered with black and white stripes and a gray stimulus card of the same size and overall luminance were placed within a uniform gray background. The right-left position of the striped card and the stripe width were varied quasi-randomly. An observer, knowing neither the position nor the size of the stripes, watched a monkey infant held 35 cm from the stimuli and judged whether the animal looked at the left or right stimulus. A look toward the striped card, as judged by the observer, was considered a correct response. At least 20 trials were presented at each of five or six stripe widths that bracketed the animals' acuity threshold. Percent correct judgements were plotted as a function of the spatial frequency of the stripes, and the spatial frequency corresponding to 75% correct was taken as the acuity threshold. Refractions were performed by an expert retinoscopist after paralysis of accommodation with 1% cyclopentolate to determine whether refractive errors, as opposed to retinal and neural factors, contributed to differences in acuity.

Results

Levels of linolenic acid in the ω-3 fatty acid deficient (safflower oil) group fell to <15% of the levels in the control (soybean oil) group in all plasma lipid classes of both adult females and infants. Linolenic acid was generally undetectable (<0.1% of total fatty acids) in the infants' plasma phospholipids (Table II). Levels of 22:6ω3, the ω-3 fatty acid prominent in retinal and brain tissue, became severely and progressively depleted in infants of the deficient group. Levels of 22:6ω3 in plasma phospholipids were

Table II. Major Polyunsaturated Fatty Acids in Plasma Phospholipids of ω-3 Fatty Acid Deficient (D) and Control (C) Rhesus Monkeys

		Mothers				Infants					
Fatty acid	Diet	Base line	At delivery	*P*, change with time within groups*	*P*, difference between diet groups‡	Birth	4 wk	8 wk	12 wk	*P*, change with time within groups*§	*P*, difference between diet groups‡§
18:2ω6	D	25.7±0.7	27.9±1.1	NS	NS	16.9±1.4	26.6±2.1	30.1±1.5	32.7±1.1	<0.001	NS
	C	(26.7±0.7)	(25.0±1.1)	NS		(17.7±1.3)	(30.3±1.4)	(30.6±2.2)	(34.7±0.9)	<0.001	
20:4ω6	D	6.3±0.5	7.0±1.0	NS	NS	16.6±2.4	12.3±1.3	10.9±0.9	10.3±0.6	<0.001	<0.005
	C	(5.9±0.5)	(4.9±1.0)	NS		(13.4±1.1)	(9.1±1.3)	(8.4±0.7)	(7.0±0.4)	<0.002	
Total ω-6	D	34.6±1.3	46.6±2.2	<0.002	<0.02	46.3±4.2	48.6±2.4	48.0±0.9	49.3±0.6	NS	<0.005
	C	(35.0±0.8)	(36.8±2.1)	NS		(38.5±1.8)	(44.0±0.9)	(42.9±1.9)	(45.9±0.8)	<0.01	
18:3ω3	D	0.10±0.00	0.05±0.03	NS	<0.001	0.00±0.00	0.01±0.01	0.00±0.00	0.01±0.01	NS	<0.001
	C	(0.16±0.03)	(0.60±0.10)	<0.005		(0.26±0.13)	(0.34±0.02)	(0.36±0.03)	(0.43±0.04)	NS	
20:5ω3	D	0.85±0.10	0.00±0.00	<0.001	<0.05	0.00±0.00	0.01±0.01	0.03±0.02	0.04±0.02	NS	<0.001
	C	(0.87±0.10)	(0.33±0.08)	<0.005		(0.27±0.05)	(0.50±0.11)	(0.39±0.05)	(0.50±0.04)	<0.05	
22:5ω3	D	0.97±0.13	0.30±0.13	<0.001	NS	0.28±0.10	0.23±0.13	0.15±0.06	0.18±0.04	NS	<0.001
	C	(1.03±0.12)	(0.96±0.27)	NS		(0.96±0.16)	(1.56±0.35)	(1.53±0.19)	(1.37±0.15)	NS	
22:6ω3	D	4.29±0.52	1.34±0.34	<0.001	NS	2.28±0.41	0.73±0.26	0.23±0.03	0.14±0.02	<0.001	<0.001
	C	(4.60±0.90)	(1.50±0.33)	<0.02		(5.40±0.95)	(3.47±0.79)	(2.67±0.18)	(2.35±0.20)	<0.01	
Total ω-3	D	6.26±0.71	1.76±0.45	<0.001	NS	2.57±0.47	1.00±0.35	0.48±0.11	0.43±0.06	<0.001	<0.001
	C	(6.81±1.13)	(3.86±0.65)	<0.05		(7.01±1.28)	(6.09±1.17)	(5.11±0.34)	(4.85±0.36)	NS	

D, ω-3 fatty acid deficient group (safflower oil diets, $n = 7$); c (with values in parentheses), control group (soybean oil diets, $n = 8$). Values for each fatty acid represent weight percent of total fatty acids (mean±SEM). Total ω-6 includes *cis*-18:2ω6, 18:3ω6, 20:2ω6, 20:3ω6, 20:4ω6, 22:4ω6, 22:5ω6; total ω-6 includes *cis*-18:3ω3, 18:4ω3, 20:3ω3, 20:4ω3, 20:5ω3, 22:5ω3, 22:6ω3.

* Results of one-way analysis of variance with repeated measures; *P* value for significance of change across time within each diet group.

‡ Results of two-way analysis of variance with repeated measures; *P* value for significance of overall difference between diet groups.

§ A few infant samples were missing at some time points (6 out of 60 samples) due to illness, failure to obtain sufficient blood, or, in two cases at birth, due to vaginal deliveries before the planned caesarian deliveries. Missing values were estimated by multiple linear regression and degrees of freedom were reduced accordingly.

reduced by 79% at 4 wk, 91% at 8 wk, and 94% at 12 wk of age, compared with values in the control group (Table II). 22:6ω3 was undetectable by 12 wk in the plasma cholesterol esters, triglycerides, and free fatty acids. Fig. 1 summarizes the levels of total ω-3 fatty acids and 22:6ω3 in the plasma phospholipids of mothers and infants at delivery and of infants at 12 wk of age. ω-6 fatty acids, particularly arachidonic acid (20:4ω6), increased significantly and replaced ω-3 fatty acids in the plasma lipids of ω-3 fatty acid deficient infants. At delivery the levels of 22:6ω3 and 20:4ω6 in umbilical cord blood were significantly higher than in maternal blood in both diet groups; that is, the fetus appeared to be preferentially supplied with these fatty acids.

Visual acuity thresholds were significantly lower in the ω-3 fatty acid deficient infants than in the control infants at all ages (Fig. 2). The difference increased between 4 and 8 wk of age. At 8 and 12 wk the mean thresholds of the deficient infants were reduced by approximately a factor of two, and were comparable to values for normal infants half their age. Control infants attained mean acuity thresholds very similar to those reported previously for normal macaque infants at these ages (13). Refractions demonstrated that all infants of both diet groups, like most human infants, were slightly farsighted. However, refractive errors were not correlated with diet or with acuity thresholds and could not account for the acuity difference between groups.

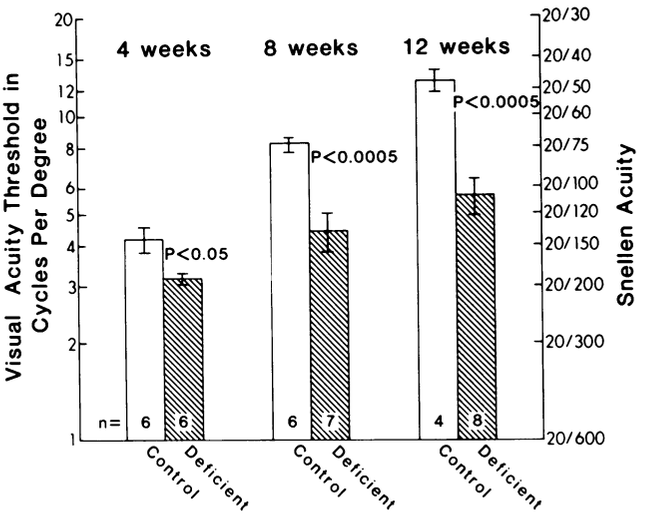

Figure 2. Visual acuity thresholds (mean±SEM) of control (soybean oil diet) and ω-3 fatty acid deficient (safflower oil diet) infant rhesus monkeys. Thresholds represent the smallest stripe width eliciting preferential looking, expressed in cycles per degree of visual angle and in the Snellen equivalents. *P* values (one-tailed) are determined by *t* test. $n = 8$ in deficient group; $n = 7$ in control group. Some infants failed to complete testing at all three ages due to illness or lack of cooperation with the testing procedure. Age was calculated by counting 160 d of gestation as day 0 for all infants.

Discussion

The existence of a human dietary requirement for ω-3 fatty acids has remained an unresolved issue (14). The present finding, that infant monkeys show a visual deficit associated with ω-3 fatty acid depletion, supports the proposed essentiality of these fatty acids in human nutrition. Human milk contains significant amounts of both 18:3ω3 and 22:6ω3. Most synthetic infant formulas contain no 22:6ω3 but high levels of 18:3ω3, however some, formulated with corn oil as the source of polyunsaturates, contain only slightly more 18:3ω3 than our deficient diet. In view of our results, the ω-3 fatty acid content of infant formulas may be an important consideration.

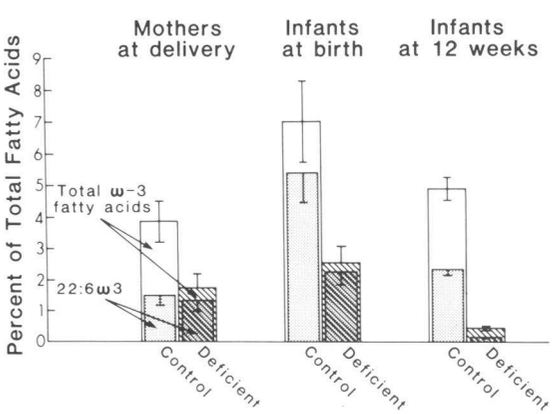

Figure 1. Levels of 22:6ω3 and total ω-3 fatty acids (mean±SEM) in plasma phospholipids of control (soybean oil diet) and ω-3 fatty acid deficient (safflower oil diet) rhesus monkeys.

Holman et al. (15) recently proposed linolenic acid deficiency as the cause of peripheral neuropathy and blurred vision in a 4-yr-old child receiving total parenteral nutrition. As in the present study, safflower oil was the source of fat. Replacement of the safflower oil emulsion with a soybean oil preparation (relatively rich in linolenic acid) was reported to correlate with disappearance of the clinical symptoms. This case report was only suggestive, however, because long-term parenteral nutrition can produce other metabolic imbalances with similar symptoms.

One aspect of our results bears on the capacity of monkeys to desaturate and elongate 18-carbon fatty acids to their longer-chain metabolites. In the soybean oil-fed adult females, the levels of 18:3ω3 quadrupled by the time of delivery, reflecting the high level of 18:3ω3 in the soybean oil diet compared with the stock diet consumed before this experiment (7.7% of total fatty acids vs. 2.3%). However, despite this increase, levels of 22:6ω3 fell progressively and to almost the same extent as in adult females fed safflower oil (Table II). This result suggests a very low capacity to convert 18:3ω3 to 22:6ω3. Unlike the soybean oil diets, the base-line stock diet contained some 22:6ω3 (0.3% of total fatty acids) plus other longer-chain ω-3 fatty acids (0.4% 20:5ω3 and 0.1% 22:5ω3). That plasma phospholipids of

females receiving this diet contained >4% of total fatty acids as 22:6ω3 suggests that this fatty acid, once present in the body, was very selectively retained.

Despite the low plasma phospholipid levels of 22:6ω3 in the adult females fed soybean oil, their infants had high levels at delivery. In both diet groups, the level of 22:6ω3 was two to four times higher in cord blood than in maternal blood. This was true despite the fact that 18:3ω3 was undetectable in the plasma phospholipids of all safflower oil infants. A similar relative enrichment of fetal over maternal blood was seen for 20:4ω6 in both groups. In contrast, the precursors of both families, 18:3ω3 and 18:2ω6, were lower in fetal than in maternal blood. Thus, the fetus is preferentially supplied with the longer-chain ω-3 and ω-6 fatty acids needed for incorporation into developing tissues. The same finding was recently reported by Crawford et al. (16) for human fetuses. The mechanism responsible for this selective enrichment is not clear. Either the fetus or placenta has an increased capacity to synthesize longer-chain fatty acids from 18:3ω3 and 18:2ω6, or the longer-chain fatty acids are preferentially transferred across the placenta from the maternal to the fetal circulation.

This study has demonstrated that nonhuman primates deprived of dietary ω-3 fatty acids during gestation and infancy show depletion of ω-3 fatty acids from plasma lipids, and that this depletion is associated with a significant impairment in the development of visual acuity. This finding provides the first evidence for a functional requirement for ω-3 fatty acids in primates. As we have ruled out refractive error as an important factor, we hypothesize that the visual loss was related to biochemical changes in the retina and brain, specifically, reduced content of 22:6ω3 in photoreceptor membranes and/or the central visual system.

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