

Biochemical and functional effects of prenatal and postnatal ω 3 fatty acid deficiency on retina and brain in rhesus monkeys

(linolenic acid/docosahexaenoic acid/essential fatty acids)

MARTHA NEURINGER*[†], WILLIAM E. CONNOR*[†], DON S. LIN*, LOUISE BARSTAD*, AND STEVEN LUCK*[†]

*Section of Clinical Nutrition and Lipid Metabolism, Department of Medicine, The Oregon Health Sciences University, Portland, OR 97201; and [†]Division of Metabolic and Immune Diseases, Oregon Regional Primate Research Center, Beaverton, OR 97006

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ABSTRACT Docosahexaenoic acid [22:6 ω 3; 22:6-(4,7,10,13,16,19)] is the major polyunsaturated fatty acid in the photoreceptor membranes of the retina and in cerebral gray matter. It must be obtained either from the diet or by synthesis from other ω 3 fatty acids, chiefly α -linolenic acid (18:3 ω 3). We tested the effect of dietary ω 3 fatty acid deprivation during gestation and postnatal development upon the fatty acid composition of the retina and cerebral cortex and upon visual function. Rhesus monkeys (*Macaca mulatta*) were fed semipurified diets very low in 18:3 ω 3 throughout pregnancy, and their infants received a similar diet from birth. A control group of females and their infants received a semipurified diet supplying ample 18:3 ω 3. In near-term fetuses and newborn infants of the deficient group, the 22:6 ω 3 content of phosphatidylethanolamine was one-half of control values in the retina and one-fourth in cerebral cortex. By 22 months of age, the content of 22:6 ω 3 in these tissues approximately doubled in control monkeys, but it failed to increase in the deficient group. Low levels of 22:6 ω 3 in the deficient animals' tissues were accompanied by a compensatory increase in longer-chain ω 6 fatty acids, particularly 22:5 ω 6. Functionally, the deficient animals had subnormal visual acuity at 4-12 weeks of age and prolonged recovery time of the dark-adapted electroretinogram after a saturating flash. Abnormally low levels of 22:6 ω 3 may produce alterations in the biophysical properties of photoreceptor and neural membranes that may underlie these functional impairments. The results of this study suggest that dietary ω 3 fatty acids are essential for normal prenatal and postnatal development of the retina and brain.

Neither linoleic acid (18:2 ω 6)[‡] nor α -linolenic acid (18:3 ω 3) can be synthesized by animals. Linoleic acid is the precursor of arachidonic acid (20:4 ω 6) and other longer-chain ω 6 fatty acids, whereas linolenic acid is the precursor of the longer-chain ω 3 fatty acids, including docosahexaenoic acid [22:6 ω 3; 22:6(4,7,10,13,16,19)]. These longer-chain fatty acids are important constituents of tissue lipids. The ω 6 and ω 3 series of fatty acids are not interconvertible.

Linoleic acid was defined as an essential fatty acid 50 years ago, because its absence from the diet leads to overt symptoms including dermatitis, kidney and liver pathology, impaired growth, and reproductive failure (1, 2). The nutritional importance of linolenic acid and the longer-chain ω 3 fatty acids, on the other hand, has remained unclear. A deficiency syndrome produced by dietary deprivation of ω 3 fatty acids has been defined for fish (3) but not for mammals.

Docosahexaenoic acid (22:6 ω 3) is the primary ω 3 fatty acid in many tissues. The highest levels of 22:6 ω 3 are found in the retina and in the cerebral cortex, specifically in the phospholipids of photoreceptor outer segment membranes and of synaptosomal membranes. With six double bonds,

22:6 ω 3 is unique in its high level of polyunsaturation. Depletion of this fatty acid from membranes might be expected to alter their physical and functional properties. In rats, dietary deprivation of ω 3 fatty acids has been associated with a decrease in the amplitude of the electroretinogram and an impairment in the ability to learn a visual discrimination (4, 5).

Holman *et al.* (6) described a clinical case of peripheral neuropathy and blurred vision that they ascribed to ω 3 fatty acid deficiency. The patient was a child maintained on long-term total parenteral nutrition with a safflower oil emulsion, very low in linolenic acid, as the only source of fat. The symptoms disappeared after the administration of a soybean oil emulsion rich in linolenic acid.

We recently demonstrated in rhesus monkeys that dietary deprivation of ω 3 fatty acids during gestation and infancy produced depletion of ω 3 fatty acids from the lipids of plasma and an impairment in the development of visual acuity during the first 3 postnatal months (7). In the present paper, we describe the alterations in fatty acid composition of the retina and brain produced by ω 3 fatty acid deprivation at different stages of fetal and postnatal development, and we assess the relative impact of maternal versus postnatal dietary manipulation on the fatty acid composition of neural tissues. We also further document changes in visual function, including visual acuity and recovery time of the electroretinogram.[§]

METHODS

Adult female rhesus monkeys (*Macaca mulatta*) were fed semipurified diets for at least 2 months before conception and throughout pregnancy. The resulting infants were fed liquid semipurified diets from birth. The composition of control and ω 3 fatty acid-deficient diets has been previously described in detail (7). For the control mothers and their infants, the fat source was soybean oil, which contains ample amounts of both linolenic and linoleic acids. For the deficient animals, the sole fat source was safflower oil, which contains very low levels of linolenic acid and high levels of linoleic acid. The resulting high ratio of linoleic to linolenic acid suppresses the conversion of linolenic acid to its longer-chain derivatives and thereby exacerbates the effect of the deficient diet. The fatty acid contents of the experimental diets are presented in Table 1. The fat content for the adult diets was 5% by weight or 13.4% of kcal, and for the infant formula diets it was 15% by weight or 30% of kcal. There were no differences between the two groups in food intake or body weight.

[‡]Fatty acid nomenclature: first number indicates length of carbon chain; second number, after colon, specifies number of double bonds; third number, after ω , gives number of carbons before first double bond, counting from the methyl end of the chain.

[§]Preliminary reports of parts of this work were presented at the Annual Meetings of the Association of American Physicians, Washington, DC, May 1984, and the Association for Research in Vision and Ophthalmology, Sarasota, FL, May 1985, and appeared in refs. 8 and 9.

Table 1. Fatty acid compositions of control and ω 3 fatty acid-deficient semipurified diets for rhesus monkeys

Fatty acid	% (wt/wt) of total fatty acids	
	Control diet (soybean oil)	Deficient diet (safflower oil)
Saturated	14.9	9.6
18:1 ω 9	23.7	13.3
18:2 ω 6	53.1	76.0
Total ω 6	53.4	76.5
18:3 ω 3	7.7	0.3
22:6 ω 3	0	0
Total ω 3	7.7	0.3
ω 6/ ω 3	7	255

Two procedures were used to assess the functional integrity of the retina and visual system. First, the visual acuity of the infants was measured behaviorally at 4, 8, and 12 weeks of age by the preferential looking method, as described in a previous paper (7). Briefly, this method takes advantage of human or animal infants' strong inherent preference for looking at patterned stimuli as a means to determine their ability to detect black and white high-contrast stripes within an unpatterned gray background. Responses to stripes of different widths are used to define a threshold for visual acuity. Second, the electroretinogram, a potential change evoked from the retina by light stimulation, was recorded with a contact lens corneal electrode while the animals were under thiamylal anesthesia. Recording and stimulating procedures have been described previously (10). For the purposes of this paper, particular attention was given to the response of the dark-adapted eye to saturating flashes, that is, those sufficiently bright to evoke a response of maximal amplitude [intensity = 0.5 millilambert-sec (1.6 cd/m²-sec)]. Flashes of this intensity must be presented at intervals of 20 sec or more to allow for full recovery of the response between flashes. In this study, these flashes were presented at intervals varying from 3.2 to 30 sec, to determine the degree to which the response was reduced by the shorter intervals and the time required for the response to recover to full amplitude.

At approximately 22 months of age, 2 animals in each diet

group were killed while they were under deep ketamine anesthesia, and tissues, including occipital and frontal cerebral cortex (gray matter) and retina, were taken for lipid fatty acid analyses. In 2 additional 22-month-old deficient animals, 20- to 30-mg biopsy samples of frontal cortex were obtained by craniotomy. Tissues were also taken from 10 fetuses and infants, 5 from each diet group, that were born prematurely, were stillborn, or died of causes unrelated to the experimental treatment. The ages of these fetuses and infants in the deficient group were 130 and 132 days postconception (average full-term gestation for rhesus monkeys = 165 days) and 0, 6, and 16 days after birth. For the control group, the ages were 160 days postconception and 11, 12, 16, and 18 days after birth. Tissues were obtained within 2 hr of death and were stored at -20°C until analyzed.

Tissue lipids were extracted by the method of Folch *et al.* (11), and thin-layer chromatography was utilized to separate the phospholipid classes of occipital and frontal cerebral cortex (12) and retina (13). The fatty acids of each phospholipid class were methylated and were then analyzed by capillary column gas-liquid chromatography (14).

Differences in the content of selected fatty acids as a function of diet and age were evaluated by 2 × 2 factorial analysis of variance and, when overall *F* values indicated significant differences, by calculation of the *F* value for simple effects of diet within each age group and simple effects of age within each diet group (15).

RESULTS

At all ages, animals of the ω 3 fatty acid-deficient group had considerably lower levels of ω 3 fatty acids in tissue phospholipids than their controls. Levels of the major fatty acids in phosphatidylethanolamine—the most prominent phospholipid containing high levels of polyunsaturated fatty acids—are presented in Table 2 for the occipital cortex, frontal cortex, and retina. Fig. 1 depicts graphically the data for occipital cortex. In near-term fetuses and infants of the deficient group, the phosphatidylethanolamine of occipital cortex contained one-fourth as much 22:6 ω 3 as in control newborns (Fig. 1A). However, in deficient tissues there was a notable compensatory increase in the longer-chain ω 6 fatty

Table 2. Major fatty acids of brain and retinal phosphatidylethanolamine in control and ω 3 fatty acid-deficient rhesus monkeys at

Fatty acid*	Occipital cortex				Frontal cortex			
	Control		Deficient		Control		Deficient	
	Perinatal (n = 5)	22 months (n = 2)	Perinatal (n = 5)	22 months (n = 2)	Perinatal (n = 5)	22 months (n = 2)	Perinatal (n = 5)	22 months (n = 4)
18:0	30.1 ± 2.9	30.5 ± 0.1	24.9 ± 5.7	26.9 ± 5.9	24.1 ± 3.2	31.4 ± 2.2 [†]	26.7 ± 2.5	29.6 ± 2.9
Total saturated	38.5 ± 2.7	38.2 ± 2.5	36.7 ± 4.5	37.4 ± 5.4	30.7 ± 3.4	39.2 ± 1.6 [†]	38.8 ± 4.1 [¶]	39.2 ± 2.2
18:1 ω 9	5.9 ± 1.2	7.9 ± 0.3	7.4 ± 1.3	7.0 ± 0.1	6.9 ± 0.8	7.1 ± 1.2	8.5 ± 1.8	6.9 ± 0.6
Total mono	10.2 ± 3.1	10.8 ± 2.2	15.0 ± 1.8	9.1 ± 0.8	12.2 ± 2.9	10.3 ± 1.6	13.9 ± 3.9	10.1 ± 1.3
20:4 ω 6	16.8 ± 2.0	7.9 ± 0.4 [‡]	18.0 ± 4.4	10.2 ± 0.9	17.9 ± 2.1	12.4 ± 0.9 [†]	16.2 ± 1.7	12.6 ± 1.5 [†]
22:4 ω 6	12.9 ± 4.0	5.3 ± 0.2 [‡]	9.8 ± 1.6	10.2 ± 0.1 [¶]	11.8 ± 1.7	9.4 ± 0.8	10.4 ± 2.2	11.6 ± 2.0
22:5 ω 6	3.7 ± 0.2	0.5 ± 0.1 [‡]	10.5 ± 2.4 [¶]	22.5 ± 2.3 ^{¶¶}	4.0 ± 1.2	1.4 ± 0.3 [†]	11.0 ± 3.2 [¶]	18.3 ± 2.5 ^{¶¶}
Total ω 6	33.7 ± 3.6	15.7 ± 0.2 [‡]	40.7 ± 5.6 [§]	45.3 ± 3.3 [¶]	35.8 ± 4.3	24.9 ± 1.5 [†]	39.9 ± 6.2	44.2 ± 4.0 [¶]
22:6 ω 3	15.0 ± 2.5	34.0 ± 0.8 [‡]	4.0 ± 2.3 [¶]	5.8 ± 0.6 [¶]	15.6 ± 3.2	22.3 ± 0.3 [†]	3.5 ± 1.6 [¶]	3.8 ± 0.4 [¶]
Total ω 3	15.2 ± 2.3	35.0 ± 0.7 [‡]	4.0 ± 2.3 [¶]	6.0 ± 0.5 [¶]	16.3 ± 3.5	22.5 ± 0.5	3.5 ± 1.6 [¶]	3.8 ± 0.4 [¶]
Total ω 6 + ω 3	48.9 ± 5.4	50.7 ± 0.5	44.7 ± 6.0	51.4 ± 2.9	52.1 ± 6.5	47.3 ± 1.0	43.4 ± 6.6	48.0 ± 3.7

All results are percent (wt/wt) of total fatty acids, presented as mean ± SD.

*Total saturated includes all saturated fatty acids with 12 to 24 carbons. Total mono includes the following monounsaturated fatty acids: 14:1, 24:1 ω 9. Total ω 6 includes *cis*-18:2 ω 6, 18:3 ω 6, 20:2 ω 6, 20:3 ω 6, 20:4 ω 6, 22:4 ω 6, and 22:5 ω 6. Total ω 3 includes *cis*-18:3 ω 3, 18:4 ω 3, 20:3 ω 3, 20:4 ω 3.

^{††}Significant difference between ages within each diet group: †, *P* < 0.05; ‡, *P* < 0.01 (two-tailed).

^{§¶}Significant difference between diet groups at a given age: §, *P* < 0.05; ¶, *P* < 0.01 (two-tailed).

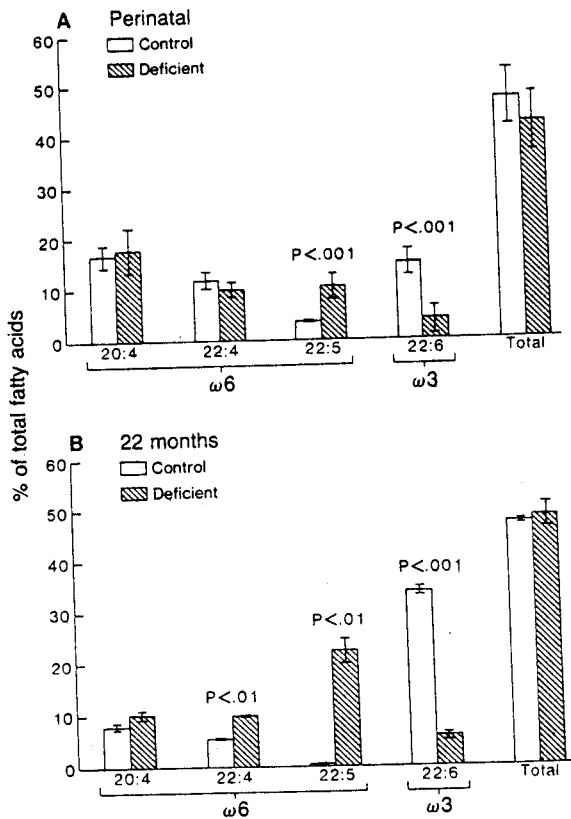


FIG. 1. Major $\omega 6$ and $\omega 3$ fatty acids of phosphatidylethanolamine in the occipital cortex of rhesus monkeys during the perinatal period (A) and at 22 months of age (B): Comparison of control (open bars) and $\omega 3$ fatty acid-deficient (hatched bars) groups. Values are expressed as percent (wt/wt) of total fatty acids (mean \pm SD). P values are given for significant differences between diet groups at each age. "Total" is the sum of the four individual fatty acids for which data are shown.

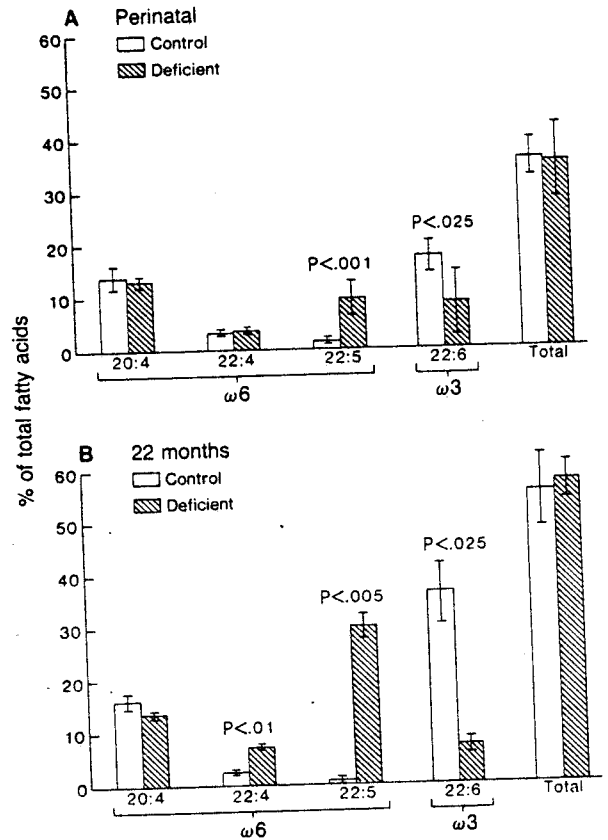


FIG. 2. Major $\omega 6$ and $\omega 3$ fatty acids of phosphatidylethanolamine in the retina of rhesus monkeys during the perinatal period (A) and at 22 months of age (B). See legend for Fig. 1.

acid, 22:5. Therefore, despite large alterations in the amount of 22:6 $\omega 3$, the sum of 20- and 22-carbon polyunsaturated fatty

acids ($\omega 3$ plus $\omega 6$) remained essentially unchanged. The same pattern was seen in frontal cortex (Table 2). As regards the retina (Fig. 2A), the perinatal deficient group had half as much 22:6 $\omega 3$ in phosphatidylethanolamine as control newborns, and they showed the same compensatory increase in 22:5.

different ages

Retina			
Control		Deficient	
Perinatal (n = 5)	22 months (n = 2)	Perinatal (n = 4)	22 months (n = 2)
29.6 \pm 6.6	23.8 \pm 6.0	30.5 \pm 1.9	28.6 \pm 1.0
42.8 \pm 5.6	30.6 \pm 4.9 [†]	45.0 \pm 8.0	32.7 \pm 2.6
7.9 \pm 1.4	5.9 \pm 1.1	8.9 \pm 1.6	4.2 \pm 0.4 [†]
11.2 \pm 1.1	8.8 \pm 1.1	12.2 \pm 3.3	5.0 \pm 0.3 [†]
13.9 \pm 2.3	16.0 \pm 1.6	13.2 \pm 1.2	13.4 \pm 0.7
3.3 \pm 0.6	2.4 \pm 0.4	3.7 \pm 0.8	7.2 \pm 0.4 ^{†¶}
1.5 \pm 0.6	0.7 \pm 0.7	9.6 \pm 3.3 [¶]	29.8 \pm 2.4 ^{†¶}
22.6 \pm 3.0	22.6 \pm 2.3	29.8 \pm 4.3 [§]	57.7 \pm 3.0 ^{†¶}
17.3 \pm 2.9	36.4 \pm 5.8 [†]	8.6 \pm 6.0 [§]	7.1 \pm 1.6 [§]
18.7 \pm 3.2	37.2 \pm 4.7 [†]	8.9 \pm 6.2 [§]	7.3 \pm 1.6 [§]
41.2 \pm 6.0	59.8 \pm 7.0 [†]	38.7 \pm 8.0	65.0 \pm 1.4 [†]

16:1 $\omega 7$, 17:1 $\omega 9$, *trans*-18:1, 18:1 $\omega 9$, 20:1 $\omega 9$, 22:1 $\omega 11$, 22:1 $\omega 9$, and 20:5 $\omega 3$, 22:5 $\omega 3$, and 22:6 $\omega 3$.

Between birth and 22 months of age, the percentage of 22:6 $\omega 3$ in the phosphatidylethanolamine of the occipital cortex more than doubled in control animals (Fig. 3A). In deficient animals, on the other hand, concentrations of 22:6 $\omega 3$ failed to increase (Fig. 3B) and at 22 months they were only one-sixth of control values (Fig. 1B). The $\omega 6$ fatty acid 22:5 declined from its already low perinatal levels in controls, whereas in the deficient animals, the proportion of this fatty acid doubled. This $\omega 6$ fatty acid reached levels 45 times greater than in controls, and thereby almost completely replaced 22:6 $\omega 3$. Another long-chain $\omega 6$ fatty acid, 22:4, was also increased in the deficient group. Thus, as the deficient animals matured, the total 20- and 22-carbon polyunsaturated fatty acids continued to be maintained at control levels despite low levels of 22:6 $\omega 3$. The same pattern of developmental changes occurred in the retina (Fig. 2B) and in frontal cortex (Table 2) between the perinatal period and 22 months of age.

Similar patterns were found in other phospholipid classes. Phosphatidylserine was most similar to phosphatidylethanolamine in that it normally contained high levels of polyunsaturated fatty acids, including 22:6 $\omega 3$; it was similarly affected by age and by $\omega 3$ fatty acid deficiency. Phosphatidylcholine and phosphatidylinositol, although they contained far less 22:6 $\omega 3$ than the other two classes, also had similar percent reductions of 22:6 $\omega 3$ content in the tissues of deficient animals.

These alterations in retina and brain fatty acid composition of the deficient animals were associated with two functional abnormalities. First, electroretinographic recordings, performed at 21 months of age, or within 1 month of the biochemical analyses, demonstrated an impairment in the recovery of the dark-adapted response to saturating flashes—i.e., those flashes sufficiently bright to elicit maximal response amplitudes. At long intervals (20 sec or more), responses were as large in deficient animals as in controls. However, the suppressive effect of shorter intervals was much greater in deficient animals (Fig. 4). At 3.2-sec intervals, responses were reduced to 74% of maximal amplitude in control animals; in deficient animals, they were reduced far more, to 51% of maximal amplitude. Fig. 4 includes individual values from the same animals from which tissues were taken at 22 months for fatty acid analyses. The mean time required for recovery of the response to 80% of its full amplitude was 3.9 sec (SEM = 0.3 sec) in the control group and 6.2 sec (SEM = 0.4 sec) in the deficient group.

The second functional deficit was demonstrated by behavioral tests of visual acuity. Deficient infants had significantly higher visual acuity thresholds (that is, poorer acuity) at 4–12 weeks of age (Fig. 5). These data, some of which were reported previously (7), now include results for six new animals. Highlighted in the figure are individual values for the animals for which biochemical results are described above. Thus, ω 3 fatty acid deficiency was accompanied by a visual sensory deficit as well as an impairment in the physiological response of the retina.

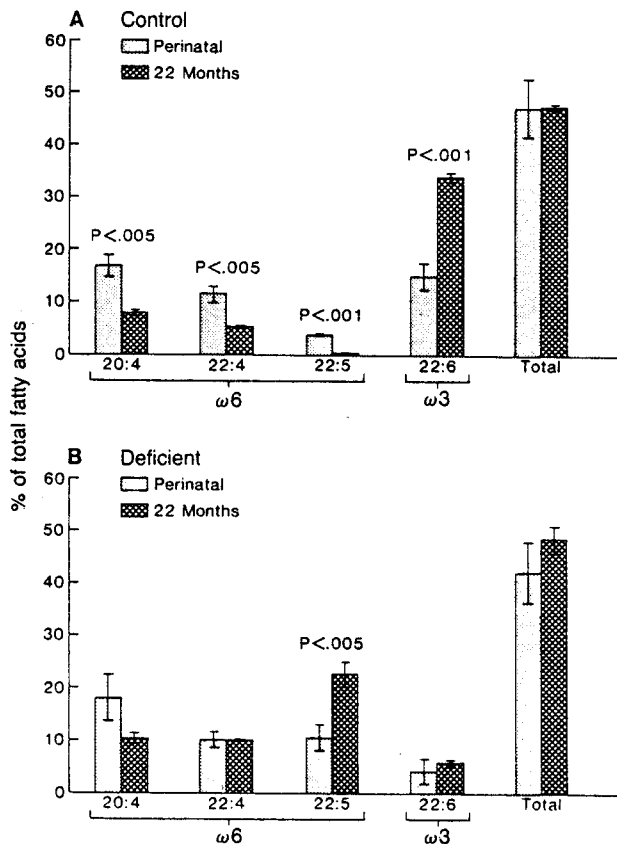


FIG. 3. Major ω 6 and ω 3 fatty acids of phosphatidylethanolamine in the occipital cortex of control (A) and ω 3 fatty acid-deficient (B) rhesus monkeys: Comparison of perinatal (stippled bars) and 22-month-old (cross-hatched bars) groups. Values are expressed as percent (wt/wt) of total fatty acids (mean \pm SD). *P* values are given for significant differences between ages within each diet group. "Total" is the sum of the four individual fatty acids for which data are shown.

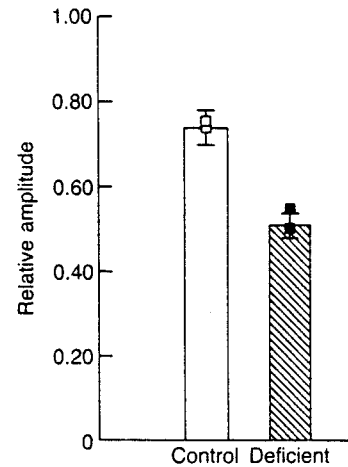


FIG. 4. The effect of short (3.2-sec) intervals between flashes on the amplitude of the dark-adapted electroretinogram B-wave response. Relative amplitude (mean \pm SEM) represents response amplitude at 3.2-sec intervals as a fraction of the maximal response amplitude obtained at intervals of 20 sec or more. Maximal amplitudes did not differ between groups, but there was a significant difference ($P < 0.01$ by Student's *t* test) in relative amplitude—i.e., the responses of deficient animals were more affected by reducing the interval between flashes. Squares indicate values for those individual animals for which retinal and brain fatty acid composition was determined at 22 months. For control group, $n = 7$; for deficient group, $n = 6$.

DISCUSSION

These results demonstrate that the high content of ω 3 fatty acids normally present in the cerebral cortex and retina is greatly reduced by a combination of maternal and postnatal dietary deprivation in rhesus monkeys. Most importantly, this depletion is associated with functional changes in the retina and visual system. Similar biochemical results were previously reported in rats for whole brain (5, 16) and retina (16) after two generations of ω 3 fatty acid deprivation.

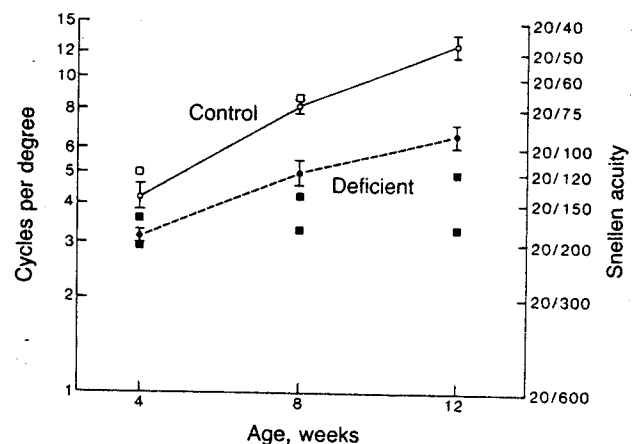


FIG. 5. Visual acuity thresholds (mean \pm SEM) as determined by the preferential looking method for control and ω 3 fatty acid-deficient infant monkeys. Thresholds are expressed in cycles per degree of visual angle and in the equivalent Snellen values. Mean values for the control group (\circ) include measurements from 8 infants at 4 and 8 weeks and 6 at 12 weeks; mean values for the deficient group (\bullet) include values for 10 subjects at 4 weeks, 11 at 8 weeks, and 12 at 12 weeks. Differences between groups were statistically significant at each age as determined by Student's *t* test ($P < 0.01$ at 4 weeks, $P < 0.001$ at 8 and 12 weeks). Squares (\square , control; \blacksquare , deficient) represent individual values for those animals for which biochemical data are presented. Acuity measurements were not obtained for one such control monkey.

