# Incorporation of docosahexaenoic acid into nerve membrane phospholipids: bridging the gap between animals and cultured cells[1–3]

*Jean-Marc Alessandri, Carine Poumès-Ballihaut, Bénédicte Langelier, Marie-Hélène Perruchot, Gilda Raguénez, Monique Lavialle, and Philippe Guesnet*

**ABSTRACT**

**Background:** Functional maturation of nervous tissues depends on membrane accretion of docosahexaenoic acid (DHA). Animal studies have shown that incorporation of dietary DHA into membrane phospholipids is dose dependent. The molecular effects of DHA are commonly studied in cultured cells, but questions remain about the physiologic connection between animal and cell models.

**Objective:** We developed a linear model for comparing the responses of rat nervous tissues to dietary DHA with the responses of human cell lines to DHA in medium.

**Design:** Rats were rendered chronically deficient in n−3 fatty acids by being reared on a peanut oil diet. DHA status was replenished in the F2 generation by using increasing supplements of a microalgal oil. Human retinoblastoma and neuroblastoma cells were dosed with unesterified DHA. DHA accumulation into phospholipids was defined by the plateau of the dose-response curve ($DHA_{max}$) and by the supplement required to produce one-half the $DHA_{max}$ ($DHA_{50}$).

**Results:** The $DHA_{max}$ values for 4 brain regions and 2 neuroblastoma lines were similar, and the value for the retinoblastoma line was similar to the retinal value. Expressing the DHA input as μmol/10 g diet and as μmol/L medium resulted in similar values for the ratio of $DHA_{max}$ to $DHA_{50}$ in the 4 brain regions and the 3 cell lines. The $DHA_{max}$-$DHA_{50}$ ratios in the ethanolamine phosphoglyceride and phosphatidylcholine fractions in retinal phospholipids were 6 and 10 times, respectively, those in the brain and cultured cells.

**Conclusions:** The dose-dependent responses of cells and the brain to DHA supplements can be compared by using $DHA_{max}$-$DHA_{50}$ ratios. We propose a counting frame that allows the comparison of the dose responses of the brain and cells to exogenous DHA. *Am J Clin Nutr* 2003;78:702–10.

**KEY WORDS** Docosahexaenoic acid, ethanolamine glycerophospholipids, phosphatidylcholine, brain, retina, hippocampus, cerebellum, striatum, cortex, neuroblastoma, retinoblastoma

## INTRODUCTION

Docosahexaenoic acid (DHA, 22:6n−3) is the major polyunsaturated fatty acid in the membranes of brain and retinal cells. The accretion of DHA is crucial for the perinatal development of nervous tissues (*see* review in reference 1). Membrane DHA originates from dietary DHA and by conversion of dietary α-linolenic acid (ALA, 18:3n−3) (*see* review in reference 2). The functional deficits that are associated with a low DHA content in developing organs have been investigated in animals fed a diet lacking n−3 fatty acids (3–6). Such n−3 deficient animals were also used to determine whether supplementing diets with ALA or DHA increases DHA concentrations in depleted nervous tissues (7–10) and normalizes the development of the brain and the visual system (11). Studies in human infants showed that adding DHA to milk replacer–formulas increases DHA concentrations in blood lipids to values as high as those found in breastfed infants (12, 13). The concurrent effect on the structure of the central nervous system, which is well established in suckling animals (14–17), may have functional outcomes, especially in preterm infants (18).

Cultured cells are now increasingly used to explore the uptake, metabolism, and molecular effects of fatty acids. Such studies generally postulate that the conditions used for cell cultures are physiologically relevant in terms of the fatty acid concentration of the culture medium. The relation between the in vivo and in vitro conditions for incorporation of DHA into membrane phospholipids has not been precisely established, although an array of specific metabolic cues, ranging from a total deficiency to a large excess, may occur in the different models. The present study was designed to compare the dose-dependent incorporation of DHA into membrane phospholipids in the developing rat retina and brain with that in human neural cells. We attempted to determine the precise relation between the cell models and the nutritional status of developing animals; ie, we tried to define the supply of exogenous DHA needed for each system while taking into account the physiologic status from deficiency to excess. We used a linearized counting frame to obtain reciprocal transposition of DHA status in animal tissues and that in cultured cells.

[1] From the Nutrition & Food Safety Laboratory, Neurobiology of Lipids, Institut National de la Recherche Agronomique, Jouy-en-Josas, France (J-MA, CP-B, BL, M-HP, ML, and PG), and the Institut Gustave Roussy, Centre National de la Recherche Scientifique, Villejuif, France (GR).

[2] Supported by the Institut National de la Recherche Agronomique, Paris, and by Blédina (Groupe Danone), Villefranche-sur-Saône, France.

[3] Address reprint requests to J-M Alessandri, INRA, LNSA, 78352 Jouy-en-Josas, France. E-mail: alessand@jouy.inra.fr.

Received January 6, 2003.

Accepted for publication April 16, 2003.

**TABLE 1**
Lipid composition of the experimental diets, which varied in docosahexaenoic acid (DHA) content and were used to study the dose-response effect in the F2 generation[1]

| | DHA content (mg/100 g diet) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 10 | 25 | 50 | 100 | 200 | 400 |
| Oil source (g/kg diet) | | | | | | | |
| Microalgal oil | 0 | 0.3 | 0.6 | 1.2 | 2.4 | 4.9 | 9.8 |
| Sunflower oil | 18.7 | 18.7 | 18.7 | 18.7 | 18.7 | 18.7 | 18.6 |
| Hydrogenated palm oil | 51.3 | 51.0 | 50.7 | 50.1 | 48.9 | 46.4 | 41.6 |
| Composition[2] (% by wt of total fatty acids) | | | | | | | |
| Saturated fatty acids | 74.4 | 74.2 | 74.6 | 74.1 | 71.8 | 69.9 | 66.7 |
| Monounsaturated fatty acids | 1.0 | 1.6 | 0.8 | 1.1 | 2.0 | 1.5 | 2.2 |
| 18:2n−6 | 24.5 | 23.9 | 24.0 | 23.8 | 24.3 | 25.0 | 24.3 |
| 18:3n−3 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| 22:6n−3 | 0.0 | 0.2 | 0.5 | 0.9 | 1.8 | 3.5 | 6.7 |

[1] The experimental diets were composed of the following (in g/kg diet): lipids, 70; casein, 220; DL-methionine, 1.6; cellulose, 20; starch, 438.4; saccharose, 220; vitamin mixture, 10; mineral mixture, 40. The caloric density was 3970 kcal/kg or 16.61 MJ/kg.
[2] Simplified (mean values from 3 batches).

## MATERIALS AND METHODS

### Experimental design (general considerations)

Gas chromatography was used to determine how efficiently rat neural tissues (retina, cerebellum, cortex, hippocampus, and striatum) incorporated dietary DHA into membrane phospholipids, and we compared the data from rat tissues with that from a human retinoblastoma cell line (Y79) and 2 neuroblastoma cell lines (IGR-N-91 and SH-SY5Y) grown in medium supplemented with DHA. The minimum in vivo DHA status ($DHA_{min}$) of nervous tissues was produced by rendering the rats deficient in n−3 fatty acids through 2 generations, and the equivalent in vitro status was produced by culturing cells in medium not supplemented with DHA. DHA concentrations in the cells and tissues were replenished by supplementation. Their specific DHA plateau values ($DHA_{max}$) and the doses of exogenous DHA required to obtain one-half the plateau value ($DHA_{50}$) were determined. Both variables were computed by plotting the reciprocals of the DHA concentrations relative to the reciprocals of the DHA doses. The $DHA_{max}$ and $DHA_{50}$ values were used to estimate the physiologic connection between DHA concentrations in the diet and those in the culture medium.

### Cell cultures

Human Y79 retinoblastoma cells and SH-SY5Y and IGR-N-91 neuroblastoma cells were cultured at 37 °C in a 5% (by vol) $CO_2$ atmosphere in RPMI (Y79) or DMEM (SH-SY5Y and IGR-N-91) supplemented with 100 mL fetal bovine serum/L and containing 2 mmol L-glutamine/L, 20 000 U penicillin/L, and 20 mg streptomycin/L (Gibco BRL, Cergy-Pontoise, France). The albumin concentration in the culture medium was ≈50 μmol/L. On the assumption that serum albumin has 6 binding sites for free fatty acids (19), the maximal concentration of unesterified DHA that may be complexed by albumin was estimated to be ≈300 μmol/L. The maximal DHA dose used was 70 μmol/L, which produced a ratio of DHA to albumin of 1.4; thus, in the present study, DHA was far from saturating all the albumin sites. Cells were transferred to media supplemented with different amounts of DHA. The DHA fatty acid sodium salts (Nu-Chek Prep, Elysian, MN) were dissolved directly in the medium to give concentrations of 7, 15, 30, or 70 μmol/L. Cells were grown for 3 d and delipidated for lipid analysis. Preliminary experiments showed that culturing the cells for up to 6 d in medium containing DHA resulted in the same phospholipid fatty acid composition as that produced by growing the cells for 3 d in the same medium (data not shown). The neuroblastoma cells were recovered by trypsination, washed with phosphate-buffered saline containing 50 μmol fatty acid–free albumin/L to remove extracellular fatty acids, and then suspended in a 7.3-g NaCl/L solution. Trypsination was not required to prepare the nonadhering Y79 retinoblastoma cells.

### Animal experiments

French regulations for the care and use of laboratory animals (nos. 87 848 and 03 056) were followed. Female Wistar rats were rendered deficient in n−3 fatty acids by being reared through 2 generations (F0 and F1) with a semipurified diet (Lesieur Alimentaire, Neuilly-sur-seine, France) containing African peanut oil as the sole source of lipids (14, 20). This oil contained 20% 18:2n−6 by wt of total fatty acids and 0.1% ALA by wt of total fatty acids. Therefore, the peanut oil diet supplied 13 g 18:2n−6/kg diet and 0.06 g ALA/kg diet. At 2 wk of age, 20 females (F1 generation) were mated over 4 d and then housed individually in polycarbonate cages for 1 wk. These matings resulted in 14 pregnancies, and those 14 females were then randomly assigned to 1 of the 7 groups (2 females per group) receiving 0–4 g DHA/kg diet (**Table 1**). At this stage, dietary 18:2n−6 was supplied in a constant amount to the females and to the 7 groups of the F2 generation by replacing the peanut oil with a mixture of sunflower and hydrogenated palm oils. Different amounts of DHA were obtained by adding a microalgal oil (DHASCO; Martek Biosciences Corporation, Columbia, MD), which contained 43% DHA by wt of total fatty acids and < 0.5% of other n−3 fatty acids by wt of total fatty acids, to the mixture of sunflower and hydrogenated palm oils (Table 1). All the diets contained 70 g total fat/kg. The diets were manufactured every 2 wk and stored at −20 °C. The F2 generation litters were equalized in size at birth so that each litter contained 8 pups, and the pups were fed their dam's milk for 3 wk. At weaning, 4 male rats from the same dietary group but from 2 different litters were retained, housed in pairs, and fed the same diet as that of their dam's group for 5 wk.

The rats were killed by decapitation when they were 8 wk old ($n$ = 4 per dietary group). The eyes were removed, and the retinas

were detached from the retinal pigment epithelium by incubating the eyecups in a calcium-free Ringer buffer (pH 7.4) containing 118 mmol NaCl/L, 4.7 mmol KCl/L, 1.17 mmol $KH_2PO_4$/L, 1.17 mmol $MgSO_4$/L, 5.6 mmol D-glucose/L, 35 mmol $NaHCO_3$/L, and 1 mmol EDTA/L with gentle stirring at room temperature (21). The retinas were pooled and stored at −80 °C. The frontal cortex, striatum, hippocampus, and cerebellum of each rat were removed on ice, weighed, frozen in liquid nitrogen, and stored at −80 °C. These brain areas differ significantly in their membrane phospholipid DHA content (5, 22). Each brain region was homogenized in 2 mL physiologic saline containing 0.02 g butyl hydroxytoluene/L, and the total lipids were extracted.

### Phospholipid fatty acid analysis

The total lipids in 1 volume of cell or tissue homogenate were extracted with 4 volumes of chloroform:methanol (2:1, vol:vol) containing 0.05 g butyl hydroxytoluene/L. The lipid bottom phase was dried under a stream of nitrogen flux, dissolved in chloroform:isopropanol (2:1, vol:vol), and stored at −80 °C. The 2 major classes of membrane phospholipid, phosphatidylcholine and ethanolamine phosphoglycerides (EPGs), were isolated by solid-phase extraction on a single cartridge (23) adapted for use with nervous tissues (24). Prepacked, 500-mg aminopropyl silica cartridges (JT Baker, Deventer, Netherlands) were washed with 3 mL hexane and equilibrated with 3 mL eluent I [isopropanol:chloroform (1:2, vol:vol)]. Each sample of total cell lipids was dissolved in 250 μL eluent I and applied to a cartridge. The cartridge was eluted with 3 mL eluent I and then with 3 mL diethylether:acetic acid (3:1, vol:vol), 1 mL acetonitrile, and 8 mL acetonitrile:*n*-propanol (3:1, vol:vol) to recover the phosphatidylcholine fraction. The cartridge was then eluted with 2 mL acetonitrile:*n*-propanol (1:1, vol:vol) and 3 mL methanol to recover the EPG fraction. Fatty acid methyl esters were produced from phosphatidylcholine and EPG by reacting each phospholipid sample for 20 min at 90 °C with 2 mL methanol containing 100 g $BF_3$/L. Two milliliters of water were then added, and the methyl esters were extracted twice with 1 mL hexane and finally taken up in isooctane. An aliquot was injected onto a gas chromatograph equipped with a retention gap and a CP WAX 52 CB bonded fused-silica capillary column (0.3 mm inside diameter and 50 m long; Chrompack, Middleburg, Netherlands). The oven temperature was programmed to increase from 79 to 140 °C at a rate of 9 °C/min and then from 140 to 205 °C at a rate of 3 °C/min. Eluted compounds were detected with a flame ionization detector, and the area under each peak was automatically integrated. Fatty acid methyl esters and dimethylacetals were identified by comparing their equivalent chain lengths with standard compounds (Nu-Chek Prep). All compositions are expressed as percentages by weight of total fatty acids.

### Linearizing model

The incorporation of DHA into the developing tissues of rats supplied with different amounts of dietary DHA describes a dose-response effect from minimum concentrations to a plateau (14). The minimum phospholipid DHA content in the nervous tissues was produced by rearing rats (from the F0 generation to the F2 generation) on a diet lacking n−3 fatty acids. This treatment has been shown to significantly impair acquisition and memory performances (25). We postulated that the dose of dietary DHA at which the DHA concentration in a given tissue equals one-half its $DHA_{max}$ defines the tissue-specific capacity for channeling exogenous DHA to its membrane phospholipids. We hypothesized that cultured cells of neural origin use exogenous DHA in a dose-response manner that is comparable with that of developing nervous tissues. We assumed that the greater the capacity of tissues and cells to take up exogenous DHA into membranes, the lower the DHA dose required to match one-half the $DHA_{max}$ in phospholipids. Precise determination of the $DHA_{max}$ and the corresponding $DHA_{50}$ would require supraphysiologic DHA intakes that could overload the capacity for phospholipid incorporation. Experimental overloading of rats and cells with DHA may cause physiologic alterations because of factors such as the toxic side effects of increased lipid peroxidation, which could distort the genuine dose-response effect. Therefore, we determined the theoretical plateau value of the dose-response curve by extrapolating the DHA concentrations in membrane phospholipids that would be obtained under conditions of DHA overloading. This was done by using a simple linear model of the incorporation of exogenous DHA into tissue and cell phospholipids. The hyperbolic dose-response curve was linearized in the same way that Michaelis-Menten enzyme kinetics are treated in the double-reciprocal Lineweaver-Burke plot. The reciprocal of the DHA concentration (1/DHA) in the phosphatidylcholine and EPG fractions was plotted against the reciprocal of the dose (1/dose). The dose was the amount of dietary DHA (mg/100 g diet) or the DHA concentration in the culture medium (μmol/L). The double-reciprocal plot results in the value of 1/dose tending toward zero as the external DHA tends toward infinite amounts, which defines the theoretical status of $DHA_{max}$. The ordinate at the origin is thus the reciprocal of the maximum DHA concentration, and the dose giving rise to twice the value of the ordinate at the origin, ie, one-half the $DHA_{max}$, gives the $DHA_{50}$.

In summary, the straight line drawn through the double-reciprocal data is described by the general equation:

$$y = y_0 + a \cdot x \quad (1)$$

where $y$ = 1/DHA, $y_0 = (1/DHA_{max})$, $x$ = (1/dose), and $a$ = slope. By definition,

$$2y_0 = y_0 + a \cdot x_{50} \quad (2)$$

Therefore,

$$a = y_0/x_{50} \quad (3)$$

Thus,

$$a = (1/DHA_{max})/(1/dose_{50}) = dose_{50}/DHA_{max} \text{ or } DHA_{50}/DHA_{max} \quad (4)$$

The ratio of $DHA_{max}$ to $DHA_{50}$ was thus directly drawn from the reciprocal of the slope. Extrapolation of the phospholipid DHA concentration resulting from a zero input is not relevant, because 1/dose and 1/DHA tend to infinity. Hence, the minimum DHA status of deficient rats was defined as the real starting point for the dose-response effect. This minimum concentration was reproduced in vitro by culturing cells in unsupplemented medium. Preliminary trials showed that the DHA concentrations in cells cultured for 3 d in 10% serum without any DHA supplement were similar to the DHA concentrations in the F2 DHA-deficient rats.

### Statistical analysis

The fit of the reciprocal data for each phosphatidylcholine and EPG fraction from tissues and cells to the linear regression ($y = y_0 + a \cdot x$) was checked by analysis of variance followed by an $F$ test. The statistical analyses were performed

**TABLE 2**
Variables for the docosahexaenoic acid (DHA) dose-response effect in ethanolamine phosphoglyceride (EPG) and phosphatidylcholine (PC) fractions from 3 human cell lines, 4 rat brain regions, and rat retinas[1]

| | $DHA_{min}$ | $DHA_{max}$ | Depletion[2] | $DHA_{50}$[3] | $DHA_{max}/DHA_{50}$ | $r^2$ | $P$[4] |
|---|---|---|---|---|---|---|---|
| | *% by wt of TFAs* | *% by wt of TFAs* | *%* | | | | |
| Rat | | | | | | | |
| Cortex | | | | | | | |
| EPG | 6.6 ± 0.5[5] | 28.8 | 77 | 18.0 | 1.6 ± 0.07 | 0.994 | <0.0001 |
| PC | 1.3 ± 0.1 | 6.3 | 79 | 21.7 | 0.3 ± 0.05 | 0.998 | <0.0001 |
| Striatum | | | | | | | |
| EPG | 6.0 ± 0.6 | 21.8 | 72 | 11.9 | 1.8 ± 0.04 | 0.996 | <0.0001 |
| PC | 1.5 ± 0.2 | 6.9 | 78 | 21.4 | 0.3 ± 0.02 | 0.988 | <0.0001 |
| Hippocampus | | | | | | | |
| EPG | 6.6 ± 0.7 | 26.2 | 75 | 16.6 | 1.6 ± 0.07 | 0.997 | <0.0001 |
| PC | 1.5 ± 0.1 | 5.5 | 73 | 14.9 | 0.4 ± 0.03 | 0.988 | <0.0001 |
| Cerebellum | | | | | | | |
| EPG | 6.6 ± 0.6 | 24.7 | 73 | 13.1 | 1.9 ± 0.05 | 0.996 | <0.0001 |
| PC | 2.0 ± 0.1 | 9.3 | 78 | 17.9 | 0.5 ± 0.02 | 0.993 | <0.0001 |
| Retina | | | | | | | |
| EPG | 11.6 ± 2.4 | 45.9 | 75 | 4.0 | 11.5 ± 1.0 | 0.978 | 0.001 |
| PC | 6.7 ± 1.2 | 24.8 | 73 | 5.0 | 5.0 ± 1.1 | 0.861 | 0.02 |
| Human | | | | | | | |
| IGR-N-91 | | | | | | | |
| EPG | 3.9 ± 0.5 | 31.8 | 88 | 6.9 | 4.6 ± 0.3 | 0.993 | 0.004 |
| PC | 0.9 ± 0.2 | 12.6 | 93 | 18.9 | 0.7 ± 0.02 | 0.997 | 0.001 |
| SH-SY5Y | | | | | | | |
| EPG | 5.9 ± 0.5 | 32.7 | 82 | 5.7 | 5.7 ± 0.5 | 0.981 | 0.01 |
| PC | 1.0 ± 0.1 | 10.6 | 91 | 25.8 | 0.4 ± 0.03 | 0.992 | 0.004 |
| Y79 | | | | | | | |
| EPG | 7.4 ± 0.6 | 39.2 | 81 | 6.6 | 5.9 ± 1.2 | 0.916 | 0.04 |
| PC | 2.6 ± 0.1 | 30.1 | 91 | 32.5 | 0.9 ± 0.1 | 0.958 | 0.003 |

[1] $n = 4$ rats or 3 cell determinations. $DHA_{min}$, DHA minimal content with no DHA supplement; $DHA_{max}$, DHA plateau value extrapolated from the dose-response effect at infinite DHA dose; $DHA_{50}$, DHA dose giving rise to one-half the DHA plateau value in EPGs or PC; TFAs, total fatty acids.
[2] Initial depletion expressed as a percentage of the $DHA_{max}$.
[3] Expressed in μmol/L culture medium (cells) or in mg/100 g diet (rats).
[4] ANOVA followed by an $F$ test for the linear regression of the double-reciprocal plot.
[5] $\bar{x}$ ± SEM.

with the use of STATVIEW+GRAPHICS software (Abacus Concepts Inc, Berkeley, CA).

## RESULTS

### Minimum docosahexaenoic acid status in developing tissues and growing cells

Minimum DHA concentrations in EPG and phosphatidylcholine fractions from n−3–deficient tissues and cells are shown in the first column of **Table 2**. DHA concentrations accounted for ≈6–7% by wt of total fatty acids in EPGs from the cortex, striatum, cerebellum, and hippocampus, and this percentage was similar to that observed in the 3 human cell lines. However, the percentage of DHA in retinal EPGs was about twice as high. The phosphatidylcholine fractions from the 4 brain areas and the 3 cell lines all had low DHA concentrations, but the phosphatidylcholine fraction from the retina had a higher DHA concentration (6.7% by wt of total fatty acids). These data indicated that the 3 human cell lines preferentially retained DHA in the EPG fraction, as did the rat tissues. In addition, the minimum DHA concentration in neuroblastoma SH-SY5Y and IGR-N-91 cells and retinoblastoma Y79 cells cultured for 3 d without a DHA supplement mimicked that in the brain of rats deprived of dietary n−3 fatty acids for 3 generations.

### $DHA_{max}$ and $DHA_{50}$ values for nervous tissues and cultured cells

DHA concentrations in EPGs from cells grown with increasing concentrations of DHA supplement were plotted against DHA concentrations in the medium. The dose-response curve for IGR cells, which is typical for the 3 cell lines, is shown in **Figure 1**. The DHA concentration in EPGs gradually increased from 3.9% by wt of total fatty acids without DHA supplement (dietary deficiency–mimicking conditions) to ≈30% in cells grown in medium containing 70 μmol DHA/L. The corresponding reciprocal plot is shown in the inset in the upper left panel of Figure 1 (linear regression; $r^2 = 0.993$). The reciprocal of the ordinate at the origin gave a $DHA_{max}$ of 31.8% by wt of total fatty acids in IGR cell EPGs. The DHA supplement concentration required to match one-half of this plateau value was 6.9 μmol/L, which gave a $DHA_{max}$-$DHA_{50}$ ratio for EPGs of 4.6. This value defined the mean increase in DHA in the EPG fraction of IGR cells per unit of DHA supplement expressed in μmol/L. The data for the phosphatidylcholine fraction are also shown in Figure 1. The $DHA_{max}$ in the phosphatidylcholine fraction was 12.6% by wt of total fatty

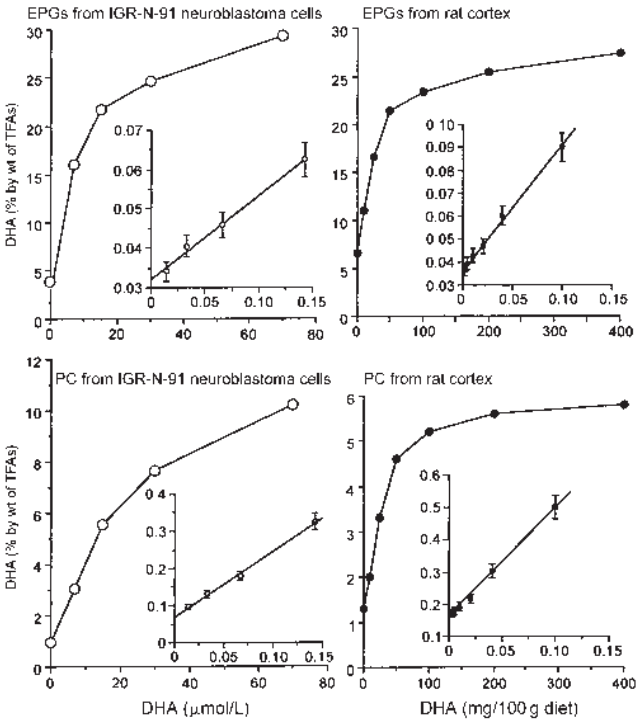


**FIGURE 1.** Mean (±SEM) docosahexaenoic acid (DHA) concentrations in ethanolamine phosphoglyceride (EPG) and phosphatidylcholine (PC) fractions from the IGR-N-91 human cell line (3 separate cultures) and the rat cortex (4 rats belonging to the same dietary group) in response to increasing DHA doses in the culture medium or the diet. Double-reciprocal data are shown in the insets. Fitting of the double-reciprocal plot to linear regression was performed by ANOVA followed by an *F* test (the corresponding *P* values are reported in Table 2). TFAs, total fatty acids.

acids, and the $DHA_{50}$ was 18.9 μmol/L, which gave a $DHA_{max}$-$DHA_{50}$ ratio for the phosphatidylcholine fraction of 0.7. Therefore, in IGR cells, the maximum incorporation of DHA into EPGs (31.8%) was 2.5 times that into phosphatidylcholine (12.6%). The $DHA_{max}$-$DHA_{50}$ ratios also indicated that the efficiency of DHA incorporation relative to DHA supplement in EPGs was 6.6 times that in the phosphatidylcholine fraction. The patterns (data not shown) for DHA incorporation into SH-SY5Y neuroblastoma and Y79 retinoblastoma cells were similar (Table 2). Among the 3 cell lines, the Y79 retinoblastoma cells had the highest $DHA_{max}$ values in both EPGs and phosphatidylcholine. The $DHA_{max}$-$DHA_{50}$ ratios for EPGs in the 3 cell lines were between 4 and 6 (Table 2), which indicated that all 3 lines displayed almost the same ability to make use of DHA supplement for uptake and phospholipid acylation.

The dose-response curves for DHA incorporation into the cortical EPG and phosphatidylcholine fractions are also shown in Figure 1. The $DHA_{max}$ values in EPGs and phosphatidylcholine were 28.8% and 6.3% by wt of total fatty acids, respectively (Table 2). The maximum DHA incorporation into the EPG fraction was thus 4.6 times that into the phosphatidylcholine fraction. The corresponding $DHA_{50}$ values in the EPG and phosphatidylcholine fractions were 18.0 and 21.7 mg DHA/100 g diet, respectively. Thus, the highest dose of dietary supplement tested (400 mg DHA/100 g diet) accounted for ≈18–22 times the $DHA_{50}$ for the cortical phospholipids. This DHA supplement dose gave rise to a DHA concentration in cortical EPGs of 27.4% by wt of total fatty acids (Figure 1), which accounted for 95% of the $DHA_{max}$ in EPGs (ie, 28.8% by wt of total fatty acids) (Table 2). Therefore, providing 95% of the theoretical $DHA_{max}$ in cortical EPGs in deficient rats required a dietary DHA dose ≥22 times the $DHA_{50}$ in EPGs. The same calculation indicated that obtaining 92% of the theoretical $DHA_{max}$ in cortical phosphatidylcholine (5.8% compared with 6.3% by wt of total fatty acids; Figure 1) required a dietary DHA intake ≈18 times the corresponding $DHA_{50}$. The profiles (data not shown) for the 3 other brain areas and the retina were very similar. All the data are summarized in Table 2.

The $DHA_{max}$ values in EPGs and phosphatidylcholine in the retina were much higher than those in the 4 brain areas (Table 2), which confirmed the high incorporation of DHA into retinal phospholipids. Moreover, the corresponding $DHA_{50}$ values in the EPG and phosphatidylcholine fractions in the retina were much lower than their counterparts in the brain areas. Hence, the $DHA_{max}$-$DHA_{50}$ ratio in EPGs was dramatically higher in the retina (11.5) than in the brain areas (1.6–1.9), which indicated that the dose-dependent accretion of DHA occurred earlier in the retina than in the brain, ie, that DHA accretion in the retina occurred during the early stages of the dose-response effect. The $DHA_{50}$ in retinal EPGs (4.0 mg/100 g diet; Table 2) indicates that rats fed the diet containing 400 mg DHA/100 g received 100 times the DHA dose required to produce one-half the $DHA_{max}$ in retinal EPGs.

Cells or tissues having different initial DHA concentrations and different $DHA_{max}$ values but the same capacity to respond to increasing DHA input for net uptake and maximum phospholipid esterification will have the same $DHA_{max}$-$DHA_{50}$ ratio. Hence, if the retina behaved like the brain areas, with a $DHA_{max}$ in EPGs of 45.9 (Table 2) but with a $DHA_{max}$-$DHA_{50}$ ratio for EPGs close to 2.0 (instead of 11.5), 23 mg DHA/100 g diet (instead of 4.0 mg/100 g diet) would be required to produce one-half the $DHA_{max}$ in retinal EPGs. The observation that brain regions of different sizes all had very similar $DHA_{max}$-$DHA_{50}$ ratios (Table 2) shows that the $DHA_{max}$-$DHA_{50}$ ratio does not depend on tissue mass. The difference in the $DHA_{max}$-$DHA_{50}$ ratios between the brain regions and the retina clearly does not reflect differences in the mass of the brain and retina. The retina has a higher $DHA_{max}$ than do the brain areas and reaches this specific plateau at a lower dietary input. This could be called the "avidity" of the retina for DHA, and the use of this term could be extended to the other tissues, so that the higher the $DHA_{max}$-$DHA_{50}$ ratio, the higher the apparent avidity for DHA. The avidities of the rat nervous tissues for DHA can be ranked as follows: cortex, hippocampus < striatum, cerebellum < retina.

### Predictive value of the linear model

The double-reciprocal model links dietary DHA input and phospholipid DHA concentration through the equations described by the ordinate at the origin ($1/DHA_{max}$) and the slope ($DHA_{50}/DHA_{max}$). We checked the predictive value of the model by computing the DHA concentrations that can be drawn from the values of the experimental DHA inputs. The predicted values were plotted against those actually measured in the rat nervous tissues and the 3 human cell lines (**Figure 2**). There was a significant linear relation between the predicted and measured DHA concentrations in the EPG and phosphatidylcholine fractions from the tissues and the cells. The tissues and the cells can be approximately superimposed on the same line, with a slope close to 1, showing that the predictive model can be applied in vivo and in vitro, regardless of the type of tissue (brain area or retina) or the type of cells (retinoblastoma cells or neuroblastoma cells).

In a symmetrical manner, we used the reciprocal plot to calculate the DHA supplement dose required to attain the $DHA_{max}$ observed

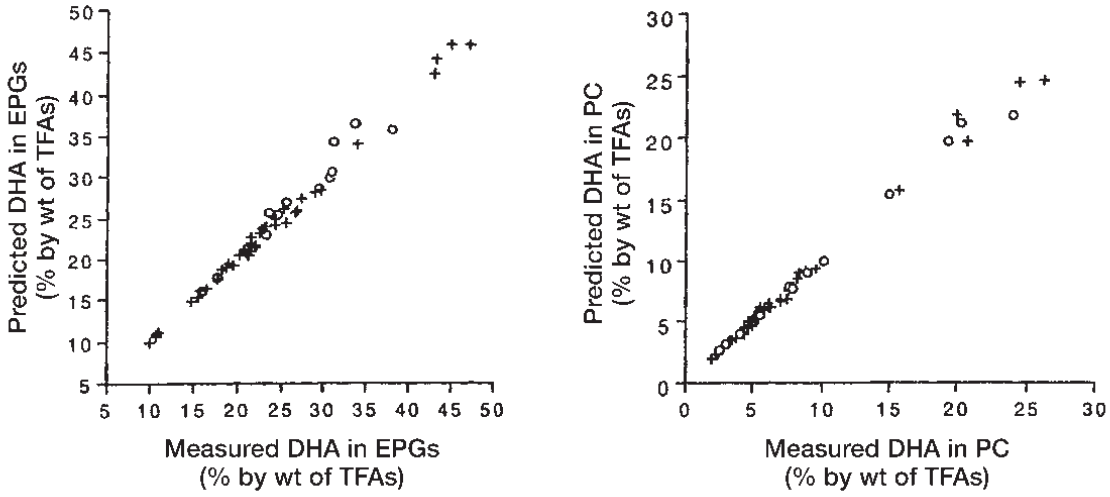


**FIGURE 2.** Correlation between predicted docosahexaenoic acid (DHA) concentrations and DHA concentrations measured in ethanolamine phosphoglyceride (EPG) and phosphatidylcholine (PC) fractions from human cells (○) and rat nervous tissues (+). The linear regression was tested for ANOVA followed by an *F* test and included all of the data from the 3 cell lines or all of the data from the rat nervous tissues (cortex, striatum, hippocampus, cerebellum, and retina). Note that one cross is the mean value ($n = 4$ rats) for 1 of the 5 tissues from rats in the same dietary group and that one circle is the mean value ($n = 3$ determinations) for 1 of the 3 cell lines. For EPGs: cells, $y = 0.67 + 0.99x$ ($r^2 = 0.943$, $P < 0.0001$); tissues, $y = 0.18 + 0.99x$ ($r^2 = 0.995$, $P < 0.0001$). For PC: cells, $y = 0.22 + 0.97x$ ($r^2 = 0.990$, $P < 0.0001$); tissues, $y = 0.09 + 0.99x$ ($r^2 = 0.993$, $P < 0.0001$). TFAs, total fatty acids.

in EPGs in the 4 rat brain areas in the cell EPG fractions instead (**Figure 3**). Each of the 3 cell lines matched the $DHA_{max}$ values in EPGs predicted for the striatum, cerebellum, hippocampus, and cortex under experimental conditions but not those predicted for the retina. The dose-response curves reconstructed in Figure 3 showed that the Y79 retinoblastoma cells tended to match the predicted DHA concentrations in EPGs from the brain areas over a narrow range of DHA doses, whereas matching the same concentrations in the IGR cell line required DHA doses 2–3 times as high. This difference in behavior reflects differences between the cell lines in their $DHA_{max}$ values in EPGs; the $DHA_{max}$ in EPGs is higher in Y79 cells than in IGR cells (Table 2).

### Bridging the gap between rat and cell models by using the $DHA_{max}$-$DHA_{50}$ ratio

Retinoblastoma and neuroblastoma cells and nervous tissues all incorporate more DHA into EPGs than into phosphatidylcholine, and the difference between their respective $DHA_{max}$-$DHA_{50}$ ratios for EPGs and phosphatidylcholine reflect the different avidity for DHA of EPG and phosphatidylcholine (Table 2). However, the cells and tissues could not be directly compared because their $DHA_{50}$ values are expressed in different units. The same holds true for the $DHA_{max}$-$DHA_{50}$ ratios for the cells and tissues. We postulated that the avidity of neural cells for DHA mimics that of brain areas and addressed the question of whether expressing $DHA_{50}$ for the nervous tissues in particular units would make a direct comparison possible. When $DHA_{50}$ was expressed as μmol/10 g diet (instead of as mg/100 g diet), the values for the $DHA_{max}$-$DHA_{50}$ ratios in the 4 brain areas were very similar to those in the 3 cell lines (**Figure 4**). The $DHA_{max}$-$DHA_{50}$ ratios for EPGs in the 4 brain areas were between 5.2 and 6.2, values similar to the $DHA_{max}$-$DHA_{50}$ ratios for EPGs in the 3 cell lines (4.6–5.9 with $DHA_{50}$ in μmol/L). The corresponding $DHA_{max}$-$DHA_{50}$ ratios for phosphatidylcholine were also in a narrow range: 1.0–1.7 in the brain areas and 0.4–0.9 in the cells. In contrast, the $DHA_{max}$-$DHA_{50}$ ratios were much higher (6-fold in the EPG fraction and 10-fold in the phosphatidylcholine fraction) in the retina than in the brain or the cultured cells (Figure 4).

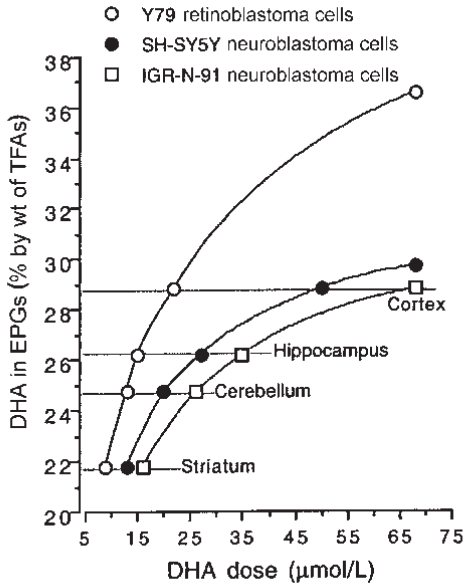


**FIGURE 3.** Counting frame for the docosahexaenoic acid (DHA) dose-response effect in ethanolamine phosphoglycerides (EPGs) reconstructed in 3 human cell lines. The DHA concentrations in EPGs indicated by horizontal lines are the plateau values ($DHA_{max}$) predicted for the 4 rat brain areas (*see* Table 2); the corresponding DHA doses are calculated from the double-reciprocal plot of the dose-response curve for each cell line. TFAs, total fatty acids.

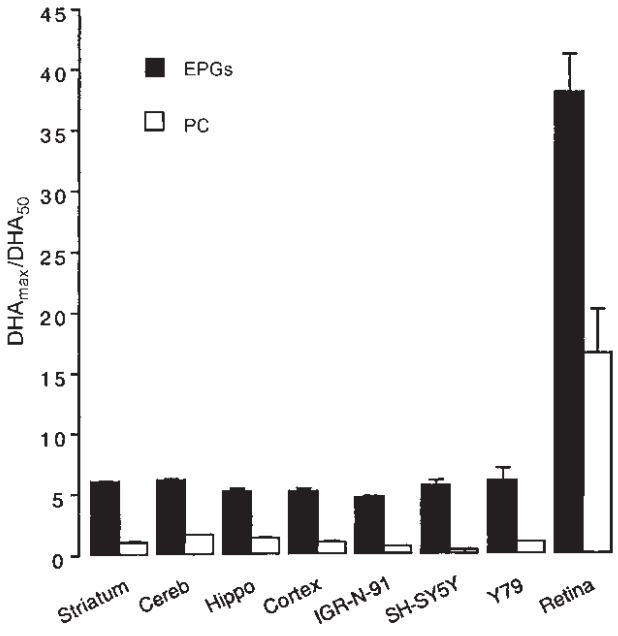


**FIGURE 4.** Comparison of the ratios of $DHA_{max}$ (plateau concentration of docosahexaenoic acid) to $DHA_{50}$ (one-half $DHA_{max}$) in ethanolamine phosphoglyceride (EPG) and phosphatidylcholine (PC) fractions from human cells and rat tissues. $DHA_{50}$ values in the human cells and the rat tissues are expressed in μmol/L (*see* Table 1) and μmol/10 g diet, respectively. In each case, the $DHA_{max}$-$DHA_{50}$ ratio is given by the inverse of the slope after linear regression of the inverted data. The error bars indicate the SD for the 1/slope value determined by ANOVA followed by an *F* test. Cereb, cerebellum; Hippo, hippocampus.

## DISCUSSION

We compared DHA dose-response curves in nervous tissue and cell phospholipids. We determined the $DHA_{min}$ resulting from n−3 fatty acid depletion, the $DHA_{max}$ produced by replenishment, and the $DHA_{50}$ in diet or medium that gave rise to one-half the $DHA_{max}$. The $DHA_{max}$-$DHA_{50}$ ratio was used to evaluate the mean gain in DHA concentration per unit of exogenous DHA. This ratio also allowed us to compare the capacity of tissues and cells to accumulate DHA in their membrane phospholipids.

### DHA status of the brain and retina: from $DHA_{min}$ to $DHA_{max}$

The DHA-depleted retina contained a higher percentage of DHA in its phospholipids than did the 4 brain regions (Table 2), which confirms that DHA is avidly sequestered by the retina (4, 26, 27). This could be due to selective uptake of systemic DHA or to deficiency-induced activation of the recycling of DHA molecular species within the eye (28–31). However, our data clearly indicate that $DHA_{min}$ concentrations in both the retinal and the brain phospholipids account for the same relative depletion, ie, ≈75% of their respective $DHA_{max}$ values (Table 2). The retina is peculiar because it replenishes its DHA status at very low input doses. This is reflected in $DHA_{50}$ values in the EPG and phosphatidylcholine fractions: values in the brain were 3–4 times those in the retina. The avidity of the retina for DHA is also reflected in the $DHA_{max}$-$DHA_{50}$ ratios for EPGs and phosphatidylcholine, which are 6 and 10 times those for the 4 brain regions studied (Table 2 and Figure 4). This seems to support the concept that the retinal pigment epithelium plays a specific role in the translocation of circulating DHA into the retina. The retinal pigment epithelium has the ability to capture plasma protein- or lipoprotein-bound DHA (possibly $HDL_2$) from the choriocapillaries and to release DHA in the interphotoreceptor matrix in an appropriate form for its uptake by retinal cells (32). This retina-specific translocation should be more efficient than that occurring in the brain, at least in terms of net efficiency in the steady state.

### Differences and similarities between nervous tissues and neural cells

The brain regions of rats fed the n−3 deficient diet throughout 3 generations and the 3 human cell lines cultured for 3 d in medium without DHA supplement all had similarly depleted DHA concentrations in their membrane phospholipids (Table 2). The Y79 retinoblastoma line retained a maximal capacity for incorporating exogenous DHA in membrane phospholipids that was higher than that of the neuroblastoma cells. On the basis of matching the $DHA_{max}$ values predicted for EPGs in the brain areas, the amount of DHA required by the 2 neuroblastoma lines was 2–3 times that required by the Y79 retinoblastoma cells (Figure 3). However, the difference in avidity for DHA between the retinoblastoma cells and the neuroblastoma cells is clearly minor in terms of $DHA_{max}$-$DHA_{50}$ ratios when compared with the difference between the retina and the brain areas. Y79 retinoblastoma cells have lost the retina-specific avidity for DHA, although their $DHA_{max}$ may approach that of retinal phospholipids in much the same way that the 2 neuroblastoma cell lines have conserved the brain $DHA_{max}$ (Table 2 and Figure 3). Neuroblastoma and retinoblastoma cells are thus well suited for taking up DHA supplements and producing phospholipids, which suggests that DHA uptake, acyl-CoA activation, and acyltransferases are not rate limiting in these cells. The parent fatty acids of DHA (ie, ALA or 22:5n−3) are partly metabolized in retinoblastoma (33) and neuroblastoma (B Langelier, MH Perruchot, G Raguénez, and JM Alessandri, unpublished observations, 2002) cells, and thus the incorporation of these fatty acids into phospholipids leads to DHA concentrations that are not as high as those obtained when preformed DHA is incorporated. Hence, the response to DHA of cultured cells, and probably also of nervous tissues, cannot be directly transposed to their response to other n−3 fatty acids.

### Connecting the in vivo and in vitro data

By comparing the $DHA_{max}$-$DHA_{50}$ ratios in brain areas and cultured cells, we found an apparent equivalence between DHA concentrations in the diet and in the culture medium. Feeding rats a diet containing 1 μmol DHA (in the form of triacylglycerols)/10 g diet and exposing cells to 1 μmol unesterified DHA/L medium produced almost identical DHA availabilities in terms of $DHA_{max}$-$DHA_{50}$ ratios (Figure 4). We assumed that the amount of DHA crossing the blood-brain barrier, which occurred in the steady state in n−3–deficient rats ingesting 1 μmol DHA/10 g diet, was approximately equivalent to the amount entering cells growing for 3 d in medium containing 1 μmol unesterified DHA/L. Whatever the mode of expression, the retina has a much higher avidity for DHA than do the brain regions (Figure 4). The diet-medium equivalence in DHA concentrations resulted from the dietary and culture conditions used in the present study. The intake of energy from lipids, the ratio of n−6 to n−3 fatty acids

**TABLE 3**
Equivalent modes of expression for the docosahexaenoic acid (DHA) dose in diet, milk, or culture medium that would produce DHA concentrations equal to 10 times the $DHA_{50}$ (one-half of the plateau value) in ethanolamine phosphoglycerides (EPGs) in the human brain and equal to 45 times the $DHA_{50}$ in EPGs in the human retina[1]

| | mg/100 g diet | μmol/10 g diet | % by wt of TFAs in milk | % of energy in milk[2] | μmol/L medium[3] |
|---|---|---|---|---|---|
| 10 × $DHA_{50}$ in brain EPGs | 180 | 55 | 0.8 | 0.4 | 55 |

[1] Note that replenishing chronically deficient animals requires a DHA supplement dose ≥22 times the $DHA_{50}$ in brain EPGs. TFAs, total fatty acids.
[2] Ratio of energy from DHA to total energy.
[3] Application to human cell lines.

in the diet, the weight and age of rats, etc, determines the fraction of DHA that is not stored or catabolized but is instead channeled to tissue phospholipids. In addition, the diet-medium transposition does not mean that the entry of DHA into the brain and cultured cells requires similar events. The translocation of systemic DHA across the blood-brain barrier may involve DHA molecular species complexed with albumin, plasma proteins, or lipoproteins (32, 34, 35). The net uptake of unesterified fatty acids by cultured cells results from a continuous flow involving fatty acid dissociation from plasma proteins, passive exchange across the phospholipid bilayer, and protein-mediated transport (36, 37). The $DHA_{max}$-$DHA_{50}$ ratio sums up these events as well as intracellular DHA trafficking, CoA acylation, and phospholipid esterification. The dose-response effect clearly shows that fetal bovine serum proteins ensure the spontaneous dissolution of unesterified DHA and its transport to cultured cells, at least over the range of concentrations tested. The DHA supplement concentration should not exceed 10–13 times the $DHA_{50}$ in EPGs in target cells. This will ensure complete replenishment of the DHA content in membrane phospholipids without exceeding the cell's metabolic requirements.

### Applicability to nutritional requirements of human and nonhuman primates

The brain, which has a lower avidity for DHA (lower $DHA_{max}$-$DHA_{50}$ ratio) than does the retina, can form the basis for translating our data to the nutritional requirements of primates. In the rat brain, the lowest $DHA_{max}$-$DHA_{50}$ ratio for EPGs occurs in the frontal cortex and the hippocampus, ie, 1.6 when the dietary supplement is expressed in mg DHA/100 g diet (Table 2). This value can serve as an arbitrary reference for defining the overall DHA requirements of nervous tissues. Because the $DHA_{max}$-$DHA_{50}$ ratios for EPGs in the rat brain and human cell lines are similar (Figure 4), we postulate that the ratio of the $DHA_{max}$ in EPGs to the DHA dietary dose required to produce one-half the $DHA_{max}$ in EPGs is also 1.6 in the primate frontal cortex. An estimate of the $DHA_{max}$ values in EPGs (expressed in g DHA/100 g total fatty acids in EPGs) is needed to calculate the corresponding $DHA_{50}$. To our knowledge, the highest published value for DHA content in EPGs in the primate frontal cortex is that for n−3–deficient rhesus monkeys in whom DHA was replenished by using fish-oil supplementation (38). The DHA concentration in EPGs from the frontal cortex of the replenished monkeys was 29.3% by wt of total fatty acids (38). Although the feeding periods, intakes of energy from lipids, and other experimental details of the rat and monkey studies were very different, we postulate that the monkey cortical EPGs at steady state had a $DHA_{50}$ of 18.3 (ie, 29.3/1.6) mg DHA/100 g diet. From the rat study, we assume that deficient monkeys would require a diet containing ≥22 times this value (≈400 mg DHA/100 g diet) to approach the $DHA_{max}$ in EPGs in the brain. The dietary dose used to replenish the deficient monkeys was 1350 mg DHA/100 g diet (38), ie, 74 times the estimated value of $DHA_{50}$. The fish oil also contained 20:5n−3, a parent fatty acid of DHA that we cannot take into account because our model does not include the metabolic conversion of n−3 series. We may express dietary DHA intake as a multiple of the $DHA_{50}$ in EPGs in the brain to facilitate the comparison of data from different nutritional studies.

A dietary intake by deficient animals of 20–75 times the $DHA_{50}$ in EPGs in the brain may influence the time required to bring the brain DHA concentration up to its plateau value but probably does not influence the plateau value itself. Human infants, who are normally not deficient in DHA at birth, should ingest ≈10 times the theoretical $DHA_{50}$ in EPGs in the brain, ie, 180 mg DHA/100 g diet. This supply may be sufficient to prevent any risk of DHA dearth, even if the diet lacks other n−3 fatty acids. If we assume that the human retina has the same avidity for DHA as that of the rat retina, a dietary supply of 180 mg DHA/100 g will provide 45 times the $DHA_{50}$ in retinal EPGs. A supply of 180 mg DHA/100 g diet through human milk or infant formula corresponds to a milk DHA content of 0.8% by wt of total fatty acids, ie, 0.4% of total energy intake. This value is physiologically relevant because increasing DHA concentrations in breast milk causes a dose-dependent increase in infant plasma and erythrocyte phospholipid DHA concentrations, which match plateau values from the moment that milk DHA exceeds 0.8 g/100 g total fatty acids (39), ie, exactly 10 times the $DHA_{50}$ that we estimated in EPGs in the brain (**Table 3**). A supply of 180 mg DHA/100 g diet is also equivalent to 55 μmol DHA/10 g diet or, for cultured cell medium, to 55 μmol DHA/L. Our counting frame indicates that human neuroblastoma cells grown in 55 μmol DHA/L medium will equal (IGR-N-91) or exceed (SH-SY5Y) the $DHA_{max}$ in EPGs in the rat cortex (Figure 3). Therefore, infants fed milk supplying 10 times the $DHA_{50}$ in EPGs in the brain should have the genuine $DHA_{max}$ in their brain phospholipids.

In conclusion, we designed a common steady-state marker of all the processes that govern the dose-dependent channeling of DHA to membrane phospholipids. The linearizing model described in the present study provides a physiologic framework that takes into account the correspondence between in vivo and in vitro conditions.

We thank Marie-Thérèse Gozzelino for technical assistance, Patrice Dahirel and Claire Maudet for animal care, and Owen Parkes and Gail Wagman for correcting the English manuscript.

The study was designed by J-MA and PG and conducted by PG and CP-B (rat study) and by BL, M-HP, and GR (cell study). The rat study was the subject of CP-B's thesis. With the assistance of PG and ML, J-MA interpreted the data and wrote the article. None of the authors had any financial or personal interests (including advisory board affiliations) in any sponsoring company or organization.

## REFERENCES


1. Lauritzen L, Hansen HS, Jùrgensen MH, Michaelsen KF. The essentiality of long chain n−3 fatty acids in relation to development and function of the brain and retina. Prog Lipid Res 2001;40:1–94.
2. Sprecher H, Luthria DL, Mohammed BS, Baykousheva SP. Reevaluation of the pathways for the biosynthesis of polyunsaturated fatty acids. J Lipid Res 1995;36:2471–7.
3. Bourre JM, François M, Youyou A, et al. The effects of dietary α-linolenic acid on the composition of nerve membranes, enzymatic activity, amplitude of electrophysiological parameters, resistance to poisons and performance of learning tasks in rats. J Nutr 1989;119: 1880–92.
4. Neuringer M, Connor WE, Lin DS, Barstad L, Luck S. Biochemical and functional effects of prenatal and postnatal n−3 fatty acid deficiency on retina and brain in rhesus monkeys. Proc Natl Acad Sci U S A 1986;83:4021–5.
5. Delion S, Chalon S, Herault J, Guilloteau D, Besnard JC, Durand G. Chronic dietary α-linolenic acid deficiency alters dopaminergic and serotoninergic neurotransmission in rats. J Nutr 1994;124:2466–76.
6. Ximenes da Silva A, Lavialle F, Gendrot G, Guesnet P, Alessandri JM, Lavialle M. Glucose transport and utilization are altered in the brain of rats deficient in n−3 polyunsaturated fatty acids. J Neurochem 2002;81:1328–37.
7. Galli C, White HB, Paoletti R. Lipid alterations and their reversion in the central nervous system of growing rats deficient in essential fatty acids. Lipids 1970;6:378–87.
8. Green P, Kamensky B, Yavin E. Replenishment of docosahexaenoic acid in n−3 fatty acid–deficient fetal rats by intraamniotic ethyldocosahexaenoate administration. J Neurosci Res 1997;48:264–72.
9. Abedin L, Lien EL, Vingrys AJ, Sinclair AJ. The effects of dietary alpha-linolenic acid compared with docosahexaenoic acid on brain, retina, liver, and heart in the guinea pig. Lipids 1999;34:475–82.
10. Schiefermeier M, Yavin E. n−3 Deficient and docosahexaenoic acid–enriched diets during critical periods of the developing prenatal rat brain. J Lipid Res 2002;43:124–31.
11. Weisinger HS, Vingrys AJ, Bui BV, Sinclair AJ. Effects of dietary n−3 fatty acid deficiency and repletion in the guinea pig retina. Invest Ophthalmol Vis Sci 1999;40:327–38.
12. Makrides M, Neumann MA, Simmer K, Gibson RA. Erythrocyte fatty acids of term infants fed either breast milk, standard formula, or formula supplemented with long-chain polyunsaturates. Lipids 1995; 30:941–8.
13. Jorgensen MH, Holmer G, Lund P, Hernell O, Michaelsen KF. Effect of formula supplemented with docosahexaenoic acid and gamma-linolenic acid on fatty acid status and visual acuity in term infants. J Pediatr Gastroenterol Nutr 1998;26:412–21.
14. Guesnet P, Alasnier C, Alessandri JM, Durand G. Modifying the n−3 fatty acid content of the maternal diet to determine the requirements of the fetal and suckling rat. Lipids 1997;32:527–34.
15. Foote KD, Hrboticky N, McKinnon MJ, Innis SM. Brain synaptosomal, liver, plasma, and red blood cell lipids in piglets fed exclusively on a vegetable-oil-containing formula with and without fish-oil supplements. Am J Clin Nutr 1990;51:1001–6.
16. Alessandri JM, Goustard B, Guesnet P, Durand G. Docosahexaenoic acid concentrations in retinal phospholipids of piglets fed an infant formula enriched with long-chain polyunsaturated fatty acids: effects of egg phospholipids and fish oils with different ratios of eicosapentaenoic acid to docosahexaenoic acid. Am J Clin Nutr 1998;67: 377–85.
17. Goustard-Langelier B, Guesnet P, Durand G, Antoine JM, Alessandri JM. n−3 And n−6 fatty acid enrichment by dietary fish oil and phospholipid sources in brain cortical areas and nonneural tissues of formula-fed piglets. Lipids 1999;34:5–16.
18. Carlson S. Long-chain polyunsaturated fatty acids and development of human infants. Acta Paediatr 1999;88(suppl):72–7.
19. Curry S, Brick P, Franks NP. Fatty acid binding to human serum albumin: new insights from crystallographic studies. Biochim Biophys Acta 1999;1441:131–40.
20. Poumès-Ballihaut C, Langelier B, Houlier F, et al. Comparative bioavailability of dietary alpha-linolenic and docosahexaenoic acids in the growing rat. Lipids 2001;36:793–800.
21. Chen H, Wiegand RD, Anderson RE. Decreased docosahexaenoic acid levels in retina and pigment epithelium of frogs fed crickets. Exp Eye Res 1992:54:885–92.
22. Poumès-Ballihaut C, Vancassel S, Chalon S, Durand G, Latge C, Guesnet P. Increasing dietary intake of docosahexaenoic acid affects long-chain polyunsaturated fatty acid composition differently according to brain areas in 2-mo-old rat. Lipids 2001;36:1071 (abstr).
23. Pietsch A, Lorenz RL. Rapid separation of the major phospholipid classes on a single aminopropyl cartridge. Lipids 1993;28:949–51.
24. Alessandri JM, Goustard-Langelier B. Alterations in fatty acid composition of tissue phospholipids in the developing retinal dystrophic rat. Lipids 2001;36:1141–52.
25. Moriguchi T, Sheaff Greiner R, Salem N Jr. Behavioral deficits associated with dietary induction of decreased brain docosahexaenoic acid concentration. J Neurochem 2000;75:2563–73.
26. Tinoco J, Miljanich P, Medwadowski B. Depletion of docosahexaenoic acid in retinal lipids of rats fed a linolenic acid–deficient, linoleic acid–containing diet. Biochim Biophys Acta 1977;486: 575–8.
27. Weisinger HS, Vingrys AJ, Sinclair AJ. Effects of dietary n−3 deficiency on the electroretinogram in the guinea pig. Ann Nutr Metab 1996;40:91–8.
28. Stinson AM, Wiegand RD, Anderson RE. Recycling of docosahexaenoic acid in rat retinas during n−3 fatty acid deficiency. J Lipid Res 1991;32:2009–17.
29. Wiegand R, Koutz C, Stinson A, Anderson R. Conservation of docosahexaenoic acid in rod outer segments of rat retina during n−3 and n−6 fatty acid deficiency. J Neurochem 1991;57:1690–9.
30. Bazan NG, Gordon WC, Rodriguez de Turco EB. Docosahexaenoic acid uptake and metabolism in photoreceptors: retinal conservation by an efficient retinal pigment epithelial cell–mediated recycling process. Adv Exp Med Biol 1992;318:295–306.
31. Rodriguez de Turco EB, Parkins N, Ershov AV, Bazan NG. Selective retinal pigment epithelial cell lipid metabolism and remodeling conserves photoreceptor docosahexaenoic acid following phagocytosis. J Neurosci Res 1999;57:479–86.
32. Wang N, Anderson RE. Transport of 22:6n−3 in the plasma and uptake into retinal pigment epithelium and retina. Exp Eye Res 1993;57:225–33.
33. Goustard-Langelier B, Alessandri JM, Raguénez G, Durand G, Courtois Y. Phospholipid incorporation and metabolic conversion of n−3 polyunsaturated fatty acids in the Y79 retinoblastoma cell line. J Neurosci Res 2000;60:678–85.
34. Li J, Wetzel MG, O'Brien PJ. Transport of n−3 fatty acids from the intestine to the retina in rats. J Lipid Res 1992;33:539–48.
35. Magret V, Elkhalil L, Nazih-Sanderson F, et al. Entry of polyunsaturated fatty acids into the brain: evidence that high-density lipoprotein–induced methylation of phosphatidylethanolamine and phospholipase A2 are involved. Biochem J 1996;316:805–11.
36. Hamilton JA, Kamp F. How are free fatty acids transported in membranes? Is it by proteins or by free diffusion through the lipids? Diabetes 1999;48:2255–69.
37. Stremmel W, Pohl J, Ring A, Herrmann T. A new concept of cellular uptake and intracellular trafficking of long-chain fatty acids. Lipids 2001;36:981–9.
38. Connor WE, Neuringer M, Lin DS. Dietary effects on brain fatty acid composition: the reversibility of n−3 fatty acid deficiency and turnover of docosahexaenoic acid in the brain, erythrocytes, and plasma of rhesus monkeys. J Lipid Res 1990;31:237–47.
39. Gibson RA, Neumann MA, Makrides M. Effect of increasing breast milk docosahexaenoic acid on plasma and erythrocyte phospholipid fatty acids and neural indices of exclusively breast fed infants. Eur J Clin Nutr 1997;51:578–84.