National Library of Canada

Bibliothèque nationale du Canada

Acquisitions and Bibliographic Services Branch

Direction des acquisitions et des services bibliographiques

395 Wellington Street
Ottawa, Ontario
K1A 0N4

395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

Canada

UNIVERSITY OF ALBERTA

Physiological Changes in Diet Fat Composition Alters Fatty Acid Content of Phosphoglycerides in Neuronal and Glial Cells During Brain Development in the Rat

by

Jacqueline A. Jumpsen

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

IN

NUTRITION

Department of Food Science and Nutrition

EDMONTON, ALBERTA

Fall, 1994

National Library of Canada

Bibliothèque nationale du Canada

Acquisitions and Bibliographic Services Branch

Direction des acquisitions et des services bibliographiques

395 Wellington Street
Ottawa, Ontario
K1A 0N4

395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

The author has granted an irrevocable non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of his/her thesis by any means and in any form or format, making this thesis available to interested persons.

L'auteur a accordé une licence irrévocable et non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de sa thèse de quelque manière et sous quelque forme que ce soit pour mettre des exemplaires de cette thèse à la disposition des personnes intéressées.

The author retains ownership of the copyright in his/her thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without his/her permission.

L'auteur conserve la propriété du droit d'auteur qui protège sa thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

ISBN 0-315-95054-4

Canadä

EXPERIMENTAL

Nov. 22, 1993

Dear Ms. Jumpsen:

Responding to your letter of today, you have my permission to use Fig. 3 from our review paper in your thesis.

Sincerely,

Peter J. Morgane, Ph.D.
Senior Scientist

PJM/av Ref: Neurosci. Biobeh. Rev. 17/91-128, 1993/

UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR: Jacqueline A. Jumpsen

TITLE OF THESIS: Physiological changes in diet fat composition alters fatty acid content of neuronal and glial cells during brain development in the rat.

DEGREE: Master of Science

YEAR THIS DEGREE GRANTED: 1994

Permission is hereby granted to THE UNIVERSITY OF ALBERTA LIBRARY to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves all other publication and other rights in association with the copyright in the thesis, and except hereinbefore provided neither the thesis nor any substantial portion thereof may be printed or otherwise reproduced in any material form whatever without the author's prior written permission.

J Jumpsen

January 31, 1994

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **Physiological Changes in Diet Fat Composition Alters Fatty Acid Content of Neuronal and Glial Cells During Brain Development in the Rat** submitted by **Jacqueline A. Jumpsen** in partial fulfillment of the requirements for the degree of Master of Science in Nutrition.

Dr. M.T. Clandinin, Supervisor

Dr. J. Van Aerde

Dr. C.J. Field

Dr. V. Dias

Date: January 18, 1994

Special thanks to:

Arlene Parrott,
for all your help

ABSTRACT

It has been suggested that the fat composition of infant formulae should provide arachidonic acid (AA) and docosahexaenoic acid (DHA) or contain increased α-linolenic acid (ALNA). To determine if these dietary regimes affect brain development, diets varying in 18:2n-6 and 18:3n-3 content with or without AA or DHA were fed to rats from birth to six weeks of age. Neuronal and glial cells were purified from the cerebellum, the frontal and hippocampal regions of the brain. Fatty acid analysis of ethanolamine-, choline-, serine- and inositol phosphoglycerides indicated that physiological changes in diet fat significantly altered brain fatty acid composition. Changes were most pronounced in ethanolamine phosphoglycerides. The effect of diet fat appeared to be greater in glial cells compared to neuronal cells. This may be an effect of the later developmental timing of gliogenesis compared to neurogenesis. The composition of fatty acids in the phosphoglycerides were distinct and most exhibited changes with age. Accretion of 18:2n-6, 18:3n-3 and 20:5n-3 however, did not respond to age. Accretion of 20:4n-6 and 22:6n-3 varied with the type of fatty acid fed. Brain regions and cell types vary in the amount and rate of DHA and AA accretion. Accretion of 22:6n-3 in all phosphoglycerides was greater when DHA was supplied in the diet. Supplementing the diet with 18:3n-3 also increased 22:6n-3 but not in all lipid classes examined. Feeding 18:3n-3 produced the greatest increase in 22:6n-3 in

phosphatidylethanolamine and phosphatidylinositol. Accretion of 20:4n-6 in all phosphoglycerides, except glial phosphatidylserine, was greater when AA was supplied in the diet. It is concluded that the supplementation of preformed AA and DHA to the diet enhances the accretion of these fatty acids in the developing rat brain.

ACKNOWLEDGEMENTS

The financial support provided by Wyeth Ayerst Research is greatly appreciated. Thanks also to Dr. Eric Lien for his suggestions and helpful discussions.

I gratefully acknowledge the statistical and graphical assistance from Dr. Goh whom also carries a large supply of patience to answer a never ending stream of questions.

To the "permanent" members of Dr. Clandinin's lab group, who were there from start to finish and are still there. Thanks for your help and support.

To Tom, for allowing me the opportunity to work on this project.

To my family, because there are so many of you, the always popular question of "when are you going to finish ?" gave me the drive and determination to finish.

To Brian Plunkie, my husband, who was there from start to finish... and is still there. Thank you.

TABLE OF CONTENTS

I. INTRODUCTION	1
LIPIDS	2
Classification	3
Membrane lipids and fatty acids	3
OMEGA-6 AND OMEGA-3 FATTY ACIDS	4
Classification	4
Sources and Requirements	5
Balance between omega-3 and omega-6	7
Excess essential fatty acids	8
ESSENTIAL FATTY ACID DEFICIENCY	9
Omega-6 fatty acids	9
Omega-3 fatty acids	10
ESSENTIALITY OF OMEGA-6 AND OMEGA-3	11
Eicosanoids	12
Development	13
EFFECT OF DIET ON MEMBRANE FATTY ACID COMPOSITION	14
BRAIN DEVELOPMENT AND VULNERABILITY	15
Timing	15
Neural Cells and Morphogenesis	16
Function	17
LIPIDS AND ESSENTIAL FATTY ACIDS IN BRAIN DEVELOPMENT	18
Fatty acid accretion in the brain	19
Essential fatty acids and retinal development	20
ESSENTIAL FATTY ACID SYNTHESIS AND TRANSPORT IN BRAIN	21
Synthesis and metabolism within brain	21
Transport from blood to brain	23
Passage from the gut	23
SOURCES OF ESSENTIAL FATTY ACIDS FOR THE FETUS AND NEONATE	24
The Placenta	24
Biomagnification	26

Research Plan 28
Rationale 28
Hypotheses 29
Research Objectives 29

2. **MATERIALS AND METHODS**
Animals and Diets 30
Tissue preparation and cell isolation 33
Cell Characterization 34
Protein determination 34
Gel Electrophoresis 35
Semi-dry electrophoretic transfer 35
Immunoblotting 36
Microscopy 37
Lipid Analysis 37
Column Chromatography 37
Thin-layer Chromatography 39
Saponification 39
Methylation 40
Reverse-phase Chromatography 40
Gas-Liquid Chromatography 40

STATISTICAL ANALYSIS 41

3. **RESULTS**
Growth Characteristics 42
Purity of Preparations 45
Omissions 45
Fatty acid composition of Glial cells 45
Phosphatidylethanolamine 46
Phosphatidylcholine 54
Phosphatidylserine 58
Phosphatidylinositol 64

Fatty acid composition of Neuronal cells 72
Phosphatidylethanolamine 72
Phosphatidylcholine 78
Phosphatidylserine 83
Phosphatidylinositol 87

4. DISCUSSION 92

5. REFERENCES

LIST OF TABLES

Table		Page
1.1	Fatty acid composition of mature milk and infant formulae.	2
1.2	N-6 and N-3 fatty acid production of eicosanoids.	13
1.3	Fatty acid accretion rates in infant brain and cerebellum.	20
2.1	Basal diet composition.	33
2.2	Fatty acid composition of mixed diets.	31
2.3	Fatty acid composition of oils.	32
3.1	Glial and neuronal cell fatty acid composition at 3 weeks of age in phosphatidylethanolamine.	50
3.2	Glial and neuronal cell fatty acid composition at 3 weeks of age in phosphatidylcholine.	57
3.3	Glial and neuronal cell fatty acid composition at 3 weeks of age in phosphatidylserine.	62
3.4	Glial and neuronal cell fatty acid composition at 3 weeks of age in phosphatidylinositol.	70

LIST OF FIGURES

Figure		Page
1.1	Structure of parent fatty acids.	5
1.2	Desaturation-elongation pathways for synthesis of long-chain unsaturated fatty acids.	6
1.3	Comparison of major developmental events in the brain of rats and humans.	15
2.1	Schematic of methods.	38
3.1	Total body weight over time.	43
3.2	Total brain weight over time.	43
3.3	Regional brain weight over time.	44
3.4	Effect of diet treatment on %16:0 in glial PE.	47
3.5	Effect of diet treatment on %22:6n-3 in glial PE.	48
3.6	Effect of diet treatment on %20:4n-6 in glial PE.	49
3.7	Effect of diet treatment on %18:1 in glial PE.	51
3.8	Effect of diet treatment on %18:0 in glial PE.	53
3.9	Effect of diet treatment on %20:4n-6 in glial PC.	55
3.10	Effect of diet treatment on %18:0 in glial PC.	56
3.11	Effect of diet treatment on %20:4n-6 in glial PS.	59
3.12	Effect of diet treatment on %22:6n-3 in glial PS.	60

		page
3.13	Effect of diet treatment on %16:0 in glial PS.	61
3.14	Effect of diet treatment on %18:1 in glial PS.	63
3.15	Effect of diet treatment on %22:6n-3 in glial PI.	66
3.16	Effect of diet treatment on %20:4n-6 in glial PI.	67
3.17	Effect of diet treatment on %16:0 in glial PI.	68
3.18	Effect of diet treatment on %18:1 in glial PI.	69
3.19	Effect of diet treatment on %18:0 in glial PI.	71
3.20	Effect of diet treatment on %22:6n-3 in neuronal PE.	74
3.21	Effect of diet treatment on %20:4n-6 in neuronal PE.	75
3.22	Effect of diet treatment on %16:0 in neuronal PE.	76
3.23	Effect of diet treatment on %18:0 in neuronal PE.	77
3.24	Effect of diet treatment on %22:6n-3 in neuronal PC.	79
3.25	Effect of diet treatment on %16:0 in neuronal PC.	80
3.26	Effect of diet treatment on %16:1 in neuronal PC.	81
3.27	Effect of diet treatment on %20:4n-6 in neuronal PC.	82
3.28	Effect of diet treatment on %22:6n-3 in neuronal PS.	84
3.29	Effect of diet treatment on %16:0 in neuronal PS.	85

		page
3.30	Effect of diet treatment on %18:0 in neuronal PS.	86
3.31	Effect of diet treatment on %22:6n-3 in neuronal PI.	89
3.32	Effect of diet treatment on %16:0 in neuronal PI.	90
3.33	Effect of diet treatment on %18:0 in neuronal PI.	91

Chapter 1.
INTRODUCTION

"For millions of years, nutrient requirements of infant mammalian species have been met through the nutrition provided by breast feeding" (Pierse, Van Aerde, Clandinin, 1988). For human infants, commercial infant formulae and the use of cow's milk exist as substitutes for human milk. In many instances, infant formulae are used when breast feeding may not be possible, as in the case of some premature infants, born at less than thirty-two weeks gestational age. Due to the early departure from intrauterine nutrition, the concern becomes whether these premature infants are receiving an adequate balance of nutrients, particularly fat.

In 1929, Burr and Burr demonstrated that fat was an essential component of the diet. In later studies, the critical components of fat were identified and termed "essential fatty acids". These were shown to be polyunsaturated fatty acids with two or more double bonds (Holman, 1968). Nearly thirty years ago, it was suggested that the dietary content of polyunsaturated essential fatty acids during early infancy may be a factor in the aetiology of certain neurological disorders (Rathbone, 1965; Clausen and Moller, 1967), as well as being a factor in behavioral and other functional deficits (Yamamoto, Hashimoto et al. 1988; Bourre, Francois, Youyou et al. 1989; Lamptey and Walker 1976; Neuringer, Connor, Lin et al. 1986).

Recently, Health and Welfare Canada recommended that infant formulae contain certain minimum levels and ratios of essential fatty acids (Food and Drug Regulations, 1991). Specifically, formulae are to contain not less than 100 mg of linolenic acid, per 100 available kilocalories, and to have a linoleic to linolenic acid ratio between 4:1 and 10:1. The inclusion of linolenic acid is to be in the form of a glyceride. The implementation of these recommendations will in essence change the composition of many infant formulas marketed in Canada. Table 1-1 shows

the fatty acid composition of mature human breast milk compared to some infant formulas. These recommendations indicate a recognition that there is an appropriate balance of dietary essential fatty acids needed for infant development and growth.

Table 1-1. Fatty acid composition of mature human milk and infant formulae.*

Fatty acid	Human milk	SMA 20	ISOMIL	SMA Preemie	ENFAMIL Premature
C12:0	3.8	14.3	24.2	14.6	10.1
C14:0	5.2	5.9	10.2	5.6	3.6
C16:0	22.5	13.1	11.0	10.3	6.8
C18:0	8.7	7.0	4.8	5.1	1.1
C18:1	39.5	39.4	26.9	34.2	22.0
C18:2	14.4	13.0	16.1	14.3	13.3
C18:3	2.0	1.0	0.3	1.2	0.4
C20:4	0.5	-	-	-	-
C22:6	0.14	-	-	-	-

*modified from Feldman et.al, 1992.

This chapter will review the essential fatty acids and the effects of dietary essential fatty acids on development, primarily of the brain.

LIPIDS

The cell and tissue structures of advanced living systems are composed of primarily protein, lipid, and polyhydric alcohols. The first two are the most important quantitatively, however, their amounts vary depending on the cell type and structure of concern (Crawford and Sinclair, 1972). The brain is one of the most lipid-concentrated organs in the body, second only to adipose tissue. Nervous tissue contains more than sixty percent lipid, which plays a role in modifying the structure, fluidity and function of brain membranes (Bourre, Bonneil, Clement et al., 1993; Dyer and Greenwood, 1991; Brenner, 1984; Stubbs and Smith, 1984; Foot, Cruz and Clandinin 1983 and 1982) . This thesis will focus on these lipids.

Classification

One classification of lipids is based on chemical composition. The main distinction in this classification is the presence or absence of glycerol as a backbone structure (White et al., 1973). Two biologically important groups of lipids containing a glycerol backbone are the neutral fats and the phosphoglycerides. Groups of lipids not containing glycerol include sphingolipids (ceramides, sphingomyelins and glycosphingolipids), aliphatic alcohols, wax esters and prostaglandins, among others. Also incorporated in this classification system are lipid compounds that complex with proteins and with carbohydrates (lipoproteins, proteolipids, lipopolysaccharides).

Membrane Lipids and Fatty Acids

In general, lipids are water insoluble as they contain predominantly nonpolar, hydrocarbon groups. However, some lipids do contain polar groups. For example, fatty acids, phospholipids, sphingolipids, bile salts and to a lesser extent, cholesterol. The combination of the water-insoluble, hydrophobic group and the water-soluble, hydrophilic group in lipids is known as amphipathic. These molecules combine in various ways to form, among other things, the lipid membrane. The positioning of these molecules may enable the membrane to limit the movement of water-soluble substances into and out of cells, one of the membranes major functions.

The major types of membrane lipids are phospholipids and glycolipids. Classification of phospholipids is based on the polar head group (choline, ethanolamine, inositol, serine). The precursors for these and other lipids are fatty acids. The two main classes of fatty acids are saturated and unsaturated. More than one-third of brain fatty acids are polyunsaturated, with a prevalence of arachidonic acid, an omega-6 fatty acid , and cervonic acid, and omega-3 fatty acid. The omega-6 and omega-3 fatty acids will encompass the major focus for this thesis.

OMEGA-6 AND OMEGA-3 FATTY ACIDS

Classification

The three main families of naturally occurring unsaturated fatty acids are the omega-9, the omega-6, and the omega-3 series. For each series, the first double bond is situated nine, six or three carbons respectively from the methyl terminal end (Fig.1-1). The omega-9 fatty acids are considered non-essential fatty acids for the reason that animals, including humans, are able to synthesize them. Mammals however, lack the enzymes required to insert double bonds at carbon atoms beyond C-9 (Rivers, Sinclair and Crawford, 1975). Hence, fatty acids belonging to the omega-6 series and the omega-3 series are essential fatty acids and must be obtained through the diet. Linoleic acid, 18:2n-6, is the parent omega-6 fatty acid and together with its derivatives, is the "primary" essential fatty acid (Conner and Neuringer, 1988 p.191). The omega-3 fatty acids are derived from the parent alpha-linolenic acid, 18:3n-3. Although a dietary requirement for 18:3n-3 has not been clearly established in the United States, a growing body of evidence indicates this series of fatty acids is also essential (Lamptey and Walker, 1976; Leprohon-Greenwood and Anderson, 1986; Bourre, Piciotti, Dumont et al., 1990). Nutrition recommendations in Canada have established a requirement for 18:3n-3 (Nutrition Recommendations. Health and Welfare Canada, 1990).

```
Omega-9 family
      Oleic acid (18:1n9)
    CH3/\/\/\/\//\/\/\/\COOH
      <-------> <------->
   mammals lack   mammals have
   enzymes to     enzymes to
   introduce      introduce double
   double bonds     bonds here
   here

Omega-6 family
     Linoleic acid (18:2n6)
     CH3\/\/\//\//\/\/\/\COOH

Omega-3 family
     Linolenic acid (18:3n3)
     CH3/\//\//\//\/\/\/\COOH
```

Figure 1.1. Structure of "parent" fatty acids

Sources and Requirements

Linoleic and alpha-linolenic acids are synthesized in plants, fungi and bacteria. The omega-6 fatty acids are found primarily in vegetable oils. Dietary sources of omega-3 fatty acids include plants from the land, phytoplankton, algae from the sea, and animal life that has consumed these lower food chain sources. When ingested by animals, linoleic and alpha-linolenic acids are converted to long chain, highly unsaturated fatty acids through stepwise chain elongation and desaturation (Fig.1.2).

	omega-9	omega-6	omega-3	
	18:0			
Δ-9 desaturase	↓			
	18:1	18:2	18:3	
Δ-6 desaturase	↓	↓	↓	
	18:2	18:3	18:4	
elongase	↓	↓	↓	
	20:2	20:3	20:4	
Δ-5 desaturase	↓	↓	↓	
	20:3	20:4	20:5	
elongase	↓	↓	↓	
	22:4	22:4	22:5	*→24:5
Δ-4 desaturase		↓	↓	↓Δ-6
		22:5	22:6 ←	24:6

Figure 1.2. Desaturation-elongation pathways for synthesis of long chain unsaturated fatty acids.
*alternate route proposed by Voss et al.,1991.

Based on the sources of these essential fatty acids, the requirements of at least 3% of energy as n-6 fatty acids and at least 0.5% of energy as n-3 fatty acids, can be met through diet, leading to sufficient amounts of the longer-chain homologues of both the omega-6 and the omega-3 series. This appears to be more so for the omega-6 fatty acids however, since a limitation may exist for the omega-3 fatty acids (Saunders and Younger, 1981).

Saunders and Younger (1981) suggested that although the conversion of linoleic acid (18:2n-6) to arachidonic acid (20:4n-6) occurs readily, there may be a limit on the conversion of linolenic acid (18:3n-3) to docosahexaenoic acid (22:6n-3) due to the limited activity of Δ-4

desaturation enzyme in humans. This was suggested when addition of 18:3n-3 to the diet did not raise the proportion of 22:6n-3 in plasma phospholipids. A similar effect was also noted by Neuringer et al.(1986) and Carlson et al., (1986). Cook (1982) noted that in the brain, omega-6 fatty acids are the preferred substrate for chain elongation; the order of preference is as follows: 18:3(6) > 16:0 > 20:4(6) > 18:3(3) > 18:2(6) > 20:3(6). Despite meeting the requirements for essential fatty acids, these studies indicate it may still be possible to exhibit low levels in the long chain homologues of each series if the right balance is not obtained between the parent fatty acids of each group.

Balance between Omega-6 and Omega-3 fatty acids

The balance between the omega-6 and the omega-3 fatty acids depends on the ratio of the parent fatty acids in the diet. The absolute amount of each is also important as there is competitive inhibition between the two series of fatty acids for the Δ-6 and Δ-5 desaturation enzymes (Tinoco, 1982; Brenner, 1981; Alling et al., 1974; Mohrhauer and Holman, 1963). In all cases where the amount of dietary linolenate is almost equal to or exceeds the levels of linoleate, the former seems to competitively inhibit the conversion of linoleate (Mohrhauer and Holman, 1963). Recently, Bourre et al. (1988), suggested that the balance between omega-3 and omega-6 fatty acids may be important in determining the interactions of these fatty acids with arachidonic acid metabolism and the formation of prostaglandins. The long-chain omega-3 fatty acids, eicosapentaenoate and docosahexaenoate, compete with arachidonate for production of their respective eicosanoids (Simopoulos, 1991; Needleman, 1979). Negligible levels of n-3 fatty acids however, shift the balance of eicosanoid production towards a more thrombic, less antiaggregatory state (Simopoulos, 1991).

The inhibitory effect of linolenic acid (18:3n-3) on the conversion of

linoleic acid (18:2n-6) to arachidonic acid (20:4n-6) is much greater than that of 18:2n-6 on the conversion of 18:3n-3 to eicosapentaenoic acid (20:5n-3) (Holman, 1964). Thus, inhibiting the conversion of 18:2n-6 to 20:4n-6 by increasing dietary 18:3n-3 reduces arachidonic acid in tissue lipids (Mohrhauer and Holman, 1963). However, this reduction in 20:4n-6 may be counterbalanced by increasing the dietary intake of this fatty acid (Sinclair, 1991 seminar). Reduction of 20:4n-6 caused by increasing 18:3n-3 in the diet was found by Anding and Hwang (1986) to be greater in liver and serum lipids than in brain lipids. Thus, the balance of essential fatty acids appears to affect various organs differently and this effect may also vary depending on the stage of development of an individual (Farrell et al., 1988).

Excess essential fatty acids

Large amounts of omega-3 fatty-acids ingested by experimental animals gives rise to adverse effects (Martinez and Ballabriga, 1987) and may increase requirements for Vitamin E (Bourre, Bonneil, Dumont, Piciotti et al., 1988). Prolonged deficit of Vitamin E can lead to neuropathy (Machlin, 1984) and thus plays an important role in neurological function. Although it is difficult to extrapolate this data to humans, it is known that young animals are more susceptible than the old (Foot, Cruz, and Clandinin, 1982) and the low-birthweight infant is especially at risk (Uauy et al., 1989). Other adverse effects of large intakes of omega-3 intake include reduced platelet aggregation, inhibition of arachidonic acid metabolism for prostaglandin formation and immunosuppression (Simopoulus, 1991). Just as large intakes of omega-3 fatty acids may lead to adverse consequences, so may high levels of omega-6 fatty acids. In a recent study (Yamamoto et al., 1988), it was concluded that alpha-linolenic acid is essential for maintaining high learning ability in rats. However, the authors also suggested that perhaps high levels of omega-6 fatty acids impair learning. It is evident that determining the

optimal essential fatty acid balance or the optimal omega-3/omega-6 fatty acid ratio for the human diet is important. For the newborn infant, toxic effects of polyunsaturated fatty acids have been reported to include alterations in immunity (McCormick et al 1977; Passwell et al 1976), and changes in myelin configuration and function (Friedman, 1980).

ESSENTIAL FATTY ACID DEFICIENCY

For most individuals essential fatty acids can be obtained through diet. In pregnancy, the fetus relies on maternal circulation and transfer of these essential fatty acids across the placenta (Bourre, Dumont, Piciotti, Pascal and Durand, 1990; Poissennet et al., 1988; Fiedman, 1980). If these requirements are not met, the fetus is at increased risk for essential fatty acid deficiency (Neuringer, Connor, VanPatten, Barstad, 1984). In fact, neonates are susceptible to essential fatty acid deficiency due to low fat reserves (Clandinin et al., 1981). For the premature infant, or the infant small for gestational age, this risk for essential fatty acid deficiency is greatly magnified and the need for the appropriate amounts and types of fatty acid is critical for proper development.

Omega-6 fatty acids

Studies in various animal species and humans have established a nutritional requirement for omega-6 fatty acids (Bourre, Piciotti, Dumont, et al., 1990; Farrell et al., 1988; Holman, 1977; FAO/WHO, 1977; Houtsmuller, 1973). Providing linoleic acid at a level of one percent of food energy is sufficient to prevent overt deficiency symptoms (Holman, 1977). However, since competition exists among the fatty acids for desaturating enzymes (Brenner, 1981), a level of at least three percent of energy should be met by omega-6 fatty acids (FAO, 1977). In a recent study by Bourre et al. (1989), which compared the requirements of omega-6 and omega-3 fatty acids in different body organs, it was found that the

demands for 18:2n-6 vary between 150 and 1200 mg/100g diet according to the organ in question (or 2.4% of calories to avoid deficiency). This estimation is a result of measuring the accretion of 18:2n-6 in various organs while increasing the amount of this fatty acid in the diet. The requirement was determined once a plateau of 18:2n-6 accretion was reached. If these requirements are not met, a deficiency occurs. Symptoms resulting from dietary deficiency include retarded growth, reproductive failure, skin and hair changes and liver pathology, (Burr and Burr, 1929; Holman, 1968) all of which can be prevented or reversed by feeding omega-6 fatty acids (Holman, Johnson and Hatch, 1982). Uauy et al.,(1989) recently suggested the recommendation of 3.0% of total energy is adequate to prevent clinical signs of deficiency but may be insufficient to assure functional and biochemical normalcy.

Omega-3 fatty acids

A comparable deficiency existing for omega-3 fatty acids has been suggested for humans (Holman, Johnson and Hatch, 1982) and demonstrated in other species (Neuringer, Conner, Van Petten, and Barstad, 1984). The requirement for omega-3 fatty acids is still controversial, but has been suggested to be at least 0.5 percent of energy (Health and Welfare Canada, 1990). Bourre et al. (1989), have indicated that a diet deficient in 18:3n-3 affects various electro-physiological and biochemical parameters in rats. The authors determined that the dietary 18:3n-3 requirement for membrane synthesis is the same regardless of the organ (200 mg/100g diet, or 0.4% of caloric energy to avoid deficiency), in contrast to that described for omega-6 fatty acids. These authors concluded the omega-6 to omega-3 ratio to be about 6:1, resulting in no "pharmacological effect". Health and Welfare Canada (1990) recommends a ratio of between 4:1 and 10:1 for infant feeding.

An increase in the conversion of oleic acid to eicosatrienoic acid (20:3n-9) is characteristic of essential fatty acid deficiency. This

increase in eicosatrienoic acid provides endogenous synthesis of polyunsaturated fatty acids from oleic acid (Holman, 1964; Mohrhauer and Holman, 1963). Thus, eicosatrienoic acid is incorporated into cellular structures (Paoletti and Galli,1972). However, this does not alleviate the deficiency of essential fatty acids (Holman, 1960), as arachidonic acid has properties that cannot be met by eicosatrienoic acid (Houtsmuller, 1973). This increase in eicosatrienoic acid does not occur in omega-3 fatty acid deficiency, but only when both omega-3 and omega-6 fatty acids are unavailable (Menon and Dhopeshwarkar, 1982). Prolonged essential fatty acid deficiency or total fat deficiency affects the balance of omega-3 and omega-6 fatty acids in the various cells in the body. It has been shown to reduce content of docosahexaenoic acid (22:6n-3) and increase percentage of docosapentaenoic acid (22:5n-6) in brain phospholipids of both rats and mice (Martins, Wennberg et al., 1984; Bourre, Francois, Youyou et al., 1989). Since humans are susceptible to essential fatty acid deficiency (Burr and Burr, 1929; Holman et al., 1982; Bjerve et al., 1987), it is important to be aware of some of the probable causes for its appearance. Some studies have shown that infants fed skim milk (Yamanaka, Clemens and Hutchinson, 1980), or individuals fed by parenteral feeds (Anonymous, 1986; Bjerve, Fisher, and Alme, 1987) are at risk for essential fatty acid deficiency.

ESSENTIALITY OF OMEGA-6 AND OMEGA-3 FATTY ACIDS

Both omega-6 and omega-3 fatty acids are required for proper development. These essential fatty acids have a role as structural components of developing membranes (Crawford and Sinclair, 1972; Clandinin et al., 1989). Thus, essential fatty acids are important in the earliest stages for the developing embryo.

Providing cells with polyunsaturated fatty acids during development is critical, otherwise functional and compositional impairment can occur to some tissues, for example the brain (Alling et al., 1973; Kuhn and

Crawford, 1986; Bourre, Piciotti, Dumont, 1990; Enslen, Milon and Malnoe, 1991). During development, a deficiency of omega-6 and/or omega-3 fatty acids has been shown to result in an alteration of a variety of CNS activities from membrane-associated enzymes and receptors (Bernsohn and Cohen, 1972; Hannah and Campagnoni, 1987; Bourre et al., 1989) to cognitive behaviors (Wainwright et al., 1991) and visual function (Bourre et al., 1989; Connor, Neuringer, Barstad and Lin, 1984; Uauy 1991 and 1992). Polyunsaturated fatty acids are a major constituent of cell membranes and tissues and are critically important to a number of biological functions (Moore, et al., 1990).

Eicosanoids

The long C20 fatty acids of both n-3 and n-6 series are involved in formation of thromboxanes, prostaglandins, and leukotrienes, collectively known as the eicosanoids. These biologically active compounds encompass a variety of functions (Simopoulus, 1991). Thromboxanes and prostaglandins are produced via the cyclooxygenase pathway while leukotrienes are produced via the lipoxygenase pathway (Leaf and Weber, 1988). The physiological properties of eicosanoids derived from eicosapentaenoic acid (EPA) differ, in some ways, from those derived from arachidonic acid (AA) (Table 1.2).

Formation of both cyclooxygenase (Wolfe, 1982) and lipoxygenase (White and Stine, 1984; Lindgren et al., 1984) metabolites of arachidonate occur in the CNS. Products of both these pathways alter neuronal activity (Palmer et al., 1981). Release of arachidonate by synaptosomes is accompanied by formation of PGE_2 and $PGF_{2\alpha}$ (Bradford et al., 1983) metabolites known to alter neurotransmitter release (Hedqvist, 1973) and possibly glial function. Thus, electrical activity may be coupled to altered arachidonate metabolism. It has also been suggested that prostaglandins may be involved in regulation of cellular growth and differentiation at several levels (Kuhn and Crawford, 1986).

	Cyclooxygenase	Lipoxygenase
AA	TXA_2 ► proaggregatory ► vasoconstriction	LTB_4 ► chemotactic ► inflammatory
	PGI_2 ► vasodilation ► antiaggregatory	LTE_4 ► vasoconstriction
	PGE_2 ► neurotransmitter release PGF_2 ► neurotransmitter release	
EPA	TXA_3 ► vasoconstriction(weak) PGI_3 ► vasodilation ► antiaggregatory	LTB_5 ►chemotactic(weak) LTE_5► antiinflammatory

Table 1.2. N-6 and N-3 fatty acid production of eicosanoids.

Development

The long chain polyunsaturated fatty acids, particularly arachidonic acid (20:4n-6) and docosahexaenoic acid (22:6n-3) are important constituents of tissue lipids (Neuringer, Connor, Lin et al., 1986) and are prominent in the cell membranes of the central nervous system. The period of development and differentiation require very large amounts of DHA for the biogenesis of cell membranes (Bazan, 1990). The highest levels of 22:6n-3 are found in the cerebral cortex and the retina, specifically in phospholipids of synaptosomal membranes and of the outer segment membranes of the photoreceptor (Clandinin, Chappell, Leong, et al., 1980b; Neuringer and Connor, 1986; Anderson, Connor, and Corliss, 1990; Sarda, Gjarib, Croset et al., 1991). In rat brain, most rapid accumulation of DHA occurs between birth and 20 days of age, correlating with the time of maximal postnatal cell differentiation (Scott and Bazan,

1989). In humans, this period of DHA accumulation occurs both before and after birth for up to 12 weeks (Clandinin et al., 1981). Based on this, it is important that large amounts of docosahexaenoic acid and arachidonic acid be available during fetal and postnatal development, specifically for the development of the central nervous system.

EFFECT OF DIET ON MEMBRANE FATTY ACID COMPOSITION

Of all the organs in the body, the brain has been viewed as most resistant to structural change by both endogenous and exogenous factors (Mohrhauer and Holman, 1963). Due to research in recent years, however, the brain has been shown to be more responsive to exogenous factors than previously considered (Sinclair, 1975; Foot et al., 1982; Bourre, Durand, Pascal and Youyou, 1989). An alteration in n-6 or n-3 series in the diet can trigger dramatic alterations in the brain (Foot et. al., 1982; Hargreaves and Clandinin, 1990) and greatly affects the pattern of tissue lipids (Svennerholm, Alling, Bruce et al., 1972). These alterations are sometimes associated with changes in the physical properties of the membrane (Foot, Cruz and Clandinin, 1982; Hargreaves and Clandinin, 1988), alterations in activities of enzymes (Hargreaves and Clandinin, 1987), receptors and carrier-mediated transport (Stubbs and Smith, 1984; Spector and Yorek, 1985) and alterations in cellular interactions (Scott, Lew, Clandinin, and Cinader, 1989). In studies by Bourre et al., (1984), Youyou et al., (1986), and Enslen et al., (1991) it was shown that alterations in fatty acid composition of brain cells and subcellular fractions could be induced through alterations of dietary fat. These alterations may ultimately contribute to brain neuronal processes (Yehuda, 1987). With recent evidence showing the brain to be responsive to alterations in exogenous factors, it becomes exceedingly important to attempt to optimize diet-induced alterations during brain development.

BRAIN DEVELOPMENT AND VULNERABILITY

Timing

The timing of birth in relation to the stage of brain maturation results in substantial difference in brain development among widely differing species (Dobbing and Sands, 1970). There are marked species differences in gross proportions, with primates showing increased cephalization and large forebrains of increasing morphological complexity. However, the sequence of broad developmental events and growth spurts is similar among different mammalian species. A comparison between rats and humand is shown (Fig 1.3).

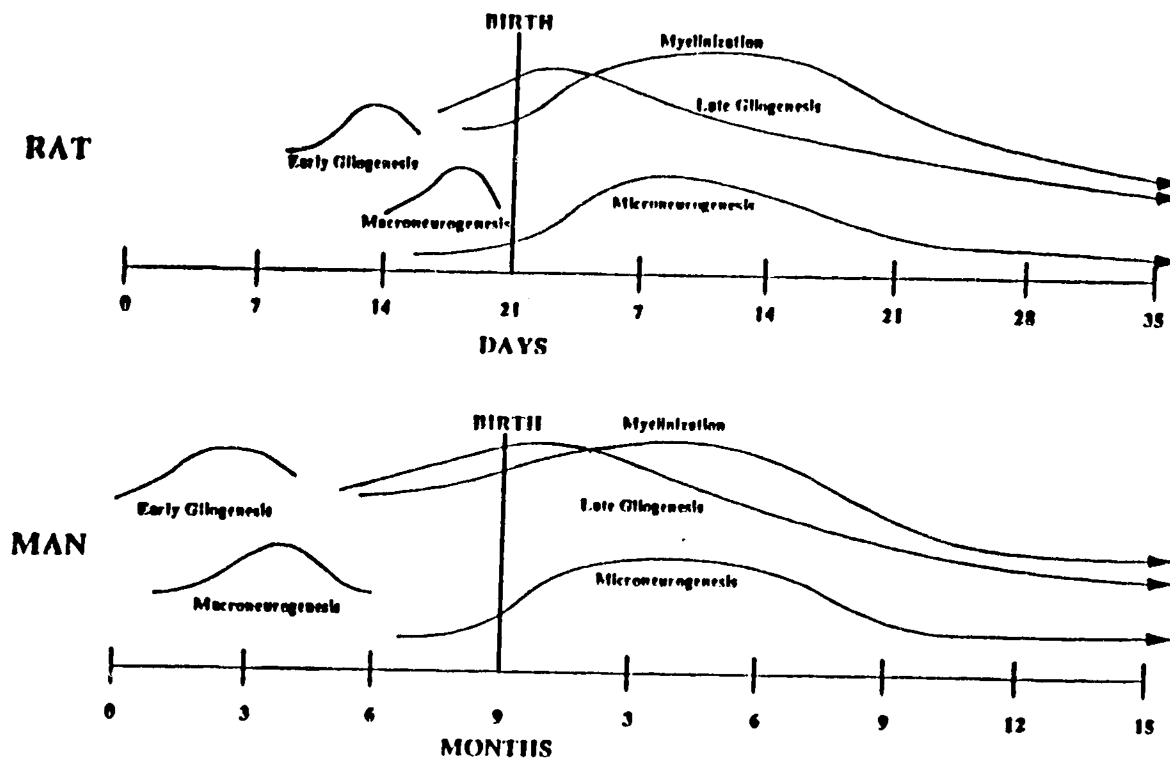


Fig.1.3. Comparison of major developmental events in rats and humans in relation to birth.
Reproduced with permission from Morgane et al., 1993.

Brain development is a sequential process in which the development of cells, regions and various brain structures does not occur uniformly like other tissues and organs (Dobbing and Sands, 1979; Herschkowitz, 1989; Dobbing, 1990). It is characterized by well-defined stages of growth, anatomically and biochemically (Gottlieb et al., 1977; Albers, 1985). There are many significant growth spurts or critical periods in fetal and neonatal life. They are critical periods of development, encompassing limited periods of time of increased metabolic activity during which a particular developmental process predominates. Figure 1-3 provides information related to timing of specific maturational events during pre- and postnatal development which are vulnerable to insult.

Neural cells and Morphogenesis

The central nervous system consists of two types of cells, neurons and glia. Neurons, or nerve cells, form the functional units of the nervous system. Production of neurons occurs over a long period, with different time schedules for formation of different neuron types. Glial cells provide various types of support functions to neurons. Gliogenesis is primarily a postnatal event (Das, 1977), however, in some brain regions it is detected before birth (Das, 1977; Rodier, 1980).

Morphogenesis of the CNS in all mammals can generally be divided into three principal stages: 1) organogenesis; 2) neuron and glial production; and 3) differentiation of immature neurons and glia (Morgane et al., 1993). This development ensues in each brain region in a specifically timed series of events, of which each becomes a critical basis for the next developmental period (Bayer, 1989). These various time scales in each brain region are complicated by the migration of cells from one region to another. Due to the brain's general lack of regeneration potential and its dependence on specialized interactions, misdirected, mistimed or absent developmental cues can perturb the systematic progression toward proper development. A disruption in the proper

formation of a certain structure or specific neuronal pathway can lead to structural aberrations (Heuther, 1990). These structural changes are irreversible. They may or may not result in a functional deficit depending on the compensatory ability of future developmental events. Thus, timing is critical.

Function

The normal functioning of the brain, at any stage of development depends on the following factors: 1)number and location of neurons; 2) degree of differentiation of these neurons: structure, metabolism; 3) ability of neurons to generate action potentials; 4) number of synapses between neurons and between neurons and their targets; 5) differentiation of synapses; 6) organization of the total network for sensory reception, information processing and action responses (Herschkowitz, 1988). Nutritional factors would be expected to interfere with developmental processes during these critical periods.

Some identifiable processes occurring during a postnatal growth spurt (Dobbing, 1972; Morgane et al., 1993) include:

1). considerable proliferation of glial cells,

2). neurogenesis of microneurons(granule cells of hippocampus, olfactory bulb and cerebellum),

3). development of several enzyme systems,

4). myelination of axons,

5). energy metabolism of the brain changes (from carbohydrates to oxidation of fats).

Although these processes occur for all animals, their specific timing of occurrence varies between different species. A comparison of the timing of two periods of brain growth and cell differentiation between rats and humans is respectively 1). between birth and 10 days postnatal; between 32 weeks gestation and birth and 2). between 11 and 20 days postnatal; between birth and 2 years of age (Herschkowitz, 1989). Despite this

difference in timing, the availability of the correct balance of lipids is required for these processes to develop fully since directly or indirectly, lipids play a role in these processes.

LIPIDS AND ESSENTIAL FATTY ACIDS IN BRAIN DEVELOPMENT

Lipids are essential for structure and function of neuronal and glial membranes. These fats also constitute the main components of the myelin sheath (Feldman, Van Aerde, and Clandinin, 1992). The myelin sheath provides essential insulation for the propagation of nerve impulses along the axon. In cases of amyelination (failure to form myelin) or dysmyelination (faults in myelin structure), serious neurological disease is noted (Davison, 1972; Baumann et al., 1972). One possible cause may be essential fatty acid deficiency, as it has been established to lead to a deficiency of myelin lipids or a delay in their formation (Svennerholm et al., 1972). Since myelin is most actively synthesized during the perinatal period and since it reaches a steady state later in development (Davison, 1972), it is critical that the correct molecules are present for assembly at the time when synthesis of myelin is most active.

It has been shown that certain cells, membranes, and phospholipids are affected differently with dietary alterations (Svennerholm et al., 1972; Foot et al., 1982; Bourre et al., 1984; Bazan et al., 1986; Anding and Hwang, 1986; Hargreaves and Clandinin, 1988). The alteration of brain synaptosomal and microsomal membrane composition by diet fat was examined by Foot et al. (1982). The composition of these membranes was compared for rats fed different types of fat. The synaptosomal membrane content of phosphatidylethanolamine, ethanolamine plasmolagen or sphingomyelin was not affected by dietary treatment. However, levels of phosphatidylcholine and cholesterol were altered by diet. A similar overall effect was noted for the microsomal membrane, with minor variations. The alterations in fatty acid composition were observed in response to dietary levels of omega-6, omega-3 and monounsaturated fatty acids. Thus, the effect of

diet modulation on membrane composition is quite specific in its effects on different phospholipids within the membrane. This may be due to the polyunsaturated fatty acids in different types of phospholipids exhibiting different rates of synthesis and turnover.

With a reduction in dietary omega-3 fatty acids, the level of 22:5n-6 has been shown to rise (Youyou et al., 1986; Enslen et al., 1991). This may be indicative that some mechanism exists to maintain a constant level or constant ratio of polyunsaturated fatty acids in the brain. In studies by Bourre et al., (1984) and Bazan et al., (1986) in which the effects of dietary deprivation of omega-3 fatty acids was considered, it was noted that despite the deprivation, the retina and the brain tenaciously retained the long-chain omega-3 fatty acid, 22:6n-3. It has also been established that the level of 22:6n-3 in brain across species is fairly constant despite the wide variation in diets (Crawford, Casperd and Sinclair, 1976; Tinoco, 1982; Anderson, Connor and Corliss, 1990). These facts suggest docosahexaenoic acid has an important functional role in brain and retina.

The effect of the dietary modulation on the composition of membranes may have different results depending on which brain process and brain region is developing. This is based on the fact that different regions of the brain develop at different times (Das, 1977; Rodier, 1980), and phospholipids within different membranes exhibit varying rates of synthesis and turnover (Foot, Cruz and Clandinin, 1982; Galli and Paoletti, 1972; Chapman, 1971).

Fatty acid accretion in the brain

It should be noted that a difference exists between prenatal growth and postnatal growth. This difference exists especially with respect to the deposition of fat and the net absorption of fat, which improves with increasing gestational age (Senterre, 1987).

During the third trimester of pregnancy, rapid synthesis of brain tissue

occurs. This rapid synthesis encompasses increases in cell size, cell type and cell number (Clandinin et al., 1980a). Lipid levels increase rapidly during this period as well, mostly due to the myelination that is taking place. Levels of 18:2n-6 and 18:3n-3 were consistently low in the brain during the last trimester of pregnancy (Clandinin et al., 1980). However, accretion rates for long-chain desaturation products, 20:4n-6 and 22:6n-3 occurred, with the absolute accretion rates of the omega-3 fatty acids, specifically 22:6n-3, being greater in the prenatal compared to the postnatal period (Table 1.3) (Clandinin et al., 1980a and 1980b). It is critical that the developing fetus obtain the correct types and amounts of fatty acids to ensure complete and proper development of the brain.

	Intra-uterine (26-41 weeks) Preterm mg/wk	Extra-uterine (0-10 weeks) Term mg/wk
Total n-6	32.8	82.4
Total n-3	14.6	5.5
Total n-9	31.2	65.5
Linoleic derivatives (n-6 - 18:2)	32.3	79.7
α-linolenic derivatives (n-3 - 18:3)	14.5	6.1

Table 1.3. Fatty acid accretion rates in infants brain and cerebellum (mg/wk).

Timing of the availability of these fatty acids is also a factor. This quantitative information indicates that large amounts of docosahexaenoic acid (22:6n-3) and arachidonic acid (20:4n-6) are required during early development when cellular differentiation and active synaptogenesis are taking place.

Essential fatty acids and development of the retina.

As cellular differentiation and active synaptogenesis occur, photoreceptor biosynthesis is also taking place. Since 22:6n-3 is found in highest levels in the retina as well as the cerebral cortex, this fatty

acid can markedly affect retina development and visual acuity.

In a recent study by Sarda et al. (1991), the effects of dietary modification on the pineal gland, a vestigial photoreceptor organ, were considered. The authors found that the fatty acid composition of this organ differs from that of brain. Although the proportion of omega-3 fatty acids was comparable, the pineal gland contained a 2.3-fold higher proportion of omega-6 fatty acids compared to brain. These authors concluded that the pineal gland, which lies outside the "blood-brain barrier", is highly sensitive to 18:2n-6, 20:4n-6 and 22:6n-3 fatty acids in the diet.

ESSENTIAL FATTY ACID SYNTHESIS AND TRANSPORT IN BRAIN

The lipid composition of the brain is unique in its high concentration of polyenoic fatty acids. In the phospholipid fraction, especially the ethanolamine and serine phospho-glycerides, these fatty acids are particularly prominent (Bernsohn and Cohen, 1972). Brain lipid fatty acids originate from three sources: 1) synthesis within the brain, 2) synthesis within other organs and transport through blood to the brain, 3) passage from the gut, with or without modification, to the brain (King et al., 1971).

Synthesis and metabolism within brain:

In a recent study by Moore et al. (1990), cultured cerebrovascular endothelia were analyzed for their role in essential fatty acid metabolism in brain. These endothelia were found to readily elongate and desaturate both linoleic (18:2n-6) and alpha-linolenic (18:3n-3) acids. The major derivative of 18:2n-6 was arachidonic acid (20:4n-6). Docosapentaenoic acid (22:5n-6) was not detected in incubations with 18:2n-6, suggesting that no delta-4 desaturase activity was present in these cerebromicrovascular endothelial cells. A similar result was noted in the

incubations with 18:3n-3. The primary product of 18:3n-3 elongation-desaturation was eicosapentaenoic acid (20:5n-3). Again, no delta-4 desaturase activity was detected with this omega-3 substrate, as labeled 22:5n-3, but no 22:6n-3 was observed. It was also noted that these endothelial cells take up preformed 20:4n-6 and 20:5n-3 when they are available in the extracellular fluid. However, Rosenthal (1987) noted that essentially all cells in culture readily and nonspecifically take up and incorporate into cellular phospholipids and triacylglycerols, free fatty acids from culture medium. This data, plus the ability of the cerebromicrovascular endothelial cells to produce and release 20:4n-6, 20:5n-3, and 22:5n-3, suggests these cells play a central role in the metabolism of the long chain essential fatty acids in the brain. This is further indicated by their strategic anatomical location, at the interface between the blood and brain parenchyma, where they have initial access to all incoming essential fatty acid precursors. Thus, it appears the cerebromicrovascular endothelia may be a potentially important site for essential fatty acid processing and production of eicosanoids.

In contrast to these results, Anderson et al. (1990), reported the presence of delta-4 desaturase activity in the brain and retina during a refeeding experiment. This study produced omega-3 deficient newly hatched chicks and then repleted their brains and retinas by feeding one of three different omega-3 fatty acids: 22:6n-3, 20:5n-3 or 18:3n-3. The authors showed that 22:6n-3 was formed from dietary 20:5n-3 and accumulated in the brain to nearly the same extent as when 22:6n-3 was fed directly in the diet. This increase in 22:6n-3 in the brain occurred despite lower levels of 22:6n-3 in the serum, suggesting that brain and retina were responsible for synthesis of the major part of 22:6n-3 that accumulated during refeeding. Whether synthesis of 22:6n-3 occurred via delta-4 desaturation or an alternate pathway (Voss, 1991) may be subject to reinterpretation. Other experimental findings also point to the de novo synthesis of 22:6n-3-containing glycerolipids (Bazan et al., 1986). A possible

explanation for the discrepancy between these studies may be that cerebromicrovascular endothelia do not have delta-4 desaturase activity, however, other cell types in the brain may have this activity. Synthesis of 20:4n-6 and 22:6n-3 within the brain is evident. However, which cells are responsible for the synthesis and metabolism of essential fatty acids remains to be confirmed.

Transport from blood to the brain:

In 1963, Mohrhauer and Holman noted brain lipids to be relatively constant and considered it to be due to the "blood-brain barrier", restricting the passage of fatty acids into the brain. Later studies, however, have shown the blood-brain barrier to offer little impediment to the passage of free fatty acids, and even suggest that the existence of the "blood-brain barrier" is doubtful (Mead and Dhopeshwarkar, 1972; Crawford and Sinclair, 1972). In the case of free fatty acids, transport occurs with little resistance from the blood to the brain.

Passage from the gut:

The general process of lipid digestion relies on a series of lipases. The first, secreted by glands in the base of the tongue, is lingual lipase. This enzyme degrades medium-chain and short-chain triacylglycerols to diacylglycerol and fatty acids. In the stomach, a lipid emulsion is formed. Gastric lipase hydrolyzes medium-chain and short-chain triacylglycerols. Once the fat enters the small intestine, the liver is stimulated to release bile and the pancreas releases pancreatic lipase in the presence of colipase. The resulting products are monoglycerides, glycerol and fatty acids. The glycerol and the short- and medium-chain fatty acids are absorbed directly into the blood at the portal vein. The long-chain fatty acids and the monoglycerides form complex micelles, which move easily into the intestinal cells. The bile

salts are removed from the micelles and the lipids are absorbed. The enterocyte contains Δ-6 and Δ-9 fatty acid desaturase activity. The role of these enzymes in remodification of diet fats fed and the composition of fatty acids absorbed is not clear but may significantly modify fatty acids transported out of the enterocyte (Garg et al., 198?). Within the enterocyte, fatty acids and monoglycerides from within the micelle are reassembled into triacylglycerols, packaged with protein as chylomicrons and transported into the blood and lymph (Linscheer and Vergroesen, 1988). Thus, fatty acids are passed from the gut to the circulation (Thomson et al., 1989).

For the developing fetus, certain brain lipid fatty acids can be synthesized by the developing brain (Menon and Dhopeshwarkar, 1982; Cook, 1978). There is also indication of high rates of lipogenesis in fetal liver (Smith and Abraham, 1970). Crastes de Paulet et al., (1992) suggest the exclusive role of placental transfer and selective secretion of 20:4n-6 and 22:6n-3, operative at the beginning of gestation, could progressively be taken over by foetal metabolism. Thus, the age of the infant at birth would be an important factor. However, the authors also state that since foetal liver desaturation-elongation chain reaction has not been clearly demonstrated under physiological conditions, this remains hypothetical (Crastes de Paulet et al., 1992).

SOURCES OF ESSENTIAL FATTY ACIDS IN THE FETUS AND THE NEONATE.

The Placenta

The placenta of the human neonate is relatively permeable to free fatty acids but not to phosphoglycerides and triacylglycerols (Feldman et al., 1992). The presence of linoleic acid (18:2n-6) in fetal plasma and its decline after birth (Robertson and Sprecher, 1968), indicates a transfer of fatty acids across the placenta, as 18:2n-6 can be aquired only through the diet. Thus, it appears that specific fatty acids pass into the fetal circulation from the maternal circulation via the placenta (Friedman,

1986). However, the free fatty acid composition of the placenta and that of maternal plasma is sufficiently different (Robertson, Sprecher and Wilcox, 1968), suggesting some components of the placental free fatty acid pool are derived from sources other than the maternal circulation. Lipoprotein lipase is present in the placenta (Friedman, 1992) and the placenta is capable of synthesizing, de novo, most lipid classes (Robertson and Sprecher, 1968). Thus, the placenta has the ability to alter some lipid classes presented to it. However, the extent to which the human placenta is able to desaturate and elongate parent EFA's is quite limited (Kuhn and Crawford, 1986).

The presence of 18:2n-6 has been found mostly in the FFA fraction of fetal circulating lipids, whereas 20:4n-6 is selectively compartmentalized into phosphoglycerides by the placenta and is exported to the fetus mostly in that form (Kuhn and Crawford, 1986). Since the placental barrier is impermeable to this lipid form, this selectivity with which the placenta distributes long-chain polyenoics may allow the feto-placental unit to preferentially retain these fatty acids, which are necessary for developing membrane systems. Concentrations of arachidonic and docosahexaenoic acids increase in the fetus as gestational age increases (Robertson and Sprecher, 1968; Menon and Dhopeshwarkar, 1982; Poissennet et al., 1988; Leaf et al., 1992). Although the mechanism responsible for this is not clear, it is suggested that either the fetus or the placenta has increased capacity to synthesize the longer chain fatty acids from parent fatty acids, or the longer chain fatty acids are preferentially transferred across the placenta from maternal to fetal circulation (Neuringer et al., 1984; Kuhn and Crawford, 1986).

Biomagnification

An earlier study by Crawford and colleagues (1976), described a process in which the relative percent of long-chain fatty acids, 20:4n-6 and 22:6n-3, compared to parent EFA, increase in phosphoglycerides progressively from maternal blood to placenta to fetal blood to fetal liver and brain. This process, coined biomagnification, hypothesizes that within the placenta specific mechanisms result in the sequestration and release of specific fatty acids to the fetal circulation. Neuringer et al., (1984) also reported that in monkey and human fetuses, the levels of 22:6n-3 and 20:4n-6 are higher in fetal blood compared to maternal blood, whereas the opposite is true for their precursors. Thus, the importance Oof these long-chain polyunsaturated fatty acids (LCPUFA) is made evident by their preferential, active transfer across the placenta to the fetus in a lipid form impermeable to the placental barrier. It has recently been proposed that a more likely mechanism of biomagnification is selective sequestering of LCPUFAs on the fetal side of the placenta (Uauy, Treen and Hoffman, 1989).

In considering the sources of fatty acids in the newborn, King and colleagues (1971) found when comparing adipose tissue triglyceride between infants and mothers, the infants were shown to have greater levels of palmitic and palmitoleic acids. The predominance of these two fatty acids in newborns indicates that glucose plays an important role in the synthesis of fat in the fetus. Embryonic and fetal lipids in early gestation are derived from maternal fatty acids that cross the placenta, but with advancing gestational age, there is a gradual shift to de novo synthesis from glucose in fetal tissue (Poissennet et al., 1988).

In summary, it is evident that some fatty acids are transferred to the fetus across the placenta . It also appears that the degree of placental and fetal synthesis of fatty acids varies with gestational age (Robertson and Sprecher, 1968; Poissennet et al., 1988). Earlier in pregnancy there is apparently a greater dependence on maternal fatty acids to provide the

fetus with lipids. This may have important implications for the low-birth weight, premature infant.

This chapter has focused on the importance of omega-6 and omega-3 fatty acids, specifically 20:4n-6 and 22:6n-3, in brain growth and development. The aim of the present research is to examine the changes in brain fatty acids over time, in different brain regions and cell types, in response to varying amounts and ratios of n-6 and n-3 fatty acids to determine the effect of diet fat on brain growth and development in the rat.

RESEARCH PLAN

Rationale

All infant formulae marketed in Canada today do not incorporate essential fatty acids of chain lengths longer than 18 carbons (Clandinin et al., 1980b). Since most of the formulas contain higher concentrations of linoleic acid and/or medium chain triglycerides compared to human milk, they do not exhibit a composition analogous to that characteristic of human milk. Based on the information presented in this first chapter, it is evident that a rationale exists for incorporation of arachidonic and docosahexaenoic fatty acids into infant formulas. This incorporation, in the correct balance, may ensure the presence of the correct amounts and types of fatty acids required by infants, particularly premature infants, to achieve proper growth and maturation. To determine this one must establish the optimum balance and amount of omega-6 and omega-3 fatty acids in these formulas. The goal of this research is to examine, in an animal model, accretion of brain fatty acids in three brain regions and two cell types, over time, in response to varying the dietary amount of n-6 and n-3 fatty acids. In this manner, fatty acid levels in potential new infant formulae may be affected.

Hypotheses

It is hypothesized that the developmental timing of accretion of 20:4n-6 and 22:6n-3 varies in brain regions and in the cell types of the brain. More specifically, it is hypothesized that:

1. the diet containing an n-6/n-3 ratio of 4:1 will decrease the amount of 20:4n-6 in the phospholipids.
2. feeding the diet containing 1%DHA will increase the amount of 22:6n-3 in brain phospholipids.
3. feeding the diet containing 1% AA will increase the amount of 20:4n-6 in brain phospholipids.
4. the mixed diet containing 1% AA and 0.7% DHA will increase both 20:4n-6 and 22:6n-3 in brain phospholipids.
5. feeding 0.7% DHA will increase 22:6n-3 with no change in 20:4n-6.

Research Objectives

These hypotheses will be tested by feeding nursing Dams and growing weanling rats nutritionally complete diets with similar n-6 to n-3 fatty acid ratios but differing in the amounts of long chain polyenes (LCP).

Chapter 2.

MATERIALS AND METHODS

Animals and Diets

Breeding pairs of Sprague-Dawley rats were obtained from the University of Alberta Vivarium. Animals were housed individually, unless breeding. During breeding, one male and three females were housed together. Following a two week 'mating' period, the females were removed to individual cages. The room conditions were kept at a temperature of 21°C with 12 hours light and 12 hours dark. Water and food were supplied *ad libitum*. On delivery of the pups, Dams were fed experimental diets. Animals were tested at five ages: birth, one week, two weeks, three weeks and six weeks of age. Animals at birth received no diet treatment and served as baseline. Animals at one, two and three weeks of age received only maternal milk. Animals at six weeks of age received the same diet as the dam for the three weeks following weaning. One entire litter was sacrificed at the same age.

Six Semi-purified diets containing 20% (w\w) fat and varying fat composition were fed. The basal diet composition is listed in Table 2.1. NSMA, n6/n3 7.3:1, was based on the fat composition of an infant formula proposed by Wyeth Ayerst (Radnor, Pennsylvania, USA). This fat blend, hereafter referred to as the SMA® fat blend, consisted of 35% oleo oil, 25% coconut oil, 23% canola oil and 17% corn oil. Remaining diets were formulated from this fat blend by addition of other triglycerides. Thus, the diet with an n6/n3 ratio of 4:1 was attained by the addition of Linseed oil to the SMA fat blend. Diets containing 1% DHA (docosahexaenoic acid), 0.7% DHA, or 1 % AA (arachidonic acid) were prepared. A mixed diet was also formulated to contain both DHA and AA at 0.7% and 1% wt/wt, respectively. The DHA and AA used in four of the six diets were triglycerides developed by a fermentation process using blue-green algae and fungi, respectively. Fatty acid composition of the mixed diets is illustrated in Table 2.2. The composition of all oils used are

Table 2.2. Fatty acid composition of diets fed.

Fatty acid (% w/w of fat)	n6/n3 7.3:1	n6/n3 4:1	1% DHA	1% AA	1%AA + 0.7%DHA	0.7% DHA
12:0	9.59	7.03	10.03	7.32	7.88	10.22
C14:0	5.82	5.12	6.38	4.83	5.69	6.21
C14:1(7)	0.28	0.23	0.26	0.12	0.19	0.12
C16:0	12.68	14.56	14.84	15.68	15.88	14.72
C16:1(7)	1.25	1.18	1.32	1.20	1.36	1.24
C18:0	7.68	8.09	6.76	9.27	8.89	7.31
C18:1(7+9)	40.72	40.39	38.73	38.89	38.77	38.32
C18:2(6)	15.84	17.61	15.98	16.24	16.79	16.04
C18:3(3)	2.17	4.30	2.28	2.29	2.25	2.34
C20:0	0.27	0.26	0.25	0.26	0.25	-
C20:1(9)	0.20	0.47	-	0.50	0.50	0.42
C20:4(6)	-	-	-	1.23	1.17	-
C20:5(3)	-	-	-	-	-	-
C22:6(3)	-	-	1.01	0.12	0.70	0.77
N6/N3	7.30	4.09	7.01	7.09	7.46	6.85

DHA=docosahexaenoic acid; AA=arachidonic acid
n6/n3=ratio of 18:2n-6/18:3n-3
numbers in brackets represent the position of double bonds from the methyl end

Table 2.3. Composition of dietary oils

Triglyceride

Fatty Acid (%w/w)	Coconut	Canola	Olein	Corn	Linseed	ARASCO®	DHASCO®
C8:0	4.11	-	-	-	-	-	-
C10:0	4.89	-	-	-	-	-	-
C12:0	42.75	-	-	-	-	-	-
C14:0	18.47	-	3.33	-	-	0.84	24.20
C16:0	11.26	4.27	25.43	11.10	6.20	16.00	25.30
C16:1	0.24	0.25	3.36	-	-	-	-
C18:0	4.05	1.83	16.73	1.89	4.78	14.00	0.44
C18:1	11.70	61.39	49.98	27.13	17.35	19.80	13.01
C18:2(6)	2.26	21.62	2.68	57.95	17.78	16.30	0.92
C18:3(6)	-	-	-	-	-	4.38	-
C18:3(3)	-	8.48	-	0.86	52.62	0.33	-
C18:4(3)	-	-	0.52	-	-	-	-
C20:0	-	0.45	-	-	0.30	-	-
C20:1	-	1.24	-	-	-	2.14	-
C20:3(9)	-	-	-	0.22	-	-	-
C20:4(6)	-	-	-	-	-	17.36	-
C20:5(3)	-	-	-	-	-	0.50	0.12
C22:0	-	0.22	-	-	0.10	1.90	-
C22:6(3)	-	-	-	-	-	0.70	27.60

listed in Table 2.3.

Table 2.1. Basal Diet Composition (per kg of diet)

Casein	270 g
Starch	200 g
Glucose	207.65 g
Non-nutritive cellulose	50 g
Vitamin mix	10 g
Mineral mix	50.85 g
Choline	2.75 g
Inositol	6.25 g
L-methionine	2.5 g
Fat mix	200 g

For each diet treatment, the brains from rat pups were analyzed at birth and at one, two, three, and six weeks of age. Pups at birth were sacrificed within twenty-four hours following delivery. The number of brains pooled for one sample varied between each age group. At birth, five brains were pooled. At both one week and two weeks of age, three brains were pooled. For three week-old and for six week-old pups, two brains and one brain were used respectively.

Rat pups were sexed prior to termination and were sacrificed by decapitation. Brains were excised and placed in ice-cold 0.32M sucrose. Stomach contents of two rats from each litter was also removed and analyzed for future determination of fatty acid composition (data not illustrated).

Tissue Preparation and Cell Isolation

A petri dish inverted on a bed of crushed ice, was covered with filter paper moistened with distilled water. Brains were weighed prior to dissection. A brain was placed on the filter paper and meningeal membranes were removed. Three regions of the brain, the frontal, cerebellum, and hippocampus, were dissected out and pooled.

Cell preparation was carried out according to a modified method of Sellinger and Azcurra (1974). Each brain region was placed in separate

beakers containing 25ml of isolation media (7.5% polyvinylpyrrolidone, 10mM $CaCl_2$; pH 4.7 at 25°C). The number of regions pooled for each sample is noted above. The pooled tissue from each region was weighed then minced with scissors. The minced tissue was poured into the barrel of a 20ml plastic syringe, fitted with a reusable filter unit (Millipore, Swinnex Disc filter holder, 25 mm) containing various nylon mesh filters. The sample was pressed, three times each, through a series of combined filters. First, a double layer of 300 μm mesh. This was followed by combinations of 300 μm and 100 μm mesh and finally 300 μm and 73 μm mesh. The final volume of filtrate was adjusted with isolation solution to 25ml and layered on a two-step gradient of 7ml 1.0M sucrose and 5.5ml 1.75M sucrose contained in a Beckmann Centrifuge tube. The gradients were centrifuged in a SW-28 rotor at 41,000 g for 30 minutes at 4°C.

Neuronal cell bodies were recovered in the pellet. The material banding at the interface of the isolation media-1.0M sucrose was discarded, as this contained mainly impure myelin. The material banding at the interface of 1.0M - 1.75M sucrose was collected as the glial cell fraction. Each cell fraction was diluted with 0.32M sucrose and briefly vortexed. The cell fractions were spun in a fixed angle rotor until pelleted, about 15-20 minutes.

CELL CHARACTERIZATION

Protein Determination

To prepare cells for Polyacrylamide Gel Electrophoresis (PAGE), 1-2 mls of electrophoresis sample buffer (2% SDS, 100mM dithiothreitol, 60mM Tris, pH 6.8) was added to pellets collected above and vortexed. Samples were boiled in a 110°C sand bath for five minutes. Chromosomal DNA was then sheared by repeatedly passing samples through a 20-gauge needle and a 26-gauge needle respectively. Samples were spun in a Beckmann TL-100 ultracentrifuge using TLS-55 rotor at 10,000g/15,000 rpm for 10 minutes. The supernatant was recovered and any pellet discarded. Relative protein

concentration of samples was determined (Lowry, 1957) using bovine serum albumin as a standard.

Gel Electrophoresis

As per the method of Laemmli (1970), a stock solution containing 30% (w/w) acrylamide and 0.8%(w/w) N,N'-bis-methylene acrylamide was used to prepare a 6% separating gel and a 3% stacking gel. The final concentrations of the gels were as follows: 0.375 M Tris-HCl (pH 8.8) and 0.1% SDS, and 0.125 M Tris-HCl (pH 6.8) and 0.1% SDS for the separation and stacking gels respectively. Both gels were polymerized by the addition of 0.05%(w/v) ammonium persulfate and 0.01%(v/v) tetramethylene-diamine (TEMED). The measurements of the separating gel were 12cm x12cm x 0.6mm I.D. The stacking gel had a length of 3cm. Samples were loaded for equal protein content of 25μg protein per mm^2 loading surface. High molecular weight standard mix (MW-sds-200 Kit, Sigma Chemical Co., St. Louis, USA), Glial Fibrillary Acidic Protein (GFAP, Boehringer Mannheim Biochemica, Canada) and Neurofilaments (Sigma Chemical Co., St. Louis, USA) were used. Electrophoresis was carried out at a constant voltage of 150 volts for four hours, using a Hoefer Studier Slab Gel unit (SE 400, Hoefer Scientific Instruments, San Francisco, California). The electrode buffer contained 0.3% Tris, 1.4% Glycine and 0.1% SDS.

Semi-Dry Electrophoretic Transfer

Transfer of proteins from the gel onto nitrocellulose membrane is achieved through electrophoretic elution (Harlow and Lane, 1988). A gel-membrane sandwhich is prepared as follows: one sheet of nitrocellulose and six sheets of absorbent paper, cut to gel-size, are soaked in transfer buffer (48mM Tris, 39mM glycine, 0.037% v/v SDS, 20% methanol). On the anode plate of carbon-plate electrodes, three layers of absorbent paper are laid followed by the nitrocellulose membrane, the polyacrylamide gel

and remaining three sheets of absorbent paper. The cathode plate is placed on top of the stack, the electrodes are connected to the power source. Running time was 1.5 hours at a current of $0.8mA/cm^2$ of gel (180mA for a 15x15cm gel). Following this, the gel was stained with Comassie blue to verify transfer.

Immunoblotting (Harlow and Lane, 1988)

The nitrocellulose membrane was rinsed several times with phosphate buffered saline (PBS), then incubated, with agitation, for one hour at 37°C in BLOTTO (5% w/v skim milk powder, 0.02% sodium azide in PBS). All remaining membrane protein binding sites were blocked with BLOTTO. The blot was incubated in primary antibody, anti-glial fibrillary aicidic protein (50μg GFAP in 10 mls 3% BSA/PBS) overnight at room temperature with agitation. The blot was washed four times in Tris-buffered saline (150mM NaCl, 50mM Tris, pH 7.5) for five minutes each. Incubation in secondary antibody, alkaline phosphatase (BioCan Scientific Inc, Toronto), 8.3μL in 25mls 3% BSA/TBS, was done for one hour with agitation at room temperature. Washing was repeated four times in TBS for five minutes each. For development of the blot, fresh substrate solution (BCIP/NBT) was prepared and used within one hour. BCIP/NBT substrate solution: 66μL of nitroblue tetrazolium (NBT) stock (0.5g NBT in 10mls of 70% dimethylformamide) was added to 10 mls alkaline phosphatase buffer (100mM NaCl, 5mM $MgCl_2$, 100mM Tris, pH 9.5), mixed well and added to 33μL bromochlorindolyl phosphate (BCIP) (0.5g BCIP in 10 mls of 100% dimethylformamide). Substrate solution, 10 mls per $15x15cm^2$ membrane, was used to develop the blot at room temperature. Agitation was applied until the bands were suitably dark, approximately 30 minutes. The reaction was stopped with a PBS rinse containing 20mM EDTA or 3% TCA.

Microscopy

Cells were collected and stained with methylene blue. Aliquots were taken for morphological examination under the light microscope (Zeiss, 1600x). Purity of the samples was also examined.

LIPID ANALYSIS

The pellet of each cell type was subjected to a modified Folch extraction (Folch, 1957). Cell lipids were extracted in 10 mls $CHCl_3$:MeOH (2:1 v\v) containing 0.05% (v\v) ethoxyquin, and 2 ml 0.1 M KCl. The mixture is left overnight 4°C to separate into two phases. The lower phase, containing neutral lipids, glycolipids and phospholipids, was removed into a 10 ml tube by pipett and evaporated in a rotary speed vacuum. The sample was dissolved in about 1.0 ml of chloroform and separation of the less polar neutral lipids from the polar lipids, phospholipids and glycolipids, was carried out by silicic acid column chromatography. This procedure does not provide complete separation of the individual polar lipid classes (Rouser, Kritchevsky and Yamamoto, 1976) and was therefore combined with thin-layer chromatography (TLC) to separate individual phospholipids, the primary focus of this thesis (Figure 2.1 glyco=glycolipids; MeOH=methanol; PLs= phospholipids; TLC=thin-layer chromatography).

Column Chromatography (Rouser et al., 1976)

A 5 ml disposable pipette (6 mm I.D) was utilized as the chromatography column. A small plug of glass wool was inserted in the bottom to retain the adsorbent. In a 50 ml beaker, a slurry of 1.3 g silicic acid (Unisil, 100-200 mesh, Clarkson Chemical Co.) in 10-15 ml chloroform was prepared and carefully added to the column by Pasteur pipet. The column was allowed to settle and the solvent level to drop to the top of the silicic acid; the column bed was washed with 10 ml of

chloroform. The height of the column was around 12 cm. With the solvent level at the top of the column, the lipid sample from the lower-phase of the Folch extract was added to the column by Pasteur pipet in 1.0-1.5 ml chloroform. The glassware containing the sample was washed with 1-2ml chloroform to ensure quantitative transfer of the sample to the column. For this size of column, elution of lipids is carried out using the following sequence and amounts of solvents: 10ml chloroform, 10ml acetone, and 10ml methanol. In the chloroform eluate, the less polar lipids, or neutral lipids (sterols, sterol esters, mono-, di-, and triglycerides, free fatty acids) are obtained. Acetone elutes cerebrosides, sulfatides and ceramide polyhexosides, the glycolipids. Phospholipids are eluted with methanol. Further separation and determination of lipid classes in each fraction is accomplished through TLC, for neutral lipids and phospholipids, and HPLC (high-performance liquid chromatography), for glycolipids (data not presented in this thesis).

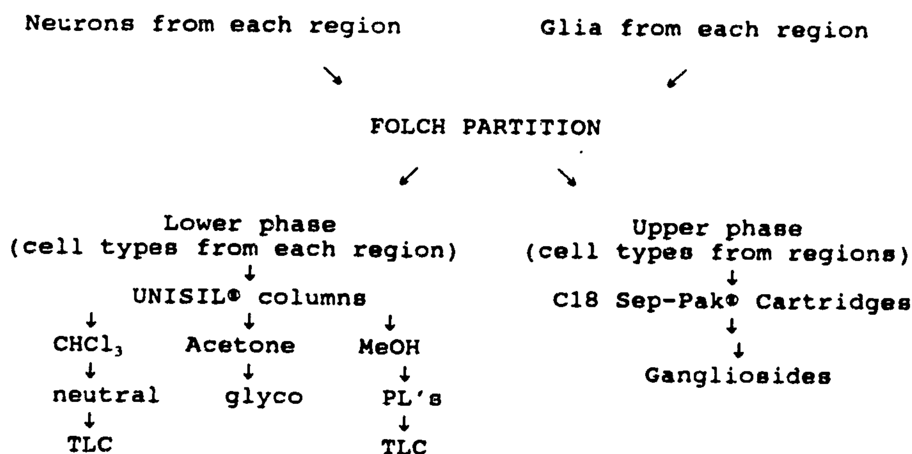


Figure 2.1. Schematic of methods.

Thin Layer Chromatography (TLC)

Thin layer chromatography plates (20x20cm, 250Um thick, Analtech, Newark, Delaware) were heat activated at 110°C for 60 minutes. Neutral lipid and phospholipid fractions were spotted on "G" plates and "H" plates respectively to obtain cholesteryl esters ("G" plates) and phosphatidylcholine, phosphatidylserine, phosphatidylinositol, and phosphatidylethanolamine ("H" plates). TLC tanks lined with Watman #1 filter paper were saturated with the solvent system sixty minutes prior to inserting plates. The "G" plate solvent system contained petroleum ether:diethyl ether:glacial acetic acid (80:20:1 / v:v:v). Plates were processed 20-25 minutes, or until the solvent front was approximately 3cm from the top of the plate.

Separation of individual phospholipids was achieved using the following system: chloroform:methanol:triethylamine:1-propanol :0.25% w/v KCl (30:9:18:25:6 by vol). "H" plates were developed approximately 90 minutes. A mixture of appropriate phospholipid standards was used to identify respective phospholipid bands. Plates were air dried for 2-3 minutes before spraying with 0.1% 8-anilino-1-napthalene-sulfonic acid (ANSA). Under UV light, lipid bands were identified and marked. Each band was scraped into a 6ml glass tube, sealed with teflon-lined caps and stored at -70°C.

Saponification

Cholesteryl ester samples from "G" plates were saponified with 1.5ml of 0.5N methanolic KOH (14.03g KOH in 500ml methanol). Five μg C17:0 internal standard was added. Tubes were capped tightly and heated in a 110°C sand bath for two hours, then cooled 30 minutes before methylation.

Methylation

All samples were methylated using the procedure described by Morrison and Smith (1961). Samples were prepared by adding 2ml of hexane and 1.5ml of 14% boron triflouride in methanol (w/v). An internal standard, C17:0 heptadecaenoic acid (Sigma Chemical Co.) was added to all fractions. Tubes were capped and heated in a sand bath at 110°C for one hour. Once cooled, 2ml of distilled water was added to wash the samples which were left overnight to separate. The upper hexane layer, containing fatty-acid methyl esters, was removed, concentrated and flushed with nitrogen. Samples were stored at -70°C.

Reverse-Phase Chromatography

The gangliosides, contained in the aqueous upper phase of the Folch extract, were isolated by Sep-Pak® C18 reverse-phase cartridges (Williams and McCluer, 1980). Before sample application, the Sep-Pak® was washed with 10ml methanol, 20ml $CHCl_3$:MeOH (2:1 v/v), and another 10ml methanol. 5 mls 0.1M-KCl was added to the sample and then applied to the Sep-Pak® cartridge. A 20ml plastic syringe fitted with tygon tubing was used to collect the sample from the cartridge. The eluate was reapplied to the column twice, each time collected in the same manner. The last collection is discarded. Salts were washed from the column in 10ml distilled water. Gangliosides were eluted in 15mls $CHCl_3$:MeOH (2:1 v/v) and concentrated in a rotary evaporator under vacuum. Samples were flushed with nitrogen and stored at -70°C.

Gas-Liquid Chromatography

Fatty-acid methyl esters were separated by gas-liquid chromatography. An automated Gas-liquid chromatograph (GLC), Varian model 6000 equipped with a Vista 8000 autosampler (Varian Instrument Company, Georgetown, Ontario) was employed. The system utilized a bonded phase fused silica

BP20 capillary column (25m x 0.25mm I.D). The carrier gas was helium with a flow rate of 1.8ml/minute using a split ratio of 100:1. The oven temperature operated on two stages. The initial temperature of 150°C to 190°C, at 20°C/minute, was held for 23 minutes. This was followed by an increase to 220°C at 2°C/minute, resulting in a total analysis time of 40 minutes. These conditions separated all saturated, mono-, di-, and polyunsaturated fatty acids from 14 to 24 carbons in chain length. A Varian Vista 654 Data System was used to analyze resolved peaks and to quantitate fatty acids. All fatty acid data is expressed as %wt/wt.

Statistical Analysis

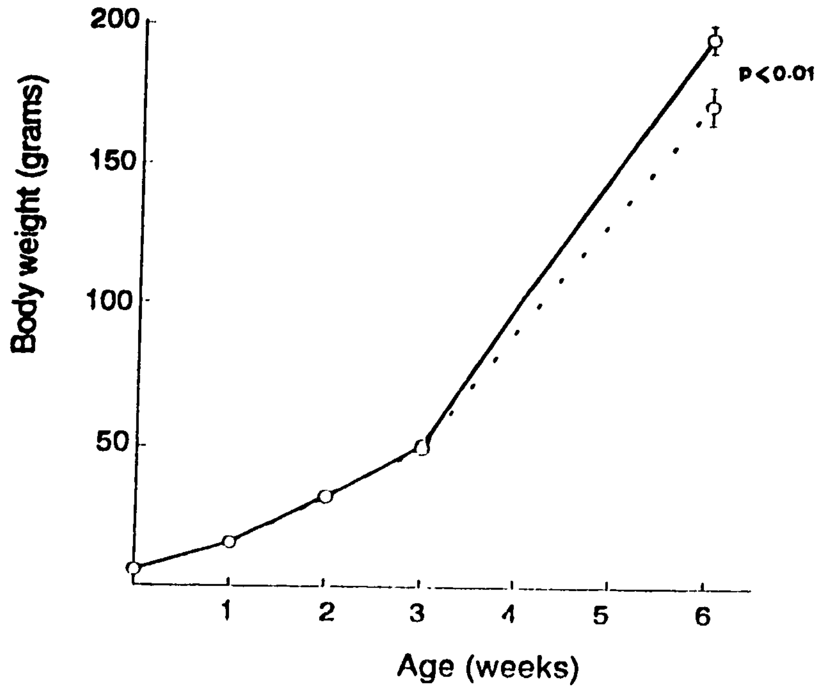
Effects of diet treatment, age, cell type and brain region were assessed by analysis of variance using SAS. Significant effects between treatments were determined by Duncan's multiple range test (Steel and Torrie, 1980) at a significance level of $p<0.01$. Significant cell type effects were tested in regions by three-way analysis of variance. Significant region effects were tested in cells by three-way analysis of variance. Correlation between 20:4n-6 and 22:6n-3 was assessed using Pearson's Correlation Coefficient ($p<0.01$). Six samples in one age group for one cell type within one region were analyzed for most comparisons.

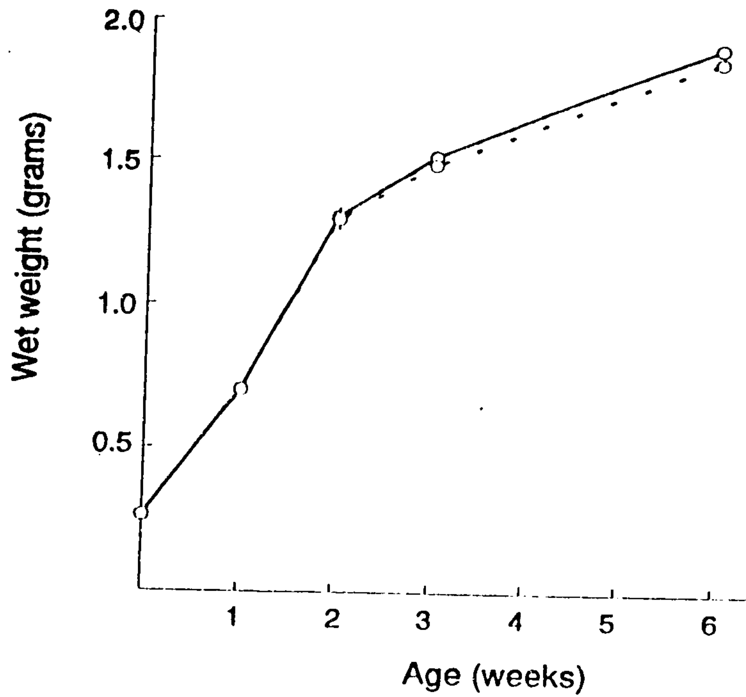
Chapter 3.

RESULTS

GROWTH CHARACTERISTICS

All litters were culled to twelve animals within 24 hours following onset of parturition. Total body weight, total brain weight and weight of each brain region is illustrated (figures 3.1, 3.2, and 3.3 respectively). Total body weight did not differ between males and females from birth to three weeks of age. A significant difference ($p<0.01$) was observed by six weeks of age. Total body weight increased 32-fold from birth to six weeks of age. Eighteen percent of this weight was attained by two weeks of age with a further eight percent increase by three weeks. More than 75% of body weight was gained in the remaining three weeks. From birth to six weeks of age total brain weight increased 7-fold. At two weeks of age, 70% of this weight was attained. In the following week, brain weight increased an additional 10%. No significant difference between males and females was observed for total brain weight. The three brain regions increased in weight with age but at different rates. The frontal region exhibited the largest weight increase in the first week. During week two the rate of weight gain was equivalent between the frontal and cerebellar regions. Weeks three through six produced a greater weight gain in the cerebellum than in the frontal or hippocampal regions. The latter two regions exhibited a similar rate of weight gain during this period. Significant difference in region weight occurred between the sexes (males greater than females) in the cerebellum ($p<0.03$) and the hippocampus ($p<0.05$) at three weeks of age and in the hippocampus ($p<0.03$) at six weeks of age. Total brain weight and weight of brain regions did not differ between animals fed the six diet treatments. No difference in fatty acid composition of neurons or glia was observed between the sexes. Thus, analyses of fatty acids were combined for males and females to test subsequent effects of the diet treatments fed.





Figures 3.1 and 3.2. Total body weight (top) and total brain weight (bottom) for male an' female Sprague Dawley rats. Means±SE. Solid lines= males dotted lines = females.

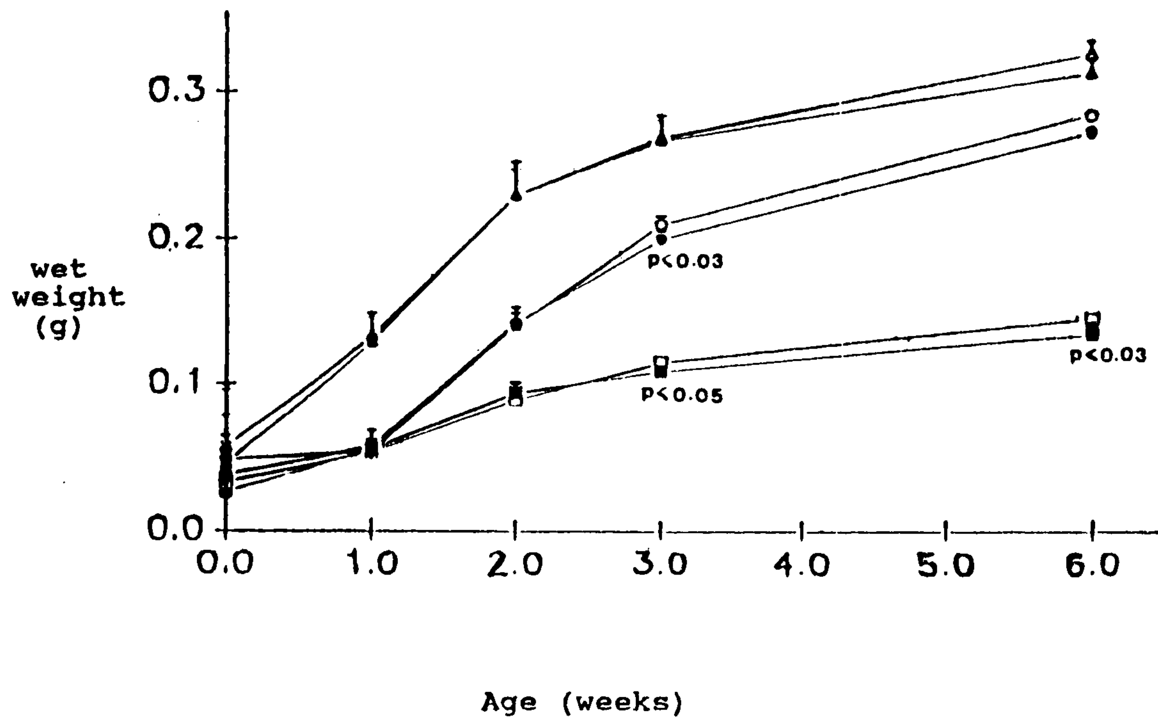


Figure 3.3. Region weights for males and females. Means ± SE. Open symbols = males; closed symbols = females. Circles = cerebellum; triangles = frontal; squares = hippocampus.

PURITY OF PREPARATIONS

Cellular preparations contained only minor cross contamination from cell membrane fragments and microvessels. Gel electrophoresis and immunoblotting were used to show that GFA protein was present only in glial cells and neurofilament was present only in neuronal cells (not illustrated). Phase contrast microscopy was used to distinguish the two cell types. Under microscopy, neurons were identified by a large nucleoli and portions of attached thick processes. Their diameter ranged from 15 to 35 µm. Glial cells were rounded cells some with short processes and a diameter between 9 and 16 µm. These observations indicate that the neuronal and glial cellular fractions contained primarily neurons and glia respectively.

OMISSIONS

In preparing samples for analysis, glycolipids, gangliosides, cholesterol esters and phosphoglycerides were separated. Only the phosphoglycerides will be discussed in the results and discussion. Data for twenty fatty acids from each phosphoglyceride was compiled. The discussion will focus on C16:0, C18:0, C16:1, C18:1, C18:2n-6, C20:4n-6, C18:3n-3, C20:5n-3, and C22:6n-3. All fatty acid data is reported as %wt/wt. C18:2n-6, C18:3n-3 and C20:5n-3 have been omitted from illustrations since no change was detected in accretion of these fatty acids. Emphasis will be given to the long-chain polyunsaturated fatty acids (LCPUFA), C20:4n-6 and C22:6n-3.

FATTY ACID COMPOSITION OF GLIAL CELLS

In phosphatidylethanolamine (PE), the major fatty acid was 18:0. Other fatty acids present in large quantities in PE included 20:4n-6, 22:6n-3 and 16:0. Phosphatdylcholine (PC) contained a high content of 16:0, 18:0, and 18:1. The fatty acid profile of brain phosphatidylserine (PS) and phosphatidylinositol (PI) showed 18:0 to be the major fatty acid in both

lipid classes. Also present in large quantities were 22:6n-3 in PS and 20:4n-6 in PI. Levels of 18:3n-3 and 20:5n-3 were negligible (<1.0% of total fatty acids) in all lipid classes.

Phosphatidylethanolamine

Age: A significant effect of age was observed for most fatty acids examined in PE of glial cells ($p<0.0003$). For 16:0, 18:0, 16:1, 18:1, 18:2n-6, 18:3n-3, 20:5n-3, 20:4n-6 and 22:6n-3 only 18:2n-6 and 20:5n-3 did not show a significant effect between age of the animals examined. After one week of age a decrease in 16:0 was observed in the frontal and hippocampal regions (Fig 3.4). Accretion of 22:6n-3 in the cerebellum and frontal region continued through to six weeks of age. In the hippocampus, maximum levels were attained by two weeks of age except in animals fed 1%DHA (Fig 3.5). In these animals 22:6n-3 reached maximum percent at three weeks of age. The relative percent of 20:4n-6 in the cerebellum decreased after one week of age in all diet treatments. In the frontal and hippocampal regions maximum 20:4n-6 was attained by two weeks of age except when animals were fed 1%AA or DHA+AA (Fig 3.6). These diet treatments prolonged accretion of 20:4n-6 until three weeks of age.

Diet: The effect of diet in glial PE composition was significant for all fatty acids examined except for 20:5n-3 ($p<0.0001$). Compared to the diet containing an n6/n3 fatty acid ratio of 4:10 the diet providing an n6/n3 ratio of 7.3:1 increased the relative percent of 16:0 (Fig 3.4) and 18:2n-6 (Table 3.1) in the cerebellum and hippocampus at three and six weeks of age and 18:1 (Fig 3.7) at all ages in the frontal and hippocampal regions. In the cerebellum, the DHA+AA diet increased 18:1 (Fig 3.7) at all ages. In all three brain regions low levels of 18:1 and of the saturates (Table 3.1) resulted from feeding the n6/n3 fatty acid ratio of 4:1. These percentages of saturates (Table 3.1) did not differ from animals fed the 0.7% DHA diet. Feeding the diet containing an n6/n3 ratio of 4:1 or the 0.7% DHA diet produced greater relative percentages of

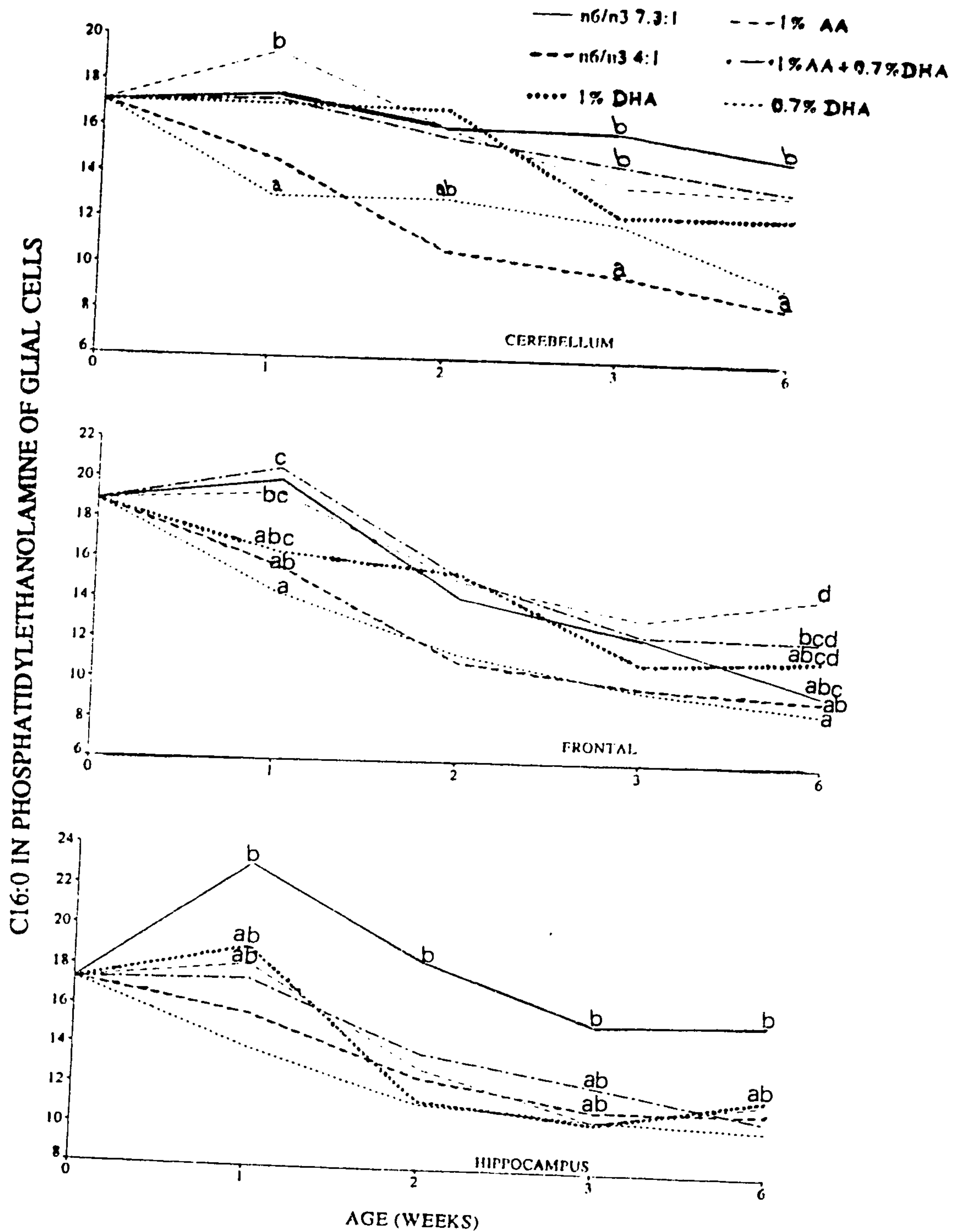


Figure 3.4. Effect of diet on % wt/wt 16:0 in glial PE. Values without a common letter within one age group differ significantly ($p<0.01$).

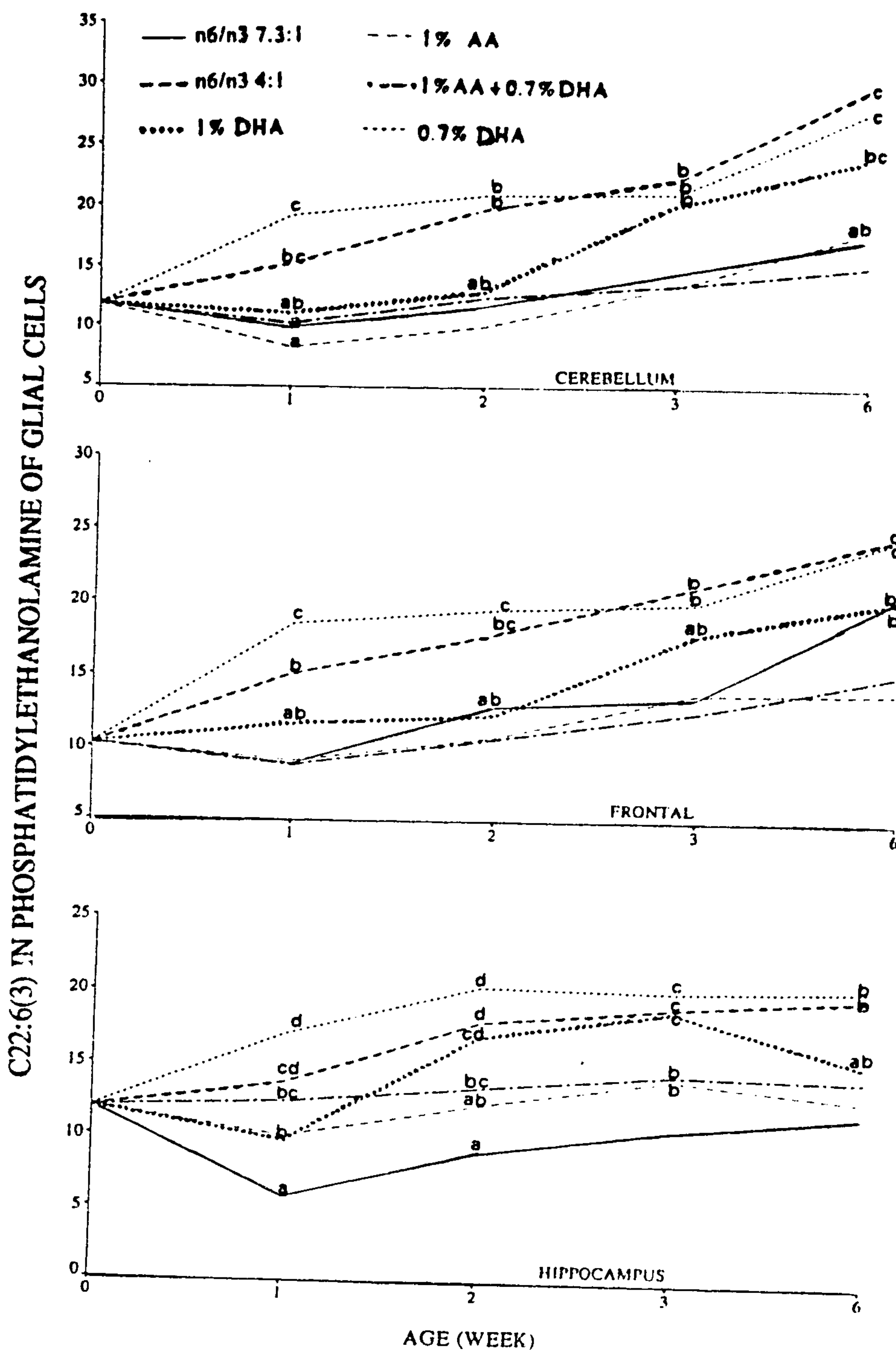


Figure 3.5. Effect of diet on % wt/wt 22:6(3) in glial PE. Values without a common letter within one age group differ significantly ($p<0.01$).

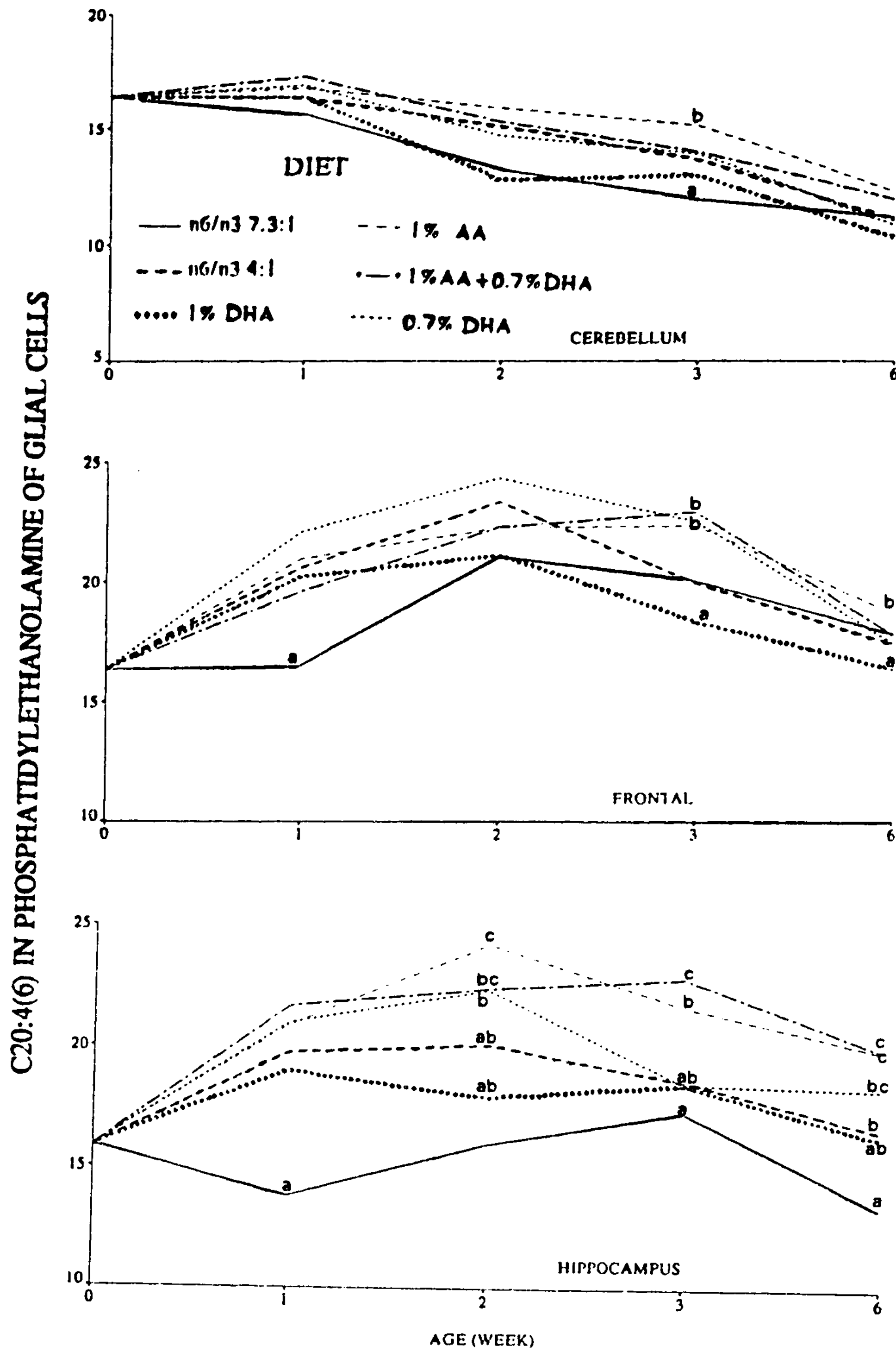


Figure 3.6. Effect of diet on % wt/wt 20:4(6) in glial PE. Values without a common letter within one age group differ significantly ($p < 0.01$).

Table 3.1. Effect of diet treatment on fatty acid composition (%w/w) at 3 weeks of age in phosphatidylethanolamine (% means ± S.D)

GLIAL cells

	CEREBELLUM						FRONTAL						HIPPOCAMPUS					
Diet	n6/n3 7.3:1	n6/n3 4:1	1% DHA	1%AA	1%AA+ .7%DHA	0.7% DHA	n6/n3 7.3:1	n6/n3 4:1	1% DHA	1%AA	1%AA+ .7%DHA	0.7% DHA	n6/n3 7.3:1	n6/n3 4:1	1%DHA	1%AA	1%AA+ .7%DHA	0.7% DHA
Σsats	42.7 ±7.2^{bc}	33.7 ±4.5^{a}	38.3 ±2.0^{ab}	41.3 ±3.0^{bc}	44.8 ±2.4^{c}	38.5 ±3.0^{ab}	44.9 ±7.0^{b}	34.3 ±9.6^{a}	38.3 ±2.0^{a}	44.2± 3.6^{b}	46.3 ±2.0^{b}	38.7 ±4.8^{a}	47.6 ±6.0^{c}	37.5 ±4.3^{a}	40.0 ±3.9^{ab}	44.6 ±2.9^{bc}	47.0 ±4.7^{c}	40.0 ±2.4^{a}
Σmonos	18.2 ±3.7^{b}	14.9 ±2.1^{a}	18.2 ±1.4^{b}	17.8 ±3.0^{b}	20.4 ±1.5^{b}	17.7 ±2.7^{b}	11.3 ±3.9	9.5± 1.6	18.2 ±1.4^{b}	7.9± 2.9	10.4 ±0.5	17.7 ±2.7^{b}	13.7 ±5.2	11.7 ±2.8	11.1 ±0.6	11.2 ±1.1	12.7 ±1.4	11.6 ±2.3
18:2(6)	2.8± 3.4^{b}	0.7± 0.2	0.8± 0.1	0.7± 0.1	0.8± 0.1	0.9± 0.1	1.3± 1.5	0.6± 0.3	0.7± 0.2	0.5± 0.1	0.6± 0.03	0.7± 0.1	2.4± 3.1	1.0± 1.5	0.5± 0.1	0.5± 0.04	0.6± 0.05	0.6± 0.02
20:4(6)	11.9 ±3.5^{a}	13.6 ±1.5^{ab}	12.9 ±1.2^{ab}	15.1 ±1.3^{b}	13.9 ±1.2^{ab}	13.8 ±2.8^{ab}	20.0 ±3.8^{ab}	20.0 ±1.8^{ab}	18.3 ±1.5^{a}	22.3 ±1.1^{b}	22.9 ±0.8^{b}	22.5 ±2.9^{b}	17.3 ±4.1^{a}	18.6 ±2.3^{ab}	18.4 ±1.0^{a}	21.7 ±3.9^{b}	22.8 ±1.7^{c}	18.5 ±3.2^{ab}
22:6(3)	14.5 ±7.3^{a}	22.1 ±4.7^{b}	20.0 ±3.3^{b}	13.4 ±1.3^{a}	13.5 ±1.7^{a}	21.0 ±2.4^{b}	13.6 ±5.3^{ab}	21.1 ±5.9^{b}	17.8 ±1.2^{ab}	13.8 ±2.2^{a}	12.6 ±1.6^{a}	20.0 ±5.7^{b}	10.7 ±4.0^{a}	19.1 ±2.6^{c}	18.8 ±1.6^{c}	14.2 ±1.4^{b}	14.5 ±2.4^{b}	20.2 ±1.3^{c}

NEURONAL cells

	CEREBELLUM						FRONTAL						HIPPOCAMPUS					
Diet	n6/n3 7.3:1	n6/n3 4:1	1% DHA	1%AA	1%AA+ .7%DHA	0.7% DHA	n6/n3 7.3:1	n6/n3 4:1	1% DHA	1%AA	1%AA + .7%DHA	0.7% DHA	n6/n3 7.3:1	n6/n3 4:1	1% DHA	1%AA	1%AA+ .7%DHA	0.7% DHA
Σsats	45.6 ±7.0^{bc}	39.2 ±6.7^{a}	39.5 ±4.7^{ab}	41.5 ±1.7^{ab}	51.5 ±2.5^{c}	37.3 ±2.2^{a}	46.5 ±7.7	42.5 ±3.0	40.5 ±4.9	46.2 ±2.0	47.5 ±13.4	43.4 ±5.2	46.3 ±5.4^{ab}	41.5 ±6.3^{a}	46.5 ±2.6^{ab}	51.4 ±2.0^{b}	49.5 ±3.1^{b}	42.7 ±3.2^{a}
Σmonos	20.2 ±4.0^{c}	13.1 ±2.9^{a}	12.4 ±1.4^{a}	14.2 ±1.4^{ab}	16.3 ±1.0^{b}	13.3 ±2.0^{ab}	16.2 ±4.7	11.4 ±2.5	13.3 ±4.7	10.7 ±0.7	15.6 ±9.9	10.0 ±0.8	21.4 ±3.7^{b}	14.0 ±4.2	11.7 ±0.8	12.4 ±0.6	11.9 ±0.6	13.3 ±2.0
18:2(6)	6.9± 5.8^{b}	1.0± 0.6	0.9± 0.1	0.9± 0.1	1.1± 0.1	0.9± 0.2	4.3± 5.5	1.0± 0.2	2.1± 2.0	1.0± 0.1	1.1± 0.1	1.0± 0.3	9.0± 6.1^{b}	1.8± 1.6	1.2± 0.5	0.8± 0.1	0.7± 0.1	0.9± 0.2
20:4(6)	9.2± 4.0^{a}	14.0 ±2.5^{bc}	11.7 ±1.5^{b}	15.5 ±0.8^{c}	14.6 ±0.8^{bc}	14.9 ±2.6^{bc}	15.0 ±5.9^{a}	18.2 ±2.1^{ab}	16.1 ±3.2^{ab}	20.9 ±1.6^{c}	19.6 ±1.4^{bc}	19.5 ±0.9^{b}	8.6± 5.7^{a}	16.4 ±2.6^{b}	18.3 ±1.1^{bcd}	20.8 ±1.1^{cd}	21.2 ±0.8^{cd}	17.7 ±3.4^{bc}
22:6(3)	7.8± 5.2^{a}	20.8 ±6.3^{b}	18.2 ±3.1^{b}	16.7 ±1.4^{b}	11.3 ±1.2^{a}	20.2 ±0.9^{b}	9.6± 5.6^{a}	18.3 ±2.6^{c}	15.1 ±4.0^{b}	12.4 ±2.6^{ab}	9.2± 1.6^{a}	17.2 ±2.5^{bc}	4.7± 3.8^{a}	14.5 ±3.2^{cd}	15.2 ±2.0^{cd}	8.5± 1.1^{b}	11.4 ±2.0^{bc}	16.4 ±3.4^{d}

Values for Σsats include 16:0 and 18:0; Values for Σmonos include 16:1 and 18:1

Values without a common superscript differ significantly. Values with the same superscript or nosuperscript did not differ significantly.

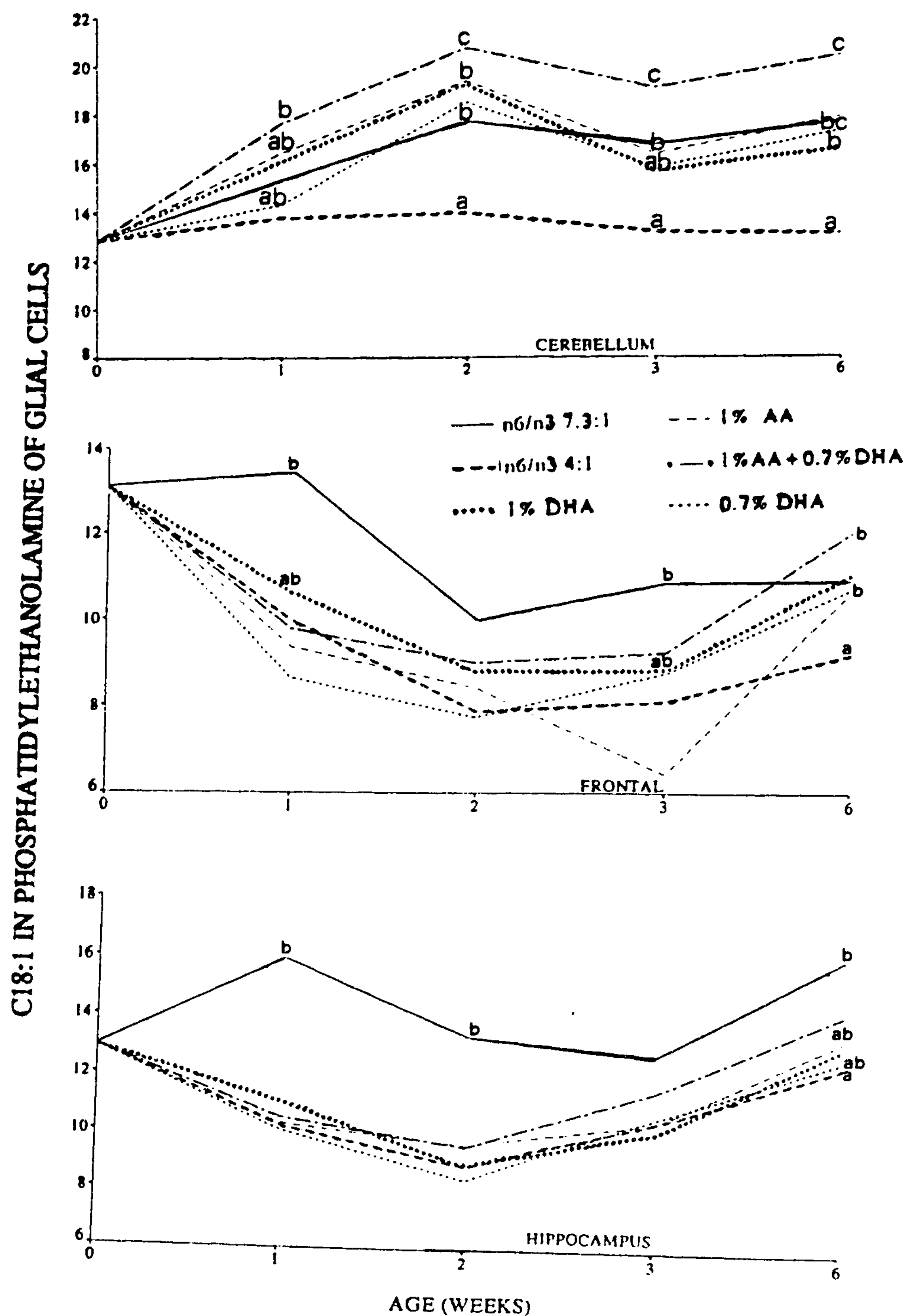


Figure 3.7. Effect of diet on % wt/wt 18:1 in glial PE. Values without a common letter within one age group differ significantly ($p < 0.01$).

22:6n-3 at all ages in all three brain regions compared to the diet supplying an n6/n3 ratio 7.3:1, the AA diet or the DHA+AA diet (Fig 3.5). At one week in the cerebellum and the hippocampus and at 1, 2 and 6 weeks in the frontal region the percent of 22:6n-3 produced by the 0.7%DHA diet was significantly greater than that resulting from the 1%DHA diet ($p<0.01$) (Fig.3.5). Feeding 1% AA resulted in significant accretion of 20:4n-6 at all ages in the hippocampus and frontal region compared to the 1% DHA diet and/or the diet providing an n6/n3 ratio of 7.3:1 (Fig 3.6). In the cerebellum, this difference was observed at three weeks of age only. The diet containing both DHA and AA increased 20:4n-6 levels at all ages but only in the hippocampus. The DHA+AA diet also raised 18:0 percent in all three regions (Fig 3.8). In the cerebellum, this occurred at all ages; in the frontal region this was observed at 1,3 and 6 weeks of age while the hippocampus exhibited this at 3 and 6 weeks of age only. The DHA+AA diet also increased the relative percent of 18:1 at all ages but only in the cerebellum (Fig 3.7). These observations indicate that diet fat alters accretion of fatty acids in glial PE and the effect of diet and age is different when comparing the accretion of fatty acids between brain regions.

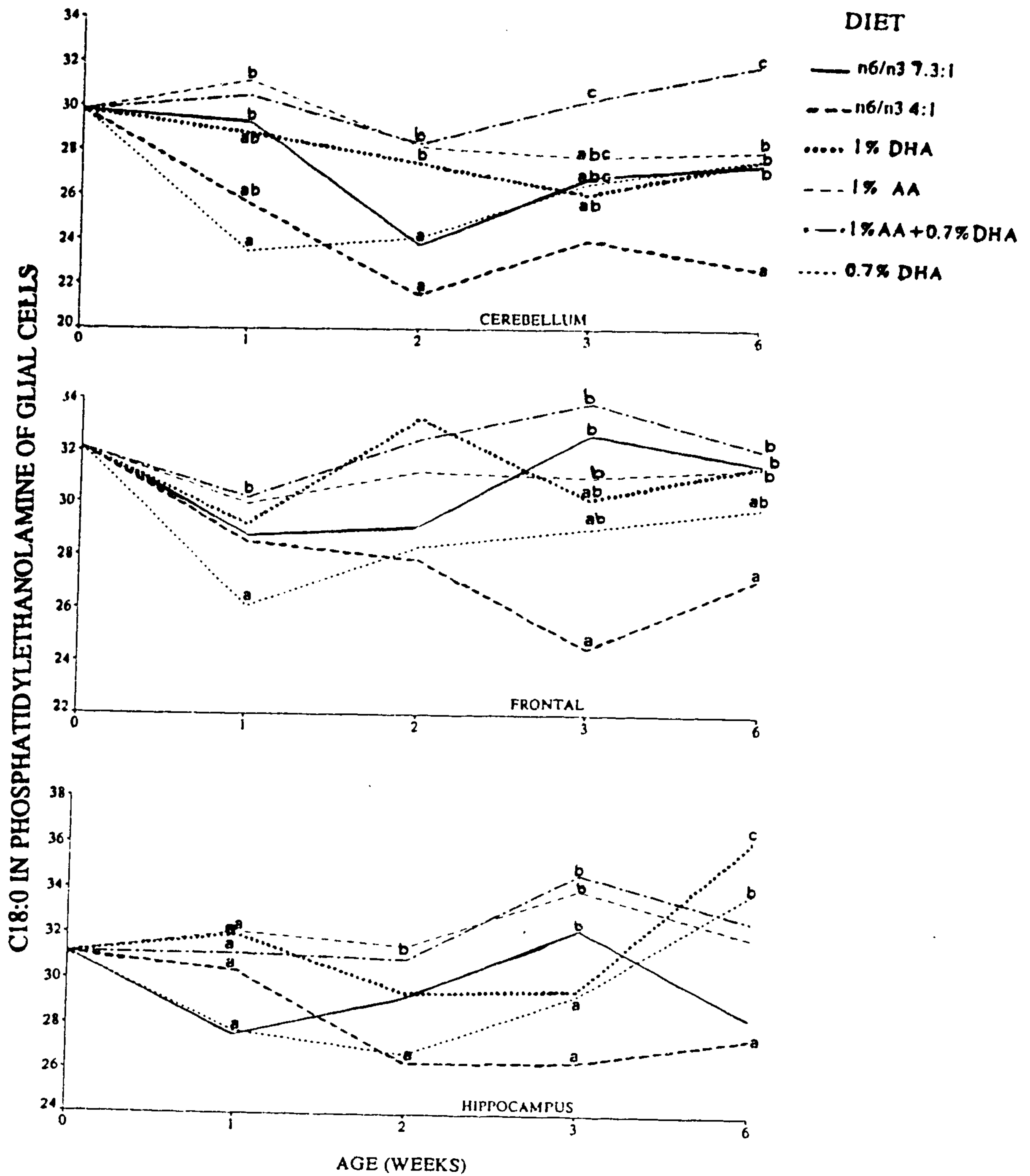


Figure 3.8. Effect of diet on % wt/wt 18:0 in glial PE. Values without a common letter within one age group differ significantly ($p<0.01$).

Phosphatidylcholine

Age: When PC was isolated from glial cells of animals at different ages an effect of age was observed for all fatty acids ($p<0.0001$) except 18:2n-6, 18:3n-3 and 20:5n-3. The relative percent of 20:4n-6 changed little in the cerebellum (Fig 3.9). Maximum 20:4n-6 was attained at three weeks in animals fed DHA+AA (Fig 3.9). In the frontal and hippocampal regions, accretion of 20:4n-6 was maximum at three weeks and two weeks of age respectively (Fig 3.9). In these two regions, feeding 1%AA resulted in the greatest accretion of 20:4n-6. In the cerebellum, 22:6n-3 increased with age. In the frontal and hippocampal regions little change was observed in the level of 22:6n-3.

Diet: An effect of diet fat was observed for all fatty acids examined in glial PC with the exception of 18:1 ($p<0.0001$). Compared to most diets the diet providing an n6/n3 ratio of 7.3:1 resulted in accretion of higher 18:0 (Fig 3.10) in the frontal and hippocampal regions and higher 18:2n-6 levels in all three regions (Table 3.2). In the cerebellar and frontal regions diet treatment resulted in different 18:2n-6 content at only one and three weeks of age. Few differences occurred in the accretion of 22:6n-3 in glial PC between diet treatments. Differences that were apparent occurred in the cerebellum at six weeks of age and in the hippocampus at three weeks of age. In both of these regions, the diet providing an n6/n3 fatty acid ratio of 4:1 resulted in high 22:6n-3 levels. A similar increase in 22:6n-3 was observed in the cerebellum of animals fed 0.7%DHA (Table 3.2). These 22:6n-3 levels were greater than that observed in animals fed DHA+AA (Table 3.2). The effect of the diet treatment containing an n6/n3 ratio of 4:1 apparently was more pronounced in glial PE than in glial PC. At one week of age the diet containing DHA+AA produced maximal 20:4n-6 levels in all three brain regions (Fig 3.9). At other ages the increase in 20:4n-6 was attained by feeding the AA diet. Low 20:4n-6 was observed in animals fed both DHA diets and the diets providing n6/n3 fatty acid ratios of 7.3:1 and 4:1 (Fig 3.9). These

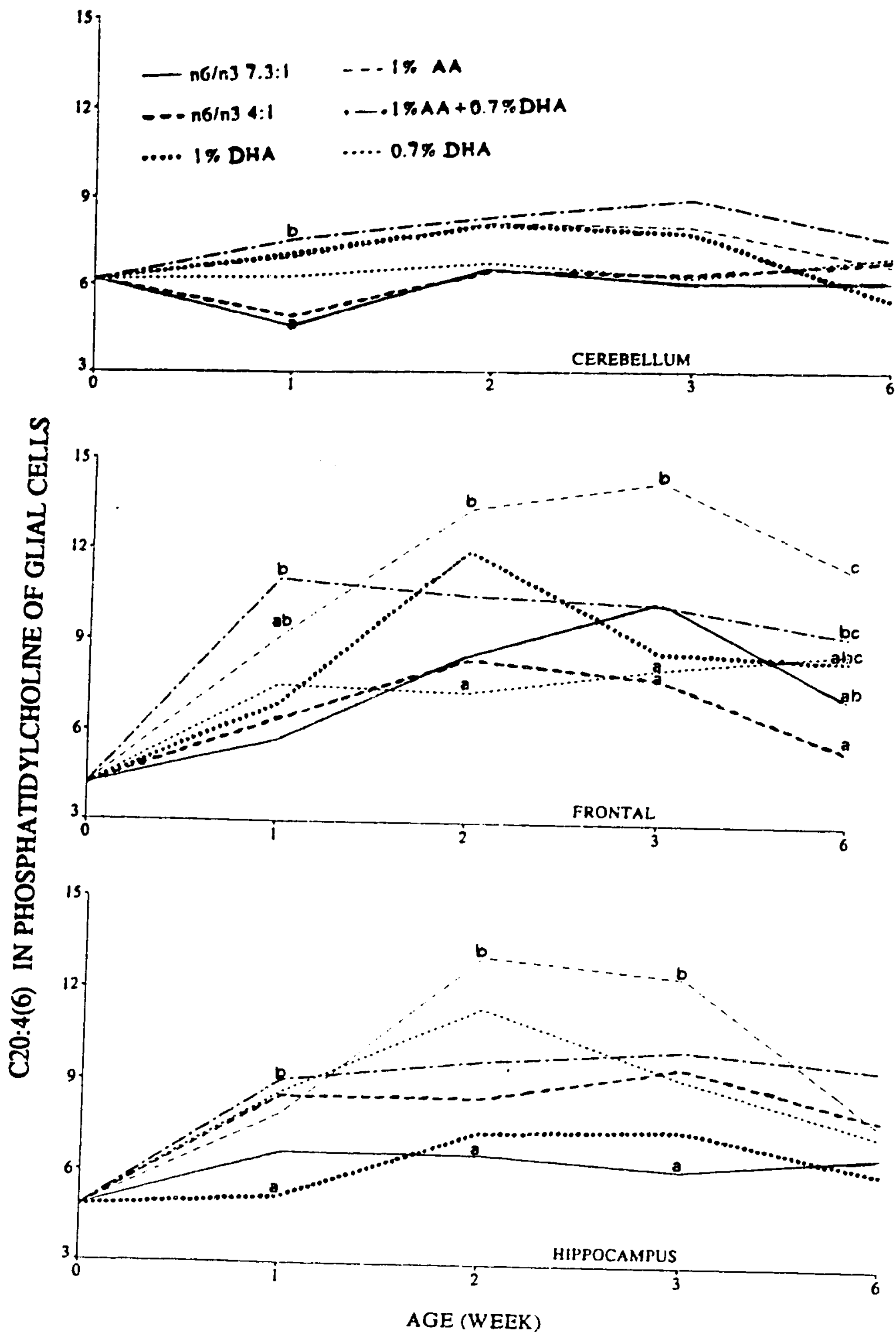


Figure 3.9. Effect of diet on % wt/wt 20:4(6) in glial PC. Values without a common letter within one age group differ significantly ($p<0.01$).

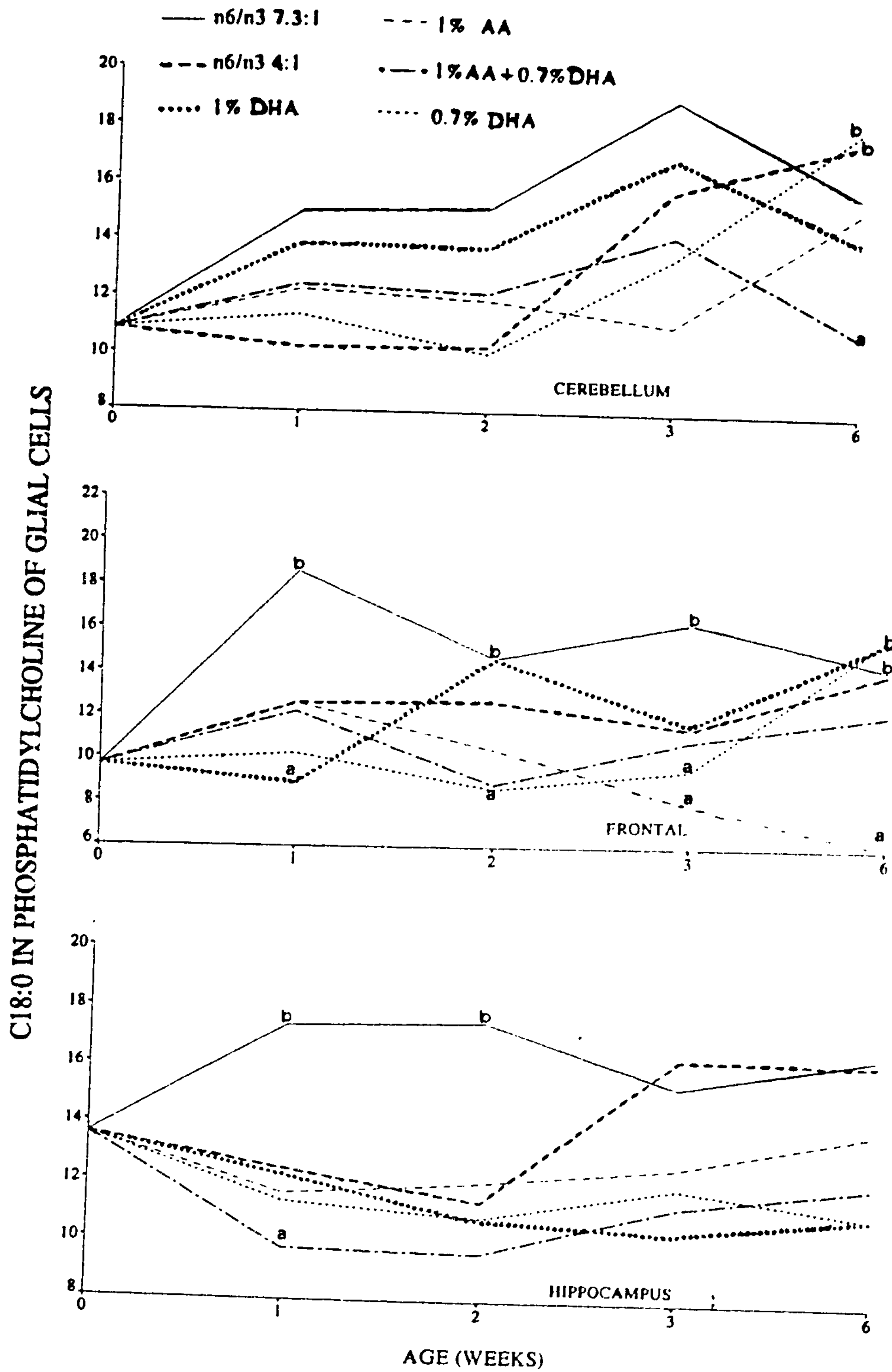


Figure 3.10. Effect of diet on % wt/wt 18:0 in glial PC. Values without a common letter within one age group differ significantly ($p<0.01$).

Table 3.2. Effect of diet treatment on fatty acid composition (%w/w) at 3 weeks of age in phosphatidylcholine (% means ± S.D)

GLIAL cells

	CEREBELLUM						FRONTAL						HIPPOCAMPUS					
Diet:	n6/n3 7.3:1	n6/n3 4:1	1% DHA	1%AA	1%AA+ .7%DHA	0.7% DHA	n6/n3 7.3:1	n6/n3 4:1	1% DHA	1%AA	1%AA+ .7%DHA	0.7% DHA	n6/n3 7.3:1	n6/n3 4:1	1% DHA	1%AA	1%AA+ .7%DHA	0.7% DHA
Σsats	57.8 ±5.8	59.2 ±6.6	56.2 ±2.8	55.7 ±3.1	55.0 ±5.5	61.1 ±7.2	56.1 ±3.3	60.6 ±8.8	57.4 ±2.7	52.4 ±7.7	55.0 ±5.5	53.2 ±20	51.6 ±9.5^{a}	56.6 ±5.2^{ab}	63.2 ±2.8^{b}	56.9 ±7.0^{ab}	58.7 ±4.3^{ab}	60.4 ±5.3^{ab}
Σmonos	24.3 ±1.3	23.5 ±1.7	24.7 ±2.7	26.5 ±3.4	24.7 ±3.0	24.1 ±4.2	23.7± 1.5^{b}	22.7 ±2.2	25.6 ±1.3^{b}	18.8 ±4.5	24.7 ±3.0	22.6 ±1.1^{b}	25.4 ±1.4	23.2 ±2.9	25.2 ±1.7	23.4 ±3.3	23.9 ±1.4	24.8 ±1.5
18:2(6)	3.9± 2.8^{a}	1.8± 0.4^{b}	2.2± 0.4^{ab}	1.3± 0.4^{b}	1.2± 0.1^{b}	1.4± 0.7^{b}	2.0± 0.6^{b}	1.5± 0.7^{ab}	1.6± 0.2^{ab}	1.1± 0.3^{a}	1.3± 0.1^{ab}	1.5± 0.2^{ab}	7.3± 7.1^{b}	1.5± 0.8	0.8± 0.6	1.1± 0.3	0.9± 0.5	1.5± 0.5
20:4(6)	6.0± 0.7	6.3± 2.4	7.8± 2.0	8.0± 1.6	8.9± 3.6	6.2± 1.9	10.3 ±3.5^{ab}	7.8± 4.2^{a}	8.7± 0.8^{ab}	14.4 ±5.1^{b}	10.3 ±2.4^{ab}	8.1± 3.5^{a}	6.2± 3.4^{a}	9.5± 4.0^{ab}	7.5± 1.5^{ab}	12.5 ±4.3^{b}	10.1 ±4.9^{ab}	9.1± 2.0^{ab}
22:6(3)	3.1± 2.0	4.8± 3.8	5.9± 1.2	4.1± 1.8	6.4± 4.0	4.0± 1.5	3.6± 5.3	2.9± 3.3	3.2± 0.4	8.0± 4.7^{b}	3.7± 1.1	2.6± 1.3	1.6± 1.2^{a}	4.8± 4.2^{b}	2.2± 1.1^{ab}	3.6± 2.2^{ab}	2.8± 0.9^{ab}	2.2± 0.8^{ab}

NEURONAL cells

	CEREBELLUM						FRONTAL						HIPPOCAMPUS					
Diet:	n6/n3 7.3:1	n6/n3 4:1	1% DHA	1%AA	1%AA+ .7%DHA	0.7% DHA	n6/n3 7.3:1	n6/n3 4:1	1% DHA	1%AA	1%AA + .7%DHA	0.7% DHA	n6/n3 7.3:1	n6/n3 4:1	1% DHA	1%AA	1%AA+ .7%DHA	0.7% DHA
Σsats	51.3 ±9.5^{a}	58.5 ±4.6^{ab}	61.3 ±4.0^{b}	57.7 ±5.8^{ab}	57.9 ±5.2^{ab}	61.5 ±2.2^{b}	47.1± 11.4^{a}	58.1± 14.5^{ab}	62.9 ±4.7^{b}	58.7 ±4.5^{ab}	63.5 ±9.1^{b}	56.7 ±4.8^{ab}	47.3 ±8.7^{a}	56.7 ±5.8^{ab}	55.4± 22.3^{ab}	60.6 ±6.5^{ab}	64.4 ±4.9^{b}	64.6 ±2.0^{b}
Σmonos	25.6 ±2.6	25.0 ±2.2	24.9 ±2.2	24.7 ±1.8	24.7 ±3.9	25.6 ±1.6	27.1 ±5.1	25.0 ±2.2	25.4 ±1.6	25.1 ±3.4	22.9 ±4.9	23.8 ±1.1	25.8 ±1.0	23.7 ±3.2	29.9± 14.6	23.1 ±2.3	22.0 ±1.9	22.3 ±1.2
18:2(6)	8.8± 6.4^{b}	1.9± 0.5	1.4± 0.7	1.5± 0.3	1.1± 0.5	1.5± 0.3	10.5± 6.8^{b}	1.7± 0.7	1.6± 0.6	1.3± 0.4	1.1± 0.6	1.8± 0.4	11.8± 6.2^{b}	2.4± 1.2	1.3± 1.0	1.3± 0.5	1.0± 0.2	1.4± 0.3
20:4(6)	3.8± 2.5^{a}	5.3± 2.0^{ab}	7.4± 3.5^{b}	7.7± 2.4^{b}	7.5± 2.7^{b}	8.8± 3.0^{b}	3.8± 2.5^{a}	5.3± 2.0^{ab}	7.4± 3.5^{b}	7.7± 2.4^{b}	7.5± 2.7^{b}	8.8± 3.0^{b}	3.3± 2.0^{a}	5.7± 2.5^{ab}	6.4± 2.5^{ab}	8.4± 3.6^{b}	6.8± 2.9^{ab}	5.8± 0.3^{ab}
22:6(3)	1.1± 0.9^{a}	3.8± 2.8^{b}	2.4± 0.8^{ab}	3.4± 1.2^{ab}	4.8± 3.3^{b}	2.6± 0.8^{ab}	1.1± 1.4	2.4± 1.5	3.0± 1.4	2.2± 0.7	2.8± 1.5	3.0± 1.8	0.5± 0.7^{a}	4.0± 3.5^{b}	1.8± 1.0^{ab}	2.5± 1.0^{ab}	1.7± 0.7^{ab}	1.6± 0.7^{ab}

Values for Σsats include 16:0 and 18:0; Values for Σmonos include 16:1 and 18:1
Values without a common superscript differ significantly. Values with the same superscript or no superscript do not differ significantly.

observations suggest fatty acid accretion in glial PC varies between brain regions. The effect of diet fat appeared to be greater in glial PC of the frontal and hippocampal regions compared to the cerebellum. An age-dependent factor was also exhibited in glial PC for most fatty acids examined.

Phosphatidylserine

Age: In PS isolated from glial cells the proportions of all fatty acids examined were affected by age ($p<0.0001$) except 18:3n-3 and 20:5n-3. A decrease in 16:1 was observed over time in all three regions (data not shown). The cerebellum exhibited a decrease in relative percent of 20:4n-6 over time. A decrease in 20:4n-6 after one week of age was observed in the hippocampus except in animals fed the diet containing n6/n3 ratio of 7.3:1 or 1%DHA (Fig 3.11). Maximum 22:6n-3 in cerebellum was attained at one week of age except in animals fed 1%DHA. For these animals, maximum 22:6n-3 was reached at six weeks of age (Fig 3.12). This response was also observed in the frontal region of animals fed 1%DHA. In the hippocampus the greatest percent of 22:6n-3 was observed at three weeks of age.

Diet: With the exception of 16:1 all fatty acids in PS in glia exhibited a diet effect ($p<0.001$). The diet supplying n6/n3 fatty acids at 7.3:1 increased 16:0 (Fig 3.13) in all three regions and increased 18:1 in the frontal and hippocampal regions (Fig 3.14). By comparison, both 16:0 and 18:1 were low when the DHA+AA diet wes fed. The diet providing an n6/n3 fatty acid ratio of 7.3:1 and the diet containing DHA+AA produced the same effects in n-6 fatty acid levels as occurred for 16:0 and 18:1 levels. The diet supplying n6/n3 fatty acids at 7.3:1 produced higher levels of n-6 fatty acids compared to the DHA+AA diet (Table 3.3 and Fig 3.11). Feeding 1% DHA resulted in the highest relative percent of 22:6n-3 in all three regions (Fig 3.12). In the cerebellum and frontal region, this was attained at six weeks of age. In the hippocampus achieved at

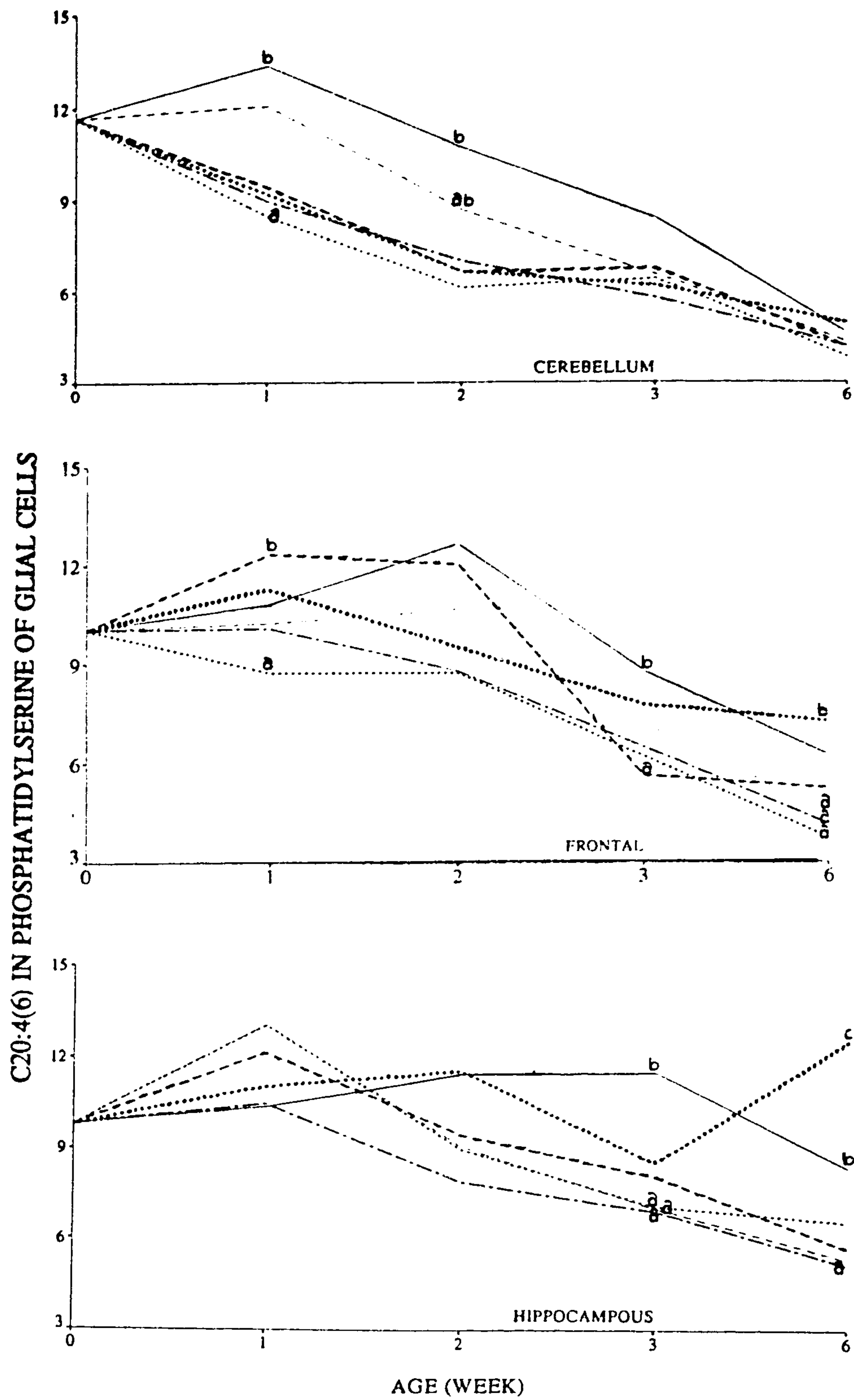


Figure 3.11. Effect of diet on % wt/wt 20:4(6) in glial PS. Values without a common letter within one age group differ significantly ($p<0.01$).

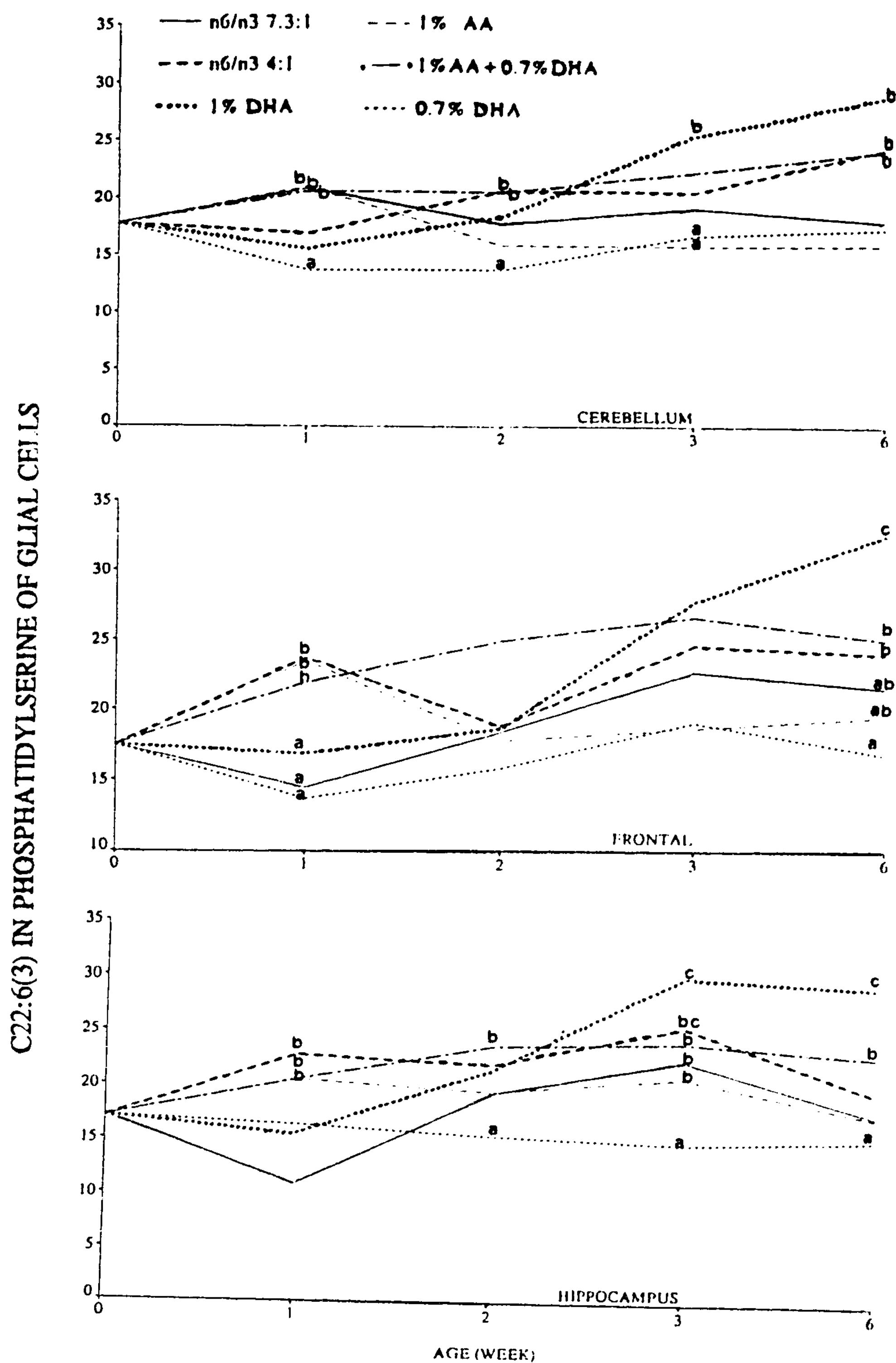


Figure 3.12. Effect of diet on % wt/wt 22:6(3) in glial PS. Values without a common letter within one age group differ significantly ($p<0.01$).

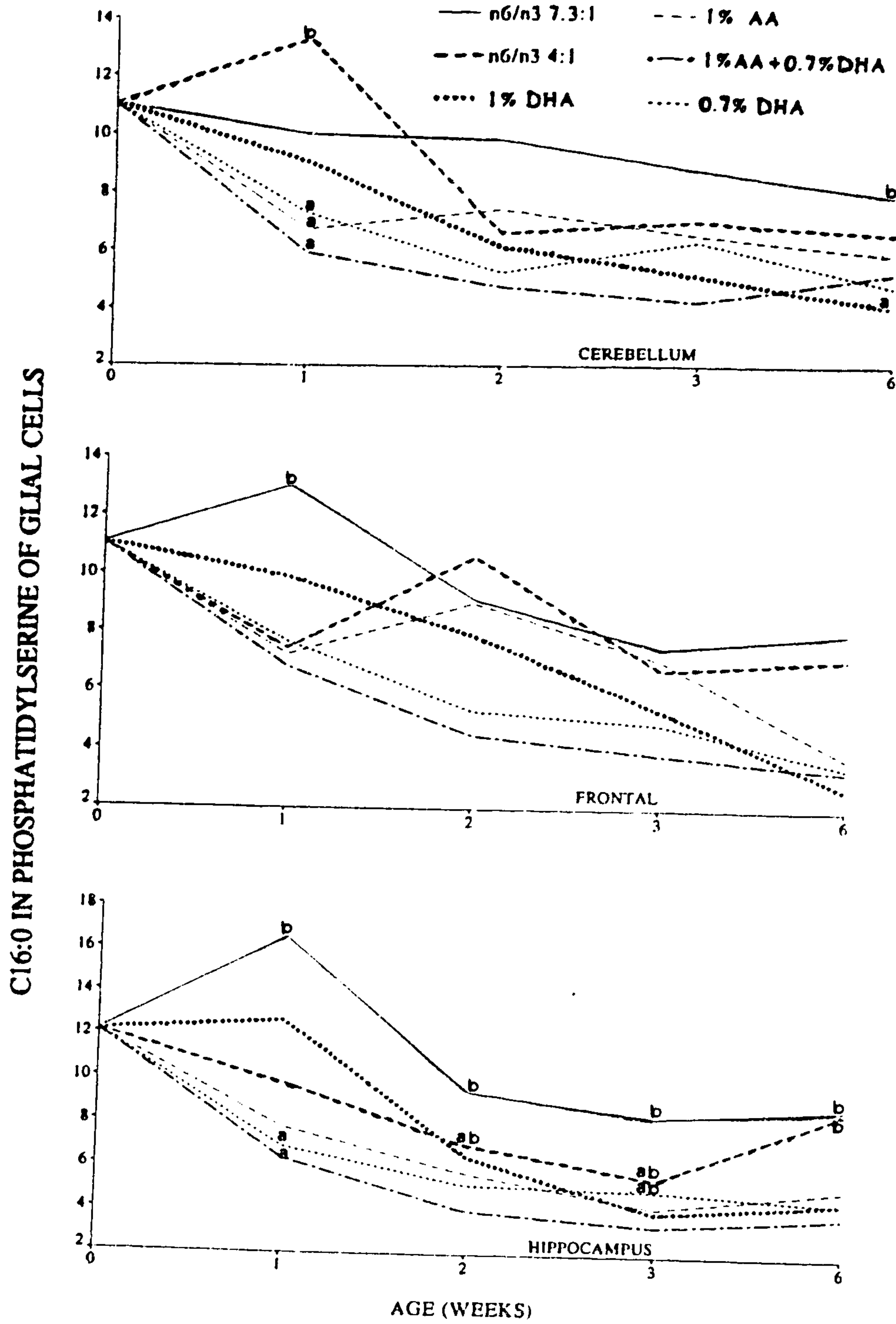


Figure 3.13. Effect of diet on % wt/wt 16:0 in glial PS. Values without a common letter within one age group differ significantly ($p<0.01$).

Table 3.3. Effect of diet treatment on fatty acid composition (%w/w) at 3 weeks of age in phosphatidylserine (% means ± S.D)

GLIAL cells

CEREBELLUM

Diet	n6/n3 7.3:1	n6/n3 4:1	1% DHA	1%AA	1%AA+ .7%DHA	0.7% DHA
Σsats	41.8 ±10.4	44.4 ±7.7	40.9 ±8.6	46.5 ±6.1	46.2 ±3.4	49.3 ±5.9
Σmonos	19.0 ±4.2	18.7 ±2.9	19.0 ±2.0	25.3 11.6	17.5 ±0.8	19.9 ±2.9
18:2(6)	1.4 ± 2.0	0.7 ± 0.5	0.5 ± 0.1	0.5 ± 0.1	0.3 ± 0.04	0.7 ± 0.5
20:4(6)	8.4 ± 4.2	6.7 ± 2.6	6.2 ± 1.5	6.5 ± 1.0	5.7 ± 0.4	6.4 ± 2.3
22:6(3)	19.0 ± 7.1^{ab}	20.4 ± 6.4^{ab}	25.4 ±7.6^{b}	15.7 ± 3.6^{a}	22.2 ± 0.7^{ab}	16.6 ±3.1^{a}

FRONTAL

Diet	n6/n3 7.3:1	n6/n3 4:1	1% DHA	1%AA	1%AA+ .7%DHA	0.7% DHA
Σsats	48.7 ±8.9^{a}	49.5 ±2.2^{a}	42.9 11.9^{a}	57.4 ±2.4^{ab}	52.4 ±1.3^{ab}	59.9 ±1.1^{b}
Σmonos	11.3 ±3.9	11.6 ±4.9	11.5 ±7.9	9.6 ± 0.6	7.67 ±0.6	9.4 ± 0.5
18:2(6)	1.0 ± 0.9	0.6 ± 0.3	1.9 ± 4.1	0.4 ± 0.03	0.3 ± 0.03	0.4 ± 0.04
20:4(6)	8.8 ± 4.1^{b}	5.6 ± 0.8^{a}	7.8 ± 3.4^{ab}	7.2 ± 0.9^{ab}	6.5 ± 0.5^{ab}	6.2 ± 0.3^{ab}
22:6(3)	22.7 ± 8.0	24.6 ± 7.2	27.7 ± 9.4	18.7 ± 1.3	26.6 ± 1.1	19.1 ± 0.9

HIPPOCAMPUS

Diet	n6/n3 7.3:1	n6/n3 4:1	1% DHA	1%AA	1%AA+ .7%DHA	0.7% DHA
Σsats	42.8 ±7.2	45.0 ±8.6	41.4 ±9.1^{ab}	53.2 ±3.6^{b}	47.2 ±5.1	56.9 ±6.2
Σmonos	12.6 ±4.4^{ab}	11.2 ±1.4^{ab}	10.1 ±0.4^{ab}	10.3 ±1.3^{ab}	9.2 ± 0.4^{a}	13.7 ±4.0^{b}
18:2(6)	0.7 ± 0.3^{d}	0.6 ± 0.3^{cd}	0.3 ± 0.05^{ab}	0.2 ± 0.04^{a}	0.2 ± 0.03^{a}	0.6 ± 0.2^{bcd}
20:4(6)	11.5 ±3.3^{b}	8.1 ± 3.1^{ab}	8.5 ± 4.2^{ab}	7.0 ± 1.1^{a}	6.9 ± 2.8^{a}	7.1 ± 1.5^{a}
22:6(3)	22.5 ±4.9^{b}	25.6 ±5.1^{bc}	30.3 ±3.6^{c}	20.9 ± 3.6^{b}	24.2 ±2.7^{b}	14.8 ±1.8^{a}

NEURONAL cells

CEREBELLUM

Diet	n6/n3 7.3:1	n6/n3 4:1	1% DHA	1%AA	1%AA+ .7%DHA	0.7% DHA
Σsats	40.7 ±8.1	46.7 ± 11.6	36.9 ±5.9	48.6 ±3.0^{ab}	46.7 ±6.9	60.6 ±5.9^{b}
Σmonos	19.2 ±3.4^{ab}	16.0 ±5.2^{ab}	16.5 ±2.1^{ab}	15.2 ±4.3^{ab}	14.7 ±1.7^{a}	20.9 ±4.9^{b}
18:2(6)	2.4 ± 4.1	1.8 ± 2.2	0.8 ± 0.3	0.6 ± 0.1	0.6 ± 0.1	0.8 ± 0.3
20:4(6)	9.7 ± 2.6^{b}	7.6 ± 2.5^{ab}	8.6 ± 2.4^{ab}	7.7 ± 1.4^{ab}	10.0 ±2.3^{b}	5.9 ± 1.1^{a}
22:6(3)	17.3 ±6.7^{b}	18.4 ±7.8^{b}	27.6 ± 4.8^{c}	17.9 ± 3.3^{b}	23.5 ±3.4^{bc}	7.7 ± 2.5^{a}

FRONTAL

Diet	n6/n3 7.3:1	n6/n3 4:1	1% DHA	1%AA	1%AA + .7%DHA	0.7% DHA
Σsats	49.7 ±5.6^{b}	52.3 ±5.0^{b}	36.4 ±4.4^{a}	55.3 ±3.0^{b}	49.2 ±5.6^{b}	56.2 ±6.1^{b}
Σmonos	16.2 ±4.6^{b}	13.9 ±3.9	13.0 ±3.0	10.2 ±2.5	9.1 ± 1.6^{a}	11.5 ±3.6
18:2(6)	2.8 ± 4.4	1.2 ± 0.6	1.4 ± 1.1	0.6 ± 0.2	0.9 ± 1.0	0.8 ± 0.3
20:4(6)	10.2 ±5.2	6.6 ± 1.7	10.8 ±2.1	8.4 ± 1.1	10.5 ± 4.1	9.1 ±2 .8
22:6(3)	13.6 ±6.5^{a}	18.4 ±6.0^{ab}	29.3 ±3.6^{c}	16.9 ± 2.1^{ab}	22.9 ±4.3^{bc}	17.2 ±7.0^{ab}

HIPPOCAMPUS

Diet	n6/n3 7.3:1	n6/n3 4:1	1% DHA	1%AA	1%AA+ .7%DHA	0.7% DHA
Σsats	46.3 ±5.7^{ab}	49.3 ±6.3^{b}	40.3 ±7.2^{a}	52.4 ±6.0^{b}	46.8 ±7.3^{ab}	50.0 ±4.8^{b}
Σmonos	15.7 ±4.2^{bc}	16.8 ±4.4^{c}	11.6 ±2.9^{ab}	10.7 ±1.9^{a}	10.3 ±0.8^{a}	15.1 ±3.4^{bc}
18:2(6)	3.0 ± 4.3	2.1 ± 0.8	1.1 ± 1.0	0.7 ± 0.2	0.5 ± 0.1	1.0 ± 0.8
20:4(6)	10.2 ±5.2	8.0 ± 2.2	10.3 ±2.7	9.8 ± 1.9	11.4 ±2.8	10.9 ±3.6
22:6(3)	11.6 ±5.9	15.8 ±6.2^{ab}	26.4 ±4.7^{c}	17.2 ±3.6^{ab}	22.2 ±4.3^{bc}	16.3 ±3.6^{ab}

Values for Σsats include 16:0 and 18:0; Values for Σmonos include 16:1 and 18:1
Values without a common superscript differ significantly. Values with the same or no superscript did not differ significantly.

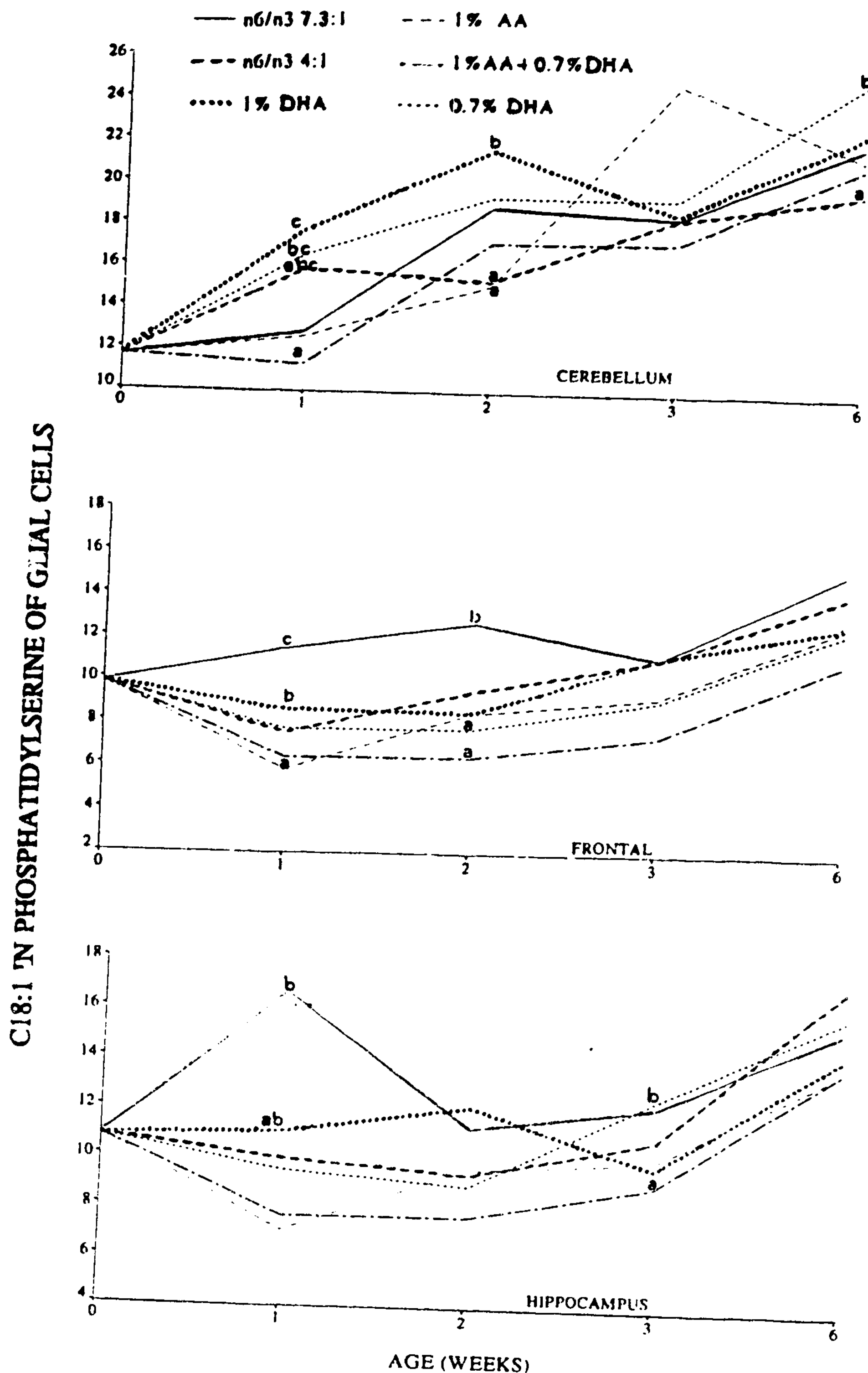


Figure 3.14. Effect of diet on % wt/wt 18:1 in glial PS. Values without a common letter within one age group differ significantly ($p<0.01$).

three weeks of age (Fig 3.12). The 0.7% DHA diet resulted in a low 22:6n-3 content in all brain regions examined (Fig 3.12). Glial PS appeared to be affected less than glial PE or PC by changes in diet fat. Fewer effects of diet on accretion of fatty acids were observed in PS. Diet plays a significant role in accretion of DHA and AA. An age-dependent factor is also present for most fatty acids in glial PS.

Phosphatidylinositol

Age: A significant effect of age was observed for all fatty acids examined in PI in glial cells ($p<0.0001$). Accretion of 22:6n-3 attained maximum levels in all three regions in animals fed 1%DHA (Fig 3.15). The age at which the peak level was observed varied between regions. By two weeks of age, the greatest relative percent of 20:4n-6 was observed in all three regions (Fig 3.16).

Diet: All fatty acids were affected by diet treatment in glial PI. Feeding an n6/n3 fatty acid ratio of 7.3:1 increased 16:0 (Fig 3.17), 18:1 (Fig 3.18) and 18:2n-6 (Table 3.4) in all three brain regions. Low 16:0, 18:1 and 18:2n-6 content resulted from feeding the AA diet or the DHA+AA diet, except in the cerebellum for the former two fatty acids. In specific comparisons, 18:1 (Fig 3.18) and 18:2n-6 (Table 3.4) were also minimized when the 0.7%DHA diet was fed. Lower 16:0 (Fig 3.17) in the cerebellum was observed in animals fed the diet providing an n6/n3 ratio of 4:1. This diet also produced low 18:0 (Fig 3.19) compared to the 0.7%DHA diet or the DHA+AA diet. Feeding the 1%DHA diet maximized accumulation of 22:6n-3 in all brain regions (Fig 3.15). Other diets raised 22:6n-3 but some variation occurred between the three brain regions. At all ages in the cerebellum the diets containing n6/n3 ratios of 7.3:1 and 4:1 increased 22:6n-3 content (Fig 3.15). Additionally, the diet supplying a ratio of 4:1 for n6/n3 fatty acids increased DHA in the frontal and hippocampal regions (Fig 3.15). This effect was not present

at all ages examined. Accretion of 22:6n-3 was low in all brain regions examined in rats fed the 0.7%DHA diet (Fig 3.15). Conversely, 22:6n-3 in brain PE was maximal in rats fed 0.7%DHA (Fig 3.5). Feeding the AA diet produced a high content of 20:4n-6 (Fig 3.16) and a low content of the C18 (Table 3.4) and C20 (not shown) n-3 fatty acids in all three regions and low 22:6n-3 content in the hippocampus (Fig 3.15). Diets that produced maximal percent of 18:2n-6 resulted in lower percent of 20:4n-6 (Table 3.4). The reverse applied for diets producing maximal levels of 20:4n-6 (Fig 3.16). In the cerebellum and hippocampus, the AA, DHA+AA and 0.7%DHA diets increased 20:4n-6 in glial PI (Fig 3.16). Levels of 20:4n-6 in the frontal region were greatest when animals were fed the AA or DHA+AA diets. These results suggest that fatty acid composition of isolated glial PI is affected over time to varying degrees in the three brain regions by physiological changes in dietary fat composition.

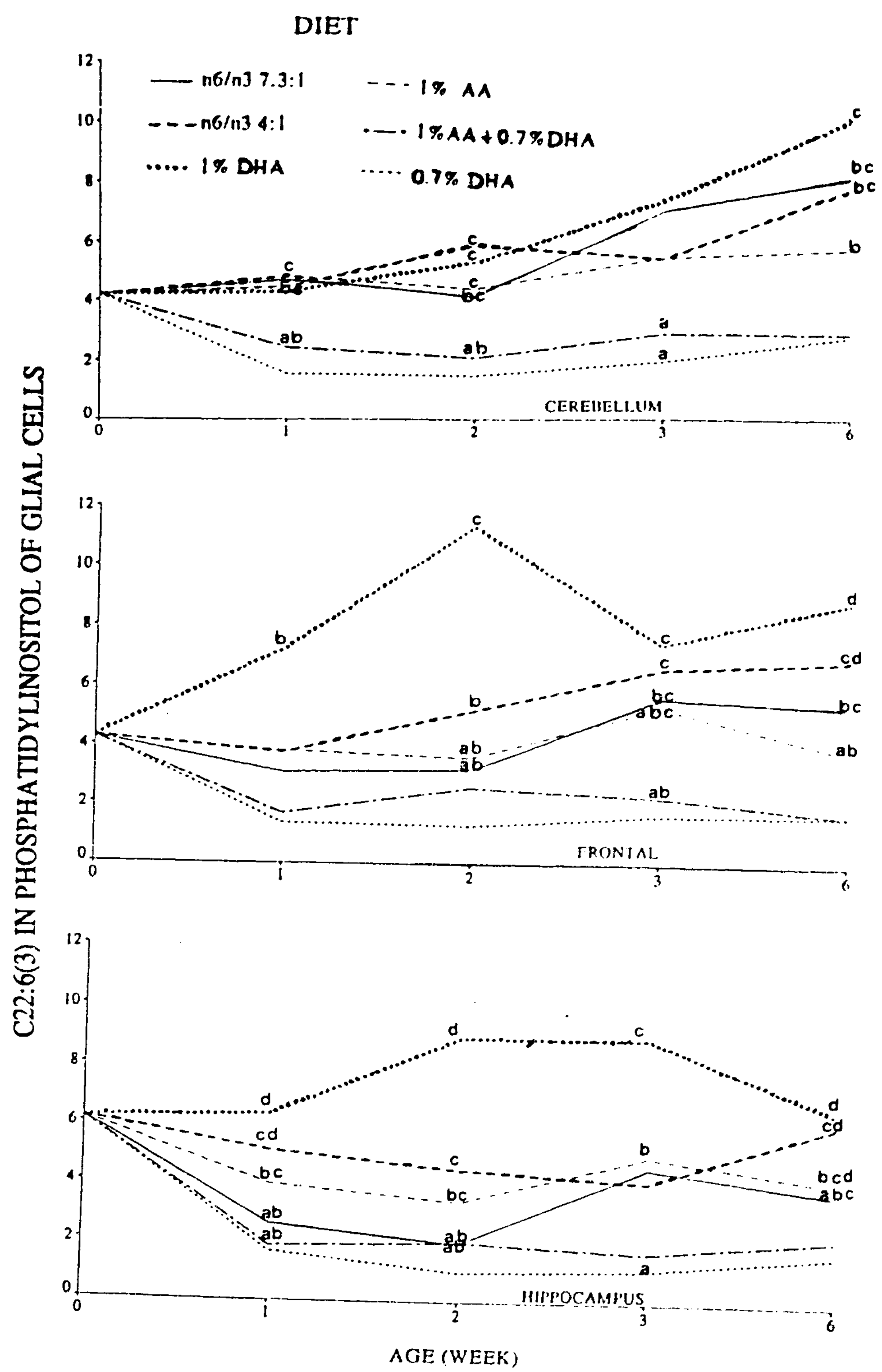


Figure 3.15. Effect of diet on % wt/wt 22:6(3) in glial PI. Values without a common letter within one age group differ significantly ($p < 0.01$).

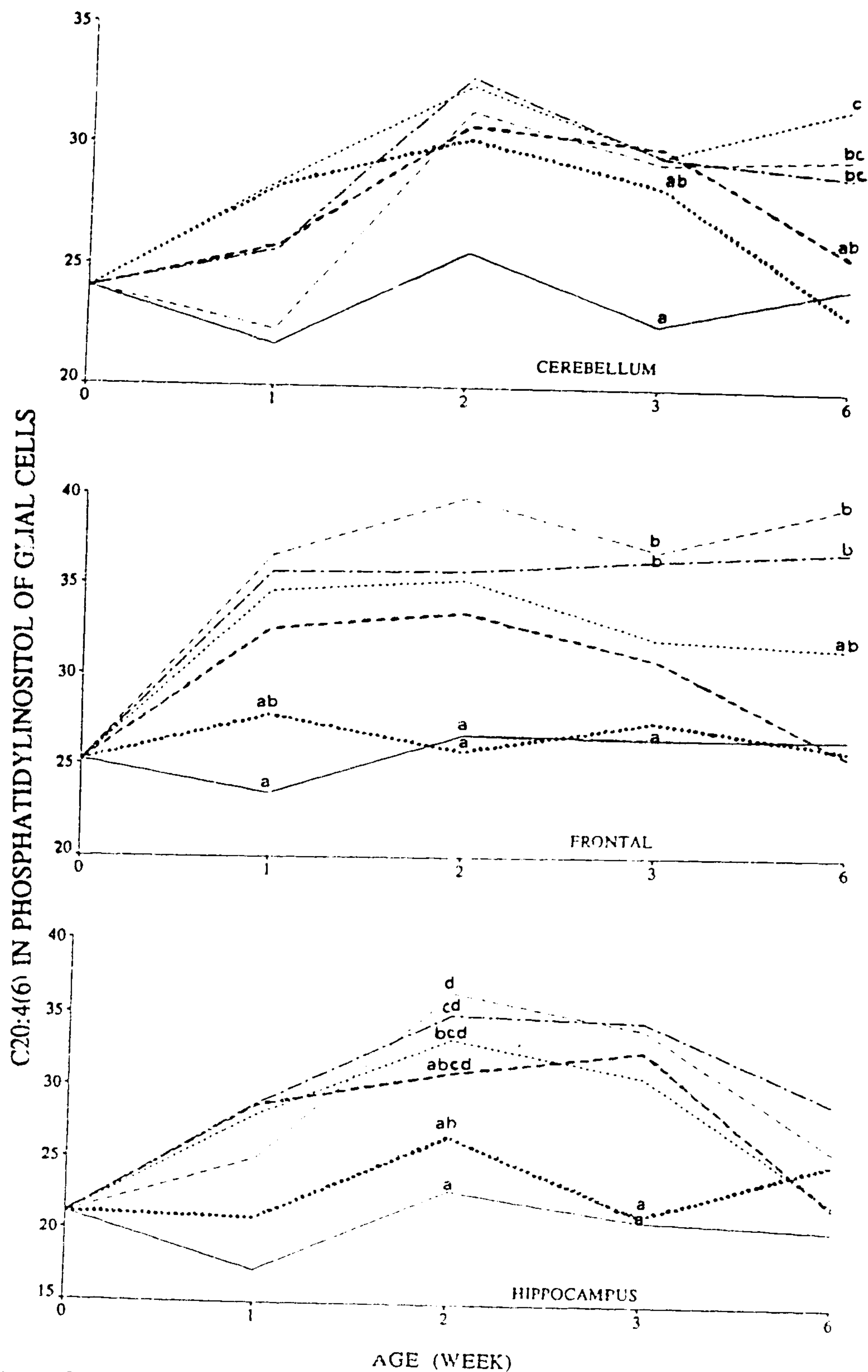


Figure 3.16. Effect of diet on % wt/wt 20:4(6) in glial PI. Values without a common letter within one age group differ significantly ($p<0.01$).

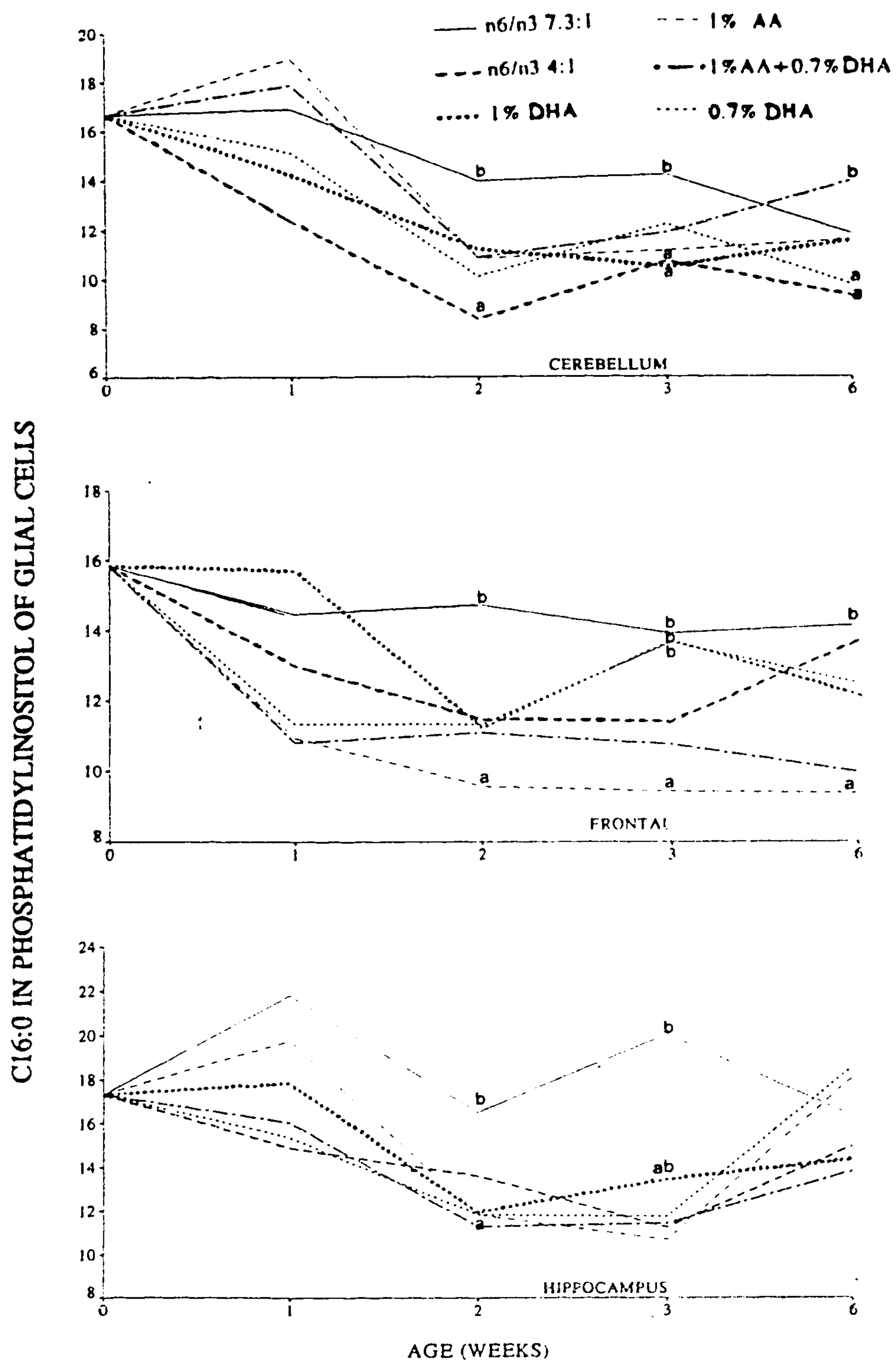


Figure 3.17. Effect of diet on % wt/wt 16:0 in glial PI. Values without a common letter within one age group differ significantly ($p < 0.01$).

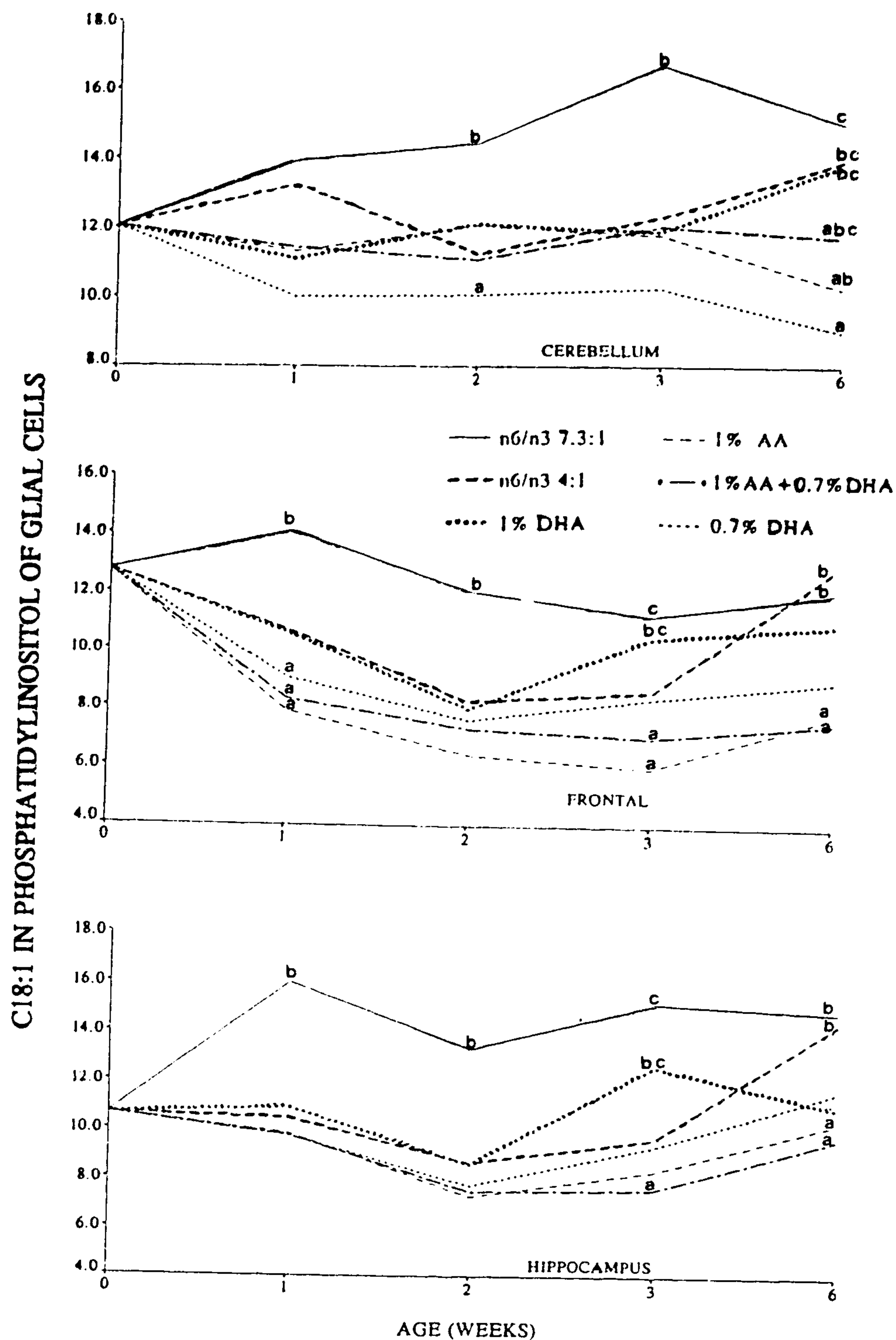


Figure 3.18. Effect of diet on % wt/wt 18:1 in glial PI. Values without a common letter within one age group differ significantly $(p<0.01)$.

Table 3.4. Effect of diet treatment on fatty acid composition (%w/w) at 3 weeks of age in phosphatidylinositol (% means ± S.D)

GLIAL cells

	CEREBELLUM						FRONTAL						HIPPOCAMPUS					
Diet	n6/n3 7.3:1	n6/n3 4:1	1% DHA	1%AA	1%AA+ .7%DHA	0.7% DHA	n6/n3 7.3:1	n6/n3 4:1	1% DHA	1%AA	1%AA+ .7%DHA	0.7% DHA	n6/n3 7.3:1	n6/n3 4:1	1%DHA	1%AA	1%AA+ .7%DHA	0.7% DHA
Σsats	43.4 ±3.7	45.8 ±1.7	46.5 ±3.8	45.9 ±2.2	51.6 ±0.8^{b}	53.2 ±0.9^{b}	46.9 ±3.7	46.5 ±3.3	46.1 ±3.2	46.9 ±2.9	51.6 ±2.8^{b}	54.6 ±1.8^{b}	49.0 ±4.2^{ab}	44.0 ±3.9^{a}	46.8 ±4.9^{a}	47.1 ±2.3^{a}	53.0 ±1.3^{bc}	55.9 ±2.4^{c}
Σmonos	17.7 ±4.4^{b}	13.2 ±2.4	12.8 ±2.7	12.5 ±1.9	7.5± 0.7	11.2 ±2.0	12.4 ±4.1^{b}	9.5± 2.5^{ab}	11.8 ±2.1^{b}	6.5± 1.7^{a}	7.5± 0.7^{a}	9.1± 1.5^{ab}	16.5 ±5.6^{c}	10.4 ±2.7^{ab}	13.7 ±4.2^{bc}	8.9 ±2.4^{abc}	8.1± 2.6^{a}	10.1 ±2.1^{ab}
18:2(6)	1.3± 0.2^{b}	1.1± 0.3^{ab}	0.7± 0.2^{a}	0.6± 0.1^{a}	0.6± 0.2^{a}	1.0± 1.0^{ab}	2.0± 2.9	0.7± 0.1	0.7± 0.1	0.4± 0.06	0.4± 0.05	0.6± 0.2	3.4± 3.8^{b}	1.0± 0.3^{ab}	1.6± 2.3^{ab}	0.4± 0.1^{a}	0.5± 0.3^{a}	0.7± 0.4^{a}
20:4(6)	22.6 ±4.2^{a}	29.9± 2.8^{b}	28.3± 4.0^{ab}	29.3± 4.7^{b}	29.5 ±5.4^{b}	29.6 ±5.7^{b}	26.4± 10.9^{a}	30.7 ±8.9^{ab}	27.3± 3.1^{ab}	36.7 ±2.9^{b}	36.2 ±1.8^{b}	31.9± 3.0^{ab}	20.8 ±7.9	32.6 ±4.1^{b}	21.1 ±4.4	34.1 ±6.2^{b}	34.0 ±5.1^{b}	30.9 ±3.7^{b}
22:6(3)	7.0± 1.9	5.4± 1.5	7.3± 2.0	5.4± 2.4	2.9± 2.0^{a}	2.0± 0.5^{a}	5.6± 3.5^{bc}	6.6± 4.2^{c}	7.4± 2.1^{c}	5.3± 1.8^{abc}	2.2± 1.0^{ab}	1.6± 0.4^{a}	4.6± 2.6^{ab}	4.1± 2.3^{ab}	8.9± 2.9^{c}	5.0± 3.6^{b}	1.7± 0.6^{ab}	1.1± 0.4^{a}

NEURONAL cells

	CEREBELLUM						FRONTAL						HIPPOCAMPUS					
Diet	n6/n3 7.3:1	n6/n3 4:1	1% DHA	1%AA	1%AA+ .7%DHA	0.7% DHA	n6/n3 7.3:1	n6/n3 4:1	1% DHA	1%AA	1%AA + .7%DHA	0.7% DHA	n6/n3 7.3:1	n6/n3 4:1	1% DHA	1%AA	1%AA+ .7%DHA	0.7% DHA
Σsats	47.0 ±4.9^{ab}	44.9 ±1.5^{a}	48.9 ±2.8^{abc}	48.9 ±1.4^{ab}	52.8 ±6.0^{c}	51.1 ±1.5^{bc}	45.2 ±2.8^{a}	46.5± 1.45^{ab}	46.8 ±5.7^{ab}	54.0 ±5.3^{c}	51.6 ±2.6^{bc}	55.1 ±4.0^{a}	43.5 ±7.7^{a}	48.5 ±4.3^{ab}	51.1 ±1.9^{b}	54.3 ±6.6^{b}	52.0 ±1.7^{b}	53.8 ±1.9^{b}
Σmonos	16.4 ±5.3^{b}	12.4 ±2.8	12.2 ±2.1	11.6 ±2.1	11.4 ±2.3	10.9 ±1.3	16.9 ±6.6^{b}	11.1 ±2.2^{ab}	14.5 ±6.0^{ab}	8.8 ±2.3^{a}	10.4 ±2.3^{a}	12.5 ±4.4^{ab}	19.6 ±6.0^{b}	14.3 ±3.8	12.2 ±2.0	10.0 ±2.2	11.7 ±3.1	14.0 ±2.6
18:2(6)	5.5± 5.3^{b}	1.8± 1.2	1.0± 0.6	0.9± 0.8	0.7± 0.2	0.7± 0.2	6.6± 5.2^{b}	1.8± 0.7	2.7± 3.2	0.6± 0.5	0.8± 0.2	1.1± 0.5	7.5± 6.3^{b}	3.0± 1.3	1.4± 1.0	1.3± 2.0	1.1± 0.5	1.5± 0.8
20:4(6)	20.6± 11.1^{a}	30.7 ±5.3^{b}	25.6± 7.8^{ab}	32.2 ±4.6^{b}	30.7 ±7.0^{b}	33.1± 2.2^{b}	20.9± 10.5	30.1 ±6.3	25.0± 8.4	30.9± 4.7	31.1 ±6.4	27.9 ±8.5	13.3± 11.6^{a}	24.5 ±8.7^{ab}	22.6 ±2.5^{ab}	22.6± 7.5^{ab}	29.3 ±7.4^{b}	25.9 ±5.1^{b}
22:6(3)	3.3± 1.9^{ab}	5.2± 2.1^{bc}	5.7± 2.3^{c}	3.3± 1.2^{ab}	1.8± 0.6^{a}	1.8± 0.5^{ab}	3.1± 2.8^{b}	4.9± 3.5^{b}	5.0± 1.5^{b}	3.0± 0.8^{ab}	2.2± 1.0^{ab}	1.8± 0.6^{a}	1.9± 1.8	3.4± 1.6	6.1± 1.3^{b}	2.7± 0.6	2.1± 1.3	1.7± 0.6

Values for Σsats include 16:0 and 18:0; Values for Σmonos include 16:1 and 18:1

Values without a common superscript differ significantly. Values with the same superscript or no superscriptr did not differ significantly.

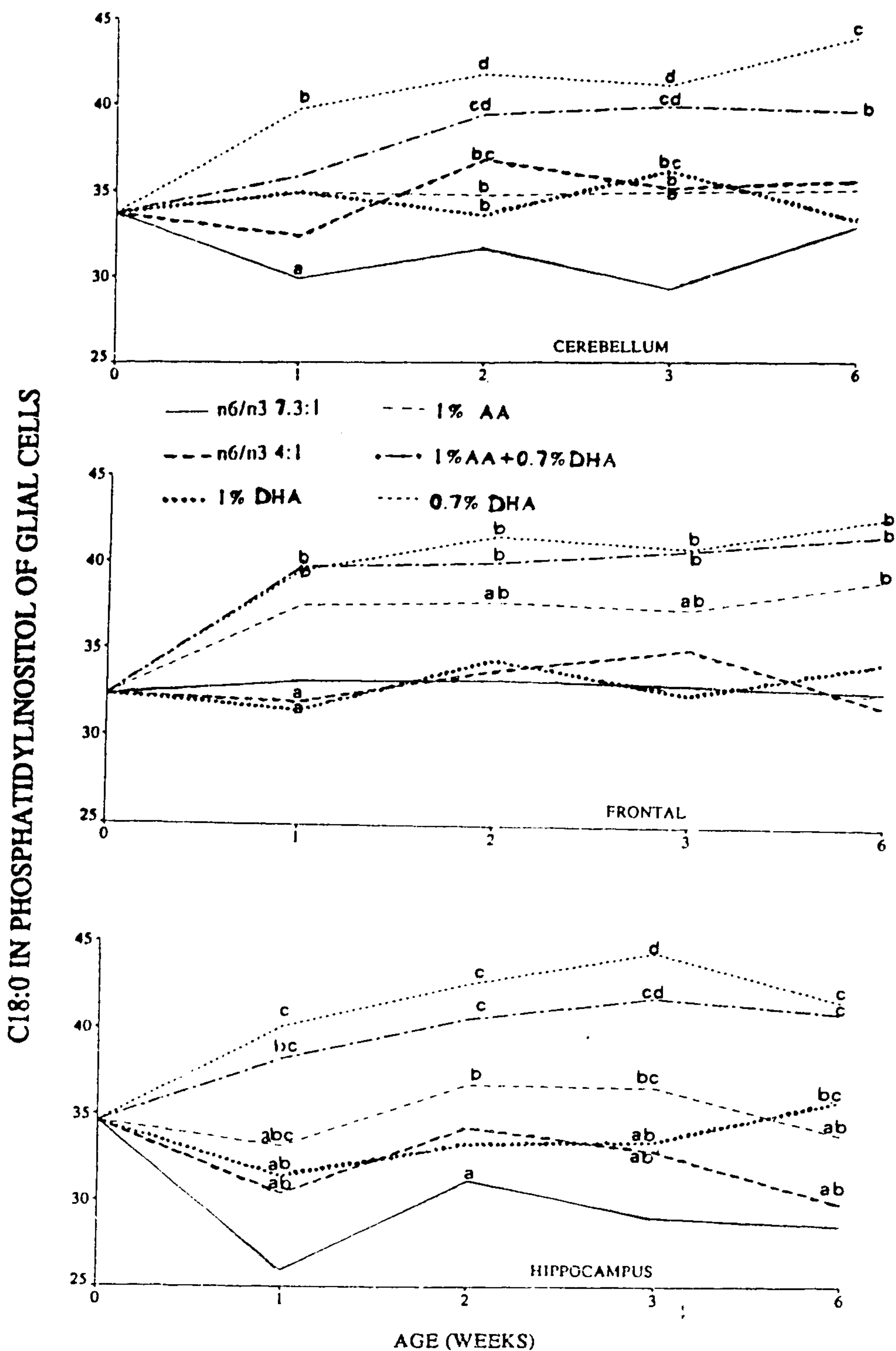


Figure 3.19. Effect of diet on % wt/wt 18:0 in glial PI. Values without a common letter within one age group differ significantly ($p < 0.01$).

FATTY ACID COMPOSITION OF NEURONAL CELLS

The major fatty acids present in each phospholipid in glial cells were also found in similar relative percents in neuronal cell phospholipids. For most comparisons, 18:3n-3 and 20:5n-3 content in the lipid classes examined was negligible (<1.0% w/w).

Phosphatidylethanolamine

Age: Accretion of fatty acids in neuronal PE was affected by the age of the animal. It is apparent that age produced a greater effect on the accretion of saturates, monoenes, 18:3n-3, 20:4n-6 and 22:6n-3 compared to 18:2n-6 and 20:5n-3 ($p<0.0001$ and $p<0.01$ respectively). The highest percent of 22:6n-3 in neuronal PE was attained at six weeks of age in the three brain regions examined (Fig 3.20) from animals fed either 0.7%DHA or the diet containing an n6/n3 fatty acid ratio of 4:1. The concentration of 20:4n-6 decreased over time in the cerebellum (Fig 3.21), except in animals fed 1%AA. In the frontal and hippocampal regions, maximum percent of 20:4n-6 was observed at two weeks of age.

Diet: The effect of diet in neuronal PE was significant for all fatty acids examined ($p<0.01$). In the cerebellum, frontal and hippocampal regions, maximum percent of monoenes (Table 3.1), 16:0 (Fig 3.22) and 18:2n-6 (Table 3.1) was attained when animals were fed the diet supplying an n6/n3 ratio of 7.3:1. Low levels of saturates occurred in animals fed primarily the diet providing an n6/n3 ratio of 4:1 or the 0.7%DHA diet (Table 3.1). In most comparisons, all other diet treatments resulted in a lower monoene content. A reduced level of 18:2n-6 was observed in animals fed primarily the AA, the DHA+AA or the 0.7% DHA diets (Table 3.1). When differences in 18:3n-3 and 20:5n-3 content between diet treatments were observed the diet containing an n6/n3 ratio of 7.3:1 resulted in increased percents of these fatty acids (data not shown). Although feeding the 7.3:1 diet increased the C18 and C20 n-3 fatty acid levels in neuronal PE, this diet produced the lowest level of 22:6n-3 in

all brain regions (Fig 3.20). Feeding the diet containing an n6/n3 ratio of 4:1 resulted in the lowest level of 18:3n-3 and the greatest accretion of 22:6n-3. The 0.7%DHA diet produced similar results for both 18:3n-3 and 22:6n-3. At one week of age the 22:6n-3 content in the cerebellum was significantly greater in animals fed the 0.7%DHA diet compared to those fed 1%DHA (Fig 3.20). In the frontal and hippocampal regions, this observation was noted at one and six weeks of age (Fig 3.20). Animals fed the AA diet exhibited a high level of 18:0 in the hippocampus, increased 18:3n-3 in the frontal and hippocampal regions and low 22:6n-3 in the hippocampus. Increased 20:4n-6 in all three brain regions developed in animals fed the AA diet compared to animals fed the diet containing the n6/n3 ratio of 7.3:1 (Fig 3.21). An increase in 20:4n-6 also occurred in the frontal and hippocampal regions when DHA+AA was fed. This diet also maximized 18:0 in neuronal PE compared to the diets containing an n6/n3 ratio of 7.3:1 and 4:1 (Fig 3.23).

In neuronal PE, the maximum level of 20:4n-6 was attained prior to the maximum level of 22:6n-3. The rate of accretion of fatty aicds in neuronal cells varied between the three brain regions.

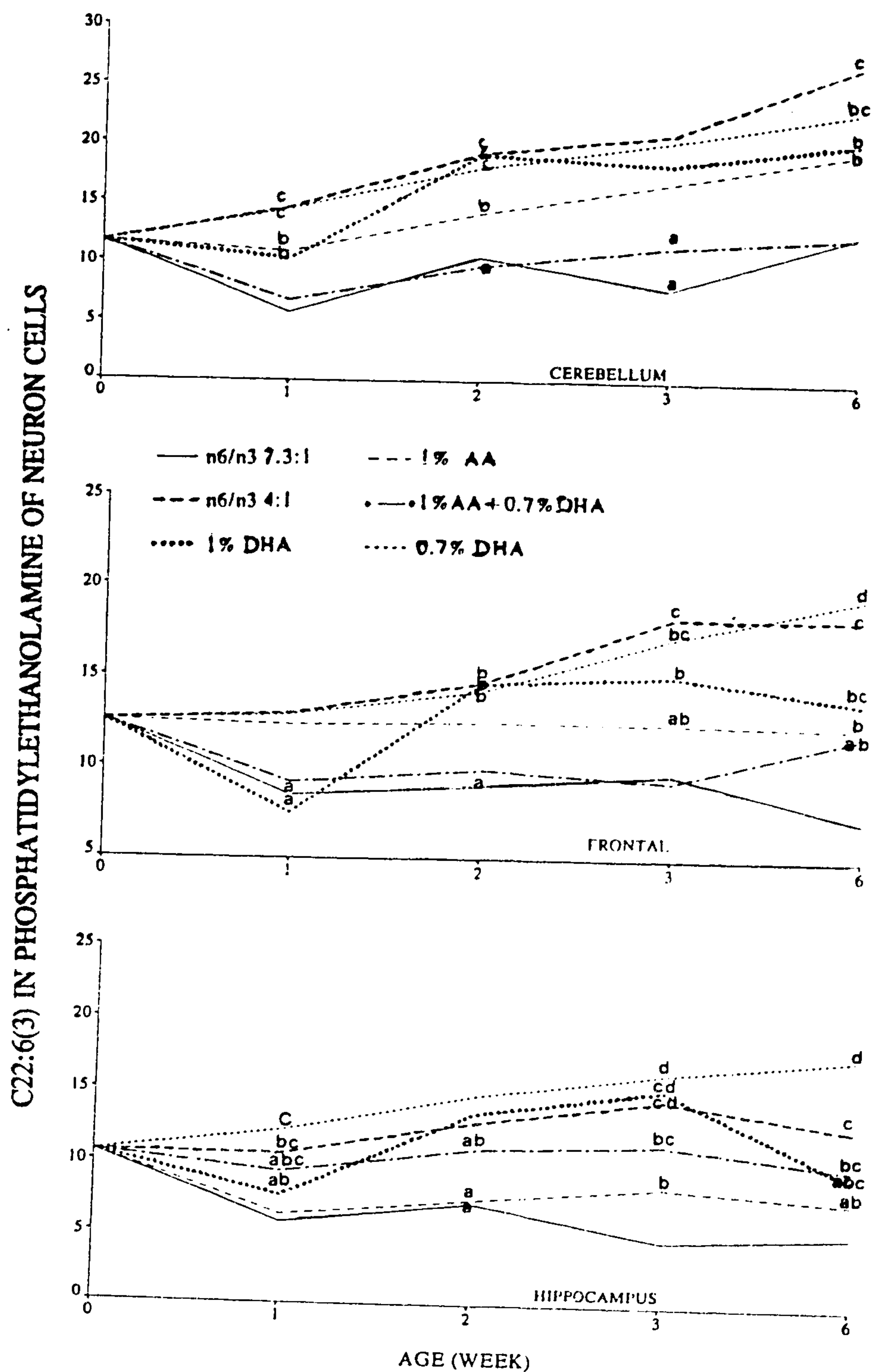


Figure 3.20. Effect of diet on % wt/wt 22:6(3) in neuronal PE.. Values without a common letter within one age group differ significantly ($p < 0.01$).

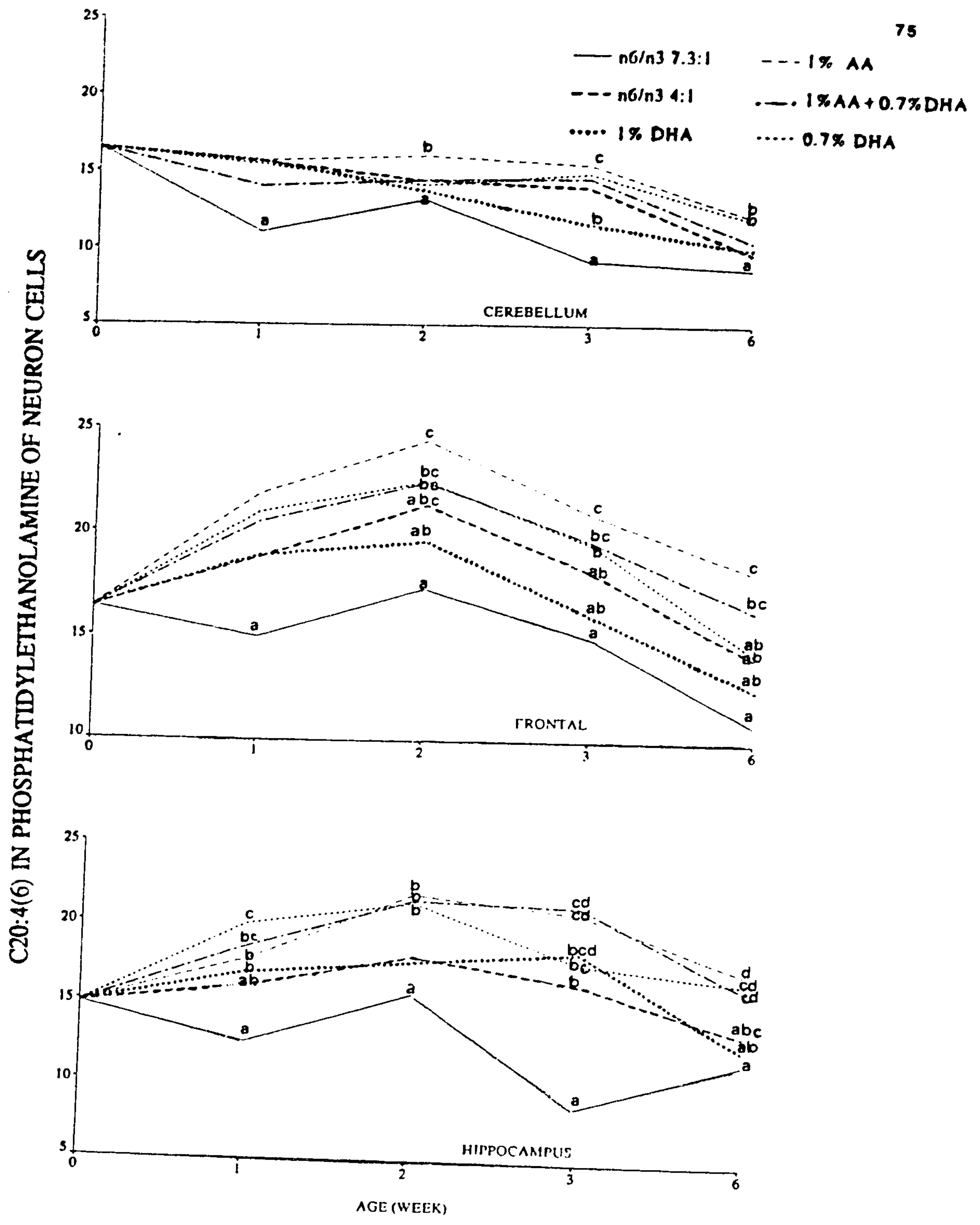


Figure 3.21. Effect of diet on % wt/wt 20:4(6) in neuronal PE.. Values without a common letter within one age group differ significantly ($p<0.01$).

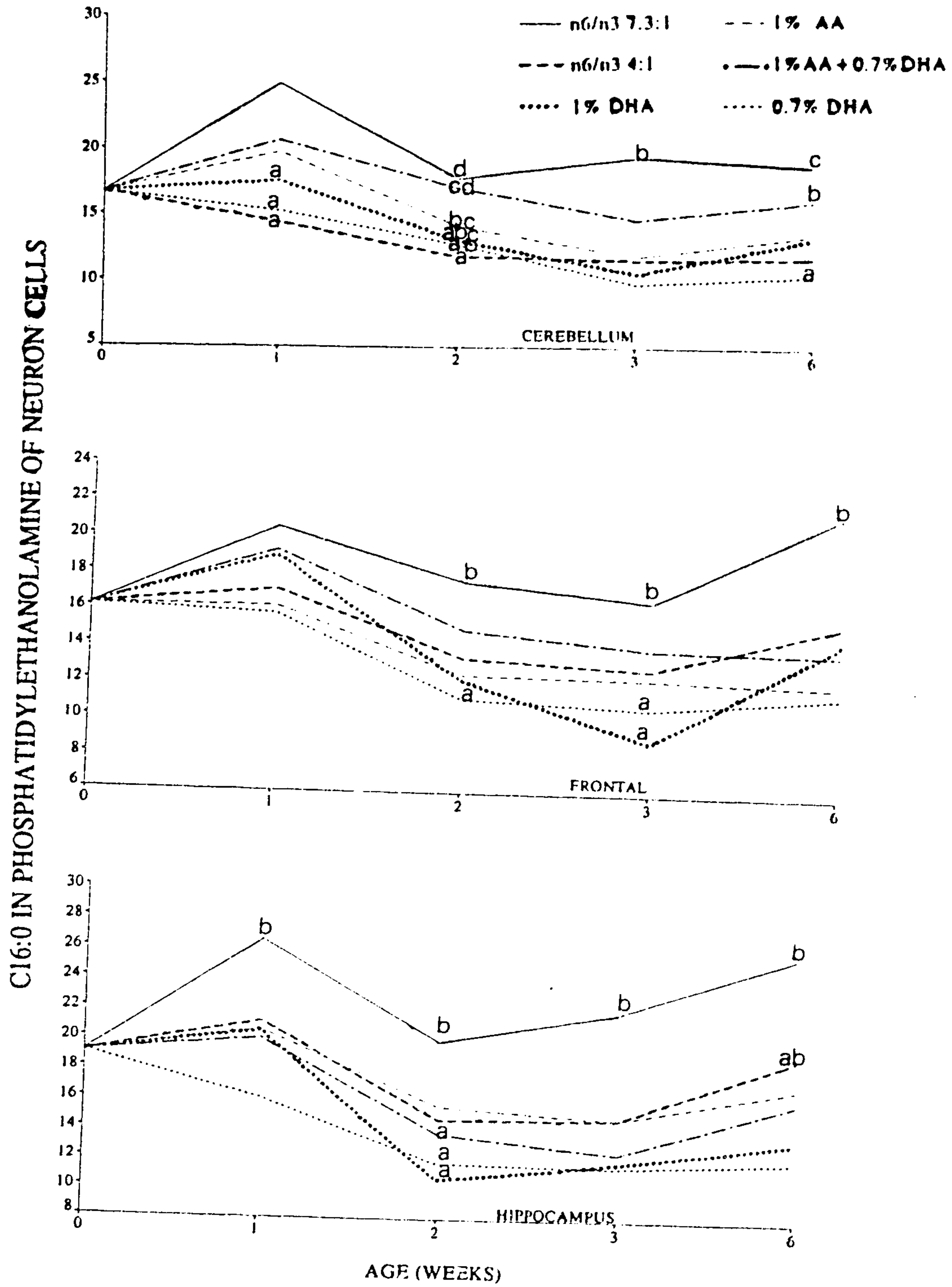


Figure 3.22. Effect of diet on % wt/wt 16:0 in neuronal PE.. Values without a common letter within one age group differ significantly ($p<0.01$).

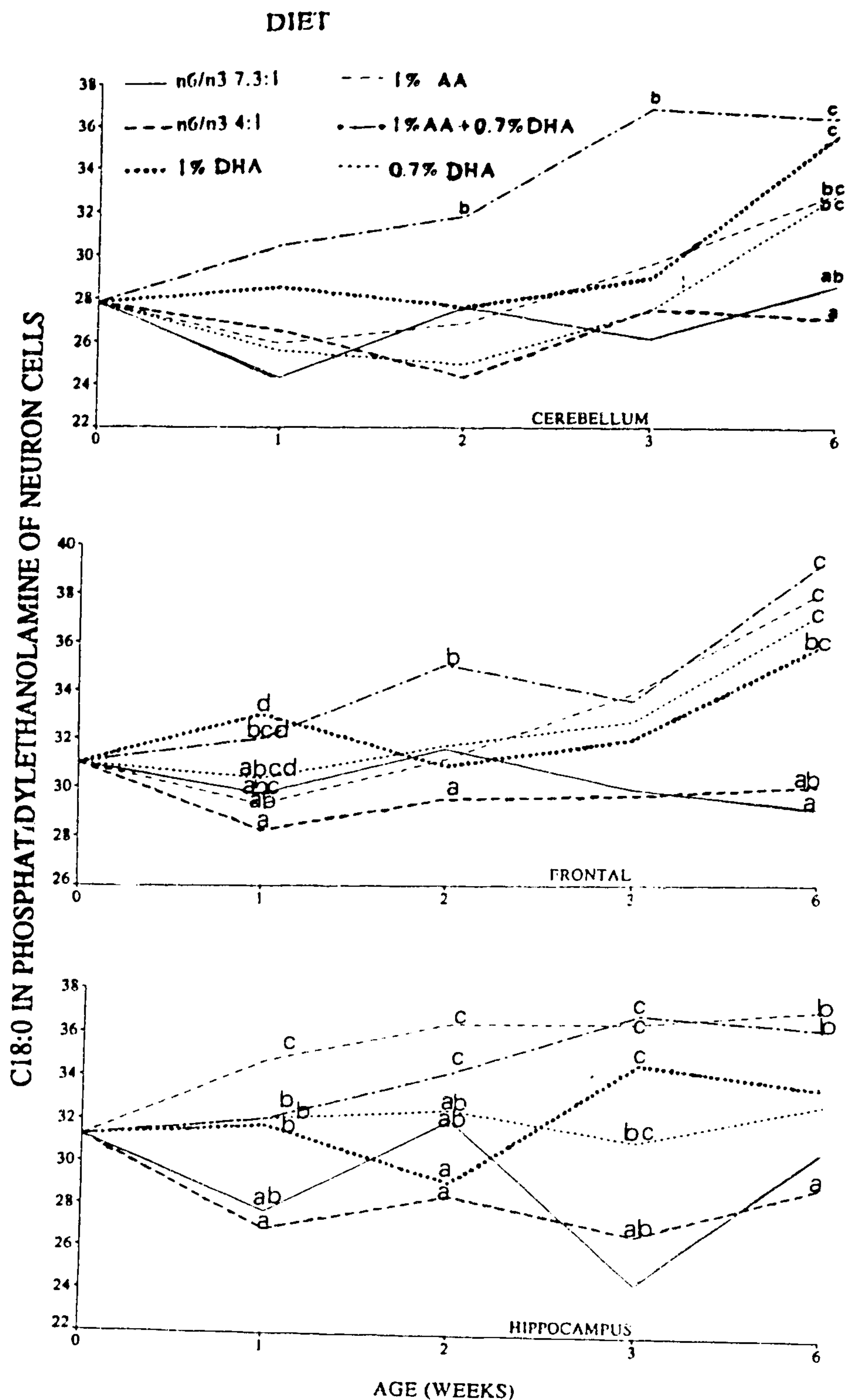


Figure 3.23. Effect of diet on % wt/wt 18:0 in neuronal PE.. Values without a common letter within one age group differ significantly ($p<0.01$).

Phosphatidylcholine

Age: None of the ages tested exhibited significant differences in the saturate and monoene content in neuronal PC. Accretion of the polyunsaturated fatty acids was affected by the age of the animal ($p<0.004$). By two weeks of age, maximum 20:4n-6 was observed in the cerebellar and frontal regions. At one week, maximum 20:4n-6 was attained in the hippocampus. In all three regions the greatest percent of 20:4n-6 was observed in animals fed DHA+AA. Feeding DHA+AA also produced maximum 22:6n-3 (Fig 3.24). In the cerebellum and frontal region, this level was attained at two weeks of age. Maximum 22:6n-3 in the hippocampus was observed at three weeks of age in animals fed the diet containing an n6/n3 ratio of 4:1.

Diet: All fatty acids examined in neuronal PC were affected by dietary fat treatment ($p<0.0001$). Animals fed the diet supplying n6/n3 fatty acids at a ratio of 7.3:1 exhibited low 16:0 in all three brain regions at all ages except at one week in the frontal region (Fig 3.25). In comparison the maximum percent of 16:0 was attained at three weeks in the cerebellum in animals fed 0.7%DHA and at three weeks in the hippocampus in animals fed 1%DHA. In the frontal region, the highest percent of 16:0 was exhibited at six weeks of age in animals fed 1%DHA (Fig 3.25). The content of 18:3n-3 and 20:5n-3 in neuronal PC increased when animals were fed the diet providing an n6/n3 ratio of 7.3:1 compared to all other diet treatments (data not shown). When diet treatments exhibited a difference in PC 18:2n-6 content, feeding the diet providing an n6/n3 ratio of 7.3:1 resulted in a higher percent of 18:2n-6 compared to the DHA+AA diet (Table 3.2). Feeding the DHA+AA diet resulted in maximum accretion of 22:6n-3 in the cerebellar and frontal regions after one week of age (Fig 3.24). In the hippocampal neurons the only difference in 22:6n-3 content occurred between diet treatments at three weeks of age (Fig 3.24 and Table 3.2). This result was also observed in glial cells in hippocampal PC (Table 3.2). In both neurons and glia in hippocampal PC the diet supplying the

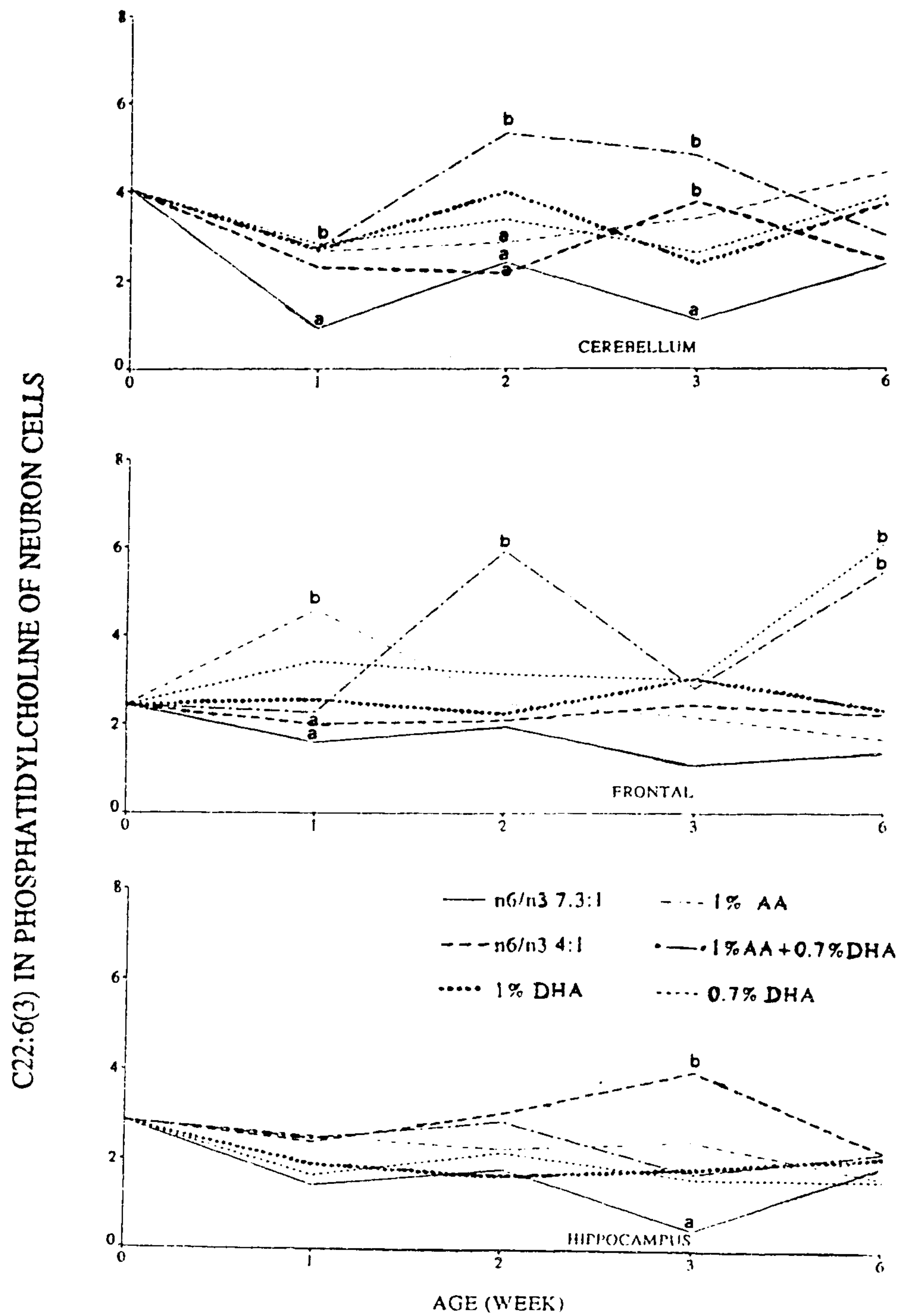


Figure 3.24. Effect of diet on % wt/wt 22:6(3) in neuronal PC.. Values without a common letter within one age group differ significantly ($p<0.01$).

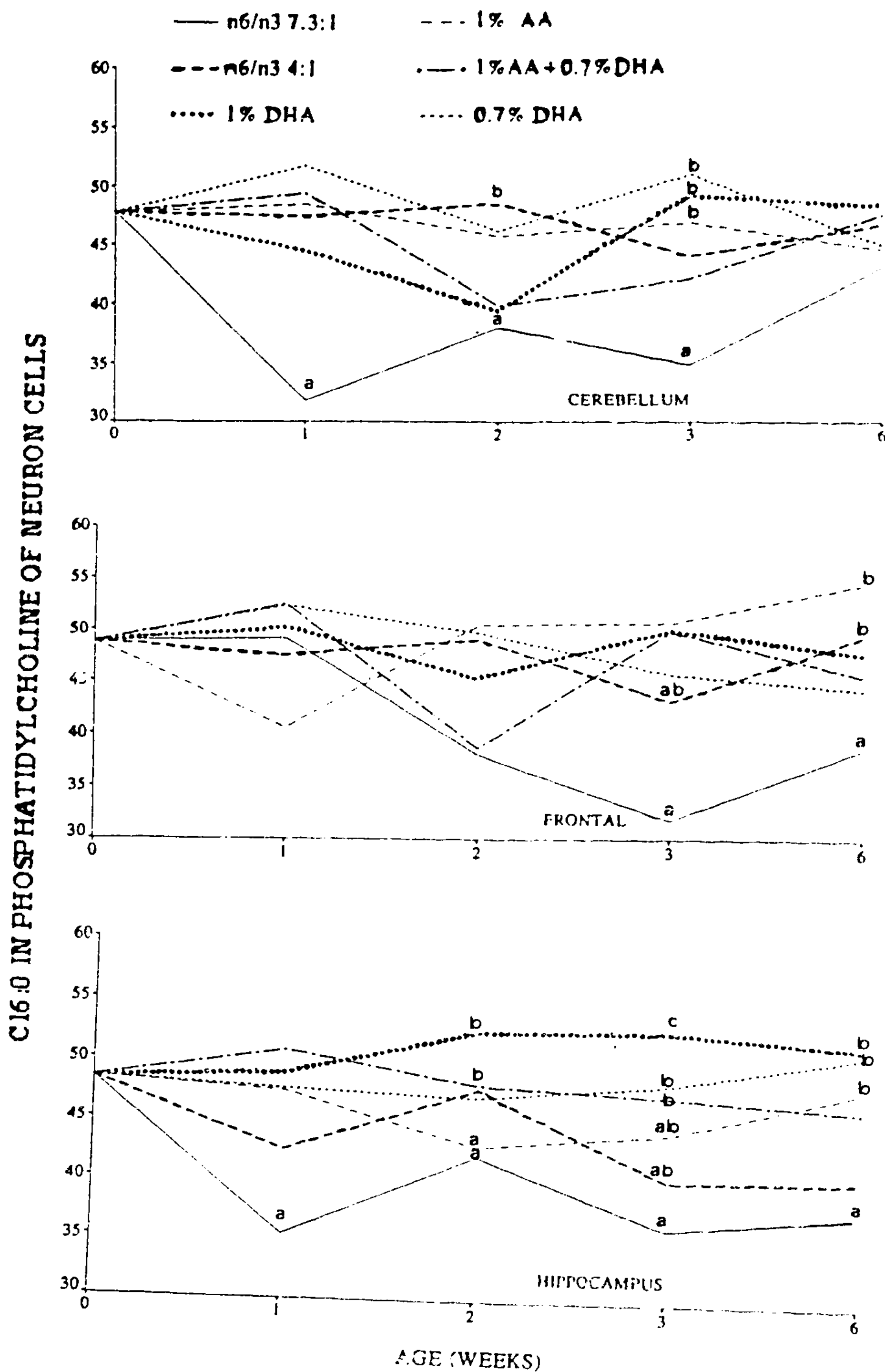


Figure 3.25. Effect of diet on % wt/wt 16:0 in neuronal PC. Values without a common letter within one age group differ significantly ($p<0.01$).

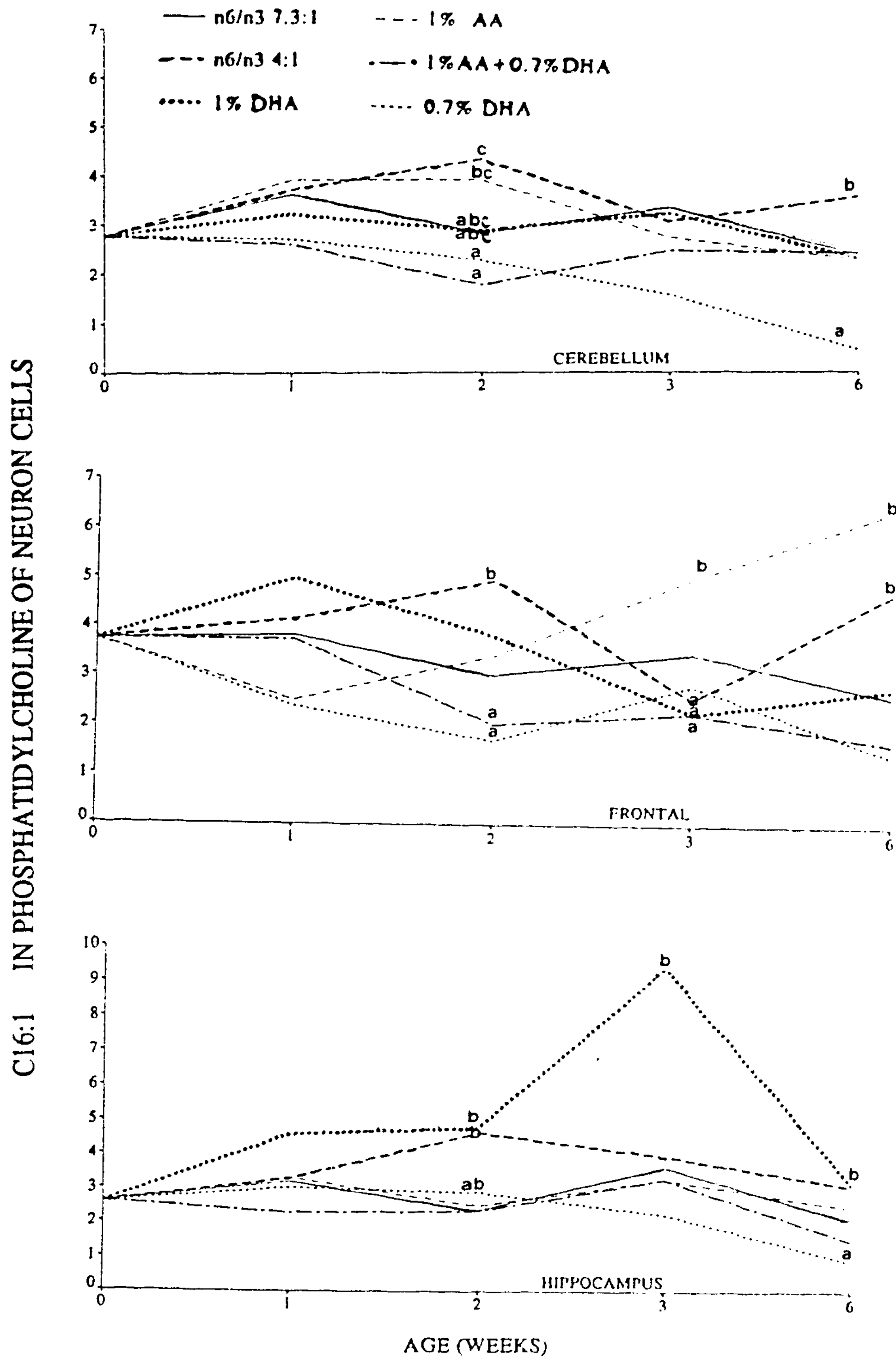


Figure 3.26. Effect of diet on % wt/wt 16:1 in neuronal PC.. Values without a common letter within one age group differ significantly ($p<0.01$).

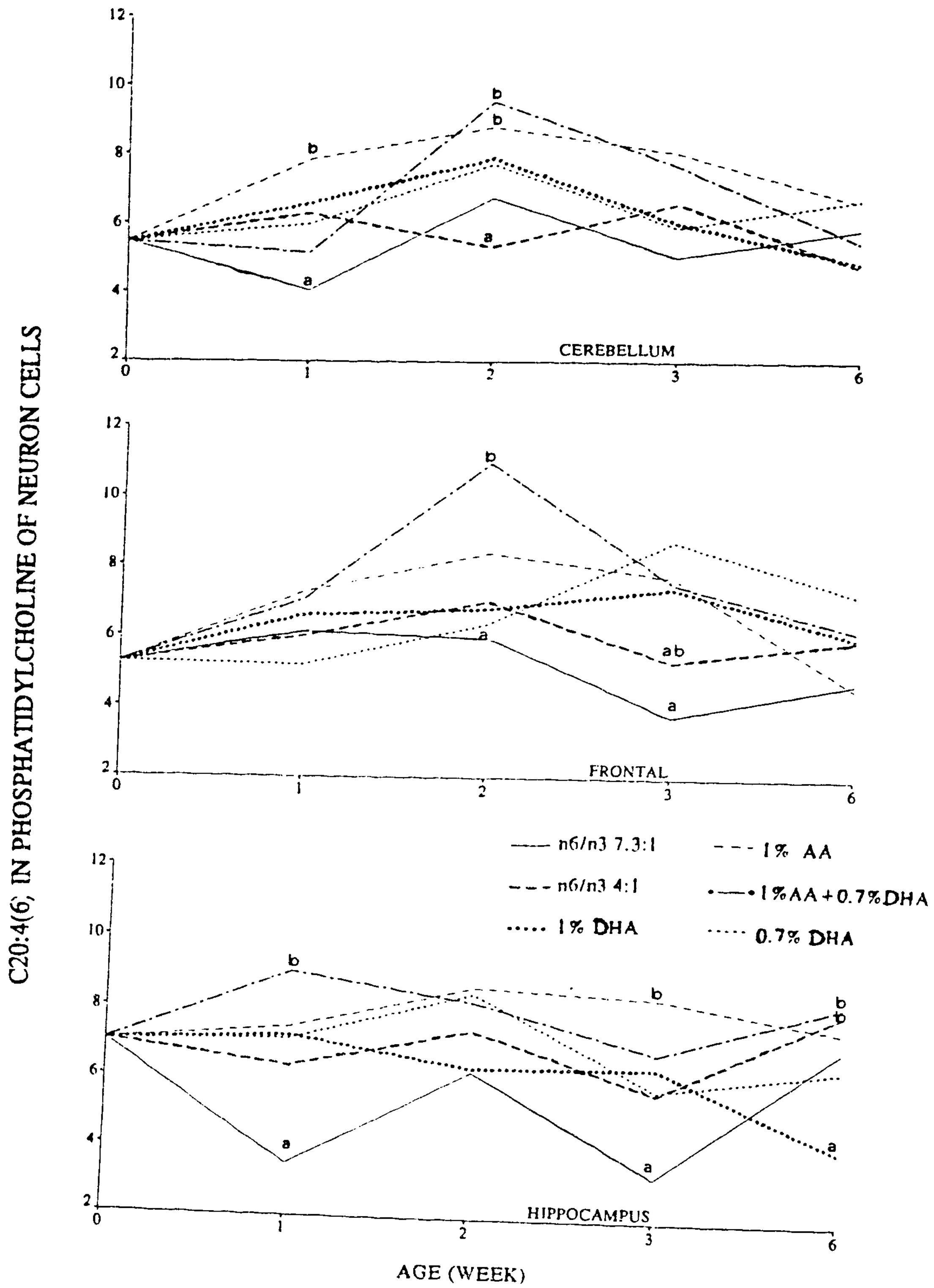


Figure 3.27. Effect of diet on % wt/wt 20:4(6) in neuronal PC.. Values without a common letter within one age group differ significantly ($p<0.01$).

ratio of 4:1 for n6/n3 fatty acids produced the increase in 22:6n-3. In all three regions low 22:6n-3 resulted when animals were fed the diet providing an n6/n3 ratio of 7.3:1 (Fig 3.24). At two weeks of age, the level of 16:1 in the cerebellum was greatest in animals fed the diet providing n6/n3 fatty acids at a ratio of 4:1 (Fig 3.26). In addition to this diet the AA diet increased the content of 16:1 in the frontal region. The maximum percent of 16:1 in neuronal PC was observed at three weeks of age in animals fed 1%DHA. A low 16:1 level in all three brain regions was observed in animals fed either 0.7%DHA or DHA+AA. The DHA+AA diet and the AA diet resulted in greater accretion of 20:4n-6 compared to the diet supplying n6/n3 fatty acids at 7.3:1 (Fig 3.27).

In neuronal PC, maximum 20:4n-6 was attained at the same age as maximum 22:6n-3 in the cerebellum and frontal region. In the hippocampus, accretion of 22:6n-3 continued beyond the age at which maximum 20:4n-6 was observed. Both age and diet played significant roles in the rate of accretion of fatty acids.

Phosphatidylserine

Age: The effect of age was significant for all fatty acids analyzed in neuronal PS except for 18:3n-3 and 20:5n-3 ($p<0.01$). The maximum concentration of 20:4n-6 was observed at one week of age in all three brain regions. The relative percent of 22:6n-3 was greatest at three weeks of age in all regions examined (Fig 3.28).

Diet: Accretion of 16:0 in neuronal PS was greater, particularly between three and six weeks of age, when diets supplying an n6/n3 ratio of 7.3:1 or 4:1 were fed compared to the DHA+AA diet or 0.7%DHA (Fig 3.29). Accumulation of 18:3n-3 and 20:5n-3 did not vary in PS in the cerebellar and frontal regions between diet treatments (data not shown). Feeding 0.7%DHA or the AA diet increased levels of 18:3n-3 and 20:5n-3 in the hippocampus compared to the DHA+AA diet or the diet containing an n6/n3

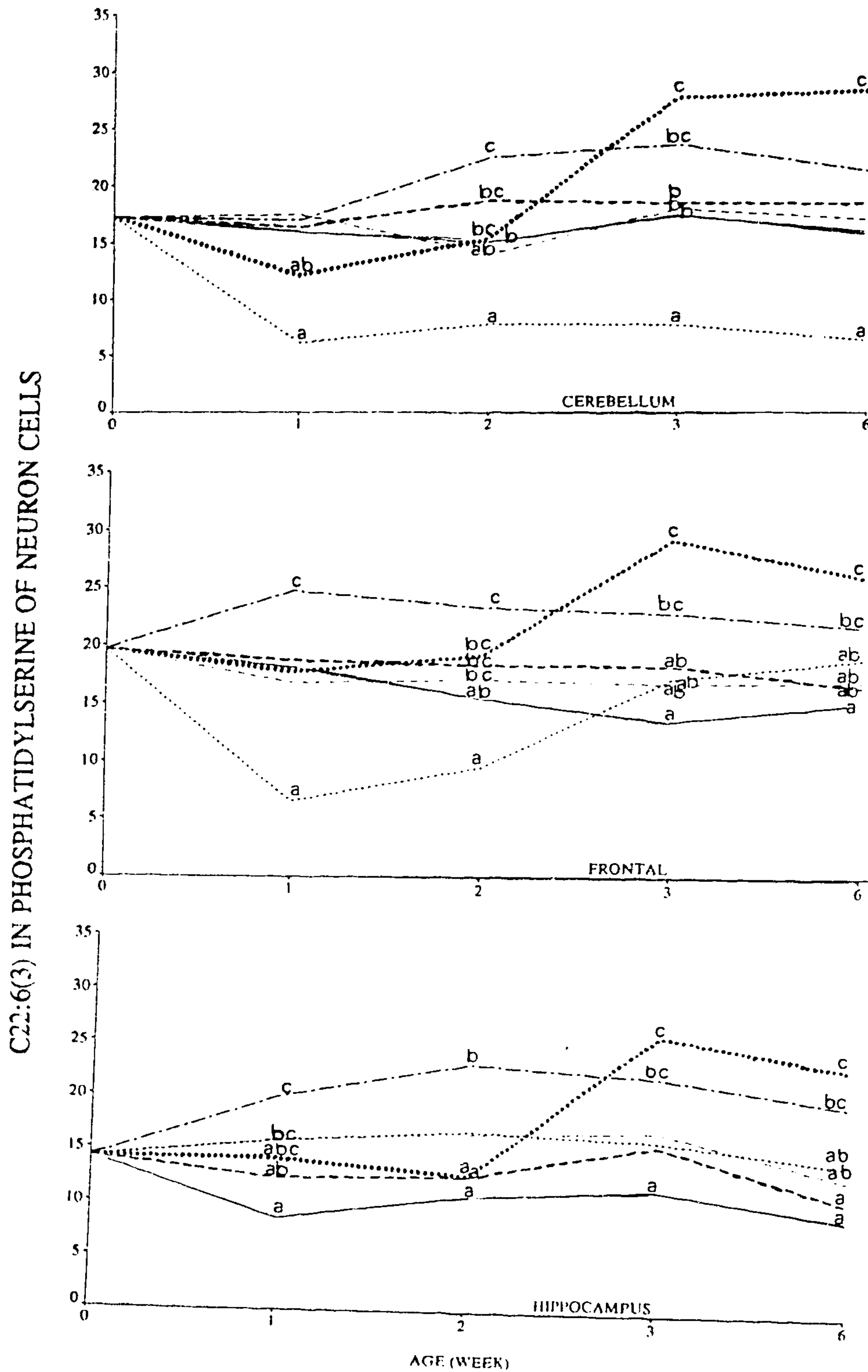


Figure 3.28. Effect of diet on % wt/wt 22:6(3) in neuronal PS. Values without a common letter within one age group differ significantly ($p < 0.01$).

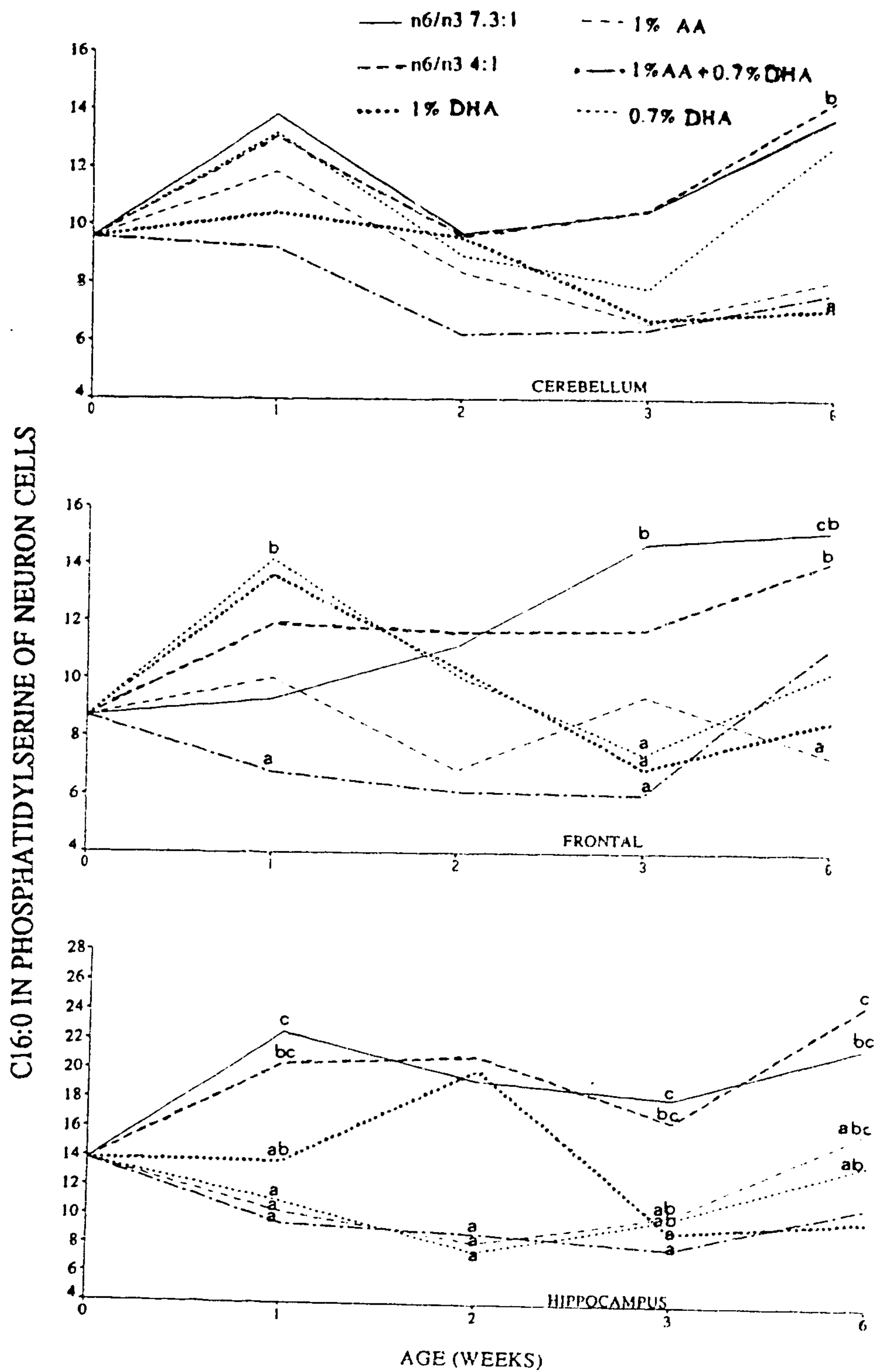


Figure 3.29. Effect of diet on % wt/wt 16:0 in neuronal PS. Values without a common letter within one age group differ significantly ($p<0.01$).

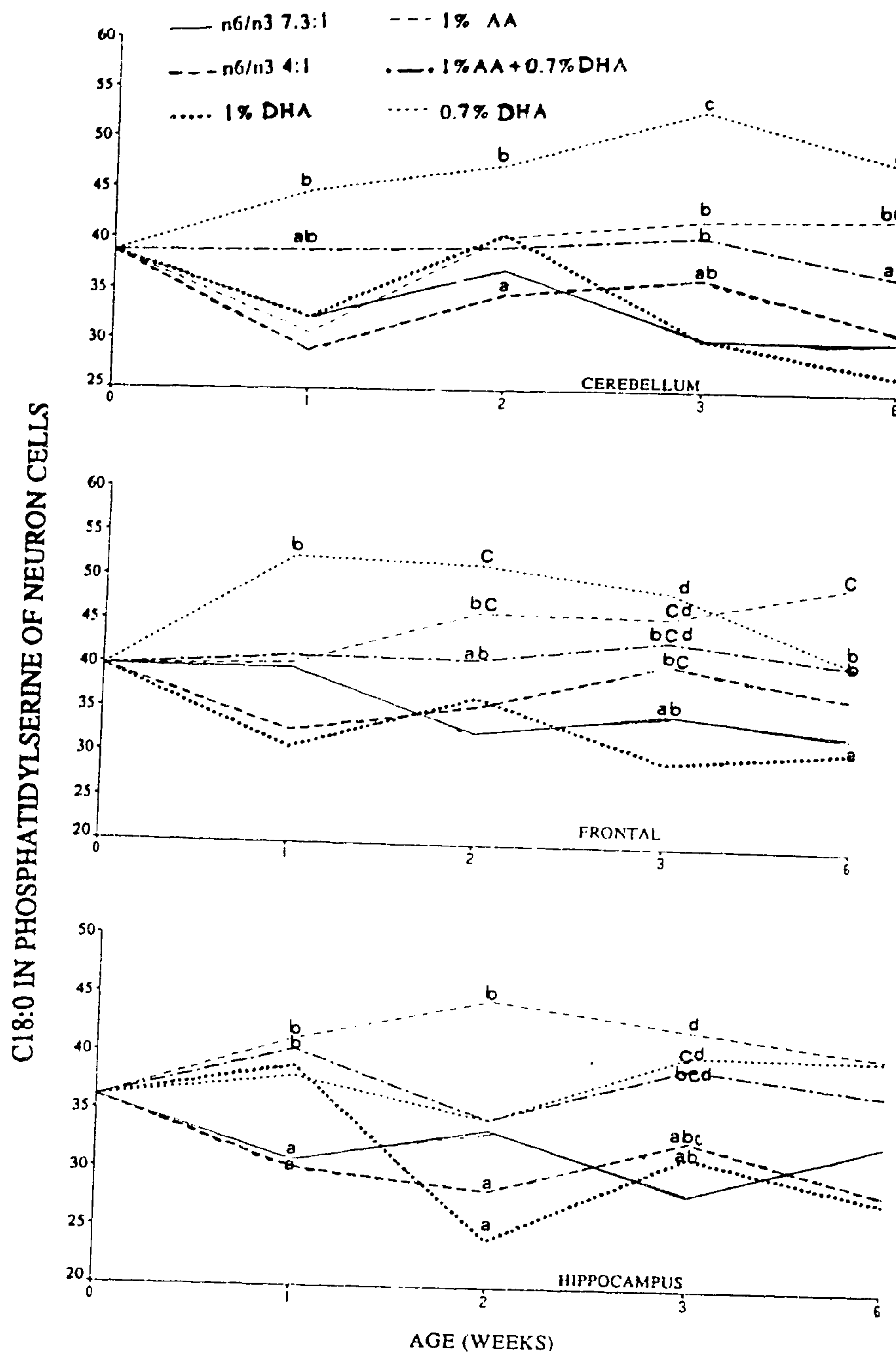


Figure 3.30. Effect of diet on % wt/wt 18:0 in neuronal PS. Values without a common letter within one age group differ significantly ($p<0.01$).

ratio of 4:1. The 18:0 content in the cerebellum and the frontal region, at three weeks and one week respectively, was maximal when animals were fed 0.7%DHA (Fig 3.30). Feeding the AA diet produced the same results at two weeks of age in the hippocampus. A low level of 18:0 was exhibited in PS for animals fed the diets containing n6/n3 ratios of 7.3:1, 4:1 or 1%DHA (Fig 3.30). Reduced levels of monoenes generally resulted from feeding the AA or the DHA+AA diets (Table 3.3). These two diets also produced low levels of 18:2n-6 (Table 3.3). Accretion of 22:6n-3 was maximal when animals were fed either the DHA+AA diet or 1%DHA. The diets supplying ratios of n6/n3 fatty acids at 7.3:1 or 4:1 produced the lowest 22:6n-3 percent in all three brain regions (Fig 3.28).

As in neuronal PE, 20:4n-6 content in neuronal PS attained its maximum earlier than the maximum 22:6n-3 was observed. Accretion of fatty acids in neuronal PS is age- and diet-dependent.

Phosphatidylinositol

Age: When fatty acids isolated from PI in neuronal cells of animals at various ages were examined, an effect of age was observed for all fatty acids ($p<0.001$) except 20:5n-3. Little change occurred in the relative percent of 22:6n-3 accrued in cerebellum and frontal neuronal PI (Fig 3.31). Maximum percent of 22:6n-3 was attained at six weeks in these two regions (Fig 3.31). In the hippocampus, 22:6n-3 decreased over time. The greatest percent of 22:6n-3 in the hippocampus was observed at birth. The greatest relative percent of 20:4n-6 occurred at two weeks of age in the cerebellar and frontal regions. In the hippocampus, this was observed at three weeks of age (data not shown).

Diet: All fatty acids in neuronal PI were affected by the diet treatment fed. The cerebellar and frontal regions accumulated high monoenes (Table 3.4), 16:0 (Fig 3.32), 18:2n-6 and 18:3n-3 and low 18:0 (Fig 3.33) when animals were fed the diet providing the n6/n3 ratio of 7.3:1. In comparisons between other diet treatments this diet produced a greater

20:5n-3 percent in the frontal and hippocampal regions. In the hippocampus accretion of monoenes (Table 3.4) and 16:0 and reduction or lack of increase in 18:0 occurred when feeding the diet supplying n6/n3 fatty acids at a ratio of 4:1. The 0.7%DHA diet also raised monoene and 16:0 percent in this region. Feeding the AA diet resulted in a lower level of 16:0 (Fig 3.32) in the frontal region at one and six weeks of age and in the hippocampus at six weeks of age and a lower 18:2n-6 (Table 3.4) and 18:3n-3 content in all brain regions examined. Maximum 18:0 (Fig 3.33) and 20:4n-6 (Table 3.4) was attained in the frontal and cerebellar regions when the AA diet or 0.7%DHA were fed. The DHA+AA diet or the 0.7%DHA diet also produced low levels of 18:2n-6 and increased 20:4n-6 (Table 3.4). These effects occurred primarily in the hippocampus. The DHA+AA and the 1%DHA diets produced higher 18:3n-3 levels in the hippocampus compared to the AA diet. The hippocampus of animals fed 1%DHA also exhibited high levels of 22:6n-3 (Fig 3.31). In the frontal and cerebellar regions a high 22:6n-3 content resulted from feeding the diet providing an n6/n3 ratio of 4:1 (Fig 3.31). In all three brain regions the 0.7%DHA diet produced the least amount of 22:6n-3 (Fig 3.31). Maximum level of 20:4n-6 was attained earlier than the maximum level of 22:6n-3 in the cerebellar and frontal regions only. In the hippocampus, the reverse was observed. It appears that many of the effects induced by changes in dietary fat affect the neuronal P[illegible] in the hippocampus differently from the frontal and cerebellar regions.

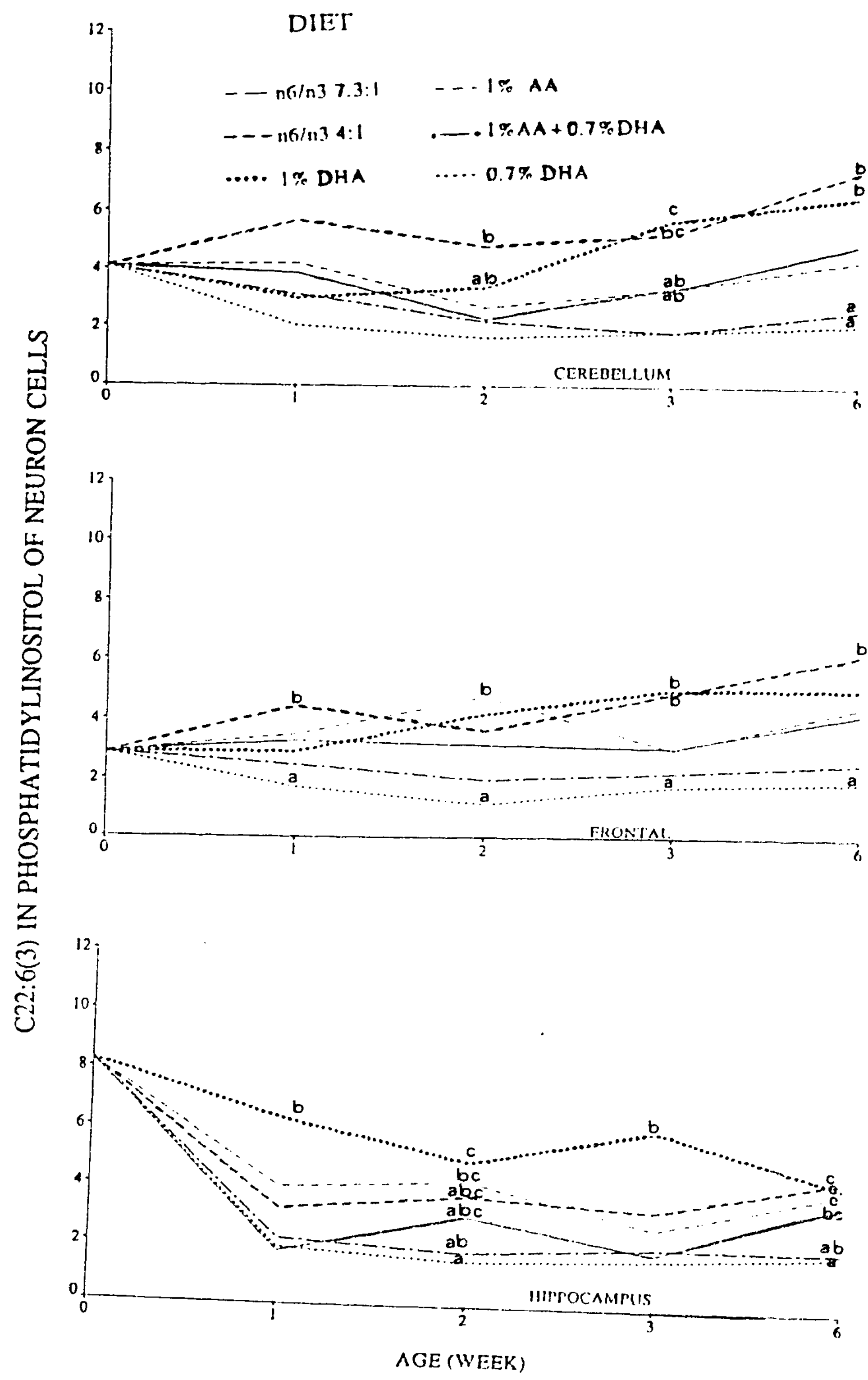


Figure 3.31. Effect of diet on % wt/wt 22:6(3) in neuronal PI. Values without a common letter within one age group differ significantly ($p<0.01$).

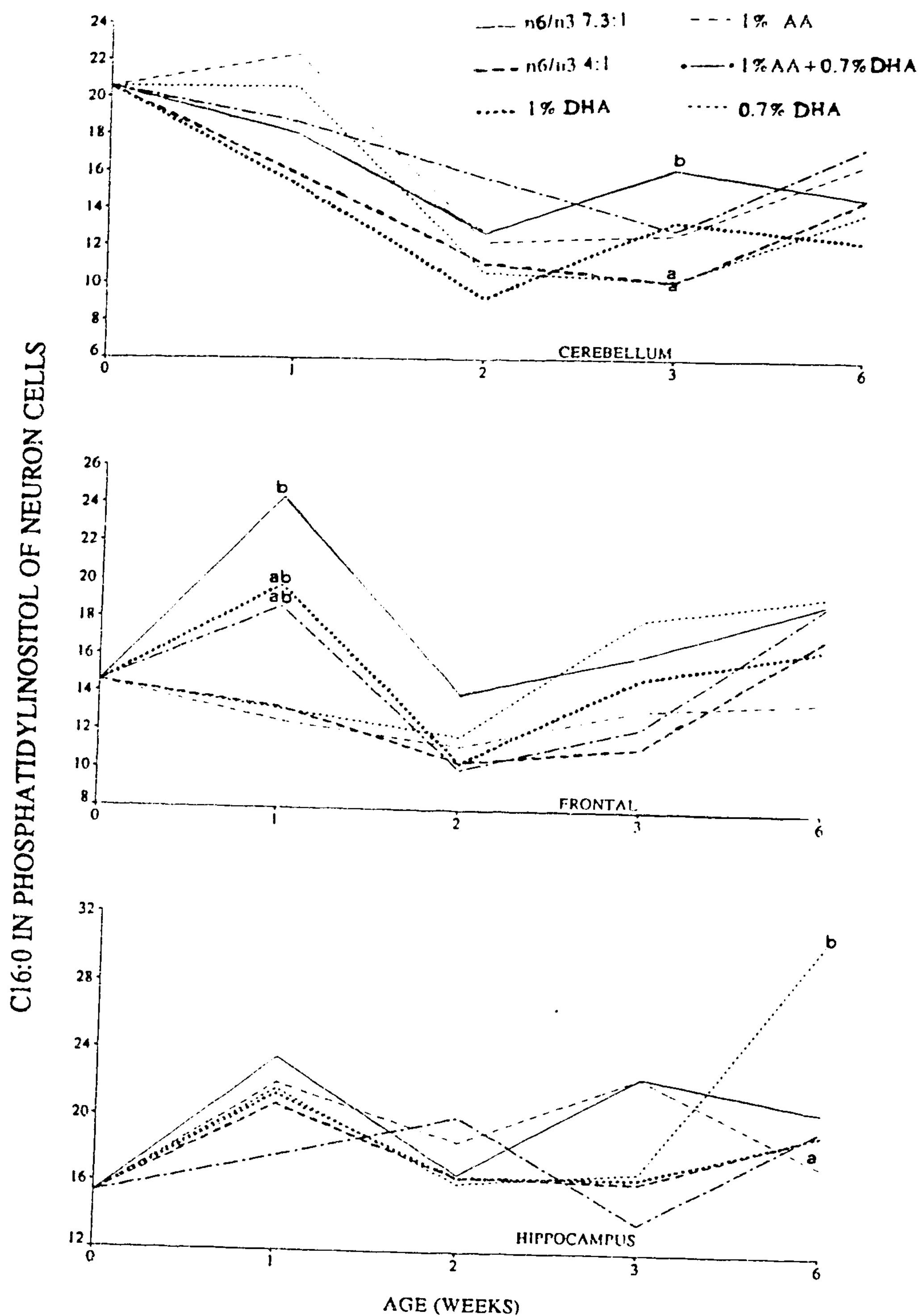


Figure 3.32. Effect of diet on % wt/wt 16:0 in neuronal PI. Values without a common letter within one age group differ significantly ($p<0.01$).

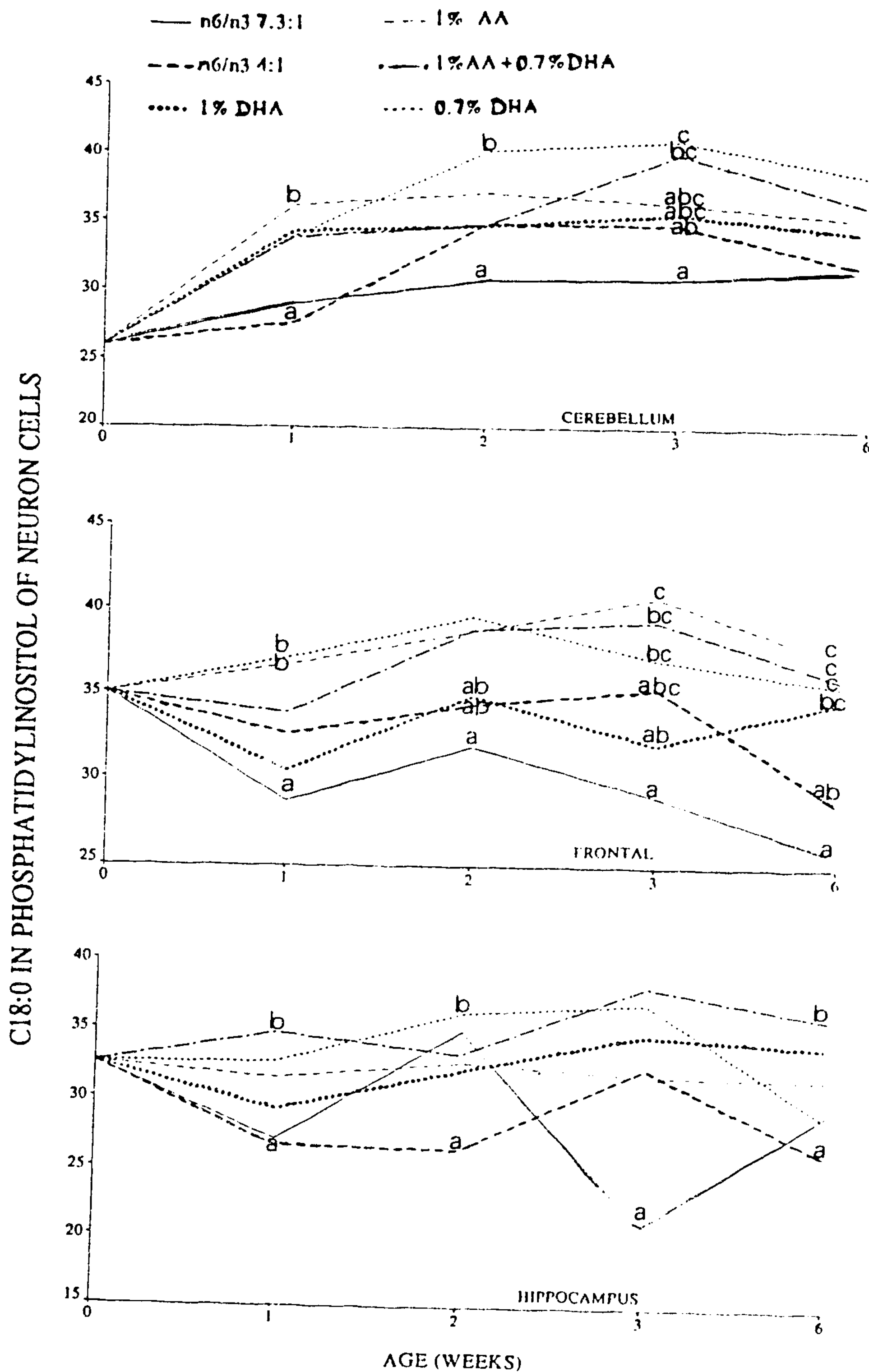


Figure 3.33. Effect of diet on % wt/wt 18:0 in neuronal PI. Values without a common letter within one age group differ significantly ($p<0.01$).

CHAPTER 4

DISCUSSION

Research on the effect of diet on brain development has generally been limited to undernutrition, malnutrition or essential fatty acid deficiency. Studies that have focused on altering n6/n3 fatty acid ratios have been limited to analyzing the brain as a whole or to considering the response at one time period only. Research in this area has not examined the effect of physiological changes in dietary fat content over time on the lipid composition of different brain regions or different cell types. To date, much of the research has predominantly examined the effects of diet on neuronal function. Much less is known about glial cell responsiveness to alterations in nutrient supply in the absence of malnutrition (Greenwood and Craig, 1987). Regional studies have focused primarily on the cerebrum, the brainstem or the cerebellum. Few studies have considered examining the hippocampus despite the relative ease of isolation and removal and the postnatal period for completion of development.

Brain phospholipid developmental patterns and its fatty acid composition can be influenced by numerous factors, particularly dietary alterations (Hargreaves and Clandinin, 1988 and 1987; Anding and Hwang, 1986; Sun and Foudin, 1985; Bourre, Pascal, Durand et al., 1984; Foot, Cruz and Clandinin, 1982). The present study demonstrates that physiological changes in dietary fat significantly alter the fatty acid composition of ethanolamine, choline, serine and inositol phosphoglycerides in neuronal and glial cells of three brain regions. More specifically, the temporal profile for the accretion of 20:4n-6 and 22:6n-3 varied between brain regions and cell types. Results from this study suggest the amounts and types of fatty acids in the diet affect accretion not only of long-chain polyunsaturated fatty acids (LCPUFA) but also of the saturates and monoenes. In this regard it is noteworthy that Zevenbergen and Houtsmuller (1989) suggested "the biosynthesis of PUFA only is not

sufficient to explain the complicated changes in fatty acid composition after feeding different dietary fats."

GROWTH CHARACTERISTICS

Body and brain weights observed in these experiments were comparable to those reported by Sinclair and Crawford (1972). No difference in brain weight between diet treatments was observed at any age. Thus it is concluded that feeding diets sufficient in essential fatty acids or supplying growing rats with preformed 20:4n-6 or 22:6n-3 did not affect body weight or total brain weight in neonatal pups. In a recent study by Leaf et al. (1992), correlations were made between circulating 20:4n-6 and 22:6n-3 and growth measurements in preterm infants. The infants in this study ranged from 24-36 weeks gestational age. Blood samples were obtained from the umbilical vein immediately after delivery of the placenta or from the infant on arrival at the neonatal unit. Thus, samples should be representative of intrauterine circulating levels of fatty acids. It has been documented that intrauterine accretion of fatty acids in human brain occurs during the last trimester (Clandinin et al., 1980 and 1981) during which time significant brain growth occurs. Leaf et al. also observed that the lower concentrations of LCPUFA were observed in very low birth weight infants and may be a factor of maternal circulation fatty acid levels, placental function or fetal metabolism. In human neonates, correlation may exist between circulating LCPUFA and growth measurements (Carlson et al., 1993; Crawford et al., 1989).

A difference in growth rate in the three regions examined indicates the timing of growth spurts varies in brain regions (Bayer, 1989; Rodier, 1980; Martinez and Ballabriga, 1978; and Das, 1977). It would seem logical that during the first week postnatal, the largest weight increase occurs in the frontal region. This brain region is responsible for basic movement and behavior (Kolb and Whishaw, 1985). During the second postnatal week in rat brain development, the cerebellum and frontal region

gain weight at equivalent rates. The cerebellum is involved in motor system function and perception (Kolb and Whishaw, 1985). Around this age movement of rat pups increases and the eyes begin to open (personal observation). Being the smallest of the three brain regions examined, the hippocampus did not gain weight to the same extent as the cerebellum or frontal region. The hippocampus continually gained weight over the ages examined. This may be the result of neurons originating gradually over a period of weeks rather than days as in most other regions (Bayer, 1989). The hippocampus plays a major role in attentional processes, memory and interaction with the environment (Kolb and Whishaw, 1985). Although weight gain slows with increasing age, the regions still exhibit a form of growth as cells migrate and differentiate within and between regions (Morgane et al., 1993). In this respect, weight gain should not be used as the sole indicator for development of the brain, growth spurts or vulnerable periods. The brain "growth" curves developed by Dobbing (1968; Dobbing and Sands, 1981) primarily represent rates of weight change over time for total brain and do not reflect many pre-brain periods of growth or the interrelationships of subregional growth (Morgane et al., 1993). Results from the present study suggest that timing in availability of fatty acids to developing brain cells and brain regions should be considered in addition to the development and growth of the brain as a whole.

AGE

Various brain regions contain unique phospholipid profiles that change during development (Sun and Foudin, 1985). The compositon of fatty acids in these lipids are also distinct and exhibit changes with age (Martinez and Ballabriga, 1987; Alling et al., 1974). In the present experiment, uptake of polyunsaturated fatty acids by rat brain occurred primarily during the pre-weaning period. This finding was consistent with that reported by Sinclair and Crawford (1972).

In both cell types, the fatty acids that consistently did not respond to changes in age included 20:5n-3 and the essential fatty acids, 18:2n-6 and 18:3n-3. The content of 20:5n-3 and 18:3n-3 in brain phosphoglycerides was negligible. The lack of deposition of 18:2n-6 detected has been reported previously by Mohrhauer and Holman (1963). Other studies have reported low levels of 18:3n-3 and 20:5n-3 (Bourre, Bonneil, Dumont et al., 1988; Carlson, Carver and House, 1986; Cook, 1982). The independent response to age may indicate that brain maintains minimal levels of these fatty acids which are attained at an early age. Amounts beyond these levels are desaturated and elongated to the more important long-chain derivatives, 20:4n-6 and 22:6n-3. Higher 22:6n-3 and lower 18:3n-3 in brain compared to 20:4n-6 and 18:2n-6 respectively, may imply a higher rate of conversion for 18:3n-3 compared to 18:2n-6 exists (Sprecher, 1989; Sanders and Rana, 1987; Brenner and Peluffo, 1966). Desaturation and elongation occurs in liver (Scott and Bazan, 1989) and in brain (Bourre et al., 1990; Cook, 1978) of neonatal rat pups. Desaturase activity has been reported to be age related (Ulmann et al., 1991; Bourre, Piciotti and Dumont, 1990). Some authors suggest desaturase activity in brain during initial postnatal brain growth is limited (Innis, 1992 ; Clandinin, Chappell, Leong et al., 1980; Sanders and Rana, 1987), particularly in premature infants (Koletzko, 1992). Bourre et al. (1990), specifically measured Δ6 desaturase activity in mice. However, these authors found the activity very high during early developent up to 21 days postnatal and questioned whether its activity after day 21 was sufficient for later brain development. In the present experiment, a large amount of 22:6n-3 was observed in brain during the preweaning period. In most comparisons, a large increase was observed at week one. An indication that some desaturation and elongation was occurring at one and two weeks of age was suggested by the large amount of 22:6n-3 observed in ethanolamine and inositol phosphoglycerides in the brain of rats fed the diet containing an n6/n3 ratio of 4:1. In this diet, no C20 or C22 fatty

acids were supplied. A recent study by Terracina et al (1992) reported that uptake of AA into brain was not affected by age. However, in the present study, incorporation of AA into brain lipids is clearly a function of age ($p<0.0001$), at least for animals up to six weeks of age.

DIET

Alteration in diet fat changed fatty acid composition of the postnatal developing brain. This has previously been observed (Yonekubo et al., 1993; Dyer and Greenwood, 1991; Hargreaves and Clandinin, 1988; Anderson and Connor, 1988; Connor, Neuringer and Lin, 1985; Galli, Trzeciak, and Paoletti, 1971). Generally the effects of diet appeared to produce larger changes in glial cells compared to neuronal cells. This may be a result of a large portion of neurogenesis being completed by birth (Balazs, Lewis and Patel, 1975) and the majority of gliogenesis and myelination occurring after birth (Morgane et al., 1993; Das, 1977). These differences may also result from the different mechanisms that exist between cell types to regulate uptake, activation and acylation of fatty acids into membranes lipids (Sprecher, 1991).

The changes in fatty acid composition observed from the diet treatments were not the same in all phosphoglycerides. Since the predominant fatty acids vary between phosphoglycerides (Sun and Foudin, 1985), supplementation of certain fatty acids will likely affect phosphoglycerides differently. The preferences of phospholipids for specific fatty acids, the location of these phospholipids (pools and domains) and transport mechanisms of fatty acids and phospholipids may play an important role (Zevenbergen and Houtsmuller, 1989). Changes were most pronounced in ethanolamine phosphoglycerides (EPG). Greater changes in EPG compared to other phosphoglycerides, were also observed by Alling et al (1974) and Selivonchick and Roots (1979). It has been suggested that in quantitative terms, ethanolamine phosphoglycerides are the most important unsaturated phospholipids of cell membranes (Martinez, 1989).

EPG also contains the highest percentage of LCPUFA (Mead, 1975).

The hypotheses and their results are as follows:

It was hypothesized that the diet containing an n6/n3 fatty acid ratio of 4:1 would reduce the amount of 20:4n-6 in phospholipids. This effect was apparent primarily in phosphatidylcholine in glia and in phosphatidylserine in both cell types, but only after two weeks of age. In most comparisons of phosphatidylethanolamine and phosphatidylcholine the 20:4n-6 content did not differ between animals fed the n6/n3 fatty acid ratio of 4:1 and animals fed the diet providing an n6/n3 ratio of 7.3:1. This would suggest that a lower n6/n3 ratio may than be required to reduce 20:4n-6 levels in brain since a similar n6/n3 ratio fed to piglets reduced 20:4n-6 in liver plasma phospholipids (Rioux and Innis, 1992). Thus, before enzyme sites are competitively blocked to inhibit conversion of 18:2n-6 in brain, the amount of dietary linolenate may need to be almost equal to or exceed the level of linoleate (Mohrhauer and Holman, 1963).

An increase in 20:4n-6 content in all phosphoglycerides was observed when animals were fed 1%AA. However, in choline-, ethanolamine-, and inositiol phosphoglyceride in animals fed 1%AA the level of 20:4n-6 did not differ from that observed in animals fed AA+DHA. This result partially verifies the third hypothesis that feeding AA+DHA will increase AA. Thus, supplementation of AA at 1% wt/wt is sufficient to increase AA levels in the developing brain. The addition of 0.7%DHA wt/wt did not interfere with the level of incorporation of 20:4n-6 and in most comparisons was just as effective at raising 20:4n-6 levels as feeding 1%AA.

Feeding the diet containing DHA+AA was hypothesized to increase levels of 20:4n-6 and 22:6n-3 in brain phospholipids. In phosphatidylethanolamine and phosphatidylinositol, the content of 20:4n-6 was raised by feeding DHA+AA but the level of 22:6n-3 was either reduced or exhibited little change. For most comparisons in phosphatidylcholine, both 20:4n-6

and 22:6n-3 levels increased. In glial phosphatidylserine, 20:4n-6 was reduced after one week of age. In neuronal phosphatidylserine, the level of 20:4n-6 peaked at two weeks of age and then decreased. The amount of 22:6n-3 increased in PS when animals were fed DHA+AA. A study by Martinez, Conde and Ballabriga (1974) examined the phosphoglyceride fatty acids during human brain development. These authors noted that in the earliest stages of life phosphatidylethanolamine and phosphatidylcholine are the dominant phosphoglycerides and as far as polyunsaturated fatty acids are concerned the n-6 series predominated in the youngest brains, especially in phosphatidylethanolamine. The polyunsaturates of the n-3 series increased with increasing age. If a similar pattern of development for lipids exists in developing rat brain, this may explain why only 20:4n-6 increased in phosphatidylethanolamine when animals were fed DHA+AA.

The brain possesses the necessary pathways to convert 18:3n-3 to 22:6n-3 (Cook, 1978; Dhopeshwarkar and Subramanian, 1976). When animals were supplemented with 18:3n-3, the level of 22:6n-3 increased significantly but only in ethanolamine and inositol phosphoglycerides. It appears however, that accretion of 22:6n-3 in all phosphoglycerides is better supported when DHA is supplied directly in the diet. This result is in agreement with that reported previously by Sinclair (1975) and Anderson, Connor and Corliss (1990) and verifies the hypothesis that feeding preformed DHA will increase 22:6n-3 in brain phospholipids.

Although the final hypothesis expected that feeding 0.7%DHA would increase 22:6n-3 with no change in 20:4n-6, this was not observed. In PE, feeding 0.7%DHA increased both DHA and AA. This implies that supplying DHA at 0.7% wt/wt is sufficient to increase the DHA content in phosphatidylethanolamine yet it is not enough to cause inhibition of the conversion of 18:2n-6 to 20:4n-6. The result of feeding 0.7%DHA on brain phophatidylcholine was to increase DHA in the cerebellum and frontal region but only after weaning. The AA level produced in brain PC of

animals fed 0.7%DHA did not differ compared to other diet treatments. Feeding 0.7%DHA resulted in a decrease in the level of 20:4n-6 and 22:6n-3 in phosphatidylserine. In phosphatidylinositol the 20:4n-6 content increased while the level of 22:6n-3 was reduced. Thus, the effect of feeding 0.7%DHA on the fatty acid composition of the phospholipid classes varied remarkably but even this small dietary change altered the composition. A highly positive correlation between AA and DHA accretion was determined for choline-, ethanolamine- and serine- phosphoglycerides using Pearson's correlation coefficient ($p<0.0004$). This relationship between AA and DHA may explain why such a large increase in 22:6n-3 level was observed in glial PC of the frontal region in animals fed AA. An increase in 20:4n-6 was also observed in animals fed AA. This observation may be explained by the positive relationship between 20:4n-6 and 22:6n-3. A negative correlation was observed between AA and DHA in phosphatidylinositol. It may be important to recall that one of the fatty acids observed in large quantities in phosphatidylinositol is 20:4n-6. Thus, this lipid class is more likely to pool 20:4n-6 than 22:6n-3.

This study focused on comparing the effects of varying the n6/n3 fatty acid ratio with or without AA or DHA on brain fatty acid composition and development. Presently, infant formulae do not incorporate essential fatty acids of chain lengths longer than 18 carbons. It has been suggested that the fat composition of infant formulas should provide arachidonic acid and docosahexaenoic acid. This study indicates that physiological changes in diet fat composition significantly alter the fatty acid content of the developing rat brain. Accretion of docosahexaenoic acid in phosphoglycerides of brain cells is better supported when DHA is supplied directly in the diet. Accretion of arachidonic acid in all phosphoglycerides, except glial phosphatidylserine, was also better supported when AA was supplied in the diet.

It has been established that the timing of development differs between

the brain cells. The majority of neurogenesis is complete prior to completion of gliogenesis. Research has also established that the temporal development of the brains regions is different. The cerebellum and hippocampus are among two of the regions to complete development postnatally. The effect of physiological changes in dietary fat on fatty acid composition of cell types and regions was made apparent in this study. In most comparisons, glial cells appeared to be affected more than neuronal cells. The degree to which these changes occurred in the regions appeared to be affected by the diet treatment fed. The functional implications of these changes are as yet, unknown.

REFERENCES

ALBERS R.W. Coordination of brain metabolism with function during development. In: Developmental Neurochemistry. R.C. Wiggins, D.W. McCandless and S.J. Enna (eds). University of Texas Press, 1985. pp 180-192.

ALLING C., Bruce A., Karlsson I., and Svennerholm L. The effects of different dietary levels of essential fatty acids on lipids of rat cerebrum during maturation. J. Neurochem. 1974; 23:1263-1270.

ANDERSON G.J., Connor W.E., and Corliss J.D. Docosahexaenoic acid is the preferred dietary w-3 fatty acid for development of the brain and retina. Pediatr. Res. 1990; 27:89-97.

ANDING R.A., and Hwang D.H. Effects of dietary linolenate on the fatty acid composition of brain lipids in rats. Lipids. 1986;21:697-701.

ANONYMOUS. Combined EFA deficiency in a patient on long term TPN. Nutr. Rev. 1986; 44:301-305.

BALAZS P., Lewis P.D. and Patel A.J. Effects of metabolic factors in brain development. In: Growth and Development of the Brain. M.A. Brazier (ed). Raven Press, New York, 1975. pp 83-115.

BAUMANN N.A., Bourre J.M., Jacque C., and Pallet S. Genetic disorders of myelination. In: Lipids, Malnutrition and the Developing Brain. Ciba Foundation Symposium. pp. 91-105. Elsevier, Amsterdam, 1972.

BAYER S. Cellular aspects of brain development. Neurotoxicology. 1989; 10:307-320.

BAZAN H.E.P., Ridenour B., Birkle D.L., and Bazan N.G. Unique metabolic features of docosahexaenate metabolism related to functional roles in the brain and retina. In: Phospholipid Research and the Nervous System. L.A. Horrocks, L. Freysz and G. Toffano (eds). Liviana Press, 1986. pp.67-78.

BAZAN N.G. Supply of polyunsaturated fatty acids and their significance in the central nervous system. In: Nutrition and the Brain. vol.8. R.J.Wurtman and J.J.Wurtman (eds). Raven Press Ltd., New York, 1990.

BERNSOHN J., and Cohen S.R. Polyenoic fatty acid metabolism of phosphoglycerides in the developing brain. In: Lipids, Malnutrition and the Developing Brain. Ciba Foundation Symposium. Elsevier, Amsterdam, 1972. pp.159-178.

BJERVE K.S., Fisher S., and Alme K. Alpha-linolenic acid deficiency in man: effect of ethyl linoleate and erythrocyte fatty acid composition and biosynthesis of prostanoids. Am. J. Clin. Nutr. 1987; 46:570-576.

BOURRE J.M., Pascal G., Durand G., Masson M., Dumont O., and Piciotti M. Alterations in fatty acid composition of rat brain cells (neurons, astrocytes, and oligodendrocytes) and of subcellular fractions (myelin and synaptosomes) induced by diet devoid of n-3 fatty acids. J. Neurochem. 1984; 43:342-348.

BOURRE J.M., Bonneil M., Dumont O., Piciotti M., Nalbone G., and Lafont H. High dietary fish oil alters the brain polyunsaturated fatty acid composition. Biochim. Biophys. Acta 1988; 960:458-461.

BOURRE J.M., Francois M., Youyou A., Dumont O., Piciotti M., Pascal G., and Durand G. 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; 119:1880-1892.

BOURRE J.M., Durand G., Pascal G., and Youyou A. Brain cell and tissue recovery in rats made deficient in n-3 fatty acids by alteration of dietary fat. J. Nutr. 1989; 119:15-22.

BOURRE J.M., Bonneil M., Dumont O., Piciotti M., Calaf R., Portugal H., Nalbone G., and Lafont H. Effect of increasing amounts of dietary fish oil on brain and liver fatty composition. Biochim. Biophys Acta. 1990; 1043:149-152.

BOURRE J.M., Dumont O., PiciottiM., Pascal G., and Durand G. Control of brain fatty acids. Upsala J of Med Sci. 1990; Suppl.48:109-131.

BOURRE J.M., Piciotti M., Dumont O., Pascal G., and Durand G. Dietary linolenic acid and polyunsaturated fatty acids in rat brain and other organs. Minimal requirements of linoleic acid. Lipids. 1990; 25:465-472.

BOURRE J.M., Bonneil M., Clement M., Dumont O., Durand G., Lafont H., Nalbone G., and Piciotti M. Function of dietary polyunsaturated fatty acids in the nervous system. Prostaglandins, Leukotrienes, and Essential Fatty Acids. 1993; 48:5-15.

BRADFORD P.G., Marinetti G.V. and Abood L.G. Stimulation of phospholipase A2 and secretion of catecholamines from brain synaptosomes by potassium and A23187. J. Neurochem. 1983;41:1684-1693.

BRENNER R.R. Nutritional and hormonal factors influencing desaturation of essential fatty acids. Prog. Lipid Res. 1981; 20:41-47.

BRENNER R.R. Effect of unsaturated acids on membrane structure and enzyme kinetics. Prog. Lipid Res. 1984;23:69-96.

BURR G.O. and Burr M.M. A new deficiency disease produced by the right exclusion of fat from the diet. J. Biol. Chem. 1929; 82:345-367.

CARLSON S.E., Carver J.D., and House S.G. High fat diets varying in ratios of polyunsaturated to saturated fatty acids and linoleic to linolenic acid: A comparison of rat neural and red cell membrane phospholipids. J. Nutr. 1986; 116:718-725.

CARLSON S.E., Werkman S.H., Rhodes P.G., and Tolley E.A. Visual-acuity development in healthy preterm infants: effect of marine-oil supplementation. Am. J. Clin. Nutr. 1993; 58: 35-42.

CHAPMAN D. The role of fatty acids in myelin and other important brain structures. In:Lipids, Malnutrition and the Developing Brain, Ciba Foundation Symposium, Elsevier, Amsterdam, 1972.

CHAPPELL J.E., Clandinin M.T., Kearny-Volpe C., et al., Fatty acid balance studies in premature infants fed human milk or formula. Effect of calcium supplementation. J. Pediat. 1986;108:439-447.

CLANDININ M.T., Chappell J.E., Leong S., Heim T., Swyer P.R., and Chance G.W. Intrauterine fatty acid accretion rates in the human brain: implications for fatty acid requirements. Early Human Development. 1980a; 4/2:121-129.

CLANDININ M.T., Chappell J.E., Leong S., Heim T., Swyer P.R., and Chance G.W. Extrauterine fatty acid accretion in infant brain: implications for fatty acid requirements. Early Human Development. 1980b; 4/2:131-138.

CLANDININ M.T., Chappell J.E., Heim T., Swyer P.R., and Chance G.W. Fatty acid utilization in perinatal de novo synthesis of tissues. Early Human Development. 1981;5:355-366.

CLANDININ M.T., Chappell J.E., and Heim T. Do low weight infants require nutrition with chain elongation-desaturation products of essential fatty acids? Prog. Lipid Res. 1982; 29:901-904.

CLANDININ M.T., Chappell J.E., and Van Aerde J.E.E. Requirements of newborn infants for long chain polyunsaturated fatty acids. Acta Paediatr. Scand. 1989; Suppl.351:63-71.

CLAUSEN J., and Moller J. Allergic encephalomyelitis induced by brain antigen after deficiency in polyunsaturated fatty acid during myelination. Acta neurol. Scand. 1967; 43:375-388.

CONNOR W.E., and Neuringer M. Importance of dietary n-3 fatty acids in retinal and brain chemistry. In: Nutritional Modulation of Neural Function. J.E. Morley, M.M. Sterman, and J.H. Walsh (eds). Academic Press Inc., San Diego, 1988. pp. 191-201.

CONNOR W.E., Neuringer M., and Lin D. The incorporation of docosahexaenoic acid into the brain of monkeys deficient in w-3 essential fatty acids. J. Clin. Invest. 1984; 73:272-276.

COOK H.W. In Vitro formation of polyunsaturated fatty acids by desaturation in rat brain: Some properties of the enzymes in developing brain and comparisons with liver. J. Neurochem. 1978; 30:1327-1334.

COOK H.W. Chain elongation in the formation of PUFA by brain: Some properties of the microsomal system. Arch Biocem Biophys. 1982; 214:695-704.

CRAWFORD M.A., Hassam A.G., Williams G., and Whitehouse W. Essential fatty acids and fetal brain growth. Lancet. 1976;I:452-453.

CRAWFORD M.A., Casperd N.M., and Sinclair A. The long-chain metabolites of linoleic and linolenic acids in liver and brain in herbivores and carnivores. Comp. Biochem. Physiol. 1976;54B: 395-401.

CRAWFORD M.A., and Sinclair A.J. Nutritional influences in the evolution of the mammalian brain. In: Lipids, Malnutrition, and the Developing Brain. Ciba Foundation Symposium. Elsevier, Amsterdam, 1972. pp.267-292.

DAS G.D. Gliogenesis during embryonic development in the rat. Experientia. 1977; 33:1648-1649.

DAVISON A.N. Biosynthesis of the myelin sheath. In: Lipids, Malnutrition, and the Developing Brain. Ciba Foundation Symposium. Elsevier, Amsterdam, 1972. pp.73-90.

DOBBING J. In: Lipids, Malnutrition and the Developing Brain. Ciba Foundation Symposium. Elsevier, Amsterdam, 1972.

DOBBING J. and Sands J. Timing of neuroblast multiplication in developing human brain. Nature. 1970; 226:639-640.

DOBBING J. and Sands J. Comparative aspects of the brain growth spurt. Early Human Development. 1979;3:79-83.

DOBBING J. Early nutrition and later achievement. Proc. Nutr. Soc. 1990;49:103-118.

DYER J.R. and Greenwood C.E. Neural 22-carbon fatty acids in the weanling rat respond rapidly and specifically to a range of dietary linoleic and α-linolenic fatty acid ratios. J. Neurochem. 1991; 56:1921-1931.

ENSLEN M., Milon H., and Malnoe A. Effect of low intake of n-3 fatty acids during development on brain phospholipid fatty acid composition and exploratory behavior in rats. Lipids. 1991; 26:203-208.

FAO/WHO. Dietary fats and oils in human nutrition: report of and expert consultation. Rome, 1977.

FARRELL P.M., Gutcher G.R., Palta M., and DeMets D. Essential fatty acid deficiency in premature infants. Am J. Clin. Nutr. 1988; 48:220-229.

FELDMAN M., Van Aerde J.E.E., and Clandinin M.T. Lipid accretion in the fetus and newborn. In: Fetal and Neonatal Physiology. R.A. Polin and W.W. Fox (eds). W.B. Saunders, Toronto. Vol.1. 1992. pp.299-314.

FRIEDMAN Z. Essential fatty acid consideration at birth in the premature neonate and the specific requirement for preformed prostaglandin precursors in the infant. Prog. Lipid Res. 1986; 25:355-364.

FRIEDMAN Z. Essential fatty acids revisited. Am J. Dis Child. 1980;134:397-408.

Food and Drug Regulations-Amendment (Schedule No. 781), Department of National Health and Welfare, Canada Gazette Part I, February 9, 1991.

FOOT M., Cruz T.F., and Clandinin M.T. Influence of dietary fat on the lipid composition of rat brain synaptosomal and microsomal membranes. Biochem. J. 1982; 208:631-641.

FOOT M., Cruz T.F., and Clandinin M.T. Effect of dietary lipid on synaptosomal acetylcholinesterase activity. Biochem J. 1983; 211:507-509.

GARG ML, Keelan M., Thomson A.B.R., and Clandinin M.T. Fatty acid desaturation in intestinal mucosa. Biochim. Biophys. Acta 1988; 958:139-141.

GOTTLIEB A., Keyder I., and Epstein H.T. Rodent brain growth stages: an analytical review. Biol. Neonate. 1977;32:166-176.

HANNAH J., and Campagnoni A.T. Effects of essential fatty acid deficiency on mouse brain development. Dev. Neurosci. 1987; 9:120-127.

HANSEN I.B., and Clausen J. The fatty acids in the human foetal brain. Scand. J. Clin. Lab Invest. 1968; 22:231-238.

HARGREAVES K.M., and Clandinin M.T. Phosphatidylethanolamine methyltransferase: evidence for influence of diet fat on selectivity of substrate for methylation in rat brain synaptic plasma membranes. Biochim. Biophys. Acta 1987: 918:97-105.

HARGREAVES K.M., and Clandinin M.T. Dietary control of diacyl phosphatidylethanolamine species in brain. MS #RPC 15271-Revision- May 5, 1988.

HARGREAVES K.M., and Clandinin M.T. Dietary lipids in relation to postnatal development of the brain. Upsala J. Med. Sci. 1990; Suppl. 48:79-95.

HARLOW E., and Lane D. Immunoblotting. In: Antibodies. A laboratory manual. Cold Spring Harbor Laboratory, U.S. 1988.

HEALTH AND WELFARE CANADA. Nutrition Recommendations. 1990.

HEDQVIST P. In: The Prostaglandins. P.W.Ramwell (ed) Plenum Press, New York. 1973. pp101-129.

HEIRD W.C., and Cooper A. Nutrition in infants and children. In: Modern Nutrition in Health and Disease. M.E. Shils and V.R. Young (eds). Lea and Febiger, Philadelphia, 1988.

HERSCHKOWITZ N. Brain development in the fetus, neonate and infant. Biol Neonate. 1988;54:1-19.

HERSCHKOWITZ N. Brain development and nutrition. In: Developmental Neurobiology. P. Evrard and A. Minkowski (eds). Nestle Nutrition Workshop Series, Raven Press, New York, 1989. pp.297-304.

HEUTHER G. Malnutrition and developing synaptic transmitter systems: lasting effects, functional implications. In: (Mal)Nutrition and the Infant Brain. N.M. vanGelder, R.F. Butterworth and B.D. Drujan (eds). Wiley-Liss New York. 1990. pp.141-156.

HOLMAN R.T. The ratio of trienoic:tetraenoic acids in tissue lipids as a measure of essential fatty acid requirement. J.Nutr. 1960; 70:405-417.

HOLMAN R.T. Nutritional and metabolic interrelationships between fatty acids. Fed Proc. 1964;23:1062-1067.

HOLMAN R.T. Essential fatty acid deficiency. In: Progress in the Chemistry of Fats and Other Lipids. R.T. Holman (ed). Pergamon Press, Oxford, England. 1968.

HOLMAN R.T., Johnson S.B., and Hatch T.F. A case of human linolenic acid deficiency involving neurological abnormalities. Am. J. Clin. Nutr. 1982; 35:617-623.

HOUTSMULLER U.M.T. Differentiation in the biological activity of polyunsaturated fatty acids. In: Dietary Lipids and Postnatal Development. Raven Press, New York. 1973. pp.145-155.

INNIS S. Effects of current infant formula on brain and retinal lipid development. Effect of formula with 2% 18:3n-3 on n-6 and n-3 fatty acid status of low birthweight infants. In: Essential Fatty Acids and Infant Nutrition. J.Ghisolfi and G. Putet (eds). John Libbey Eurotext, Paris, 1992. pp31-43.

KING K.C., Adam P.A.J., Laskowski D.E., and Schwartz R. Sources of fatty acid in the newborn. Pediatrics. 1971; 47:192-198.

KOLB B., and Whishaw I.Q. Fundamentals of Human Neuropsychology. W.H. Freeman and Co., New York, 1985.

KOLETZKO B. Long-chain polyunsaturated fatty acids in the diets of premature infants. In: Polyunsaturated Fatty Acids in Human Nutrition. Workshop series, vol.28, Nestle Ltd, Raven Press, New York, 1992. pp 135-146.

KUHN D.C., and Crawford M. Placental essential fatty acid transport and prostaglandin synthesis. Prog. Lipid Res. 1986; 25:345-353.

LAEMMLI U.K. Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. Nature 1970; 229: 680-685.

LAMPTEY M.S., and Walker B.L. A possible essential role for dietary linolenic acid in the development of the young rat. J. Nutr. 1976;106:86-93.

LEAF A., and Weber P.C. Cardiovascular effects of n-3 fatty acids. N. Engl.J. Med. 1988;318 :549-547.

LEAF A.A., Leighfield M.J., Co-teloe K.L., and Crawford M.A. Long chain polyunsaturated fatty acids and fetal growth. Early Human Development. 1992;30.183-191.

LEPROHON-GREENWOOD C.E., and Anderson H.G. An overview of the mechanisms by which diet affects brain function. Food Technology 1986; :132-138.

LINDGREN J.A., Hulting A., Dahlen S.E., Hokfelt T., Werner S., and Samuelsson . Evidence for the occurrence of leukotrienes (LT) in the central nervous system and for a neuroendocrine role of LT. Soc. Neurosci. Abs. 1984; 10:1129.

LINSCHEER W.G., and Vergroesen A.J. Lipids. In:Nutrition in Health and Disease. M.E. Shils and V.E. Young (eds). Lea and Febiger, Philadelphia, 1988.

LOWRY O.H., Rosebrough W.J., Farr A.L., and Randall R.J. Protein measurement with Folin phenol reagent. J. Biol Chem 1957;93:265-275.

MACHLIN L.J. Vitamin E. In: Handbook of Vitamins: Nutritional, Biochemical and Clinical Aspects. L.J. Machlin (ed). Marcel Dekker Inc. New York, 1984.

MARTINEZ M., and Ballabriga A. Effects of parenteral nutrition withe high doses of linoleate on the developing human liver and brain. Lipids. 1987; 22:133-138.

MARTINEZ M. Dietary polyunsaturated fatty acids in relation to neural development. In: Dietary N-3 and N-6 Fatty Acids: Biological Effects and Nutritional Essentiality. C.Galli and A.P. Simopoulos (eds). Plenum Press, New York, 1989. pp123-133.

MARTINS F.M., Wennberg A., Meurling S., Kihlberg R., and Lindmark L. Serum lipid and fatty acid composition of tissues in rats on total parenteral nutrition (TPN). Lipids. 1984; 19:728-737.

McCORMICK J.H., Neill W.A., Sim A.K. Immunosuppressive effect of linoleic acid. Lancet. 1977;2:508.

MEAD J.F. Lipid composition and brain function. In: The Essential Fatty Acids. Proceedings of the Miles Symposium '75. The Nutrition Society of Canada. .V.Hawkins (ed). Miles Laboratories Ltd. pp73-83.

MEAD J.F., and Dhopeshwarkar G.A. Types of fatty acids in brain lipids, their derivation and function. In: Lipids, Malnutrition and the Developing Brain. Ciba Foundation Symposium. Elsevier, Amsterdam, 1972. pp. 59-72.

MENON N.K., and Dhopeshwarkar G.A. Essential fatty acid deficiency and brain development. Prog. Lipid Res. 1982; 21:309-326.

MOHRHAUER H., and Holman R.T. Alteration of the fatty acid composition of brain lipids by varying levels of dietary essential fatty acids. J. Neurochem. 1963: 10:523-530.

MOORE S.A., Yoder E., and Spector A.A. Role of the blood-brain barrier in the formation of long chain w-3 and w-6 fatty acids from essential fatty acid precursors. J. Neurochem. 1990; 55:391-402.

MORGANE P.J., Austin-LaFrance R., Bronzino J., Tonkiss J., Diaz-Cintra S., Cintra L., Kemper T., and Galler J.R. Prenatal malnutrition and development of the brain. Neurosci and Biobehav. Rev. 1993; 17:91-128.

MORRISON W.R. and Smith L.M. Preparation of fatty acid methyl esters and dimethyl acetals from lipids with boron-trifluoride -methanol. J.Lipid Res., 1964;5:600-608.

NEEDLEMAN P., Raz A., Minkes M.S., Ferrendelli J.A. and Sprecher H. Triene prostaglandins: Prostacyclin and thromboxane biosynthesis and unique biological properties. Proc. Natl. Acad. Sci. 1979;76:944-948.

NEURINGER M., and Connor W.E. n-3 fatty acids in the brain and retina; evidence for their essentiality. Nutr. Rev. 1986;44:285-294.

NEURINGER M., Connor W.E., Lin D.S., Barstad L., and Luck S. Biochemical and functional effects of prenatal and postnatal w-3 fatty acid deficiency on retina and brain in rhesus monkeys. Proc. Natl. Acad. Sci. 1986; 83:4021-4025.

NEURINGER M., Connor W.E., Van Petten C., and Barstad L. Dietary omega-3 fatty acid deficiency and visual loss in infant rhesus monkeys. J. Clin. Invest. 1984; 73:272-276.

PALMER M.R., Mathews W.R., Hoffer B.J. and Murphy R.C. Electro-physiological response of cerebellar Purkinje neurons to leukotriene B4 and D4. J. Pharmacol Exp Ther. 1981; 219:91-96.

PAOLETTI R., and Galli C. Effects of essential fatty acid deficiency on the CNS in the growing rat. In: Lipids, Malnutrition and the Developing Brain. Ciba Foundation Symposium. Elsevier, Amsterdam, 1972. pp.121-140.

PASSWELL J.H., David R., Katznelson D., and Cohen B.E. Pigment deposition in the reticuloendothelial system after fat emulsion infusion. Arch. Dis. Child. 1976;51:366-367.

PASTERNAK C.A. Lipid changes in membranes during growth and development. In: Lipid Metabolism in Mammals. F. Snyder (ed). Plenum Press, New York. 1977.

PIERSE P., Van Aerde J.A., and Clandinin M.T. Nutritional value of human milk. Progress in Food and Nutrition Sci. 1988; 12:421-447.

POISSONNET C.M., LaVelle M., and Burdi A.R. Growth and development of adipose tissue. J. Pediat. 1988;113:1-9.

PURVIS J.M., Clandinin M.T., and Hacker R.R. Chain elongation-desaturation of linoleic acid during the development of the pig. Implications for the supply of polyenoic fatty acids to the developing brain. Comp. Biochem. Physiol. 1983; 75B:199-204.

RATHBONE L. The effect of diet on the fatty acid composition of serum brain, brain mitochondria and myelin in the rat. Biochem. J. 1965; 97:620-628.

RIOUX F.M., and Innis S.M. Arachidonic acid concentrations in plasma and liver phospholipid and cholesterol esters of piglets raised on formulas with different linoleic and linolenic acid contents. Am J Clin Nutr. 1992;56:106-112.

RIVERS J.P.W., Sinclair A.J., and Crawford M.A. Inability of the cat to desaturate essential fatty acids. Nature. 1975; 258:171 -173.

ROBERTSON A.F. and Sprecher H. A review of human placental lipid metabolism and transport. Acta Paediatr. Scand. 1968;183(Suppl):2-18.

ROBERTSON A.F., Sprecher H., and Wilcox J. Free fatty acid patterns of human maternal plasma, perfused placenta and umbilical cord plasma. Nature. 1968;217:378-379.

RODIER P.M. Chronology of Neuron development: animal studies and their clinical implications. Develop. Med. Child.Neurol. 1980; 22:525-545.

ROSENTHAL M.D. Fatty acid metabolism of isolated mammalian cells. Prog. Lipid Res. 1987; 26:87-124.

ROUSER G., Kritchevsky G., and Yamamoto A. Column chromatographic and associated procedures for separation and determination of phosphatides and glycolipids. In: Lipid Chromatographic Analysis. G.V. Marinetti (ed) Marcel Dekker Inc., New York, 1976.

SANDERS T.A.B. and Rana S.K. Comparison of the metabolism of linoleic and linolenic acids in the fetal rat. Ann. Nutr. Metab. 1987;31:349-353.

SARDA N., Gharib A., Croset M., Moliere P., and Lagarde M. Fatty acid composition of the rat pineal gland. Dietary modification. Biochim. Biophys. Acta. 1991; 1081:75-78.

SAUNDERS T.A.B., and Younger K.M. The effects of dietary supplements on the fatty acid composition of platelets and plasma choline phospholipids. Br. J. Nutr. 1981; 45:613-616.

SENTERRE J. Nutrition of premature babies. In: Feeding the Sick Infant. L. Stern (ed). Nestle Nutrition Workshop Series. Raven Press, New York, 1987. pp.191-197.

SCOTT B.L., and Bazan N.G. Membrane docosahexaenoate is supplied to the developing brain and retina by the liver. Proc. Natl. Acad. Sci. 1989;86:2903-2907.

SCOTT B., Lew J., Clandinin M.T., and Cinader B. Dietary fat influences electric membrane properties of neurons in cell culture. Cell Molec. Neurobiol. 1989;9:105-113.

SELIVONCHICK D.P. and Roots B.I. Isolated brain capillary endothelia: Influence of various levels of essential fatty acids on the acyl group composition of glycerophospholipids. Lipids. 1979; 14:66-69.

SELLINGER O.Z., and Azcurra J.M. Bulk separation of neuronal cell bodies and glial cells in the absence of added digestive enzymes. In: Research Methods in Neurochemistry. N. Marks and R. Rodnight (eds). Plenum Press, New York, 1974. pp3-38.

SIMOPOULUS A. Omega-3 fatty acids in health and disease and in growth and development. Am. J. Clin. Nutr. 1991;54:438-463.

SINCLAIR A.J. and Crawford M.A. The accumulation of arachidonate and docosahexaenoate in the developing rat brain. J. Neurochem. 1972;19:1753-1758.

SINCLAIR A.J. Incorporation of radioactive polyunsaturated fatty acids into liver and brain of developing rat. Lipids. 1975;10:175-184.

SMITH S., and Abraham S. Fatty acid synthesis in th developing mouse liver. Arch. Bioch. Biophy. 1970; 136:112-121.

SPECTOR A.A., and Yorek M.A. Membrane lipid composition and cellular function. J. Lipid Res. 1985; 26:1015-1035.

SPRECHER H. In: Dietary n-3 and n-6 Fatty Acids: Biological Effects and Nutritional Essentiality. C. Galli and A.P. Simopoulos (eds). Plenum Press, New York, 1989. pp69-79.

STEEL R.G.D., and Torrie J.H. Principals and procedures of statistics. Second Edition, McGraw-Hill Book Co. Inc., New York, 1970, chps 8 and 9.

STUBBS C.D., and Smith A.D. The modification of the mammalian membrane polyunsaturated fatty acid composition in relation to membrane fluidity and function. Biochim. Biophys. Acta. 1984; 79:89-137.

SUN G.Y., and Foudin L.L. In: Phospholipids in Nervous Tissue. J. Eichberg (ed). Wiley and Sons, New York, 1985 pp79-134.

SVENNERHOLM L., Alling C., Bruce A., Karlsson I., and Sapia O. Effects on offspring of maternal malnutrition in the rat. In: Lipids, Malnutrition and the Developing Brain. Ciba Foundation Symposium. Elsevier, Amsterdam, 1972. pp. 141-157.

TERRACINA L., Brunetti M., Avellini L., Evalina DeMedio G., Trovarelli G., and Gaiti A. Arachidonic and palmitic acid utilization in aged rat brain areas. Molec. Cell. Biochem. 1992; 115:35-42.

TINOCO J. Dietary requirements and functions of α-linolenic acid in animals. Prog. Lipid Res. 1982; 21:1-45.

THOMSON A.B.R., Keelan M., Garg M.L. and Clandinin M.T. Intestinal aspects of lipid absorption: in review. Can.J.Physiol.Pharm. 1989;67:179-191.

UAUY R., Treen M., and Hoffman D.R. Essential fatty acid metabolism and requirements during development. Seminars in Perinatology. 1989; 13:118-130.

UAUY R.D., Birch D., Birch E., and Hoffman D.R. Effect of dietary w3 fatty acids on eye and brain development in very-low-birth- weight neonates. In: Health Effect of W3 Polyunsaturated Fatty Acids in Seafoods. A.P. Simopoulos, R.R. Kifer, R.E. Martin and S.M.Barlow (eds). Karger, Switzerland. 1991. World Rev Nutr Diet. pp.506-537 .

UAUY R., Birch E., Birch D., and Peirano P. Visual and brain function measurements in studies of n-3 fatty acid requirements of infants. J. Pediatr. 1992; 120:77-90.

ULMANN L., Blond J.P., Maniongui C., Poisson J.P., Durand G., Bezard J., and Pascal G. Effects of age and dietary essential fatty acids on desaturase activities and on fatty acid composition of liver microsomal phospholipids of adult rats. Lipids. 1991; 26:127-133.

VOSS A., Reinhart M., Sankarappa S., and Sprecher H. The metabolism of 7,10,13,16,19-Docosapentaenoic acid to 4,7,10,13,16,19,-Docosahexaenoic acid in rat liver is independent of a 4-desaturase. J. Biol. Chem. 1991;266:19995-20000.

WAINWRIGHT P.E., Huang Y.S., Bulman-Fleming B., Mills D.E., Redden P., and McCutcheon D. The role of n-3 essential fatty acids in brain and behavioral development: a cross-fostering study in the mouse. Lipids. 1991; 26:37-45.

WHITE A., Handler P., and Smith E.L. In: Principles of Biochemistry, 5th ed., McGraw-Hill, New York, 1973. p.1296.

WHITE H.L., and Stine L. Arachidonate lipoxygenase activity in rat brain synaptosomal preparations. Soc. Neurosci. Abs. 1984; 10:1130.

WILLIAMS M.A. and McCleur R.H. The use of Sep-Pak® C18 cartridges during the isolation of gagliosides. J. Neurochem. 1980; 35:266-269.

WOLFE L.S. Eicosanoids, Prostaglandins, Thromboxanes, Leukotrienes and other derivatives of Carbon-20 unsaturated fatty acids. J. Neurochem. 1982; 38:1-14.

YAMAMOTO N., Hashimoto A., Takemoto Y., Okuyama H., Nomura M., Kitajima R., Togashi T., and Tamai Y. Effect of the dietary α-linolenate/linoleate balance on lipid compositions and learning ability of rats. II. Discrimination process, extinction process, and glycolipid compositons. J. Lipid Res. 1988; 28:144-151.

YAMANAKA W.K., Clemens G.W., and Hutchinson, M.L. Essential fatty acid deficiency in humans. Prog. Lipid Res. 1980; 19:187-215.

YEHUDA S. Nutrients, brain biochemistry and behavior: a possible role for the neuronal membrane. Intern. J. Neurosci. 1987; 35:21-36.

YONEKUBO A., Honda S., Okano m., Takahashi K., and Yamamoto Y. Dietary fish oil alters rat milk composition and liver and brain fatty acid composition of fetal and neonatal rats. J. Nutr., 1993; 123:1703-1708.

YOUYOU A., Durand G., Pascal G., Piciotti M., Dumont O., and Bourre J.M. Recovery of altered fatty acid composition induced by diet devoid of n-3 fatty acids in myelin, synaptosomes, mitochondria and microsomes of developing rat brain. J. Neurochem. 1986;46:224-228.

ZEVENBERGEN J.L., and Houtsmuller U.M.T. Effect of dietary fats on linoleic acid metabolism. A radiolabel study in rats. BBA 1989; 1002:312-323.