THE DIETARY ESSENTIALITY OF n-3 POLYUNSATURATED

FATTY ACIDS IN INFANT NUTRITION

by

LUCILLE DIANNE ARBUCKLE

B.H.E., University of Manitoba, 1987

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF

THE REQUIREMENT FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE STUDIES

(School of Family & Nutritional Sciences)

We accept this thesis as conforming

to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

June 1993

© Lucille Dianne Arbuckle, 1993

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

(Signature)

Department of ___Human Nutrition___ School of Family &
The University of British Columbia Nutritional Science
Vancouver, Canada

Date ___June 17, 1993___

ABSTRACT

Docosahexaenoic acid (22:6n-3) and arachidonic acid (20:4n-6) are deposited in large amounts in membrane phospholipids of the developing central nervous system (CNS). High levels of 22:6n-3 are found in synaptic terminals and retina, and are important for normal visual development and function. 20:4n-6 and 22:6n-3 are supplied in human milk. In contrast, infants fed formula rely completely on endogenous synthesis of 20:4n-6 and 22:6n-3 from linoleic (18:2n-6) and α-linolenic (18:3n-3) acid, respectively. Levels of 22:6n-3 in the blood lipids of infants fed formula are lower than in infants fed human milk. Concern over the supply of 22:6n-3 led to clinical trials in which premature infants were fed formulas containing fish oils as a source of 22:6n-3.

Piglets, which have a similar lipid metabolism and perinatal timing of the brain growth spurt to humans, have a lower percentage of 22:6n-3 in blood, liver and CNS tissues when fed formula with 30% of fatty acids as 18:2n-6 and 0.8% 18:3n-3, compared to sow milk. It was hypothesized that the low blood and tissue 22:6n-3 in formula-fed piglets was due to inappropriate quantities and/or ratios of dietary 18:2n-6 and 18:3n-3 limiting the synthesis of 22:6n-3. Thus, the main objectives of this thesis were to determine: (1) if 22:6n-3 is an essential dietary nutrient for the term gestation piglet, (2) if appropriate quantities and ratios of 18:2n-6 and 18:3n-3 in formula will support CNS membrane accretion of 20:4n-6 and 22:6n-3, comparable to piglets fed varying amounts of 22:6n-3 in natural milk, and (3) if lower blood phospholipid 22:6n-3 consistently reflects reduced 22:6n-3 in the CNS.

Initial studies (Experiment I) showed that formula with 4% 18:3n-3 supported a similar percentage of 22:6n-3 in piglet liver and CNS membrane lipids to sow milk, but was associated with lower brain weight. Deposition of 22:6n-3 in brain was influenced by the formula 18:3n-3 content. The 18:2n-6:18:3n-3 ratio (22:1 and 37:1) seemed to be important, however, when formulas contained 1% 18:3n-3. Low levels of fish oil in formula, similar to those used in clinical trials, were effective in supplying 22:6n-3 to the developing piglet brain (Experiment II). The efficacy of 18:3n-3 in supporting the deposition of 22:6n-3 in the brain was estimated to be at least 20% that of dietary 20:5n-3 plus 22:6n-3.

With increasing dietary fish oil, however, levels of eicosapentaenoic acid (20:5n-3) increased and 20:4n-6 decreased in plasma, liver and retina, but not brain (Experiment III). This suggests regulatory mechanisms may exist to maintain relatively constant levels of 20:4n-6 and 20:5n-3 in brain.

Milk 22:6n-3 varies with maternal intake of 22:6n-3. The effect of milk 22:6n-3 content was studied in piglets fed milk with 0.1% or 1.5% 22:6n-3 obtained from sows fed usual pig diets containing vegetable fats without or with fish oil, respectively (Experiment IV). Consumption of 1.5 vs 0.1% 22:6n-3 from sow milk resulted in 300% higher 22:6n-3 in liver and blood phospholipids and 11% higher 22:6n-3 in cerebrum of nursing piglets. Despite similar milk 20:4n-6, the % 20:4n-6 in tissues other than the brain was lower in piglets fed high 22:6n-3 sow milk. Thus, high intakes of n-3 fatty acids decrease 20:4n-6 in piglet liver and blood lipids. The blood phospholipid % 22:6n-3 in piglets fed formulas containing 18:2n-6 and 18:3n-3 but not their long-chain derivatives, was lower than in piglets fed 22:6n-3 in natural milk, consistent with published findings in formula-fed infants. However, in contrast to circulating lipids, formulas with 4% 18:3n-3 maintained similar levels of 22:6n-3 in the piglet CNS compared to milk. These studies show that blood phospholipid 22:6n-3 and 20:4n-6 are not specific indices of effects in CNS lipids.

This thesis has shown (1) 22:6n-3 is not essential in the diet of the term piglet, if adequate 18:3n-3 is given, (2) fish oils are an effective source of 22:6n-3 for deposition in the developing brain, (3) high dietary n-3 fatty acids interfere with 20:4n-6 metabolism, and (4) blood lipid 20:4n-6 and 22:6n-3 do not accurately reflect CNS fatty acids.

TABLE OF CONTENTS

ABBREVIATIONS

ANOVA	analysis of variance
apo	apoprotein
BF_3	boron trifluoride
C	carbon chain
°C	degrees celsius
CNS	central nervous system
EDTA	ethylenediamine tetraacetic acid
EPL	ethanolamine phospholipids
g	gram
GLC	gas liquid chromatography
h	hour
HCl	hydrochloric acid
HDL	high density lipoprotein
HPTLC	high-performance thin-layer chromatography
IgG	immunoglobulin G
K_2CO_3	potassium carbonate
KCl	potassium chloride
kg	kilogram
L	litre
LCP	long-chain polyunsaturated fatty acids: 20:4, 22:4, 22:5n-6; 20:5, 22:5, 22:6n-3
LDL	low density lipoprotein
m	metre
meq	milliequivalents
$MgCl_2$	magnesium chloride
μg	microgram
μl	microlitre

μmol	micromole
mg	milligram
mmol	millimole
ml	millilitre
mm	millimetre
min	minute
M	molar
mol	mole
mRNA	messenger ribonucleic acid
NaCl	sodium chloride
PC	phosphatidylcholine
PI	phosphatidylinositol
PL	phospholipid
PS	phosphatidylserine
p	statistical probability of mean differences not existing
R^2	coefficient of multiple determinations
RBC	red blood cell
SEM	standard error of mean
TLC	thin-layer chromatography
Tris HCl	tris(hydroxymethyl)aminomethane hydrochloride
VLDL	very low density lipoprotein
v	volume
vs	versus
wt	weight

LIST OF TABLES

LIST OF FIGURES

Foreward[1]

Experiment I has been accepted for publication:

Arbuckle, L.D., MacKinnon, M.J. & Innis, S.M. (1993) Formula 18:2(n-6) and 18:3(n-3) content and ratio influence long-chain polyunsaturated fatty acids in the developing piglet liver and central nervous system. *J. Nutr.* (in press)

Part of the work in Experiment I has been published:

Arbuckle, L.D., Rioux, F.M., MacKinnon, M.J. & Innis, S.M. (1991) Formula α-linolenic (18:3(n-3)) and linoleic (18:2(n-6)) acid influence neonatal piglet liver and brain saturated fatty acids, as well as docosahexaenoic acid (22:6(n-3)). *Biochim. Biophys. Acta* 1125: 262-267.

Experiment II has been published:

Arbuckle, L.D. & Innis, S.M. (1992) Docosahexaenoic acid in developing brain and retina of piglets fed high or low α-linolenate formula with and without fish oil. *Lipids* 27: 89-93.
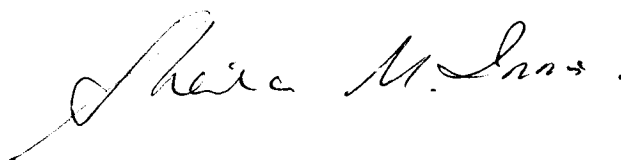
Experiment III has been published:

Arbuckle, L.D., Rioux, F.M., MacKinnon, M.J., Hrboticky, N. & Innis, S.M. (1991) Response of (n-3) and (n-6) fatty acids in piglet brain, liver and plasma to increasing, but low, fish oil supplementation of formula. *J. Nutr.* 121: 1536-1547.

Experiment IV has been accepted for publication:

Arbuckle, L.D. & Innis, S.M. (1993) Docosahexaenoic acid is transferred through maternal diet to milk and to tissues of natural milk-fed piglets. *J. Nutr.* (in press).

[1]The author's contribution to these publications included animal care, tissue preparation, fatty acid analysis of all tissues (with technical assistance for red blood cell and liver phospholipid fatty acid analysis) and statistical analysis, in consultation with Murray MacKinnon from the Statistical Consulting Service of the Research Centre. The manuscripts were written with consultation and editorial assistance from the research supervisor, Dr. Sheila M. Innis.

ACKNOWLEDGEMENTS

1 INTRODUCTION

Large amounts of long chain (20 and 22-carbon) polyunsaturated fatty acids (LCP), particularly arachidonic

acid (20:4n-6) and docosahexaenoic acid (22:6n-3) are deposited in the central nervous system (CNS) during

development (Sastry 1985). Rapid rates of LCP accretion in the CNS coincide with the period of neuronal

differentiation and maturation (Sastry 1985). This period of rapid brain growth in the human, as in the piglet,

occurs perinatally, *i.e.* during the last trimester of gestation and throughout the first months after birth (Dobbing

& Sands 1979). The equivalent developmental period in the rat is postnatal, and in the monkey and guinea pig

is before birth, as shown in **Figure 1.1**. Particularly high levels of LCP, especially 22:6n-3, are found in the retina

and synaptic nerve endings (**Table 1.1**). Within the retina, 22:6n-3 is concentrated in the rod outer segments and

represents 35 − 60% of the fatty acids in ethanolamine phospholipids (EPL). Reduced levels of 22:6n-3 in brain

and/or retina lipids has been associated with altered learning (reviewed by Wainwright 1992) and visual function

(Bourre et al 1989, Neuringer et al 1984, Neuringer et al 1986) in animals deprived of dietary α-linolenic acid

(18:3n-3).
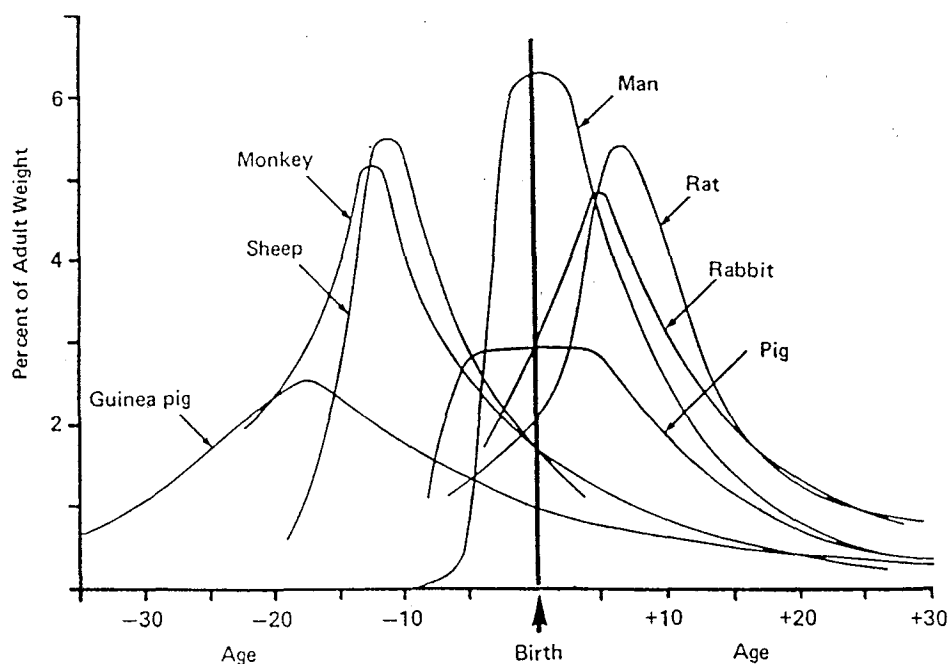
Figure 1.1 The brain growth spurt of 7 mammalian species expressed as first-order velocity curves of the increase
in weight with age. The rates are weight gain as a percentage of adult weight for each unit of time. The units
of time for each species are: guinea pig: days; rhesus monkeys: 4 days; sheep: 5 days; man: months; rat: days;
rabbit: 2 days; pig: weeks. Reproduced with permission from Dobbing & Sands 1979.

1

Table 1.1

Polyunsaturated fatty acid composition of cell membranes and organelles of rat central and peripheral nervous systems.

	Total Polyunsaturated Fatty Acids	20:4n-6	22:6n-3
	(% total fatty acids)		
Neurons	32	15	8
Synaptosomes	33	18	12
Oligodendrocytes	20	9	5
Myelin	15	9	5
Astrocytes	29	10	11
Capillaries	35	16	10
Mitochondria	30	16	12
Microsomes	29	11	12
Retina	45	5	35
Photoreceptor membrane	60	4	56
Peripheral nerve	10	7	2
Schwann Cells	22	11	5

Adapted from Bourre et al 1991. Animals were fed a standard lab chow diet, containing both n-6 and n-3 fatty acids.

LCP constitute about 2 − 4% of the total fatty acids in human and sow milk. Infant formulas, in contrast to milk, are devoid of 20:4n-6 and 22:6n-3, but do contain the 18-carbon (C18) dietary essential fatty acids, linoleic acid (18:2n-6) and 18:3n-3. Synthesis of 20:4n-6 and 22:6n-3 occurs by desaturation and elongation of 18:2n-6 and 18:3n-3, respectively (Sprecher 1981, Voss et al 1991), and is influenced by both the amount and balance of 18:2n-6 and 18:3n-3 in the diet (Holman 1986). Infants fed formula must rely completely on endogenous synthesis of LCP from 18:2n-6 and 18:3n-3 for accretion in all membrane lipids. Levels of 22:6n-3 are lower in the plasma and red blood cell (RBC) phospholipids of infants fed formula than in infants fed human milk (Carlson et al 1986, Clark et al 1992, Innis et al 1990, Putnam et al 1982). This is of concern because lower circulating levels of 22:6n-3 in premature infants have been associated with altered visual function (Birch et al 1992, Carlson et al 1989, Carlson et al 1989a, Uauy et al 1990). It has been speculated that the desaturase enzymes in the newborn are immature and that 22:6n-3 may therefore be an essential dietary nutrient for the

newborn (Carlson et al 1986, Clandinin et al 1981). However, the extent to which plasma and RBC phospholipid 22:6n-3 and 20:4n-6 reflect deposition in CNS lipids is not known. This concern over the supply of 22:6n-3 led to clinical trials in which premature infants were fed formulas containing fish oils (Carlson et al 1987, Carlson et al 1991, Liu et al 1987, Uauy et al 1990). Most fish oils, however, also contain high levels of eicosapentaenoic acid (20:5n-3) and this can result in replacement of 20:4n-6 by 20:5n-3 in tissue phospholipids.

Piglets have lower levels of 22:6n-3 in blood, liver and CNS tissues when fed formula containing 30% 18:2n-6[1] and 0.8% 18:3n-3, rather than sow milk (Hrboticky et al 1989, Hrboticky et al 1990, Hrboticky et al 1991). The low blood and tissue lipid 22:6n-3 in these formula-fed piglets may have been due to inappropriate quantities and/or ratios of dietary 18:2n-6 and 18:3n-3 limiting the synthesis of 22:6n-3. Thus, it is possible that 22:6n-3 is not essential in the diet of the newborn infant.

1.1 Essential fatty acid metabolism

The fatty acid nomenclature gives the number of carbon atoms in the fatty acid followed by a colon and the number of unsaturated carbon atoms. The notation "n" (equivalent to the Greek symbol ω) is used to indicate the unsaturated fatty acid series and is based on the position of the first double bond counting the number of carbons from the methyl (CH_3) end of the fatty acid. For example, linoleic acid, 18:2n-6, has 18 carbon atoms and 2 double bonds, with the first double bond occurring between the sixth and seventh carbon from the CH_3 end of the fatty acid chain; α-linolenic acid, 18:3n-3 has 18 carbons and 3 double bonds, with the first double bond occurring between the third and fourth carbon from the CH_3 end of the fatty acid chain (**Figure 1.2**). Fatty acids with double bonds at either the n-3 or n-6 positions cannot be synthesized by animals. They are, therefore, essential nutrients which must be supplied in the human diet (reviewed by Tinoco 1982). These fatty acids can be metabolized further by (1) desaturation and elongation to LCP: 20:4, 22:4 and 22:5n-6; 20:5, 22:5 and 22:6n-3, (2) β-oxidation for energy, or (3) direct esterification into phospholipids, triglycerides or cholesterol esters (**Figure 1.3**).

---

[1]30% 18:2n-6 is an abbreviation for 30% (wt/wt) of fatty acids as 18:2n-6. This is consistently used throughout the thesis, unless specified as % of energy.

linoleic acid, 18:2n-6

linolenic acid, 18:3n-3
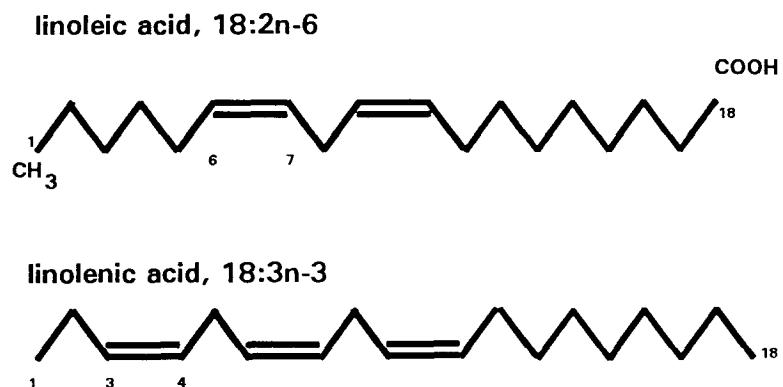
Figure 1.2 Schematic representation of linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3) with the position of the unsaturated carbon atoms shown.
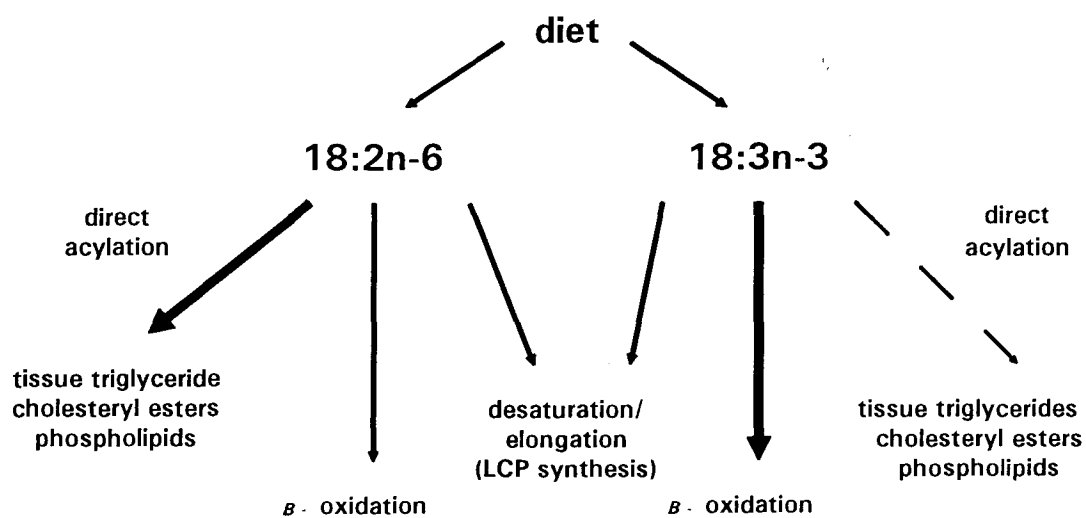
Figure 1.3 Schematic representation of the major pathways of n-6 and n-3 fatty acid metabolism. The intensity of the arrow indicates the relative importance of the metabolic pathways in the absence of n-6 and/or n-3 fatty acid deficiency. Adapted from Innis 1991.

1.1.1 Desaturation and elongation

It is now well known that in animals, the C18 unsaturated fatty acids are desaturated and elongated to C20 and C22 LCP in the microsomal membranes of many tissues. Evidence suggests, however, that the microsomal fraction of brain cells from the developing rat accounts for less than half the total brain homogenate desaturase activity (Cook 1978). The proposed pathways of desaturation and elongation are shown in **Figure 1.4**. The Δ6 desaturase introduces a double bond between C6 and C7 (*i.e.* 6 carbons from the carboxyl terminus) and is considered to be the rate-limiting step in the synthesis of 20:4n-6 from 18:2n-6 (Hassam 1976). After an elongation step, further desaturation to 20:4n-6 or 20:5n-3 is catalyzed by Δ5 desaturase which inserts a double bond between C5 and C6. In rat liver, the substrate fatty acid for Δ5 desaturase, 20:3n-6, can be either CoA-bound or phospholipid-bound, indicating that two different enzymes may exist (Pugh & Kates 1977). Although never purified, Δ4 desaturase was believed to catalyze the last step in the synthesis of 22:6n-3 from 18:3n-3. Recent findings imply, however, that the synthesis of 22:6n-3, and possibly of 22:5n-6, in rat hepatocytes is independent of a specific Δ4 desaturase enzyme (Voss et al 1991). The studies show chain elongation of 22:5n-3 to 24:5n-3 and subsequent desaturation at position 6 to yield 24:6n-3. Significant accumulation of 24:6n-3 has also been found in retinoblastoma cells which are capable of synthesizing 22:6n-3 from 18:3n-3 (Rosenthal et al 1991). Chain shortening, *i.e.* partial β-oxidation, of 24:6n-3 to 22:6n-3 may occur in the peroxisome. It is not yet known whether the same Δ6 desaturase is used for the two reactions: *i.e.* 18:3n-3 → 18:4n-3 and 24:5n-3 → 24:6n-3. Competitive enzyme studies however, have shown that 18:3n-3 strongly inhibits the desaturation of 24:5n-3 to 24:6n-3 with liver microsomes; therefore 22:6n-3 synthesis may be reduced as intracellular levels of 18:3n-3 increase (Sprecher 1992).

The desaturases and chain-elongases are common enzyme systems for the fatty acids of different series (n-9, n-6, n-3 series). In *in vitro* studies, the affinity of Δ6 desaturase for 18:3n-3 is higher than for 18:2n-6 and oleic acid (18:1n-9) (Brenner & Peluffo 1966). The inhibitory effect, *in vitro*, of 18:3n-3 on the conversion of 18:2n-6 to 20:4n-6 is much greater than that of 18:2n-6 on the conversion of 18:3n-3 to 20:5n-3 (Holman 1964). This competition among potential substrates for a given desaturase enzyme suggests that the balance, as well as the absolute amounts, of dietary 18:2n-6 and 18:3n-3 may impact on the synthesis of 20:4n-6 and 22:6n-3.

The desaturase enzymes are also inhibited by their reaction products, such as 20:4n-6, 20:5n-3 and 22:6n-3 (Brenner et al 1969, Garg et al 1988, Grønn et al 1992). End-product inhibition, as well as competition for acylation (Voss et al 1992) results in increased deposition of 20:5n-3 and 22:6n-3 and decreased n-6 LCP in tissue lipids of animals consuming dietary fish oils, which contain high levels of 20:5n-3 and 22:6n-3 (Bourre et al 1988, Bourre et al 1990, Huang et al 1992, Philbrick et al 1987, Swanson et al 1988, Wainwright et al 1992, Yeh et al 1990).

18:2n-6   18:3n-3

↓   Δ6 desaturation   ↓

18:3n-6   18:4n-3

↓   elongation   ↓

20:3n-6   20:4n-3

↓   Δ5 desaturation   ↓

20:4n-6   20:5n-3

↓   elongation   ↓
                                                              elongation
22:4n-6   22:5n-3 — — — — —→ 24:5n-3

↓   Δ4 desaturation?   ↓   ↓ Δ6 desaturation
                                                    β-oxidation
22:5n-6   22:6n-3 ←— — — — — 24:6n-3
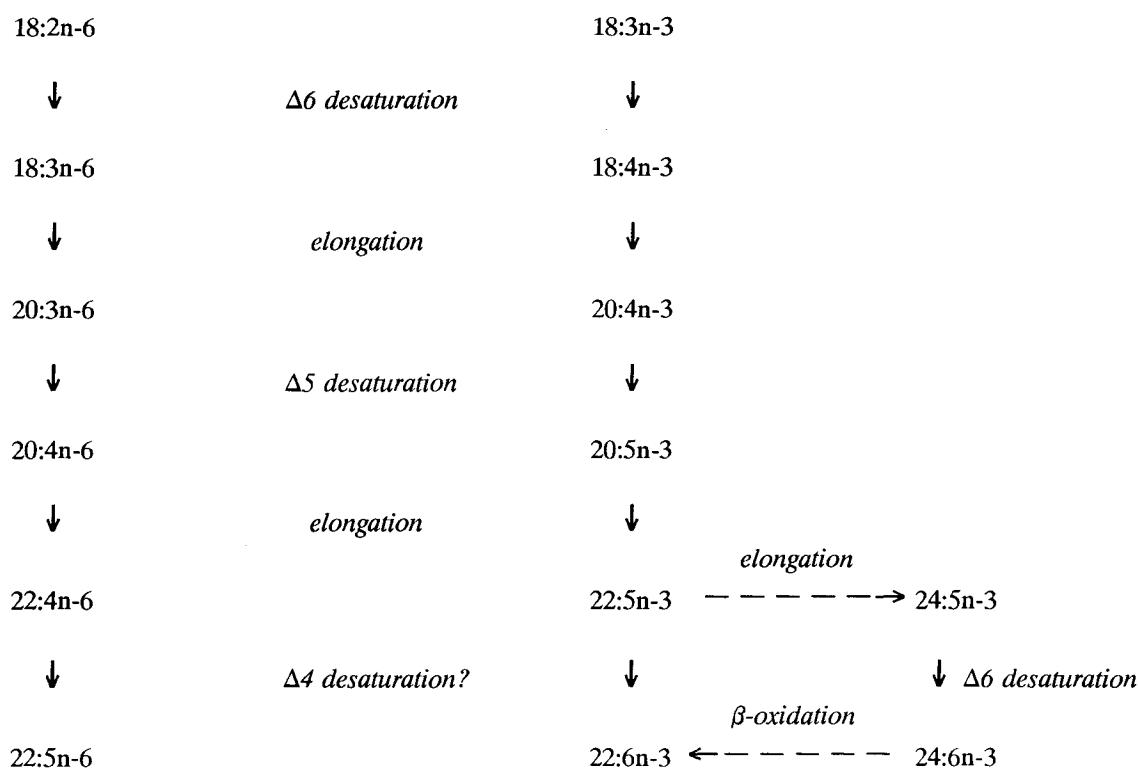
Figure 1.4 Schematic representation of the desaturation/elongation pathway of 18:2n-6 and 18:3n-3 in the synthesis of long chain polyunsaturated fatty acids.

1.1.2 Oxidation

Studies of *in vivo* utilization of fatty acids suggest that 18:3n-3 enters pathways of β-oxidation more extensively than 18:2n-6, 18:1, n-3 or n-6 LCP (Anderson & Connor 1988, Dhopeshwarkar & Subramanian 1975, Leyton et al 1987). Differences in body pool size and specific activity *in vivo* of the 18:2n-6 and 18:3n-3 substrates, however, should also be considered in the interpretation of these data. Carnitine palmitoyltransferase

6

regulates the entry of long chain fatty acyl-CoA into the mitochondria for β-oxidation. The activity of this enzyme is highest towards 18:3n-3, and is relatively lower for stearic acid (18:0), and n-3 or n-6 LCP (Gavino & Gavino 1991, Clouet et al 1989). The fast oxidation rate and ready desaturation of 18:3n-3, as well as the low activity of acyltransferase with 18:3n-3 (Lands et al 1982), could explain the low proportion of 18:3n-3 in structural lipids compared to 18:2n-6. In contrast, 18:2n-6 is present at relatively high levels in tissue lipids and reflects the diet 18:2n-6 content (Bourre et al 1990a). Differences in the oxidation of the C18 fatty acids compared to LCP also occur; in the comparative study of Leyton et al (1987a), only 14% of the [^{14}C] from [^{14}C]20:4n-6 was recovered in CO_2 compared to 48% [^{14}C] recovered from [^{14}C]18:2n-6.

1.1.3 Esterification

The acylation of substrate fatty acids, mainly into phosphatidylcholine (PC), EPL and triglyceride, has been shown to compete with the desaturation reaction (Brenner & Peluffo 1966). In rat liver lipids, 18:2n-6 and, to a lesser extent, 18:3n-3 were found to be incorporated into triglyceride. The triglycerides represent a store of fatty acids for energy, but a proportion may also be transferred directly or indirectly to structural pools. For example, liver triglyceride of suckling rats contained about 25% LCP, compared to less than 8% in adult rats (Chen & Cunnane 1992, Sinclair & Crawford 1972) and could potentially serve as direct substrates for acylation of phospholipids (Cook 1981). The incorporation of 18:2n-6 and 18:3n-3 into liver triglyceride, which has a rapid turnover rate, allows greater availability of these fatty acids, particularly n-3 fatty acids (Hagve & Christophersen 1984), for β-oxidation or further desaturation and elongation to LCP for membrane lipids. Unlike the C18 fatty acids, preferential acylation of 20:4n-6 and 22:6n-3 into phospholipids rather than triglycerides has also been shown (Hagve & Christophersen 1984, Sinclair 1975). This, together with lower oxidation rates for LCP described in section 1.1.2, provides a reasonable biochemical explanation for repeated demonstrations that dietary 18:2n-6 and 18:3n-3 are not equivalent in either their dietary or metabolic effects, to their longer-chain, more unsaturated products (reviewed by Innis 1991).

LCP are thought to be incorporated predominantly through deacylation of existing phospholipid by phospholipases, then reacylation of lysophospholipid by acyltransferases (reviewed by MacDonald & Sprecher 1991). Distinctive phospholipid fatty acyl composition results from selective esterification related to a number of acyltransferases, transacylases and phospholipases, each with its own substrate specificities (reviewed by

Rosenthal 1987). For example, large amounts of 20:4n-6 but only low levels of 20:5n-3 are found in most animal tissue lipids (reviewed by Innis 1991). Liver 1-acyl-GPC acyltransferase has a high selectivity for unsaturated fatty acids, but does not appear to discriminate among the C20 LCP of the n-6 and n-3 series (Lands et al 1982). Findings by Hagve and Christophersen (1984), however, suggest a specificity of phospholipases for 18:3n-3 and 20:5n-3 in preference to 18:2n-6 or 20:4n-6. Chain elongation of both 20:4n-6 and 20:5n-3 by liver microsomes is now believed to proceed at similar rates (Sprecher 1991), but selective activation of 20:4n-6 by arachidonyl-CoA synthetase (reviewed by MacDonald & Sprecher 1991), more extensive oxidation or esterification into triglycerides of 20:5n-3 (Hagve & Christophersen 1984), and active chain shortening of 22:4n-6 to 20:4n-6 (Hagve & Christophersen 1984, Voss et al 1992) would also contribute to tissue phospholipid fatty acid compositional differences. Partial replacement of 20:4n-6 by 20:5n-3 at the *sn*-2 position of liver phospholipids occurs when high amounts of fish oil are fed (Voss et al 1992). Therefore, the reduced levels of 20:4n-6 in tissue lipids of animals fed diets with fish oil (Bourre et al 1988, Bourre et al 1990, Huang et al 1992, Philbrick et al 1987, Swanson et al 1988, Wainwright et al 1992, Yeh et al 1990) may be due to competition for acylation and/or inhibition of Δ6 desaturase, as discussed in section 1.1.1.

In summary, relative rates of desaturation and elongation, oxidation and esterification of various n-6 and n-3 fatty acids depend on the absolute quantity and balance of the C18 fatty acid precursor and/or presence of C20 or C22 end-products in the diet. The possible importance of the ratio of 18:2n-6:18:3n-3 and the amount of C20 or C22 LCP must be considered in establishing dietary requirements for n-3 and n-6 fatty acids for the newborn. The inhibitory effects of end-products (20:4n-6, 20:5n-3, 22:6n-3) on n-6 and n-3 fatty acid metabolism must also be recognized if supplementation is with only one series of fatty acids (*i.e.* either n-6 or n-3). Finally, due to differences in rates of oxidation and specificity of enzymes for esterification, and deacylation/reacylation, the relative efficacy of 18:3n-3 as a dietary source of n-3 fatty acids to achieve adequate desaturation/elongation to 22:6n-3 must be determined.

1.2 LCP and Neuronal Development

The development of neurons in the CNS can be divided into four phases (reviewed by Herschkowitz 1988). The first phase is the formation and proliferation of precursor cells of neurons (*i.e.* neuroblasts). The second phase, neurogenesis, is the differentiation of the neuroblasts into postmitotic immature neurons. The third phase involves the migration of immature neurons from their sites of production to their final locations. Growth cones are found at the distal end of developing neurites and have been implicated in the guidance of growing axons toward their targets (reviewed by Arenander & de Vellis 1989). The fourth phase includes dendritic development, formation of synaptic contacts and the synthesis of myelin. The first three phases are virtually complete at time of birth in the human infant. The maturation of the settled neurons with the development of characteristic membrane properties and synaptic specialization extends from birth to one year. This period extends beyond one year of age for most parts of the human cerebral cortex (reviewed by Rees 1978).

Studies have shown that LCP may be important at the time of neurite elongation and synaptogenesis. For example, growth cones are enriched with 20:4n-6 (Martin & Bazan 1992) which may possibly be important in signal transduction systems that depend on the release of 20:4n-6 from the membrane (Garafalo & Pfenninger 1986). The fatty acid composition of growth cones, with the exception of 20:4n-6, becomes similar to that of the synaptic membrane by the beginning of synaptogenesis (Martin & Bazan 1992). These findings imply that delivery and incorporation of specific fatty acids, including 22:6n-3, to nerve growth cones may be prerequisites for the formation of mature synapses (Martin & Bazan 1992). In addition, a role of membrane lipids, including 20:4n-6 and 22:6n-3, for regulating the initiation, consolidation and final assembly of synaptic contacts during neuronal differentiation, has been suggested (Bourre et al 1983, Giesing & Zilliken 1980, Yavin et al 1975).

The total fatty acid content of human brain increases 4 to 5-fold during the period of brain maturation, due to the rapid accretion of structural lipids (Sastry 1985). Maximum rates of fatty acid accretion in the human are reached towards the end of gestation, with more moderate rates of increase in the content of 22:6n-3 continuing after birth until at least two years of age (Martinez 1992). Although the absolute amount of fatty acids in brain increases with development, there are differences in the rates of assimilation of 20:4n-6 and 22:6n-3 when expressed as a percentage of total fatty acids (Hrboticky et al 1990, Martinez 1992). In ethanolamine phospholipids, 22:6n-3 as percent of total fatty acids increases, whereas 20:4n-6 as a percentage decreases in

9

human cerebrum, retina and liver during the last trimester of gestation (Martinez 1992). A developmental increase in the percentage of n-3 LCP and decrease in the percentage of n-6 LCP is also seen postnatally in the retina and brain of monkeys (Neuringer et al 1986) and brain synaptosomal membranes of nursing piglets (Hrboticky et al 1989, Hrboticky et al 1990) and rodents (Foot et al 1982). This relative enrichment of brain and retina 22:6n-3 is consistent with the timing of rapid formation of synapses and dendritic spines and the rapid development of photoreceptor cells.

Superimposed on these postnatal developmental changes in membrane fatty acids, the overall composition of membranes is, to a certain extent, modified by dietary fat. Young rats born with low levels of 22:6n-3 in brain, but cross-fostered to dams fed diets with 5.3% 18:3n-3, achieved levels of 22:6n-3 in the brain, similar to the natural young from these dams (Walker 1967). Differences in brain, synaptic plasma membrane, retina and liver 22:6n-3 have also been shown between term gestation piglets fed formula containing 15% of energy as 18:2n-6 and 0.4% of energy as 18:3n-3 and piglets fed sow milk for 15 − 25 days. Newborn monkeys fed a diet deficient in 18:3n-3 from birth had lower RBC phospholipid 22:6n-3 than monkeys fed a diet with 2.1% of energy as 18:3n-3 (Connor et al 1992). The retina EPL 22:6n-3 composition in 3 year old deficient monkeys was only 29.4% that of the value in monkeys fed the adequate diet with 2.1% of energy as 18:3n-3. This difference in 22:6n-3 was accompanied by altered visual function. The deficient animals also drank twice as much water and excreted 2-fold the volume of urine compared to the control monkeys (Connor et al 1992). A recently reported account of autopsy analysis of 20 term human infants who died within 43 weeks of birth indicated a lower proportion of 22:6n-3 in the cerebral cortex of infants who had been fed formula rather than human milk (Farquharson et al 1992). Together, this information suggests the dietary fatty acids received in the immediate postnatal period influence the phospholipid fatty acid composition of the brain, its synaptic plasma membranes (Farquharson et al 1992, Hrboticky et al 1989, Hrboticky et al 1990, Walker 1967) and the retina (Connor et al 1992, Hrboticky et al 1991). These studies emphasize the necessity of providing an adequate and optimal dietary intake of n-3 fatty acids directly after birth.

1.3 The role of LCP

The LCP, particularly 20:4n-6 and 22:6n-3 are found in large amounts in membrane phospholipids and together with other membrane components, they impart specific structure to membranes (Sastry 1985). Singer and Nicholson's *fluid mosaic model* of membrane structure (1972) is shown in **Figure 1.5**. The matrix or continuous part of a membrane structure is a polar lipid bilayer. The most abundant lipids are phospholipids,
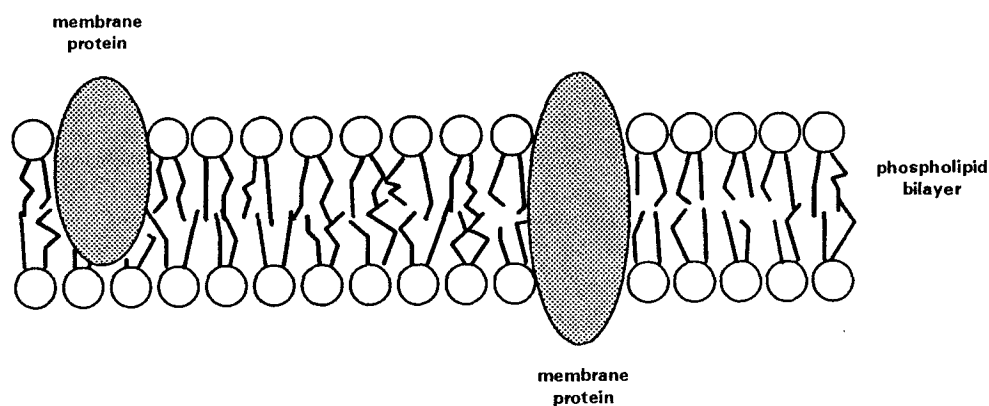
Figure 1.5 Schematic representation of Singer & Nicolson's *fluid mosaic model* (1972) of the structure of cell membranes.

which contain two fatty acids esterified to a polar headgroup, most usually PC, EPL, phosphatidyl-serine (PS) or -inositol (PI). There appears to be some specificity of fatty acids associated with particular phospholipid head groups: 22:6n-3 is primarily found in EPL and PS, whereas 20:4n-6 is more prominent in the PI fraction of the brain and retina (Sastry 1985, Fliesler & Anderson 1983). Phospholipid class asymmetry in plasma membranes and intracellular organelles has also been described in a variety of cells (reviewed in Houslay & Stanley 1982). A common feature is that the amino-containing phospholipids, PS and EPL, are limited chiefly to the cytosolic side, whereas PC is located on the extra-cytosolic surface (Cullis & Hope 1985). The small amount of EPL that does exist in the outer layer contains predominantly monounsaturated fatty acids while polyunsaturated fatty acids are more prominent in EPL in the inner surface (reviewed by Salem et al 1986).

Interspersed in the lipid bilayer are proteins, which may be transport systems, receptors, or enzymes. LCP may possibly affect membrane protein function through an overall effect on membrane physical properties or a specific effect, for example, by preferential association of 22:6n-3-containing phospholipids with particular membrane proteins (Weidmann et al 1988). The carbon chain length and degree of unsaturation of fatty acids

can influence the structure of membranes: the highly unsaturated LCP increase the disarrangement of the membrane. This may change the microenvironment of the particular proteins and thus the functional components. For example, alterations in neuronal membrane n-6 and/or n-3 fatty acid composition have been found to influence membrane-associated events, such as $Na^+ K^+$ ATPase activity (Sun & Sun 1974, Bourre et al 1989), acetylcholinesterase transition temperature (Hrboticky et al 1989), the regulation of neurotransmitter and drug receptor binding (Loh & Law 1980), and ethanol (John et al 1980) and neurotoxin (Bourre et al 1989) tolerance. Alterations in anesthetic potency, however, have been associated with 20:4n-6, specifically in PI, with no apparent effect of 22:6n-3 (Evers et al 1986). It is plausible, therefore, that LCP of each series may have a specific function(s).

In addition to their role as structural components of biological membranes, long chain n-3 (20:5n-3) as well as n-6 (20:3n-6, 20:4n-6) fatty acids are precursors for the synthesis of prostaglandins, prostacyclins, thromboxanes, and leukotrienes (reviewed by Smith 1989). Eicosanoids (Smith 1989) and 20:4n-6 second messenger systems (Axelrod et al 1988) are also important in normal brain metabolism. In addition, a possible important role of 20:4n-6 in growth has recently been suggested, based on studies which found a positive correlation of plasma phospholipid 20:4n-6 (Carlson et al 1992, Leaf et al 1992) and triglyceride 20:4n-6 (Koletzko & Braun 1991) with body weight in premature infants. This statistical relationship of circulating lipid 20:4n-6 and growth, however, does not necessarily imply a direct effect of 20:4n-6 on growth. It is plausible that differences in blood lipid 20:4n-6 may be symptomatic of, for example, differences in growth related to nutritional status of possibly zinc or protein.

1.3.1 The role of n-3 fatty acids in visual function

In the retina, 22:6n-3 is especially concentrated in the outer segments of photoreceptor cells and represents 35 − 60% of the fatty acids in EPL and PS, a proportion higher than in any other tissue (Anderson et al 1974, Stone et al 1979). The lipid environment of the rod outer segment accommodates a shift in the equilibrium of conformational states of the membrane protein rhodopsin, which allows binding of a G-protein and the subsequent initiation of an enzyme cascade, thereby directly facilitating the process of visual signal transduction (Brown & Gibson 1992). This may possibly occur as a result of the coupling of membrane thickness changes, which are related to the presence of 22:6n-3, to the conformational change of rhodopsin (Dratz & Holte

1992). Docosahexaenoyl-phospholipids (Brown & Gibson 1992), particularly PS (reviewed by Salem et al 1986), are now known to be essential for optimum rhodopsin activity.

The importance of 22:6n-3 in the visual process has also been investigated using the electroretinogram (ERG) and behavioral measures of visual acuity. The ERG measures the retinal evoked response to flashes of light. The activity of the photoreceptors is specifically seen in the a wave, an early component of the ERG response, whereas the b wave is representative of the total electrical activity of the neural cells (Fliesler & Anderson 1983). The ERG is a standard measure of retinal function whereas visual acuity evaluates function of both the retina and central visual pathways. Second or third-generation rats fed diets deficient in n-3 fatty acids (Bourre et al 1989, Watanabe et al 1987, Nouvelot et al 1985) had reductions in both retinal 22:6n-3 levels and ERG amplitudes at high light intensity. The amplitude of the a and b waves in 4-week old rats fed diets with 0.01% of energy as 18:3n-3 was 38% and 45%, respectively, that of animals fed 0.3% of energy as 18:3n-3 (Bourre et al 1989). The differences in ERG response between the two groups of rats were less pronounced by 6 weeks and, with the exception of a persistent effect on the a wave at the highest stimulus intensity, had virtually disappeared by adulthood (Bourre et al 1989). Studies in rhesus monkeys have shown lower plasma, RBC, retina and brain phospholipid levels of 22:6n-3 in monkeys fed a diet with 0.08% of energy as 18:3n-3, when compared to monkeys fed a soybean oil (control) diet with about 2.1% of energy as 18:3n-3 and no preformed 22:6n-3 (Connor et al 1984). A 50% decrease in retina 22:6n-3, with compensatory increased levels of C22 n-6 fatty acids, particularly 22:5n-6, was associated with abnormalities in ERG recordings in these monkeys (Neuringer et al 1986). The a wave amplitudes of n-3 fatty acid deficient monkeys were lower at 3 − 4 months of age; however, as in the rat (Bourre et al 1989), differences in response amplitude from the control disappeared over time (Neuringer & Connor 1989). The timing of the ERG response in the monkeys was also altered, with significant delays in the peak latencies (i.e. time to the b wave peak) of both rod and cone responses. A more specialized test of ERG function revealed a marked effect of n-3 fatty acid deficiency on the speed of recovery of the dark-adapted ERG response after a bright flash of light. This effect was present at 3 months and increased in magnitude with age, unlike the effects on amplitude (Neuringer & Connor 1989). The visual acuity of these monkey infants, tested directly with behavioral methods, was 50% lower than in the control monkeys at 1 − 3 months of age (Neuringer et al 1986). The decreased visual acuity may have been due entirely to effects on the

retina, or may also have involved changes in the visual pathways of the CNS. ERG abnormalities were not found to be reversible by feeding fish oil, despite restoration of retinal 22:6n-3 content to normal (Connor et al 1992) or even supranormal levels (Connor & Neuringer 1988). This may indicate that early functional delays in visual development in 18:3n-3 deficient animals may have long-term effects.

Transient differences in rod ERG thresholds and amplitudes have recently been reported in premature infants fed formula with 0.2% of energy as 18:3n-3 compared to those fed formulas with 0.5% of energy as n-3 LCP from fish oil or human milk (Uauy et al 1990). Measurements of visual acuity showed that these fish oil-supplemented infants matched the performance of human milk-fed infants at 4 months corrected postnatal age (Birch et al 1992). Visual acuity in premature infants fed formulas containing 0.2 or 1.2% of energy as 18:3n-3, however, was significantly below the milk-fed and fish oil-supplemented group (Birch et al 1992). In a similar study, visual acuity, measured by the Teller Acuity Card procedure, was significantly lower in premature infants fed formulas with 1.5% of energy as 18:3n-3 rather than formulas containing 0.5% of energy as n-3 LCP (Carlson et al 1992). These early differences in visual acuity, however, were not present between 6 − 12 months corrected postnatal age (Carlson et al 1992). The physiological significance of this transient increase in the rate of visual acuity development in premature infants is not known.

1.3.2 The role of n-3 fatty acids in learning ability and behavior

Studies on the learning ability and behavior of animals specifically depleted of n-3 fatty acids have recently been reviewed (Wainwright 1992). Studies have compared rats fed safflower oil (containing about 0.1% of fatty acids as 18:3n-3) as the dietary fat with those fed soybean or perilla oils, which are relatively rich in 18:3n-3 (approximately 8% and 65%, respectively) for two or more generations. The n-3 fatty acid-deficient rats showed lower frequencies of behaviors usually interpreted as exploratory (Enslen et al 1987, Lamptey & Walker 1976) and took longer to learn discrimination problems (Bourre et al 1989, Lamptey & Walker 1976, Yamamoto et al 1987). More recently, the level of 22:6n-3 in the brain and liver was significantly higher in young rats weaned to their mother's diet containing 1.3% of energy as 18:3n-3; the learning ability, evaluated by a swimming test, was superior to that of rats fed n-3 fatty acid-deficient diets (Yonekubo et al 1993). The interpretation of the effects on cognitive function is complicated by the use of a visual (black vs white, or brightness) discrimination or shock avoidance because differences in learning ability could have resulted from altered retinal function (Bourre

et al 1989, Neuringer et al 1986) or pain tolerance (Yehuda et al 1986), respectively. Independent of visual function, however, it took longer to extinguish the learned response in the n-3 fatty acid-deficient rats, when the food reinforcement was removed (Yamamoto et al 1988).

The effect of pre- and post-natal dietary fatty acid composition on behavioral development and functional consequences in the adult animal have been studied by use of cross-fostering designs (Wainwright et al 1991). Such studies have indicated a small developmental acceleration in eye opening in mice receiving n-3 fatty acids before birth when compared to deficient animals. However, n-3 LCP supplementation during gestation and lactation had no apparent long-term effects on visual discrimination learning or visual acuity in later adulthood (Wainwright et al 1991). In rats borne and nursed by dams fed diets with 0.01% of energy as 18:3n-3, a complete recovery of synaptic plasma membrane and myelin fatty acid composition, similar to control chow-fed animals, was observed when animals were fed a diet with 0.9% of energy as 18:3n-3 from weaning (21 days) to 16 weeks of age (Enslen et al 1987). However, exploratory behavior in the 18:3n-3 deficient animals weaned to diets with either 0.01 or 0.9% of energy as 18:3n-3 was significantly lower than control animals or those raised exclusively on diets providing 0.9% of energy as 18:3n-3. Thus, the lack of n-3 fatty acids during the early postnatal days may have a detrimental effect on normal behavioral development. However, altered visual perception cannot be ruled out since exploratory activity may be modulated by light.

In summary, LCP appear to play a role in normal neuronal development. Limitations in the supply of n-3 fatty acids in prenatal and early postnatal life may result in functional perturbations in the CNS. Of particular concern is that alterations in visual function (Connor & Neuringer 1988, Connor et al 1992) or behavioral development (Enslen et al 1991) may not be reversible after tissue levels of 22:6n-3 are restored to normal. Normal tissue levels of 22:6n-3 and 20:4n-6 may be necessary during infancy to support the development of proper CNS function. Although the long term effects of dietary deprivation of n-3 fatty acids during CNS development are unknown, it is clearly important to ensure an appropriate supply and balance of n-6 and n-3 fatty acids to support CNS deposition of 22:6n-3 and 20:4n-6 in this vulnerable period.

1.4 Origin and metabolism of n-6 and n-3 fatty acids in the developing tissues

Desaturase activity is present in developing fetal rat brain and liver (Bourre et al 1990b, Clandinin et al 1985, Cook & Spence 1973, Cook & Spence 1973a, Purvis et al 1983, Sanders & Rana 1987, Strouvé-Vallet

15

& Pascaud 1971). However, the available data suggest that the fetus acquires much of the LCP used in new tissue growth by transfer from the mother (reviewed by Innis 1991 and by Neuringer & Connor 1986). The exact mechanism for the assimilation of these fatty acids during prenatal life is not known, but human infants born at term are well provided with LCP in the liver, as well as the brain (Martinez & Ballabriga 1987). Sufficient tissue levels of LCP at birth, however, do not protect animals from decreases in brain 22:6n-3 if they are subsequently fed a diet deficient in n-3 fatty acids (Hrboticky et al 1989, Hrboticky et al 1990, Connor et al 1992, Walker 1967). This knowledge emphasizes the need for an adequate dietary intake of n-6 and n-3 fatty acids in early postnatal life. The potential sources of n-6 and n-3 LCP for the developing CNS are (1) synthesis *in situ* in the CNS by desaturation and elongation of dietary 18:2n-6 and 18:3n-3 derived from plasma, (2) uptake of LCP from plasma following synthesis in some other tissue, and/or (3) uptake from plasma of preformed LCP derived from the diet. The relative importance of each to the accumulation of LCP in the developing CNS is not yet clear (reviewed by Innis 1991).

1.4.1 *In situ* CNS synthesis of LCP

Δ6 Desaturase enzyme activity in brain is very high at birth, but decreases after about 7 days in the developing rat (Bourre et al 1990b, Cook 1978, Strouvé-Vallet & Pascaud 1971). A major portion of the label of ^{14}C-18:3n-3 and ^{14}C-18:2n-6 administered to rats orally (Sinclair 1975) was found in LCP of brain tissues. More complete removal of any effect of liver metabolism by intracranial injection of labelled 18:2n-6 and 18:3n-3 into the brains of suckling rats has shown the synthesis of 20:4n-6 and 22:6n-3, respectively, in the developing brain (Dhopeshwarkar & Subramanian 1976, Dhopeshwarkar & Subramanian 1976a). Data from recent studies show conversion of 18:3n-3 to 22:6n-3 within the rat retina when [^{14}C]18:3n-3 was introduced intravitreally (Wetzel et al 1991). Thus, the brain and retina have the capacity for synthesis of LCP from C18 essential fatty acids derived from the diet.

Cultured capillary endothelial cells take up similar amounts of 18:2n-6 and 18:3n-3, however the rate of desaturation and elongation of 18:3n-3 in these cells far exceeds that of 18:2n-6 (Moore et al 1990). The major products are 20:4n-6 and 20:5n-3, which are both incorporated into endothelial cell lipids and released into the culture medium. Preformed 20:4n-6 and 20:5n-3 are also taken up by the endothelial cells when they are available in the extracellular fluid (Moore et al 1990). The brain capillary endothelial cells are almost completely

surrounded by processes of the astrocytes, whose long extensions make contact with several other types of brain cells, including neurons. Astrocytes can take up preformed 20:4n-6 and 20:5n-3, or 18:2n-6 and 18:3n-3, convert them to 20:4n-6 and 22:6n-3 and release these elongation/desaturation products into the interstitium of the brain for uptake and incorporation into neuronal phospholipids (Moore et al 1991). Neurons themselves, however, are not capable of producing significant amounts of 22:6n-3 or 22:5n-6 (Moore et al 1991).

1.4.2 Synthesis in non-neural organs

The liver has been implicated as a potential source of LCP for uptake by the developing CNS (Scott & Bazan 1989, Wetzel et al 1990, Li et al 1991, Wang et al 1992). In liver, $\Delta6$ desaturase enzyme activity has been shown to be similar to (Cook 1978, Strouvé-Vallet & Pascaud 1971) or about 3-fold higher (Bourre et al 1990, Sanders & Rana 1987) than in developing rat brain. In contrast to the brain, desaturase activity in the rat liver is maintained (Bourre et al 1990b, Strouvé-Vallet & Pascaud 1971) and possibly even increased (Cook 1978) after weaning to standard chow diets. Introducing 18:2n-6 and 18:3n-3 through the gut or the peritoneal cavity results in substantial elongation and desaturation of these fatty acids in the liver (Nouvelot et al 1986, Scott & Bazan 1989, Sinclair & Crawford 1972). $\Delta6$ Desaturase enzyme activity has also been shown in homogenates of rat jejunum and ileum, although enzyme activities were highest in liver relative to the activity in these intestinal sites (Garg et al 1988a). The intestinal mucosa, however, is a relatively large organ and may therefore contribute substantially to the supply of LCP to other organs, through lipoproteins. Thus, the liver and intestine, as well as the brain, are potentially important sites of 18:3n-3 desaturation during early postnatal development.

1.4.3 Uptake of LCP from plasma (from diet or endogenous synthesis)

A number of *in vivo* studies have provided evidence that brain uptake of 18:3n-3 may be greater than for 18:2n-6, and higher for 20:4n-6 and 22:6n-3 than for the C18 essential fatty acids (reviewed by Innis 1991). LCP are incorporated into brain lipids *in vivo* (Anderson & Connor 1988, Sinclair 1975) and 20:4n-6 is found in brain phospholipid soon after intracerebral injection of the fatty acid (Yau & Sun 1974). Other *in vivo* (Wetzel et al 1991) and *in vitro* (Scott et al 1987) studies have shown developing retinas also incorporate exogenous radiolabeled 22:6n-3 into their lipids.

In considering the delivery of LCP to the brain, it has been suggested that newly synthesized 22:6n-3 is acylated into phospholipids and secreted by liver into blood in the form of lipoproteins (Scott & Bazan 1989). Information is not yet available, however, to describe the presence of a specific receptor(s) in the brain for a particular fatty acid or lipoprotein pool in the plasma. Nouvelot et al (1986) demonstrated that increased amounts of 18:3n-3 in the dams' diet resulted in elevated levels of 22:6n-3-HDL in suckling rats and suggested that HDL may be involved in delivery of 22:6n-3 to the rat brain. More recently, a significant progressive increase in the proportion of 20:4n-6 and 22:6n-3 has been shown in serum phospholipids of nursing piglets in the order VLDL < LDL < HDL (Innis et al 1993). A number of lipases and lipoprotein receptors have been identified in brain, any or all of which might play a role in the transfer of plasma fatty acids to the developing CNS membrane lipids (reviewed by Innis 1991, Moore et al 1991).

Selective uptake of C22 LCP may possibly occur in the brain and retina. Chain length alone, however, is not sufficient for the selectivity shown by these two tissues, as shown by the absence of 22:1 in the retina, despite 2 to 8-fold greater circulating levels of 22:1 than 22:6n-3 (Wang et al 1992). Therefore, the degree of unsaturation and acyl chain length of the fatty acid are both important determinants in the selective uptake mechanism. The accumulation of 22:5n-6 in CNS tissues when 22:6n-3 is not available (*i.e.* n-3 fatty acid deficiency) suggests that the carrier may not be series specific and recognizes both n-6 and n-3 C22 LCP (Innis et al 1993). In contrast to C22 LCP, a specific mechanism has been proposed for the uptake of 20:4n-6 into the brain (Innis et al 1993). This would be consistent with the maintenance of high levels of 20:4n-6 and lack of 20:5n-3 accumulation in the brain and brain capillary endothelial cells in the presence of high circulating 20:5n-3, as found with moderate levels of fish oil supplementation (Bourre et al 1988, Bourre et al 1990, Kálmán et al 1992).

The role of the blood-brain barrier in the uptake and retention of LCP is not presently known. The manipulation of dietary essential fatty acids and LCP has been shown to result in changes in the composition of acyl groups of phospholipids in isolated brain capillary endothelial cells (Brown et al 1984, Homayoun et al 1988, Kálmán et al 1992), similar to those seen in brain cells and subcellular fractions (Bourre et al 1984). This similarity to CNS tissues rather than plasma lipids suggests that the brain capillary endothelium is potentially an important site for specific uptake mechanisms of C18 essential fatty acids and/or LCP. However, the possibility of non-specific uptake of fatty acids into brain capillary endothelial cells, followed by formation of complex lipids

unique to the CNS, cannot be ruled out. In this regard, it has been suggested that the synthesis of triglyceride through diacylglycerol acyltransferase in glial plasma membranes may be important in transporting and remodelling fatty acids from plasma prior to transport into brain cells (Lin et al 1988).

1.5 Dietary supply of n-6 and n-3 fatty acids to the infant

Fat represents the major portion of energy in most human milk and infant formulas, usually about 45 − 50% of energy (reviewed by Jensen 1989). About 98% of the fat is triglycerides; phospholipids and plant lecithins make up the major portion of the remaining 2% of the fat in human milk and formula, respectively (Innis 1992, Jensen 1989).

1.5.1 Natural Milk

Human milk usually contains about 8 − 16% 18:2n-6, 0.5% 18:3n-3, 0.5 − 0.7% 20:4n-6 and 0.2 − 0.4% 22:6n-3. The content of 18:2n-6 and 18:3n-3 in human milk reflects the quantity of these fatty acids in the maternal diet (Innis 1992). Human milk 20:4n-6, however, does not seem to be related to the milk 18:2n-6 (Innis & Kuhnlein 1988, reviewed by Jensen 1989 and Jensen et al 1992, Sanders & Reddy 1992), or to high dietary or milk levels of n-3 LCP (Harris et al 1984, Henderson et al 1992, Innis & Kuhnlein 1988). Whether the content of 20:4n-6 in milk is regulated by some specific metabolic process in the mammary tissue is not known.

Levels of 22:6n-3 in milk as low as 0.1% of fatty acids have been reported for women consuming vegan diets devoid of LCP (Sanders & Reddy 1992). Levels as high as 1.3 − 1.4% of fatty acids, however, have been found in the milk of women consuming diets high in marine lipid (Innis & Kuhnlein 1988) or taking fish oil supplements (Harris et al 1984). These wide variations in human milk 22:6n-3, which seem to be due at least in part to differences in the mother's intake of preformed 22:6n-3 (Innis 1992, Jensen 1989, Henderson et al 1992, Sanders & Reddy 1992), also seem to be reflected in the RBC fatty acid composition of the nursing infant (Henderson et al 1992, Sanders & Reddy 1992). Intakes of fish oil over the wide range of 3 − 40% of dietary energy have been shown to increase 22:6n-3 in the milk of lactating rodents (Huang et al 1992, Wainwright et al 1992, Yeh et al 1990) and rabbits (Lin et al 1991) and to increase blood (Yeh et al 1990), brain (Wainwright et al 1992), retina (Lin et al 1991) and organ (Huang et al 1992) lipid 22:6n-3 in the suckling young.

Infants fed human milk, therefore, are supplied with a wide dietary range of 18:2n-6 and n-3 LCP, because of differences in the mother's dietary fat practices. It is not known if the differences in intake of 22:6n-3 over the range found in human milk (*i.e.* 0.1 − 1.4% of fatty acids) are reflected in CNS 22:6n-3. Furthermore, the wide variability in human milk fatty acids poses considerable problems in attempting to establish dietary fatty acid requirements from the infant fed human milk (Innis 1992).

1.5.2 Infant formula

In contrast to human milk, infant formulas in North America contain varying amounts and ratios of 18:2n-6 and 18:3n-3, and no 20:4n-6 or 22:6n-3. Infants fed formula, therefore, must rely completely on endogenous synthesis of LCP for deposition in membrane lipids. LCP synthesis is influenced by both the amount and balance of 18:2n-6 and 18:3n-3 in the diet (Holman 1986).

The fat in infant formulas is provided as a blend of vegetable and oleo oils, usually corn, safflower, soybean, coconut and palm oil. Corn and soybean oils provide similar large amounts of 18:2n-6, about 50 − 60% of fatty acids, but differ substantially with regard to 18:3n-3, providing 1.1 and 7% 18:3n-3, respectively (Table 2.1). Traditionally, many powdered infant formulas contained corn rather than soybean as the only unsaturated oil because of the susceptibility of the large amounts of 18:3n-3 in soybean oil to oxidative degradation. Formulas which rely on soybean oil as the principal source of 18:2n-6 have 18:2n-6:18:3n-3 ratios within the range of 4:1 to 10:1. In contrast, the high 18:2n-6 and low 18:3n-3 of corn oil result in high 18:2n-6:18:3n-3 ratios in formula (usually over 30:1) (Innis 1992, Ponder et al 1992).

Term gestation infants receiving formulas containing 0.6 − 4.8% 18:3n-3 as the only dietary source of n-3 fatty acids, with 13 − 45% 18:2n-6, have significantly lower 22:6n-3 in plasma and RBC phospholipids than infants receiving human milk containing preformed 22:6n-3 (Clark et al 1992, Olegard & Svennerholm 1971, Ponder et al 1992, Putnam et al 1982). It has been suggested that these differences may be due to immaturity of desaturase enzymes in the newborn infant (Carlson et al 1986). As higher dietary intakes of 22:6n-3 from milk are reflected in the RBC 22:6n-3 of nursing infants (Henderson et al 1992, Sanders & Reddy 1992), it is plausible that the lower circulating 22:6n-3 in formula-fed infants may merely reflect the absence of a dietary supply of 22:6n-3. It is also possible, however, that the low amounts of 18:3n-3 and/or high 18:2n-6:18:3n-3 ratio in some

20

formulas may not support adequate synthesis and incorporation of 22:6n-3 into tissues. Whether the lower blood lipid 22:6n-3 in term gestation infants receiving formulas devoid of 22:6n-3 is an accurate predictor of lower synthesis and deposition of 22:6n-3 in developing CNS tissues is not known.

Neonatal piglets fed formulas with 0.8% fatty acids 18:3n-3 from corn oil, in an 18:2n-6:18:3n-3 ratio of 37:1, had a significantly lower proportion of 22:6n-3 in plasma and RBC (Hrboticky et al 1990), as well as in liver, brain (Hrboticky et al 1989, Hrboticky et al 1990) and retina phospholipids (Hrboticky et al 1991), than piglets fed sow milk. Incorporation of n-6 LCP, particularly 20:4n-6 and 22:5n-6, in CNS membranes of formula-fed piglets indicates that the desaturase enzymes are functional during this perinatal period (Hrboticky et al 1989, Hrboticky et al 1990, Hrboticky et al 1991). Lower brain 22:6n-3 has been reported from autopsy data of infants fed formula rather than human milk (Farquharson et al 1992). Although significant differences were not found among the formula-fed groups, the lowest levels of 22:6n-3 in the brain appeared to occur in those infants fed formula with an 18:2n-6:18:3n-3 ratio of 40:1, compared to 10:1, particularly after 20 weeks of age. Thus, the decreased 22:6n-3 in the CNS tissues of piglets and human infants may have resulted from an inappropriate fatty acid blend, such as high 18:2n-6:18:3n-3 ratio, or from an inadequate supply of 18:3n-3, rather than the previously speculated fatty acid desaturase enzyme immaturity (Carlson et al 1986, Clandinin et al 1981, Putnam et al 1982).

The effect of the formula content of 18:3n-3, or 18:2n-6:18:3n-3 ratio, on the plasma and RBC levels of 22:6n-3 in term infants has recently been studied (Clark et al 1992, Ponder et al 1992). Circulating levels of 22:6n-3 and 22:5n-6 did not differ in 8 week old infants fed formulas with 0.8% or 4.8% of fatty acids as 18:3n-3 in 18:2n-6:18:3n-3 ratios of 30:1 or 7:1, respectively, from birth (Ponder et al 1992). These findings are similar to earlier studies in infants who had received formulas with 1.2% and 5% 18:3n-3 in dietary 18:2n-6:18:3n-3 ratios of 11:1 and 9:1, respectively, for 4½ to 6 months (Putnam et al 1982). Two separate reasons may explain the similar low levels of 22:6n-3 in the blood lipids of infants fed the different formulas. Firstly, it is plausible that 0.8% of fatty acids as 18:3n-3 may be too low to support sufficient 22:6n-3 synthesis. Secondly, high amounts of 18:2n-6 (i.e. 34% and 45%) (Ponder et al 1992, Putnam et al 1982) may inhibit desaturation of 18:3n-3 in these infants. Consistent with this suggestion, 50% lower levels of 22:6n-3 in liver have been reported from autopsy data of full-term infants who received an intravenous lipid emulsion containing 45% 18:2n-6 and 8% 18:3n-3 for 4 to 12 days, compared to milk-fed infants (Martinez & Ballabriga 1987). Infants fed formula with 0.7% 18:3n-3,

21

and an 18:2n-6:18:3n-3 ratio of 19:1, had significantly lower RBC total lipid 22:6n-3 than infants fed formula with either 1.1 or 3.4% 18:3n-3, in 18:2n-6:18:3n-3 ratios of 4:1 (Clark et al 1992). The results of these studies suggest that blood lipid 22:6n-3 may be influenced by the amount and balance of 18:2n-6 and 18:3n-3 in infant formula. However, studies in an appropriate animal model are required to determine if the effects of formula 18:3n-3 and 18:2n-6 content and balance on blood lipids consistently extend to CNS n-3 and n-6 fatty acids.

In summary, lower circulating lipid levels of 22:6n-3 in term gestation infants fed formulas with varying amounts of 18:2n-6 and 18:3n-3 and no LCP, compared to those fed human milk, cannot be assumed to be indicative of dietary essentiality of 22:6n-3. There is evidence to suggest that the lower circulating 22:6n-3 in infants may be related to the absence of a dietary source of 22:6n-3. The low circulating and CNS phospholipid 22:6n-3 in piglets fed formula with 0.8% 18:3n-3 and 30% 18:2n-6 may possibly have been due to an inadequate amount of formula 18:3n-3 (*i.e.* 0.8% of fatty acids = 0.4% of energy) and/or high 18:2n-6:18:3n-3 ratios (*i.e.* 37:1), thus limiting adequate synthesis and incorporation of 22:6n-3.

1.5.3 Fish oil supplementation

The early speculation of dietary essentiality of 22:6n-3 led to a series of clinical trials in which formulas were supplemented with fish oils (Carlson et al 1987, Carlson et al 1989, Carlson et al 1991, Liu et al 1987, Uauy et al 1990). The use of fish oils as a source of 22:6n-3, however, also entails addition of high levels of 20:5n-3 to the formula; this can result in replacement of 20:4n-6 by 20:5n-3 in tissue phosholipid (Voss et al 1992). The addition of fish oils to preterm formulas results in an increase in the plasma and RBC phospholipid 22:6n-3 (Carlson et al 1987, Carlson et al 1991, Uauy et al 1990) and supports a transient increase in the maturation of normal visual acuity in premature infants fed formulas with 0.2% (Birch et al 1992) or about 1.5% of energy as 18:3n-3 (Birch et al 1992, Carlson et al 1992). The increased blood phospholipid 22:6n-3 in infants fed the formula with fish oil, however, was accompanied by increased plasma and RBC phospholipid 20:5n-3 and reduced 20:4n-6 (Carlson et al 1992a) and poorer growth (Carlson et al 1992b) and psychomotor development (Carlson et al 1992).

Although the functional significance of the high amounts of 20:4n-6 in membrane lipids of the liver and brain is poorly understood, 20:4n-6 is known to be an important substrate for eicosanoid synthesis. Furthermore, diet-induced changes in hepatic membrane lipids result in alterations in receptor function and membrane-

associated enzyme activities (reviewed by Stubbs & Smith 1984), and the metabolism of xenobiotics (Christon et al 1988). Evaluation of the deposition of LCP in developing tissues of infants given a dietary source of n-3 LCP in the absence of a dietary source of n-6 LCP is therefore important.

1.6 Plasma and RBC phospholipid fatty acids

Clinical studies have traditionally relied on measures of plasma and RBC phospholipid n-6 and n-3 fatty acids. The specificity of these measure as an index of deficiency in the developing organs of infants with different dietary, and thus circulating n-6 and n-3 LCP is not known. Plasma phospholipids are components of lipoproteins and consist primarily of PC and lysophosphatidylcholine. Plasma phospholipids represent 4 − 8% (wt/wt) of chylomicrons, 10 − 20% of VLDL, 30% of LDL and 50% of HDL (reviewed by Scanu 1986). The fatty acid composition of plasma phospholipids reflects the transport of LCP absorbed from the diet (in chylomicrons and intestinal VLDL) and LCP secreted into the plasma (in endogenous VLDL, LDL and HDL) following synthesis from C18 precursors in organs such as the liver. Differences in the proportion of LCP have been shown in different serum lipoprotein fractions of animals (Innis et al 1993, Nouvelot et al 1986) and in the fed versus fasted state of nursing piglets (Innis 1992b). Two-fold higher levels of 22:6n-3 in fasted compared to fed piglets may reflect the low dietary intake of 22:6n-3 and relatively low amounts in chylomicrons and intestinal VLDL post absorption, and an increased contribution from the liver of VLDL, LDL and HDL in fasting plasma. Progressive enrichment of 22:6n-3 and 20:4n-6 also occurs as VLDL < LDL < HDL (Innis et al 1993). Changes in plasma phospholipid fatty acid composition may be related to short-term fluctuations in the fat absorbed from the diet and transported in lipoproteins, as well as the rate of fatty acid uptake by the developing organs. It is unlikely, therefore, that measurement of plasma fatty acids would give an accurate indication of changes in CNS fatty acids.

Mature RBCs are incapable of *de novo* phospholipid synthesis or chain elongation or desaturation of fatty acids (Dise et al 1980). Therefore, the turnover of RBC fatty acids *in situ* is limited to exchange with plasma lipoproteins (Mulder & Van Deenen 1965) and deacylation of endogenous phospholipid followed by reacylation of the resulting lysophospholipid on the cytoplasmic side of the membrane (Dise et al 1980). It has been suggested that only those phospholipids occupying the outer half of the RBC bilayer are exchangeable (Dise et al 1980). PC is mainly located in the outer monolayer (reviewed in Houslay & Stanley 1982). Similar changes

in fatty acid composition in the plasma phospholipid and RBC PC, therefore, probably reflect the renewal of RBC PC by exchange with plasma. Most of the EPL and all of the PS, which are enriched in 22:6n-3, are located in the inner membrane and would be influenced by deacylation and reacylation on the cytosolic surface, as well as the turnover of the RBC population. In addition, Marinetti & Cattieu (1982) identified a very small population of EPL molecules on the outer half of the human RBC membrane that had a 4-fold more rapid turnover of their fatty acids than the remaining EPL molecules.

Selective transfer of 20:4n-6 into 1-acyl-PC of RBCs by the acyl-CoA:lysophospholipid acyltransferase has been reported (Kaya et al 1984). The incorporation of n-3 LCP into the RBC membrane is also believed to be specific, with increases in PC, EPL, and PS, but not in PI and sphingomyelin, following dietary supplementation with fish oil (Cartwright et al 1985). It seems reasonable to conclude that the fatty acid composition of mature RBCs is related to the fatty acids available for exchange, and the specificities of the enzymatic and exchange processes involved in red blood cell membrane turnover (reviewed by Innis 1992).

In summary, 20:4n-6 and 22:6n-3 are lower in plasma and RBC membrane phospholipids of term (Clark et al 1992, Olegard & Svennerholm 1971, Ponder et al 1992, Putnam et al 1982) and preterm infants fed formula rather than human milk (Carlson et al 1986, Innis et al 1990, Uauy et al 1990). Based on these findings, it is generally believed that high dietary intakes of 18:2n-6 and 18:3n-3 cannot compensate for lack of LCP in formula. The fatty acid composition of plasma and RBCs is influenced by diet and organ uptake; thus, the degree to which their analysis can reflect fatty acid changes in the CNS, liver or other organs, is uncertain. Therefore, information regarding the adequacy of formula n-6 and n-3 fatty acids for deposition of LCP in the developing CNS requires extrapolation from studies in the young of other species.

1.7 Significance of study

Surveys of infant formula usage by Canadian mothers has indicated that approximately 30% of infants receive formula during the first days of life in the hospital, at which time 18% are exclusively formula fed. Over 50% of infants receive formula as the sole "milk" by 4 months of age (Table 6.1). The studies outlined in this thesis are particularly relevant in that the formula blends used very closely resembled the most popular infant formulas on the Canadian market.

The supplementation of infant formulas with preformed LCP has not been shown to be required nutritionally and may potentially have adverse effects. Feeding preformed 20:4n-6 and 22:6n-3 bypasses all the regulatory steps of desaturation. The membrane composition can only be controlled via acyltransferases and oxidation, thereby increasing the risk of attaining inappropriate tissue composition. Therefore, a logical alternative would be to investigate the possibility of *de novo* synthesis of LCP by the infant when given 18:2n-6 and 18:3n-3 in appropriate amounts. If the infant is able to synthesize 20:4n-6 and 22:6n-3, providing 18:2n-6 and 18:3n-3 in the formula may allow for control of LCP synthesis and lead to appropriate membrane composition. This information will be of importance in the development of guidelines for use of oil blends by industry in the manufacture of infant formula, and can be extended to the improvement of intravenous lipid compositions. These studies will also contribute substantially to the fundamental knowledge on the dietary requirement for 18:3n-3 and/or 22:6n-3 in the newborn.

Clinical trials involved the supplementation of infant formulas with n-3 LCP from fish oils as a means to maintain blood phospholipid 22:6n-3 in premature infants. The effects of these levels of fish oil supplementation on the pattern of n-6 LCP or n-3 LCP accretion in brain, however, are not known. Therefore, the studies in Experiment III were undertaken to investigate any potential detrimental effects of fish oil supplementation on the CNS, particularly with regard to 20:4n-6 and 20:5n-3.

Current approaches to determine the dietary adequacy of n-6 and n-3 fatty acids include the analysis of the fatty acid composition of human milk and/or infant plasma and RBCs. Whether or not these are valid measures of the dietary requirement for n-6 and n-3 fatty acids during growth and development needs to be clarified. Therefore, studies were carried to out to compare plasma and RBC phospholipid fatty acids with liver and CNS tissue 20:4n-6 and 22:6n-3 in piglets fed different formula and milk diets.

1.8 Rationale

In the last 5 – 10 years several publications have suggested that the fat in infant formulas does not meet the needs of infants for normal membrane LCP accretion (Carlson et al 1986, Clandinin et al 1980, Putnam et al 1982). The demonstration of lower LCP in plasma and RBC phospholipid of infants fed formula rather than human milk (Carlson et al 1986, Clark et al 1992, Ponder et al 1992, Putnam et al 1982) has led to concern over

the supply of LCP for membrane lipid accretion in the CNS of infants fed formula, and to the speculation that

n-3 LCP are essential dietary nutrients for the newborn. Accretion of n-6 LCP in the brain and retina of the

neonatal piglet, however, indicated that the newborn is capable of elongation/desaturation of 18:2n-6. The low

levels of n-3 LCP seen in blood phospholipids of term infants (Clark et al 1992, Olegard & Svennerholm 1971,

Ponder et al 1992, Putnam et al 1982) and in blood, liver and CNS tissues of piglets (Hrboticky et al 1989,

Hrboticky et al 1990, Hrboticky et al 1991) may be due, then to inappropriate quantities and/or ratios of 18:2n-6

and 18:3n-3 currently used in some infant formulas which may limit the synthesis of 22:6n-3. There is no evidence

as yet that 22:6n-3 is an essential dietary fatty acid for the term infant.

Although RBCs and plasma are accessible for study in the human infant, their limitations as indicators

of CNS lipids are evident. This emphasizes the need for an appropriate animal model in which direct analysis

of organs such as the brain, retina and liver can be undertaken. The piglet offers several advantages over other

species for the study of lipid nutrition relevant to the term gestation human infant. These include the similar

perinatal timing of the brain growth spurt (Figure 1.1) and brain myelination, similar intestinal development, fat

digestion and absorption and pathways of lipid and lipoprotein metabolism (reviewed by Innis 1993). As well,

the lipid composition of sow milk, including 18:2n-6, 18:3n-3 and LCP, is very close to that of human milk

(Hrboticky et al 1990).

Of experimental importance, the piglet is easily raised from birth on formula, when supplemented with

serum-derived immunoglobulins for the first 4 days of life (Innis 1993). This negates the need for any sow milk

(colostrum) in the diet of the piglet, and therefore any difference in brain lipid accretion is likely to be largely

due to formula intake alone. The normal suckling duration is only 20 − 25 days and during this relatively short

time, brain and body weight increase by c.a. 50% and 500%, respectively. This provides substantial tissue and

tissue growth to allow analyses for the effects of diet on the fatty acid composition of highly purified CNS cellular

or subcellular membranes (Innis 1993).

The tissues selected for study to evaluate the dietary adequacy of n-3 fatty acids in the neonatal piglet

were the cerebrum, synaptic plasma membranes, retina, liver, plasma and RBCs. The initial studies analyzed

the fatty acid composition of cerebrum total lipid to allow comparison to other studies of n-3 fatty acid

requirement. Ethanolamine phospholipids of synaptic plasma membrane and retina were analyzed because this

phospholipid fraction of these specific CNS tissues is enriched in 22:6n-3. The liver has been implicated as a

possible source of 22:6n-3 for the developing brain and is therefore of interest in biochemical measures of fatty acids in the newborn. Plasma and RBC phospholipid fatty acids were measured in the piglets to allow comparison and possible interpretation of findings in clinical trials which rely on these measures.

Studies in Experiment I were carried out to determine if levels of LCP in the CNS (cerebrum and retina) equivalent to that of piglets fed sow milk could be achieved by feeding piglets an appropriate amount and balance of 18:2n-6 and 18:3n-3. The studies in Experiment II determined whether fish oil supplementation of formula, at the level used in clinical trials (Uauy et al 1990) was effective in supplying 22:6n-3 for deposition in developing CNS lipids. Increasing, but low, levels of fish oil were added to formulas in Experiment III in order to determine the LCP response in CNS tissues and liver, as well as the plasma phospholipid. The levels of fish oil used were similar to those used in clinical trials with premature infants.

In order to evaluate whether feeding formulas with 18:2n-6 and 18:3n-3 can result in "normal" levels of 22:6n-3 in the CNS of piglets, comparison was made to a group of piglets consuming sow milk in Experiment I. Analysis of the milk lipid, however, found very low levels of 22:6n-3 (0.1% of milk fatty acids) in the milk of sows fed usual pig diets containing only vegetable fats. Milk 22:6n-3 levels vary with maternal intake of preformed 22:6n-3, primarily from fish lipids (Henderson et al 1992, Innis 1992, Harris et al 1984). Therefore, "normal" milk-fed infants would receive widely different 22:6n-3 intakes from milk, depending on their mother's diet. Studies in Experiment IV were undertaken to determine if tissue levels of 22:6n-3 would be increased in piglets in response to higher 22:6n-3 intakes from milk, and to provide a range of milk intake and tissue 22:6n-3 compositions within which the adequacy of feeding formulas with 18:2n-6 and 18:3n-3 could be evaluated. Ethanolamine phospholipid (and PC) fatty acids were analyzed in all organs and tissues for the final evaluation and comparisons among piglets fed different formula and milk diets. This was based on a greater sensitivity of measures in EPL rather than total lipid, which was determined in Experiment I,

1.9 Research Hypothesis

Docosahexaenoic acid (22:6n-3) is *not* an essential dietary nutrient for the term gestation piglet

27

1.10 Thesis Objective

To use piglets as an appropriate animal model to determine if:

appropriate quantities and ratios of 18:2n-6 and 18:3n-3 in infant formula will support normal membrane accretion of 20:4n-6 and 22:6n-3 in the CNS.

1.11 Specific aims of thesis

Experiment I: to determine if

- increasing the amount of 18:3n-3 in formula from 1 to 4% fatty acids increases CNS and liver 22:6n-3
- the amount of 18:2n-6 (18:2n-6:18:3n-3 ratio) in formula influences deposition of n-6 and n-3 LCP in piglet tissues

Experiment II: To determine whether fish oil supplementation of formula is effective in supplying 22:6n-3 for deposition in CNS lipids.

Experiment III:

- to determine the relationship between the amount of n-3 LCP in formula and the composition of n-3 and n-6 LCP in piglet tissues
- to define inter-tissue differences in n-3 and n-6 LCP compositions in response to increasing amounts of n-3 LCP in formula

Experiment IV: To determine the effect of maternal dietary n-3 fatty acid intake on the fatty acid composition of milk and the developing CNS, liver and plasma phospholipids of nursing piglets.

To use the data from **Experiment I** and **Experiment IV** to:

- evaluate the effects of feeding formula with low or high 18:3n-3 and no 22:6n-3 on deposition of 22:6n-3 in the developing CNS, in comparison to the range of tissue 22:6n-3 compositions found in natural milk-fed piglets.
- compare the relationship between the effects of the different milk and formula diets on the plasma and RBC phospholipid fatty acids and the effect on the liver and CNS lipid fatty acids.

2 MATERIALS AND METHODS

2.1 Chemicals

Enzymatic kits for assays of total cholesterol and triglycerides and of unesterified cholesterol were obtained from Biopacific Diagnostics, North Vancouver, British Columbia, and Boehringer Mannheim Canada Ltd., Laval, Quebec, respectively. The iron-dextran complex, Pigtran 200, was from Tuco Products Co., Orangeville, Ontario. All other chemicals were reagent grade and purchased from Sigma Chemical Co., St. Louis, Missouri, and BDH Chemicals Canada Ltd., Vancouver, British Columbia. Authentic lipid standards for thin-layer chromatography (TLC) and gas liquid chromatography (GLC) were purchased from Sigma Chemical Co., St. Louis, Missouri and NuChek Prep, Elysian, Missouri.

2.2 Equipment

Pig immunoglobulin was concentrated with a Pellicon ultrafiltration system with a 100 000 nominal molecular wt limit filter from Millipore Corp., Bedford, Massachusetts and lyophilized using a Dura-Top bulk tray drier and Dura-Dry condenser module from FTS Systems, Inc., Stone Ridge, New York. Oil was added to formulas in Experiment III by sonication with Sonifier Cell Disrupter 350 from Branson Ultrasonics Corp., Danbury, Connecticut. Pig liver was homogenized in an electric blender from Philips Electronic Industries, Ltd., Vancouver, British Columbia. Liver and retina were homogenized in a 2 ml Potter-Elvehjem tissue grinder with PTFE pestle (Fisher Scientific, Vancouver, Canada) using a Con-Torque power unit for tissue homogenization from Eberbach Corp., Ann Arbor, Michigan. Cerebrum homogenization was done with a 40 ml Dounce tissue grinder, Wheaton "200", (pestles "A" and "B" with respective clearances of 0.025 - 0.075 and 0.063 - 0.088 mm), from VWR Scientific Canada Ltd., London, Ontario.

Low-speed centrifugation ($<3,000g$) was performed in a J-6B low-speed centrifuge with a swinging basket rotor from Beckman Instruments (Canada), Inc., Mississauga, Ontario. Centrifugation at $12,000g$ was performed in a B-20 International Refrigerated Centrifuge, using a model 870 rotor, (International Equipment Co.), purchased from Fisher Scientific, Vancouver, British Columbia. Centrifugation at $100,000g$ was done in ultracentrifuges from Beckman Instruments (Canada), Inc., (model numbers L7-55, L8-55 and L8-70) using 7 Beckman fixed-angle rotors (types 42.1 TI, 50.2 TI and 60 TI). Fatty acid methyl esters were separated and quantified by GLC using a dual-column 6000 gas liquid chromatograph from Varian Canada, Inc., Mississauga,

Ontario, equipped with flame ionization detection and a Varian 654 data system, or a Varian 3400 with an IBM computer system using Varian "STAR" software. Glass capillary SP 2330 columns (30m x 0.2 mm) from Supelco Canada Ltd., Oakville, Ontario, were used.

2.3 Pig serum-derived immunoglobulin

Pig serum-derived immunoglobulin was prepared according to the method of Drew and Owen (1988). Porcine blood for this procedure was obtained from Intercontinental Packers Ltd., Vancouver, British Columbia. The blood was collected in 6×20 L pails containing 200 ml of 2.5% (wt/v) sodium citrate as anticoagulant. The blood was centrifuged at 1,000g for 5 min to remove RBCs. Fibrin was precipitated by the addition of 60 mL of calcium phosphate powder mixed in a small amount of water. After overnight precipitation, the resulting clot was chopped up and centrifuged (1,000g x 5 min). Serum was then fractionated using sodium polyphosphate glass (Calgon), a mixture of $Na_{15}P_{13}O_{40}$-$Na_{20}P_{18}O_{55}$. One hundred millilitres of a solution containing 11.4% (wt/v) sodium polyphosphate glass and 8.5% (wt/v) NaCl was added to each litre of serum with constant stirring. After pH adjustment to 3.95 with 3 M HCl, the mixture was stirred for 10 min and allowed to equilibrate overnight. The mixture was then centrifuged at 3,000g for 5 min and the supernatant was subsequently filtered through sand and glass wool. The immunoglobulin in the supernatant was concentrated using a Pellicon ultrafiltration system with a 100,000 nominal molecular wt limit filter. The concentrated fraction was lyophilized and stored at -70°C. The Department of Animal Science, University of British Columbia, generously quantified IgG in our immunoglobulin preparation using the double antibody sandwich enzyme linked immunosorbent assay (ELISA) (Voller et al 1976).

2.4 Animals and Diets

Male Yorkshire piglets of normal term gestation and of minimum 1.0 kg weight, were obtained from the University of British Columbia, Department of Animal Science and Pitt Ineffable Growers Ltd., Pitt Meadows, British Columbia. Piglets receiving sow milk and used as a reference group for those fed formula, were kept on the farm and suckled from sows fed a typical 16% protein lactation feed (Ritchie-Smith Inc., Abbotsford, British Columbia) with about 2.5% (wt/wt) vegetable oils (soybean and canola oils) added. Milk samples were taken from two sows, at 1 and 2 weeks lactation, frozen immediately, transported on ice to The Research Centre,

and stored at -70°C until analysis. Seven piglets were taken at random from different litters at 15 or 16 days post partum. This represents about two-thirds of the normal 20 – 25 day suckling period.

All piglets designated for formula feeding (Experiment I, II, III) were taken from the sow immediately after birth, prior to receiving colostrum. Six to 8 non-littermate piglets for each of the formulas studied were bottle-fed immunoglobulin-supplemented formulas within 2 h of birth, and transported in sterilized cages to a germ-free animal containment unit at The Research Centre. Provision of passive immunity to the colostrum-deprived formula-fed piglets was continued with pig serum-derived immunoglobulin for the first 4 days of life. The pig immunoglobulin was added to the formulas to give an IgG content of 20 mg/ml (i.e. 40 mg immunoglobulin preparation of 50% purity IgG/ml formula) for the first 48 h after birth and 5 mg/ml from 48 h to 4 days after birth. This supplementation was based on previous published studies (Drew & Owen 1988, Hrboticky et al 1989). The contribution of fatty acids from immunoglobulins previously prepared by this method, at the high level of supplementation, was 0.065 mg per ml formula, containing 0.013 and 0.003% n-6 and n-3 LCP, respectively (Hrboticky et al 1989). Animals were housed in large plastic bins lined with woodshavings and given free access to drinking water. For the first 48 h, the piglets were housed together, and bottle-fed by hand every 3 hours. Subsequently, they were housed in pairs, and bottle-fed by hand every 3 hours from 0700 – 2300 h until 15 or 16 days of age. Room temperature was maintained at 27 – 29°C. Spot heat lamps were attached above the cages to provide 24 h light and heat for the first 7 days. An 0700 – 1900 h light/1900-0700 h dark cycle was maintained from 8 – 15 days to parallel lighting conditions of sow milk-fed piglets. The animals were weighed every 4 days. All the formula and sow milk-fed piglets received an intra-muscular injection of 100 mg of an iron dextran complex on day 3 postpartum. The procedures used were approved by the University of British Columbia Animal Care Committee and conformed to the guidelines of the Canadian Council on Animal Care.

The macronutrient composition of the formulas (prepared and donated by Ross Laboratories, Columbus, Ohio) was based on that of infant formula, but modified to resemble the composition of sow milk (Table 2.1) and to meet National Research Council requirements (1979) for the growing pig (Table 6.2). The fat contributed about 50% of dietary energy, similar to that provided by sow milk. The formula fats were blends of soy, coconut, corn, canola, high-oleic acid sunflower, and/or marine oils. The fatty acid composition and blend of oils used in the formulas are in Table 2.2. After opening, the liquid, ready-to-feed formulas were kept at 4°C for no longer than 24 h.

Table 2.1

Macronutrient composition of sow milk[1] and experimental formulas[2]

| | Sow milk | Formula (% fatty acids) 18:2n-6/18:3n-3 | | | | |
		16/1 + FO[3]	16/1	16/4	30/1	35/4
Protein (g/L)	55	56	55	55	55	59
Fat (g/L)	50-60	60	59	59	60	60
Lactose (g/L)	58	58	58	58	72	55
Energy (kcal/L)	1000	992	995	987	1050	992

[1]Values for sow milk from Hrboticky et al 1989.
[2]Values for formula determined by Ross Laboratories, Columbus, Ohio.
[3]FO, fish oil added to formula in manufacturing process.

Table 2.2

Fatty acid composition of oils[1] and blend of oils[2] used in pig formulas

	Canola	Coconut	Corn	High-Oleic Safflower	Soybean	Marine
Fatty acids			*(% of total fatty acids)*			
≤ 14:0	0.1	77	-	-	-	10
16:0	4	10	13	4	10	20
16:1	0.3	-	0.1	-	0.1	13
18:0	2	3	2	5	4	4
18:1n-9	61	7	25	80	22	14
18:2n-6	21	2	58	7	55	1
18:3n-3	9	-	1.1	0.1	7	1
20:4n-6	-	-	-	-	-	1.1
20:5n-3	-	-	-	-	-	18
22:6n-3	-	-	-	-	-	9
Formula Fat Blends			*% (v/v)*			
16/1 + FO[3]	-	41	8	41	8	2
16/1	-	42	8	42	8	-
16/4	35	40	-	15	10	-
30/1	-	40	60	-	-	-
35/4	-	40	-	-	60	-

[1]adapted from Innis 1991
[2]information on oil blends was provided by Ross Laboratories, Columbus, Ohio
[3]16/1 + FO formula: 16% fatty acids 18:2n-6 / 1% 18:3n-3 plus fish oil

2.4.1 Experiment I: Influence of formula 18:2n-6 and 18:3n-3 content and ratio on n-6 and n-3 fatty acids in developing piglet CNS and liver.

Competition between 18:2n-6 and 18:3n-3 for desaturation implies that not only the quantity, but also the balance, of 18:2n-6 and 18:3n-3 in infant formula may influence levels of n-3 and n-6 LCP in the CNS and liver. This was investigated by feeding formulas with different vegetable oil blends to achieve low (16% of fatty acids) or high (30 or 35%) 18:2n-6 and low (1%) or high (4%) 18:3n-3. Each level of 18:2n-6 was fed with low (1%) or high (4%) 18:3n-3 to allow study of possible independent or interactive effects of formula 18:2n-6 and 18:3n-3 on tissue fatty acid composition. The formulas with (% 18:2n-6 / % 18:3n-3) 16/1, 30/1, 16/4 and 35/4 had 18:2n-6:18:3n-3 ratios of 22:1, 37:1, 4:1 and 8:1, respectively. Piglets fed sow milk were included as the reference for comparison. The fatty acid composition of sow milk and of the four formulas is in **Table 2.3**. The amount of saturated fatty acids was similar in all the formulas, but the percentage of C ≤ 14 and 18:1 were changed inversely with 18:3n-3 and 18:2n-6, respectively. The formulas differed from sow milk in their higher percentage of fatty acids with C ≤ 14, lower % 16:0 and absence of n-3 and n-6 LCP.

Table 2.3
Fatty acid composition[1] of sow milk and formulas in Experiment 1

Fatty Acid	Sow Milk[2]	Formula (% fatty acids) 18:2n-6/18:3n-3			
		16/1	16/4	30/1	35/4
		(% of fatty acids)			
Σ C $\leq 14:0$[3]	4.0	32.2	28.3	38.9	27.5
16:0	30.5	8.1	7.9	10.4	10.4
18:0	4.4	3.4	3.8	2.8	4.6
18:1	37.5	38.6	40.1	17.3	17.2
18:2n-6	11.1	15.6	16.6	29.5	35.1
18:3n-3	1.1	0.7	3.9	0.8	4.5
20:4n-6	0.5	nd[4]	nd	nd	nd
20:5n-3	0.1	nd	nd	nd	nd
22:5n-3	0.2	nd	nd	nd	nd
22:6n-3	0.1	nd	nd	nd	nd
18:2n-6:18:3n-3	10:1	22:1	4:1	37:1	8:1

[1]Fatty acid composition was determined by gas liquid chromatography after direct transesterification (Lepage & Roy 1986) as described in Materials and Methods.
[2]Values for sow milk are the average of 2 milk samples from different sows collected at 1 and 2 wk post partum lactation.
[3]total of 10:0, 12:0 and 14:0.
[4]nd, not detected.

2.4.2 Experiment II: Efficacy of formula 18:3n-3 compared to n-3 LCP in supporting deposition of 22:6n-3 in CNS lipids.

The objective of this study was to determine if formula containing either 18:3n-3 as the only n-3 fatty acid, in amounts approximately equal to and four-fold greater than the total n-3 fatty acids in milk, or similar 18:3n-3 to milk plus n-3 LCP from fish oil, could support equivalent deposition of 22:6n-3 in the CNS to sow milk. The formulas supplied similar amounts of 18:2n-6 (16%), but with low (1%) or high (4%) 18:3n-3 (16/1, 16/4 formulas, respectively) or low 18:3n-3 with fish oil to provide 20:5n-3, 22:5n-3 and 22:6n-3 in amounts of 0.4, 0.1 and 0.3% of the total formula fatty acids, respectively (16/1+FO formula). The fatty acid composition of the sow milk and formulas is given in **Table 2.4**. The four-fold greater 18:3n-3 was based on the work of Anderson et al (1990), which suggests 18:3n-3 was about three to four times less effective than 20:5n-3 and 22:6n-3 in supporting brain n-3 fatty acid deposition. Synaptic plasma membrane and retina EPL fatty acids were analyzed because these membranes are especially enriched in 22:6n-3.

Table 2.4
Fatty acid composition[1] of sow milk and formulas in Experiment II

Fatty acids	Sow milk	Formula (% fatty acids) 18:2n-6/18:3n-3		
		16/1	16/4	16/1 + FO[2]
		(% of fatty acids)		
Σ C $\leq$ 14:0[3]	4.0	32.2	28.3	34.6
16:0	30.5	8.1	7.9	7.8
18:0	4.4	3.4	3.8	3.3
18:1	37.5	38.6	40.1	38.4
18:2n-6	11.1	15.6	16.6	15.5
18:3n-3	1.1	0.7	3.9	0.6
20:4n-6	0.5	nd[4]	nd	nd
20:5n-3	0.1	nd	nd	0.4
22:5n-3	0.2	nd	nd	0.1
22:6n-3	0.1	nd	nd	0.3
18:2n-6:18:3n-3	10:1	22.3	4.3	25.8

[1]Fatty acid composition of formulas was determined by gas liquid chromatography after direct transesterification (Lepage & Roy 1986), as described in Materials and Methods.
[2]FO, fish oil
[3]total 10:0, 12:0 and 14:0
[4]nd, not detected

2.4.3 Experiment III: The relationship of formula n-3 LCP to n-3 and n-6 LCP in piglet tissues.

The effect of supplementing formula with increasing amounts of fish oil on n-3 and n-6 LCP in the brain

and its synaptic plasma membranes, retina, plasma and liver of piglets was investigated. The formula fat was

a 60/40 blend of corn-coconut oil (i.e. formula 30/1 in Experiment I) supplemented with 0 g (F diet), 2 g (FO1

diet) or 6 g (FO3 diet) of refined menhaden oil (ICN Biomedicals Canada, Ltd., Montreal, Quebec) per litre

of formula. The oil was dispersed in the formula by sonication using a Sonifier Cell Disrupter 350 with an output

control of 7, percent cycle of 75 and a 1/2 inch tip for four 20 second intervals, exactly as described for studies

in the preterm infant (Carlson et al 1987). The macronutrient and fatty acid composition of the formulas is in

Table 2.5. Addition of fish oil to the formula caused a small increase in energy density and % 16:1 and 20:4n-6.

FO1 and FO3 diets had 0.8 and 2.3% n-3 LCP in formula fatty acids, respectively; the levels of 20:5n-3 were two-

fold that of 22:6n-3. FO1 and FO3 diets were prepared fresh daily and stored at 4°C for no longer than 24 h.

Table 2.5
Macronutrient and major fatty acid composition of formulas fed to piglets in Experiment III[1]

	F[2]	FO1[2]	FO3[2]
Protein (g/l)	55	55	55
Fat (g/l)	60	62	66
Lactose (g/l)	72	72	72
Energy (Kcal/l)	1050	1069	1104
Fatty Acids *(% of fatty acids)*			
Σ C ≤ 14:0[3]	38.9	38.4	36.6
16:0	10.4	10.5	11.0
16:1	nd[4]	0.4	2.4
18:0	2.8	2.9	3.5
18:1	17.3	17.4	16.7
18:2n-6	29.4	28.8	26.5
18:3n-3	0.8	0.8	0.8
20:4n-6	nd	tr	0.1
20:5n-3	nd	0.5	1.4
22:5n-3	nd	tr	0.2
22:6n-3	nd	0.3	0.7

[1]Fatty acid composition determined by gas liquid chromatography after direct transesterification (Lepage & Roy 1986), as described in Materials and Methods
[2]F, 30/1 formula from Experiment I; FO1, 30/1 formula + 2 g menhaden oil/L; FO3, 30/1 formula + 6 g menhaden oil/L added by sonication
[3]total of 10:0, 12:0 and 14:0
[4]nd = not detected; tr = trace levels: 0.01 − 0.05% of total fatty acids

2.4.4 Experiment IV: The effect of maternal diet n-3 fatty acids on fatty acid composition of milk and the fatty acids of the developing CNS and liver of piglets.

Whether addition of fish oil to the sow's diet would increase 22:6n-3 in milk, and if this would be accompanied by altered assimilation of n-3 and n-6 LCP into brain and synaptic plasma membranes, retina and liver of natural milk-fed piglets, was studied. Three pregnant Yorkshire sows (from Peter Hill Holdings, Langley, British Columbia) were fed a usual pig diet with 5% (wt/wt) soybean oil until 4 days before term gestation, then 4% (wt/wt) soybean oil plus 1% (wt/wt) fish oil for the remainder of the study. The fish oil contained 24% of fatty acids as 22:6n-3, 6% 20:5n-3 and 2% 20:4n-6, and was a gift from Ross Laboratories, Columbus, Ohio. Six male piglets suckled by the sows fed the fish oil diet (2 piglets per sow) were taken for study at 15 or 16 days of age. Each piglet was nursed exclusively by its mother from birth and received no other food. Milk from each of the three fish oil-fed sows was obtained at 1 and 2 weeks post-partum, frozen immediately, then transported on ice to The Research Centre and stored at -70°C until analysis. The fatty acid content of the sow diet with 2.5% (wt/wt) vegetable oil added (soybean and canola oils) (from Experiment I) and of the diet with 4% (wt/wt) soybean oil plus 1% (wt/wt) fish oil is in **Table 2.6**. The notable difference in fatty acid content of the two sow diets was higher 18:2n-6 and n-3 LCP, particularly 22:6n-3. Saturated fatty acids (C ≤ 14, 16:0 and 18:0) and oleic acid (18:1) were slightly lower and 18:3n-3 was higher in the diet supplemented with fish oil (Table 2.6).

2.5 Tissue Collection and Preparation

At 15 or 16 days of age and within 1.5 to 2 h of the last feed, 10 ml of blood was collected from the piglets by cardiac puncture with 15% (wt/v) EDTA in saline as the anticoagulant, using a 2-way stop-valve and syringes. Ten ml of KCl (20 meq/10 ml) was then injected into the heart, killing the animal instantly. The piglets were decapitated and the heads immediately transferred to ice. All the following procedures were carried out at 4°C or on ice.

Organs and tissues collected and prepared for study included the cerebrum, synaptic plasma membrane, retina, liver, plasma and red blood cell. Additional data for triglyceride and cholesterol ester fatty acids of plasma and liver, and for PC fractions have been included in the Appendix (**Tables 6.3 − 6.21**) for completeness. The focus of this thesis was on the adequacy of formula fatty acids for the deposition of LCP in membrane phospholipids. It is beyond the scope of this thesis to discuss each lipid class in detail.

Table 2.6

Fatty acid content of sow diets containing vegetable oil only[1] or vegetable and fish oil[2].

Fatty acid	Vegetable oil	Vegetable oil + fish oil
	(% of fatty acids)	
Σ C$\leq$14:0[3]	1.0	0.5 $\pm$ 0.0
16:0	17.7	12.7 $\pm$ 0.3
16:1	0.8	0.7 $\pm$ 0.1
18:0	6.0	2.9 $\pm$ 0.1
18:1	31.2	23.1 $\pm$ 1.6
18:2n-6	35.4	47.7 $\pm$ 1.1
18:3n-3	5.3	7.2 $\pm$ 0.2
20:4n-6	tr[4]	0.3 $\pm$ 0.0
20:5n-3	tr	0.7 $\pm$ 0.1
22:5n-3	tr	0.2 $\pm$ 0.0
22:6n-3	tr	2.2 $\pm$ 0.3

[1]Values for the diet with vegetable oil are an average of duplicate analysis.
[2]Values for the fish oil diet are mean $\pm$ SEM of samples taken from 6 different diet/oil mixes prepared throughout the study period.
[3]total of 10:0, 12:0 and 14:0
[4]tr = trace levels: 0.01 − 0.05% of total fatty acids.

2.5.1 Cerebrum

The cerebrum was removed within 5 min of death, visible vasculature and connective tissue was removed and cerebrum weight recorded. The cerebrum was placed in a chilled beaker and minced with scissors. The cerebrum was homogenized in sections (6 − 8 g each) in 5 v/wt of 0.32 M sucrose-Tris HCl buffer (15 mM Tris HCl, 1mM EDTA, 1 mM $MgCl_2$, 1.5 mM glutathione, pH 7.4). Homogenization was with a 40 ml Dounce tissue grinder, using 5 strokes with the loose (B) pestle and 8 strokes with the tight (A) pestle. The homogenates were combined, and the total volume and total cerebrum wt homogenized were recorded and the ml homogenate/g cerebrum calculated. Aliquots of cerebrum homogenate were stored at -70°C until analysis.

2.5.2 Synaptic Plasma Membrane

A volume of cerebrum homogenate equivalent to 30 g of cerebrum was used for the isolation of synaptic plasma membranes, following the method of Cruz & Gurd (1978). Briefly, the 5% (wt/v) homogenate was diluted

to 9% (wt/v) by the addition of 4 mls homogenizing buffer per g of cerebrum used, and centrifuged at 3,000g

for 10 min. The supernatant was collected and centrifuged at 10,500g for 20 min. The resulting pellet was washed

3 times in 4.5 mls homogenizing buffer/g cerebrum. This involved resuspending the pellet with 5 strokes of the

tight pestle in a Dounce tissue grinder and subsequent centrifugation at 15,000g for 20 min. The washed pellet

was then suspended in 8 volumes of Tris HCl buffer with 5 strokes of the tight pestle of the Dounce tissue grinder.

After standing for 30 min at 4°C, the suspension was centrifuged at 97,000g for 25 min. The pellet was suspended

with 5 strokes of the tight pestle in 72 mls of 34% (wt/v) sucrose. Six mls of the suspension were aliquoted into

30 ml ultracentrifuge tubes. A discontinuous sucrose gradient was set up by carefully layering 5 mls of 28.5%

(wt/v) sucrose, then 5 mls of 24.0% (wt/v) sucrose, and finally 2 mls of 10.0% (wt/v) sucrose over the suspension.

After balancing the tubes on a scale, they were centrifuged at 97,000g for 110 min. Using a glass syringe with

a blunt needle, the 28.5%/34.0% (wt/v) density interface band was collected and resuspended in about 50 mls

of resuspension buffer (15 mM Tris HCl, 1 mM EDTA, 1 mM $MgCl_2$, 1.5 mM glutathione, pH 7.4), using 5

strokes of the tight pestle. The volume of the suspension was adjusted to 200 ml and centrifuged at 97,000g for

30 min. The synaptic plasma membrane pellet was collected and resuspended in 5 mls of resuspension buffer,

using a 5 ml Potter-Elvehjem tissue grinder with pestle. The final volume was recorded and aliquots of synaptic

plasma membranes were stored at -70°C until analysis.

The purity of synaptic plasma membrane preparations isolated by this procedure was previously

determined in this laboratory, using acetylcholinesterase and 5'-nucleotidase as marker enzymes. Marker enzymes

were not analyzed in the membrane preparations reported in this thesis. However, the fatty acid composition

of synaptic plasma membranes isolated from piglets fed sow milk and the 30/1 formula was very similar to that

of synaptic plasma membranes from respective piglet groups in previous studies which assessed membrane purity

(Hrboticky et al 1989).

2.5.3 Retina

Whole eyes were dissected within 15 min of death and kept on ice until retinas were removed with a

gentle stream of ice-cold saline. Retinas were stored at -70°C until analysis. Preceding lipid extraction, retinas

were thawed on ice and homogenized in 1 ml of 0.9% (wt/v) NaCl. Homogenization was achieved with 5 − 6

up and down strokes in a chilled 2 ml Potter-Elvehjem tissue grinder, using a Con-Torque power unit. Three 10 μl samples were aliquoted for protein determination (Lowry et al 1951).

2.5.4 Liver

The whole liver was immediately removed, perfused with ice-cold saline to remove the blood, blotted and weighed. In all but Experiment III, the whole liver was homogenized in a Philips electric blender with 50 ml of ice cold saline plus 15% (wt/v) EDTA. Liver samples of about 1 g were taken from piglets used in Experiment III. Aliquots of crude liver homogenate and liver samples (Experiment III) were stored at -70°C until analysis. A volume of thawed crude homogenate or liver sample equivalent to approximately 1 g of liver was homogenized in 2 ml saline in a chilled 2 ml Potter-Elvehjem tissue grinder, using a Con-Torque power unit. An aliquot was used for the determination of protein concentration (Lowry et al 1951) and the remaining liver homogenate was extracted for lipid (see Section 2.6).

2.5.5 Blood

Plasma was separated from RBCs by centrifugation (3,000g for 5 min at 4°C). RBCs were washed three times with an equal volume of 0.9% (wt/v) NaCl with 15% (wt/v) EDTA. Aliquots of plasma and packed RBC were stored at -70°C until analysis.

2.6 Lipid Extraction

Sow milk (for fatty acid compositional analyses of phospholipid and the sn-2 position of triglyceride), cerebrum, synaptic plasma membrane, liver and retina homogenates, and plasma total lipids were extracted using the method of Folch et al (1957). RBC lipids were extracted by the procedure of Rose and Oklander (1965) using chloroform/isopropanol, because the use of isopropanol in place of methanol avoids attracting heme pigments. The total lipid extracts from cerebrum, synaptic plasma membrane and retina were redissolved in chloroform-methanol (2/1, v/v) and aliquoted for determination of total fatty acid composition, total cholesterol and lipid phosphorus content and for TLC. Liver lipid extract and plasma were aliquoted for total and free cholesterol, triglyceride and lipid phosphorus assays. Liver and RBC lipid extracts were aliquoted for TLC. All aliquots of lipid extract were stored at -70°C until analysis.

2.7 Tissue Protein and Lipid Content

2.7.1 Protein Content

Aliquots of cerebrum, liver, synaptic plasma membrane and retina homogenates were assayed according to Lowry et al (1951) with bovine serum albumin as the standard.

2.7.2 Lipid Content

Aliquots from total lipid extracts of all tissues for cholesterol determination were first dried down under nitrogen gas, then reconstituted in 50 μl isopropyl alcohol. Total cholesterol levels were quantified with an enzymatic kit. Quality control samples were run with each assay. The intraassay and interassay coefficients of variation were $\leq$ 3%. Plasma aliquots and standards for triglyceride and free cholesterol were made up to 50 μl volume with distilled water and assayed using enzymatic kits with glycerol and cholesterol, respectively, as standards. Hepatic triglyceride and free cholesterol levels were assayed similarly in an aliquot of total liver lipid extract after resuspension of the dried lipid in 50 μl isopropyl alcohol. The standards in this case were similarly prepared, and for the assay of triglyceride was triolein rather than glycerol. Phospholipid phosphorus was assayed after digestion of an aliquot of the tissue lipid extracts with 70% perchloric acid (Chen et al 1956).

2.8 Fatty Acid Composition

2.8.1 Sow milk

Frozen milk samples were thawed in cold water and lipase activity prevented by rapidly heating to and maintaining at 80°C for 1 min (Bitman et al 1983). A 100 μl aliquot of sow milk, equivalent to about 600 μg of lipid, and 500 μg of C17:0 internal standard were methylated with 2 ml methanol-benzene (4/1, v/v) by a modification of the direct transesterification method of Lepage & Roy (1986). While vortexing, 200 μl acetyl-chloride was added, then tubes capped tightly and heated at 100°C for 1 h. During this hour, the tubes were vortexed at 15 min intervals. The sample was neutralized by the addition of 5 ml of 6% (wt/v) K_2CO_3, then recapped and shook for 3 minutes. Methyl esters were recovered twice with 4 ml pentane. The pooled pentane layers were dried under nitrogen gas and stored at -70°C for later GLC analysis.

The fatty acid composition of milk phospholipid and the *sn*-2 position of triglyceride were analyzed in the following way. Aliquots of milk lipid extract equivalent to about 2 mg lipid were spotted on silica gel plates

(F-254 0.5 mm) under a gentle stream of nitrogen gas. Milk triglyceride and phospholipid were separated from other lipid classes by TLC, using petroleum ether-diethyl ether-glacial acetic acid (85/15/3, v/v/v) as a solvent system (Innis & Clandinin 1981). Phospholipid remained at the origin and was visualized under ultraviolet light. Triglyceride was identified by an authentic standard which was run simultaneously and visualized in iodine. These lipid classes were scraped and eluted from the silica with chloroform-methanol (2/1, v/v). The distribution of fatty acids at the sn-2 position of sow milk triglyceride was analyzed by a modified method of Kuksis (1984). Briefly, triglyceride was dissolved in 200 μl of petroleum ether and vortexed. After the addition of 600 μl of 1 M Tris buffer (pH 8.0), 100 μl of 1 M $CaCl_2$ and 50 μl of 0.19 M taurocholic acid, the mixture was sonicated, then vortexed. Incubation at 37°C for 20 min with pancreatic lipase (100 μL of 20 mg/ml) allowed hydrolysis of the sn-1 and -3 positions of the triglyceride. The lipids were extracted from the reaction mixture with 2 ml diethyl ether and purified by TLC on pre-run PK6F Whatman hard plates. The plates were developed in hexane-diethyl ether-acetic acid (60/40/1.5, v/v/v). The sn-2 monoglycerides were identified in relation to a monoolein lipid standard. Following addition of C17:0 internal standard, the sn-2 monoglyceride was methylated with 2 ml methanol-benzene (4/1, v/v) by a modification of the direct transesterification method of Lepage & Roy (1986), as described for milk total lipid.

2.8.2 Formula

As for sow milk, 100 μl of formula, equivalent to about 600 μg of lipid, and 500 μg C17:0 internal standard were vortexed in 2 ml methanol-benzene (4/1, v/v). Fatty acid methyl esters were prepared by the direct methylation procedure described in section 2.8.1.

2.8.3 Cerebrum

A volume of total lipid extract equivalent to about 500 μg of cerebrum total lipid and 50 μg of C17:0 standard were dried under nitrogen gas. Total fatty acids were determined by transmethylation in 1 ml methanolic HCl (1/5, v/v) at 100°C for 90 min. The methyl esters were partitioned twice with 3 ml saline and 4 ml pentane. The pooled pentane layers were dried under nitrogen gas and stored at -70°C for later GLC analysis. Whole cerebrum homogenate contains cerebroside and sulfatide which are not separated from EPL by one-dimensional

41

chromatography. Therefore, two-dimensional chromatography (Pollet et al 1978) was used to eliminate contamination of EPL with sulphatide and cerebroside. Approximately 750 μg of total lipid extract was spotted on 10 x 10 cm high-performance thin-layer chromatography (HPTLC) 60 F-254 Merck plates, under a gentle stream of nitrogen gas. The plate was developed in chloroform-methanol-water (70/30/4, v/v/v) from the bottom to the top of the plate. The plate was rotated counterclockwise 90° and placed in the second solvent system, chloroform-methanol (20/80, v/v); the solvent was allowed to run two-thirds the distance of the plate. In the same direction, the plate was then developed in chloroform-methanol (60/30, v/v) from the bottom to the top of the plate. Finally, the plate was rotated clockwise back to its original position and developed in chloroform-methanol (60/30, v/v). The plates were dried for 15 min in the fume hood between each development. PC was separated from other phospholipid classes on HPTLC plates using chloroform-methanol-acetic acid-water (50/37.5/3.5/2, v/v/v/v) (Holub & Skeaff 1987) as the solvent system. The EPL and PC fractions were visualized under ultraviolet light after spraying with 2'7'-dichlorofluorescein and identified using authentic standards. Following scraping and elution with chloroform-methanol (2/1, v/v), fatty acids of EPL and PC were methylated at 100°C in 1 ml 14% boron trifluoride (BF_3)-methanol (Morrison & Smith 1961). EPL includes ether-containing analogues (i.e. alkyl- and alkenyl-acyl- phosphoethanolamine), which are transesterified more slowly than the more common diacyl derivatives (Christie 1982). Therefore, EPL was methylated for 30 minutes, and PC for 10 minutes. Methyl esters were recovered by pentane and saline partition as described above and stored at -70°C for later GLC analysis.

2.8.4 Synaptic Plasma Membrane and Retina

The total fatty acid composition of synaptic plasma membrane and retina were determined as described in Section 2.8.3. The separation of phospholipid classes was achieved on HPTLC plates using a solvent system of chloroform-methanol-acetic acid-water (50/37.5/3.5/2, v/v/v/v) (Holub & Skeaff 1987). The atmosphere inside the developing tank was allowed to saturate with solvent vapour for one hour before the plates were added; plates were then developed for 2½ hours. These conditions were strictly controlled for each set of plates. The bands corresponding to EPL and PC were identified using authentic standards and were processed as described above.

42

2.8.5 Liver, Plasma and RBC

Total phospholipid, triglyceride and cholesterol esters of plasma and liver lipid extracts were separated by TLC using a solvent system of petroleum ether-diethyl ether-glacial acetic acid (85/15/3, v/v/v) (Innis & Clandinin 1981). Complete bands corresponding to triglyceride and cholesterol esters were visualized, scraped and eluted as above. Triglyceride was transmethylated at 100°C for 30 min in 1 ml BF_3-benzene-methanol (25/20/55, v/v/v). Cholesterol esters were transmethylated at 100°C for 45 min in 1 ml BF_3-benzene-methanol (35/30/35, v/v/v). Half of the liver phospholipid band and the entire plasma phospholipid band were collected and prepared for subsequent transmethylation in 1 ml methanol-HCl (5/1, v/v) for 5 min at 100°C. The other half of the liver phospholipid band was scraped, eluted and dried down under nitrogen gas. The liver phospholipid extract and RBC lipid extract were separated into individual phospholipid classes by TLC on silica gel plates (F-254, 0.5 mm) using a chloroform-methanol-acetic acid-water (25/15/4/2/, v/v/v/v) solvent system (Skipski et al 1964). EPL and PC fractions were collected, eluted, transmethylated in 1 ml BF_3 for 10 min at 100°C, and stored at -70°C for GLC analysis.

2.8.6 Gas Liquid Chromatography

Fatty acid methyl esters were separated and quantified by GLC. Separation was achieved on 30 m × 0.25 mm nonbonded, fused silica capillary SP 2330 columns. Helium was used as the carrier gas at a column flow of 1 ml/min and inlet pressure of 15 pounds per square inch. The inlet splitter was set at 10 to 1. Samples were injected at 80°C and the oven temperature programmed to remain at 80°C for 2 min, then increase to 170°C at 20°C/min, stabilize for 25 min, rise to 195°C at 20°C/min, and stabilize for 18 min. The column was then heated to 245°C at 20°C/min and stabilized for 20 min prior to subsequent analyses. The injectors and detectors were set at 240° and 260°C, respectively. Fatty acid methyl esters were identified by comparison of retention times with those of authentic standards. The identification of fatty acids did not include mass spectrometry.

Identified fatty acids were comprised of the n-3 series 18:3, 20:5, 22:5 and 22:6; the n-6 series 18:2, 18:3, 20:2, 20:3, 20:4, 22:4, 22:5; the n-7 series 16:1; the n-9 series 20:1, 22:1; and 10:0, 12:0, 14:0, 16:0, 18:0, 20:0, 22:0 saturated fatty acids. Values for 18:1 fatty acid represented total isomers. Peak areas, wt percent fatty acid composition (of identified fatty acids) and μg values based on the amount of internal standard (C17) were computed by a chromatography data system (Varian 654 data system or a Varian 3400 with an IBM computer

system using Varian "STAR" software). The response of the detector to equivalent weightf of fatty acids across the range of C16 to C22 fatty acids was similar. The consistency of the flame ionization detector response was checked periodically by injecting a calibration mixture of equivalent amounts of C13, C17 and C23, and known authentic fatty acid methyl ester standard mixtures. The variance in response across the C16 to C23 fatty acids did not exceed 5% and was within the range of interassay reproducibility. Across the C8 to C14 fatty acids, the flame ionization detector response differed from the C13 and the C17 standards. Therefore, correction factors were used for the lower response for C8 (0.75) and for C10, C12 and C14 fatty acids (0.895) in milk and formula fatty acid analyses.

2.9 Statistical analyses

Data for all experiments was analyzed using the Number Cruncher Statistical System, version 5.01 (Kaysville, Utah), under the supervision of the Statistical Consulting Service of The Research Centre.

2.9.1 Experiment I

Two-way ANOVA (Montgomery 1984) was used to examine the effect of varying levels of 18:2n-6 and 18:3n-3 in the formula, and their interaction with respect to effects on the tissue fatty acid composition. Where an interaction between 18:2n-6 and 18:3n-3 was found ($p < 0.05$), post hoc contrasts were used to study the effect of 18:2n-6 level in the low or high 18:3n-3 formulas. These formal tests of differences were based on least squares means and standard errors calculated from the ANOVA. Bonferroni corrections (0.05 / 6 contrasts) were used to control the chance of error per experiment instead of per test (*i.e.* to keep the level of significance as low as intended), in determining which differences were of statistical significance ($p < 0.008$). Based on the small numbers of animals involved, the primary aim was not to infer too much from the data and therefore to reduce the chance of falsely claiming significant differences between groups (*i.e.* conserve the type I error).

Regression analysis was also used to study the relationship between the tissue % 22:6n-3 and the ratio of 18:2n-6:18:3n-3 in the formula. R^2 from the two-way ANOVA and regression analyses were compared to give an indication of whether the variability in 22:6n-3 in tissues from the different groups was best explained by the level of 18:3n-3 or by the 18:2n-6:18:3n-3 ratio of the formula. When a significant difference ($p < 0.05$) between

44

the sow milk-fed and formula-fed piglet groups was found by one-way ANOVA, mean levels of the fatty acid were then compared using post hoc contrasts with a Bonferroni correction applied to set the level for statistical significance for the four contrasts ($p < 0.013$) (Montgomery 1984).

2.9.2 Experiment II

Differences in the mean levels of each tissue fatty acid among the sow milk and formula groups were investigated using one-way ANOVA. As described above, post hoc contrasts with Bonferroni corrections (0.05/6 contrasts) within a fatty acid were used to determine which of these differences were statistically significant ($p < 0.008$).

2.9.3 Experiment III

The changes in phospholipid LCP composition with increasing fish oil intake were characterized, using orthogonal polynomial analyses (Montgomery 1984), as quadratic or linear. Analysis of variance was used to test for differences in the response to the three levels (0, 2, 6 g/L) of fish oil. When the results of the one-way ANOVA showed a significant difference ($p < 0.05$) in the levels of the tissue fatty acid under consideration, two orthogonal polynomial contrasts were used to describe the nature of the change in fatty acid level among F, FO1 and FO3 diets (Montgomery 1984). The first contrast was used to determine if there was an overall increase or decrease in the response to increasing dietary n-3 LCP. A second independent contrast was used to determine whether the intermediate dose (FO1 diet) response was inconsistent with a linear increase or decrease from F diet to FO3 diet, and thus representative of a curvilinear (quadratic) trend.

2.9.4 Experiment IV

Analyses for statistically significant differences ($p < 0.05$) between the milk fatty acid composition and the tissue fatty acid composition of piglets suckling milk from sows fed diets with and without fish oil were done using unpaired Student's t test.

2.10 Evaluation of the effects of feeding formula with low 18:3n-3 and no 22:6n-3 on deposition of 22:6n-3 in the developing liver and CNS and comparison of relationship between effects on circulating lipids with that on the liver and CNS lipids.

The natural milk consumed by piglets nursed by two groups of sows fed diets containing vegetable oil without or with fish oil had 0.1% and 1.5% fatty acids 22:6n-3, a range compatible with that in human populations. The adequacy of formulas containing either 16 or $\geq 30\%$ 18:2n-6 and either 1 or 4% 18:3n-3, with no 22:6n-3 (Experiment I), with regard to deposition of 20:4n-6 and 22:6n-3 in specific phospholipids (EPL and PC) of the developing CNS and liver was analyzed. One-way ANOVA was used to compare the mean levels of tissue 20:4n-6, 22:6n-3 and 22:5n-6 of the formula groups to those of piglets receiving milk from sows fed a vegetable oil diet. An independent one-way ANOVA was used for a similar comparison to piglets receiving milk from sows fed the fish oil diet. Bonferroni corrections (0.05 / 4 contrasts) within a fatty acid were used to set the level of statistical significance ($p < 0.013$).

The relationship between the plasma and RBC phospholipid 20:4n-6 and 22:6n-3 and the liver and CNS phospholipids was analyzed in piglets fed the different milks (Experiment IV) and formulas (Experiment I) to determine if circulating fatty acids are a specific measure of the 20:4n-6 and 22:6n-3 status of developing organs.

3 RESULTS

3.1 Experiment I: Influence of formula 18:2n-6 and 18:3n-3 content and ratio on major saturated, monounsaturated, n-6 and n-3 fatty acids in the developing piglet CNS and liver

3.1.1 Growth

There was no significant difference ($p > 0.05$) in body or liver wt between the groups of 15 day-old piglets fed formula or those fed sow milk (**Table 3.1**). Statistical analysis by two-way ANOVA found significantly lower cerebrum wt and cerebrum:body wt ratio in piglets fed formulas with 4% 18:3n-3 (16/4 and 35/4 formulas) rather than 1% 18:3n-3 (16/1 and 30/1 formulas). The cerebrum wt of piglets fed the 35/4 formula was significantly lower than that of piglets fed sow milk ($p < 0.013$). The differences among formula-fed piglets could not be attributed to differences in birth weight or in formula intake. The formula intake was similar among all groups from days 0 − 5, 6 − 10, 11 − 15, and over the entire 15 day study period.

Table 3.1

Birth weight, body and organ weights of 15 day-old piglets and formula intakes

| | | Formula (% fatty acids) 18:2n-6/18:3n-3 | | | |
	Sow milk ($n = 7$)	16/1 ($n = 8$)	16/4 ($n = 7$)	30/1 ($n = 6$)	35/4 ($n = 6$)
Birth wt (kg)	NA[1]	1.4 ± 0.1	1.6 ± 0.1	1.4 ± 0.2	1.4 ± 0.1
Body wt (kg)	4.1 ± 0.4	4.5 ± 0.3	4.4 ± 0.3	4.3 ± 0.4	4.3 ± 0.4
Liver wt (g)	118 ± 13	121 ± 9	123 ± 7	118 ± 10	116 ± 11
Cerebrum wt[†] (g)	35.0 ± 1.0	35.0 ± 1.0	32.3 ± 0.6	34.4 ± 1.0	31.7 ± 0.8*
Cerebrum:body wt (x100)[†]	0.9 ± 0.0	0.8 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	0.7 ± 0.1
Formula intake (L)					
day 0 − 5	NA	2.6 ± 0.2	2.5 ± 0.1	2.6 ± 0.2	2.4 ± 0.2
day 6 − 10	NA	4.0 ± 0.4	3.4 ± 0.3	3.5 ± 0.3	3.2 ± 0.4
day 11 − 15	NA	4.0 ± 0.4	4.0 ± 0.4	3.7 ± 0.4	3.8 ± 0.4
Total volume	NA	10.7 ± 0.9	9.9 ± 0.6	9.7 ± 0.9	9.4 ± 0.9

[1]NA, not available; n = number of animals/group
[†]significant negative effect of formula 18:3n-3 ($p < 0.03$), determined by two-way ANOVA. *significantly different from piglets fed sow milk ($p < 0.013$), determined by one-way ANOVA and post hoc contrast with Bonferroni correction.

3.1.2 Plasma and tissue lipid and protein content

There was no evidence of a significant effect of milk or formula fatty acids on plasma and liver triglyceride concentration, or liver and cerebrum protein concentration of piglets (**Table 3.2**). Nor were the concentrations of phospholipid and total cholesterol and the phospholipid:cholesterol ratio in piglet cerebrum, synaptic plasma membrane and retina significantly affected by dietary intake of milk or formula. All the groups of piglets fed formula had significantly lower concentrations of total and free cholesterol in plasma ($p \leq 0.0002$) and liver ($p \leq 0.0006$) than piglets fed sow milk. These findings probably reflect the higher dietary intake of cholesterol from sow milk, compared to negligible amounts of cholesterol in formula (Jones et al 1990). The liver phospholipid concentration was significantly lower in piglets fed the 16/1, 16/4 and 30/1 formulas than in reference piglets; however, because of the lower concentration of cholesterol in these groups, there was no significant difference in the hepatic phospholipid:cholesterol (μmol:μmol) ratio among the piglet groups.

3.1.3 Piglet tissue fatty acids

The significant effects on tissue fatty acid composition are described within each section as follows: (1) effects due to the amount of 18:2n-6 in the formula, (2) effects due to the amount of 18:3n-3 in the formula, and (3) interactive effects of formula 18:2n-6 and 18:3n-3, *i.e.* the magnitude of the effect due to the amount of 18:2n-6 in the formula is different, depending on the accompanying amount of 18:3n-3 in the formula (1% or 4% 18:3n-3) (from **Table 3.3**). Where a significant interaction was found, individual group comparisons are given (from **Figures 3.1, 3.2, 3.4**). Comparisons to the piglets fed sow milk are then described (from Fig. 3.1, 3.2, 3.3).

3.1.3.1 Liver and CNS tissue saturated and monounsaturated fatty acids
3.1.3.1.1 Levels of 16:0 in tissue membrane lipids

There was no evidence of a significant effect of the amount of 18:2n-6 or 18:3n-3 in the formula on synaptic plasma membrane and retina EPL % 16:0. The percentage of 16:0 in liver phospholipid, however, was significantly lower ($p < 0.0001$) in piglets fed the formula containing 4% 18:3n-3 (16/4 and 35/4) compared to 1% 18:3n-3 (16/1 and 30/1). The effect of diet on cerebrum total lipid % 16:0 was different from that on the

Table 3.2

Lipid and protein content of plasma, liver, cerebrum, synaptic plasma membrane and retina of 15 day-old piglets fed sow milk or formulas with varying amounts of 18:2n-6 and 18:3n-3 in Experiment I[1]

	Sow milk	Formula (% fatty acids) 18:2n-6/18:3n-3			
		16/1	16/4	30/1	35/4
Plasma		*(mmol/L plasma)*			
Triglyceride	1.2 ± 0.1	1.2 ± 0.3	1.3 ± 0.2	1.2 ± 0.2	1.4 ± 0.3
Total cholesterol	5.5 ± 0.8[a]	2.8 ± 0.3	2.8 ± 0.1	2.7 ± 0.3	2.5 ± 0.2
Free cholesterol	2.2 ± 0.4[a]	1.2 ± 0.1	1.1 ± 0.1	0.9 ± 0.1	0.9 ± 0.1
Liver		*(μmol/g liver)*			
Triglyceride	6.8 ± 0.8	5.2 ± 0.8	4.3 ± 0.4	6.5 ± 1.2	5.2 ± 0.4
Total cholesterol	7.9 ± 0.7[a]	4.6 ± 0.4	5.3 ± 0.2	5.1 ± 0.2	5.5 ± 0.1
Free cholesterol	6.0 ± 0.4[a]	3.3 ± 0.4	2.8 ± 0.3	2.6 ± 0.1	2.8 ± 0.1
Phospholipid	38.7 ± 3.2[a]	27.6 ± 2.2[b]	28.2 ± 2.2[b]	28.0 ± 1.9[b]	33.4 ± 2.1[ab]
PL:cholesterol	5.0 ± 0.4	6.1 ± 0.3	5.3 ± 0.4	5.5 ± 0.3	6.1 ± 0.4
		(mg/ g liver)			
Protein	49.5 ± 4.5	47.0 ± 1.8	44.1 ± 2.9	50.6 ± 3.1	44.6 ± 2.5
Cerebrum		*(μmol/mg protein)*			
Phospholipid	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	0.6 ± 0.0
Total cholesterol	0.4 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0
PL:cholesterol	1.6 ± 0.1	1.5 ± 0.1	1.6 ± 0.0	1.6 ± 0.1	1.6 ± 0.0
		(mg/g cerebrum)			
Protein	86.8 ± 1.5	80.0 ± 1.8	83.5 ± 3.3	86.9 ± 1.6	87.5 ± 1.6
Synaptic Plasma Membrane		*(μmol/mg protein)*			
Phospholipid	1.4 ± 0.0	1.3 ± 0.0	1.4 ± 0.1	1.6 ± 0.1	1.4 ± 0.1
Total Cholesterol	0.8 ± 0.0	0.8 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	0.9 ± 0.0
PL:cholesterol	1.6 ± 0.1	1.7 ± 0.0	1.6 ± 0.1	1.7 ± 0.0	1.6 ± 0.0
Retina		*(μmol/mg protein)*			
Phospholipid	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
Total cholesterol	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
PL:cholesterol	2.7 ± 0.6	3.2 ± 0.1	3.3 ± 0.0	3.2 ± 0.0	3.2 ± 0.1

[1]Piglets were housed as described in Materials and Methods. Piglets received sow milk from sows fed a usual pig diet with c.a. 2.5% (wt/wt) vegetable oil. Quantitative analyses of triglyceride, cholesterol, phospholipid and protein were performed as described in Materials and Methods. Values are means ± SEM, $n \geq 6$ for all groups. PL, phospholipid. The PL:cholesterol ratio is μmol:μmol. Significant differences were determined using one-way ANOVA and post hoc contrasts with Bonferroni correction. Values within a row with a different superscript letter are significantly different, $(p < 0.005)$.

Table 3.3

Results of 2-way ANOVA for significant differences due to interactive or independent effect of formula 18:2n-6 and 18:3n-3 content

Tissue fatty acid	Liver Phospholipid		Cerebrum Total Lipid		Synaptic Plasma Membrane EPL[1]		Retina EPL	
	(effect)	(*p*-value)	(effect)	(*p*-value)	(effect)	(*p*-value)	(effect)	(*p*-value)
16:0	−18:3n-3[2]	< 0.0001	18:2n-6 × 18:3n-3[3]	0.05		NS[4]		NS
18:0		NS	+18:2n-6 +18:3n-3	0.03 0.02		NS	18:2n-6 × 18:3n-3	0.04
Σ saturated fatty acids[5]	+18:2n-6 −18:3n-3	0.01 0.0001	18:2n-6 × 18:3n-3	0.03		NS	−18:3n-3	0.05
18:1	−18:2n-6	< 0.0001	−18:2n-6 −18:3n-3	0.0006 0.0029	−18:2n-6	0.03	−18:2n-6	< 0.0001
Σ monounsaturated fatty acids[6]	−18:2n-6	< 0.0001	−18:2n-6 −18:3n-3	< 0.0001 0.0007	−18:2n-6	0.02	−18:2n-6	< 0.0001
18:2n-6	+18:2n-6	< 0.0001	18:2n-6 × 18:3n-3	0.0002	+18:2n-6	0.002	+18:2n-6 +18:3n-3	< 0.0001 0.0003
20:4n-6	−18:3n-3	0.02	+18:2n-6	0.0007	−18:3n-3	0.02	−18:3n-3	0.0008
22:4n-6	18:2n-6 × 18:3n-3	< 0.0001	+18:2n-6	0.003	−18:3n-3	0.004	18:2n-6 × 18:3n-3	0.0012
22:5n-6	+18:2n-6 −18:3n-3	0.0081 < 0.0001	18:2n-6 × 18:3n-3	0.03	18:2n-6 × 18:3n-3	0.05	18:2n-6 × 18:3n-3	0.0002
22:5n-3	+18:3n-3	< 0.0001	+18:3n-3	< 0.0001	18:2n-6 × 18:3n-3	0.02	+18:3n-3	0.01
22:6n-3	−18:2n-6 +18:3n-3	0.02 < 0.0001	+18:3n-3	< 0.0001	+18:3n-3	0.0002	+18:3n-3	0.0001
22:6n-3: 22:5n-6	18:2n-6 × 18:3n-3	0.05	−18:2n-6 +18:3n-3	0.05 < 0.0001	18:2n-6 × 18:3n-3	0.05	+18:3n-3	< 0.0001

[1]EPL, ethanolamine phospholipids
[2]independent effects of formula 18:2n-6 and 18:3n-3 designated as either positive (+) or negative (−)
[3]18:2n-6 × 18:3n-3 indicates significant interactive effect of formula 18:2n-6 and 18:3n-3
[4]NS, not statistically significant, *p* > 0.05
[5]total saturated fatty acids: 14:0, 16:0, 18:0, 20:0 and 22:0
[6]total monounsaturated fatty acids: 14:1, 16:1, 18:1, 20:1 and 22:1.
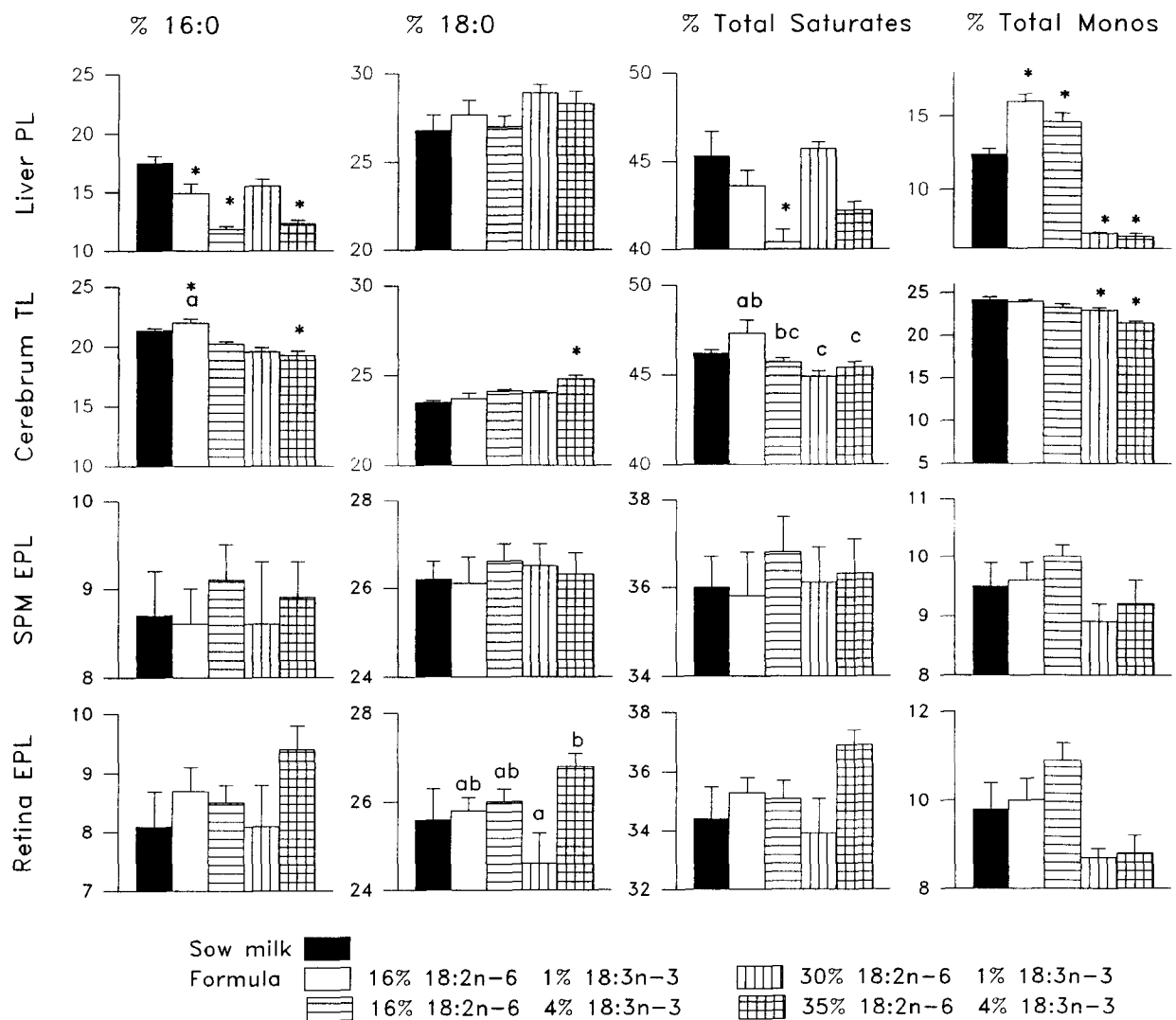
Figure 3.1 Major saturated and monounsaturated fatty acids (% total fatty acids) (mean ± SEM) in liver phospholipid (PL), brain total lipid (TL), synaptic plasma membrane ethanolamine phospholipids (SPM EPL) and retina EPL in 15 day-old piglets fed sow milk (solid bars) or formulas containing (% 18:2n-6 / % 18:3n-3): 16/1 (open bars), 16/4 (horizontal bars), 30/1 (vertical bars), or 35/4 (crossed bars). Individual group comparisons were determined using post hoc contrasts with Bonferroni correction, where the p-value from Table 3.3 indicated a significant interaction of the 18:2n-6 and 18:3n-3 (*i.e.* 18:2n-6 × 18:3n-3). Significant independent effects of the formula 18:2n-6 and 18:3n-3 content are given in Table 3.3, and not indicated in the figure. Values for formula-fed piglets with a different superscript letter are significantly different, $p < 0.008$. *indicates values significantly different ($p < 0.013$) from piglets fed sow milk.

liver in that it was influenced by the accompanying amount of 18:2n-6 in the formula ($p < 0.05$). Piglets fed the formulas containing 30 and 35% 18:2n-6 had significantly lower levels of 16:0 in cerebrum total lipid than piglets fed the formulas containing 16% 18:2n-6. In contrast to the results in the liver, there was no significant difference in cerebrum % 16:0 in piglets fed the 35/4 and 30/1 formulas, but the level of 16:0 in cerebrum was significantly lower in piglets fed the 16/4 compared to the 16/1 formula.

Compared to the piglets fed sow milk, the percentage of 16:0 in liver phospholipid was significantly lower in piglets fed the 16/1, 16/4 and 35/4 formulas. Cerebrum total lipid % 16:0 was significantly higher than the reference milk-fed group in the piglets fed the 16/1 formula, and significantly lower in piglets fed the 35/4 formula.

3.1.3.1.2 Levels of 18:0 in tissue membrane lipids

There was no evidence of a significant effect of formula 18:2n-6 or 18:3n-3 content on the percentage of 18:0 in liver phospholipid or synaptic plasma membrane EPL (Table 3.3). Unlike the % 16:0 which was lower, the level of 18:0 in cerebrum total lipid was significantly higher in piglets fed the formulas containing 30 or 35% 18:2n-6 rather than 16% 18:2n-6 ($p < 0.03$). Levels were also higher in piglets fed the formulas containing 4% 18:3n-3 compared to those fed formulas with 1% 18:3n-3 ($p < 0.02$). A similar effect of 4% 18:3n-3 in formula was seen on the percentage of 18:0 in retina EPL, but was dependent on the amount of 18:2n-6 in the formula ($p < 0.04$). The proportion of 18:0 was significantly higher in retina EPL of piglets fed the 35/4 formula compared to piglets fed the 30/1 formula, but not significantly different between piglets fed the 16/4 and the 16/1 formula.

The levels of 18:0 in all tissues of formula-fed piglets were not significantly different from piglets fed sow milk. The only exception was a significantly higher level of 18:0 in cerebrum total lipid of piglets fed the 35/4 formula.

3.1.3.1.3 Levels of total saturated fatty acids in tissue membrane lipids

There was no significant effect of the amount of 18:2n-6 or 18:3n-3 in the formula on the percentage of total saturated fatty acids in synaptic plasma membrane EPL. The proportion of total saturated fatty acids

in liver phospholipid was significantly higher in piglets fed the formulas containing 30 or 35% 18:2n-6 rather than 16% 18:2n-6 ($p \leq 0.0001$). Piglets fed the formulas containing 4% 18:3n-3 had significantly lower total saturated fatty acids in both liver phospholipid ($p < 0.01$) and retina EPL ($p < 0.05$) than piglets fed the formulas containing 1% 18:3n-3. The effect of formula 18:2n-6 and 18:3n-3 on the level of total saturated fatty acids in the cerebrum was inter-dependent; the level of saturated fatty acids was significantly lower in pigets fed the 30/1 compared to the 16/1 formula, but not significantly different in piglets fed the 16/4 and 35/4 formulas.

Piglets fed the 16/4 formula had significantly lower liver phospholipid total saturated fatty acids than piglets fed sow milk (Fig. 3.1). The level of total saturated fatty acids was not significantly different in the CNS tissues of piglets fed formula or sow milk.

3.1.3.1.4 Levels of total monounsaturated fatty acids in tissue lipids

Consistent significant changes in the proportion of total monounsaturated fatty acids, predominantly 18:1, were seen in all tissues, in response to the formula 18:2n-6 content (Table 3.3). To manipulate the amount of 18:2n-6 in the formula, however, the amount of 18:1 in the formula was altered inversely (Table 2.3). Therefore, the changes in tissue levels of monounsaturated fatty acids, attributed to formula 18:2n-6, are probably most appropriately discussed with respect to the amount of 18:1 in the formula. The percentage of 18:1 and total monounsaturated fatty acids was significantly lower in liver phospholipid ($p < 0.0001$), cerebrum total lipid ($p \leq 0.0006$), synaptic plasma membrane ($p < 0.03$) and retina EPL ($p < 0.0001$) in piglets fed the formula containing high amounts of 18:2n-6 (i.e. low 18:1) than it was in piglets fed formulas containing low amounts of 18:2n-6 (i.e. high 18:1). The only tissue to show an effect of formula 18:3n-3, however, was cerebrum total lipid, with significantly lower levels of 18:1 and total monounsaturated fatty acids in piglets fed the formulas containing 4% 18:3n-3 (16/4 and 35/4) than in piglets fed formulas containing 1% 18:3n-3 (16/1 and 30/1).

The percentage of total monounsaturated fatty acids was significantly lower in liver phospholipid and cerebrum total lipid of piglets fed the 30/1 and 35/4 formulas (*i.e.* low 18:1 formulas) than in sow milk-fed piglets. Levels of monounsaturated fatty acids were significantly higher than reference piglets in liver phospholipid of piglets fed the 16/1 and 16/4 formulas (*i.e.* high 18:1 formulas).

53
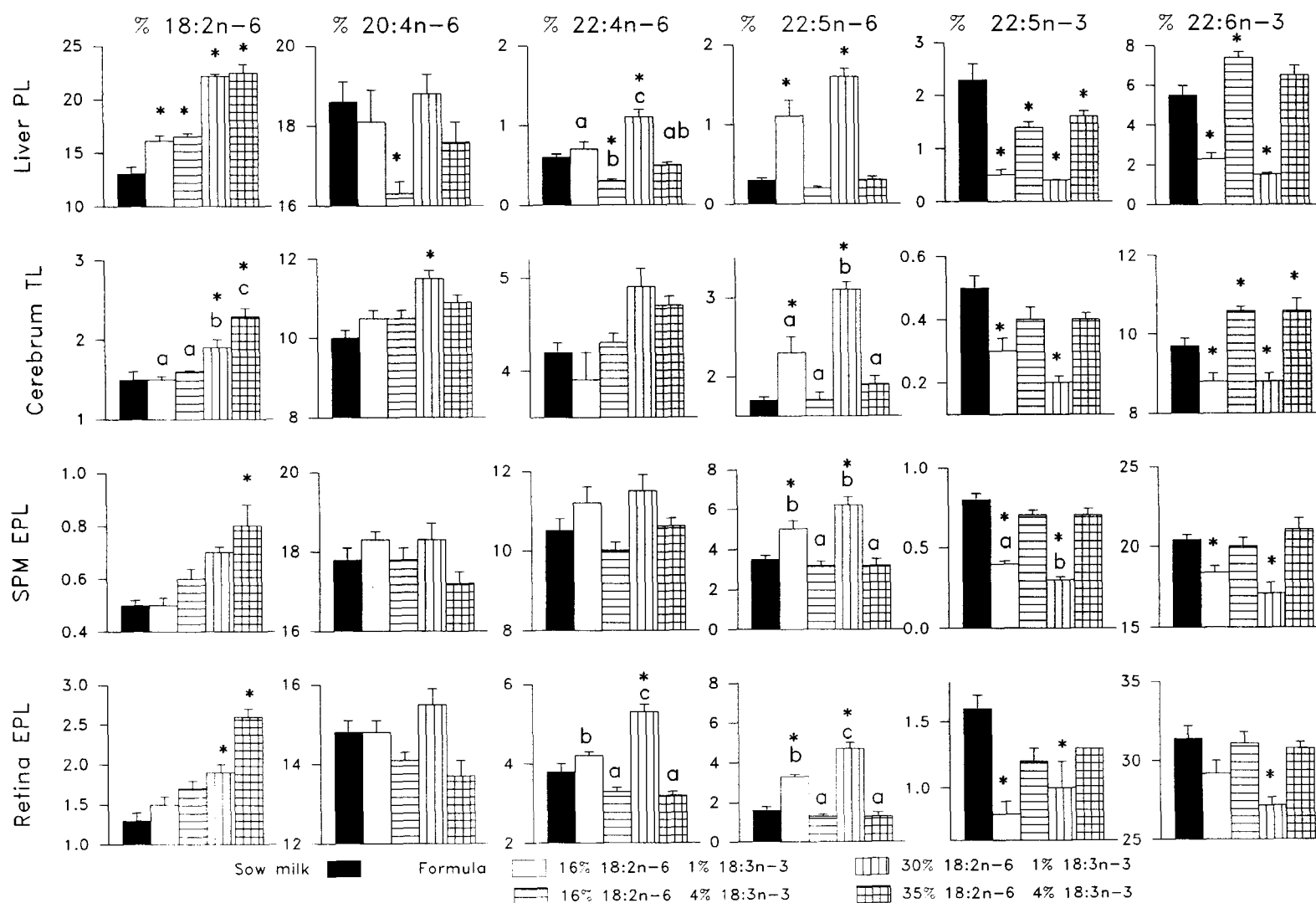
Figure 3.2 Major n-6 and n-3 fatty acids (% fatty acids) (mean ± SEM) in liver phospholipid (PL), cerebrum total lipid (TL), synaptic plasma membrane ethanolamine phospholipids (SPM EPL) and retina EPL from 15 day-old piglets fed sow milk (closed bars) or formulas containing (% 18:2n-6 / % 18:3n-3): 16/1 (open bars), 16/4 (horizontal bars), 30/1 (vertical bars), or 35/4 (crossed bars). Individual group comparisons were determined using post hoc contrasts with Bonferroni correction, where the p-value from Table 3.3 indicated a significant interaction of the 18:2n-6 and 18:3n-3 (*i.e.* 18:2n-6 × 18:3n-3). Significant independent effects of the formula 18:2n-6 and 18:3n-3 content are given in Table 3.3 and are not indicated in the figures. Values for piglets fed formula with a different superscript letter are significantly different, $p < 0.008$. *indicates values significantly different from piglets receiving sow milk, $p < 0.013$.

54

3.1.3.2 Liver and CNS tissue n-6 fatty acids.

3.1.3.2.1 Levels of 18:2n-6 in tissue membrane lipids

High amounts (30 and 35%) of 18:2n-6 in the formula fed to piglets resulted in significantly higher levels of 18:2n-6 in liver phospholipid ($p < 0.0001$), synaptic plasma membrane ($p < 0.002$) and retina EPL ($p < 0.0001$) over those in piglets fed low amounts (16%) of dietary 18:2n-6. Piglets fed the formulas containing 4% 18:3n-3 (16/4 and 35/4) had significantly higher levels of 18:2n-6 in the retina EPL ($p < 0.0003$) than piglets fed the formulas supplying 1% 18:3n-3 (16/1 and 30/1). In cerebrum total lipid, the effects of the formula 18:2n-6 and 18:3n-3 on the percentage of 18:2n-6 were inter-dependent ($p < 0.0002$). Levels of 18:2n-6 in cerebrum total lipid were significantly higher in both piglet groups fed the formulas containing high amounts of 18:2n-6 (30/1 and 35/4) than in those fed the formulas containing low amounts of 18:2n-6 (16/1 and 16/4). Although the level of 18:2n-6 in cerebrum total lipid was significantly lower in piglets fed the 30/1 formula than in those fed the 35/4 formula, there was no significant difference in piglets fed the 16/1 and 16/4 formulas.

The proportion of 18:2n-6 was significantly higher in the liver phospholipid of all the groups of formula-fed piglets compared to piglets fed sow milk. The percentage of 18:2n-6 was also significantly increased in CNS tissues in piglets fed the formula with 30 or 35% 18:2n-6 over that in piglets fed sow milk. The only exception to this general increase occurred in the synaptic plasma membrane EPL of piglets fed the 30/1 formula. In this case, the level of 18:2n-6 was not significantly different from the sow milk-fed reference group ($p = 0.02$) (Fig. 3.2).

3.1.3.2.2 Levels of 20:4n-6 in tissue membrane lipids

The effect of dietary fatty acids on the level of 20:4n-6 in the cerebrum differed from that in the liver and other CNS tissues among formula-fed piglets. Cerebrum total lipid % 20:4n-6 was significantly higher in piglets fed the formulas with 30 or 35% 18:2n-6 than in piglets fed the formulas containing 16% 18:2n-6 ($p < 0.0007$). In contrast to the cerebrum, the level of 20:4n-6 in liver phospholipid, synaptic plasma membrane and retina EPL was not influenced by formula 18:2n-6, but was significantly lower in piglets fed formulas supplying 4% 18:3n-3 (16/4 and 35/4) than in piglets fed the formulas containing 1% 18:3n-3 (16/1 and 30/1).

The percentage of 20:4n-6 in the liver and CNS tissues from the four groups of piglets fed formula was not significantly different from that of piglets fed sow milk. Two exceptions, however, were significantly higher

20:4n-6 in the cerebrum total lipid of piglets fed the 30/1 formula, and significantly lower % 20:4n-6 in liver phospholipid of piglets fed the 16/1 formula, than in the sow milk-fed piglets.

3.1.3.2.3. Levels of 22:4n-6 in tissue membrane lipids

The changes in levels of 22:4n-6 in the cerebrum and synaptic plasma membrane closely paralleled the changes in 20:4n-6 found in these tissues. The percentage of 22:4n-6 was significantly higher in cerebrum total lipid in piglets fed formulas with 30 or 35% 18:2n-6 than in piglets fed the formulas containing 16% 18:2n-6 ($p < 0.003$). Levels of 22:4n-6 were significantly lower in synaptic plasma membrane EPL in piglets fed formulas containing 4% 18:3n-3 compared to those fed formulas containing 1% 18:3n-3 ($p < 0.004$). The effects of formula 18:2n-6 and 18:3n-3 on 22:4n-6 composition in the liver and retina, however, were inter-dependent. The % 22:4n-6 was significantly lower in piglets fed the 16/4 and 35/4 formulas compared with those fed the 16/1 and 30/1 formulas, and significantly higher in piglets fed the 30/1 compared to the 16/1 formula (Fig. 3.2).

The level of 22:4n-6 was significantly higher than sow milk-fed piglets in liver phospholipid and retina EPL in piglets fed the 30/1 formula and significantly lower in liver phospholipid in piglets fed the 16/4 formula.

3.1.3.2.4 Levels of 22:5n-6 in tissue membrane lipids

Piglets fed the formula with 30 or 35% 18:2n-6 had significantly higher levels of 22:5n-6 in liver phospholipid than piglets fed formulas containing 16% 18:2n-6 ($p < 0.008$). The percentage of 22:5n-6 in liver phospholipid was also significantly higher in piglets fed the formulas supplying 1% 18:3n-3 (16/1 and 30/1) than in those fed formulas containing 4% 18:3n-3 (16/4 and 35/4) ($p < 0.0001$). Similar, but inter-dependent effects of the amount of 18:2n-6 and 18:3n-3 in formula were seen in cerebrum total lipid ($p \leq 0.03$), synaptic plasma membrane ($p \leq 0.05$) and retina EPL ($p \leq 0.0002$). The level of 22:5n-6 in CNS tissues was significantly higher in piglets fed the 16/1 and 30/1 formulas compared to those fed the 16/4 and 35/4 formulas. The only exception was no significant difference ($p = 0.009$) in synaptic plasma membrane EPL between piglets fed the 16/1 formula and the 30/1 formula. As in the liver, the level of 22:5n-6 in CNS tissues was significantly higher in piglets fed the 30/1 formula than in those fed the 16/1 formula; however, there was no significant difference between piglets fed the 16/4 and 35/4 formulas.

Tissue levels of 22:5n-6 are known to increase in animals fed n-3 fatty acid-deficient diets. Piglets fed

the formulas with 1% 18:3n-3 (16/1 and 30/1) had significantly higher tissue levels of 22:5n-6 than piglets fed

sow milk. In contrast, there was no evidence of a significant difference in the level of 22:5n-6 in piglets fed

formulas containing 4% 18:3n-3 (16/4 and 35/4) and those fed sow milk.

3.1.3.2.5 Predicted response of 20:4n-6 in liver and plasma phospholipid using the Lands equation (1991)

Levels of 20:4n-6 as a percentage of total LCP in piglet plasma and liver phosholipid were predicted by

the hyperbolic equation of Lands (1991), based on the % of energy from 18:2n-6 and 18:3n-3 in the formula.

This equation closely approximated the actual measured 20:4n-6 as a percentage of total LCP found in piglets

fed the formulas with 8, 9 or 15% of energy as 18:2n-6 (**Figure 3.3**). The predicted value for the plasma and

liver phospholipid 20:4n-6 of piglets fed the formula with 8% of energy as 18:2n-6 (*i.e.* 16/1 formula) was within

the range of the SEM (about 10% of the observed mean) of the measured value. These equations developed

by Lands et al (1990) are based on experimental data from rats fed diets containing 0 to 13% of dietary energy

from 18:2n-6 and 0 to 12% of energy from 18:3n-3. There was a discrepancy in the observed and predicted values

for both liver and plasma phospholipid of piglets fed the formula with 19% energy 18:2n-6 (*i.e.* 35/4 formula)

(Fig. 3.3). The deviation of the predicted from the actual values was of similar magnitude for both plasma and

liver phospholipid.
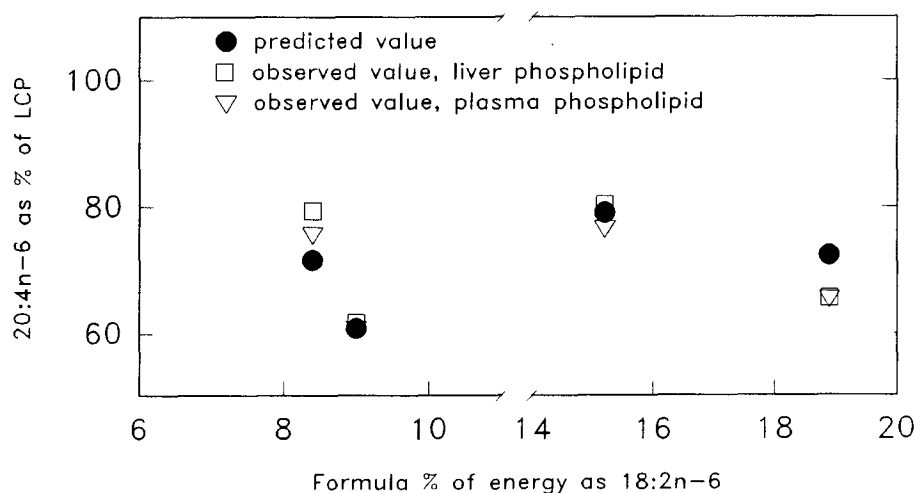
Figure 3.3 Observed values for liver (open squares) and plasma (open triangle) phospholipid (PL) 20:4n-6 as a percentage of total LCP in piglets fed the formulas with (% of dietary energy) 8% (16/1 formula), 9% (16/4 formula), 15% (30/1 formula) or 19% 18:2n-6 (35/4 formula) compared to values predicted (closed circles) by the equation of Lands (1991): 100/ [1 + (0.07/energy % 18:2n-6) [1 + (energy % 18:3n-3/0.05 + energy % other fatty acids/2 + energy % 18:2n-6/0.50)]]. The break in the axis designates the upper limit for which the equations were devised (*i.e.* 13% of dietary energy as 18:2n-6) (Lands et al 1990).

3.1.3.3 Liver and CNS tissue n-3 fatty acids

3.1.3.3.1 Levels of 18:3n-3 and 20:5n-3 in tissue membrane lipids

There was no significant effect of diet on the level of 18:3n-3 or 20:5n-3 in the synaptic plasma membrane and retina EPL (data not shown). Formula containing 4% 18:3n-3 was associated with a significantly higher proportion of 18:3n-3 in liver phospholipid and cerebrum total lipid than formula containing 1% 18:3n-3 ($p <$ 0.04); however, the level of 18:3n-3 in the liver and CNS tissue fatty acids was consistently $\leq$ 0.3% (data not shown). Levels of 20:5n-3 were $\leq$ 0.2% in CNS tissues (data not shown), irrespective of the formula or sow milk fed. The percentage of 20:5n-3 in liver phospholipid was influenced inter-dependently by formula 18:2n-6 and 18:3n-3 ($p <$ 0.0001). Piglets fed the 16/4 formula had a significantly higher level of 20:5n-3 ($p <$ 0.0001) than piglets fed the 35/4 formula (0.8 $\pm$ 0.1, 0.3 $\pm$ 0.0 %, respectively). As well, the % 20:5n-3 in the liver phospholipid of piglets fed the 16/4 and 35/4 formulas was significantly higher than in piglets fed the 30/1 and 16/1 formulas ($<$ 0.1% of fatty acids).

3.1.3.3.2 Levels of 22:5n-3 in tissue membrane lipids

The level of 22:5n-3 was significantly lower in liver phospholipid ($p <$ 0.0001), cerebrum total lipid ($p <$ 0.0001) and retina EPL ($p <$ 0.01) of piglets fed the formulas containing 1% 18:3n-3 than in piglets fed the formulas supplying 4% 18:3n-3. The synaptic plasma membrane EPL % 22:5n-3 was influenced inter-dependently by the formula 18:2n-6 and 18:3n-3 content ($p <$ 0.02). The proportion of 22:5n-3 in synaptic plasma membrane EPL was significantly lower in piglets fed the 16/1 and 30/1 formulas compared with those fed the 16/4 and 35/4 formulas. It was also lower in piglets fed the 30/1 formula compared with piglets fed the 16/1 formula (Fig. 3.2).

Piglets fed the 16/1 and 30/1 formulas had significantly lower levels of 22:5n-3 than sow milk-fed piglets in the liver and CNS tissues. Liver phospholipid % 22:5n-3 was also significantly lower in animals fed the 16/4 and 35/4 formulas than in the milk-fed reference group (Fig. 3.2).

3.1.3.3.3 Levels of 22:6n-3 in tissue membrane lipids

The liver phospholipid, but not the CNS tissue 22:6n-3 was significantly lower in the piglets fed the 30/1 and 35/4 formulas compared with those fed the 16/1 and 16/4 formulas ($p < 0.02$). The percentage of 22:6n-3 was significantly higher ($p \leq 0.0002$) in the liver and CNS tissues of piglets fed the formulas containing 4% 18:3n-3 compared to 1% 18:3n-3.

Piglets fed the 30/1 and 16/1 formulas had significantly lower tissue levels of 22:6n-3 than piglets fed sow milk. An exception was retina EPL % 22:6n-3 of piglets fed the 16/1 formula, which was not significantly different from sow milk-fed piglets. The level of 22:6n-3 in liver phospholipid of piglets fed the 16/4 formula, and in cerebrum total lipid of piglets fed the 35/4 and 16/4 formulas, was significantly higher than in reference piglets (Fig. 3.2).

3.1.3.4 Liver and CNS tissue 22:6n-3:22:5n-6 ratio

Piglets fed high amounts (30 and 35%) of 18:2n-6 in formula had a significantly lower 22:6n-3:22:5n-6 ratio in cerebrum total lipid than piglets fed the formula with low amounts (16%) of 18:2n-6 ($p < 0.05$). The ratio of 22:6n-3 and 22:5n-6 in retina EPL was significantly lower in piglets fed the formulas containing 1% 18:3n-3 compared with those fed formulas containing 4% 18:3n-3 ($p < 0.0001$). Inter-dependent effects of the formula 18:2n-6 and 18:3n-3 were found for synaptic plasma membrane EPL and liver phospholipid; the 22:6n-3:22:5n-6 ratio was significantly higher in piglets fed the 16/4 and 35/4 formulas compared with piglets fed the 16/1 and 30/1 formulas. The ratio of 22:6n-3 to 22:5n-6 was significantly lower in liver phospholipid of piglets fed the the 35/4 formula compared with those fed the 16/4 formula.

The ratio of 22:6n-3 and 22:5n-6 in tissue lipids has been proposed as a sensitive indicator of the dietary n-3 fatty acid adequacy (Galli et al 1974). The 22:6n-3:22:5n-6 ratio in the liver and CNS tissue lipids was not significantly different between piglets fed the formulas with 4% 18:3n-3 and piglets fed sow milk, except for a significantly higher 22:6n-3:22:5n-6 ratio in liver phospholipid of piglets fed the 16/4 formula. In contrast, the tissue lipid 22:6n-3:22:5n-6 ratio was significantly lower than sow milk-fed piglets, in liver and CNS tissues of piglets fed the formulas containing 1% 18:3n-3 (16/1 and 30/1).
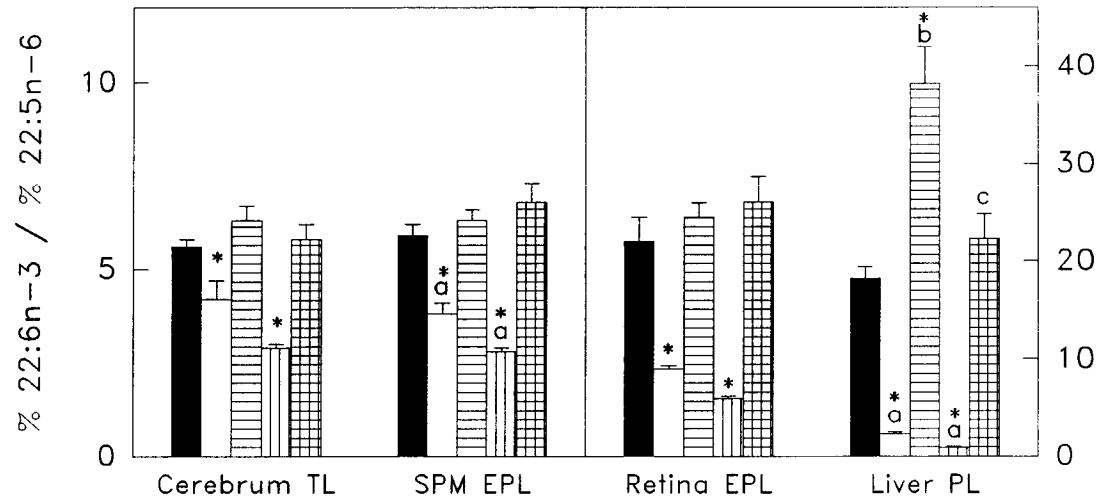
Figure 3.4 Ratio of 22:6n-3 to 22:5n-6 (mean ± SEM) in cerebrum total lipid (TL), synaptic plasma membrane ethanolamine phospholipids (SPM EPL), retina EPL and liver phospholipid (PL) fatty acids from 15 day-old piglets fed sow milk (solid bars), or formulas containing (% 18:2n-6 / % 18:3n-3): 16/1 (open bars), 16/4 (horizontal bars), 30/1 (vertical bars), or 35/4 (crossed bars). Individual group comparisons were determined using post hoc contrasts with Bonferroni correction, where the p-value indicated a significant interaction between the formula 18:2n-6 and 18:3n-3 (*i.e.* 18:2n-6 × 18:3n-3). Values for piglets fed formula within a tissue with a different superscript letter are significantly different, $p < 0.008$. *indicates values significantly different from piglets receiving sow milk, $p < 0.013$.

3.1.4 Relative importance of 18:3n-3 content compared to 18:2n-6:18:3n-3 ratio in formula on piglet tissue 22:6n-3 level

The relationship between the proportion of 22:6n-3 in the piglet tissues and 18:2n-6:18:3n-3 ratios in the formula fed is in **Figure 3.5**. Evidence of a significant ($p \leq 0.0002$) inverse linear relationship was found between the level of 22:6n-3 in the piglet liver, cerebrum, synaptic plasma membrane and retina and the formula 18:2n-6:18:3n-3 ratio. The R^2, derived from the two-way ANOVA and regression analyses indicated that 81% of the variability in cerebrum total lipid 22:6n-3 could be explained by the formula 18:3n-3 content, whereas 65% of the variability could be explained by the 18:2n-6:18:3n-3 ratio of the formula. The variability in tissue 22:6n-3 accounted for by the amount of 18:3n-3 in the formula or the 18:2n-6:18:3n-3 ratio, respectively, was 91% vs 83% for liver phospholipid, 57% vs 50% for synaptic plasma membrane EPL, and 58% vs 55% for retina EPL. These results suggest that the dietary 18:2n-6:18:3n-3 ratio may be relatively more important in synaptic plasma membrane, retina and liver than in cerebrum total lipid, where the effect of the formula 18:3n-3 content is most prominent.

60
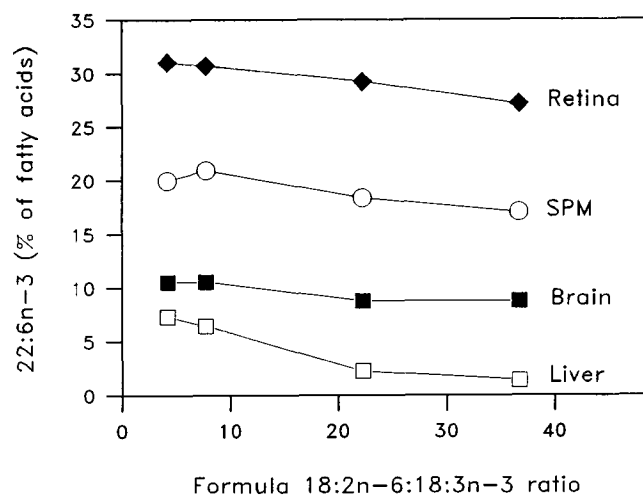
Figure 3.5 Relationship between the 18:2n-6:18:3n-3 ratio in the formulas fed and the % 22:6n-3 in fatty acids of liver phospholipid (open squares), cerebrum total lipid (closed squares), synaptic plasma membrane ethanolamine phospholipids (open circles) and retina ethanolamine phospholipids (closed diamonds) in 15 day-old piglets. Points represent mean ± SEM (error bars do not signify because the SEM is smaller than the symbol shown).

3.2 Experiment II: Effect of feeding formula containing low or high 18:3n-3, or low 18:3n-3 plus fish oil on tissue n-6 and n-3 fatty acids

3.2.1 Growth

No significant differences ($p > 0.05$) were found in body wt: (mean ± SEM) 4.1 ± 0.4, 4.5 ± 0.3, 4.4 ± 0.3 and 4.3 ± 0.3 kg, or in cerebrum wt: 35.0 ± 1.0, 35.0 ± 1.0, 32.3 ± 0.6 and 33.3 ± 1.1 g, in 15 day-old piglets fed sow milk, 16/1, 16/4 or 16/1+FO formulas, respectively.

3.2.2 Piglet tissue n-6 and n-3 LCP

The n-6 and n-3 LCP composition of synaptic plasma membrane and retina EPL of piglets fed sow milk or formulas containing 16% 18:2n-6 with 0.7% 18:3n-3 (16/1 formula), 3.9% 18:3n-3 (16/4 formula), or 0.6% 18:3n-3 plus 0.8% n-3 LCP (16/1+FO formula) are given in **Figure 3.6**. The results show, as in Experiment I (Fig. 3.2), significantly higher levels of 22:4n-6 and 22:5n-6 and lower 22:6n-3 in the synaptic plasma membrane EPL of piglets fed the 16/1 rather than 16/4 formula or sow milk. The inclusion of fish oil in the formula resulted in a significantly higher percentage of 22:6n-3 in the piglet synaptic plasma membrane EPL than in piglets

61
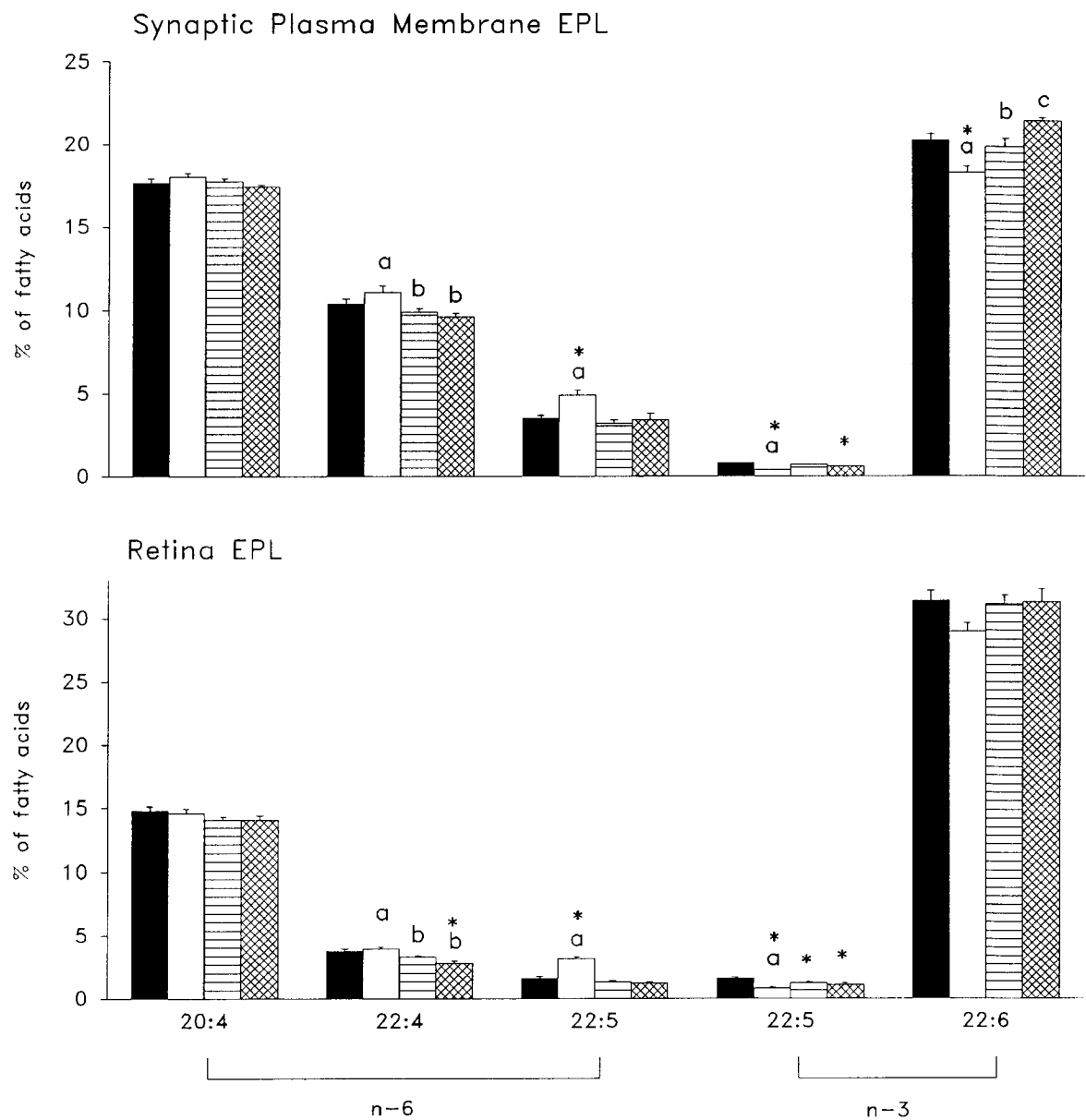
Figure 3.6 Effect of dietary 18:3n-3 and/or n-3 LCP on n-6 and n-3 LCP composition of synaptic plasma membrane and retina ethanolamine phospholipids (EPL) in 15 day-old piglets. The piglets were fed sow milk (closed bars) or formulas containing 16% 18:2n-6 with: 0.7% of fatty acids as 18:3n-3 (16/1, open bars), 3.9% 18:3n-3 (16/4, horizontal bars), or 0.6% 18:3n-3 + 0.4, 0.1 and 0.3% of fatty acids as 20:5n-3, 22:5n-3 and 22:6n-3, respectively (16/1 + FO, hatched bars). Values are mean ± SEM (n = 6, 6 and 5, respectively). Values for formula-fed piglets with a different superscript ([abc]) are significantly different, (p < 0.017); *indicates values significantly different from piglets receiving sow milk, p < 0.017.

62

fed the 16/1 and 16/4 formulas. The level of 22:6n-3 in piglets fed the 16/1 + FO formula or the 16/4 formula, however, was not significantly different from that of piglets fed sow milk. No significant differences were found in retina EPL 22:6n-3 by one-way ANOVA.

Feeding the 16/1+FO formula resulted in a significantly lower percentage of 22:4n-6 in retina EPL, compared to piglets receiving sow milk or the 16/1 formula. The proportion of 20:4n-6 in the synaptic plasma membrane and retina EPL, however, remained constant, irrespective of milk or formula feeding.

3.2.3 Efficacy of 18:3n-3 and fish oil as a source of n-3 fatty acids for deposition of 22:6n-3 in CNS lipids

The results of this study show that piglets fed formula containing 4% of fatty acids as 18:3n-3, and formula with 0.6% 18:3n-3 plus 0.8% n-3 LCP from fish oil achieve levels of 22:6n-3 in the growing synaptic plasma membranes and retina similar to that of piglets fed sow milk. Thus, dietary 18:3n-3 is at least 20% as efficient as n-3 LCP (*i.e.* 0.8/4 × 100%) in providing a source of n-3 fatty acids for synthesis and deposition of 22:6n-3 in developing CNS lipids.

3.3 Experiment III: The relationship of formula n-3 LCP to the piglet tissue saturated, monounsaturated, n-6 and n-3 fatty acid composition

3.3.1 Growth

The mean piglet birth wt, body and brain wt at 15 days and the intake of formula containing 30% 18:2n-6 and 0.8% 18:3n-3 (F), formula + 2 g fish oil/L (FO1) and formula + 6 g fish oil/L (FO3) are given in **Table 3.4**. No significant differences ($p > 0.05$) were found in body weight at birth, or at 15 days of age. The brain wt of FO3-fed piglets was significantly less ($p < 0.001$) than that of all other groups. The brain:body wt ratio of the FO3-fed group, however, was similar to that of the other piglet groups. The reason for the lower brain wt, and any possible relationship to the lower formula intake of the FO3-fed group compared with the group fed the F diet during the first 5 days after birth, is unclear. The formula intake did not differ among the groups for days 6 − 10, 11 − 15, or when considered as the cumulative 15-day intake.

Table 3.4

Birth weight, body and brain[1] weights and formula intake of 15 day-old piglets fed formula (F) or formula supplemented with 2 (FO1) or 6 (FO3) g of fish oil/L[2]

	F	FO1	FO3
Birth wt (kg)	1.5 ± 0.1	1.3 ± 0.1	1.2 ± 0.1
Body wt (kg)	4.4 ± 0.3	3.9 ± 0.3	3.7 ± 0.2
Brain wt (g)	42.2 ± 1.0	39.5 ± 1.3	35.8 ± 0.9[a]
Brain:body wt (x 100)	0.9 ± 0.1	1.0 ± 0.1	1.0 ± 0.1
Formula intake (L)			
day 0 − 5	2.8 ± 0.3[a]	2.7 ± 0.2[ab]	2.1 ± 0.2[b]
day 6 − 10	3.5 ± 0.4	3.3 ± 0.3	2.9 ± 0.2
day 11 − 15	3.8 ± 0.5	3.1 ± 0.2	3.0 ± 0.2
Total volume	10.0 ± 1.1	9.1 ± 0.6	8.0 ± 0.5

[1]Weight of entire brain, including the cerebrum, cerebellum and brain stem
[2]F, formula containing 30% 18:2n-6 and 0.8% 18:3n-3; FO1, formula + 2 g menhaden oil/L; FO3, formula + 6 g menhaden oil/L. Values are means ± SEM, $n=6$ for all groups. Significant differences were determined using one-way ANOVA and post hoc contrasts with Bonferroni correction. Values with a different superscript letter are significantly different, $p < 0.017$.

3.3.2 The effect of formula n-3 LCP content on saturated, monounsaturated, n-6 and n-3 fatty acids in piglet plasma, liver and CNS tissues

3.3.2.1 Piglet plasma and tissue saturated and monounsaturated fatty acids

The effect of the formulas on the total saturated and monounsaturated fatty acid composition of the piglet tissues is shown in **Figure 3.7**. The addition of fish oil to the formula had no significant effect on the saturated or monounsaturated fatty acids in the tissues of piglets fed any of the formulas, except for the level of monounsaturated fatty acids in brain total lipid and retina EPL, which showed a linear decrease ($p < 0.009$) with increased formula n-3 LCP.
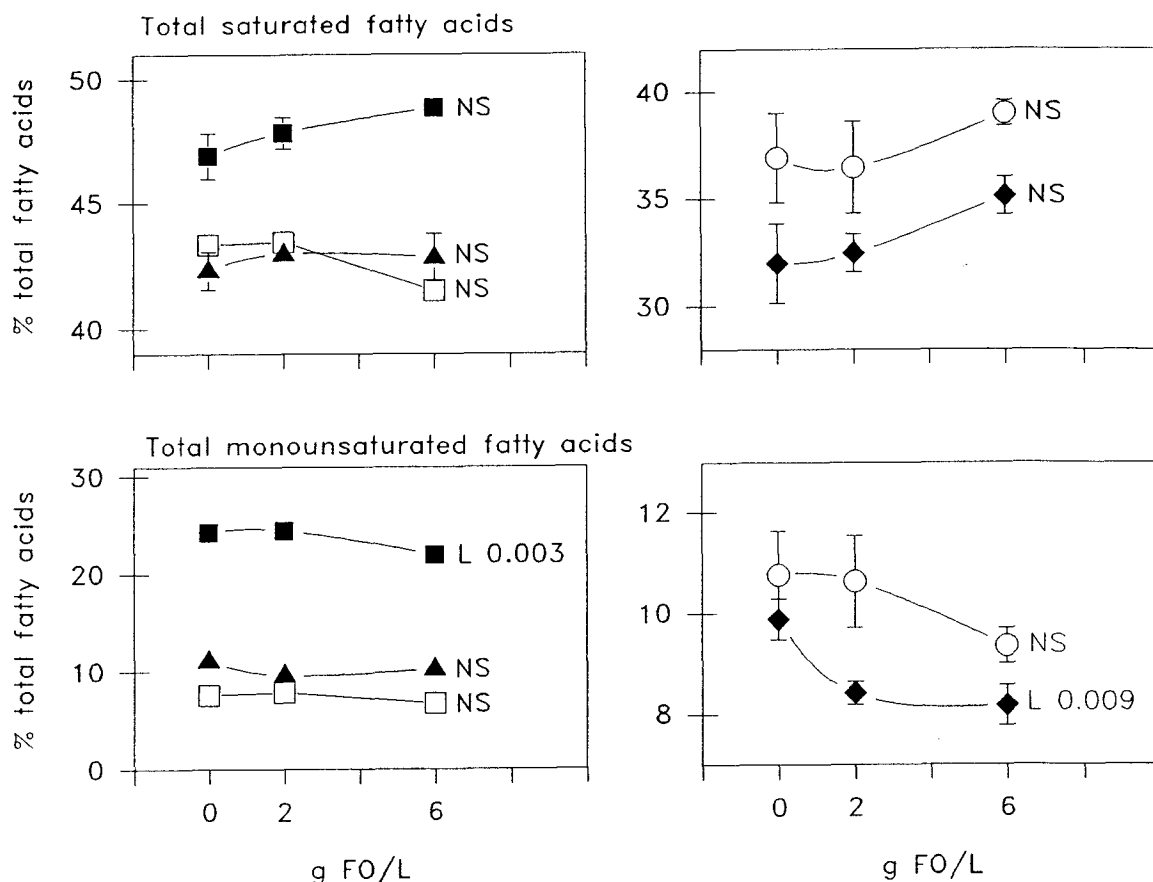
Figure 3.7 Effect of increasing fish oil addition [in g fish oil (FO)/L] to formula on % total saturated and monounsaturated fatty acids in piglet plasma (closed triangle) and liver (open square) total phospholipid, brain total lipid (closed square), and synaptic plasma membrane (open circle) and retina (closed diamond) phosphatidylethanolamine. Points represent mean ± SEM (where error bars do not signify, the SEM is smaller than the symbol shown). p-values of orthogonal contrast analyses are given and results designated as linear (L), quadratic (Q) or not statistically significant ($p > 0.05$) (NS).

3.3.2.2 Piglet plasma and tissue n-6 LCP

The orthogonal contrast analyses demonstrated a significant linear decrease in the plasma phospholipid, brain total lipid, synaptic plasma membrane and retina EPL total n-6 LCP, and a non-linear (quadratic) decrease in the liver phospholipid total n-6 LCP with increased addition of fish oil to the formula (**Figure 3.8**). The statistical analysis to describe the nature of the significant changes in individual n-6 LCP showed that the decrease in the plasma and liver phospholipid 22:4n-6 and 22:5n-6 was curvilinear (quadratic); the decrease for the liver phospholipid and retina EPL % 20:4n-6 was linear ($p \leq 0.0001$) from the F to FO3 group. These results indicate that maximum potential depletion of plasma, liver and retina n-6 LCP had not been reached.

In contrast, the brain total lipid and synaptic plasma membrane EPL % 20:4n-6 was not significantly altered by the addition of fish oil to the formula. The brain total lipid, synaptic plasma membrane EPL and retina EPL percentage of 22:4n-6 and the synaptic plasma membrane EPL 22:5n-6 showed significant linear decreases

with the increase from 0 to 6 g/L fish oil in the formula. Non-linear (quadratic) decreases in the percentage of 22:5n-6 in brain total lipid and retina EPL also occurred and are explained by a smaller decrease in 22:5n-6 between the FO1 and FO3 dietary groups than between the F- and FO1-fed groups. The greater decrease between the F- and FO1-fed groups is probably a correction of the compensatory increase in n-6 LCP caused by an inadequate supply of n-3 fatty acids to support CNS 22:6n-3 accretion in piglets fed the F (30% 18:2n-6, 1% 18:3n-3) formula, as determined in Experiment I (Fig. 3.2).

3.3.2.3 Piglet plasma and tissue n-3 LCP

Increasing the level of n-3 LCP in the formula resulted in a significant quadratic, rather than linear, increase in the proportion of total n-3 LCP, 20:5n-3, 22:5n-3 and 22:6n-3 in the plasma and liver phospholipid and brain total lipid, and in the percentage of total n-3 LCP and 22:6n-3 in retina EPL (**Figure 3.9**). Exceptions were the liver phospholipid 22:5n-3 and brain total lipid 20:5n-3, which showed linear ($p < 0.0001$, $p < 0.009$, respectively) increases. The percent total n-3 LCP, 22:5n-3 and 22:6n-3 increased linearly ($p < 0.001$) in the synaptic plasma membrane EPL with increasing n-3 LCP in the formula. The shape of the curve (quadratic) describing the response of the tissue n-3 LCP to the dietary n-3 LCP for the brain differed from that for the other tissues. In plasma and liver phospholipid and retina EPL, the individual and total n-3 LCP showed an immediate and greater increase between the F and FO1, than between the FO1 and FO3 dietary groups. In contrast, an apparent lag in response of the brain is suggested by the greater increase in brain 22:5n-3, 22:6n-3 and total n-3 LCP from the FO1 to FO3 than from the F to FO1 dietary groups. The significantly higher levels of 22:6n-3 and total n-3 LCP in the plasma and liver phospholipid, brain total lipid and synaptic plasma membrane, but not retina EPL in piglets fed the FO3 compared to FO1 formula suggests that tissue saturation with n-3 LCP was not attained with the FO3 diet. Thus, feeding higher amounts of fish oil could lead to excess accumulation of these fatty acids in tissues other than the retina.

No significant difference was found in the synaptic plasma membrane EPL percentage of 20:5n-3 among the piglets fed the three formula diets; however, a significant linear increase in 20:5n-3 was found in retina EPL (Fig. 3.9). The 20:5n-3 represented $< 0.5\%$ of the fatty acids in the retina, as well as in the synaptic plasma membrane EPL and brain total lipid. It seems unlikely, therefore, that the small, although significant, diet-related changes in brain total lipid and retina EPL % 20:5n-3 were of any physiological significance.

Total n−6 LCP
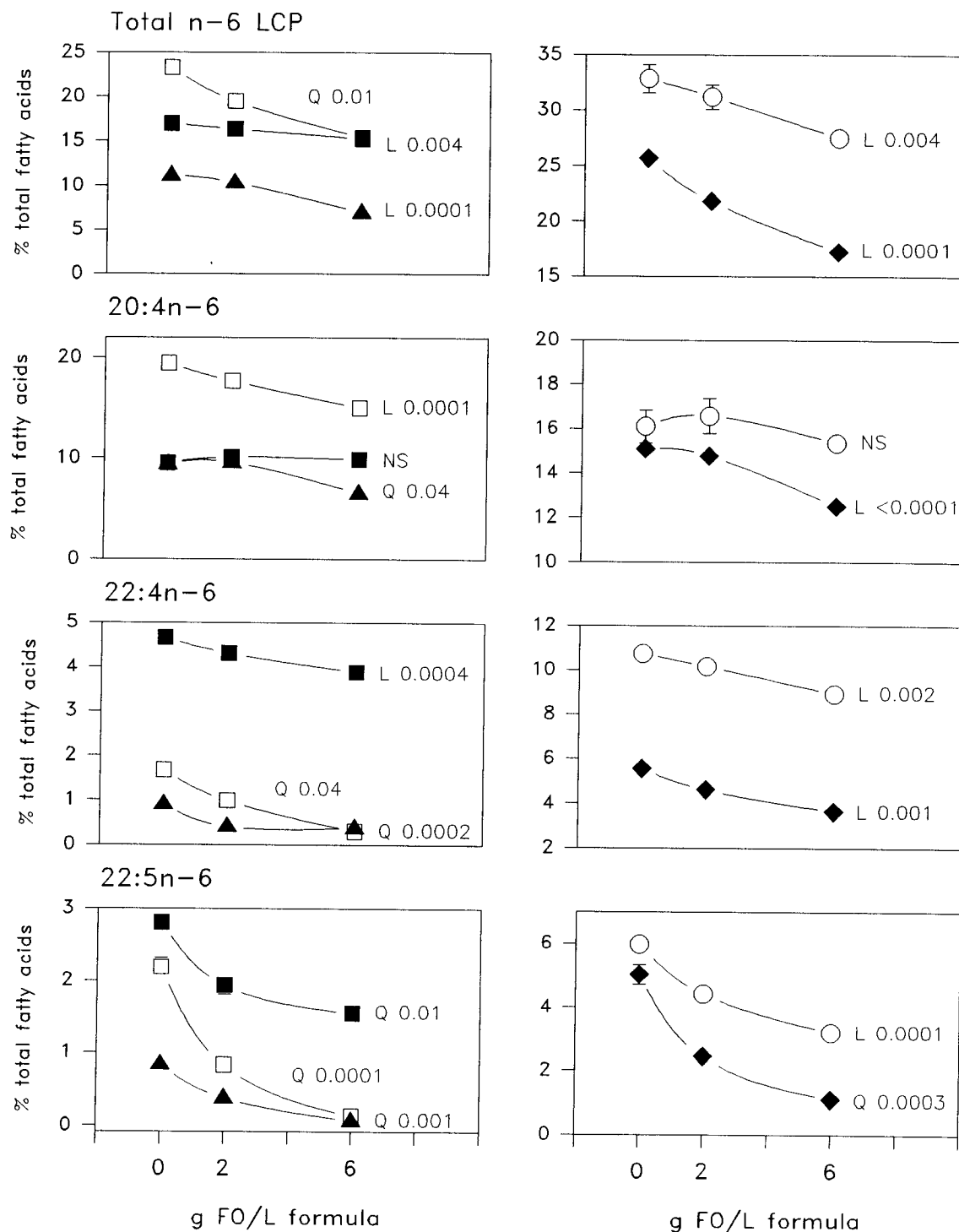
Figure 3.8 Effect of increasing fish oil addition [in g fish oil (FO)/L] to formula on % n-6 long chain polyunsaturated fatty acids (LCP) in piglet plasma (closed triangle) and liver (open square) total phospholipid, brain total lipid (closed square), and synaptic plasma membrane (open circle) and retina (closed diamond) phosphatidylethanolamine. Points represent mean ± SEM (where error bars do not signify, the SEM is smaller than the symbol shown). p-values of orthogonal contrast analyses are given and results designated as linear (L), quadratic (Q) or not statistically significant ($p > 0.05$) (NS)

Total n−3 LCP

15

% of fatty acids

10

5

0

□ Q 0.0002
■ Q 0.004
▲ Q 0.006

40

30

20

10

♦ Q 0.0006

○ L 0.001

20:5n−3

3

% of fatty acids

2

1

0

▲ Q 0.03

□ Q 0.003

■ L 0.009

0.4

0.2

0.0

♦ L 0.0001
○ NS

22:5n−3

2

% of fatty acids

1

0

□ L 0.0001

▲ Q 0.04

■ Q 0.01

2.0

1.5

1.0

0.5

0.0

♦ NS

○ L 0.0001

22:6n−3

10

% of fatty acids

5

0

Q 0.008

Q 0.0001

▲ Q 0.0008

40

30

20

10

♦ Q 0.0004

○ L 0.0001

0   2   6

g FO/L formula

0   2   6

g FO/L formula
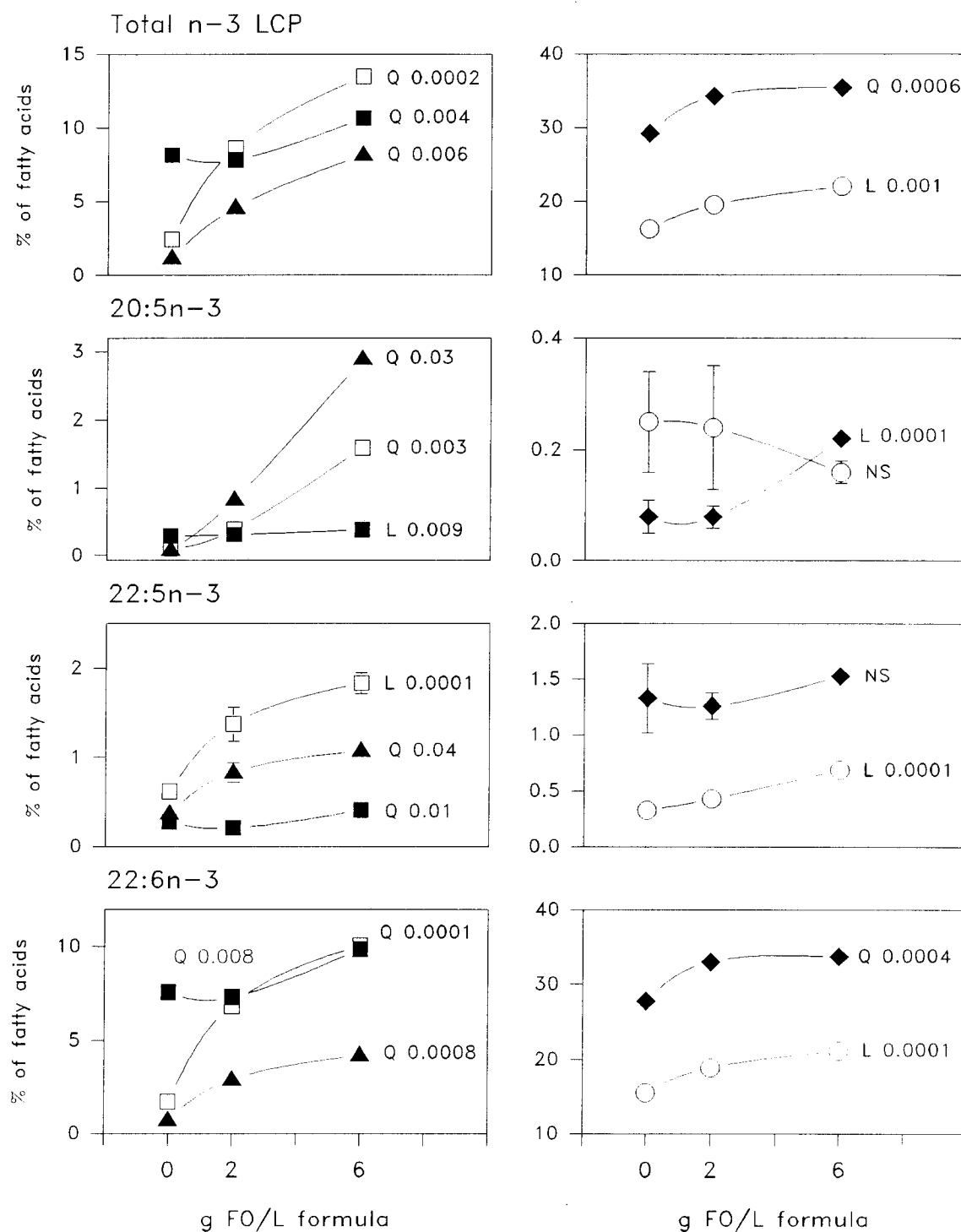
Figure 3.9 Effect of increasing fish oil addition [in g fish oil (FO)/L] to formula on % n-3 long chain polyunsaturated fatty acids (LCP) in piglet plasma (closed triangle) and liver (open square) total phospholipid, brain total lipid (closed square), and synaptic plasma membrane (open circle) and retina (closed diamond) phosphatidylethanolamine. Points represent mean ± SEM (where error bars do not signify, the SEM is smaller than the symbol shown). p-values of orthogonal contrast analyses are given and results designated as linear (L), quadratic (Q) or not statistically significant ($p > 0.05$) (NS).

3.3.2.4 Piglet plasma and tissue total LCP

The similar total LCP in the synaptic plasma membrane EPL (**Figure 3.10**) among all the formula-fed piglet groups, provides evidence for homeostatic control of membrane unsaturation, irrespective of the amount of n-3 fatty acids in the diets. In contrast, the total LCP levels of the plasma and liver phospholipid showed a significant linear increase and a curvilinear (quadratic) increase in brain total lipid in response to the increase in fish oil from the F to FO3 diet. The significantly higher proportion of total LCP in brain total lipid of piglets fed the FO3 rather than the FO1 diet, but not of piglets fed the FO1 rather than the F diet (quadratic change), indicates a lag (threshold) in response. The similar level of total LCP in the plasma and liver phospholipid of the FO3- and FO1-fed piglets may be interpreted as evidence of equivalent reduction in the n-6 LCP with the increase in the diet-derived n-3 LCP in these lipids. In contrast to other tissues, the retina EPL showed a non-linear decrease in total LCP with the increase in dietary fish oil (Fig. 3.9). The quadratic change in retina EPL was characterized by an increase in total LCP between the F and FO1 dietary groups, followed by a decrease from the FO1 to FO3 group. The lower total LCP in retina EPL of piglets fed the FO3 diet reflects the continuing linear decrease in n-6 LCP concomitant with a plateau in levels of 22:6n-3 in response to increasing dietary n-3 LCP.
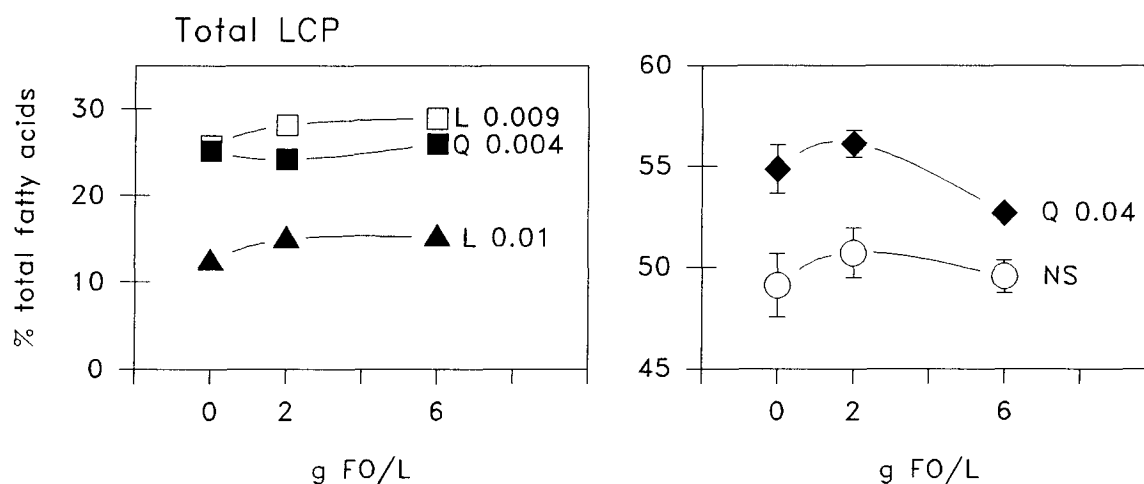
Figure 3.10 Effect of increasing fish oil addition [in g fish oil (FO)/L] to formula on total n-6 plus n-3 long chain polyunsaturated fatty acids (LCP) in piglet plasma (closed triangle) and liver (open square) total phospholipid, brain total lipid (closed square), and synaptic plasma membrane (open circle) and retina (closed diamond) phosphatidylethanolamine. Points represent mean ± SEM (where error bars do not signify, the SEM is smaller than the symbol shown). *p*-values of orthogonal contrast analyses are given and results designated as linear (L), quadratic (Q) or not statistically significant (*p* > 0.05) (NS).

3.4 Experiment IV: The effect of maternal dietary n-3 fatty acids on the fatty acid composition of milk and the developing CNS and liver of piglets

3.4.1 Milk fatty acid composition in response to maternal diet.

The notable difference in the fatty acid content of the two sow diets was the higher 18:2n-6 and n-3 LCP, particularly 22:6n-3, in the diet supplemented with fish oil (Table 2.6). Feeding 1% (wt/wt) of the diet as fish oil to the lactating sows resulted in 1.5 ± 0.1% of fatty acids as 22:6n-3 compared to 0.1% 22:6n-3 in the milk total lipid of sows fed only vegetable oil ($p < 0.0001$) (**Table 3.5**). The levels of 18:2n-6, 18:3n-3 and 20:5n-3 were also about 2-fold higher, and 18:1 lower in the milk of sows fed the diet with fish oil when compared to the milk from sows fed only vegetable oil. Levels of 16:0, 18:0 and 20:4n-6 in milk, however, were not altered by adding fish oil to the sow diet. As in the total lipid, the proportion of 22:6n-3 was higher in the *sn*-2 position of triglyceride, and in phospholipid of the milk from sows fed diets with, rather than without fish oil (Table 3.5). Although no difference was seen in the level of 20:4n-6 in milk total lipid, the milk of sows fed diets with fish oil had markedly lower levels of 20:4n-6 in milk phospholipid (1.9 vs 4.8% in milk from sows fed diets with and without fish oil, respectively). In contrast to the milk LCP, the differences in the percentage of 18:2n-6 and 18:3n-3 found in the milk total lipid were found in the *sn*-2 position of triglyceride, but were not found in milk phospholipid, which had similar levels of 18:2n-6 and 18:3n-3 between the two groups.

3.4.2 Growth

The differences in the fatty acid composition of milk suckled was not associated with any significant differences in body wt of the piglets at 15 days of age; the body wt and cerebrum wt of the 15 day-old piglets nursed by sows fed diets with vegetable oil only or with added fish oil was (mean ± SEM) 4.1 ± 0.4 and 4.8 ± 0.2 kg, and 35.0 ± 1.0 and 33.9 ± 0.5 g, respectively. Cerebrum wt as a percentage of body wt, however, was significantly lower ($p < 0.03$) in piglets suckling from sows fed the diets with (0.71 ± 0.03%) compared to without (0.88 ± 0.05%) fish oil.

Table 3.5
Fatty acid composition of sow milk total lipid, sn-2 monoglyceride of triglyceride and phospholipid from sows fed usual diets with and without added fish oil

Fatty acid	Total Lipid		sn-2 position of Triglyceride		Total Phospholipid	
	Reference milk[1]	Fish oil milk[2]	Reference milk[2]	Fish oil milk	Reference milk[2]	Fish oil milk
			(% of milk fatty acids)			
Σ C $\leq$ 14:0[3]	4.0	2.7 $\pm$ 0.1	4.3 $\pm$ 0.2	3.3 $\pm$ 0.2	0.6 $\pm$ 0.4	0.3 $\pm$ 0.1
16:0	27.6 − 1.2	28.6 $\pm$ 1.4	57.3 $\pm$ 2.1	56.9 $\pm$ 2.3	19.2 $\pm$ 2.0	19.7 $\pm$ 1.0
16:1	8.3 − 9.7	7.6 $\pm$ 0.5	8.5 $\pm$ 0.3	6.4 $\pm$ 0.1	2.0 $\pm$ 0.4	1.6 $\pm$ 0.2
18:0	4.4 − 4.5	4.5 $\pm$ 0.2	3.7 $\pm$ 0.2	4.8 $\pm$ 0.7	23.0 $\pm$ 0.5	22.2 $\pm$ 0.8
18:1n-9	34.3 − 40.8	28.6 $\pm$ 2.9	16.9 $\pm$ 1.7	9.5 $\pm$ 2.1	25.9 $\pm$ 1.9	24.9 $\pm$ 3.2
18:2n-6	10.8 − 11.3	20.7 $\pm$ 1.3	4.9 $\pm$ 0.3	12.2 $\pm$ 0.5	10.3 $\pm$ 0.7	11.4 $\pm$ 1.7
18:3n-3	1.1	2.6 $\pm$ 0.2	0.3 $\pm$ 0.0	1.5 $\pm$ 0.1	0.4 $\pm$ 0.1	0.5 $\pm$ 0.1
20:4n-6	0.5 − 0.6	0.6 $\pm$ 0.0	0.9 $\pm$ 0.1	0.6 $\pm$ 0.1	4.8 $\pm$ 0.9	1.9 $\pm$ 0.5
20:5n-3	0.0 − 0.1	0.4 $\pm$ 0.0	tr	0.2 $\pm$ 0.0	0.3 $\pm$ 0.0	0.7 $\pm$ 0.1
22:5n-3	0.2	0.4 $\pm$ 0.0	0.2 $\pm$ 0.1	0.4 $\pm$ 0.1	1.5 $\pm$ 0.1	1.0 $\pm$ 0.2
22:6n-3	0.1	1.5 $\pm$ 0.1	tr	2.0 $\pm$ 0.2	0.4 $\pm$ 0.1	3.1 $\pm$ 0.5

[1]Values for reference sow milk (from Experiment I) are 2 milk samples, collected at wk 1 and wk 2 lactation from sows fed diet containing 2.5% (wt/wt) vegetable oil (canola and soybean oil) from Pitt Ineffable Growers, Ltd., Pitt Meadows, British Columbia
[2]Values are mean $\pm$ SEM for 6 samples of sow milk (from Peter Hill Holdings, Ltd., Langley, British Columbia), 3 samples collected at 1 wk and 3 samples collected at 2 wk of lactation from sows fed a usual pig diet with (Fish oil milk) or without (Reference milk) 1% (wt/wt) fish oil added
[3]total of 10:0, 12:0 and 14:0

3.4.3 Piglet plasma and liver lipid content

The well-known hypotriglyceridemic effect of 20:5n-3 and 22:6n-3 in dietary fish oil, attributed to a reduction in liver triglyceride production and VLDL secretion (Nestel et al 1984), was found in the liver and plasma ($p = 0.05$) of the piglets fed the milk with high, rather than low, 22:6n-3 (mean ± SEM) (4.4 ± 0.6 vs 6.8 ± 0.8 μmol/g liver, 0.8 ± 0.2 vs 1.2 ± 0.1 mmol/L plasma). There was no significant difference ($p > 0.05$) in liver and plasma total cholesterol (6.7 ± 0.6 vs 7.8 ± 0.7 μmol/g liver, 3.7 ± 0.5 vs 5.5 ± 0.8 mmol/L plasma) or free cholesterol (5.3 ± 0.4 vs 6.0 ± 0.4 μmol/g liver, 2.0 ± 0.4 vs 2.2 ± 0.4 mmol/L plasma) between piglets suckling milk from sows fed diets with or without fish oil, respectively.

3.4.4 Piglet plasma and tissue fatty acid composition

The % fatty acid composition of major saturated, monounsaturated, n-6 and n-3 fatty acids in the plasma, liver and CNS tissues of 15 day-old piglets nursed by the two groups of sows is in **Table 3.6**.

3.4.4.1 Piglet plasma and tissue saturated and monounsaturated fatty acids

Although the levels of 16:0 in the sows' milk were similar (Table 3.5), the % 16:0 was significantly lower in the liver phospholipid and higher in retina total lipid of piglets nursed by sows fed diets with fish oil rather than vegetable oil only (Table 3.6). The level of 18:0 was significantly increased in the plasma and cerebrum of piglets suckling milk from sows fed diets with fish oil compared to without it. The percentage of 18:1 in plasma and liver lipids of piglets nursed by sows fed fish oil was significantly lower than in piglets nursed by sows not fed fish oil, reflecting the lower dietary intake of 18:1 in the milk (Table 3.5).

3.4.4.2 Piglet plasma and tissue n-6 fatty acids

The 2-fold higher level of 18:2n-6 in milk total lipid of sows fed the diet with added fish oil rather than with only vegetable oil had no significant effect on the percentage of 18:2n-6 in any of the piglet tissue lipids reported, except for a small but significant increase in 18:2n-6 from 13.1 to 14.6% of fatty acids in liver phospholipid (Table 3.6). Significantly higher levels of 18:2n-6, however, were found in the triglyceride lipid fraction of plasma (19.5 ± 1.2 vs 13.2 ± 0.2) and liver (21.1 ± 0.3 vs 16.0 ± 1.4) and in the cholesterol esters of plasma (48.6 ± 1.5 vs 31.9 ± 1.0) and liver (33.6 ± 1.9 vs 22.2 ± 1.6) of piglets suckling milk from sows fed

Table 3.6

Fatty acids of plasma and tissues in 15 day-old piglets suckling milk from sows fed diets with vegetable oil and without or with 1% (wt/wt) fish oil[1]

Fatty acid	Plasma Phospholipid		Liver Phospholipid		Cerebrum Total Lipid		Synaptic Plasma Membrane Total Lipid		Retina Total Lipid	
	Vegetable oil	Vegetable oil + fish oil	Vegetable oil	Vegetable oil + fish oil	Vegetable oil	Vegetable oil + fish oil	Vegetable oil	Vegetable oil + fish oil	Vegetable oil	Vegetable oil + fish oil
	(% of fatty acids)									
16:0	23.7 ± 0.8	23.8 ± 1.2	17.5 ± 0.6	15.5 ± 0.4*	21.3 ± 0.2	21.7 ± 0.2	24.7 ± 0.7	24.8 ± 0.3	20.0 ± 0.4	21.4 ± 0.3*
18:0	20.5 ± 0.4	22.6 ± 0.6*	26.8 ± 0.9	25.4 ± 0.3	23.5 ± 0.1	24.4 ± 0.2*	26.1 ± 0.3	26.2 ± 0.2	23.7 ± 0.4	24.7 ± 0.2
18:1	14.9 ± 0.2	9.7 ± 0.6*	10.9 ± 0.4	6.9 ± 0.4**	21.0 ± 0.4	21.3 ± 0.2	14.5 ± 0.2	14.1 ± 0.2	16.8 ± 0.4	16.2 ± 0.2
18:2n-6	20.8 ± 0.1	19.7 ± 0.8	13.1 ± 0.6	14.6 ± 0.1*	1.5 ± 0.1	1.5 ± 0.0	0.8 ± 0.1	0.7 ± 0.1	2.4 ± 0.1	2.3 ± 0.1
20:2n-6	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.1	0.4 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	0.1 ± 0.0**
20:3n-6	0.6 ± 0.1	1.1 ± 0.1*	0.7 ± 0.1	1.0 ± 0.1	0.6 ± 0.0	0.7 ± 0.0	0.5 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	0.4 ± 0.0
20:4n-6	11.1 ± 0.5	9.2 ± 0.4*	18.6 ± 0.5	15.4 ± 0.2**	10.3 ± 0.2	10.2 ± 0.1	9.8 ± 0.3	9.4 ± 0.2	9.9 ± 0.4	9.3 ± 0.1
22:4n-6	0.4 ± 0.0	0.6 ± 0.1	0.6 ± 0.0	0.3 ± 0.0**	4.3 ± 0.1	3.5 ± 0.1*	4.9 ± 0.3	4.6 ± 0.1	2.0 ± 0.1	1.6 ± 0.0*
22:5n-6	0.1 ± 0.0	0.2 ± 0.0*	0.3 ± 0.0	0.2 ± 0.0**	1.7 ± 0.0	1.6 ± 0.1*	2.3 ± 0.2	2.1 ± 0.0	0.9 ± 0.1	0.5 ± 0.0*
18:3n-3	0.1 ± 0.0	0.5 ± 0.0**	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.1	nd*	nd[2]	nd	nd	nd
20:5n-3	0.4 ± 0.1	1.1 ± 0.1**	0.3 ± 0.1	1.1 ± 0.1**	0.2 ± 0.1	0.1 ± 0.0	nd	tr*	tr	0.2 ± 0.0**
22:5n-3	1.8 ± 0.2	1.2 ± 0.2	2.3 ± 0.3	1.4 ± 0.2*	0.5 ± 0.0	0.3 ± 0.0*	0.4 ± 0.0	0.3 ± 0.0*	0.7 ± 0.1	0.7 ± 0.0
22:6n-3	2.6 ± 0.2	7.9 ± 0.2**	5.5 ± 0.5	15.8 ± 0.6**	9.7 ± 0.2	10.9 ± 0.2*	13.3 ± 0.5	14.7 ± 0.1*	19.0 ± 0.8	19.9 ± 0.2

[1]Values (mean ± SEM, $n = 6$) significantly different from piglets nursed by sows fed diet with vegetable oil only, *$p < 0.05$, **$p < 0.0001$.

[2]nd = not detected, tr = trace levels: 0.01 − 0.05% of total fatty acids.

the diets with, rather than without fish oil, respectively. The level of 20:3n-6 and 22:5n-6 in plasma phospholipids was significantly higher in piglets nursed by sows fed diets containing vegetable oil only rather than with added fish oil. The proportion of 22:4n-6 and 22:5n-6 was significantly lower in the liver and cerebrum, and 20:2n-6, 22:4n-6 and 22:5n-6 were significantly lower in the retina of piglets suckled by sows fed fish oil compared to those nursed by sows fed vegetable oil only (Table 3.6).

Although the level of 20:4n-6 in the milk total lipid was not altered by the sows' diets (Table 3.5), piglets fed milk from sows fed fish oil had significantly lower 20:4n-6 in the plasma and liver phospholipid than piglets suckled by sows fed the diet without fish oil. Unlike the circulating and liver lipids, no significant differences were found in the levels of 20:4n-6 in the CNS tissues between the two groups of piglets (Table 3.6, **Figure 3.11**).

3.4.4.3 Piglet plasma and tissue n-3 fatty acids

The 2-fold higher proportion of 18:3n-3 in the milk of sows fed fish oil (Table 3.5) was associated with significantly higher levels of 18:3n-3 in the nursing piglet plasma phospholipid than in piglets nursed by sows not fed fish oil. This difference in 18:3n-3 between piglets receiving milk from sows fed diets with or without fish oil was not found in any other tissue (Table 3.6, Tables 6.3 - 6.21) or lipid class (Tables 6.12, 6.13, 6.17, 6.18). Piglets receiving the higher level of 20:5n-3 in milk had significantly higher levels of 20:5n-3 in plasma and liver phospholipid and retina total lipid (p < 0.0001) than piglets suckled by sows fed only vegetable oil. Piglet cerebrum total lipid 20:5n-3 was not altered by the composition of the sow milk. Although significant increases were seen in 20:5n-3 in the synaptic plasma membrane and retina total lipid, the proportion of 20:5n-3 was always $\leq$ 0.2% of fatty acids. Despite a similar percentage of 22:5n-3 in the milk total lipid fatty acids, piglets nursed by sows fed fish oil had significantly lower (p < 0.05) levels of 22:5n-3 in liver, cerebrum and cerebrum synaptic plasma membranes, than piglets nursed by sows not fed fish oil.

Levels of 22:6n-3 were about 300% higher in plasma and liver phospholipid (p < 0.0001) of piglets nursed by sows fed fish oil than in piglets nursed by sows fed the diet with only vegetable oil (Table 3.6, Fig. 3.11). A smaller (11%), but significant (p < 0.05) increase in 22:6n-3 was also found in the cerebrum and synaptic plasma membrane. A similar trend which was not of statistical significance (p = 0.38) was found in the retina.
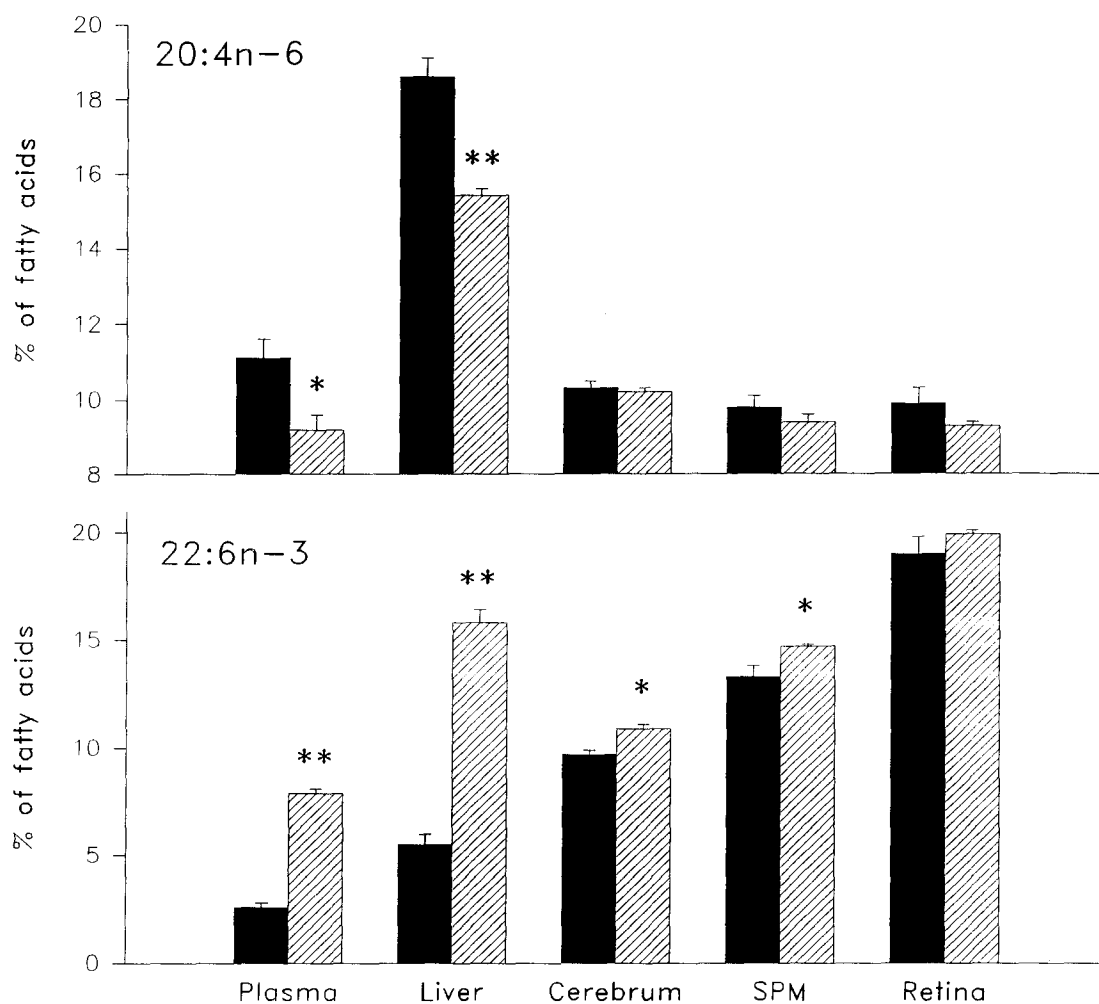
Figure 3.11 Levels of 20:4n-6 and 22:6n-3 in plasma and liver phospholipid, and cerebrum, synaptic plasma membrane and retina total lipid of 15 day-old piglets suckling milk from sows fed diets with vegetable oils without (n=7, solid bars) or with 1 g% (wt/wt) fish oil added (n=6, hatched bars). Values represent mean ± SEM. Values for piglets suckling milk from sows fed fish oil are significantly different from piglets suckling milk from sows fed only vegetable oils, *p < 0.05 and **p < 0.0001, by unpaired Student's t test.

3.5 Dietary adequacy of formula 18:2n-6 and 18:3n-3 for synthesis and deposition of 20:4n-6 and 22:6n-3 in piglet tissues in relation to the range of tissue fatty acid compositions of piglets fed different milks

3.5.1 Liver and CNS tissue 20:4n-6

Tissue 20:4n-6 levels were not significantly different in any formula-fed group compared to piglets fed milk containing 0.1% 22:6n-3 (**Figure 3.12**). Compared to the piglets fed milk containing 1.5% 22:6n-3, the level of 20:4n-6 was significantly higher in synaptic plasma membrane and retina EPL in piglets fed the 16/1 and 30/1 formulas, and in synaptic plasma membrane PC in piglets fed the 30/1 formula; the proportion of 20:4n-6 was significantly lower in retina EPL and PC in piglets fed the 16/4 formula and in retina PC in those fed the 35/4 formula.

Liver EPL and PC % 20:4n-6 was significantly lower in piglets receiving formula with 4% 18:3n-3 compared to piglets nursed by sows fed diets with vegetable oil; there was no evidence of a significant difference, however, when compared to piglets receiving milk from sows fed diets with fish oil added. An exception was the percent 20:4n-6 in liver PC of piglets fed the 35/4 formula, which was similar to levels in both natural milk-fed groups. Piglets fed formulas with 1% 18:3n-3 had significantly higher levels of 20:4n-6 in liver EPL and PC than piglets receiving milk from sows fed diets with or without fish oil. An exception was liver EPL % 20:4n-6 in the piglets fed the 16/1 formula, which was not significantly different than in piglets receiving milk from sows fed diets with vegetable oil only.

3.5.2 Liver and CNS tissue 22:6n-3

Levels of 22:6n-3 were significantly lower in all tissues except synaptic plasma membrane PC and cerebrum EPL of piglets fed the 30/1 formula than in piglets receiving milk from sows fed diets with vegetable oil (**Figure 3.13**). Piglets fed the 16/1 formula had significantly lower levels of 22:6n-3 in retina EPL, cerebrum EPL and PC and synaptic plasma membrane and liver PC than those receiving milk from sows fed diets with vegetable oil added. There was no evidence of a significant difference between the levels of 22:6n-3 in any tissue studied from piglets fed formulas with 4% 18:3n-3 and piglets fed sow milk with 0.1% 22:6n-3.

When compared to piglets consuming milk from sows fed the diet with fish oil, the piglets fed the formulas with 1% 18:3n-3 had significantly lower levels of 22:6n-3 in liver EPL and PC and all CNS tissues. The proportion of 22:6n-3 was significantly lower in cerebrum and liver EPL and PC of piglets fed formulas with 4%

76
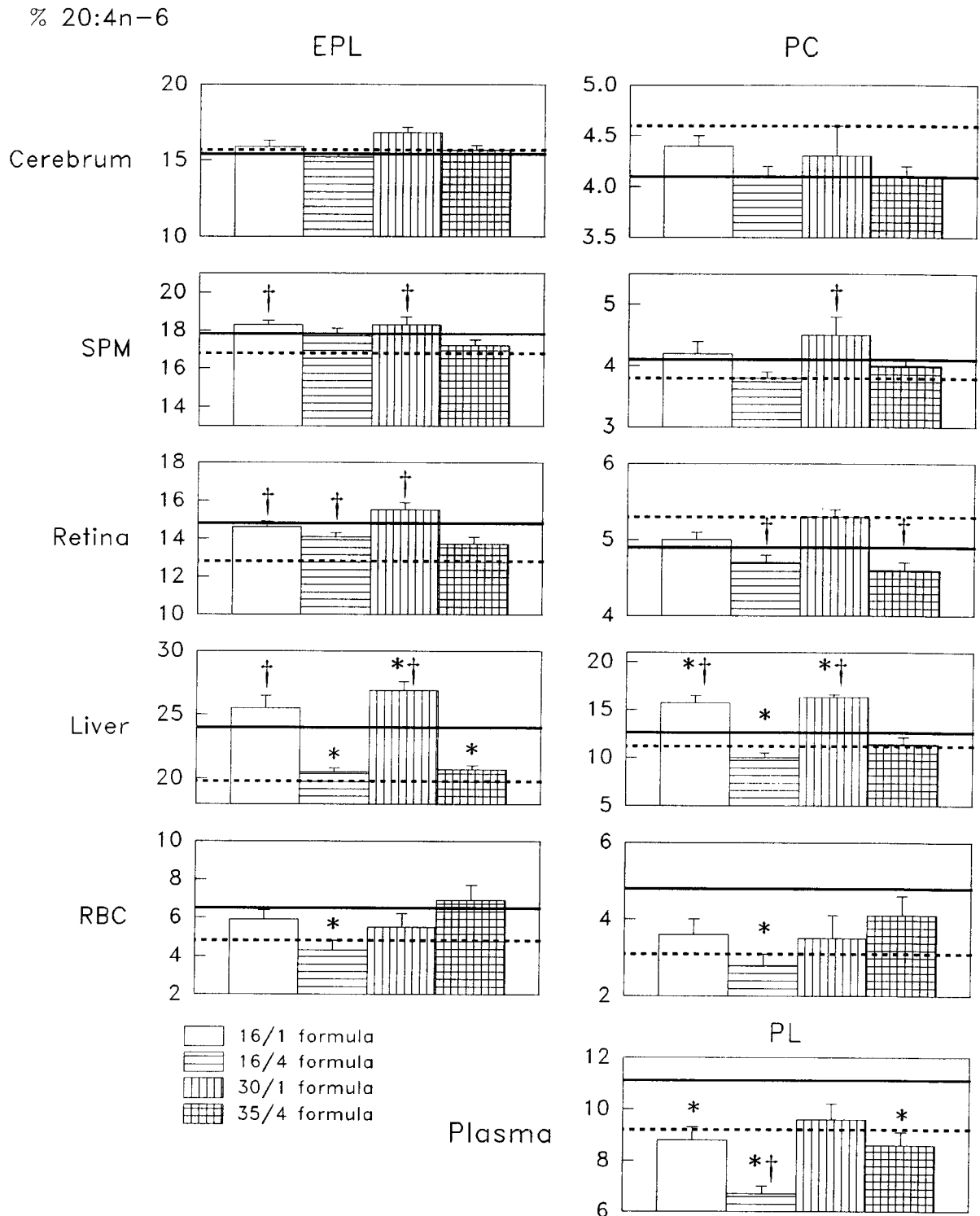
Figure 3.12 Levels of 20:4n-6 (% of fatty acids) in cerebrum, synaptic plasma membrane (SPM), retina, liver and red blood cell (RBC) ethanolamine phospholipids (EPL) and phosphatidylcholine (PC) and plasma phospholipid (PL) in 15 day-old piglets. The piglets were fed formulas containing (%18:2n-6/%18:3n-3): 16/1 (open bars), 16/4 (horizontal bars), 30/1 (vertical bars) or 35/4 (crossed bars). Values are mean ± SEM. Mean values for piglets receiving natural milk from sows fed diets with vegetable oil only (solid line) or with added fish oil (dashed line) are shown. The SEM of values for piglets fed natural milk represent < 7% of the mean value and are not included on the graph. Values for the formula-fed piglets are significantly different from piglets fed natural milk from sows fed diets with vegetable oil only (*p < 0.013) or with added fish oil ($^{\dagger}p$ < 0.013).

77
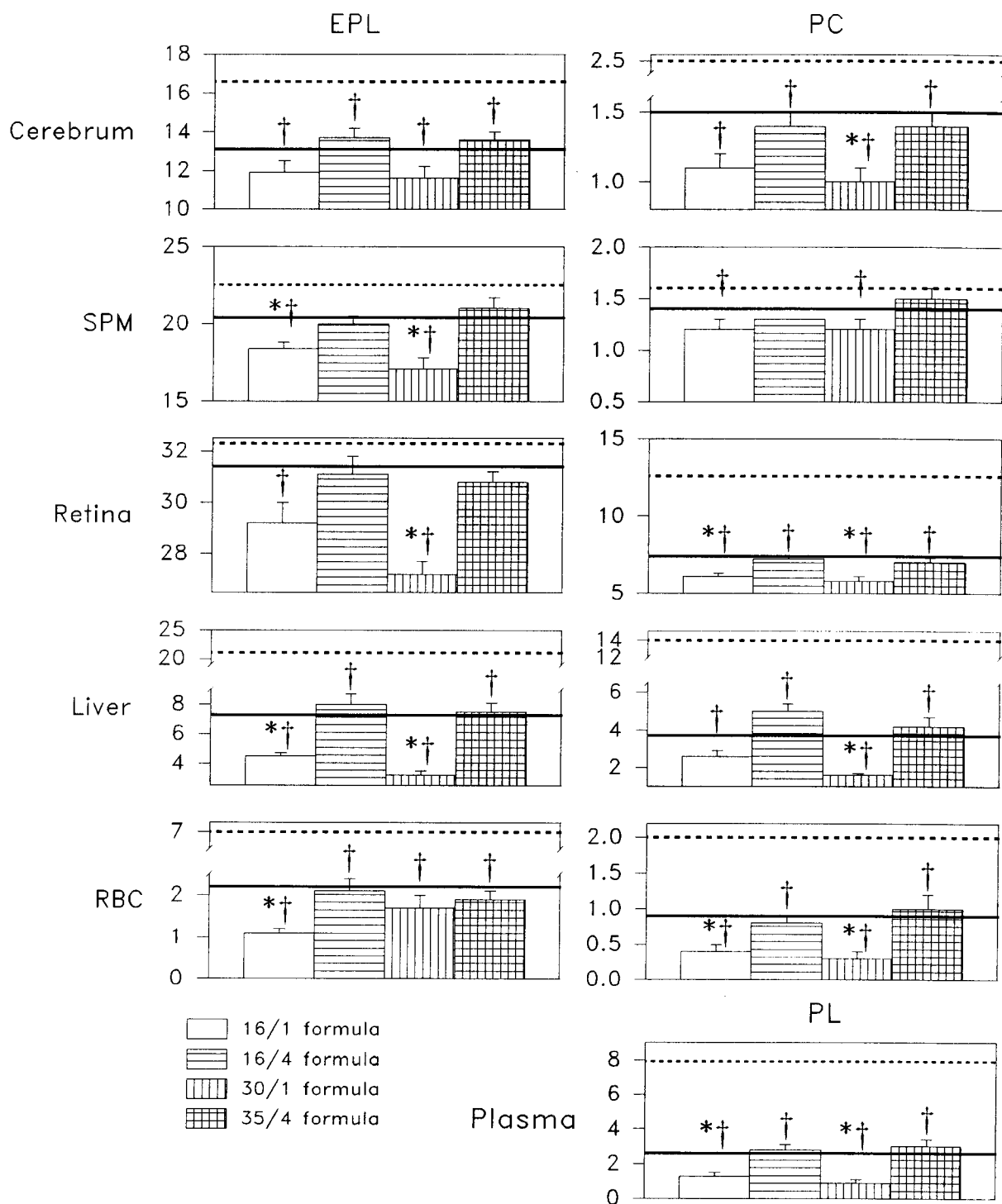
Figure 3.13 Levels of 22:6n-3 (% of fatty acids) in cerebrum, synaptic plasma membrane (SPM), retina, liver and red blood cell (RBC) ethanolamine phospholipids (EPL) and phosphatidylcholine (PC) and plasma phospholipid (PL) in 15 day-old piglets. The piglets were fed formulas containing (%18:2n-6/%18:3n-3): 16/1 (open bars), 16/4 (horizontal bars), 30/1 (vertical bars) or 35/4 (crossed bars). Values are mean ± SEM. Mean values for piglets receiving natural milk from sows fed diets with vegetable oil only (solid line) or with added fish oil (dashed line) are shown. The SEM of values for piglets fed natural milk represent ≤ 10% of the mean value and are not included on the graph. Values for the formula-fed piglets are significantly different from piglets fed natural milk from sows fed diets with vegetable oil only ($*p < 0.013$) or with added fish oil ($^{\dagger}p < 0.013$).

18:3n-3. The level of 22:6n-3 in retina EPL and synaptic plasma membrane EPL and PC in piglets fed the formulas with 4% 18:3n-3, however, was not significantly different from that of piglets receiving milk from sows fed diets with fish oil. One exception was significantly lower % 22:6n-3 in synaptic plasma membrane EPL of piglets fed the 16/4 formula, compared to those receiving milk with 1.5% 22:6n-3.

3.6 Comparison of the relationship between the effects of milk and formula fatty acids on the circulating lipids with that on the liver and CNS lipids

The biochemical assessment of the adequacy of dietary n-6 and n-3 fatty acids in infants fed formulas varying in the amount and balance of 18:n-6 and 18:3n-3 or with fish oil, is limited to plasma and RBC fatty acids. Knowledge of the sensitivity and specificity of these measures as indices of the adequacy of 20:4n-6 and 22:6n-3 in the liver and CNS tissues is fundamental in the interpretation of the effect of milk and formula feeding on the circulating lipids of infants.

Plasma phospholipid % 20:4n-6 in piglets fed the 16/1, 16/4 and 35/4 formulas was significantly lower than in piglets fed milk fom sows fed diets with vegetable oil only (Fig. 3.12). In contrast, there was no evidence of a significant difference in cerebrum, synaptic plasma membrane and retina EPL and PC % 20:4n-6 in piglets fed formula, compared to piglets nursed by sows fed diets with vegetable oil. Therefore, changes in the plasma phospholipid % 20:4n-6 did not reflect the maintenance of 20:4n-6 in CNS tissues, which was irrespective of the amount of 18:2n-6 or 18:3n-3 in the formula fed. The level of 20:4n-6 in RBC EPL and PC, as well as in the CNS, of piglets fed the 16/1, 30/1 and 35/4 formulas was not significantly different from piglets fed natural milk. However, piglets fed the 16/1 formula had significantly lower levels of 20:4n-6 in RBC EPL and PC, but similar 20:4n-6 in CNS tissues compared to piglets fed milk from sows fed diets with vegetable oil. The differences in plasma phospholipid % 20:4n-6 in formula-fed and milk-fed piglets were of a similar magnitude and direction to those found in liver EPL and PC. However, there was no evidence of a significant difference in plasma phospholipid 20:4n-6, whereas liver EPL and PC 20:4n-6 was significantly higher in piglets fed the 30/1 formula rather than sow milk. The changes in RBC EPL and PC 20:4n-6 composition reflected the changes in liver EPL and PC in piglets fed the 16/4 formula, with significantly lower levels of 20:4n-6 than piglets fed sow milk containing 0.1% 22:6n-3. However, the measurement of 20:4n-6 in RBC EPL and PC did not indicate the significantly higher levels of 20:4n-6 in liver EPL or PC in piglets fed the 16/1 and 30/1 formulas.

79

The level of 22:6n-3 in plasma phospholipid and RBC EPL and PC of formula-fed piglets was significantly lower than piglets nursed by sows fed diets with fish oil (Fig. 3.13). However, there was no evidence of a significant difference in synaptic plasma membrane EPL and PC and retina EPL % 22:6n-3 in piglets fed the formulas with 4% 18:n-3 and those fed natural milk. The plasma phospholipid and RBC EPL and PC percentage of 22:6n-3 did reflect the trend of lower 22:6n-3 in liver EPL and PC of piglets fed the formulas with 1% 18:3n-3 than of piglets fed natural milk, and the significantly lower proportion of 22:6n-3 in liver EPL and PC of all formula-fed piglets, compared to piglets nursed by sows fed diets with fish oil. The significantly higher proportion of 22:5n-6 in plasma phospholipid and RBC PC in piglets fed the formulas containing 1% 18:3n-3 rather than sow milk was consistent with the changes in the cerebrum, synaptic plasma membrane, retina and liver EPL and PC 22:5n-6 (**Figure 3.14**). RBC EPL % 22:5n-6 in piglets fed the 16/1 formula, however, was not significantly different from piglets fed sow milk.

% 22:5n−6
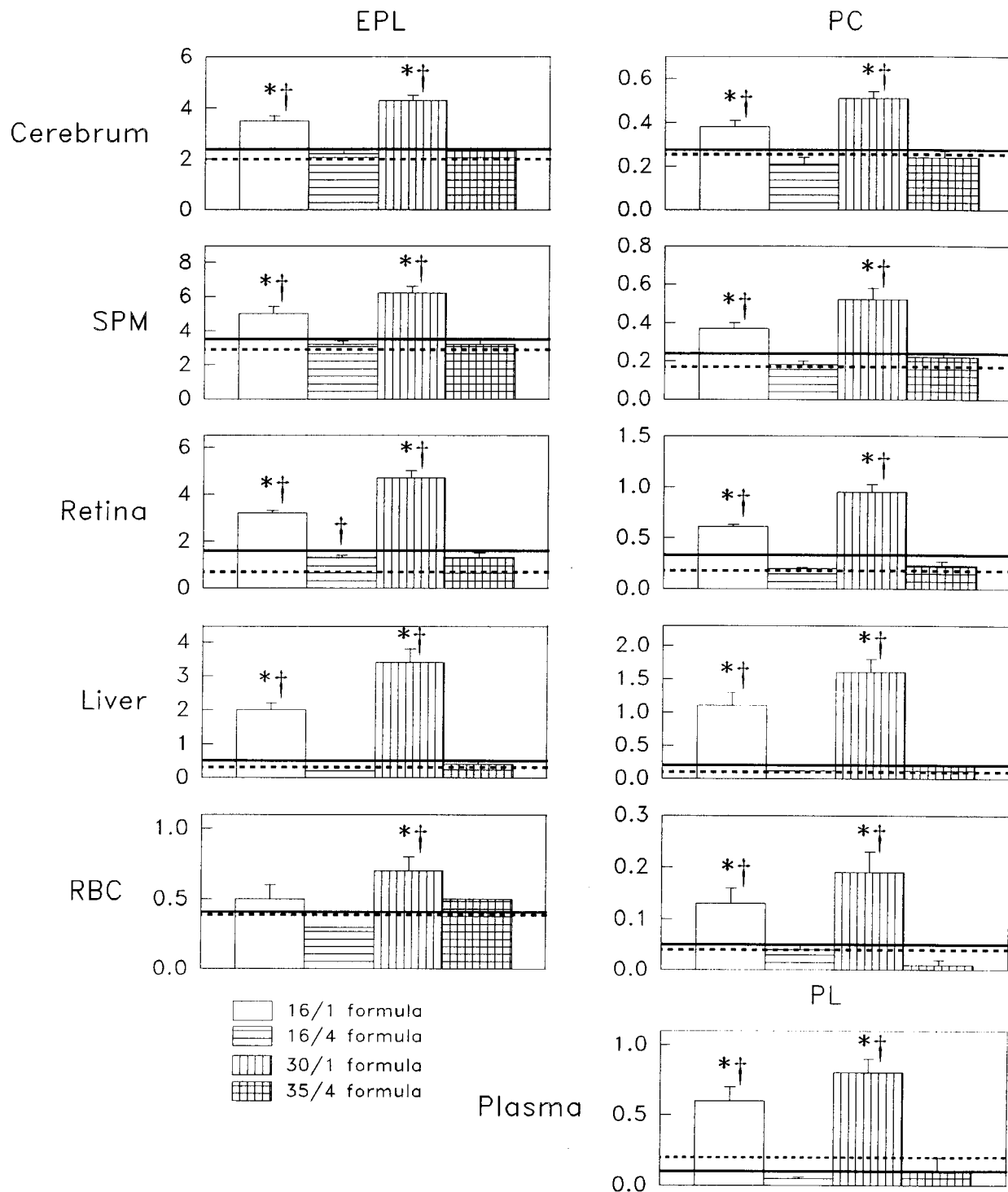
Figure 3.14 Levels of 22:5n-6 (% of fatty acids) in cerebrum, synaptic plasma membrane (SPM) and retina, liver and red blood cell (RBC) ethanolamine phospholipids (EPL) and phosphatidylcholine (PC) and plasma phospholipid (PL) in 15 day-old piglets. The piglets were fed formulas containing (%18:2n-6/%18:3n-3): 16/1 (open bars), 16/4 (horizontal bars), 30/1 (vertical bars) or 35/4 (crossed bars). Values are mean ± SEM. Mean values for piglets receiving natural milk from sows fed diets with vegetable oil only (solid line) or with added fish oil (dashed line) are shown. The SEM of values for piglets fed natural milk represent ≤ 12% of the mean value and are not included on the graph. Values for the formula-fed piglets are significantly different from piglets fed natural milk from sows fed diets with vegetable oil only (*p < 0.013) or with added fish oil (†p < 0.013).

81

4 DISCUSSION

4.1 Effect of formula 18:2n-6 and 18:3n-3 content on liver and CNS phospholipid n-3 and n-6 fatty acids

The higher levels of 22:6n-3 in the CNS and liver phospholipid of piglets fed formulas with 4% rather than 1% 18:3n-3 (Fig. 3.1) provide evidence that young piglets can synthesize 22:6n-3 from 18:3n-3. Weanling rodents (Bourre et al 1989, reviewed in Innis 1991) and young non-human primates (Connor & Neuringer 1988, Neuringer et al 1986) also accumulate 22:6n-3 in cerebrum and liver when fed diets with about 1 − 2% of energy as 18:3n-3 but no 22:6n-3. The lower level of 22:6n-3 in tissue lipids of piglets fed the formulas containing 1% of fatty acids as 18:3n-3 was accompanied by higher 22:5n-6 and lower 22:6n-3:22:5n-6 ratios. The ratio of the percentage of 22:6n-3 and of 22:5n-6 has been suggested to be a more sensitive indicator of the dietary n-3 fatty acid adequacy than the percentage of 22:6n-3 or 22:5n-6 alone (Galli et al 1974). This increase in 22:5n-6 in tissue membranes is a characteristic finding in n-3 fatty acid-deficient animals (reviewed in Innis 1991). The reciprocal replacement of 22:6n-3 with 22:5n-6 resulted in similar levels of total LCP in the cerebrum total lipid (Table 6.3) and synaptic plasma membrane EPL (Table 6.7) among the piglet groups. This finding is consistent with previously published reports of constant percent and milligram quantities of total LCP in the brain (Hrboticky et al 1990, reviewed by Innis 1991) and synaptic plasma membrane (Hrboticky et al 1989) of piglets fed formula containing 30% 18:2n-6 and 0.8% 18:3n-3, or sow milk. As levels of 20:4n-6 are relatively constant in the piglet brain, the maintenance of total LCP in cerebral membranes is primarily due to changes in C22 LCP.

Large amounts of dietary 18:3n-3 may result in competitive inhibition of the desaturation of 18:2n-6 (Holman 1986). The proportion of 20:4n-6 in the liver, synaptic plasma membrane and retina was significantly lower in piglets fed the 16/4 and 35/4 formulas rather than the 16/1 and 30/1 formulas, despite amounts of 18:2n-6 in formula well above the 3% of energy often suggested as the dietary requirement for n-6 fatty acids (Bourre et al 1989, Bourre et al 1990, Mohrhauer & Holman 1963, Mohrhauer & Holman 1963a). Equations have been derived from studies in rats to predict the liver and plasma phospholipid 20:4n-6 as a percentage of total LCP at dietary intakes of up to about 13% of energy as 18:2n-6 and 18:3n-3 (Lands et al 1990, Lands 1991). The inhibition of 20:4n-6 incorporation into the piglet liver and plasma phospholipid accompanying the increase in formula 18:3n-3 content was accurately predicted by the Lands equation (1991) (Fig. 3.3), suggesting that general competitive interactions in desaturation, metabolic selectivities for fatty acid synthesis and esterification into glycerolipids are similar in rats and piglets. At about 19% of energy as 18:2n-6, however, the equation

predicted the tissue 20:4n-6 levels less accurately than at 8, 9 or 15% of energy as 18:2n-6 (Fig. 3.3). The equation

published by Lands (1991) can be used to estimate the dietary levels of 18:2n-6 and 18:3n-3, between 1 and 13%

of energy, that would achieve liver phospholipid 20:4n-6 similar to that in piglets fed sow milk. Such estimates

suggest that the "control" level of 20:4n-6 as a percentage of the total LCP could be achieved by feeding formula

with about 1 − 1.5% of energy as 18:3n-3, in an 18:2n-6:18:3n-3 ratio of about 6:1 to 8:1 (**Figure 4.1**). These

absolute and relative amounts are similar to the dietary intakes proposed to achieve adequate levels of 22:6n-3

in developing tissues (Innis 1991, Neuringer & Connor 1986).
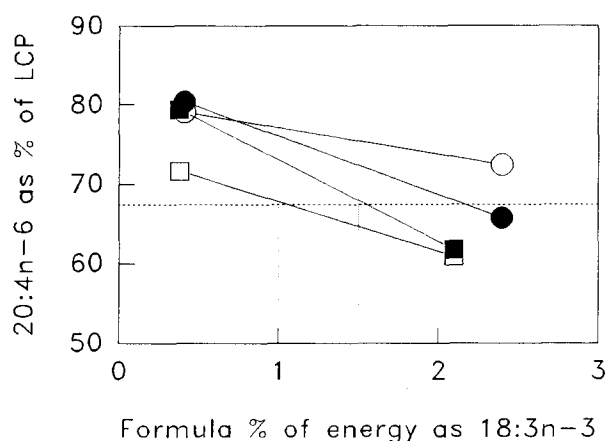
Figure 4.1 Liver phospholipid 20:4n-6 as a percentage of LCP observed (closed symbols) and predicted (open symbols) from Lands equation (Lands 1991) in piglets fed formulas containing 0.4 or 2.1% of energy as 18:3n-3 with either 8 and 9% of energy as 18:2n-6 (squares) (16/1 and 16/4 formulas) or 15 and 19% of energy as 18:2n-6 (circles) (30/1 and 35/4 formulas) compared to observed values in piglets fed reference sow milk (dashed line).

4.2 The relative importance of 18:3n-3 content compared to 18:2n-6:18:3n-3 ratio in formula on piglet tissue levels

of 22:6n-3

The effect of the 18:3n-3 content, and 18:2n-6:18:3n-3 ratio in formula, on the ability of the newborn to

synthesize and incorporate 22:6n-3 into tissues, was of particular interest in these studies. The piglet formulas

with 4% of fatty acids as 18:3n-3 had 18:2n-6:18:3n-3 ratios of 4:1 and 8:1; the formulas with 1% 18:3n-3 had ratios

of 22:1 and 37:1. This encompasses the range found in many term infant formulas (Innis 1992, Ponder et al 1992).

The higher 22:6n-3 in tissues of piglets fed the 16/4 and 35/4 compared to the 16/1 and 30/1 formulas could

be explained by the low 18:2n-6:18:3n-3 ratio, facilitating desaturation of 18:3n-3 to 22:6n-3 by decreasing

competition by 18:2n-6 for the desaturase enzymes. The 18:2n-6:18:3n-3 ratio and the absolute amount of 18:3n-3

in the formula are closely associated. Some evidence as to the predominant influencing factor, however, can be obtained from the R^2 when both the two-way ANOVA and regression analyses show highly significant effects of the formula 18:2n-6 and 18:3n-3 content on the % 22:6n-3 in the tissue lipids. For example, feeding the formulas with 4% rather than 1% 18:3n-3 explained 81% of the variability, whereas the low rather than high ratio (4:1 and 8:1 vs 22:1 and 37:1) accounted for only 65% of the difference in 22:6n-3 in piglet cerebrum total lipid. These results suggest the higher amount of 18:3n-3 in formula (2.1 and 2.4% vs 0.4% of energy) was a major factor in determining the proportion of 22:6n-3 in the developing piglet cerebrum. The relative importance of the amount of 18:3n-3 and the 18:2n-6:18:3n-3 ratio on the level of 22:6n-3 in the synaptic plasma membrane, retina and liver, however, appeared to be quite similar. The percentage of 22:6n-3 was lower and 22:5n-6 was higher in the tissues of piglets fed the 30/1 rather than 16/1 formula, with 18:2n-6:18:3n-3 ratios of 37:1 and 22:1, respectively. Consistent with this, studies by others have provided evidence that rat cerebrum (Alling et al 1974, Sanders et al 1984), synaptosome (Bourre et al 1989, Bourre et al 1990a) and retina (Bourre et al 1989, Bourre et al 1990a, Sanders et al 1984) lipid 22:6n-3 and 22:5n-6 levels depend on the dietary 18:2n-6:18:3n-3 ratio when the dietary 18:3n-3 intake is marginal (*i.e.* 0.2 − 0.4% of energy) or deficient. The level of 22:6n-3 in brain was similar among rats fed diets with 0.3% of energy as 18:3n-3 and 18:2n-6:18:3n-3 ratios between about 4:1 and 10:1 (Bourre et al 1989), but appeared to decrease with dietary 18:2n-6:18:3n-3 ratios above 19:1. The proportion of 22:6n-3 in the retina of these animals did not differ with dietary 18:2n-6:18:3n-3 ratios between about 1:1 and 6:1, but decreased at ratios between about 10:1 and 20:1 (Bourre et al 1989). Together with the data for piglets provided by this study, it seems that ratios of 18:2n-6:18:3n-3 above about 8:1 − 10:1 may further decrease the low levels of 22:6n-3 in CNS tissues of rapidly growing animals which result from feeding diets containing deficient or marginal amounts of 18:3n-3.

In summary, the results of these studies show formulas containing 4% of fatty acids as 18:3n-3 (2.1 and 2.4% of energy) provide adequate n-3 fatty acids to achieve similar levels of 22:6n-3 in CNS membrane phospholipids to that found in piglets fed sow milk from birth. Therefore, 22:6n-3 is not essential in the diet of the term gestation piglet, if adequate 18:3n-3 is provided. The levels of 18:3n-3 fed in these studies, however, did not support comparable brain weight to sow milk-fed piglets. It remains to be determined whether less than 2% of energy as 18:3n-3 can provide adequate deposition of 22:6n-3 in the CNS and maintain normal brain growth.

The relative efficacy of dietary 18:3n-3 as a source of n-3 fatty acids for deposition of 22:6n-3 in CNS membrane lipids is at least 20% that of preformed dietary n-3 LCP, as shown in Experiment II. The ratio of 22:6n-3 to 22:5n-6 in synaptic membranes and retina was a more sensitive index of the formula n-3 fatty acid adequacy than the percentage of 22:6n-3 or 22:5n-6 alone, or the 22:6n-3:22:5n-6 ratio in cerebrum total lipid. Deposition of 22:6n-3 in cerebrum seemed to be influenced by the quantity of 18:3n-3 in the diet more than the 18:2n-6:18:3n-3 ratio. The ratio of 18:2n-6:18:3n-3 in the formula, however, was more important when the formula 18:3n-3 was limiting (ie. 0.4% of energy). The effect of high formula 18:3n-3 in reducing levels of 20:4n-6 in piglet liver and plasma phospholipid could be predicted by theoretical published calculations. The accuracy of the predicted compared to observed values was better for the formula with 8, 9 or 15% of energy as 18:2n-6 rather than 19% of energy as 18:2n-6.

4.3 Effect of n-3 fatty acids in formula and milk on brain growth

Studies in the rat (Anding & Hwang 1986, Bourre et al 1989, Mohrhauer & Holman 1963, 1963a), chick (Lin et al 1991a) and human (Bjerve et al 1988, Farquharson et al 1992) have evidenced that the dietary requirement for 18:3n-3 for maximum tissue levels of 22:6n-3 is in the range of 0.75 to 1% of dietary energy. The piglets fed the formulas with 2.1% or 2.4% of energy as 18:3n-3 had comparable tissue levels of 22:6n-3 to that of piglets fed sow milk. This intake of 18:3n-3, however, was associated with a lower cerebrum weight. Similarly, the brain weight in piglets fed formulas with 1.2% of energy as n-3 LCP from fish oil (FO3 diet in Experiment III) was significantly lower than in piglets receiving sow milk with about 0.7% of energy as 18:3n-3 and 0.1% of energy as n-3 LCP. This is believed to be the first report of an adverse effect of excess dietary 18:3n-3 on brain growth. Results from several other studies, however, are available to suggest that high intakes of n-3 LCP may decrease brain growth. For example, high maternal intakes of n-3 LCP from fish oil throughout gestation and lactation decreased brain weight in 10 day-old mouse pups (Wainwright et al 1989, Wainwright et al 1992). Recent studies have also reported lower head circumference in premature infants fed formula with about 0.5% of energy as n-3 LCP from fish oil than in infants fed a similar formula without fish oil (Carlson et al 1992a).

The cerebrum lipid and protein content of all piglets fed sow milk or formula were similar (Table 3.2). Therefore, feeding high n-3 fatty acids resulted in smaller cerebrums with consistent lipid and protein composition. The cerebrum 22:6n-3 content was 73.0 ± 5.5, 77.2 ± 2.7 and 75.6 ± 3.1 mg in piglets fed the 16/4 and 35/4

formulas or sow milk containing 1.5% of fatty acids as 22:6n-3, respectively, and 66.0 ± 3.6, 62.9 ± 2.2, and 67.5

± 2.9 in piglets fed the 16/1 and 30/1 formulas or sow milk with 0.1% 22:6n-3, respectively. The higher amount

of 22:6n-3 was the only notable difference in the cerebrum of piglets fed high compared to low n-3 fatty acids.

An important role of 22:6n-3 is to maintain membrane fluidity and permeability, and possibly the function of

several membrane-bound enzymes and transport systems (Spector & Yorek 1985, reviewed by Stubbs & Smith

1984, Yorek et al 1984). It is possible, therefore, that the lower cerebrum weight in piglets may be explained

by the increased amount of 22:6n-3 in the cerebrum membranes which could alter some transport process of the

blood brain barrier.

The 20:4n-6 composition and content of the cerebrum and synaptic plasma membranes does not appear

to play a role in the reduced cerebrum growth because levels were not altered by the high dietary n-3 fatty acids.

However, the level of 20:4n-6 in membranes does not have to vary to elicit a functional change. For example,

isolated brain capillary endothelial cells from weanling rats fed 10% (wt/wt) fish oil for 4 weeks showed a similar

maintenance of 20:4n-6 accompanying the increased 22:6n-3 and decreased 22:4n-6 and 22:5n-6 (Kálmán et al

1992). Although the percentage of 20:4n-6 in the brain was similar, the amount of total [^{14}C]20:4n-6 metabolites,

such as eicosanoids, in these cells was markedly reduced (50%) in response to fish oil supplementation (Kálmán

et al 1992). Furthermore, prostacyclin production is known to be inhibited by 22:6n-3 in endothelial cells

(Hadjiagapiou & Spector 1987). It is conceivable, therefore, that altered eicosanoid metabolism in piglets fed

high amounts of 18:3n-3 (or n-3 LCP) in formula may affect the functions of the blood brain barrier system,

thereby affecting growth. Alternatively, it is possible that the proportion of 20:4n-6 was altered in some specific

cell membrane or intracellular membranes not analyzed in the studies in this thesis. One example may be cell

nuclei which are fundamental to brain growth and metabolism.

Given the enrichment of brain membranes with highly unsaturated LCP such as 22:6n-3, and the high

oxygen consumption of the brain, it is reasonable to believe the CNS is susceptible to oxidative damage.

Antioxidant systems such as vitamin E, selenium-dependent glutathione peroxidase or catalase exist in brain and

other organs to prevent oxidative damage. The vitamin E content in the formulas fed to the piglets was twice

the NRC requirement for pigs (Table 6.2). The vitamin E requirement, however, is determined primarily by the

concentration and composition of polyunsaturated fatty acids in the tissues, and therefore is related to the

polyunsaturated fatty acid content of the diet (Nutrition Recommendations 1990). Thus, it is not known whether

the amount of vitamin E was adequate in formulas with high amounts of 18:3n-3, or n-3 LCP from fish oil. Also, selenium deficiency is known to occur in some piglets in the lower mainland of British Columbia and the formulas fed to the piglets did not contain selenium. Thus, the reduced cerebrum growth in piglets fed high n-3 fatty acids may be related to potentially compromised vitamin E and/or selenium-dependent antioxidant systems in the brain.

4.4 The relationship of formula n-3 LCP with tissue n-3 and n-6 LCP

The inclusion of fish oil in formula with 16% 18:2n-6 and 0.6% 18:3n-3 (Experiment II) provided 0.8% n-3 LCP to piglets and resulted in significantly higher levels of 22:6n-3 in the synaptic plasma membrane EPL, and significantly lower levels of 22:4n-6 in retina EPL, than in piglets fed sow milk (Fig. 3.6). Clinical studies have also shown that addition of fish oils to formula is an effective way to provide 22:6n-3 to infants fed formula (Carlson et al 1987, Carlson et al 1989, Liu et al 1987, Uauy et al 1990). However, most fish oils usually contain significant amounts of 20:5n-3 and little n-6 LCP. Studies in premature infants fed formulas with 0.2 and 0.5% of energy as n-3 LCP from marine oil have shown decreased 20:4n-6:20:5n-3 ratios in plasma and RBC phospholipids (Carlson et al 1987, Carlson et al 1991, Liu et al 1987, Uauy et al 1990). Both 20:5n-3 and 22:6n-3 are known to inhibit synthesis of 20:4n-6 (Grønn et al 1992). The inclusion of large amounts of fish oil (3 − 6% of energy as n-3 LCP) in the diet of animals has been shown to result in replacement of 20:4n-6 with 20:5n-3 in structural lipids of the brain, retina (Connor & Neuringer 1988, Philbrick et al 1987) and liver (Bourre et al 1988, Bourre et al 1990). The effect of including fish oil in formula over the low range of supplementation used in clinical trials, on liver and CNS membrane lipids, however, was not previously known. Feeding formulas with increasing, but low, levels of fish oil to piglets resulted in significantly higher 20:5n-3 and lower 20:4n-6 in the piglet plasma and liver phospholipid (Fig. 3.8, 3.9). A similar increase in 20:5n-3 (and 22:6n-3) and decrease in 20:4n-6 in plasma and liver phospholipids of piglets fed sow milk with 0.6% of fatty acids as 20:4n-6 and 1.5% 22:6n-3 was shown in Experiment IV (Table 3.6). These results demonstrate that the intake of 20:4n-6 in milk does not protect against the effects of 20:5n-3 and 22:6n-3 on 20:4n-6 synthesis and/or acylation.

The liver phospholipid level of 20:4n-6 in piglets fed the FO3 formula (1.2% of energy as n-3 LCP) was about 20% lower than in piglets fed the F formula. The magnitude of difference is similar to that reported for liver EPL and PC of rats fed increasing dietary n-3 LCP from 0 to 1.4% of energy (Huang et al 1992). The lower proportion of 20:4n-6 and higher 20:5n-3 in the liver may be explained by the well-known inhibition of 18:2n-6

87

desaturation by n-3 LCP, as well as competition by the exogenous 20:5n-3 for incorporation in phospholipids (Hagve et al 1988). The orthogonal contrast analyses gave no evidence that maximum saturation of 20:5n-3 or depletion of 20:4n-6 had occurred in the liver or plasma phospholipid or retina EPL. Thus, greater changes can be expected to occur when higher levels of fish oil are fed. Indeed, 20:4n-6 in liver lipids was decreased by about 55%, 75% and 83% in response to dietary fish oil intakes of 4% (Bourre et al 1990, Huang et al 1992), 5.5% (Barzanti et al 1990, Huang et al 1992), and 6.4% of energy, respectively (Bourre et al 1988). As well, lower proportions of 20:4n-6 in retina phospholipids of rats (Philbrick et al 1987), rabbits (Lin et al 1991) and monkeys (Connor & Neuringer 1988) have been found in association with diets containing n-3 LCP from fish oils at 1.4 to 6% of energy.

Despite the marked changes in plasma and liver 20:5n-3 and 20:4n-6 found in piglets in Experiment III, no changes were found in the proportion of 20:5n-3 or 20:4n-6 in brain total lipid or synaptic plasma membrane EPL. A significant ($p < 0.009$) linear increase was found in the brain total lipid % 20:5n-3 of piglets fed the FO3 compared to FO1 or F diet (Fig. 3.8). The accumulation of 20:5n-3 in the brain of piglets fed the FO3 diet, however, did not result in levels of 20:5n-3 greater than 0.3% of fatty acids. The minimal amounts of 20:5n-3 deposited in the brain and synaptic plasma membrane EPL of piglets fed the FO3 diet suggests that 20:5n-3 was excluded from the brain at the relatively modest plasma phospholipid level of 20:5n-3 of about 2.9 ± 0.1% of fatty acids. Alternatively, the low levels of 20:5n-3 in brain could be explained by uptake and rapid oxidation or elongation and desaturation to 22:6n-3. Hepatic peroxisomal β-oxidation of 20:5n-3 (Hagve & Christophersen 1986) is known and may provide an explanation for the relatively low retention of dietary 20:5n-3. Whether or not the peroxisomes known to be present in brain (Singh et al 1989) are important in preventing accumulation of 20:5n-3 in CNS lipids is unknown. All of the n-3 LCP in the brain total lipid showed a significant increase with the increase in fish oil in the formula. This suggests that higher dietary intakes of fish oil, such as those used in studies with rodents and monkeys (Bourre et al 1988, Connor & Neuringer 1988, Swanson et al 1988), could override potential uptake regulation, or exceed the capacity of peroxisomal oxidation or elongation/desaturation pathways. Possible pharmacological doses of fish oil (3 − 6% of energy) could therefore result in significant excess accumulation of 20:5n-3 in brain.

The level of 20:4n-6 in the brain total lipid and synaptic plasma membrane EPL was constant over an increasing dietary intake of n-3 LCP from 0 (F diet) to 1.2% of energy (FO3 diet). Similarly, high levels of n-3

LCP in natural milk (Experiment IV) did not significantly alter the proportion of 20:4n-6 in the piglet brain and synaptic plasma membrane total lipids (Fig. 3.10). Bourre and associates (1990) also found no significant difference in brain total lipid % 20:4n-6 in adult rats fed n-3 LCP up to 1.6% of dietary energy. In contrast, a decrease of about 10 − 15% in the level of 20:4n-6 in brain EPL (Philbrick et al 1987, Wainwright et al 1992) and PC (Wainwright et al 1992) in rodents fed diets with about 1.4% of energy as n-3 LCP has been reported. In some cases, however, the rats were fed only 1.3% of energy as 18:2n-6 in the diet (Philbrick et al 1987), compared to 15% of energy in the piglets and about 8% of energy in other rat studies (Bourre et al 1990). This low level of 18:2n-6 may have been insufficient to allow desaturation/elongation of 18:2n-6 in the presence of large amounts of n-3 LCP. Although the energy density of n-3 LCP is similar to that of the FO3 diet used here, the rat diet in these studies (Philbrick et al 1987, Wainwright et al 1992) contained less total fat (c.a. 20% of dietary energy) and thus a higher percentage of fat as n-3 LCP than did the FO3 piglet diet. Therefore, the greater susceptibility of brain 20:4n-6 to diet-induced changes observed in these rats may be explained by lower amounts of dietary 18:2n-6 and/or different absolute and relative amounts of n-3 LCP fed, as well as possible effects of species differences.

The ability to maintain brain 20:4n-6 at dietary n-3 LCP intakes of about ≤ 1.5% of energy could be explained by the presence of specific, high affinity transport systems for 20:4n-6 into brain, such as that postulated for α-fetoprotein-bound fatty acids (Uriel et al 1987). Highly specific CNS glycerophospholipid acyltransferase reactions, such as arachidonyl-CoA synthetase (Morand et al 1987, reviewed by Sastry 1985) could also be important to the maintenance of the structural lipid composition of nervous tissues. Retroconversion of 22:4n-6 to 20:4n-6 has been shown for brain (Singh et al 1989), suggesting that 22:4n-6 may serve as a pool of n-6 fatty acids for maintenance of optimal 20:4n-6 levels. The significantly lower percentage of 22:4n-6 in the brain of piglets fed the FO3 rather than any other diet (Fig. 3.8) is consistent with this suggestion. Of interest, in Experiment I, the changes in the level of 20:4n-6 in the cerebrum total lipid and synaptic plasma membrane EPL of piglets fed formula were similar to changes in 22:4n-6. Changes in the proportion of 22:4n-6 in the piglet liver phospholipid and retina EPL, in contrast, paralleled the changes in 22:5n-6. Whether this reflects a greater contribution of 22:4n-6 to the maintenance of 20:4n-6 levels in cerebral tissues compared to a role of 22:4n-6 in maintaining overall membrane unsaturation in extra-cerebral tissues is not known. Alternatively, it is possible that the significant decrease in the brain percentage of 22:4n-6 and 22:5n-6 in piglets in the FO3 group, compared

to piglets fed the n-3 fatty acid-deficient F formula represented a return to normal levels with the concomitant elevation of brain 22:6n-3.

The results of these studies with piglets show important differences in the incorporation of dietary n-3 LCP from fish oil into growing piglet brain and retina when compared to liver or plasma, but a general similarity between the effects on plasma and liver. The greatest increase in the tissue levels of 22:6n-3 occurred between piglets in the n-3 fatty acid-deficient F group compared to the FO1 group (Fig. 3.9); smaller increases were found between the FO1 and FO3 diet groups. An important exception was the brain total lipid % 22:6n-3, where an apparent lag in 22:6n-3 was found. That is, the increase from the FO1 to FO3 group was greater than from the F to FO1 group. Others have shown no change in brain 22:6n-3 when dietary n-3 LCP was increased from 0 to 0.6% of energy, but a large increase in brain 22:6n-3, similar to that in piglets fed the FO3 diet, when dietary n-3 LCP was increased from 0.6 to 1.6% of energy (Bourre et al 1990). Although brain phosholipid 22:6n-3 was higher in weanling rats fed 1.4% compared to 0% of energy as n-3 LCP, the difference was not of statistical significance (Philbrick et al 1987) and others have found no difference in brain 22:6n-3 among rodents fed from 1.4% to 4% of energy as n-3 LCP (Bourre et al 1990, Wainwright et al 1992). This lack of effect of increasing dietary n-3 LCP on the brain 22:6n-3 may be interpreted as evidence that brain 22:6n-3 was already maximal. By extrapolation from the studies with rodents, it seems likely that the level of 22:6n-3 in the brain total lipid of piglets fed the FO3 diet was maximal, thus no further increase would be expected with fish oil supplementation up to about 4% of energy. The difference in the response between the brain and liver percentage of 22:6n-3 to fish oil supplementation possibly suggests the presence of specific uptake mechanisms which regulate the entry of 22:6n-3 into the brain, which are absent from liver. Levels of 22:6n-3 in the piglet retina EPL appeared to plateau between the FO1 (0.4% of energy as n-3 LCP) and FO3 (1.2% of energy) diets (Fig. 3.9), and seemed to be more tightly controlled than in other CNS tissues studied. This finding is consistent with selective uptake and retention of 22:6n-3 in the retina, which is compatible with the probable specific role of 22:6n-3 in the visual process (reviewed by Neuringer & Connor 1989). Diets with $\geq$ 4% of energy as n-3 LCP, however, have been found to result in levels of 22:6n-3 in brain and retina above those in control animals receiving diets with 2.1% of energy as 18:3n-3 from soybean oil (Connor et al 1990). Possibly, the very high levels of 22:6n-3 in the retina following repletion with fish oil may be a rebound effect in n-3 fatty acid -deficient animals. Alternatively, such high intakes may represent a pharmacological rather than nutritional change at which normal regulatory processes of uptake are no longer functional.

In summary, providing 22:6n-3 from fish oil was effective in supplying 22:6n-3 to the developing CNS. Constant levels of 20:4n-6 and 20:5n-3 were maintained in the brain over an increasing dietary intake of n-3 LCP from 0 to 1.2% of energy. This occurred despite circulating levels of 20:5n-3 at 2.9% of fatty acids, similar to levels in plasma PC of preterm infants fed fish oil (Carlson et al 1991). The level of 20:5n-3 was increased and 20:4n-6 was decreased in the liver, as well as the plasma phospholipid, of piglets fed increasing levels of dietary fish oil. These differences between the brain, and the liver and plasma could conceivably be explained by selective high affinity mechanisms for uptake of 20:4n-6 into brain, but not into liver. The increase of 20:5n-3 in the liver was not linear with increasing dietary n-3 LCP. This may possibly suggest regulatory mechanisms such as hepatic peroxisomal oxidation of 20:5n-3, or desaturation/elongation to 22:6n-3 to limit the accumulation of 20:5n-3 in the liver phospholipid.

4.5 The effect of maternal diet on the fatty acid composition of milk and the developing liver and CNS fatty acid composition in piglets

4.5.1 Fatty acid composition of milk lipid

Data have been published to suggest that high dietary intakes of 22:6n-3 from fish oil or marine lipids increase 22:6n-3 in human milk (Harris et al 1984, Henderson et al 1992, Innis & Kuhnlein 1988). The findings in Experiment IV confirm the transfer of 22:6n-3 from maternal diet to sow milk. Substantial amounts of 22:6n-3 (1.5% of fatty acids), and 20:5n-3 (0.4% of fatty acids) were secreted in the milk of sows fed 1% (wt/wt) fish oil providing c.a. 35 mg 22:6n-3 and 10 mg 20:5n-3/kg body wt/day. The relative proportions of 22:6n-3 and 20:5n-3 in the maternal diet and in the sow milk were similar, about 3:1. The percentages of 22:6n-3 and 20:5n-3 found in the sow milk (Table 3.5) are similar to those reported for the milk of women taking fish oil supplements (providing c.a. 17 mg 22:6n-3 and 30 mg 20:5n-3/kg body wt/day) (Harris et al 1984), or consuming diets high in marine lipids (Innis & Kuhnlein 1988). Somewhat lower levels of 22:6n-3 (0.7% of milk fatty acids) have recently been reported in the milk of women taking fish oil supplements (providing c.a. 12 mg 22:6n-3 and 18 mg 20:5n-3/kg body wt/day) for 21 days (Henderson et al 1992).

The sow milk composition of 18:2n-6 and 18:3n-3 (Table 3.5) reflected the differences in the quantity of these fatty acids in the maternal diet (Table 2.6). Similar data concerning human milk have been published (Harris et al 1984, Innis 1992, Innis & Kuhnlein 1988, Jensen 1989, Jensen et al 1992, Sanders & Reddy 1992).

91

Despite the difference in dietary intake and milk levels of 18:2n-6 and n-3 fatty acids between the two groups of sows, the proportion of 20:4n-6 in the sow milk total lipid was relatively constant at about 0.6% of fatty acids. Constant levels of 20:4n-6 (0.6% of fatty acids) have also been found in rabbit milk following dietary supplementation with 2% (wt/wt) fish oil (Lin et al 1991). Similarly, human milk 20:4n-6 does not seem to be related to the milk 18:2n-6 (Innis 1992, Innis & Kuhnlein 1988, Jensen 1989, Jensen et al 1992, Sanders & Reddy 1992), or to the maternal dietary or milk levels of 22:6n-3 and 20:5n-3 (Harris et al. 1984, Innis 1992, Innis & Kuhnlein 1988). Whether the content of 20:4n-6 in milk total lipid is regulated by some specific metabolic process in the mammary tissue is not known. In contrast, the level of 20:4n-6 in milk phospholipid was lower in the milk from sows fed diets with, rather than without fish oil (1.9% vs 4.8% of fatty acids, respectively). This finding suggests an effect of high n-3 LCP on phospholipid % 20:4n-6 in mammary epithelial cells, typical to that seen in other extra-cerebral tissues. That is, the lower proportion of 20:4n-6 in milk phospholipid may be explained by competition for acylation by the high 22:6n-3 and/or 20:5n-3 in the milk phospholipid of sows fed the diet with added fish oil (3.1% 22:6n-3 and 0.7% 20:5n-3) rather than vegetable oil only (0.4% 22:6n-3 and 0.3% 20:5n-3). It is not known, however, whether these changes in milk phospholipid fatty acids, which represent < 2% of milk fatty acids (Jensen 1989), are of significance to the nursing infant.

4.5.2 Piglet tissue fatty acid composition

The results of Experiment IV show 22:6n-3 was transferred from maternal diet to milk and from the milk to the plasma phospholipid of the nursing piglet. Furthermore, a high compared to very low intake of 22:6n-3 from milk was accompanied by higher levels of 22:6n-3 in the liver and cerebrum, but not retina, of the suckling animal. The increase in 22:6n-3 was several orders of magnitude greater in the plasma and liver phospholipids than in the cerebrum and its synaptic plasma membranes. These results are consistent with the known resistance of cerebrum and retina 22:6n-3, relative to other organs, to dietary n-3 fatty acid manipulation (Stubbs & Smith 1984). Published studies have reported that 22:6n-3 represents 15.7 $\pm$ 0.2% compared to 20.6 $\pm$ 0.9% retina total lipid fatty acids in newborn and adult pigs, respectively (Hrboticky et al 1991). A similar increase in n-3 fatty acids was found in brain synaptosomal membranes from these animals (Hrboticky et al 1989). These results suggest that, as in the human infant (Martinez 1992), the newborn piglet retina and brain is compositionally immature at birth and therefore vulnerable to manipulation by the postnatal dietary supply of n-3 fatty acids

(Hrboticky et al 1989, Hrboticky et al 1991). The significantly higher levels of 22:4n-6 and 22:5n-6 in the cerebrum and retina of piglets fed the sow milk low in 22:6n-3 (Table 3.6) is a characteristic finding in animals fed diets deficient in n-3 fatty acids (reviewed by Innis 1991). These results, therefore, may suggest that piglets suckling milk containing 0.1% of fatty acids as 22:6n-3, with 1.1% as 18:3n-3, may have received an inadequate dietary supply of n-3 fatty acids.

Levels of 22:6n-3 in human milk plateau after several days of fish oil supplementation (Henderson et al 1992). Therefore, the sow diet was modified for 4 days before birth to ensure maximum effects of fish oil supplementation on 22:6n-3 in the milk fed to newborn piglets. The degree to which differences in placental transfer of 22:6n-3 during the 4 days prior to birth (gestation about 116 days) contributed to differences in 22:6n-3 in the CNS of the piglets is unknown. The brain weight and 22:6n-3 content (mean ± SEM) was 25.0 ± 1.0 g and 50.5 ± 2.4 mg, respectively, in newborn piglets ($n=2$) and 39.8 ± 1.0 g and 76.6 ± 3.1 mg, respectively, in 15 day-old piglets from sows fed the diet with only vegetable oil added. This represents 40 and 50% increases, respectively, in brain weight and 22:6n-3 content from birth to 15 days. The increase in piglet body weight between birth and 15 days of age was also very large, about 300%. It seems unlikely, therefore, that the growth during the last 4 days of gestation would contribute considerably to changes in CNS 22:6n-3 composition.

In summary, the results of this study confirm the dependency of milk 22:6n-3 on the maternal dietary intake of 22:6n-3. These studies show that an increase in the milk 22:6n-3 from 0.1 to 1.5% of fatty acids led to increased 22:6n-3 in the circulating and liver phospholipids, and cerebrum and synaptic plasma membrane lipids of natural milk-fed piglets. The considerable effect of maternal dietary fat composition on milk fatty acids in the sow, and resultant differences in the proportion of 22:6n-3 in the developing piglet tissue and blood lipids, emphasize the difficulty of extrapolating dietary essential fatty acid requirements from the knowledge of the fatty acid composition of milk. The results also raise the questions of what the optimal level of 22:6n-3 is in the developing brain, what optimal levels are in milk, and whether adequate levels are achieved in the developing young fed milk containing very low amounts of 22:6n-3.

4.6 Effect of n-3 fatty acids in formula and milk on tissue levels of saturated and monounsaturated fatty acids

4.6.1 Piglet tissue saturated fatty acids

Saturated fatty acids can be synthesized *de novo* from acetyl CoA. The amount and composition of saturated fatty acids in membrane lipids play a role in determining the physical properties of structural membranes. The percentage of 16:0 in the liver phospholipid was lower in piglets fed the formula with high 18:3n-3 (Fig 3.1, Table 3.3), the formula with n-3 LCP from fish oil (Experiment III, data not shown), or milk with high levels of n-3 LCP (Table 3.6). This decrease in the percentage of 16:0 was accompanied by an increase in the proportion of total LCP in piglets fed the formulas containing 4% 18:3n-3 and those fed milk with 1.5% 22:6n-3, compared to the respective comparison groups. However, quantitative differences in liver phospholipid 16:0 did exist between piglets fed the 16/1 versus 16/4 and between piglets fed the 30/1 versus 35/4 formulas (data not shown). These results may suggest that high dietary intakes of n-3 fatty acids from either formula or natural milk may interfere with saturated fatty acid metabolism. The decrease in liver phospholipid % 16:0 could possibly be related to decreased synthesis and/or increased utilization of 16:0. Several possible explanations are available. The formulas in Experiment I all contained similar low levels of 16:0 and 18:0 (8 and 4% of fatty acids, respectively), suggesting that the relatively high proportions of these fatty acids in the phospholipids of brain and liver are likely to have originated from *de novo* synthesis, or possibly from chain elongation of the formula 14:0. The quantity of fatty acids of $C \leq 14$ was 5 − 10% lower in the formulas with 4% 18:3n-3 than in the formulas with 1% 18:3n-3 (Table 2.3). The medium chain fatty acids of C8 − C12 are known to be rapidly oxidized to acetyl-CoA. The acetyl-CoA produced by β-oxidation may be a source of C2 units for *de novo* synthesis of fatty acids, or for use in elongation of 14:0 to 16:0, and 16:0 to 18:0 (Bach & Babayan 1982). Whether the difference in the amount of fatty acids of $C \leq 14$ in the formulas with 1 compared to 4% 18:3n-3 could explain the difference in saturated fatty acid metabolism in the piglets is unclear.

De novo synthesis of 16:0 is known to be inhibited by 18:3n-3, as a result of both short-term inhibition of acetyl CoA carboxylase (Bloch & Vance 1977) and by a long-term adaptive response in which the activity and synthesis of acetyl CoA carboxylase (Abraham et al 1983, Toussant et al 1981) and fatty acid synthetase (Abraham et al 1983, Clarke et al 1977, Toussant et al 1981) are decreased. Dietary n-3 LCP from shellfish or as pure 22:6n-3 have also been shown to decrease the activity of acetyl-CoA carboxylase, glucose-6-phosphate dehydrogenase and fatty acid synthetase in rat liver (Clarke et al 1976, Iritani et al 1980, Williams et al 1989).

LCP have been shown to depress fatty acid synthetase mRNA by inhibiting fatty acid synthetase gene transcription, specifically in the liver (reviewed by Clarke & Abraham 1992). Thus, it is plausible that lower proportions of 16:0 in liver phospholipid may be explained by inhibition of fatty acid synthesis by the high dietary intakes of n-3 fatty acids.

In contrast to the findings reported in this thesis, the level of saturated fatty acids in adult rat liver did not differ as a result of feeding increasing dietary 18:3n-3 (Hwang et al 1986) or n-3 LCP from fish oil (Bourre et al 1988, Bourre et al 1990). The discrepancy between the current data with piglets and those reported for adult rodents (Bourre et al 1988, Bourre et al 1990, Hwang et al 1986) may be related to more pronounced effects evident in a rapidly growing animal with greater fatty acid needs for tissue growth than a mature animal.

Unlike the liver, cerebrum total lipid percentage of 16:0 and of total saturated fatty acids were not altered in piglets fed formula with high 18:3n-3 (Fig. 3.1, Table 3.3), formula with high n-3 LCP (Fig. 3.7) or sow milk with high n-3 LCP (Table 3.6). An exception was a significantly lower percentage of 16:0 in cerebrum total lipid of piglets fed the 16/4 compared to 16/1 formula (Fig. 3.1). Published studies have shown the dietary 18:1 content may influence the tissue levels of other fatty acids (Wall et al 1992). It is reasonable to assume, therefore, that the high 18:1 content (40% of fatty acids) of the 16/4 formula may have contributed to the differences in the level of 16:0 in cerebrum total lipid, which were not seen when 4% 18:3n-3 was fed with low (17%) 18:1. In contrast to the cerebrum, no changes were seen in the saturated fatty acid composition of the synaptic plasma membrane at the high dietary intakes of n-3 fatty acids (Table 3.3, Table 3.6, Fig. 3.7). The cerebrum total lipid, however, included the structural lipids of myelin which contain large amounts of saturated and monounsaturated fatty acids (reviewed by Sastry 1985). The analyses of cerebrum total lipid in these studies, therefore, reflects the developmental increase in the relative amount of myelin to the neural membranes, as well as maturation or diet-modifications in the fatty acid composition of myelin and all other brain membranes from birth to 15 days of age. The change in saturated fatty acids of cerebrum total lipid, but not synaptic plasma membrane, suggests that the differences in cerebrum total lipid may have resulted from changes in the myelin component of brain. It has been suggested that all of the saturated fatty acids required by the brain for membrane growth are synthesized *in situ* (Marbois et al 1992). The findings in piglets in this thesis suggest that the deposition of fatty acids in brain may be modified by the dietary n-3 fatty acid intake.

4.6.2 Piglet tissue monounsaturated fatty acids

Significant changes in the total monounsaturated fatty acids, predominantly 18:1, of liver, cerebrum, synaptic plasma membrane and retina were seen in response to the amount of 18:2n-6 in the formula. The amount of 18:2n-6 in the formula was adjusted by a reciprocal change in the amount of 18:1 (Table 2.3). Therefore, the changes in tissue 18:1 composition are probably most appropriately discussed with respect to formula content of 18:1. Levels of monounsaturated fatty acids in liver phospholipid and cerebrum total lipid were about 50% and 5% lower, respectively, in piglets fed the formula containing low (17% of fatty acids) 18:1 (i.e. 30/1 and 35/4 formulas) rather than high 18:1 (40% fatty acids) (i.e. 16/1 and 16/4 formulas) (Fig. 3.1, Table 3.3). These values are very similar to the 60% and 8% lower proportion of total monounsaturated fatty acids recently reported in liver phospholipid and brain total lipid of 18 day-old piglets fed formulas with 11% compared to 41% 18:1 (Wall et al 1992). Possibly, the more limited effect of the formula 18:1 content on the cerebrum than liver is explained by de novo fatty acid synthesis by the oligodendrocytes, which are known to synthesize large amounts of myelin lipid containing particularly high 18:1 (reviewed by Sastry 1985) during the first three weeks after birth in the piglet (Sweasy et al 1976). Levels of monounsaturated fatty acids in cerebrum total lipid were also lower in piglets fed the formulas with high 18:3n-3 (Fig. 3.1, Table 3.3) or increasing fish oil supplementation in formula (Fig 3.7). There was no evidence, however, of a significant effect of high n-3 fatty acids on the proportion of total monounsaturated fatty acids in the synaptic plasma membrane EPL of these piglets. Others have reported that brain EPL percent 18:1 of suckling mice was significantly higher when dietary n-3 LCP was increased from 1.4 to 2.2% of energy; however, no difference was seen when n-3 LCP was increased from 0 to 1.4% of energy (Wainwright et al 1992). Possibly, the discrepancy in these results is explained by a pharmacological response at 2.2% of energy as n-3 LCP, or by the differences in the stage of brain development and myelination between the postnatal piglet and mouse (Dobbing & Sands 1979).

In summary, formula and milk n-3 fatty acids have been shown to alter the levels of saturated fatty acids in the piglet cerebrum, retina and liver. It is possible that the lower proportion of 16:0 in liver phospholipid may be explained by inhibition of de novo fatty acid synthesis by high dietary n-3 fatty acids. Liver and CNS monounsaturated fatty acids were increased with higher amounts of dietary 18:1. Changes were found in the cerebrum total lipid monounsaturated fatty acids in response to high dietary intakes of n-3 fatty acids, which may

possibly be explained by changes in the myelin component of brain, rather than the grey matter. The determination of essential fatty acid requirements, specifically 18:3n-3, should take into consideration the possible influences on the saturated and monounsaturated fatty acids which also play a role in determining the properties of structural membranes of important organs such as the liver.

4.7 Approaches to study the dietary adequacy of n-3 fatty acids in infant nutrition

The amounts and relative ratios of 18:2n-6 and 18:3n-3 in infant formulas that provide the best outcomes cannot be determined with certainty at present. The approaches used to study dietary adequacy of n-3 fatty acids in infant nutrition that have been addressed in this thesis include (1) milk fatty acid composition, (2) body and organ weight and tissue fatty acid composition (3) plasma and RBC fatty acid composition.

4.7.1 Milk fatty acid composition

The composition of mature human milk has been used in the development of recommendations for the lipid composition of infant formula. There are a number of factors which must be considered when using human milk as a model for other infant diets. For example, the fatty acid pattern of human (reviewed in Innis 1992 and in Jensen 1989) and, as shown in these studies, sow milk is influenced by the composition of the maternal diet. The dependence of milk 22:6n-3 on maternal diet can result in levels of 22:6n-3 as low as 0.1% of fatty acids in the milk of women and sows consuming diets devoid of animal products and as high as 1.4% of fatty acids in the milk of women and sows consuming diets of high marine lipid content. It is not known if current intakes of 22:6n-3 from milk represent optimal intakes for deposition of 22:6n-3 in the developing CNS. Secondly, the ratio of total n-6:n-3 fatty acids in human milk is about 4:1 to 10:1 (reviewed by Jensen 1989). However, natural milk contains preformed LCP, which are more biologically active than the precursors 18:2n-6 and 18:3n-3 supplied in formulas. The dietary 18:2n-6:18:3n-3 ratio in formula is therefore not equivalent to the total n-6:n-3 fatty acid ratio in human milk with preformed LCP. It would be useful to know the relative biological activity of 18:3n-3 to n-3 LCP, *i.e.* the amount of 18:3n-3 needed in formula to achieve equivalent metabolic effects to that of dietary 22:6n-3. From the studies in this thesis, the biological activity of 18:3n-3 as a source of 22:6n-3 can be estimated to be at least 20% that of n-3 LCP. Published studies have suggested this number may be as high as 33%

(Anderson et al 1990). In human milk, the total n-3 fatty acid content is normally 0.7 − 1.3% of energy, with the n-3 LCP accounting for about half of this. Assuming a biological equivalency of 20 and 33%, formulas providing 2.1 − 3.9% of energy or 1.4 − 2.6% of energy as 18:3n-3 could theoretically support deposition of 22:6n-3 in the CNS, similar to that achieved by human milk with typical amounts of preformed LCP.

4.7.2 Levels of 22:6n-3 in CNS tissues

Changes in the total lipid fatty acid composition of the brain or cerebrum are the most commonly reported measures in animal studies of fatty acid requirements. These analyses in the initial studies allowed comparison of the findings in piglets to studies of other species. The fatty acid composition of cerebrum EPL was subsequently determined to allow comparison of the effects in different piglet tissues and organs, in a specific phospholipid fraction. It is notable that changes in cerebrum EPL percentage of n-3 LCP were similar to those found in cerebrum total lipids. In contrast, more subtle differences between the formula groups were detected only in the synaptic plasma membrane and retina. For example, the percentage of 22:6n-3 was significantly lower in piglet synaptic plasma membrane EPL when the ratio of 18:2n-6:18:3n-3 in the formula fed was increased from 22:1 (16/1 formula) to 37:1 (30/1 formula). Significant differences in 22:6n-3 composition between these two piglet groups were not evident in cerebrum total lipid or cerebrum EPL.

The studies in Experiment IV have shown a range of tissue 22:6n-3 composition exists among natural milk-fed piglets. It seems reasonable, therefore, to evaluate the deposition of 22:6n-3 in CNS tissues of piglets fed formulas containing 18:2n-6 and 18:3n-3, but no long-chain derivatives, within this range. Otherwise, the interpretation of the dietary adequacy of 18:3n-3 depends on the natural milk-fed group used for comparison. In this regard, levels of 22:6n-3 in cerebrum EPL of piglets fed formulas containing 4% 18:3n-3 fall within the range of natural milk-fed piglets (Fig. 3.13). However, if comparison is made to piglets consuming milk with 1.5% 22:6n-3, all formulas would be considered inadequate to support deposition of 22:6n-3 in the cerebrum, comparable to natural milk.

Together, these findings suggest that the sensitivity of measures of tissue LCP content as a method to evaluate the adequacy of the dietary 18:3n-3 supply depends on the organ, membrane and phospholipid fraction chosen for study, as well as the reference group used for comparison. For example, compositional changes in synaptic plasma membrane and retina EPL, rather than cerebrum total lipid or EPL appear to be more sensitive

to dietary changes of 18:3n-3. Therefore, the comparison of levels of 22:6n-3 in specific tissues of the CNS, such as synaptic plasma membrane and retina EPL are probably most appropriate to evaluate the adequacy of formula 18:3n-3 during growth and development.

4.7.3 Plasma and RBC phospholipid LCP: Comparison to liver, cerebrum and retina

Clinical studies on the adequacy of infant n-6 and n-3 fatty acid nutrition have traditionally relied on comparative measures of plasma and RBC phospholipid fatty acids (Carlson et al 1986, Clark et al 1992, Innis et al 1990, Ponder et al 1992, Putnam et al 1982, Sanders et al 1992, Uauy et al 1990). Therefore, changes in the proportion of 22:6n-3 and 20:4n-6 in plasma phospholipid and RBC EPL and PC in response to the different milk and formula diets were compared to assess the accuracy and potential limitations of these measures as indices of the adequacy of n-6 and n-3 fatty acids in developing organs.

The significantly lower 22:6n-3 and higher 22:5n-6 in the RBC and plasma phospholipids of piglets fed formulas with 1% 18:3n-3 reflected the changes in the liver and CNS and appear, therefore, to be valid indicators of dietary n-3 fatty acid deficiency (Fig. 3.13 and 3.14). The circulating levels of 22:6n-3 (and 22:5n-6) in piglets fed formulas with 4% 18:3n-3 and sow milk with very low levels of 22:6n-3 (0.1% of fatty acids) were also similar, as were levels of 22:6n-3 in the liver and CNS of these piglet groups. It is important to note, however, that the plasma and RBC phospholipid levels of 22:6n-3 showed a clear response to the dietary intake of 22:6n-3 (Fig. 3.13), with 2 to 3-fold greater levels in piglets receiving milk with 1.5% compared to 0.1% 22:6n-3. The plasma and RBC phospholipid percentage of 22:6n-3 in piglets fed formulas containing 18:2n-6 and 18:3n-3, but not their long-chain derivatives, was lower than in piglets fed 1.5% 22:6n-3 in natural milk. Therefore, based on lower blood phospholipid 22:6n-3, all formulas fed to the piglets would be considered inadequate in n-3 fatty acids. However, in contrast to blood lipids, feeding formulas with 4% 18:3n-3 maintained similar levels of 22:6n-3 in the CNS to natural milk. It is possible, therefore, that the lower plasma and RBC phospholipid 22:6n-3 reported in infants fed formulas rather than human milk (Carlson et al 1987, Innis 1991, Liu et al 1987, Sanders & Reddy 1992, Uauy et al 1990) may reflect the difference in dietary intake of 22:6n-3, which is negligible in infant formula and typically 0.4% from human milk fatty acids (Innis 1992), but may not indicate lower levels in the CNS.

Levels of 20:4n-6 in circulating and liver phospholipids were significantly lower in piglets fed the milk with high rather than low 22:6n-3 (Fig. 3.12), despite similar intakes of 20:4n-6 from the milk (Table 3.5). Piglets

fed formulas containing 18:2n-6 but devoid of 20:4n-6 had a similar proportion of 20:4n-6 in RBC EPL and PC to piglets receiving 20:4n-6 from sow milk. Therefore, circulating lipid levels of 20:4n-6 do not reflect differences in the dietary 20:4n-6 supply, but do seem to depend on the intake and type of n-3 fatty acids in the diet. Although marked differences in 20:4n-6 are evident in plasma and liver, this does not seem to alter the deposition of 20:4n-6 in the developing piglet cerebrum. The assimilation and avid retention of 22:6n-3 and 20:4n-6 in cerebrum, at different intakes of n-3 fatty acids from formula, was not consistently or accurately reflected by the fatty acid composition of the plasma or RBC. These differences could be explained by selective, high affinity mechanisms for uptake of 20:4n-6 and C22 n-6 and n-3 fatty acids into brain, but not into RBCs or liver (reviewed by Innis 1992).

In summary, low circulating 22:6n-3, when accompanied by increased 22:5n-6, seems to be a valid indicator of low 22:6n-3 in the liver and CNS of term piglets fed formulas deficient in 18:3n-3. Low plasma and RBC phospholipid 22:6n-3 not accompanied by increased 22:5n-6, however, may merely reflect differences in dietary intake of 22:6n-3 when comparison is made to piglets receiving high amounts of 22:6n-3 in milk. Differences in circulating 20:4n-6 do not reflect changes in CNS lipid 20:4n-6. Thus, the response of 22:6n-3 and 20:4n-6 in piglet plasma and RBC is not a valid measure of adequacy of 22:6n-3 and 20:4n-6 in the developing CNS. This would preclude the use of plasma and RBC phospholipid 22:6n-3 and 20:4n-6 as an indicator of differences in CNS 22:6n-3 and 20:4n-6 in formula-fed compared to milk-fed piglets or in human infants fed diets varying in fatty acid composition.

4.8 Concluding remarks

The major findings of this thesis are: (1) 22:6n-3 is not essential in the diet of the term gestation piglet, if adequate 18:3n-3 is provided, (2) adequate synthesis and deposition of 22:6n-3 depends on both the absolute amount of 18:3n-3 and ratios of 18:2n-6:18:3n-3 in the formula, (3) high intakes of 18:3n-3 and n-3 LCP may adversely affect brain growth and possibly saturated fatty acid metabolism, (4) differences in the amount of 22:6n-3 in milk, related to maternal dietary intake of 22:6n-3, extend to the liver lipids and, to a lesser extent, the CNS of the nursing young, (5) diet-related differences in plasma and RBC fatty acids do not accurately reflect changes in CNS 22:6n-3 and 20:4n-6.

Lower cerebrum weight in animals fed high dietary 18:3n-3 has not been previously reported. Therefore, it is important to determine whether a similar effect on brain weight occurs in other species, or whether this effect is specific to piglets from the lower mainland of British Columbia, with potentially compromised vitamin E and/or selenium status. If observed in other species, the mechanism responsible for this effect on growth, seen specifically in the brain and not other organs, needs to be studied. It is possible that the adverse effects on growth may be related to fatty acid changes in a specific organelle or membrane, for example, microsomes, mitochondria or nuclei, that may not be detected in the analyses of cerebrum total lipids.

The oil blends and 18:2n-6 and 18:3n-3 content of the formulas fed to piglets were selected to be relevant to commercially available infant formulas. Adequate synthesis and deposition of 22:6n-3 in CNS tissues occurred when formula contained 2.1% of energy, but not 0.4% of energy as 18:3n-3, compared to milk-fed animals. The next logical step, particularly given the potential adverse effects of high 18:3n-3 on brain growth, would be to determine the lowest level of dietary 18:3n-3 required to support comparable deposition of 22:6n-3 in the CNS, in an 18:2n-6:18:3n-3 ratio of about 8:1. The requirement for 18:3n-3 was shown to be $> 0.4\%$ of energy; however, tissue levels of 22:6n-3 were higher when the 18:2n-6:18:3n-3 ratio was lowered from 37:1 to 22:1. It remains to be determined if 0.4% of energy as 18:3n-3 can support adequate synthesis of 22:6n-3 at dietary ratios of 4:1 and 8:1.

Studies have reported an interaction between dietary saturated and monounsaturated fatty acids and the desaturation (Brenner & Peluffo 1966, Garg et al 1989) and tissue composition (Garg et al 1990, Wall et al 1992) of n-6 and n-3 fatty acids. However, this is believed to be the first report of a potential interaction between the dietary intake of 18:3n-3 and deposition of 16:0 in growing tissues. Further studies are required to determine how the level and balance of n-6 and n-3 fatty acids alter the metabolism and incorporation of saturated and monounsaturated fatty acids into brain and liver lipids. One possible area of research could be the potential inhibitory effects of formula 18:3n-3 on *de novo* fatty acid synthesis.

Limitations in several of the measures currently used to evaluate the adequacy of dietary n-3 fatty acids have been revealed in these studies. For example, (1) milk 22:6n-3 levels in pigs vary with maternal dietary intake of 22:6n-3, as they are known to do in humans. Milk levels of 22:6n-3, therefore do not necessarily reflect the specific needs of the piglet, (2) plasma and RBC 22:6n-3 may reflect the dietary intake of the nursing young and

are not specific indices of brain 22:6n-3 in piglets, and (3) evaluation depends on which comparison group is considered "normal". One of the major difficulties in assessing optimal versus normal or typical levels of 22:6n-3 in the brain or retina is the lack of a physiological measurement. It is possible that there is a small window of 22:6n-3 composition in CNS membranes above and below which some membrane function may be altered. Further advances in the understanding of n-6 and n-3 fatty acids in growth and development will require functional measures of 20:4n-6 and 22:6n-3 which accurately reflect deficiency or excess and adequacy of n-6 and n-3 LCP in growing membranes.

These studies have shown differences in liver and cerebrum 22:6n-3 composition due to high maternal intakes of fish oil during lactation. The most rapid rate of assimilation of 22:6n-3 and 20:4n-6 in the brain and retina, however, occurs in the human during the last trimester of gestation (Martinez 1992). It is not known to what extent maternal diet throughout gestation affects fetal and newborn CNS levels of 22:6n-3. Therefore, it would be useful to examine the CNS 22:6n-3 content and organ weights in fetal and newborn piglets from sows fed diets differing in 22:6n-3 throughout gestation. Liver and CNS fatty acid composition could be compared in fetal pigs at the beginning of the third trimester (about 80 days gestation), at birth and after nursing for 5, 15 and 25 days. The sow diet would be manipulated to achieve milk 22:6n-3 levels of about 0.1, 0.4 and 1.4% of fatty acids, representing levels found in human milk from women consuming vegan, omnivorous and high marine lipid diets, respectively. Such a study would show whether different fetal and infant reserves of fatty acids are associated with maternal 22:6n-3 intakes, or whether selective mechanisms ensure an adequate supply and/or prevent an excessive supply of LCP to the developing fetus.

The differences shown in milk 22:6n-3 were in response to extremely low and high intakes of 22:6n-3 from vegan and fish oil diets, respectively. Milk 20:4n-6 is believed to be independent of dietary 18:2n-6 and 20:4n-6 intake; however, studies of human populations with low and high intakes of 20:4n-6 have not been done. It is plausible that levels of 20:4n-6 in sow milk may be manipulated by including increasing levels of 20:4n-6 in the sows' diet. These studies, however, would require large amounts of 20:4n-6, for which there is no commercially feasible source at present.

In conclusion, this thesis has contributed to the fundamental knowledge on the dietary requirement of 18:3n-3 in the newborn. The information should be useful in determining acceptable quantities and ratios of 18:2n-6 and 18:3n-3 in infant formula which will support membrane accretion of 20:4n-6 and 22:6n-3 in the CNS,

similar to milk-fed comparison groups. Knowledge of the interactive effects of dietary 18:2n-6 and 18:3n-3 on CNS tissue LCP may also be applied to the design of intravenous lipids administered to infants. Possible species differences between piglets and humans in lipid metabolism, or due to differences in rate of growth however, do need to be considered. The adequacy of potential 18:2n-6:18:3n-3 contents and ratio should be verified with biochemical and functional studies in infants. The finding that 22:6n-3 is not an essential dietary nutrient for the term gestation piglet may negate the need for the addition of fish oil to formulas, thereby avoiding potential detrimental effects on growth and development. Although adequate synthesis of 22:6n-3 and deposition in the CNS has been demonstrated, the relative importance of *in situ* synthesis of 22:6n-3 in the brain or uptake of 22:6n-3 synthesized in the liver, intestine, or some other organ is not known. Further studies are required to identify the ligand and mechanism whereby 22:6n-3 that is synthesized in extra-cerebral tissues may be delivered to and selectively taken up by the developing brain.

5 REFERENCES

Abraham, S., Hillyard, L.A., Lin, C.Y. & Schwartz, R.S. (1983) Effect of specific dietary faty acids on lipogenesis in the livers and mammary glands of lactating mice. *Lipids* 18: 820-829.

Alling, C., Bruce, Å., Karlsson, I. & Svennerholm, L. (1974) The effect of different dietary levels of essential fatty acids on lipids of rat cerebrum during maturation. *J. Neurochem.* 23: 1263-1270.

Anderson, G.J. & Connor, W.E. (1988) Uptake of fatty acids by the developing rat brain. *Lipids* 23: 286-290.

Anderson, G.J., Connor, W.E. & Corliss, J.D. (1990) Docosahexaenoic acid is the preferred dietary n-3 fatty acid for the development of the brain and retina. *Pediatr. Res.* 27: 89-97.

Anderson, R.E., Benolken, R.M., Dudley, P.A., Landis, D.J. & Wheeler, T.G. (1974) Polyunsaturated fatty acids of photoreceptor membranes. *Exp. Eye Res.* 18: 205-213.

Anding, R.H. & Hwang, D.H. (1986) Effects of dietary linolenate on the fatty acid composition of brain lipids in rats. *Lipids* 21: 697-701.

Arenander, A.T. & de Vellis, J. (1989) Development of the nervous system. In: *Basic Neurochemistry: Molecular, Cellular, and Medical Aspects*, 4th Edn. (Siegel, G., Agranoff, B., Albers, R.W. & Molinoff, P., eds.), pp. 479-506, Raven Press, New York, NY.

Axelrod, J., Burch, R.M. & Jelsema, C.L. (1988) Receptor-mediated activation of phospholipase A_2 via GTP-binding proteins: arachidonic acid and its metabolites as second messengers. *Trends Neurosci.* 11: 117-123.

Bach, A.C. & Babayan, V. (1982) Medium-chain triglycerides: an update. *Am. J. Clin. Nutr.* 43: 745-751.

Barzanti, V., Maranexi, M., Solaini, G. & Turchetto, E. (1990) Dietary lipid effects on microsome fatty acid composition of liver and brain, on liver glucose-6-phosphatase, and on brain 5'-nucleotidase activity in the rat. *J. Nutr. Biochem.* 1: 305-309.

Birch, E.E., Birch, D.G., Hoffman, D.R. & Uauy, R. (1992) Dietary essential fatty acid supply and visual acuity development. *Invest. Ophthmal. Vis. Sci.* 33: 3242-3253.

Bitman, J., Wood, L., Hamosh, M. & Mehta, N.R. (1983) Comparison of the lipid composition of breast milk from mothers of term and preterm infants. *Am. J. Clin. Nutr.* 38: 300-312.

Bjerve, K.S., Thoresen, L., Børsting, S. (1988) N-3 fatty acids: a new essential growth factor in man. *J. Parent. Ent. Nutr.* 12: 521-525.

Bloch, K. & Vance, D. (1977) Control mechanisms in the synthesis of saturated faty acids. *Ann. Rev. Biochem.* 46: 263-298.

Bourre, J.M., Faivre, A., Dumont, O., Nouvelot, A., Loudes, C., Puymirat, J. & Tixier-Vidal, A. (1983) Effect of polyunsaturated fatty acids on fetal mouse brain cells in culture in a chemically defined medium. *J. Neurochem.* 41: 1234-1242.

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

Bourre, J.M., Bonnell, M., Dumont, O., Piciotti, M., Nalbone, G. & Lafont, H. (1988) High dietary fish oil alters the brain polyunsaturated fatty acid composition. *Biochim. Biophys. Acta* 960: 458-461.

Bourre, J.M., Francois, M., Youyou, A., Dumont, O., Piciotti, M., Pascal, G. & Durand, G. (1989) The effects of dietary α-linolenic acid on the composition of nerve membranes, enzymatic activity, amplitude of electrophysiological paramaters, resistance to poisons and performance of learning tasks in rats. *J. Nutr.* 119: 1880-1892.

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

Bourre, J.M., Piciotti, M., Dumont, O., Pascal, G. & Durand, G. (1990a) Dietary linoleic acid and polyunsaturated fatty acids in rat brain and other organs. Minimal requirements of linoleic acid. *Lipids* 25: 465-472.

Bourre, J.M., Piciotti, M. & Dumont, O. (1990b) Δ6 Desaturase in brain and liver during development and aging. *Lipids* 25: 354-356.

Bourre, J.M., Dumont, O., Piciotti, M., Clément, M., Chaudière, J., Bonneil, M., Nabone, G., Lafont, J., Pascal, G. & Durand, G. (1991) Essentiality of ω3 fatty acids for brain structure and function. In: *Health Effects of ω3 Polyunsaturated Fatty Acids in Seafoods*. World Rev. Nutr. Diet, 66: 103-117, Karger, Basel, Switzerland.

Brenner, R.R. & Peluffo, R.O. (1966) Effect of saturated and unsaturated fatty acids on the desaturation in vitro of pamitic, stearic, oleic, linoleic and linolenic acids. *J. Biol. Chem.* 241: 5213-5219.

Brenner, R.R., Peluffo, R.O., Nervi, A.M. & Detomás, M.E. (1969) Competitive effect of α- and γ-linolenyl-CoA and arachidonyl-CoA in linoleyl-CoA desaturation to γ-linolenyl-CoA. *Biochim. Biophys. Acta* 176: 420-422.

Brown, M.L., Marshal, L.A. & Johnson, P.A. (1984) Alteration in cerebral and microvascular prostaglandin synthesis by manipulation of dietary essential fatty acids. *J. Neurochem.* 43: 1392-1400.

Brown, M.F. & Gibson, N.J. (1992) Biological functon of docsahexaenoic acid in the retinal rod disk membranes. In: *Essential Fatty Acids and Eicosanoids: invited papers from the Third International Conference* (Gibson, R.A. & Sinclair, A.J., eds.) pp. 134-138, Am. Oil Chemists' Soc., Champaign, IL.

Carlson, S.E., Rhodes, P.G. & Ferguson, M.G. (1986) Docosahexaenoic acid status of preterm infants at birth and following feeding with human milk or formula. *Am. J. Clin. Nutr.* 44: 798-804.

Carlson, S.E., Rhodes, P.G., Rao, V.S. & Goldgar, D.E. (1987) Effect of fish oil supplementation on the n-3 fatty acid content of red blood cell membranes in preterm infants. *Pediatr. Res.* 21: 507-510.

Carlson, S.E., Cooke, R.J., Werkman, S.H. & Peeples, J.M. (1989) Docosahexaenoate (DHA) and eicosapentaenoate (EPA) supplementation of preterm infants: Effects on phospholipid DHA and visual acuity. *Fed. Proc.* 3:A1056 (abstr.).

Carlson, S.E., Cooke, R.J., Peeples, J.M., Werkman, S.H. & Tolley, E.A. (1989) Docosahexaenoate (DHA) and eicosapentaenoate (EPA) status of preterm infants: relationship to visual acuity in n-3 supplemented and unsupplemented infants. *Pediatr. Res.* 25: 285A (abstr.).

Carlson, S.E., Cooke, R.J., Rhodes, P.G., Peeples, J.M., Werkman, S.H. & Tolley, E.A. (1991) Long-term feeding of formulas high in linolenic acid and marine oil to very low birth weight infants: phospholipid fatty acids. *Pediatr. Res.* 30: 404-412.

Carlson, S.E., Werkman, S.H., Peeples, J.M., Cooke, R.J., Tolley, E.A. & Wilson, W.M. (1992) Growth and development of very low birth weight infants in relation to N-3 and N-6 essential fatty acid status. In: *Essential Fatty Acids and Eicosanoids: invited papers from the Third International Conference* (Gibson, R.A. & Sinclair, A.J., eds.) pp. 192-196, Am. Oil Chemists' Soc., Champaign, IL.

Carlson, S.E., Werkman, S.H., Peeples, J.M., Cooke, R.J. & Wilson, W.M. (1992a) Plasma phospholipid arachidonic acid and growth and development of preterm infants. In: *Recent Advances in Infant Feeding:* symposium Leidschendam 1990, (Koletzko, B., Okken, A., Rey, J., Salle, B & Van Biervliet, J.P. eds.), pp. 22-27. Georg Thieme Verlag, Stuttgart; New York.

Carlson, S.E., Cooke, R.J., Werkman, S.H. & Tolley, E.A. (1992b) First year growth of preterm infants fed standard compared to marine oil n-3 supplemented formula. *Lipids* 27: 901-907.

Cartwright, I.J., Pockley, A.G., Galloway, J.H., Greaves, M. & Preston, F.E. (1985) The effects of dietary ʊ-3 polyunsaturated fatty acids on erythrocyte deformability and blood viscosity in healthy volunteers. *Atherosclerosis* 55: 267-281.

Chen, P.S., Toribara, T.Y. & Warner, H. (1956) Microdetermination of phosphorus. *Anal. Chem.* 28: 1756-1758.

Chen, Z.-U. & Cunnane, S.C. (1992) Early postnatal development in the rat is characterized by accumulation of highly unsaturated triacylglycerols. *Pediatr. Res.* 31: 47-51.

Christie, W.W. (1982) *Lipid Analysis*, 2nd Edn. Pergamon Press, Oxford, England.

Christon, R. Fernandez, Y., Cambon-Gros, C., Periquet, A., Deltour, P., Leger, C.L. & Mitjavila, S. (1988) The effect of dietary essential fatty acid deficiency on the composition and properties of the liver microsomal membrane of rats. *J. Nutr.* 118: 1311-1318.

Clandinin, M.T., Chappell, J.E., Leong, S., Heim, T., Swyer, P.R. & Chance, G.W. (1980) Extrauterine fatty acid accretion in infant brain: implications for fatty acid requirements. *Early Hum. Dev.* 4: 131-138.

Clandinin, M.T., Chappell, J.E. & Heim, T. (1981) Do low birthweight infants require nutrition with long chain elongation-desaturation products of essential fatty acids? *Prog. Lipid Res.* 20: 901-904.

Clandinin, M.T., Wong, K. & Hacker, R. (1985) Synthesis of chain elongation-desaturation products of linoleic acid by liver and brain microsomes during development of the pig. *Biochem. J.* 226: 305-309.

Clark, K.J., Makrides, M., Neumann, M.A. & Gibson, R.A. (1992) Determination of the optimal ratio of linoleic acid to α-linolenic acid in infant formulas. *J. Pediatr.* 120: S151-158.

Clarke, S.D., Rosmos, D.R. & Leveille, G.A. (1976) Specific inhibition of hepatic fatty acid synthesis exerted by dietary linoleate and linolenate in essential fatty acid adequate rats. *Lipids* 11: 485-490.

Clarke, S.D., Romsos, D.R. & Leveille, G.A. (1977) Differential effects of dietary methyl esters of long-chain saturated and polyunsaturated fatty acid on rat liver and adipose tissue lipogenesis. *J. Nutr.* 107: 1170-1181.

Clarke, S.D. & Abraham, S. (1992) Gene expression: nutrient control of pre- and posttranscriptional events. *FASEB J.* 6: 3146-3152.

Clouet, P., Niot, I. & Bézard, J. (1989) Pathway of α-linolenic acid through the mitochondrial outer membrane in the rat liver and influence on the rate of oxidation. *Biochem. J.* 263: 867-873.

Connor, W.E., Neuringer, M., Barstad, L. & Lin, D.A. (1984) Dietary deprivation of linolenic acid in rhesus monkeys: effects on plasma and tissue fatty acid composition and on visual function. *Trans. Assn. Am. Phys.* 97: 1-9.

Connor, W.E. & Neuringer, M. (1988) The effects of n-3 fatty acid deficiency and repletion upon the fatty acid composition and function of the brain and retina. In: *Biological Membranes: Aberrations in Membrane Structure and Function*, pp. 275-294, Alan R. Liss, New York, NY.

Connor, W.E., Lin, D.S., Neuringer, M., Reisbick, S. & Anderson, G.J. (1992) The comparative importance of prenatal and postnatal n-3 fatty acid deficiency: repletion at birth and later. In: *Essential Fatty Acids and Eicosanoids: invited papers from the Third International Congress.* (Sinclair, A. & Gibson, R., eds.), pp. 156-160, Am. Oil Chemists' Soc., Champaign, IL.

Cook, H.W. & Spence, M.W. (1973) Formation of monoenoic fatty acids by desaturation in rat brain homogenate. Some properties of the enzyme system of 10-day-old brain. *J. Biol. Chem.* 248: 1786-1793.

Cook, H.W. & Spence, M.W. (1973a) Formation of monoenoic fatty acids by desaturation in rat brain homogenate. Effects of age, fasting, and refeeding, and comparison with liver enzyme. *J. Biol. Chem.* 248: 1793-1796.

Cook, H.W. (1978) *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.* 30: 1327-1334.

Cook, H.W. (1981) Metabolism of triacylglycerol in developing rat brain. Neurochem. Res. 6: 1217-1229.

Cruz, T.F. & Gurd, J.W. (1978) Reaction of synaptic plasma membrane sialoglycoproteins with intrinsic sialidase and wheat germ agglutinin. *J. Biol. Chem.* 253: 7314-7318.

Cullis, P.R. & Hope, M.J. (1985) Physical properties and functional roles of lipids in membranes. In: *Biochemistry of Lipids and Membranes*, (Vance, D.E. & Vance, J.E., eds.), pp. 25-72. The Benjamins/Cummings Publishing Co., Inc., Menlo Park, California.

Dhopeshwarkar, G.A. & Subramanian, C. (1975) Metabolism of linolenic acid in developing brain: I: incorporation of radioactivity from 1-^{14}C linolenic acid into brain fatty acids. *Lipids* 10: 238-241.

Dhopeshwarkar, G.A. & Subramanian, C. (1976) Intracranial conversion of linoleic acid to arachidonic acid: evidence for lack of Δ8 desaturase in the brain. *J. Neurochem.* 26: 1175-1179.

Dhopeshwarkar, G.A. & Subramanian, C. (1976a) Biosynthesis of polyunsaturated fatty acids in the developing brain: 1. Metabolic transformations of intracranially administered 1-^{14}C linolenic acid. *Lipids* 11: 67-71.

Dise, C.A., Goodman, D.B.P. & Rasmussen, H. (1980) Definition of the pathway for membrane phospholipid fatty acid turnover in human erythrocytes. *J. Lipid Res.* 21: 292-300.

Dobbing, J. & Sands, J. (1979) Comparative aspects of the brain growth spurt. *Early Human Dev.* 3: 79-83.

Dratz, E.A. & Holte, L.L. (1992) The molecular spring model for the function of docosahexaenoic acid (22:6ω-3) in biological membranes. In: *Essential Fatty Acids and Eicosanoids: invited papers from the Third International Conference* (Gibson, R.A. & Sinclair, A.J., eds.) pp. 122-127, Am. Oil Chemists' Soc., Champaign, IL.

Drew, M.D. & Owen, B.D. (1988) The provision of passive immunity to colostrum deprived piglets by bovine or porcine serum immunoglobulins. *Can. J. Animal Sci.* 68: 1277-1284.

Enslen, M., Milon, H. & Malnöe, A. (1991) Effect of low intake of n-3 fatty acids during development on brain phospholipid composition and exploratory behavior in rats. *Lipids* 3: 203-205.

Evers, A.S., Elliot, W.J., Lefkowith, J.B. & Needleman, P. (1986) Manipulation of rat brain fatty acid composition alters volatile anesthetic potency. *J. Clin. Invest.* 77: 1028-1033.

Farquharson, J., Cockburn, F., Patrick, W.A., Jamieson, E.C. & Logan, R.W. (1992) Infant cerebral cortex phospholipid fatty-acid composition and diet. *Lancet* 340: 810-813.

Fliesler, S.J. & Anderson, R.E. (1983) Chemistry and metabolism of lipids in the vertebrate retina. *Prog. Lipid Res.* 22: 79-131.

Folch. J., Lees, M. & Stanley, G.H.S. (1957) A simple method for total lipid extraction and purification. *J. Biol. Chem.* 226: 497-509.

Foot, M., Cruz, T.F. & Clandinin, M.T. (1982) Influence of dietary fat on the lipid composition of rat brain synaptosomal and microsomal membranes. *Biochem. J.* 208: 631-640.

Galli, D., Agradi, E. & Paoletti, R. (1974) The (*n*-6) pentaene:(*n*-3) hexane fatty acid ratio as an index of linolenic acid deficiency. *Biochim. Biophys. Acta* 369: 142-145.

Garg, M.L., Sebokova, E., Thomson, A.B.R. & Clandinin, M.T. (1988) Δ6-Desaturase activity in liver microsomes of rats fed diets enriched with cholesterol and/or ϖ3 fatty acids. *Biochem. J.* 249: 351-356.

Garg, M.L., Keelan, M., Thomson, A.B. & Clandinin, M.T. (1988a) Fatty acid desaturation in the intestinal mucosa. *Biochim. Biophys. Acta* 958: 139-141.

Garg, M.L., Wierzbicki, A.A., Thomson, A.B.R. & Clandinin, M.T. (1989) Dietary saturated fat levels alter the competition between α-linolenic and linoleic acid. *Lipids* 24: 334-339.

Garg, M.L., Thomson, A.B.R. & Clandinin, M.T. (1990) Interactions of saturated, n-6 and n-3 polyunsaturated fatty acids to modulate arachidonic acid metabolism. *J. Lipid Res.* 31: 271-277.

Garofalo, R.S. & Pfenninger, K.H. (1986) Phosphatidylinositol turnover in nerve growth cones isolated from the CNS. *J. Cell Biol.* 130: 454 (abstr).

Gavino, G.R. & Gavino, V.C. (1991) Rat liver outer mitochondrial carnitine palmitoyltransferase activity towards long-chain polyunsaturated fatty acids and their CoA esters. *Lipids* 26: 266-270.

Giesing, M. & Zilliken, F. (1980) Lipid metabolism of developing central nervous tissues in organotypic cultures III. Ganglionic control of glycerolipids and fatty acids in cortex grey matter. *Neurochem. Res.* 5: 257-267.

Grønn, M., Christensen, E., Hagve, T.A. & Christophersen, B.O. (1992) Effects of dietary purified eicosapentaenoic acid (20:5(*n*-3)) and docosahexaenoic acid (22:6(*n*-3)) on fatty acid desaturation and oxidation in isolated rat liver cells. *Biochim. Biophys. Acta* 1125: 35-43.

Hadjiagapiou, C. & Spector, A.A. (1987) Docosahexaenoic acid metabolism and effect on prostacyclin production in endothelial cells. *Arch. Bioch. Biophys.* 253: 1-12.

Hagve, T.A. & Christophersen, B.O. (1983) Linolenic acid desaturation and chain elongation and rapid turnover of phospholipid *n*-3 fatty acids in isolated rat liver cells. *Biochim. Biophys. Acta* 753: 339-349.

Hagve, T.A. & Christophersen, B.O. (1984) Effect of dietary fats on arachidonic acid and eicosapentanoic acid biosynthesis and conversion to C22 fatty acids in isolated rat liver cells. *Biochim. Biophys. Acta* 796: 205-217.

Hagve, T.A. & Christophersen, B.O. (1986) Evidence for peroxisomal retroconversion of adrenic acid (22:4(n-6)) and docosahexaenoic acids (22:6(n-3)) in isolated liver cells. *Biochim. Biophys. Acta* 875: 165-173.

Hagve, T.A., Christensen E., Grønn, M. and Christophersen, B.O. (1988) Regulation of the metabolism of polyunsaturated fatty acids. *Scand. J. Clin. Lab. Invest.* 48: Suppl. 191: 33-46.

Harris, W.S., Connor, W.E. & Lindsey, S. (1984) Will dietary ω-3 fatty acids change the composition of human milk? *Am. J. Clin. Nutr.* 40: 780-785.

Hassam, A.G. (1976) The availability of long-chain polyenoic fatty acids for brain development in the rat. *Biochem. Soc. Trans.* 4: 233-234.

Henderson, R.A., Jensen, R.G., Lammi-keefe, C.J., Ferris, A.M. & Dardick, K.R. (1992) Effect of fish oil on the fatty acid composition of human milk and maternal and infant erythrocytes. *Lipids* 27: 863-869.

Herschkowitz, N. (1988) Brain development in the fetus, neonate and infant. *Biol. Neonate.* 54: 1-19.

Holman, R.T. (1964) Nutritional and metabolic interrelationships between fatty acids. *Fed. Proc.* 23: 1062-1067.

Holman, R.T. (1986) Nutritional and biochemical evidences of acyl interaction with respect to essential polyunsaturated fatty acids. *Prog. Lipid Res.* 25: 29-39.

Holub, B.J. & Skeaff, C.M. (1987) Nutritional regulation of cellular phosphatidylinositol. *Methods Enzymol.* 141: 234-244.

Homayoun, P., Durand, G., Pascal, G. & Bourre, J.M. (1988) Alterations in fatty acid composition of adult rat brain capillaries and choroid plexus induced by a diet deficient in n-3 fatty acids: slow recovery after substitution with a nondeficient diet. *J. Neurochem.* 51: 45-48.

Houslay, M.D. & Stanley, K.K. (1982) *Dynamics of Biological Membranes: Influence on synthesis, structure and function.* John Wiley & Sons Ltd., New York, NY.

Hrboticky, N., MacKinnon, M.J., Puterman, M.L. & Innis, S.M. (1989) Effect of linoleic acid-rich infant formula on brain synaptosomal lipid accretion and enzyme thermotropic behavior in the piglet. *J. Lipid Res.* 30: 1173-1184.

Hrboticky, N., MacKinnon, M.J. & Innis, S.M. (1990) Effect of vegetable oil formula rich in linoleic acid on tissue fatty acid accretion in the brain, liver, plasma and erythrocytes of infant piglets. *Am. J. Clin. Nutr.* 51: 173-182.

Hrboticky, N., MacKinnon, M.J. & Innis, S.M. (1991) Retina fatty acid composition of piglets fed from birth with a linoleic acid-rich vegetable-oil formula for infants. *Am. J. Clin. Nutr.* 53: 483-490.

Huang, Y.S., Wainwright, P.E., Redden, P.R., Mills, D.E., Bulman-Fleming, B. & Horrobin, D.F. (1992) Effect of maternal dietary fats with variable n-3/n-6 ratios on tissue fatty acid composition in suckling mice. *Lipids* 27: 104-110.

Hwang, D.H., Boudreau, M. & Chanmugam, P. (1988) Dietary linolenic acid and longer-chain n-3 fatty acids: comparison of effects on arachidonic acid metabolism in rats. *J. Nutr.* 118: 427-437.

Infant Nutrition, A study of Canadian mothers (1984) *Cogemine Marketing Consulting and Research*, Montréal, Québec.

Innis, S.M. & Clandinin, M.T. (1981) Dynamic modulation of mitochondrial inner-membrane lipids in rat heart by dietary fat. *Biochem. J.* 193: 155-167.

Innis, S.M. & Kuhnlein, H.V. (1988) Long-chain n-3 fatty acids in breast milk of Inuit women consuming traditional foods. *Early Hum. Dev.* 18: 185-189.

Innis, S.M., Foote, K.D., MacKinnon, M.J. & King, D.J. (1990) Plasma and red blood cell fatty acids of low-birth-weight infants fed their mother's expressed breast milk or preterm-infant formula. *Am. J. Clin. Nutr.* 51: 994-1000.

Innis, S.M. (1991) Essential fatty acids in growth and development. *Prog. Lipid Res.* 30: 39-103.

Innis, S.M. (1992) Human milk and formula fatty acids. *J. Pediatr.* 120: S56-61.

Innis, S.M. (1992a) Tissue omega-6 and omega-3 fatty acid relationships during differing intakes of saturated, monounsaturated, omega-6 and omega-3 fatty acids from breast milks and formulae in the newborn. In: *Essential Fatty Acids and Eicosanoids: invited papers from the Third International Congress* (Gibson, R.A. & Sinclair, A.J., eds.) pp. 183-191, Am. Oil Chemists' Soc., Champaign, IL.

Innis, S.M. (1992b) Plasma and red blood cell fatty acids as indexes of essential fatty acids in the developing organs of infants fed with milk or formulas. *J. Pediatr.* 120: S78-86.

Innis, S.M. (1993) The colostrum-deprived piglets as a model for study of infant lipid nutrition. *J. Nutr.* 123: 386-390.

Innis, S.M., Arbuckle, L.D. & Wall, K.M. (1993) Insights into possible mechanisms of essential fatty acid uptake into developing brain from studies of diet, circulating lipid, liver and brain N-6 and N-3 fatty acids. In: *Lipids, Learning and the Brain* (Dobbing, J., ed.), Ross Laboratories, Columbus, OH, in press.

Iritani N., Inoguchi, K., Endo, M., Fukuda, E & Morita, M. (1980) Identification of shellfish fatty acids and their effects on lipogenic enzymes. *Biochim. Biophys. Acta* 618: 378-382.

Jensen, R.G. (1989) Lipids in human milk - composition and fat soluble vitamins. In: *Textbook of Gastroenterology and Nutrition in Infancy*, 2nd Ed. (Lebenthal, E. ed.) pp. 157-208. Raven Press, New York.

Jensen, R.G., Lammi-Keefe, C.J., Henderson, R.A., Bush, V.J. & Ferris, A.M. (1992) Effect of dietary intake of n-6 and n-3 fatty acids on the fatty acid composition of human milk in North America. *J. Pediatr.* 120: S87-S92.

John, G.R., Littleton, J.M. & Jones, P.A. (1980) Membrane lipids and ethanol tolerance in the mouse. The influence of dietary fatty acid composition. *Life Sci.* 27: 545-555.

Jones, P.J.H., Hrboticky, N., Hahn, P. & Innis, S.M. (1990) Comparison of breast-feeding and formula feeding on intestinal and hepatic cholesterol metabolism in neonatal pigs. *Am. J. Clin. Nutr.* 51: 979-984.

Kálmán, J., Geese, Á., Farkas, T., Joó, F., Telegdy, G. & Lajtha, A. (1992) Dietary manipulation with high marine fish oil intake of fatty acid composition and arachidonic acid metabolism in rat cerebral microvessels. *Neurochem. Res.* 17: 167-172.

Kaya, K. Miura, T. & Kubota, K. (1984) Different incorporation rates of arachidonic acid into alkenylacyl- alkylacyl- and diacylphosphatidylethanolamine of rat erythrocytes. *Biochim. Biophys. Acta* 796: 304-311.

Koblin, D.D., Dong, D.E., Deady, J.E. & Eger, E.I. (1980) Alteration of synaptic membrane fatty acid composition and anesthetic requirement. *J. Pharmacol. Exp. Ther.* 212: 546-552.

Koletzko, B. & Braun, M. (1991) Arachidonic acid and early human growth: is there a relation? *Ann. Nutr. Metab.* 35: 128-131.

Kuksis, A. (1984) Quantitative and positional analysis of fatty acids. In: *Lipid Research Methodology*, pp. 77-131, Alan R. Liss, Inc., New York, NY.

Lamptey, M.S. & Walker, B.L. (1976) A possible role for dietary linoleic acid in the development of the young rat. *J. Nutr.* 106: 86-93.

Lands, W.E., Inoue, M., Sugiura, Y. & Okuyama, H. (1982) Selective incorporation of polyunsaturated fatty acids into phosphatidylcholine by rat liver microsomes. *J. Biol. Chem.* 257: 14968-14972.

Lands, E.M., Morris, A. & Libelt, B. (1990) Quantitative effects of dietary polyunsaturated fats on the composition of fatty acids in rat tissues. *Lipids* 25: 505-516.

Lands, E.M. (1991) Dose-response relationships for ω3/ω6 effects. In: *Health Effects of ω3 Polyunsaturated Fatty Acids in Seafoods*, (Simopoulos, A.P., Kifer, R.R., Martin, R.E. & Barlow, S.M. eds). World Rev Nutr Diet. 66: 177-194, Basel, Karger.

Leaf, A.A., Leighfield, M.J., Ghebremeskel, K., Costeloe, K.L. & Crawford, M. (1992) Polyunsaturated fatty acids in plasma choline phosphoglycerides of preterm infants. *Third International Congress on Essential Fatty Acids and Eicosanoids*, Adelaide 1992, p. 77 (abstr.).

Lepage, G. & Roy, C.C. (1986) Direct transesterification of classes of lipids in a one-step reaction. *J. Lipid Res.* 27: 114-120.

Leyton, J., Drury, P.J. & Crawford, M.A. (1987) In vivo incorporation of labeled fatty acids in rat liver lipids after oral administration. *Lipids* 22: 553-558.

Leyton, J., Drury, P.J. & Crawford, M.A. (1987a) Differential oxidation of saturated and unsaturated fatty acids in vivo in the rat. *Brit. J. Nutr.* 57: 383-393.

Li, J., Wetzel, M.G. & O'Brien, P.J. (1992) Transport of n-3 fatty acids from the intestine to the retina in rats. *J. Lipid Res.* 33: 539-548.

Lin, D.S., Anderson, G.J. & Connor, W.E. (1991) High levels of the (*n*-6) fatty acid 4,7,10,13,16-docosapentaenoate in the retinas of rabbits are reduced by feeding dietary fish oil from birth to adult life. *J. Nutr.* 121: 1924-1931.

Lin, D.S., Connor, W.E. & Anderson, G.J. (1991a) The incorporation of n-3 and n-6 essential fatty acids into the chick embryo from egg yolks having vastly different fatty acid compositions. *Pediatr. Res.* 29: 601-605.

Lin, T.N., MacQuarrie, R. & Sun, G.Y. (1988) Arachidonic acid uptake by phospholipids and triacylglycerols of rat brain subcellular membranes. *Lipids* 23: 942-947.

Liu, C.C.F., Carlson, S.E., Rhodes, P.G., Rao, V.S. & Meydrech, E.F. (1987) Increase in plasma phospholipid docosahexaenoic and eicosapentaenoic acids as a reflection of their intake and mode of administration. *Pediatr. Res.* 22: 292-296.

Loh, H.H. & Law, P.Y. (1980) The role of membrane lipids in receptor mechanisms. *Ann. Rev. Pharmacol. Toxicol.* 20: 201-234.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R.J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193: 265-275.

MacDonald, J.I.S. & Sprecher, H. (1991) Review: Phospholipid fatty acid remodeling in mammalian cells. *Biochim. Biophys. Acta* 1084: 105-121.

Marbois, B.N., Ajie, H.O., Korsak, R.A., Sensharma, D.K. & Edmond, J. (1992) The origin of palmitic acid in brain of the developing rat. *Lipids* 27: 587-592.

Marinetti, G.V. & Cattieu, K. (1982) Asymmetric metabolism of phosphatidylethanolamine in the human red cell membrane. *J. Biol. Chem.* 10: 245-248.

Martin, R.E. & Bazan, N.G. (1992) Changing fatty acid content of growth cone lipids prior to synaptogenesis. *J. Neurochem.* 59: 318-325.

Martinez, M. & Ballabriga, A. (1987) Effects of parenteral nutrition with high doses of linoleate on the developing human liver and brain. *Lipids* 22: 133-138.

Martinez, M. (1992) Tissue levels of polyunsaturated fatty acids during early human development. *J. Pediatr.* 120: S129-138.

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

Mohrhauer, H. & Holman, R.T. (1963a) The effect of dose levels of essential fatty acids upon fatty acid composition of the rat liver. *J. Lipid Res.* 4: 151-159.

Montgomery, D.C. (1984) *Design and Analysis of Experiments.* J. Wiley & Sons, New York, USA.

Moore, S.A., Yoder, E., Murphy, S., Dutton, G.R. & Spector, A.A. (1991) Astrocytes, not neurons, produce docosahexaenoic acid (22:6ω3) and arachidonic acid (20:4ω6). *J. Neurochem.* 56: 518-524.

Moore, S.A., Yoder, E. & Spector, A.A. (1990) Role of the blood-brain barrier in the formation of long-chain ω-3 and ω-6 fatty acids from essential fatty acid precursors. *J. Neurochem.* 55: 391-402.

Morand, O., Carré, J.B., Homayoun, P., Niel, E., Baumann, N. & Bourre J.M. (1987) Arachidonyl-coenzyme A synthetase and nonspecific acyl-coenzyme A synthetase activities in purified rat brain microvessels. *J. Neurochem.* 48: 1150-1156.

Morrison, W.R. & Smith, L.M. (1961) Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. *J. Lipid Res.* 5: 600-608.

Mulder, E. & Van Deenan, L.L.M. (1965) Metabolism of red-cell lipids I: incorporation *in vitro* of fatty acids into phospholipids from mature erythrocytes. *Biochim. Biophys. Acta* 106: 106-117.

Nestel, P.J., Connor, W.E., Reardon, M.R., Connor, S., Wong, S. & Boston, R. (1984) Suppression by diets rich in fish oil of very low density lipoprotein production in man. *J. Clin. Invest.* 74: 72-89.

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

Neuringer, M., Connor, W.E., Lin, D.S., Barstad, L. & Luck, S. (1986) Biochemical and functional effects of prenatal and postnatal n-3 fatty acid deficiency on retina and brain in rhesus monkey. *Proc. Natl. Acad. Sci. USA* 83: 4021-4025.

Neuringer, M. & Connor, W.E. (1989) Omega-3 fatty acids in the retina. In: *Dietary ω3 and ω6 Fatty Acids: Biological Effects and Nutritional Essentiality* (Galli, C. & Simopoulos, A.P. eds.) pp. 177-190, Plenum Press, New York.

Nouvelot, A., Dedonder, E., Dewailly, P. & Bourre, J.M. (1985) Influence des n-3 exogenes sur la compositin en acides gras polyinsatures de la retine, aspects structural et physiologique. *Cah. Nutr. Diet.* 20: 123-125.

Nouvelot, A., Delbart, C. & Bourre, J.M. (1986) Hepatic metabolism of dietary alpha-linolenic acid in suckling ras, and its possible importance in polyunsaturated fatty acid uptake by the brain. *Ann. Nutr. Metab.* 30: 316-323.

NRC, Sub-committee on Swine Nutrition (1979) *Nutrient Requirements of Swine.* 8th Edn., pp. 22-23, Natl. Acad. Press, Washington, DC.

Nutrition Recommendations (1990) *The Report of the Scientific Review Committee.* Health & Welfare Canada, pp. 40-52.

Olegård, R. & Svennerholm, L. (1971) Effects of diet on fatty acid composition of plasma and red cell phosphoglycerides in three-month-old infants. *Acta Pædiat. Scand.* 60: 505-511.

Philbrick, D.-J., Mahadevappa, V.G., Ackman, R.G. & Holub, B.J. (1987) Ingestion of fish oil or a derived n-3 fatty acid concentrate containing eicosapentaenoic acid (EPA) affects fatty acid compositions of individual phospholipids of rat brain, sciatic nerve and retina. *J. Nutr.* 117: 1663-1670.

Pollet, S., Ermidou, S., Le Saux, F., Monge, M. & Baumann, N. (1978) Microanalysis of brain lipids: multiple two-dimensional thin-layer chromatography. *J. Lipid Res.* 19: 916-921.

Ponder, D.L., Innis, S.M., Benson, J.D. & Siegman, J.S. (1992) Docosahexaenoic acid status of term infants fed breast milk or infant formula containing soy oil or corn oil. *Pediatr. Res.* 32: 683-688.

Pugh, E.L. & Kates, M. (1977) Direct desaturation of eicosatrienoyl lecithin to arachidonoyl lecithin by rat liver microsomes. *J. Biol. Chem.* 252: 68-73.

Purvis, J.M., Clandinin, M.T. & Hacker, R.R. (1983) 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.* 75B: 199-204.

Putnam, J.C., Carlson, S.E., Devoe, P.W. & Barness, L.A. (1982) The effect of variations in dietary fatty acids on the fatty acid composition of erythrocyte phosphatidylcholine and phosphatidylethanolamine in human infants. *Am. J. Clin. Nutr.* 36: 106-114.

Rees, R.P. (1978) The morphology of interneuronal synaptogenesis: a review. *Fed. Proceed.* 37: 2000-2009.

Rose, H.G. & Oklander, M. (1965) Improved procedure for the extraction of lipids from human erythrocytes. *J. Lipid Res.* 6: 428-431.

Rosenthal, M.D. (1987) Fatty acid metabolism in isolated mammalian cells. *Prog. Lipid Res.* 26: 87-124.

Rosenthal, M.D., Garcia, M.C., Jones, M.R. & Sprecher, H. (1991) Retroconversion and Δ^4 desaturation of docosatetraenoate (22:4(n-6)) and docosapentaenoate (22:5(n-3)) by human cells in culture. *Biochim. Biophys. Acta* 1083: 29-36.

Salem, N. Jr., Hee-Yong, K. & Yergey, J.A. (1986) Docosahexaenoic acid: membrane function and metabolism. In: *Health Effects of Polyunsaturated Fatty Acids in Seafoods* (Simopoulos, A.P., Kifer, R.R. & Martin, R.E., eds.) pp. 263-318. Academic Press, Inc., Orlando, Florida.

Sanders, T.A.B., Mistry, M. & Naismith, D.J. (1984) The influence of a maternal diet rich in linoleic acid on brain and retinal docosahexaenoic acid in the rat. *Brit. J. Nutr.* 51: 57-66.

Sanders, T.A.B. & Rana, S.K. (1987) Comparison of the metabolism of linoleic and linolenic acids in the fetal rat. *Ann. Nutr. Metab.* 31: 349-353.

Sanders, T.A.B. & Reddy, S. (1992) The influence of a vegetarian diet on the fatty acid composition of human milk and the essential fatty acid status of the infant. *J. Pediatr.* 120: S71-77.

Sastry, P.S. (1985) Lipids of nervous tissue: composition and metabolism. *Prog. Lipid Res.* 24: 69-176.

Scanu, A.M. (1986) Plasma lipoproteins: an overview. In: *Biochemistry and Biology of Plasma Lipoproteins*, (Scanu, A.M. & Spector, A.A., eds.), pp. 1-51, Marcel Dekker, New York, NY.

Scott, B.L., Reddy, T.S. & Bazan, N.G. (1987) Docosahexaenoate metabolism and fatty acid composition in developing retinas of normal and _rd_ mutant mice. _Exp. Eye Res._ 44: 101-113.

Scott, B.L. & Bazan, N.G. (1989) Membrane docosahexaenoate is supplied to the developing brain and retina by the liver. _Proc. Nat. Acad. Sci. USA_ 86: 2903-2907.

Sinclair, A.J. & Crawford, M.A. (1972) The accumulation of arachidonate and docosahexaenoate in the developing rat brain. _J. Neurochem._ 19: 1753-1758.

Sinclair, A.J. (1975) Incorporation of radioactive polyunsaturated fatty acids into liver and brain of developing rat. _Lipids_ 10: 175-184.

Singer, S.J. & Nicolson, G.L. (1972) The fluid mosaic model of the structure of membranes. _Science_ 175: 720-731.

Singh, H. Usher, S. & Poulos, A. (1989) Mitochondrial and peroxisomal beta-oxidation of stearic and lignoceric acid by rat brain. _J. Neurochem._ 53: 1711-1718.

Skipski, V.P., Peterson, R.F. & Barkley, M. (1964) Quantitative analysis of phospholipids by thin-layer chromatography. _Biochem. J._ 90: 374-378.

Smith, W.L. (1989) Review article: the eicosanoids and their biochemical mechanisms of action. _Biochem. J._ 259: 315-324.

Spector, A.A. & Yorek, M.A. (1985) Membrane lipid composition and cellular function. _J. Lipid Res._ 26: 1015-1035.

Sprecher, H. (1981) Biochemistry of essential fatty acids. _Prog. Lipid Res._ 20: 13-22.

Sprecher, H. (1991) Enzyme activities affecting tissue lipid fatty acid composition. In: _Health Effects of ω3 Polyunsaturated Fatty Acids in Seafoods_. World Rev. Nutr. Diet, 66: 166-176, Karger, Basel, Switzerland.

Sprecher, H. (1992) Interconversions between 20- and 22-carbon n-3 and n-6 fatty acids _via_ 4-desaturase independent pathways. In: _Essential Fatty Acids and Eicosanoids: invited papers from the Third International Congress_ (Gibson, R.A. & Sinclair, A.J., eds.) pp. 18-22, Am. Oil Chemists' Soc., Champaign, IL.

Stone, W.L., Farnsworth, C.C. & Dratz, E.A. (1979) A reinvestigation of the fatty acid content of bovine, rat and frog retinal rod outer segment. _Exp. Eye Res._ 28: 387-397.

Strouvé-Vallet, C. & Pascaud, M. (1971) Désaturation de l'acide linoléique par les microsomes du foie et du cerveau du rat en développement. _Biochimie_ 53: 699-703.

Stubbs, C.D. & Smith, A.D. (1984) The modification of mammalian membrane polyunsaturated fatty acid composition in relation to membrane fluidity and function. _Biochim. Biophys. Acta_ 779: 89-137.

Sun, G.Y. & Sun, A.Y. (1974) Synaptosomal plasma membranes: acyl group composition of phosphoglycerides and (Na^+ + K^+) ATPase activity during fatty acid deficiency. _J. Neurochem._ 22: 15-18.

Swanson, J.E., Black, J.M. & Kinsella, J.E. (1988) Dietary menhaden oil: effects on the rate and magnitude of modification of phospholipid fatty acid composition of mouse heart and brain. _Brit. J. Nutr._ 59: 535-545.

Sweasy, D., Patterson, D.S.P. & Glancy, E.M. (1976) Biphasic myelination and the fatty acid composition of cerebrosides and cholesterol esters in the developing central nervous system of the domestic pig. _J. Neurochem._ 27: 375-380.

Tinoco, J. (1982) Dietary requirements and functions of alpha-linolenic acid in animals. *Prog. Lipid Res.* 21: 1-45.

Toussant, M.J., Wilson, M.D. & Clarke, S.D. (1981) Coordinate suppression of liver acetyl-CoA carboxylase and fatty acid synthetase by polyunsaturated fat. *J. Nutr.* 111: 146-153.

Uauy, R.D., Birch, D.G., Birch, E.E., Tyson, J.E. & Hoffman, D.R. (1990) Effect of omega-3 fatty acids on retinal function of very low birth weight neonates. *Pediatr. Res.* 28: 485-492.

Uriel, J., Naval, J. & Laborda, J. (1987) α-Fetoprotein-mediated transfer of arachidonic acid into cultured cloned cells derived from a rat rhabdomyosarcoma. *J. Biol. Chem.* 262: 3579-3585.

Voller, A., Bidwell, D.E. & Bartlett, A. (1976) Enzyme immunoassays in diagnostic medicine. *Bull. W.H.O.* 53: 55-65.

Voss, A., Reinhart, M., Sankarappa, S. & Sprecher, H. (1991) 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.* 266: 19995-20000.

Voss, A., Reinhart, M., Sankarappa, S. & Sprecher, H. (1992) Differences in the interconversion between 20- and 22-carbon (n-3) and (n-6) polyunsaturated fatty acids in rat liver. *Biochim. Biophys. Acta* 1127: 33-40.

Wainwright, P.E. (1992) Do essential fatty acids play a role in brain and behavioral development? *Neuroscience and Behavioral Reviews* 16: 193-205.

Wainwright, P.E., Huang, Y.S., Bulman-Fleming, B., Dalby, D., Mills, D.E., Redden, P. & McCutcheon, D. (1992) The effects of dietary n-3/n-6 ratio on brain development in the mouse: a dose response study with long-chain n-3 fatty acids. *Lipids* 27: 98-103.

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

Wainwright, P.E., Huang, Y.S., Mills, D.E., Ward, R.P. & McCutcheon, D. (1989) Interactive effects of prenatal ethanol and n-3 fatty acid supplementation on brain development in mice. *Lipids* 24: 989-997.

Walker, B.L. (1967) Maternal diet and brain fatty acids in young rats. *Lipids* 2: 497-500.

Wall, K.M., Diersen-Schade, D. & Innis, S.M. (1992) Nonessential fatty acids in formula fat blends influence fatty acid metabolism and composition in plasma and organ lipid classes in piglets. *Lipids* 27: 1024-1031.

Wang, N., Wiegand, R.D. & Anderson, R.E. (1992) Uptake of 22-carbon fatty acids into rat retina and brain. *Exp. Eye Res.* 54: 933-939.

Watanabe, I., Kato, M., Anuma, H., Hashimoto, A., Naito, Y., Moriuchi, A. & Okuyama, H. (1987) Effect of dietary alpha-linolenate/linoleate balance on the lipid composition and electroretinographic responses in rats. *Adv. Biosci.* 62: 563-570.

Weidmann, T.S., Pates, R.D., Beach, J.M., Salmon, A. & Brown, M.F. (1988) Lipid-protein interactions mediate the photochemical function of rhodopsin. *Biochem.* 27: 6469-6474.

Wetzel, M.G., Richard, J.L., Alvarez, A., Anderson, R.E. & O'Brien, P.J. (1991) Metabolism of linolenic acid and docosahexaenoic acid in rat retinas and rod outer segments. *Exp. Eye Res.* 53: 437-446.

Williams, M.A., Tinoco, J., Yang, Y.-T., Bird, M.I. & Hincenbergs, I. (1989) Feeding pure docosahexaenoate or arachidonate decreases plasma triacylglycerol secretion in rats. *Lipids* 24: 753-758.

Yamamoto, N., Saitoh, M., Moriuchi, A., Nomura, M. & Okuyama, H. (1987) Effect of dietary α-linolenate/linoleate balance on brain lipid compositions and learning ability of rats. *J. Lipid Res.* 28: 144-151.

Yamamoto, N., Hashimoto, A., Takemoto, Y., Okuyama, H., Nomura, M., Kitajima, R., Togashi, T. & Tamai, Y. (1988) Effect of the dietary α-linolenate/linoleate balance on lipid compositions and learning ability of rats. II. Discrimination process, extinction process, and glycolipid compositions. *J. Lipid Res.* 29: 1013-1021.

Yau, T.M. & Sun, G.Y. (1974) The metabolism of 1-^{14}C arachidonic acid in the neutral glycerides and phosphoglyceride of mouse brain. J. Neurochem. 23: 99-104.

Yavin, E., Yavin, Z. & Menkes, J.H. (1975) Polyunsaturated fatty acid metabolism in neuroblastoma cells in culture. *J. Neurochem.* 24: 71-77.

Yeh, Y.-Y., Winters, B.L. & Yeh, S.-M. (1990) Enrichment of (*n*-3) fatty acids of suckling rats by maternal dietary menhaden oil. *J. Nutr.* 120: 436-443.

Yehuda, S., Leprohon-Greenwood, C.E., Dixon, L.M., Coscina, D.V. (1986) Effects of dietary fat on pain threshold, thermoregulation and motor activity in rats. *Pharmacol. Biochem. Behav.* 24: 1775-1777.

Yonekubo, A., Honda, S., Okano, M. & Yamamoto, Y. (1993) Effect of dietary safflower oil or soybean oil on the milk composition of the maternal rat and tissue fatty acid composition and learning ability of postnatal rats. *Biosci. Biotech. Biochem.* 57: 253-259.

Yorek, M.A., Strom, D.K. & Spector, A.A. (1984) Effect of membrane polyunsaturation on carrier-mediated transport in cultured retinoblastoma cells: alterations in taurine uptake. *J. Neurochem.* 42: 254-261.

Table 6.1
Patterns of infant feeding in Canada from birth to 6 months[1]
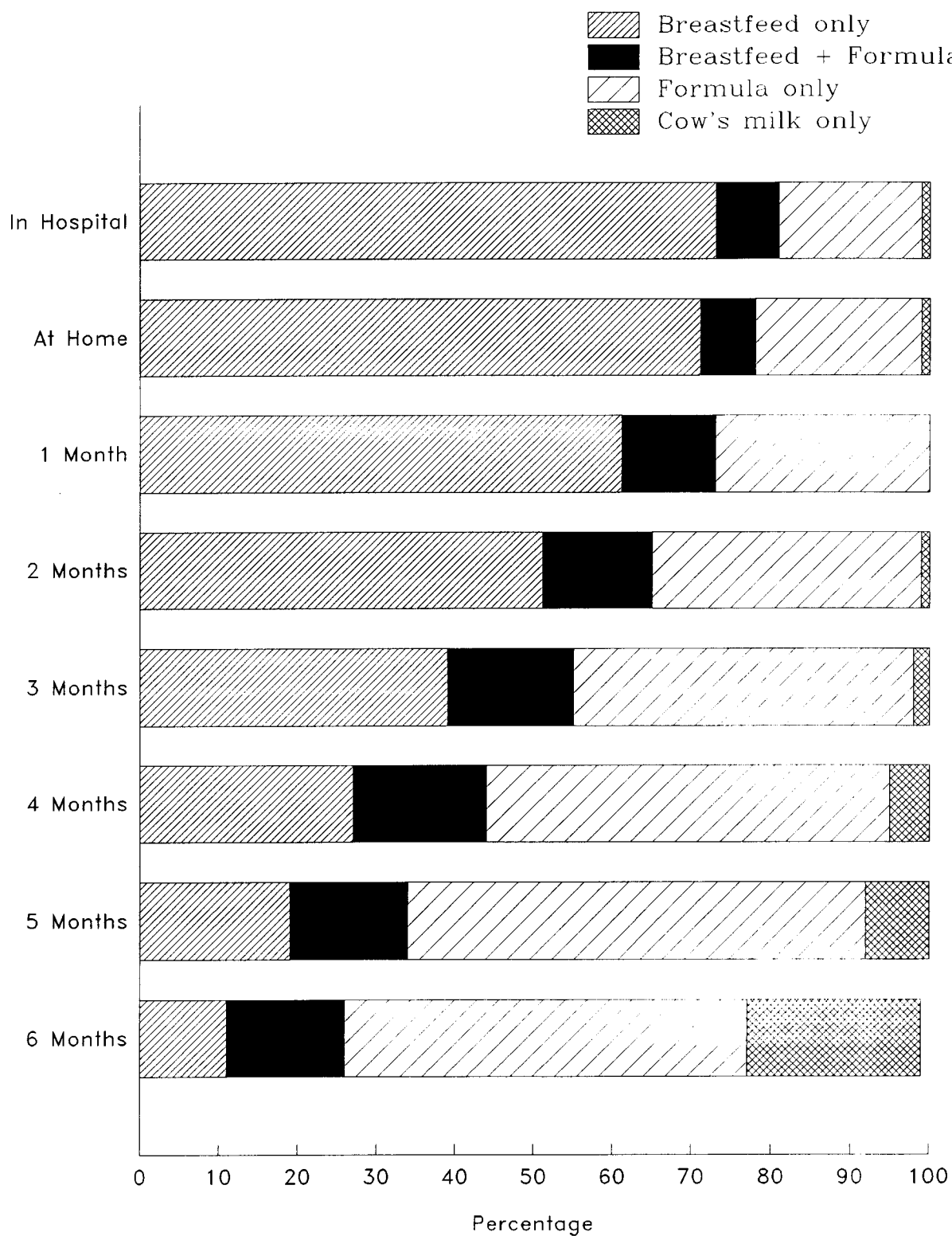
[1]from Infant Nutrition: A study of Canadian mothers (1984).

117

Table 6.2
National Research Council requirements for pigs[1]

Mineral	NRC Requirement (mg/dL)
Calcium	255
Phosphorus	200
Magnesium	11.1
Iron	4.2
Zinc	2.3
Copper	0.17
Manganese	0.11

Vitamin	NRC Requirement (per dL formula)
A	61 I.U.
D	6.1 I.U.
E	0.31 I.U.
K	6 μg
C	no requirement
B1	0.037 mg
B2	0.083 mg
B12	0.061 μg
Niacin	0.61 mg
Pyridoxine	40 μg
Folate	1.7 μg
Pantothenic Acid	0.37 mg
Biotin	0.33 μg
Choline	30 mg

Amino Acid	NRC Requirement (g/dL)
Lysine	0.36
Arginine	0.09
Histidine	0.09
Isoleucine	0.23
Leucine	0.28
Methionine + Cystine	0.21
Phenylalanine + Tyrosine	0.33
Threonine	0.21
Tryptophan	0.06
Valine	0.23

[1]from NRC, Sub-committee on Swine Nutrition (1979).

Table 6.3

Fatty acid composition of cerebrum total lipid in 15-day-old piglets fed sow milk or formula with varying % 18:2n-6 / % 18:3n-3[1]

Fatty acid	Reference milk ($n=7$)	Fish oil milk ($n=6$)	16/1 + FO ($n=5$)	16/1 ($n=8$)	16/4 ($n=7$)	30/1 (n=6)	35/4 (n=6)
16:0	21.3 ± 0.2	21.8 ± 0.2	19.7 ± 0.7	21.9 ± 0.4	20.2 ± 0.2	19.5 ± 0.4	19.2 ± 0.4
18:0	23.5 ± 0.1	24.4 ± 0.1	24.0 ± 0.2	23.7 ± 0.3	24.1 ± 0.1	24.0 ± 0.1	24.8 ± 0.2
Σ sats[2]	46.2 ± 0.2	47.9 ± 0.1	44.9 ± 0.5	47.3 ± 0.7	45.7 ± 0.2	44.9 ± 0.3	45.4 ± 0.3
16:1	2.1 ± 0.1	1.0 ± 0.0	1.6 ± 0.1	1.8 ± 0.2	1.7 ± 0.1	1.5 ± 0.1	1.4 ± 0.1
18:1	21.0 ± 0.4	21.3 ± 0.2	21.2 ± 0.3	21.0 ± 0.2	20.5 ± 0.4	20.3 ± 0.3	19.0 ± 0.2
Σ monos[3]	24.1 ± 0.4	23.2 ± 0.2	23.8 ± 0.3	23.9 ± 0.2	23.2 ± 0.4	22.9 ± 0.3	21.4 ± 0.2
18:2n-6	1.5 ± 0.1	1.5 ± 0.0	1.5 ± 0.1	1.5 ± 0.0	1.6 ± 0.0	1.9 ± 0.1	2.3 ± 0.1
18:3n-6	0.7 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.7 ± 0.0
20:2n-6	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.4 ± 0.0	0.4 ± 0.0
20:3n-6	0.6 ± 0.0	0.7 ± 0.0	0.8 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.9 ± 0.0
20:4n-6	10.3 ± 0.2	10.2 ± 0.1	10.9 ± 0.1	10.5 ± 0.2	10.5 ± 0.2	11.5 ± 0.2	10.9 ± 0.2
22:4n-6	4.3 ± 0.1	3.5 ± 0.1	3.7 ± 0.1	3.9 ± 0.3	4.3 ± 0.1	4.9 ± 0.2	4.7 ± 0.1
22:5n-6	1.7 ± 0.0	1.6 ± 0.1	1.9 ± 0.2	2.3 ± 0.2	1.7 ± 0.1	3.1 ± 0.1	1.9 ± 0.1
Σ n-6 LCP[4]	16.3 ± 0.2	15.2 ± 0.2	16.5 ± 0.3	16.6 ± 0.5	16.5 ± 0.2	19.5 ± 0.2	17.5 ± 0.3
18:3n-3	0.1 ± 0.1	nd[5]	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	tr[6]	tr
20:5n-3	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.0
22:5n-3	0.5 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	0.4 ± 0.0
22:6n-3	9.7 ± 0.2	10.9 ± 0.2	11.1 ± 0.2	8.8 ± 0.2	10.6 ± 0.1	8.8 ± 0.2	10.6 ± 0.3
Σ n-3 LCP[7]	10.4 ± 0.2	11.3 ± 0.2	11.6 ± 0.2	9.2 ± 0.3	11.3 ± 0.2	9.2 ± 0.2	11.3 ± 0.3
22:6n-3/22:5n-6	5.6 ± 0.2	7.0 ± 0.2	6.3 ± 0.7	4.2 ± 0.5	6.3 ± 0.4	2.9 ± 0.1	5.8 ± 0.4
Σ LCP	26.7 ± 0.4	26.6 ± 0.3	28.0 ± 0.4	25.8 ± 0.6	27.8 ± 0.3	28.6 ± 0.2	28.8 ± 0.4
UI[8]	160 ± 2	159 ± 1	169 ± 2	154 ± 3	166 ± 1	167 ± 1	171 ± 2
MChL[9]	18.4 ± 0.0	18.4 ± 0.0	18.5 ± 0.0	18.4 ± 0.0	18.5 ± 0.0	18.6 ± 0.0	18.6 ± 0.0

[1]Piglets were fed and housed as described in Materials and Methods. Piglets received reference milk from sows fed usual pig diets with c.a. 2.5% (wt/wt) vegetable oil. Piglets received fish oil milk from sows fed usual pig diets with 5% (wt/wt) soybean oil throughout gestation and 4% (wt/wt) soybean oil plus 1% (wt/wt) fish oil from 4 days pre- to 15 days post-partum. Fatty acids were prepared and analyzed as described in Materials and Methods. n = number of animals per group. [2]Σ sats, sum of saturated fatty acids: 16:0, 18:0, 20:0 and 22:0. [3]Σ monos, sum of monounsaturated fatty acids: 16:1, 18:1, 20:1 and 22:1. [4]Σ n-6 LCP, sum of 20:4n-6, 22:4n-6 and 22:5n-6. [5]Σ n-3 LCP, sum of 20:5n-3, 22:5n-3 and 22:6n-3. [6]nd, not detected. [7]tr, trace levels 0.01 − 0.05% of total fatty acids. [8]UI, unsaturation index = Σ (number of double bonds × % fatty acid). [9]MChL, mean chain length of fatty acids = Σ (number of carbons × % fatty acid).

Table 6.4
Fatty acid composition of cerebrum ethanolamine phospholipids (EPL) in 15-day-old piglets fed sow milk or formulas with varying % 18:2n-6 / % 18:3n-3 [1]

Fatty acid	Reference milk (n=6)	Fish oil milk (n=6)	16/1 + FO (n=5)	16/1 (n=8)	16/4 (n=7)	30/1 (n=6)	35/4 (n=6)
16:0	10.4 ± 0.6	8.5 ± 0.4	8.3 ± 0.6	10.5 ± 0.7	10.6 ± 0.4	9.3 ± 0.4	10.3 ± 0.5
18:0	28.7 ± 0.6	23.9 ± 0.7	23.6 ± 0.8	27.5 ± 0.9	29.6 ± 0.8	25.8 ± 0.9	26.9 ± 0.8
Σ sats	41.0 ± 1.2	33.1 ± 1.2	33.9 ± 0.9	39.6 ± 1.2	42.0 ± 1.0	36.5 ± 1.2	39.2 ± 1.0
16:1	0.5 ± 0.1	0.6 ± 0.0	0.3 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1
18:1	14.6 ± 0.6	18.4 ± 0.5	19.9 ± 1.5	14.9 ± 0.5	13.5 ± 0.7	15.5 ± 0.4	13.9 ± 0.6
Σ monos	17.0 ± 0.7	20.4 ± 0.5	21.4 ± 1.6	17.5 ± 0.5	15.9 ± 0.7	17.9 ± 0.6	16.7 ± 0.7
18:2n-6	0.9 ± 0.1	0.7 ± 0.0	0.5 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	1.3 ± 0.1	1.4 ± 0.0
18:3n-6	nd	nd	tr	0.1 ± 0.1	tr	tr	0.1 ± 0.1
20:2n-6	0.2 ± 0.0	tr	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.4 ± 0.0
20:3n-6	0.6 ± 0.1	0.5 ± 0.0	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.7 ± 0.0	0.8 ± 0.0
20:4n-6	15.4 ± 0.5	15.7 ± 0.5	16.1 ± 0.7	15.9 ± 0.4	15.2 ± 0.3	16.8 ± 0.4	15.7 ± 0.3
22:4n-6	8.6 ± 0.3	10.3 ± 0.3	9.1 ± 0.6	9.5 ± 0.2	8.3 ± 0.3	10.0 ± 0.4	9.0 ± 0.3
22:5n-6	2.4 ± 0.1	2.0 ± 0.1	2.7 ± 0.4	3.5 ± 0.2	2.2 ± 0.2	4.3 ± 0.2	2.3 ± 0.1
Σ n-6 LCP	26.4 ± 0.8	27.9 ± 0.7	28.0 ± 1.3	28.8 ± 0.6	25.8 ± 0.6	31.1 ± 0.8	26.9 ± 0.5
18:3n-3	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	tr	0.1 ± 0.0	0.1 ± 0.0
20:5n-3	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
22:5n-3	0.7 ± 0.0	0.5 ± 0.0	0.6 ± 0.1	0.3 ± 0.0	0.6 ± 0.0	0.3 ± 0.0	0.6 ± 0.0
22:6n-3	13.1 ± 0.5	16.6 ± 0.6	14.4 ± 0.7	11.9 ± 0.6	13.7 ± 0.5	11.6 ± 0.6	13.6 ± 0.4
Σ n-3 LCP	13.9 ± 0.5	17.2 ± 0.6	15.1 ± 0.7	12.3 ± 0.6	14.5 ± 0.5	12.0 ± 0.6	14.5 ± 0.5
22:6n-3/22:5n-6	5.6 ± 0.3	8.5 ± 0.3	6.1 ± 1.1	3.5 ± 0.3	6.4 ± 0.4	2.7 ± 0.2	6.1 ± 0.5
Σ LCP	40.2 ± 1.2	45.1 ± 1.2	43.1 ± 1.9	41.1 ± 1.0	40.2 ± 1.0	43.2 ± 1.2	41.3 ± 0.8
UI	212 ± 6	240 ± 6	230 ± 8	214 ± 5	212 ± 5	224 ± 6	219 ± 4
MChL	19.3 ± 0.0	19.5 ± 0.1	19.3 ± 0.1	19.4 ± 0.1	19.3 ± 0.1	19.5 ± 0.1	19.4 ± 0.0

[1] See footnote to Table 6.3.

Table 6.5

Fatty acid composition of cerebrum phosphatidylcholine (PC) in 15-day-old piglets fed sow milk or formula with varying % 18:2n-6 / % 18:3n-3[1]

Fatty acid	Reference milk ($n=7$)	Fish oil milk ($n=6$)	16/1 + FO ($n=5$)	16/1 ($n=8$)	16/4 ($n=7$)	30/1 ($n=6$)	35/4 ($n=6$)
16:0	40.5 ± 1.3	39.9 ± 0.7	39.0 ± 0.8	42.0 ± 0.8	43.2 ± 0.5	42.1 ± 1.1	43.6 ± 1.1
18:0	13.7 ± 0.5	14.2 ± 0.4	16.7 ± 1.0	13.7 ± 0.3	13.8 ± 0.4	13.6 ± 0.6	13.9 ± 0.3
Σ sats	56.0 ± 1.2	55.2 ± 0.4	56.9 ± 0.7	57.2 ± 0.8	59.1 ± 0.5	57.5 ± 1.3	60.0 ± 1.0
16:1	1.4 ± 0.1	1.4 ± 0.1	1.2 ± 0.1	1.4 ± 0.1	1.3 ± 0.0	1.4 ± 0.1	1.4 ± 0.1
18:1	33.0 ± 1.2	31.8 ± 0.4	31.6 ± 0.8	31.3 ± 0.6	29.8 ± 0.5	30.8 ± 1.4	27.6 ± 0.7
Σ monos	35.2 ± 1.2	34.0 ± 0.5	33.5 ± 0.6	33.5 ± 0.6	31.9 ± 0.5	32.8 ± 1.4	29.8 ± 0.6
18:2n-6	1.6 ± 0.1	1.6 ± 0.2	1.8 ± 0.1	1.8 ± 0.1	1.9 ± 0.1	2.2 ± 0.1	3.0 ± 0.1
18:3n-6	tr	tr	tr	tr	tr	tr	tr
20:2n-6	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.4 ± 0.0
20:3n-6	0.4 ± 0.0	0.5 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.6 ± 0.0
20:4n-6	4.1 ± 0.1	4.6 ± 0.2	4.3 ± 0.1	4.4 ± 0.1	4.1 ± 0.1	4.3 ± 0.3	4.1 ± 0.2
22:4n-6	0.6 ± 0.0	0.8 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.5 ± 0.1
22:5n-6	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	0.5 ± 0.0	0.2 ± 0.0
Σ n-6 LCP	4.9 ± 0.2	5.6 ± 0.2	5.2 ± 0.2	5.5 ± 0.2	4.9 ± 0.2	5.5 ± 0.3	4.9 ± 0.3
18:3n-3	tr	0.1 ± 0.1	tr	nd	tr	tr	tr
20:5n-3	0.1 ± 0.0	tr	tr	tr	tr	tr	tr
22:5n-3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	tr	0.1 ± 0.0	tr	0.1 ± 0.0
22:6n-3	1.5 ± 0.1	2.5 ± 0.2	1.6 ± 0.1	1.1 ± 0.1	1.4 ± 0.1	1.0 ± 0.1	1.4 ± 0.1
Σ n-3 LCP	1.7 ± 0.1	2.6 ± 0.1	1.7 ± 0.1	1.2 ± 0.1	1.5 ± 0.1	1.1 ± 0.1	1.5 ± 0.1
22:6n-3/22:5n-6	5.5 ± 0.3	9.9 ± 1.1	9.7 ± 2.7	3.1 ± 0.2	7.6 ± 0.8	2.0 ± 0.1	6.0 ± 0.6
Σ LCP	6.6 ± 0.2	8.2 ± 0.4	6.9 ± 0.2	6.6 ± 0.3	6.4 ± 0.3	6.6 ± 0.4	6.4 ± 0.4
UI	70 ± 2	78 ± 1	71 ± 1	69 ± 2	67 ± 1	69 ± 2	68 ± 2
MChL	17.5 ± 0.0	17.5 ± 0.0	17.5 ± 0.0	17.4 ± 0.0	17.4 ± 0.0	17.4 ± 0.0	17.4 ± 0.1

[1]See footnote Table 6.3

Table 6.6

Fatty acid composition of synaptic plasma membrane total lipid in 15-day-old piglets fed sow milk or formulas with varying % 18:2n-6 / % 18:3n-3 [1]

Fatty acid	Reference milk (n=7)	Fish oil milk (n=6)	16/1 + FO (n=5)	16/1 (n=6)	16/4 (n=6)	30/1 (n=5)	35/4 (n=6)
16:0	24.7 ± 0.7	24.8 ± 0.3	24.4 ± 0.1	23.3 ± 0.4	23.1 ± 0.6	25.7 ± 0.8	24.4 ± 0.2
18:0	26.1 ± 0.3	26.2 ± 0.2	25.3 ± 0.2	26.1 ± 0.4	26.6 ± 0.5	25.1 ± 0.7	26.4 ± 0.4
Σ sats	51.6 ± 0.9	52.0 ± 0.3	50.8 ± 0.2	50.2 ± 0.6	50.6 ± 0.9	52.1 ± 0.7	51.9 ± 0.4
16:1	0.6 ± 0.0	0.6 ± 0.1	0.5 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	0.4 ± 0.0
18:1	14.5 ± 0.2	14.1 ± 0.2	14.0 ± 0.3	14.4 ± 0.3	14.0 ± 0.3	12.8 ± 0.4	12.9 ± 0.2
Σ monos	15.5 ± 0.2	15.0 ± 0.1	14.9 ± 0.3	15.3 ± 0.3	14.9 ± 0.3	13.7 ± 0.4	13.6 ± 0.2
18:2n-6	0.8 ± 0.1	0.7 ± 0.1	0.8 ± 0.0	0.8 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	1.3 ± 0.1
18:3n-6	0.1 ± 0.0	tr	tr	0.1 ± 0.0	tr	tr	tr
20:2n-6	0.2 ± 0.0	0.1 ± 0.0	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.1
20:3n-6	0.6 ± 0.0	0.6 ± 0.0	0.7 ± 0.0	0.6 ± 0.0	0.7 ± 0.0	0.6 ± 0.0	0.8 ± 0.0
20:4n-6	9.8 ± 0.3	9.5 ± 0.2	9.8 ± 0.1	10.4 ± 0.2	10.1 ± 0.3	10.5 ± 0.3	9.9 ± 0.2
22:4n-6	4.9 ± 0.3	4.6 ± 0.1	5.0 ± 0.1	5.7 ± 0.2	5.3 ± 0.2	5.5 ± 0.2	5.3 ± 0.1
22:5n-6	2.3 ± 0.2	2.1 ± 0.0	2.4 ± 0.2	3.3 ± 0.2	2.3 ± 0.2	3.8 ± 0.2	2.4 ± 0.2
Σ n-6 LCP	17.0 ± 0.7	16.1 ± 0.2	17.1 ± 0.3	19.4 ± 0.5	17.7 ± 0.5	19.8 ± 0.6	17.6 ± 0.4
18:3n-3	nd	nd	nd	nd	nd	tr	nd
20:5n-3	nd	tr	0.1 ± 0.1	nd	0.1 ± 0.1	tr	nd
22:5n-3	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.1	0.2 ± 0.1	0.4 ± 0.0
22:6n-3	13.3 ± 0.5	14.7 ± 0.1	14.6 ± 0.3	12.9 ± 0.3	14.2 ± 0.3	11.7 ± 0.3	13.8 ± 0.2
Σ n-3 LCP	13.7 ± 0.5	15.1 ± 0.1	15.1 ± 0.4	13.1 ± 0.3	14.7 ± 0.3	11.9 ± 0.3	14.2 ± 0.2
22:6n-3/22:5n-6	5.8 ± 0.2	7.0 ± 0.2	6.4 ± 0.8	4.0 ± 0.4	6.4 ± 0.5	3.1 ± 0.1	5.8 ± 0.5
Σ LCP	30.7 ± 1.2	31.2 ± 0.2	32.2 ± 0.3	32.5 ± 0.3	32.4 ± 0.8	31.7 ± 0.8	31.9 ± 0.3
UI	172 ± 5	175 ± 1	180 ± 1	179 ± 1	180 ± 4	173 ± 4	177 ± 1
MChL	18.5 ± 0.1	18.6 ± 0.0	18.6 ± 0.0	18.6 ± 0.0	18.6 ± 0.0	18.5 ± 0.1	18.6 ± 0.0

[1]See footnote Table 6.3

Table 6.7

Fatty acid composition (% total fatty acids) of synaptic plasma membrane ethanolamine phospholipids (EPL) in 15-day-old piglets fed sow milk or formulas varying in % 18:2n-6 / % 18:3n-3[1]

Fatty acid	Reference milk (n=7)	Fish oil milk (n=6)	16/1 + FO (n=5)	16/1 (n=6)	16/4 (n=6)	30/1 (n=5)	35/4 (n=6)
16:0	8.7 ± 0.5	7.3 ± 0.7	8.4 ± 0.3	8.6 ± 0.4	9.1 ± 0.4	8.6 ± 0.7	8.9 ± 0.4
18:0	26.2 ± 0.4	26.7 ± 0.9	26.5 ± 0.2	26.1 ± 0.6	26.6 ± 0.4	26.5 ± 0.5	26.3 ± 0.5
Σ sats	36.0 ± 0.7	34.9 ± 0.8	35.7 ± 0.3	35.8 ± 1.0	36.8 ± 0.8	36.1 ± 0.8	36.3 ± 0.8
16:1	0.3 ± 0.0	0.2 ± 0.1	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
18:1	8.7 ± 0.3	9.6 ± 0.3	8.9 ± 0.4	8.8 ± 0.3	9.2 ± 0.2	8.2 ± 0.3	8.5 ± 0.4
Σ monos	9.5 ± 0.4	10.4 ± 0.3	9.7 ± 0.4	9.6 ± 0.3	10.0 ± 0.2	8.9 ± 0.3	9.2 ± 0.4
18:2n-6	0.5 ± 0.0	0.3 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	0.6 ± 0.0	0.7 ± 0.0	0.8 ± 0.1
18:3n-6	0.1 ± 0.0	nd	0.1 ± 0.0	tr	tr	0.1 ± 0.1	0.1 ± 0.0
20:2n-6	0.1 ± 0.0	tr	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
20:3n-6	0.6 ± 0.0	0.4 ± 0.1	0.8 ± 0.0	0.6 ± 0.1	0.7 ± 0.0	0.7 ± 0.0	0.8 ± 0.1
20:4n-6	17.8 ± 0.3	16.8 ± 0.4	17.6 ± 0.2	18.3 ± 0.2	17.8 ± 0.3	18.3 ± 0.4	17.2 ± 0.3
22:4n-6	10.5 ± 0.3	9.7 ± 0.4	9.7 ± 0.2	11.2 ± 0.4	10.0 ± 0.2	11.5 ± 0.4	10.6 ± 0.2
22:5n-6	3.5 ± 0.2	2.9 ± 0.1	3.4 ± 0.4	5.0 ± 0.4	3.2 ± 0.2	6.2 ± 0.4	3.2 ± 0.3
Σ n-6 LCP	31.9 ± 0.6	29.3 ± 0.5	30.7 ± 0.5	34.5 ± 0.8	31.0 ± 0.6	36.0 ± 0.5	31.0 ± 0.5
18:3n-3	0.1 ± 0.0	0.2 ± 0.0	tr	tr	tr	tr	tr
20:5n-3	tr	0.2 ± 0.0	tr	tr	tr	tr	tr
22:5n-3	0.8 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.4 ± 0.0	0.7 ± 0.0	0.3 ± 0.0	0.7 ± 0.0
22:6n-3	20.4 ± 0.3	22.5 ± 0.7	21.7 ± 0.2	18.4 ± 0.4	20.0 ± 0.5	17.1 ± 0.7	21.0 ± 0.7
Σ n-3 LCP	21.3 ± 0.4	23.2 ± 0.7	22.3 ± 0.1	18.8 ± 0.4	20.7 ± 0.5	17.4 ± 0.7	21.7 ± 0.7
22:6n-3/22:5n-6	5.9 ± 0.3	7.9 ± 0.2	6.7 ± 0.7	3.8 ± 0.3	6.3 ± 0.3	2.8 ± 0.1	6.8 ± 0.5
Σ LCP	53.2 ± 0.9	52.5 ± 1.1	53.0 ± 0.5	53.3 ± 1.0	51.7 ± 1.0	53.4 ± 1.0	52.7 ± 1.0
UI	271 ± 4	271 ± 6	273 ± 2	268 ± 5	265 ± 5	267 ± 5	270 ± 5
MChL	19.5 ± 0.1	19.3 ± 0.2	19.5 ± 0.0	19.5 ± 0.1	19.4 ± 0.1	19.5 ± 0.1	19.5 ± 0.1

[1]See footnote Table 6.3

Table 6.8

Fatty acid composition (% of total fatty acids) in synaptic plasma membrane phosphatidylcholine (PC) in 15-day-old piglets fed sow milk or formulas with varying % 18:2n-6 / % 18:3n-3 [1]

Fatty acid	Reference milk (n=7)	Fish oil milk (n=6)	16/1 + FO (n=5)	16/1 (n=6)	16/4 (n=6)	30/1 (n=5)	35/4 (n=6)
16:0	52.3 ± 0.9	48.5 ± 1.1	53.0 ± 0.6	51.9 ± 0.6	52.9 ± 0.5	50.6 ± 1.7	53.4 ± 0.7
18:0	11.7 ± 0.4	13.5 ± 0.3	11.1 ± 0.2	11.7 ± 0.1	11.6 ± 0.3	12.7 ± 0.7	11.5 ± 0.1
Σ sats	65.4 ± 0.7	63.0 ± 1.4	66.1 ± 0.6	65.1 ± 0.7	66.5 ± 0.4	65.0 ± 1.5	67.2 ± 0.7
16:1	1.1 ± 0.1	1.0 ± 0.1	0.9 ± 0.0	0.8 ± 0.0	0.9 ± 0.0	0.9 ± 0.1	0.9 ± 0.0
18:1	24.3 ± 0.5	26.3 ± 1.3	24.1 ± 0.5	24.8 ± 0.5	23.9 ± 0.3	23.2 ± 0.8	21.8 ± 0.3
Σ monos	25.9 ± 0.5	28.1 ± 1.2	25.6 ± 0.5	26.1 ± 0.5	25.2 ± 0.3	24.5 ± 0.8	23.1 ± 0.3
18:2n-6	1.1 ± 0.1	1.0 ± 0.4	1.3 ± 0.1	1.3 ± 0.0	1.4 ± 0.1	1.8 ± 0.1	2.0 ± 0.1
18:3n-6	0.1 ± 0.0	tr	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0
20:2n-6	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.4 ± 0.0	0.4 ± 0.0
20:3n-6	0.4 ± 0.0	0.3 ± 0.1	0.5 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	0.5 ± 0.1	0.5 ± 0.0
20:4n-6	4.1 ± 0.2	3.8 ± 0.1	3.8 ± 0.1	4.2 ± 0.2	3.8 ± 0.1	4.5 ± 0.3	4.0 ± 0.1
22:4n-6	0.5 ± 0.0	0.5 ± 0.1	0.4 ± 0.0	0.5 ± 0.0	0.4 ± 0.0	0.6 ± 0.1	0.5 ± 0.0
22:5n-6	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	0.5 ± 0.1	0.2 ± 0.0
Σ n-6 LCP	4.8 ± 0.2	4.5 ± 0.2	4.4 ± 0.1	5.1 ± 0.2	4.4 ± 0.1	5.7 ± 0.4	4.7 ± 0.2
18:3n-3	0.1 ± 0.0	0.2 ± 0.1	tr	tr	tr	0.1 ± 0.0	0.1 ± 0.0
20:5n-3	nd	0.1 ± 0.0	tr	nd	nd	tr	nd
22:5n-3	tr	0.1 ± 0.0	tr	nd	tr	nd	tr
22:6n-3	1.4 ± 0.1	1.6 ± 0.1	1.5 ± 0.1	1.2 ± 0.1	1.3 ± 0.0	1.2 ± 0.1	1.5 ± 0.1
Σ n-3 LCP	1.5 ± 0.1	1.8 ± 0.1	1.6 ± 0.1	1.2 ± 0.1	1.3 ± 0.0	1.2 ± 0.1	1.5 ± 0.1
22:6n-3/22:5n-6	6.0 ± 0.3	8.7 ± 0.7	7.3 ± 0.7	3.4 ± 0.3	7.3 ± 0.8	2.3 ± 0.1	6.8 ± 0.5
Σ LCP	6.3 ± 0.3	6.3 ± 0.3	6.0 ± 0.1	6.3 ± 0.3	5.7 ± 0.1	6.9 ± 0.5	6.2 ± 0.3
UI	58 ± 2	61 ± 2	57 ± 1	58 ± 2	56 ± 1	61 ± 4	58 ± 2
MChL	16.9 ± 0.1	17.0 ± 0.1	17.0 ± 0.0	17.0 ± 0.0	17.0 ± 0.0	17.0 ± 0.1	16.9 ± 0.0

[1]See footnote Table 6.3

Table 6.9
Fatty acid composition (% total fatty acids) of retina total lipid in 15-day-old piglets fed sow milk or formulas varying in % 18:2n-6 / % 18:3n-3[1]

Fatty acid	Reference milk (n=7)	Fish oil Milk (n=6)	16/1 + FO (n=5)	16/1 (n=6)	16/4 (n=6)	30/1 (n=5)	35/4 (n=6)
16:0	20.0 ± 0.4	21.4 ± 0.3	19.5 ± 0.4	21.0 ± 0.3	19.4 ± 0.5	20.8 ± 0.6	21.2 ± 1.1
18:0	23.7 ± 0.4	24.7 ± 0.2	23.4 ± 0.3	24.6 ± 0.1	24.2 ± 0.3	24.0 ± 0.6	24.8 ± 0.4
Σ sats	44.6 ± 0.7	47.3 ± 0.4	43.8 ± 0.7	47.1 ± 0.3	44.5 ± 0.7	46.5 ± 0.8	47.3 ± 1.2
16:1	0.9 ± 0.2	0.7 ± 0.0	0.4 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	0.7 ± 0.2	0.5 ± 0.1
18:1	16.8 ± 0.4	16.2 ± 0.2	17.5 ± 0.2	17.8 ± 0.2	17.8 ± 0.2	15.9 ± 0.2	15.0 ± 0.2
Σ monos	18.8 ± 0.4	17.5 ± 0.2	18.6 ± 0.2	19.7 ± 0.3	19.2 ± 0.2	17.4 ± 0.3	16.4 ± 0.1
18:2n-6	2.4 ± 0.1	2.3 ± 0.1	2.8 ± 0.1	2.7 ± 0.2	3.0 ± 0.1	3.9 ± 0.2	4.6 ± 0.2
18:3n-6	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.0
20:2n-6	0.3 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.3 ± 0.1	0.2 ± 0.0	0.5 ± 0.0	0.3 ± 0.1
20:3n-6	0.5 ± 0.2	0.4 ± 0.0	0.5 ± 0.0	0.5 ± 0.1	0.4 ± 0.0	0.4 ± 0.0	0.3 ± 0.1
20:4n-6	9.9 ± 0.4	9.3 ± 0.1	10.3 ± 0.2	9.7 ± 0.1	10.2 ± 0.3	10.6 ± 0.2	9.7 ± 0.2
22:4n-6	2.0 ± 0.1	1.6 ± 0.0	1.6 ± 0.1	2.4 ± 0.2	1.5 ± 0.3	2.6 ± 0.1	1.7 ± 0.2
22:5n-6	0.9 ± 0.1	0.5 ± 0.0	0.8 ± 0.1	1.6 ± 0.2	0.8 ± 0.1	2.3 ± 0.1	0.7 ± 0.1
Σ n-6 LCP	12.8 ± 0.5	11.5 ± 0.2	12.7 ± 0.3	13.7 ± 0.4	12.5 ± 0.6	15.5 ± 0.3	12.1 ± 0.4
18:3n-3	nd	nd	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1
20:5n-3	tr	0.2 ± 0.0	tr	nd	0.1 ± 0.0	tr	tr
22:5n-3	0.7 ± 0.1	0.7 ± 0.0	0.8 ± 0.1	0.3 ± 0.0	0.7 ± 0.1	0.2 ± 0.0	0.7 ± 0.1
22:6n-3	19.0 ± 0.8	19.9 ± 0.2	20.3 ± 0.7	15.5 ± 0.3	18.9 ± 0.7	15.2 ± 0.7	17.9 ± 0.9
Σ n-3 LCP	19.8 ± 0.9	20.8 ± 0.2	21.2 ± 0.7	15.8 ± 0.3	19.7 ± 0.7	15.4 ± 0.7	18.6 ± 1.0
22:6n-3/22:5n-6	22.3 ± 2.8	37.3 ± 1.4	28.1 ± 3.4	10.0 ± 1.0	25.3 ± 3.2	6.6 ± 0.1	28.3 ± 5.3
Σ LCP	32.6 ± 0.6	32.3 ± 0.3	33.8 ± 0.9	29.5 ± 0.6	32.2 ± 1.1	30.9 ± 0.8	30.7 ± 1.3
UI	199 ± 5	194 ± 2	204 ± 5	179 ± 3	197 ± 5	185 ± 5	189 ± 7
MChL	18.8 ± 0.1	18.7 ± 0.0	18.8 ± 0.0	18.6 ± 0.0	18.8 ± 0.1	18.7 ± 0.1	18.7 ± 0.1

[1]See footnote Table 6.3

Table 6.10

Fatty acid composition (% total fatty acids) of retina ethanolamine phospholipids (EPL) in 15-day-old piglets fed sow milk or formulas varying in % 18:2n-6 / % 18:3n-3[1]

Fatty acid	Reference milk (n=8)	Fish oil Milk (n=6)	16/1 + FO (n=5)	16/1 (n=6)	16/4 (n=7)	30/1 (n=6)	35/4 (n=6)
16:0	8.1 ± 0.6	9.4 ± 0.4	9.3 ± 0.7	8.7 ± 0.4	8.5 ± 0.3	8.1 ± 0.7	9.4 ± 0.4
18:0	25.6 ± 0.7	27.9 ± 0.7	26.7 ± 0.2	25.8 ± 0.3	26.0 ± 0.3	24.6 ± 0.7	26.8 ± 0.3
Σ sats	34.4 ± 1.1	37.7 ± 1.0	36.6 ± 0.8	35.3 ± 0.5	35.1 ± 0.6	33.9 ± 1.2	36.9 ± 0.5
16:1	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.0	0.3 ± 0.1	0.1 ± 0.0
18:1	9.1 ± 0.5	9.8 ± 0.3	9.5 ± 0.2	9.5 ± 0.4	10.3 ± 0.4	8.1 ± 0.2	8.4 ± 0.3
Σ monos	9.8 ± 0.6	10.1 ± 0.3	10.0 ± 0.2	10.0 ± 0.5	10.9 ± 0.4	8.7 ± 0.2	8.8 ± 0.4
18:2n-6	1.3 ± 0.1	0.9 ± 0.0	1.6 ± 0.2	1.5 ± 0.1	1.7 ± 0.1	1.9 ± 0.1	2.6 ± 0.1
18:3n-6	0.1 ± 0.0	tr	tr	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
20:2n-6	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.0	0.4 ± 0.1	0.3 ± 0.0
20:3n-6	0.3 ± 0.0	0.2 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0
20:4n-6	14.8 ± 0.3	12.8 ± 0.2	14.1 ± 0.3	14.6 ± 0.3	14.1 ± 0.2	15.5 ± 0.4	13.7 ± 0.4
22:4n-6	3.8 ± 0.2	3.7 ± 0.5	2.9 ± 0.2	4.0 ± 0.1	3.3 ± 0.1	5.3 ± 0.2	3.2 ± 0.1
22:5n-6	1.6 ± 0.2	0.7 ± 0.0	1.2 ± 0.1	3.2 ± 0.1	1.3 ± 0.1	4.7 ± 0.3	1.3 ± 0.2
Σ n-6 LCP	20.2 ± 0.6	17.3 ± 0.4	18.1 ± 0.5	21.8 ± 0.4	18.7 ± 0.3	25.5 ± 0.5	18.2 ± 0.2
18:3n-3	0.1 ± 0.0	0.1 ± 0.0	tr	tr	tr	tr	tr
20:5n-3	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	nd	0.1 ± 0.0	0.1 ± 0.1	tr
22:5n-3	1.6 ± 0.1	1.0 ± 0.0	1.1 ± 0.1	0.8 ± 0.1	1.2 ± 0.1	1.0 ± 0.2	1.3 ± 0.0
22:6n-3	31.4 ± 0.8	32.3 ± 0.8	31.3 ± 1.0	29.2 ± 0.8	31.1 ± 0.7	27.2 ± 0.5	30.8 ± 0.4
Σ n-3 LCP	33.1 ± 0.9	33.3 ± 0.8	32.5 ± 1.0	29.9 ± 0.8	32.4 ± 0.6	28.3 ± 0.7	32.1 ± 0.4
22:6n-3/22:5n-6	22.0 ± 2.5	46.1 ± 2.6	28.5 ± 4.2	9.1 ± 0.3	24.5 ± 1.5	5.9 ± 0.3	26.1 ± 2.6
Σ LCP	53.3 ± 1.1	50.6 ± 0.9	50.6 ± 0.9	51.8 ± 1.0	51.0 ± 0.5	53.8 ± 0.9	50.3 ± 0.5
UI	294 ± 6	282 ± 5	282 ± 5	284 ± 5	284 ± 3	291 ± 5	282 ± 3
MChL	18.0 ± 0.1	17.8 ± 0.1	17.9 ± 0.1	17.9 ± 0.1	17.8 ± 0.1	18.2 ± 0.0	18.1 ± 0.1

[1]See footnote Table 6.3

Table 6.11
Fatty acid composition (% total fatty acids) of retina phosphatidylcholine (PC) in 15-day-old piglets fed sow milk or formulas varying in % 18:2n-6 / % 18:3n-3[1]

Fatty acid	Reference milk ($n=7$)	Fish oil milk ($n=6$)	16/1 + FO ($n=5$)	16/1 ($n=6$)	16/4 ($n=7$)	30/1 ($n=8$)	35/4 ($n=6$)
16:0	37.7 ± 1.1	32.1 ± 0.8	40.0 ± 0.7	37.8 ± 0.5	38.0 ± 0.6	37.6 ± 1.1	39.8 ± 0.5
18:0	18.2 ± 0.2	19.6 ± 0.4	18.0 ± 0.4	18.6 ± 0.4	18.7 ± 0.4	18.4 ± 0.2	18.8 ± 0.1
Σ sats	56.4 ± 1.3	52.0 ± 0.5	58.6 ± 0.6	57.0 ± 0.3	57.2 ± 0.3	56.5 ± 0.9	59.0 ± 0.4
16:1	1.1 ± 0.1	0.9 ± 0.0	0.5 ± 0.0	0.4 ± 0.0	0.6 ± 0.2	1.1 ± 0.2	0.4 ± 0.0
18:1	24.7 ± 0.4	24.2 ± 0.3	24.4 ± 0.2	25.7 ± 0.5	24.6 ± 0.4	23.0 ± 0.2	21.3 ± 0.2
Σ monos	26.3 ± 0.5	25.6 ± 0.3	25.4 ± 0.3	26.6 ± 0.5	25.6 ± 0.4	24.5 ± 0.4	22.0 ± 0.2
18:2n-6	2.6 ± 0.1	2.5 ± 0.1	3.0 ± 0.1	3.1 ± 0.2	3.4 ± 0.1	4.4 ± 0.2	5.2 ± 0.2
18:3n-6	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.1	0.4 ± 0.0
20:2n-6	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.5 ± 0.0	0.4 ± 0.0
20:3n-6	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	0.4 ± 0.0
20:4n-6	4.9 ± 0.3	5.3 ± 0.1	4.5 ± 0.2	5.0 ± 0.1	4.7 ± 0.1	5.3 ± 0.1	4.6 ± 0.1
22:4n-6	0.6 ± 0.1	0.4 ± 0.0	0.3 ± 0.0	0.6 ± 0.0	0.4 ± 0.0	0.9 ± 0.1	0.4 ± 0.0
22:5n-6	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.6 ± 0.0	0.2 ± 0.0	1.0 ± 0.1	0.2 ± 0.0
Σ n-6 LCP	5.8 ± 0.4	5.8 ± 0.1	5.0 ± 0.1	6.3 ± 0.1	5.3 ± 0.1	7.1 ± 0.3	5.3 ± 0.1
18:3n-3	tr	0.1 ± 0.0	tr	tr	0.1 ± 0.0	tr	0.1 ± 0.0
20:5n-3	0.1 ± 0.0	0.2 ± 0.0	tr	tr	tr	0.1 ± 0.1	tr
22:5n-3	0.5 ± 0.0	0.3 ± 0.1	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0
22:6n-3	7.4 ± 0.4	12.6 ± 0.2	6.9 ± 0.2	6.1 ± 0.2	7.2 ± 0.2	5.8 ± 0.3	7.0 ± 0.3
Σ n-3 LCP	7.9 ± 0.5	13.0 ± 0.3	7.2 ± 0.2	6.2 ± 0.2	7.5 ± 0.2	6.2 ± 0.3	7.3 ± 0.3
22:6n-3/22:5n-6	24.3 ± 3.3	81.1 ± 11.2	50.5 ± 16.5	9.9 ± 0.3	36.2 ± 2.0	6.4 ± 0.4	33.7 ± 6.1
Σ LCP	13.7 ± 0.7	18.9 ± 0.3	12.2 ± 0.3	12.5 ± 0.3	12.8 ± 0.3	13.3 ± 0.6	12.5 ± 0.4
UI	105 ± 4	135 ± 2	97 ± 2	98 ± 1	101 ± 1	103 ± 3	100 ± 2
MChL	17.7 ± 0.1	18.0 ± 0.0	17.6 ± 0.0	17.7 ± 0.0	17.7 ± 0.0	17.7 ± 0.0	17.6 ± 0.0

[1]See footnote Table 6.3

Table 6.12
Fatty acid composition (% total fatty acids) of liver triglyceride in 15-day-old piglets fed sow milk or formulas varying in % 18:2n-6 / % 18:3n-3[1]

Fatty acid	Reference milk ($n=5$)	Fish oil Milk ($n=6$)	16/1 + FO ($n=5$)	16/1 ($n=6$)	16/4 ($n=7$)	30/1 ($n=6$)	35/4 ($n=6$)
16:0	27.7 ± 1.5	27.4 ± 0.4	16.2 ± 0.5	16.7 ± 0.7	14.3 ± 0.5	21.6 ± 0.5	15.1 ± 0.4
18:0	6.5 ± 1.1	6.0 ± 0.5	6.0 ± 0.4	6.2 ± 0.3	6.0 ± 0.5	6.9 ± 0.7	5.9 ± 0.4
Σ sats	36.8 ± 1.8	34.7 ± 0.7	28.5 ± 1.6	28.7 ± 1.1	29.5 ± 1.6	40.2 ± 0.8	28.5 ± 1.2
16:1	5.6 ± 0.7	4.7 ± 0.6	0.5 ± 0.0	0.6 ± 0.2	0.5 ± 0.1	0.5 ± 0.1	0.3 ± 0.0
18:1	29.1 ± 1.4	21.5 ± 0.8	41.4 ± 0.8	44.5 ± 0.9	38.8 ± 0.9	16.8 ± 0.5	16.7 ± 0.4
Σ monos	35.5 ± 1.7	26.6 ± 1.3	42.4 ± 0.8	45.8 ± 0.8	40.1 ± 0.8	17.6 ± 0.5	17.2 ± 0.4
18:2n-6	16.0 ± 1.4	21.1 ± 0.3	19.2 ± 0.3	17.6 ± 0.8	19.1 ± 0.6	32.0 ± 0.8	40.6 ± 0.5
18:3n-6	0.7 ± 0.3	0.5 ± 0.0	0.5 ± 0.1	0.6 ± 0.1	0.6 ± 0.0	1.0 ± 0.2	1.0 ± 0.1
20:2n-6	0.4 ± 0.1	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.6 ± 0.1	0.5 ± 0.0
20:3n-6	0.3 ± 0.1	0.5 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.6 ± 0.1	0.5 ± 0.0
20:4n-6	5.4 ± 0.8	5.7 ± 0.3	4.2 ± 0.5	4.4 ± 0.3	4.5 ± 0.6	5.8 ± 0.5	5.5 ± 0.6
22:4n-6	0.4 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	0.4 ± 0.1	0.2 ± 0.0	0.6 ± 0.1	0.4 ± 0.0
22:5n-6	0.1 ± 0.1	0.3 ± 0.0	tr	0.2 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	0.1 ± 0.0
Σ n-6 LCP	5.9 ± 0.9	6.3 ± 0.3	4.4 ± 0.5	4.9 ± 0.3	4.8 ± 0.6	6.7 ± 0.6	6.0 ± 0.6
18:3n-3	1.4 ± 0.2	1.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	2.8 ± 0.2	0.3 ± 0.0	3.6 ± 0.1
20:5n-3	0.4 ± 0.0	1.6 ± 0.3	0.8 ± 0.1	tr	0.4 ± 0.1	tr	0.3 ± 0.0
22:5n-3	0.8 ± 0.1	1.0 ± 0.1	0.5 ± 0.1	0.2 ± 0.0	0.5 ± 0.1	0.2 ± 0.0	0.6 ± 0.1
22:6n-3	0.7 ± 0.1	5.2 ± 0.6	1.4 ± 0.2	0.2 ± 0.0	0.9 ± 0.1	0.2 ± 0.0	0.9 ± 0.1
Σ n-3 LCP	1.9 ± 0.2	7.8 ± 0.9	2.8 ± 0.4	0.4 ± 0.1	1.8 ± 0.2	0.4 ± 0.0	1.8 ± 0.2
22:6n-3/22:5n-6	6.7 ± 1.8	20.4 ± 1.0	38.5 ± 5.8	1.4 ± 0.2	11.2 ± 1.3	0.7 ± 0.1	7.2 ± 0.8
Σ LCP	7.8 ± 1.0	14.1 ± 1.0	7.1 ± 0.8	5.3 ± 0.4	6.6 ± 0.8	7.1 ± 0.6	7.7 ± 0.8
UI	110 ± 4	147 ± 4	118 ± 5	108 ± 2	119 ± 4	118 ± 3	148 ± 4
MChL	17.4 ± 0.1	17.7 ± 0.1	17.5 ± 0.1	17.5 ± 0.0	17.5 ± 0.1	17.2 ± 0.1	17.6 ± 0.1

[1]See footnote Table 6.3

Table 6.13

Fatty acid composition (% total fatty acids) of liver cholesterol ester in 15-day-old piglets fed sow milk or formulas varying in % 18:2n-6 / % 18:3n-3

Fatty acid	Reference milk (n=7)	Fish oil milk (n=6)	16/1 + FO (n=5)	16/1 (n=7)	16/4 (n=7)	30/1 (n=6)	35/4 (n=6)
16:0	23.8 ± 1.0	23.7 ± 1.5	14.8 ± 0.7	16.5 ± 1.2	15.1 ± 0.7	19.9 ± 1.4	17.1 ± 0.7
18:0	8.1 ± 1.3	7.0 ± 0.7	6.7 ± 0.7	8.8 ± 0.8	6.5 ± 0.3	9.0 ± 0.8	7.1 ± 0.4
Σ sats	34.3 ± 1.6	33.9 ± 2.5	24.4 ± 1.4	29.4 ± 2.4	26.9 ± 1.1	33.3 ± 2.5	28.9 ± 1.2
16:1	5.0 ± 0.8	3.3 ± 0.4	2.1 ± 0.2	1.6 ± 0.4	1.5 ± 0.3	1.6 ± 0.2	1.1 ± 0.2
18:1	31.9 ± 1.6	14.8 ± 1.1	31.1 ± 2.4	33.1 ± 1.8	31.2 ± 1.0	18.7 ± 0.5	16.2 ± 0.9
Σ monos	33.6 ± 4.3	19.9 ± 1.5	35.6 ± 2.5	36.4 ± 1.3	33.9 ± 1.1	21.9 ± 0.7	18.3 ± 0.8
18:2n-6	22.2 ± 1.6	33.6 ± 1.9	31.5 ± 1.3	27.9 ± 2.0	32.5 ± 2.3	38.6 ± 3.2	42.7 ± 0.8
18:3n-6	0.6 ± 0.1	0.8 ± 0.1	1.1 ± 0.2	0.8 ± 0.1	1.0 ± 0.0	1.1 ± 0.1	1.0 ± 0.1
20:2n-6	0.5 ± 0.1	0.7 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.3 ± 0.0	0.3 ± 0.1	0.5 ± 0.0
20:3n-6	0.6 ± 0.1	0.8 ± 0.1	0.3 ± 0.0	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.1	0.5 ± 0.1
20:4n-6	3.8 ± 0.5	5.1 ± 0.2	2.1 ± 0.2	2.8 ± 0.3	1.8 ± 0.1	2.3 ± 0.2	2.9 ± 0.3
22:4n-6	0.3 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.0	0.3 ± 0.1
22:5n-6	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.2 ± 0.1
Σ n-6 LCP	4.1 ± 0.5	5.3 ± 0.2	2.6 ± 0.2	3.2 ± 0.3	2.3 ± 0.2	2.6 ± 0.2	3.4 ± 0.3
18:3n-3	1.1 ± 0.1	1.0 ± 0.4	0.2 ± 0.1	0.5 ± 0.1	1.9 ± 0.2	0.3 ± 0.0	2.0 ± 0.1
20:5n-3	0.2 ± 0.1	1.2 ± 0.1	1.0 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.1
22:5n-3	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.1 ± 0.0	1.2 ± 0.4	0.1 ± 0.0	0.6 ± 0.2
22:6n-3	0.5 ± 0.0	1.9 ± 0.2	0.9 ± 0.1	0.6 ± 0.2	1.3 ± 0.4	0.7 ± 0.1	1.3 ± 0.2
Σ n-3 LCP	0.9 ± 0.1	3.4 ± 0.4	2.3 ± 0.1	0.8 ± 0.2	2.7 ± 0.8	1.0 ± 0.2	2.0 ± 0.3
22:6n-3/22:5n-6	6.3 ± 1.8	20.5 ± 9.3	6.7 ± 0.9	3.4 ± 1.2	4.5 ± 1.3	5.2 ± 1.2	7.3 ± 2.7
Σ LCP	5.1 ± 0.6	8.8 ± 0.6	4.9 ± 0.4	3.6 ± 0.3	5.0 ± 0.9	3.5 ± 0.2	5.4 ± 0.5
UI	109 ± 2	138 ± 5	128 ± 2	119 ± 3	136 ± 8	126 ± 3	142 ± 3
MChL	17.4 ± 0.2	17.7 ± 0.1	17.7 ± 0.0	17.7 ± 0.0	18.3 ± 0.5	17.5 ± 0.1	17.8 ± 0.0

[1]See footnote Table 6.3

Table 6.14
Fatty acid composition (% total fatty acids) of liver total phospholipid in 15-day-old piglets fed sow milk or formulas varying in % 18:2n-6 / % 18:3n-3[1]

Fatty acid	Reference milk (*n*=7)	Fish oil Milk (*n*=6)	16/1 + FO (*n*=5)	16/1 (*n*=8)	16/4 (*n*=7)	30/1 (*n*=6)	35/4 (*n*=6)
16:0	17.5 ± 0.6	15.5 ± 0.4	13.0 ± 0.3	14.9 ± 0.8	11.8 ± 0.3	15.5 ± 0.6	12.3 ± 0.3
18:0	26.8 ± 0.9	25.4 ± 0.3	26.2 ± 0.6	27.7 ± 0.8	27.0 ± 0.6	28.9 ± 0.5	28.3 ± 0.7
Σ sats	45.3 ± 1.4	41.2 ± 0.3	39.8 ± 0.4	43.6 ± 0.9	40.4 ± 0.7	45.7 ± 0.4	42.2 ± 0.5
16:1	1.2 ± 0.1	0.9 ± 0.1	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	tr
18:1	10.9 ± 0.4	6.9 ± 0.4	13.1 ± 0.5	14.9 ± 0.5	14.3 ± 0.6	6.7 ± 0.1	6.6 ± 0.2
Σ monos	12.4 ± 0.4	7.9 ± 0.5	13.4 ± 0.4	16.0 ± 0.5	14.6 ± 0.6	7.0 ± 0.1	6.8 ± 0.2
18:2n-6	13.1 ± 0.6	14.6 ± 0.1	16.1 ± 0.7	16.2 ± 0.5	16.6 ± 0.3	22.2 ± 0.2	22.5 ± 0.8
18:3n-6	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
20:2n-6	0.3 ± 0.1	0.4 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	0.4 ± 0.0	0.9 ± 0.1	0.8 ± 0.1
20:3n-6	0.7 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	0.5 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.6 ± 0.0
20:4n-6	18.6 ± 0.5	15.4 ± 0.2	15.1 ± 0.5	18.1 ± 0.8	16.3 ± 0.3	18.8 ± 0.5	17.6 ± 0.5
22:4n-6	0.6 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.7 ± 0.1	0.3 ± 0.0	1.1 ± 0.1	0.5 ± 0.0
22:5n-6	0.3 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	1.1 ± 0.2	0.2 ± 0.0	1.6 ± 0.1	0.3 ± 0.0
Σ n-6 LCP	19.5 ± 0.5	16.0 ± 0.2	15.5 ± 0.5	19.9 ± 1.0	16.8 ± 0.3	21.5 ± 0.5	18.4 ± 0.5
18:3n-3	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.1	0.1 ± 0.0	0.2 ± 0.1
20:5n-3	0.4 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	tr	0.8 ± 0.1	tr	0.3 ± 0.0
22:5n-3	2.3 ± 0.3	1.4 ± 0.2	1.5 ± 0.1	0.5 ± 0.1	1.4 ± 0.1	0.4 ± 0.0	1.6 ± 0.1
22:6n-3	5.5 ± 0.5	15.8 ± 0.6	10.4 ± 0.4	2.3 ± 0.3	7.4 ± 0.3	1.5 ± 0.1	6.5 ± 0.5
Σ n-3 LCP	8.1 ± 0.8	18.2 ± 0.7	13.1 ± 0.3	2.9 ± 0.4	9.5 ± 0.2	1.9 ± 0.1	8.4 ± 0.5
22:6n-3/22:5n-6	18.2 ± 1.2	74.1 ± 2.2	94.2 ± 8.3	2.3 ± 0.2	38.2 ± 3.8	0.9 ± 0.1	22.3 ± 2.5
Σ LCP	27.6 ± 1.2	34.2 ± 0.6	28.6 ± 0.7	22.8 ± 1.3	26.4 ± 0.4	23.4 ± 0.5	26.8 ± 0.9
UI	167 ± 6	213 ± 3	188 ± 2	149 ± 6	175 ± 1	154 ± 2	178 ± 3
MChL	19.7 ± 0.1	18.7 ± 0.0	18.5 ± 0.0	19.2 ± 0.2	19.6 ± 0.2	19.6 ± 0.0	19.8 ± 0.0

[1]See footnote Table 6.3

130

Table 6.15

Fatty acid composition (% total fatty acids) of liver ethanolamine phospholipids (EPL) in 15-day-old piglets fed sow milk or formulas with varying % 18:2n-6 / % 18:3n-3[1]

Fatty acid	Reference milk (n=7)	Fish oil milk (n=6)	16/1 + FO (n=5)	16/1 (n=8)	16/4 (n=7)	30/1 (n=6)	35/4 (n=6)
16:0	10.4 ± 0.5	10.2 ± 0.4	9.3 ± 0.2	7.8 ± 0.2	8.8 ± 0.3	7.9 ± 0.3	9.7 ± 0.5
18:0	31.8 ± 0.6	26.5 ± 0.6	26.8 ± 1.0	27.8 ± 1.0	31.1 ± 1.2	29.0 ± 1.7	32.6 ± 1.3
Σ sats	43.1 ± 0.5	37.2 ± 0.8	36.9 ± 1.1	36.6 ± 1.2	40.6 ± 1.2	37.6 ± 1.7	42.9 ± 1.3
16:1	0.7 ± 0.0	0.6 ± 0.1	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
18:1	9.8 ± 0.6	5.8 ± 0.4	12.2 ± 0.5	13.8 ± 0.6	15.7 ± 0.5	7.0 ± 0.3	8.9 ± 0.6
Σ monos	11.0 ± 0.6	6.7 ± 0.4	12.7 ± 0.5	14.4 ± 0.6	16.2 ± 0.5	7.3 ± 0.3	9.2 ± 0.6
18:2n-6	8.7 ± 0.5	10.0 ± 1.0	15.1 ± 1.5	13.0 ± 1.0	10.6 ± 0.4	16.8 ± 0.8	15.3 ± 0.6
18:3n-6	0.1 ± 0.0	tr	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
20:2n-6	0.3 ± 0.1	0.4 ± 0.0	0.9 ± 0.1	0.7 ± 0.1	0.3 ± 0.0	1.0 ± 0.2	0.6 ± 0.1
20:3n-6	0.5 ± 0.1	0.7 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.1	0.4 ± 0.0
20:4n-6	24.0 ± 0.4	19.8 ± 0.5	18.3 ± 0.8	25.5 ± 1.0	20.5 ± 0.3	26.9 ± 0.7	20.7 ± 0.3
22:4n-6	0.7 ± 0.2	0.5 ± 0.0	0.4 ± 0.0	1.5 ± 0.1	0.5 ± 0.1	2.4 ± 0.2	0.7 ± 0.2
22:5n-6	0.5 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	2.0 ± 0.2	0.2 ± 0.0	3.4 ± 0.4	0.4 ± 0.1
Σ n-6 LCP	25.1 ± 0.5	20.6 ± 0.5	18.8 ± 0.8	29.0 ± 0.9	21.2 ± 0.3	32.7 ± 1.1	21.8 ± 0.4
18:3n-3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.2
20:5n-3	0.4 ± 0.1	1.4 ± 0.1	1.5 ± 0.1	0.1 ± 0.0	0.9 ± 0.1	tr	0.4 ± 0.0
22:5n-3	2.8 ± 0.3	1.9 ± 0.1	1.5 ± 0.2	0.9 ± 0.1	1.3 ± 0.1	0.7 ± 0.1	1.5 ± 0.2
22:6n-3	7.3 ± 0.6	21.1 ± 0.6	11.9 ± 0.9	4.5 ± 0.2	8.0 ± 0.7	3.2 ± 0.3	7.5 ± 0.6
Σ n-3 LCP	10.5 ± 0.9	24.4 ± 0.7	14.9 ± 1.1	5.6 ± 0.2	10.2 ± 0.7	3.9 ± 0.3	9.4 ± 0.6
22:6n-3/22:5n-6	16.2 ± 1.9	79.2 ± 2.5	99.4 ± 7.2	2.5 ± 0.2	34.1 ± 2.4	1.0 ± 0.1	22.0 ± 2.6
Σ LCP	35.6 ± 1.2	45.0 ± 0.6	33.6 ± 1.8	34.6 ± 1.0	31.4 ± 0.8	36.6 ± 1.1	31.2 ± 1.0
UI	192 ± 5	255 ± 3	208 ± 7	195 ± 4	185 ± 5	202 ± 5	185 ± 5
MChL	18.7 ± 0.1	19.2 ± 0.0	18.9 ± 0.1	18.8 ± 0.0	18.7 ± 0.0	18.8 ± 0.0	18.7 ± 0.0

[1]See footnote Table 6.3

Table 6.16

Fatty acid composition (% total fatty acid) of liver phosphatidylcholine (PC) in 15-day-old piglets fed sow milk or formulas with varying % 18:2n-6 / % 18:3n-3

Fatty acid	Reference milk (n=7)	Fish oil milk (n=6)	16/1 + FO (n=5)	16/1 (n=7)	16/4 (n=7)	30/1 (n=6)	35/4 (n=6)
16:0	23.0 ± 0.8	20.9 ± 0.6	16.2 ± 0.3	15.6 ± 0.7	17.0 ± 0.3	16.9 ± 0.3	18.1 ± 0.4
18:0	26.2 ± 1.7	24.4 ± 0.5	26.1 ± 0.7	26.5 ± 0.8	27.4 ± 1.1	27.7 ± 0.4	29.1 ± 1.0
Σ sats	50.1 ± 2.2	45.8 ± 0.4	42.9 ± 0.6	43.1 ± 0.5	45.5 ± 1.3	45.5 ± 0.2	48.4 ± 0.5
16:1	1.2 ± 0.1	1.0 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
18:1	15.6 ± 1.4	8.1 ± 0.4	15.7 ± 0.6	17.8 ± 0.7	17.4 ± 0.6	8.4 ± 0.2	7.3 ± 0.2
Σ monos	17.0 ± 1.4	9.3 ± 0.5	16.2 ± 0.6	18.2 ± 0.7	17.9 ± 0.7	8.7 ± 0.2	7.5 ± 0.2
18:2n-6	12.6 ± 1.0	15.2 ± 0.2	17.3 ± 1.0	16.1 ± 0.8	17.6 ± 0.5	22.8 ± 0.4	24.9 ± 1.1
18:3n-6	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
20:2n-6	0.3 ± 0.0	0.4 ± 0.0	0.4 ± 0.1	0.5 ± 0.0	0.3 ± 0.0	0.7 ± 0.1	0.6 ± 0.0
20:3n-6	0.7 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	0.5 ± 0.0
20:4n-6	12.6 ± 0.3	11.2 ± 0.4	10.4 ± 0.5	15.7 ± 0.8	10.0 ± 0.5	16.3 ± 0.3	11.4 ± 0.7
22:4n-6	0.4 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.8 ± 0.1	0.2 ± 0.1	1.1 ± 0.1	0.2 ± 0.0
22:5n-6	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	1.1 ± 0.2	0.2 ± 0.0	1.6 ± 0.2	0.2 ± 0.0
Σ n-6 LCP	13.2 ± 0.3	11.4 ± 0.4	10.6 ± 0.5	17.6 ± 1.0	10.4 ± 0.5	19.0 ± 0.3	11.9 ± 0.7
18:3n-3	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.5 ± 0.1	0.1 ± 0.0	0.4 ± 0.0
20:5n-3	0.3 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	0.1 ± 0.0	0.7 ± 0.1	tr	0.3 ± 0.0
22:5n-3	1.6 ± 0.2	1.3 ± 0.1	1.2 ± 0.1	0.6 ± 0.2	1.0 ± 0.1	0.4 ± 0.0	1.1 ± 0.1
22:6n-3	3.7 ± 0.4	14.0 ± 0.4	8.9 ± 0.5	2.6 ± 0.3	5.0 ± 0.4	1.6 ± 0.1	4.2 ± 0.5
Σ n-3 LCP	5.7 ± 0.6	16.4 ± 0.4	11.2 ± 0.5	3.3 ± 0.5	6.7 ± 0.5	2.1 ± 0.1	5.6 ± 0.5
22:6n-3/22:5n-6	19.2 ± 1.8	144.9 ± 30.7	144.0 ± 24.1	2.5 ± 0.2	30.0 ± 1.6	1.0 ± 0.1	21.5 ± 2.4
Σ LCP	18.8 ± 0.9	27.8 ± 0.1	21.8 ± 0.9	20.9 ± 1.3	17.1 ± 1.0	21.1 ± 0.3	17.5 ± 1.1
UI	131 ± 6	187 ± 1	163 ± 3	145 ± 4	139 ± 5	149 ± 1	142 ± 3
MChL	18.1 ± 0.0	18.5 ± 0.0	18.4 ± 0.0	18.3 ± 0.1	18.2 ± 0.0	18.2 ± 0.0	18.1 ± 0.0

[1]See footnote Table 6.3

Table 6.17
Fatty acid composition (% total fatty acids) of plasma triglyceride in 15-day-old piglets fed sow milk or formulas with varying % 18:2n-6 / % 18:3n-3[1]

Fatty acid	Reference milk (n=3)	Fish oil milk (n=6)	16/1 + FO (n=5)	16/1 (n=6)	16/4 (n=6)	30/1 (n=6)	35/4 (n=5)
16:0	29.2 ± 1.8	28.9 ± 2.0	14.4 ± 1.4	12.3 ± 1.0	12.9 ± 0.6	18.2 ± 0.3	14.9 ± 0.5
18:0	5.0 ± 0.6	8.0 ± 1.6	5.8 ± 0.9	5.9 ± 0.3	5.6 ± 0.4	5.5 ± 0.5	5.6 ± 0.3
Σ sats	36.8 ± 2.1	39.4 ± 3.3	27.2 ± 2.1	24.6 ± 1.2	24.5 ± 1.3	35.0 ± 1.2	27.2 ± 0.7
16:1	6.8 ± 0.6	5.6 ± 0.6	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.0	0.4 ± 0.1
18:1	35.8 ± 2.4	26.9 ± 1.8	48.5 ± 2.2	51.4 ±1.3	48.6 ± 0.6	22.5 ± 1.0	20.9 ± 0.9
Σ monos	43.3 ± 2.0	33.9 ± 2.2	49.5 ± 2.1	52.1 ± 1.4	49.9 ± 0.6	23.3 ± 1.1	21.6 ± 1.0
18:2n-6	13.2 ± 0.2	19.5 ± 1.2	18.4 ± 0.5	19.2 ± 0.6	18.9 ± 0.6	36.1 ± 0.8	41.6 ± 0.8
18:3n-6	0.3 ± 0.1	0.1 ± 0.1	0.2 ± 0.0	0.3 ± 0.0	0.4 ± 0.1	0.7 ± 0.1	0.7 ± 0.1
20:2n-6	0.5 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	0.3 ± 0.2	0.1 ± 0.1	0.3 ± 0.0	0.3 ± 0.1
20:3n-6	0.2 ± 0.0	0.2 ± 0.0	tr	0.2 ± 0.2	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.1
20:4n-6	2.3 ± 0.3	1.5 ± 0.3	0.5 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	2.2 ± 0.4	2.1 ± 0.4
22:4n-6	0.2 ± 0.0	0.4 ± 0.2	0.2 ± 0.1	0.4 ± 0.0	0.4 ± 0.1	0.5 ± 0.2	0.2 ± 0.0
22:5n-6	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0
Σ n-6 LCP	2.5 ± 0.3	2.1 ± 0.3	0.7 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	2.9 ± 0.6	2.3 ± 0.4
18:3n-3	1.4 ± 0.1	1.5 ± 0.3	1.1 ± 0.2	1.2 ± 0.1	3.9 ± 0.3	0.9 ± 0.3	4.7 ± 0.1
20:5n-3	0.3 ± 0.0	0.4 ± 0.2	0.5 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	tr	0.2 ± 0.1
22:5n-3	0.4 ± 0.1	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.2	0.2 ± 0.0	0.1 ± 0.0	0.4 ± 0.1
22:6n-3	0.2 ± 0.0	2.0 ± 0.5	1.0 ± 0.2	0.2 ± 0.1	0.4 ± 0.1	0.1 ± 0.1	0.4 ± 0.1
Σ n-3 LCP	0.9 ± 0.1	2.7 ± 0.7	1.8 ± 0.1	0.4 ± 0.4	0.7 ± 0.2	0.3 ± 0.1	1.0 ± 0.2
22:6n-3/22:5n-6	4.1 ± 1.4	14.5 ± 2.1	13.9 ± 6.4	3.3 ± 1.0	5.1 ± 1.2	0.5 ± 0.2	5.3 ± 1.1
Σ LCP	3.4 ± 0.3	4.8 ± 0.9	2.4 ± 0.2	1.6 ± 0.5	1.8 ± 0.3	3.2 ± 0.7	3.3 ± 0.6
UI	85 ± 3	97 ± 8	104 ± 2	103 ± 1	109 ± 3	114 ± 3	136 ± 2
MChL	17.3 ± 0.1	17.5 ± 0.1	17.5 ± 0.1	17.5 ± 0.0	17.6 ± 0.1	17.4 ± 0.1	17.6 ± 0.0

[1]See footnote Table 6.3

Table 6.18

Fatty acid composition (% total fatty acids) of plasma cholesterol esters in 15-day-old piglets fed sow milk or formulas with varying % 18:2n-6 / % 18:3n-3[1]

Fatty acid	Reference milk (n=4)	Fish oil milk (n=6)	16/1 + FO (n=3)	16/1 (n=7)	16/4 (n=7)	30/1 (n=6)	35/4 (n=6)
16:0	23.0 ± 1.6	17.8 ± 1.5	12.4 ± 0.7	11.4 ± 0.3	10.9 ± 0.3	11.3 ± 0.5	11.1 ± 0.2
18:0	4.0 ± 0.3	2.9 ± 0.4	2.1 ± 0.1	2.0 ± 0.2	1.9 ± 0.1	2.0 ± 0.2	2.0 ± 0.2
Σ sats	27.5 ± 1.6	22.0 ± 2.0	16.3 ± 1.0	15.3 ± 0.5	14.8 ± 0.3	15.6 ± 0.9	14.9 ± 0.4
16:1	6.4 ± 0.4	3.1 ± 0.2	0.9 ± 0.1	1.2 ± 0.1	1.1 ± 0.2	1.0 ± 0.1	0.8 ± 0.1
18:1	27.5 ± 2.6	16.5 ± 1.2	30.6 ± 1.2	30.4 ± 0.3	26.2 ± 0.4	13.2 ± 0.4	11.4 ± 0.3
Σ monos	34.0 ± 2.3	20.1 ± 1.4	31.8 ± 1.2	31.7 ± 0.4	27.6 ± 0.4	14.4 ± 0.5	12.4 ± 0.4
18:2n-6	31.9 ± 1.0	48.6 ± 1.5	46.3 ± 2.2	47.3 ± 1.1	49.3 ± 0.6	63.9 ± 1.3	64.6 ± 0.4
18:3n-6	0.9 ± 0.0	0.5 ± 0.0	0.5 ± 0.2	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.7 ± 0.1
20:2n-6	nd	tr	tr	nd	tr	nd	nd
20:3n-6	0.2 ± 0.0	0.4 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
20:4n-6	3.6 ± 0.2	4.1 ± 0.3	2.5 ± 0.2	3.5 ± 0.4	3.2 ± 0.2	4.2 ± 0.3	4.2 ± 0.4
22:4n-6	0.3 ± 0.1	0.8 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.3 ± 0.1	0.1 ± 0.0	0.2 ± 0.1
22:5n-6	nd	nd	0.1 ± 0.0	nd	tr	tr	nd
Σ n-6 LCP	3.9 ± 0.2	4.9 ± 0.4	2.8 ± 0.3	3.6 ± 0.5	3.5 ± 0.2	4.3 ± 0.3	4.4 ± 0.4
18:3n-3	1.3 ± 0.0	1.4 ± 0.1	0.6 ± 0.0	0.6 ± 0.0	3.0 ± 0.1	0.3 ± 0.0	2.1 ± 0.1
20:5n-3	0.2 ± 0.0	0.6 ± 0.1	0.9 ± 0.1	tr	0.2 ± 0.0	tr	0.2 ± 0.0
22:5n-3	nd	tr	tr	tr	tr	tr	nd
22:6n-3	0.2 ± 0.0	0.9 ± 0.1	0.5 ± 0.0	0.4 ± 0.1	0.4 ± 0.0	0.3 ± 0.1	0.5 ± 0.1
Σ n-3 LCP	0.4 ± 0.1	1.5 ± 0.2	1.5 ± 0.1	0.2 ± 0.1	0.6 ± 0.1	0.4 ± 0.1	0.6 ± 0.2
22:6n-3/22:5n-6	NA[2]	NA	NA	NA	NA	NA	NA
Σ LCP	4.3 ± 0.2	6.3 ± 0.5	4.3 ± 0.3	3.8 ± 0.5	4.1 ± 0.3	4.7 ± 0.3	5.1 ± 0.4
UI	116 ± 2	149 ± 4	147 ± 3	147 ± 1	155 ± 1	165 ± 2	171 ± 2
MChL	17.7 ± 01	17.8 ± 0.0	17.9 ± 0.0	17.9 ± 0.0	17.9 ± 0.0	17.9 ± 0.0	17.9 ± 0.1

[1]See footnote Table 6.3
[2]NA, not available, due to negligible % 22:5n-6

134

Table 6.19

Fatty acid composition (% total fatty acids) of plasma total phospholipid in 15-day-old piglets fed sow milk or formulas with varying % 18:2n-6 / % 18:3n-3[1]

Fatty acid	Reference milk (n=5)	Fish oil milk (n=6)	16/1 + FO (n=5)	16/1 (n=5)	16/4 (n=7)	30/1 (n=6)	35/4 (n=6)
16:0	23.7 ± 0.8	23.8 ± 1.2	16.9 ± 0.9	16.7 ± 0.3	15.5 ± 0.3	19.2 ± 0.9	17.0 ± 0.4
18:0	20.5 ± 0.4	22.6 ± 0.6	23.6 ± 0.8	22.9 ± 0.4	23.3 ± 0.6	24.3 ± 0.8	25.7 ± 0.5
Σ sats	45.4 ± 0.7	48.6 ± 1.0	42.6 ± 0.6	41.6 ± 0.5	41.2 ± 0.7	46.3 ± 0.9	45.3 ± 0.6
16:1	1.3 ± 0.1	0.4 ± 0.1	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
18:1	14.9 ± 0.2	9.7 ± 0.6	19.4 ± 0.5	20.8 ± 0.3	19.3 ± 0.5	8.9 ± 0.2	8.2 ± 0.2
Σ monos	16.5 ± 0.3	10.2 ± 0.6	19.9 ± 0.5	21.3 ± 0.3	19.7 ± 0.4	8.4 ± 0.6	8.4 ± 0.2
18:2n-6	20.8 ± 0.1	19.7 ± 0.8	24.1 ± 0.6	25.1 ± 0.8	26.1 ± 0.4	31.8 ± 1.2	32.4 ± 0.7
18:3n-6	0.1 ± 0.0	tr	tr	tr	tr	tr	tr
20:2n-6	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.1	0.2 ± 0.0	0.9 ± 0.3	0.3 ± 0.1	0.4 ± 0.0
20:3n-6	0.6 ± 0.1	1.1 ± 0.1	0.6 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.0	0.3 ± 0.0
20:4n-6	11.1 ± 0.5	9.2 ± 0.4	6.0 ± 0.4	8.8 ± 0.5	6.7 ± 0.3	9.6 ± 0.6	8.6 ± 0.5
22:4n-6	0.4 ± 0.0	0.6 ± 0.1	0.1 ± 0.0	0.5 ± 0.1	0.2 ± 0.0	0.8 ± 0.1	0.3 ± 0.0
22:5n-6	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.6 ± 0.1	tr	0.8 ± 0.1	0.1 ± 0.1
Σ n-6 LCP	11.6 ± 0.6	10.0 ± 0.3	6.2 ± 0.4	9.9 ± 0.6	7.0 ± 0.4	11.2 ± 0.6	9.0 ± 0.6
18:3n-3	0.1 ± 0.0	0.5 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	1.1 ± 0.3	0.2 ± 0.0	0.8 ± 0.2
20:5n-3	0.4 ± 0.1	1.1 ± 0.1	1.7 ± 0.1	0.1 ± 0.0	0.4 ± 0.1	tr	0.2 ± 0.0
22:5n-3	1.8 ± 0.2	1.2 ± 0.2	0.8 ± 0.1	0.4 ± 0.1	0.7 ± 0.1	0.3 ± 0.1	0.9 ± 0.1
22:6n-3	2.6 ± 0.2	7.9 ± 0.4	4.3 ± 0.4	1.3 ± 0.1	2.8 ± 0.2	0.9 ± 0.1	3.0 ± 0.3
Σ n-3 LCP	4.8 ± 0.4	10.1 ± 0.4	6.8 ± 0.4	1.8 ± 0.2	4.0 ± 0.3	1.3 ± 0.2	4.1 ± 0.4
22:6n-3/22:5n-6	20.1 ± 1.4	33.3 ± 3.1	61.4 ± 8.2	2.4 ± 0.2	54.1 ± 9.0	1.2 ± 0.2	19.3 ± 6.2
Σ LCP	16.4 ± 0.8	20.2 ± 0.5	13.0 ± 0.8	11.6 ± 0.8	11.0 ± 0.6	12.5 ± 0.7	13.1 ± 0.9
UI	134 ± 3	154 ± 3	134 ± 3	124 ± 2	129 ± 3	128 ± 2	137 ± 4
MChL	18.0 ± 0.0	18.2 ± 0.1	18.0 ± 0.0	18.0 ± 0.0	18.0 ± 0.0	18.0 ± 0.0	18.1 ± 0.0

[1]See footnote Table 6.3

Table 6.20

Fatty acid composition (% total fatty acids) of red blood cell (RBC) ethanolamine phospholipids (EPL) in 15-day-old piglets fed sow milk or formulas with varying % 18:2n-6 / % 18:3n-3[1]

Fatty acid	Reference milk (n = 11)	Fish oil milk (n = 6)	16/1 + FO (n = 3)	16/1 (n = 5)	16/4 (n = 7)	30/1 (n = 5)	35/4 (n = 5)
16:0	24.0 ± 0.6	24.6 ± 0.7	20.5 ± 0.1	17.7 ± 0.6	19.1 ± 0.4	22.9 ± 0.7	21.4 ± 0.6
18:0	6.7 ± 0.2	6.5 ± 0.3	7.9 ± 0.1	8.1 ± 0.1	8.3 ± 0.2	9.2 ± 0.6	11.1 ± 1.0
Σ sats	31.6 ± 0.6	32.3 ± 0.7	29.7 ± 0.1	27.1 ± 0.9	28.9 ± 0.2	33.8 ± 1.3	34.2 ± 1.0
16:1	2.1 ± 0.3	2.0 ± 0.1	0.5 ± 0.1	0.8 ± 0.2	0.8 ± 0.1	1.0 ± 0.1	0.9 ± 0.1
18:1	36.7 ± 0.9	32.7 ± 0.9	47.2 ± 0.5	46.6 ± 0.3	45.6 ± 0.5	32.4 ± 0.8	26.8 ± 1.8
Σ monos	39.1 ± 0.8	35.0 ± 1.0	48.0 ± 0.5	47.9 ± 0.4	47.0 ± 0.5	34.0 ± 0.7	28.1 ± 2.0
18:2n-6	15.7 ± 0.4	16.1 ± 0.4	13.1 ± 0.2	14.8 ± 0.8	14.4 ± 0.7	21.9 ± 0.5	24.5 ± 0.8
18:3n-6	tr	tr	tr	tr	tr	tr	tr
20:2n-6	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	0.4 ± 0.0	0.6 ± 0.0	0.7 ± 0.0
20:3n-6	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0
20:4n-6	6.5 ± 0.2	4.8 ± 0.2	3.3 ± 0.2	5.9 ± 0.5	4.3 ± 0.5	5.5 ± 0.7	6.9 ± 0.8
22:4n-6	0.8 ± 0.1	1.1 ± 0.1	0.3 ± 0.0	1.0 ± 0.2	0.4 ± 0.0	0.7 ± 0.3	0.8 ± 0.1
22:5n-6	0.4 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.5 ± 0.1	0.3 ± 0.0	0.7 ± 0.1	0.5 ± 0.0
Σ n-6 LCP	7.7 ± 0.2	6.3 ± 0.3	3.8 ± 0.3	7.4 ± 0.7	5.1 ± 0.6	7.0 ± 1.0	8.1 ± 0.9
18:3n-3	0.5 ± 0.1	0.6 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.8 ± 0.1	0.1 ± 0.0	0.9 ± 0.1
20:5n-3	0.4 ± 0.1	0.4 ± 0.0	0.4 ± 0.1	tr	0.1 ± 0.0	tr	0.1 ± 0.0
22:5n-3	2.2 ± 0.1	1.8 ± 0.0	1.2 ± 0.2	0.5 ± 0.1	0.9 ± 0.1	0.6 ± 0.1	1.1 ± 0.1
22:6n-3	2.2 ± 0.1	7.0 ± 0.2	2.8 ± 0.4	1.1 ± 0.1	2.1 ± 0.3	1.7 ± 0.3	1.9 ± 0.2
Σ n-3 LCP	4.8 ± 0.2	9.1 ± 0.3	4.4 ± 0.6	1.7 ± 0.2	3.1 ± 0.4	2.3 ± 0.4	3.2 ± 0.3
22:6n-3/22:5n-6	5.4 ± 0.5	16.9 ± 1.9	11.1 ± 0.6	2.4 ± 0.1	6.2 ± 0.7	2.4 ± 0.3	4.0 ± 0.5
Σ LCP	12.5 ± 0.2	15.4 ± 0.3	8.2 ± 0.7	9.1 ± 0.9	8.2 ± 0.9	9.3 ± 1.1	11.3 ± 1.1
UI	131 ± 1	149 ± 1	117 ± 3	120 ± 4	118 ± 3	122 ± 6	133 ± 4
MChL	17.9 ± 0.0	18.0 ± 0.0	17.9 ± 0.0	17.9 ± 0.0	17.9 ± 0.0	17.8 ± 0.0	17.9 ± 0.0

[1]See footnote Table 6.3

Table 6.21

Fatty acid composition (% total fatty acids) of red blood cell (RBC) phosphatidylcholine (PC) in 15-day-old piglets fed sow milk or formulas with varying % 18:2n-6 / % 18:3n-3[1]

Fatty acid	Reference milk ($n=9$)	Fish oil Milk ($n=6$)	16/1 + FO ($n=3$)	16/1 ($n=6$)	16/4 ($n=6$)	30/1 ($n=5$)	35/4 ($n=5$)
16:0	31.9 ± 1.2	38.4 ± 0.4	31.8 ± 0.6	31.3 ± 0.8	31.0 ± 0.7	35.2 ± 1.2	32.4 ± 0.2
18:0	13.3 ± 1.1	9.5 ± 0.3	10.8 ± 0.2	9.9 ± 0.3	11.1 ± 0.3	12.2 ± 0.6	13.6 ± 0.3
Σ sats	46.2 ± 1.5	49.8 ± 0.4	44.6 ± 0.5	43.3 ± 0.7	44.8 ± 0.8	49.9 ± 1.4	49.1 ± 0.6
16:1	1.2 ± 0.1	1.1 ± 0.1	0.3 ± 0.0	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.0	0.3 ± 0.0
18:1	25.1 ± 0.8	19.7 ± 0.2	28.6 ± 0.8	29.8 ± 0.5	27.2 ± 1.1	17.5 ± 1.0	16.0 ± 0.3
Σ monos	26.4 ± 0.8	21.2 ± 0.3	29.1 ± 0.8	30.4 ± 0.5	27.9 ± 1.2	18.0 ± 1.1	16.6 ± 0.3
18:2n-6	19.4 ± 1.1	21.6 ± 0.5	21.4 ± 1.3	21.0 ± 0.6	21.1 ± 0.5	26.9 ± 1.9	26.4 ± 0.8
18:3n-6	nd	nd	tr	tr	tr	tr	tr
20:2n-6	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.0	0.3 ± 0.1	0.3 ± 0.0	0.5 ± 0.1	0.7 ± 0.1
20:3n-6	0.4 ± 0.0	0.4 ± 0.0	0.3 ± 0. 0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
20:4n-6	4.8 ± 0.3	3.1 ± 0.2	2.1 ± 0.2	3.6 ± 0.4	2.8 ± 0.3	3.5 ± 0.6	4.1 ± 0.5
22:4n-6	0.2 ± 0.1	0.4 ± 0.1	nd	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.7 ± 0.1
22:5n-6	tr	tr	tr	0.1 ± 0.0	tr	0.2 ± 0.0	tr
Σ n-6 LCP	5.0 ± 0.3	3.5 ± 0.3	2.1 ± 0.2	4.0 ± 0.4	3.1 ± 0.3	4.0 ± 0.7	4.9 ± 0.5
18:3n-3	0.5 ± 0.0	0.5 ± 0.0	0.3 ± 0.0	0.3 ± 0.1	1.3 ± 0.1	0.2 ± 0.0	0.8 ± 0.0
20:5n-3	0.2 ± 0.0	0.5 ± 0.0	0.7 ± 0.0	tr	0.2 ± 0.0	nd	0.1 ± 0.0
22:5n-3	0.6 ± 0.1	0.3 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	0.3 ± 0.0
22:6n-3	0.9 ± 0.1	2.0 ± 0.1	1.0 ± 0.2	0.4 ± 0.1	0.8 ± 0.1	0.3 ± 0.1	1.0 ± 0.2
Σ n-3 LCP	1.6 ± 0.2	2.8 ± 0.2	2.0 ± 0.2	0.5 ± 0.1	1.2 ± 0.1	0.3 ± 0.1	1.3 ± 0.2
22:6n-3/22:5n-6	24.5 ± 5.0	48.1 ± 4.3	23.1 ± 4.5	2.9 ± 0.3	16.5 ± 2.6	1.3 ± 0.1	13.3 ± 0.3
Σ LCP	6.7 ± 0.5	6.3 ± 0.4	4.1 ± 0.3	4.5 ± 0.5	4.3 ± 0.4	4.3 ± 0.7	6.2 ± 0.7
UI	98 ± 4	98 ± 2	93 ± 2	93 ± 2	95 ± 1	92 ± 5	101 ± 2
MChL	17.5 ± 0.1	17.5 ± 0.0	17.5 ± 0.0	17.5 ± 0.0	17.5 ± 0.0	17.4 ± 0.0	17.6 ± 0.0

[1]See footnote Table 6.3