INFLUENCE OF DIETARY FATTY ACIDS AND POSITIONAL DISTRIBUTION OF DIETARY FATTY ACIDS ON PLASMA LIPIDS, VISUAL ACUITY AND COGNITIVE DEVELOPMENT IN TERM INFANTS

by

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ABSTRACT

Arachidonic acid (20:4n-6) and 22:6n-3 are incorporated into the CNS during development. Deficiency of 18:3n-3 during development reduces 22:6n-3 and alters function of the retina and brain. As of 1999, infant formulas in North America contain 18:2n-6 and 18:3n-3, but not 20:4n-6 or 22:6n-3, whereas formulas in Europe, Asia and South American may contain lon-chain fatty acids from sources that include fish oils, egg phospholipids and single cell oils. It is not known if 20:4n-6 and 22:6n-3 are essential nutrients for normal brain and retina development in term infants. Human milk TG has 16:0 preferentially esterified at the *sn*-2 position, whereas in infant formula 16:0 is predominantly at the *sn*-1,3 position. The effect of milk and formula TG fatty acid distribution on plasma lipid fatty acids is not known. These studies determined whether preferential looking acuity or novelty preference differs between breast-fed infants and infants fed formula with 18:3n-3 (1% of energy) and no 20:4n-6 or 22:6n-3. The influence of the milk and formula TG fatty acid distribution on the distribution of fatty acids in plasma lipids and lipoproteins was also determined in a randomized double-blind study with infants fed formula with 16:0 preferentially esterified at the 2 position, a standard formula or breast-fed. These studies found no association between visual acuity and erythrocyte or plasma PL 22:6n-3 at three mo, and no difference in visual acuity at any age to 18 mo between breast-fed and formula-fed infants. 16:0 was found to be higher in the plasma TG 2 position of the breast-fed infants and the infants fed the synthesized TG formula than infants fed the standard formula. These studies also showed about 50% of the 2 position 16:0 of milk and synthesized TG formula was conserved through digestion, absorption, reesterification to TG and secretion in chylomicron TG. Furthermore, the distribution of TG in milk and formula did influence plasma lipids, notably, 18:3n-3. Analysis of the lipoprotein lipids showed high 20:4n-6 and 22:6n-3 in lysoPL, 18:2n-6 and 18:3n-3 in albumin fatty acids, and 20:4n-6 and 22:6n-3 in HDL PL. The role of these lipids in n-6 and n-3 fatty acid delivery to brain remains to be determined.

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ABBREVIATIONS

ANCOVA	analysis of covariance
ANOVA	analysis of variance
apo	apolipoprotein
BSSL	bile-salt stimulated lipase
C	carbon chain
CE	cholesterol ester
CETP	cholesterol ester transfer protein
CLD	cytoplasmic lipid droplets
cm	centimetre
CM	chylomicron
CNS	central nervous system
cyc/deg	cycles per degree
d	day
Δ	delta
EDTA	ethylenediamine tetraacetic acid
EFA	essential fatty acids (18:2n-6 and 18:3n-3)
FAS	fatty acid synthetase
FFA	unesterified fatty acid
FPL	forced-choice preferential looking
g	gram
GLC	gas-liquid chromatography
h	hour
HDL	high-density lipoprotein
kg	kilogram

L	litre
LCAT	lecithin cholesterol acyltransferase
LCPUFA	long-chain polyunsaturated fatty acids
LDL	low density lipoprotein
LRP	lipoprotein related protein
LysoPL	lysophospholipid
MCFA	medium-chain fatty acids
MEq	milliequivalents
MFGM	milk fat globule membrane
µg	microgram
mg	milligram
MG	monoacylglycerol
mmol	millimole
mL	millilitre
M	molar
mo	month
NaCl	sodium chloride
PC	phosphatidylcholine
PE	phosphatidylethanolamine
PL	phospholipid
P	statistical probability of mean differences not existing
s	second
SD	standard deviation
SEM	standard error of mean
TG	triacylglycerol
TLC	thin-layer chromatography

VEP	visual evoked potential
VLDL	very low density lipoprotein
wk	week
yr	year

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FOREWARD

Study I has been published:

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Innis SM, Dyer R & Nelson CM. (1994) Evidence that palmitic acid is absorbed as *sn*-2 monoacylglycerols from human milk breast-fed infants. *Lipids* 29: 541-546.

Part of Study II has been accepted for publication:

Nelson CM & Innis SM. Plasma lipoprotein fatty acids are altered by the positional distribution of fatty acids in infant formulae triacylglycerols and human milk. *Am J Clin Nutr*. (accepted 1999)

Nelson CM & Innis SM. Arachidonic acid and docosahexaenoic acid are distributed differently among lipoproteins, lysophospholipids and unesterified fatty acids of breast-fed and formula-fed infants. *Am J Clin Nutr* (submitted 1999)

Part of Study III has been published:

Innis SM, Nelson CM, Lwanga D, Rioux FM & Waslen P. (1996) Feeding formula without arachidonic acid and docosahexaenoic acid has no effect on preferential looking acuity or recognition memory in healthy full-term infants at 9 mo of age. *Am J Clin Nutr* 64: 40-46.

Nelson CM & Innis SM. Infant preferential looking acuity and novelty preference are influenced by genetic and environmental factors at 9 months of age. *Infancy* (submitted 1999).

1. INTRODUCTION

Fat is essential in an infant's diet as it provides a concentrated source of energy needed to support rapid growth. A significant portion of energy in human milk and infant formula comes from fat, usually about 45% to 50% of total kilocalories, despite fat representing only 3-5% of the total milk volume (Gaull 1982, Innis 1992, Jensen 1995). Dietary fat is also important as it provides precursors for the synthesis of eicosanoids, steroid hormones, cell membrane lipids and bile acids. Dietary fat acts as a carrier for the fat soluble vitamins, A D E and K. In recent years, studies with infants have shown that not just the quantity of fat, but also the quality (ie fatty acid composition) is important. One area of current interest in fatty acids relates to the role of the n-6 and n-3 polyunsaturated fatty acids in central nervous system (CNS) development and function. The importance of the distribution of fatty acids in dietary triacylglycerol and its subsequent influence on digestion, absorption and transportation of fatty acids is also an important area to infant nutrition.

1.1. Fatty Acid Metabolism

1.1.1. Nomenclature

Fatty acids are carboxylic acids that have the following basic structure (reviewed by Laposta 1995):

Methyl end$\rightarrow CH_3CH_nCOOH \leftarrow$Carboxyl end

The carboxyl end is the reactive end and is the site where fatty acids covalently bind with other molecules, such as glycerol, for the synthesis of triacylglycerols (TG) or phospholipids (PL). The most common nomenclature for fatty acids utilises the base name of the hydrocarbon of the same configuration. For example, the 4-carbon fatty acid butyric acid is named after the hydrocarbon butyrate. A common convention for denoting the number and position of double bonds in a fatty

acid starts with the number of carbon atoms followed by a colon, then the number of unsaturated carbon atoms (**Table 6.1**). The final number indicates the position of the first unsaturated carbon atom from the methyl (CH_3) end. Using this convention, the saturated fatty acid palmitic acid is represented as 16:0, indicating that there are 16 carbon atoms and no double bonds (**Figure 1.1**). The unsaturated fatty acid linoleic acid is designated 18:2n-6 with the first number, 18, indicating a total number of 18 carbon atoms in the fatty acid and the number after the colon, 2, representing the number of unsaturated bonds. The position of the first unsaturated carbon from the methyl end of the fatty acid is designated by the n-6, indicating that it is positioned between carbon six and seven from the methyl end. Linolenic acid (18:3n-3) has 18 carbon atoms and three double bonds with the first double bond three carbons from the methyl end.

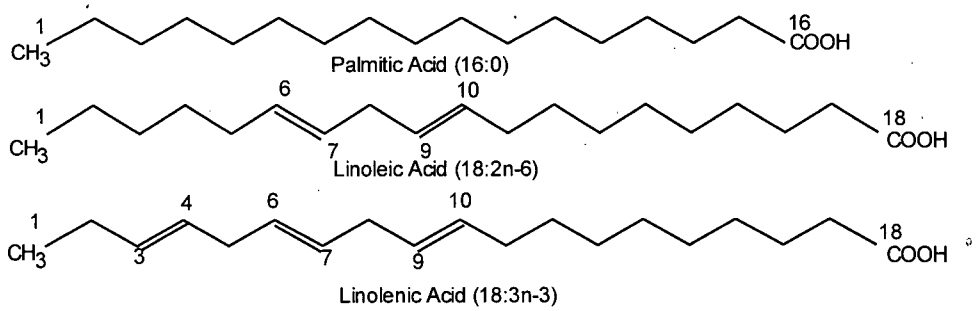


Figure 1.1 - Schematic representation of palmitic acid (16:0), linoleic acid (18:2n-6) and linolenic acid (18:3n-3) with the position of the unsaturated carbon atoms shown.

Fatty acids are grouped based on the number of double bonds that occur in the carbon chain. The saturated fatty acids have no double bonds; monounsaturated fatty acids have one double bond; polyunsaturated fatty acids have two or more double bonds. For the unsaturated fatty acids, they can be further classified into "families" by the position of the first double bond from the

methyl end of the carbon chain. The most common fatty acid families are the n-3, n-6, n-9 and n-7 groups. When the relative position of a fatty acid must be accurately indicated in TG or PL, the "*sn*" or stereochemical numbering system is used, as recommended by the International Union of Pure and Applied Chemistry. The distinction between carbon position is not arbitrary, but defined by enzymatic reactivity. A Fischer projection formula, indicating the *sn* position of fatty acids in TG is shown in **Figure 1.2**

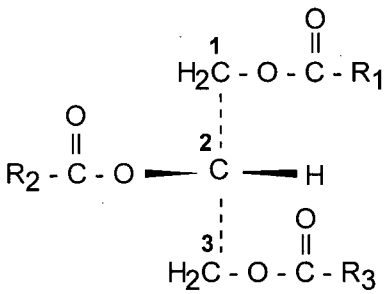


Figure 1.2 – Fisher projection formula for triacyl - *sn* - glycerol

1.1.2. Synthesis

Synthesis of fatty acids occurs in many tissues including kidney, lung, mammary gland and liver. In humans, the liver is the primary organ for the *de novo* synthesis of fatty acids and in lactating women, the mammary gland synthesizes significant amounts of fatty acid for milk production. As the regulation of lipogenesis in the liver is different from the mammary gland, the mechanisms involved in fatty acid synthesis in the mammary gland will be dealt with in Section 1.3.1. The liver converts excess glucose not being used to meet energy requirements or in the production of glycogen to fatty acids (Volpe 1978). Starvation, diabetes and a high fat diet all result in inhibition of *de novo* lipogenesis due to the action of glucagon and the relative lack of insulin (Volpe 1978, Bloch & Vance 1977). The main pathway for synthesis of fatty acids is in the liver cytosol, with glucose as the primary substrate (**Figure 1.3**). Acetyl-CoA is generated in the

mitochondria and must move to the cytosol for fatty acid synthesis. As acetyl-CoA cannot pass through the mitochondrial membrane, it is transported as citrate to the cytosol, where citrate is converted back to acetyl-CoA and oxaloacetate. Acetyl-CoA supplies the first two-carbon unit of the fatty acid. The remaining two carbon units used in the synthesis of fatty acids are supplied by malonyl-CoA. The pathway repeats seven times, with successive addition of two carbon units, resulting in a 16 carbon (16:0, palmitate) product. The product of hepatic *de novo* fatty acid synthesis in mammals is predominantly 16:0, with minor amounts of stearate (18:0) formed. The synthesized fatty acids can be metabolized to other fatty acids through chain elongation and desaturation primarily in the liver (Section 1.1.3) or brain (Section 1.4.3.2.3), be oxidized for energy (Section 1.1.4), or esterified (Section 1.1.5) to a glycerol backbone for the formation of TG or PL (Jeffcoat 1979). Arachidonic acid, eicosapentaenoic acid (20:5n-3) and dihomo-γ-linolenic acid (20:3n-6) can be converted to eicosanoids which include prostaglandins, thromboxanes, leukotrienes and lipoxins. The eicosanoids have a wide range of physiological and pharmacological effects that include vasoconstriction, platelet aggregation, or conversely inhibition of platelet aggregation and may have a role in inflammatory disorders such as asthma.

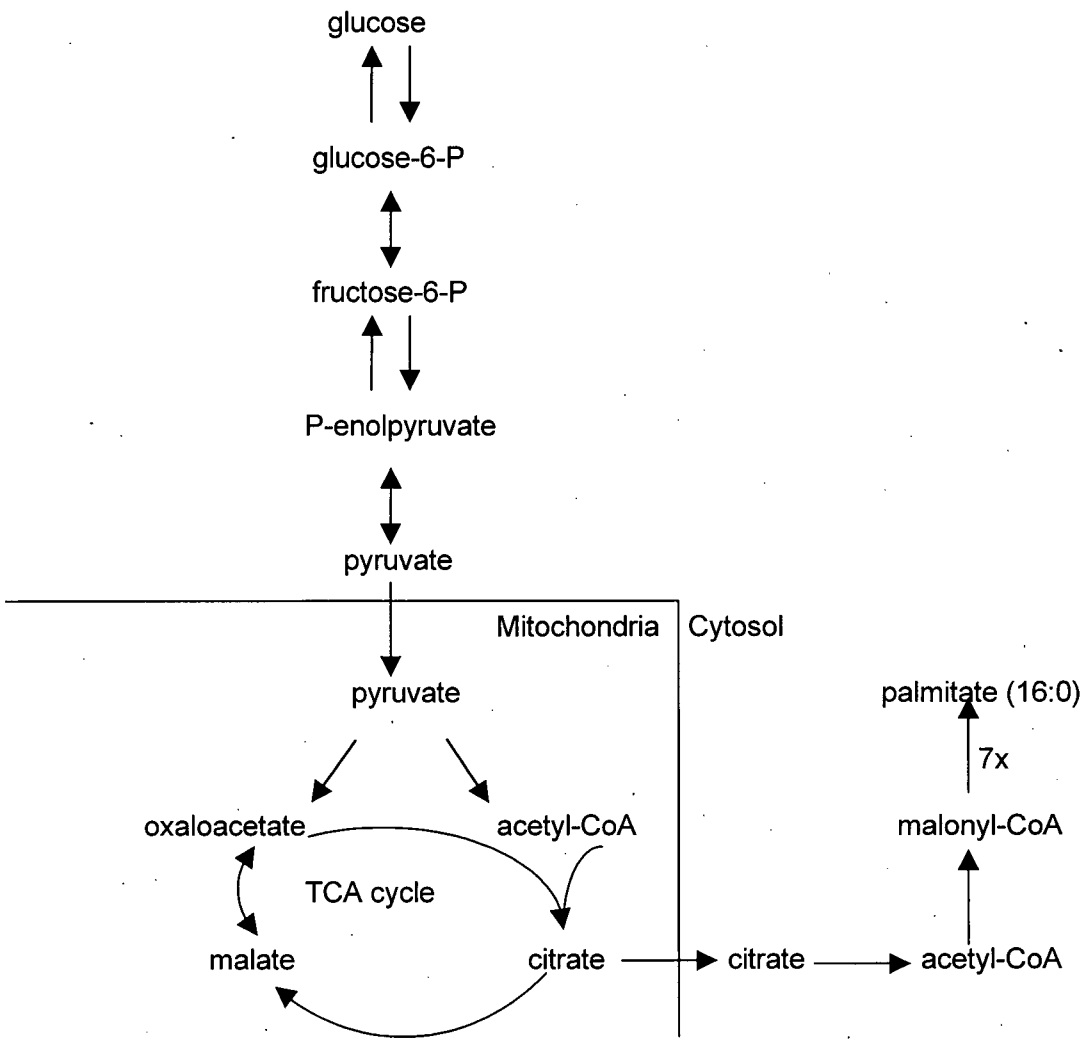


Figure 1.3 - Pathway of *de novo* fatty acid synthesis from glucose.

1.1.3. Desaturation and elongation

The desaturation and chain elongation of fatty acids was reviewed by Cook (1991). Desaturation involves the removal of two hydrogen atoms from the fatty acid carbon chain, whereas elongation is the addition of two carbon units. Mammals lack the Δ 12 and Δ 15 desaturase enzymes and consequently are incapable of inserting an unsaturated bond beyond carbon number 12 and 15, respectively, from the carboxy terminal of a fatty acid carbon chain. These fatty acids have critical roles in normal cell metabolism. Thus, the fatty acids linoleic (18:2n -

6) and linolenic acid (18:3n-3) are considered essential in the diet (Burr & Burr 1929, Burr & Burr1930, Gaull 1982). Common oil sources of these fatty acids are listed in the Appendix (Section 6, Table 6.1). As an additional point of interest, recent *in vitro* (Luthria et al 1997) and *in vivo* (Cunnane et al 1995) research has determined in rats that 18:2n-6 can be synthesized from 14:2n-6 or 16:2n-6 and 18:3n-3 can be formed from 16:3n-3. It is not known what contribution these pathways make to the tissue levels of 18:3n-3 and 18:2n-6, but it is likely minimal, given the limited availability in the diet of 14:2n-6, 16:2n-6 and 16:3n-3.

Key mammalian fatty acid desaturation enzymes are the Δ9, Δ6 and Δ5 desaturases. The Δ symbol indicates the carbon position that the enzyme introduces the first double bond relative to the COOH group. The Δ9 desaturase is the predominant desaturation enzyme for long chain saturated fatty acids and is associated with the endoplasmic reticulum. The saturated fatty acids myristic acid (14:0), 16:0 and 18:0 are all good substrates for the Δ9 desaturase, resulting in the formation of the monounsaturated fatty acids 9-tetradecanoic acid (14:1n-9), 9-hexadecaenoic acid (16:1n-9) and oleic acid (18:1n-9). The fatty acids 18:3n-3, 18:2n-6 and 18:1 compete as substrates for the Δ6 desaturase, with 18:3n-3 being the preferred substrate, followed by 18:2n-6 and then 18:1n-9 (Cook et al 1983).

There are two systems for fatty acid chain elongation, one in the endoplasmic reticulum and the other in the mitochondria. The endoplasmic reticulum system uses 2 carbon units donated by malonyl-CoA and saturated fatty acids from capric acid (C10:0) and longer. The endoplasmic reticulum system appears to be the primary metabolic source of saturated fatty acids and LCPUFA longer than 16 carbons during growth and maturation. The final elongation step in the synthesis of 22:6n-3 (**Figure 1.4**) has recently been determined to be a regulatory step, similar to that seen with Δ6-desaturase enzyme (Luthria et al 1997). Elongation in the mitochondria is less active than the

elongation system in the endoplasmic reticulum and uses acetyl-CoA as the two carbon donor instead of malonyl-CoA as is the case in the endoplasmic reticulum (Seubert & Podack 1973). The mitochondrial system also differs from the endoplasmic reticulum system in that saturated and monounsaturated fatty acids are more readily elongated by mitochondria than LCPUFA.

The desaturation and elongation of the essential fatty acids, 18:2n-6 and 18:3n-3 results in the formation of 20 and 22 carbon fatty acids, including 20:4n-6, 20:5n-3 and 22:6n-3 (Figure 1.4). Recent studies have shown that the synthesis of 20:4n-6 and 22:6n-3 requires not only desaturation and elongation of 18:2n-6 and 18:3n-3, respectively, but also chain shortening through oxidation (Voss et al 1991, Mohammed et al 1995, Sprecher et al 1995, Moore et al 1995, Sauerwald et al 1997). A similar pathway for the synthesis of 22:6n-3 has also been confirmed in the retinal pigment epithelium (Wang et al 1993). The continued oxidation of 22:6n-3 in the peroxisomes seems to be prevented as a result of low reductase activity, allowing 22:6n-3 to be transported out of the peroxisomes into microsomes for esterification into PL (Baykousheva et al 1995, Luthria et al 1996). The continued oxidation of 20:4n-6 in the peroxisomes results in the conversion of oxidation by-products to 18:2n-6 (Luthria et al 1997a). The quantitative significance of this pathway is not known.

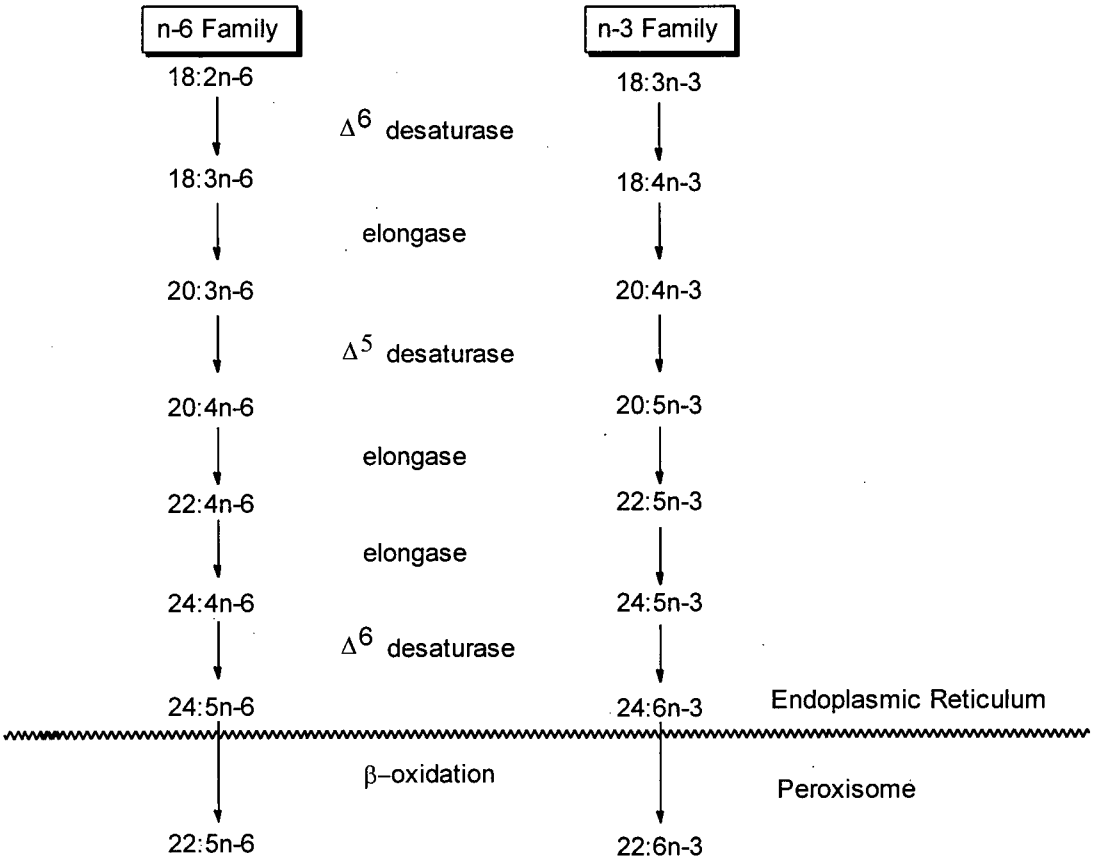


Figure 1.4 - Schematic representation of the pathway of desaturation and elongation of 18:2n-6 and 18:3n-3 for the synthesis of longer chain polyunsaturated fatty acids.

1.1.4. Oxidation

During the fasting state, the oxidation of fatty acids provides a significant source of energy. There are various pathways of oxidation including α,β and ω oxidation. Whereas β-oxidation occurs mainly in mitochondria and peroxisomes (cell organelles), α and ω-oxidation occur in the endoplasmic reticulum. Under normal conditions, α and ω-pathways of fatty acid oxidation are considered to make insignificant contributions to the oxidation of fatty acids, whereas β-oxidation is considered the most important pathway for fatty acid oxidation (Schulz 1991).

1.1.4.1. Mitochondrial ß-oxidation

Fatty acids are oxidized for energy in the mitochondria by the ß-oxidation pathway (reviewed by Bennett 1994). There is also evidence that, in the fed state, 18:3n-3 is more readily oxidized than 18:2n-6 (Clouet 1989, Mantzioris et al 1995) and that 18:2n-6 and 18:3n-3 are more readily oxidized than 20:4n-6 and 22:6n-3 (Leyton 1987). In order for long chain fatty acids to undergo ß-oxidation in the mitochondria, they must first cross the mitochondrial membrane using a carnitine-dependent transport system. Short (<C8) and medium-chain (C8-C12) fatty acids are not dependent on the carnitine shuttle system to cross the mitochondrial membrane. Once in the mitochondria, ß-oxidation of fatty acids involves cyclical removal of two carbon units, starting at the COOH end and eventually resulting in the generation of acetyl-CoA. Energy is conserved through the linking of the oxidative process with an electron transport chain. Very-long-chain fatty acids (>C20) are not ß-oxidized in the mitochondria due to the lack of the activating enzyme long-chain acyl CoA synthetase. Instead, these fatty acids are oxidized in the peroxisomes (Section 1.1.4.2). Under conditions such as starvation or diabetes, ß-oxidation of fatty acids results in rapid production of acetyl-CoA that condenses to form the ketone bodies acetoacetate and 3-hydroxybutyrate. These ketones can be used as a source of energy in skeletal muscle and brain (Webber 1977), but not kidney or red blood cell. Although the majority of ß-oxidation occurs in the

liver, enzymes of ß-oxidation have also been detected in the developing rat cortex (Reichmann et al 1988). The significance of this has not been fully determined given that the brain relies primarily on carbohydrates as a source of energy.

1.1.4.2. Peroxisomal ß-oxidation

Peroxisomal ß-oxidation has been reviewed by Reddy (1994). Enzymes present in the peroxisomes, but not the mitochondria, such as the long-chain acyl-CoA synthetase enzyme, function to chain-shorten the very-long chain (>C20) fatty acids through oxidative processes. For example, the final stages in the synthesis of 20:4n-6 and 22:6n-3 have recently been determined to involve the peroxisomal ß-oxidation of 22:4n-6 and 24:6n-3, respectively (Sprecher et al 1995). Although peroxisomes are capable of metabolizing the medium- (C8-C12) and long-chain (C14-C20) fatty acids, their oxidation occurs more readily in the mitochondria. The short-chain (<C8) fatty acids are not ß-oxidized in the peroxisomes. The oxidation of the very-long-chain fatty acids does not go to completion in the peroxisome. Rather, after these fatty acids have been chain shortened in the peroxisomes, they are transported either to the mitochondria for further oxidation, or to the ER for esterification into PL (Schulz 1991). The metabolic steps resulting in the production of 20:4n-6 and 22:6n-3 from 24-carbon precursors are classified as anabolic metabolism, whereas ß-oxidation is traditionally considered to be catabolic. Recent work by Luthria et al (1997a) confirmed and extended earlier findings (Sprecher 1968) that 20:4n-6 could be ß-oxidized to 14:2n-6, resulting in subsequent resynthesis to 18:2n-6. It is not known how significant this pathway is, given that 20:4n-6 is preferentially esterified into PL, rather than being further ß-oxidized (Lands et al 1982).

In contrast to the mitochondria, carnitine is not required for the transportation of LCPUFA across the peroxisomal membrane. Furthermore, as peroxisomal ß-oxidation is not coupled to an energy conserving electron-transfer-chain, it is considered only half as efficient in conserving

energy as mitochondrial ß-oxidation. The energy generated from peroxisomal ß-oxidation is lost as heat, making this metabolic route important in thermogenesis.

1.1.4.3. Omega Oxidation

Omega (ω) oxidation is most commonly seen in situations where medium-chain (C8-C14) fatty acids have been consumed, such as the preterm infant receiving a preterm formula product with medium chain fatty acids. The ω-oxidation process occurs in liver microsomes and involves removal of the fatty acid's methyl carbon. The products are dicarboxylic acids that cannot be further metabolized and are excreted in urine.

1.1.4.4. Alpha Oxidation

Alpha (α) oxidation involves the removal of single carbon units from the COOH end of the fatty acid chain. In mammals, α-oxidation occurs primarily in the brain mitochondrial and microsomal fractions and involves very-long-chain fatty acids. One product of α-oxidation of fatty acids in the brain is sphingolipids.

1.1.5. Esterification

Fatty acids can be esterified to a glycerol molecule and form TG for storage in adipose tissue, or PL for cellular structural components. Triacylglycerols can be synthesized via *de novo* synthesis with the phosphatidic pathway or via the monoacylglycerol pathway. The phosphatidic acid pathway, also known as the *sn*-glycero-3-phosphate pathway, is associated with the rough endoplasmic reticulum (reviewed by Lehner 1996). In the intestine, this pathway predominates when monoacylglycerols are not available, such as in the fasting state. The rate-limiting step of *de novo* TG synthesis occurs with the initial esterification of glycerol-3-phosphate with a saturated fatty acyl-CoA, catalyzed by glycerol-3-phosphate acyltransferase, resulting in 1-acylglycerol-3-phosphate (**Figure 1.5**). The addition of a second unsaturated fatty acyl-CoA results in the

formation of 1,2-diacylglycerol phosphate. For the completed synthesis of a TG, a third activated acyl group is esterified to the 1,2-diacylglycerol. Overall, the phosphatidic acid pathway results in a TG with an unsaturated fatty acid in the *sn*-2 position.

The monoacylglycerol (MG) pathway was first identified in the enterocytes of the small intestine (Breckenridge & Kuksis 1975, Tso & Fujimoto 1991), but has since been identified in the liver as well (reviewed by Lehner 1996). This pathway is found in the smooth endoplasmic reticulum and predominates in the intestine in the fed state. The intestinal monoacylglycerol pathway is discussed in more detail in Section 1.4.2. The first step in the MG pathway is catalyzed by the monoacylglycerol acyltransferase (MGAT) enzyme. In the liver MGAT reesterifies the 2-MG to a 1,2-diacylglycerol (Coleman & Haynes 1984, Coleman et al 1986). The hepatic MGAT appears to show preference for re-esterification of 2-monolinolenylglycerol and 2-monolinolenoylglycerol, suggesting that this enzyme may have a role in the conservation of 18:2n-6 and 18:3n-3 (Xia et al 1993), allowing for other MG species to be oxidized.

Phospholipids are formed following the same initial biochemical steps in the phosphatidic acid pathway as for *de novo* synthesis of TG. However, the pathway changes when 1,2-diacylglycerol phosphate and cytidine diphosphate choline (CDP-choline), form phosphatidylcholine through the action of phosphocholine diacylglycerol phosphate transferase. Other PL classes include phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and cardiolipin.

Higher proportions of LCPUFA are found in the PL than in the TG or CE, probably due to specificity of the acyltransferases (Iritani et al 1984). Most PL have a saturated fatty acid in the *sn*-1 position but an unsaturated fatty acid in the *sn*-2 position, due to the action of 1-acylglycerol 3-phosphate acyltransferase that esterifies LCPUFA into the two position of PL (Lands et al 1982).

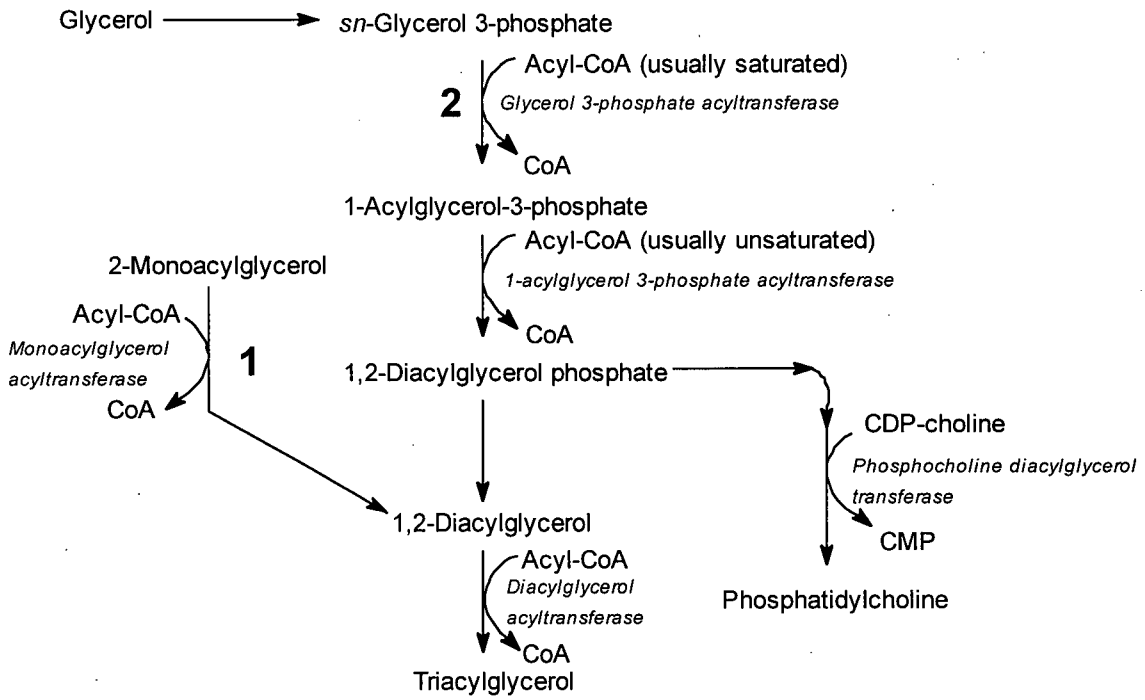


Figure 1.5 - Biosynthesis of triacylglycerol and phospholipid. 1 Monoacylglycerol pathway; 2 Phosphatidic acid pathway. Adapted from Mayes 1996.

1.2. Development of the Human Central Nervous System

The central nervous system includes both the brain and the visual system. The growth and development of the human CNS begins shortly after conception and continues for a few years after birth. During the prenatal period, brain weight increases to about 350 g by term gestation (Blinkov & Glezer 1968). After birth, the brain continues to grow, reaching approximately 1300 g at 20 yr of age, although the majority of the postnatal growth occurs during the first three or four yr. Not all animals have the same degree of CNS maturity, defined by the degree of myelination, at birth as the human infant. For example, in rats and mice myelination and accumulation of 22:6n-3 occurs primarily after birth (Crawford et al 1973), whereas in humans and pigs myelination (Dobbing & Sands 1979) occurs approximately equally in the prenatal and postnatal periods. Accumulation of 22:6n-3 is quantitatively most significant after birth (Martinez 1992). Within a species, the different regions of the brain do not all mature at the same time period, but rather maturation of some regions occurs predominantly prenatally and for other regions predominantly postnatally (Rodier 1980). Thus, research on CNS development in animals must consider the species used and their stage of CNS development at the time of the experiment before extrapolating results to infants.

1.2.1. Brain development

The development of the nervous system can be divided into six phases: 1) neurogenesis, 2) the migration of neurons, 3) aggregation of neuronal bodies, 4) differentiation of neurons into axons and dendrites, 5) synaptogenesis, and 6) pruning back of connections and cells through selective cell death (Spreen et al 1995, Cowan 1979). The first CNS structure to appear is the neural tube, which in the human infant is visible one mo after conception. Neural cells proliferate at a rate of 250,000 new cells per min (Cowan 1979). As the neural cells are generated, they migrate in waves on a preordained route towards what will become the cerebral cortex, with brain layers developing from the inside-out (Dodd & Jessell 1988). By the sixth mo of gestation, neurogenesis

and migration within the cortex is largely complete, with small amounts occurring until birth (Rabinowicz 1986). After the neuronal cells have migrated, synaptogenesis begins with the generation of axons and dendrites that branch and become myelinated. The cortical areas are the last to myelinate and may be influenced the most by experience from the outside world. At the time of birth, the growth spurt of the spinal column, brain stem and large parts of the forebrain are almost finished (reviewed by Casaer 1993).

After birth, the brain continues to grow, increasing in size from an average weight of 350 g at birth to 1000 g by the end of the first postnatal yr. The cerebellum has its maximum growth spurt starting around the time of birth and continuing for the first postnatal yr (Casaer 1993). Glial cells continue to divide and multiply during the first six mo of postnatal life. The visual system begins to myelinate about the 40^{th} gestational wk and is complete a few mo after birth (see Section 1.2.3). By the end of the infant's second yr, myelination of the cerebrum is largely complete (reviewed by Spreen 1995). The brain continues to grow and myelinate at a much slower rate for 12 or 15 yr, reaching a final brain weight of approximately 1275 g and 1400 g in the female and male, respectively (Rabinowicz 1986).

1.2.2. Cognitive assessment

Despite careful anatomic observations of myelination, correlation of the degree of myelination with developmental behavioral data in normal infants and animals has not been possible. Early attempts at assessment of cognitive development and function in children focused on quantifying and predicting intelligence, but these efforts were impeded by a lack of understanding of the concept "intelligence". Charles Spearman (1923) proposed that every intellectual function could be reduced to a single factor "g", which he believed was a measure of abstract reasoning ability (Lewis et al 1986). Others, however, postulated that intelligence is a multifaceted paradigm consisting of factors such as language ability, memory, reasoning, creativity,

ability to adapt and mental speed (reviewed in Berk 1997). Despite lacking a clear understanding of what "intelligence" was, the first test of cognitive function for children was developed in 1905 by Alfred Binet who developed a screening tool to objectively assess the abilities of public school students (Sattler 1992). The Binet test focused on functions such as memory, judgement and abstraction and was the first test to have age-defined norms. In 1916, the Binet test was standardized by researchers at Stanford University, resulting in a new test called the Stanford-Binet test. From this test, the child's "mental quotient" was calculated by dividing mental age by chronological age. The mental quotient was later renamed the intelligence quotient (IQ).

Today, despite continuing controversy about the concept of what intelligence is, efforts to quantify and predict infant intelligence continue. This practice, when used for reasons other than diagnostic purposes, is controversial. Arguments against the use of "intelligence" tests include their lack of predictive ability from infancy to childhood, a lack of understanding of what the test is measuring, the lack of control for the influence of socioeconomic status, the lack of association between IQ and life-success and the fact that summing cognitive abilities into an IQ score masks individual variability (Barrett & Depinet 1991, Neisworth & Bagnato 1992). There is also concern that classifying an infant's ability by IQ tests could become a "self-fulfilling prophecy".

Currently, many behavioral techniques are employed to evaluate CNS development in the infant and child. As with tests of intelligence, developmental tests attempt to assess the infant's current level of functioning, but developmental tests differ from intelligence tests in that they attempt to inventory the developmental milestones exhibited at different ages (Bayley 1993). Thus, a developmental test will categorize an assessed ability as existing or not existing, while intelligence tests assess varying degrees of ability. Standardized assessments of infant development attempt to derive a score that can be compared with a pre-defined age-appropriate norm allowing an infant's performance to be compared with the performance of other infants on the

same measure. To this end, the majority of the standardized developmental tests are scaled such that the mean of any age group is 100, with a standard deviation of 15. Thus, two-thirds of the population will score between 85 and 115. The intelligence quotient (IQ), therefore, is an assessment of relative standing within a population, not of absolute achievement. Examples of psychological assessments include the Weschler Intelligence Scale for Children and the Stanford-Binet, while tests of development include the Gesell Developmental Schedules and the Bayley Scales of Infant Development (BSID). The BSID, first developed in 1933 by Nancy Bayley, is the most commonly used tool for the assessment of infants in both clinical and research settings (Gilbride & Regalado 1996). The BSID is composed of items grouped into motor items or mental items. Raw scores that are calculated based on the number of passed and failed items are converted into a Mental Development Index (MDI) and a Psychomotor Development Index (PDI). These indexes provide normalized standard scores derived from a national stratified sample of normal infants (Birk 1997, Bornstein & Lamb 1992). The scores achieved on the BSID tend to be stable over different ages, for the same child, with correlations r= 0.5 (Wilson 1983). However, this means that half of the variance in performance on the MDI and PDI is likely attributable to external factors.

A concern of standardized infant developmental tests is that they seem to have little ability to predict intelligence in childhood (Rose et al 1986). This lack of association may be explained by the reliance of infant tests on measures of motor and perceptual skill, which are different from the intellectual functions commonly assessed by childhood tests such as verbal ability. Tests of habituation and dishabituation to visual stimuli with infants considered at-risk (eg. preterm, small for gestational age) seem to have better abilities to predict performance on later tests of cognitive function. Correlations between scores achieved in tests of habituation-dishabituation and performance in childhood on age-appropriate test of cognitive function are in the range of r=0.3 to

0.6 (McCall & Lamb 1993, Rose & Feldman 1995). The rationale behind the habituation-dishabituation procedure is that as a stimulus is repeatedly presented to an infant, the infant's attention to the stimulus declines. It is thought that the decline in response to the stimulus reflects the acquisition of an internal representation of the stimulus. One example of a more commonly used test of habituation/dishabituation is the Fagan Test of Infant Intelligence (Fagan & Detterman 1992).

In habituation-dishabituation test procedures, there are two methods for presenting a stimulus to the infant. The first is called the fixed-trial procedure where a stimulus is provided to the infant for a fixed interval and these intervals are repeated for a fixed number of trials (Millar & Weir 1995). The trials continue regardless of the infant's attention to the stimulus. The advantage of this method is that sessions can be conducted quickly, they are uniform in length and there is the same number of data entries for each infant. One disadvantage is that the procedure is less sensitive to individual differences in looking because not all infants actually habituate to the stimulus during the fixed time (Millar & Weir 1995).

The second and preferred habituation method is known as the infant-control procedure (Millar & Weir 1995). It adapts the number and length of stimulus exposures to the infant's own behavior toward the stimulus. The stimulus is presented in a discrete number of trials until the infant's attention declines, which is a sign that the infant has habituated. Commonly, the criterion for habituation is a looking period that is 50% of the length of the proceeding look. The Fagan test is composed of parts that utilize both the fixed-trial and the infant-control procedure.

It is not known if the habituation-dishabituation procedure is a measure of infant memory (Colombo 1993) or the ability to disengage attention (McCall 1994). The correlations between infant habituation-dishabituation measures and memory in later childhood are much smaller than

the correlations to measures of inhibition of attention (Sigman et al 1991). Tests of habituation-dishabituation are generally not standardized and although they do have moderately higher correlations with performance in later IQ tests, it is only a small increase. As a result, and given that it is not clear what these tests are measuring, it is important not to overinterpret the results obtained.

1.2.3. Visual system development and structures

The development of the visual system has been reviewed by Hickey & Peduzzi (1987). The eye begins to develop in the human embryo about the 22nd d after conception. It is formed from forebrain invagination and from the ectoderm and the mesoderm. The ectoderm forms the lens and retina. The lens separates from the ectoderm at the 5th wk of gestation and by the 8th wk is more similar in structure to the adult lens. The cornea develops from the ectoderm and the neural crest cells. The optic nerve begins development at the 6th week, with myelination beginning at the 5th mo of gestation. Myelination continues during the postnatal period reaching adult levels when the child is about two yr old. In the area of the retina, the macula, containing the fovea, develops at a slower rate than the retina, finishing it's development in humans four mo after birth (Hickey & Peduzzi 1987). Non-human primates, often used in experiments on dietary fatty acids and visual development, have a postnatal visual development that occurs at a rate four times as fast as in human infants (Boothe et al 1985). Thus, infant acuity at four mo is equivalent to non-human primate acuity at about one mo.

The improvement in visual acuity during the first few months of life is primarily attributed to retinal development (reviewed by Odom 1984). When looking at an object, the image of the object is projected in the eye and falls on the fovea, an area within the retina that contains only cones (reviewed by Carlson 1998). The foveal area of the retina is responsible for visual acuity, but has decreased sensitivity in low light situations. The extrafoveal areas of the retina, which contain the

rods, compensate for this limitation by providing high sensitivity in low light situations. The foveal area does not complete its development until the 4th mo after birth, suggesting that neonatal visual acuity prior to four mo of age may be mainly due to the extrafoveal areas of the retina (Abramov et al 1982). Overall, the most rapid improvement in visual acuity is during the first 6-8 mo after birth, with slower improvements up to age 12 yr (Hamer & Mayer 1994).

The retina consists of three main layers that are, in order, the photoreceptor (rods and cones), bipolar and ganglion layers. When light falls on the retina, a conformational change occurs in the retinal pigment rhodopsin, resulting in the photoreceptors becoming hyperpolarized and sending a signal to the horizontal and bipolar cells. Bipolar cells give a direct pathway through the retina while horizontal cells provide a more lateral pathway across the retina. Synaptic contact between bipolar cells and ganglion cells results in continuing transfer of the visual signal. The axons and ganglion cells join and exit the retina at the optic nerve. The signal continues along the optic nerve to the lateral geniculate nucleus and primary visual cortex.

In summary, the development of the visual system of the human infant is rapid during the first 6-8 months and fully mature at about 12 years of age. Visual perception is a complicated process that involves many layers of tissue and structures. Alterations in visual ability could be due to an effect at any anatomical level from the eye to the cerebral structures.

1.2.4. Assessment of visual acuity

Assessing visual ability in the infant can be a challenge as infants are unable to communicate what they see as an adult can. It is known that infants demonstrate visual preference for some objects (Fantz 1961), indicating that there are elements of perception and differentiation shown by the infant. Infants are able to follow a horizontally or vertically moving object between 50

and 55 d of age and are able to follow an object moving in a circle at 2.5 mo. Perception of form seems to be apparent by two or three mo of age (Fantz 1961). Preference for colour over gray is seen by four mo (reviewed in Adams 1997) and by six mo the infant can discriminate between colours.

For many years, infant vision was assessed using behavioural methods that involved determining whether the infant could fixate and follow a light or an object (reviewed by McDonald 1986). However, these methods did not provide a quantifiable measure that could be compared with a standard. In the 1960s, standardized methods were developed that were able to give a quantifiable measure of visual acuity. Visual acuity is defined as the ability to visually discriminate detail and can be further defined as either recognition or resolution acuity. Recognition acuity is the ability to distinguish the detail of a stimulus from other stimuli such as the letter charts used with the Snellen eye test. Resolution acuity is the ability to discriminate individual elements in a repetitive pattern such as a black and white grating (stripe) pattern. Methods for assessing resolution acuity are most often used in the research setting. The major reason for assessing resolution acuity, rather than one of the many other visual functions such as colour detection or contrast sensitivity function, is predominantly that tests of resolution acuity are relatively easy and accessible to the non-ophthalmologically trained researcher. Resolution acuity is also known as grating acuity, referring to the black and white grating pattern used in the test.

Three tests used to assess resolution acuity in preverbal infants include optokinetic nystagmus, preferential looking acuity and visual evoked potential (McDonald 1986). In the optokinetic nystagmus test, a rotating grating pattern is presented to the infant. The infant's eye reacts to the stimulus as a saccade (eye jerk). If the grating pattern is not seen by the infant, their eyes do not saccade. While this test is easy to administer, it lacks objectivity. The preferential looking technique is a behavioural method for studying sensory and perceptual aspects of vision in

infants (reviewed by Dobson 1994). Acuity as measured by preferential looking techniques primarily assesses the function of the foveal area of the retina; thus, it is questionable how accurate results are using this measure until the fovea matures at four mo. The preferential looking procedure is based on the idea that when infants are given the choice, they prefer to look at patterned stimuli rather than unpatterned stimuli. The forced-choice preferential looking method involves presenting the infant with stimuli either to the left or right of a central peephole. The observer, blinded to the position of the gratings, notes if the infant looks to the left or right. The infant's acuity limit is the point where the observer was able to guess the location of the gratings correctly (based on the infant's looking behavior) with an accuracy of 75%. Early forced-choice preferential looking procedures involved 60 to 100 looking trials, which was not practical for infants. The Teller Acuity Card procedure was developed as a more appropriate test for infants and takes only three to five minutes to complete (McDonald et al 1985).

Another common method for assessing acuity of infants is the visual evoked potential (VEP), which involves placing electrodes on an infant's head and presenting visual stimuli, such as an alternating black and white checkerboard pattern on a television monitor. The stimuli are detected by the retina and the response transmitted down the optic nerve to the primary visual cortex, making VEP specifically a measure of retinal function. Infant acuity measured by VEP is known to consistently be higher than acuity measured by preferential looking methods (reviewed in Dobson 1994). Possible reasons for this include: 1) VEP uses a dynamic stimulus while preferential looking uses a static stimulus; 2) the retinal location for the preferential looking stimulus is peripheral while VEP is a test of central retinal function; 3) VEP is a measure of sensory development, while preferential looking is a measure of behavioral response based on cognitive and motor functions; 4) an infant may not prefer the patterned stimulus of the preferential looking test, which says nothing of whether the infant can see the pattern (Salapatek 1975). Similar

differences in acuity have been reported for the Teller Acuity Card Procedure and VEP (Riddell et al 1997). However, the degree of difference in acuity measured by the VEP *versus* the Teller Acuity Card test decreases with increasing infant age, with differences stabilizing at 6 mo of age, around the time of foveal maturation. The VEP is usually done in the dark and may be assessing rod function, while the Teller Acuity Card test is done in full light and is assessing cone function. It is important that tests are only compared and contrasted in their ability to accurately assess function when they are assessing the same function. This does not appear to be the case for preferential looking methods, such as the Teller Acuity Card Procedure and VEP methods.

1.2.5. Role of fatty acids in CNS function

The central nervous system is second only to adipose tissue in containing the highest concentration of lipid by weight of any organ in the body. However, while adipose tissue is predominantly TG, CNS lipid is predominantly PL (Sastry 1985). Additionally, the fatty acid composition of the CNS is very different from PL in most other tissues.

1.2.5.1. Fatty acids and visual system structure and function

The n-6 and n-3 fatty acid composition of PL in the CNS is distinctly different from that found in other organs. High amounts of 22:6n-3 are found in the retina (Fliesler & Anderson 1983), especially in the phosphatidylethanolamine fraction, with levels of n-6 LCPUFA generally lower than n-3 LCPUFA (Craig-Schmidt et al 1996), except in cases of prolonged n-3 fatty acid deficiency where there is a significant increase in the n-6 LCPUFA (Anderson et al 1994). The fatty acid composition and metabolism of 22:6n-3 in rods has been extensively studied using the frog retina (Fliesler & Anderson 1983, Boesze-Battaglia & Albert 1989, Gordon & Bazan 1990, Wiegand et al 1991). The appropriateness of the frog as a model for the human eye may be questionable. The frog retina metabolizes 22:6n-3 primarily in the rods, with almost no 22:6n-3 in the cones, while the human retina metabolizes more 22:6n-3 in the cones than the rods (Rodriguez de Turco et al

1990). A comparison of fatty acids from the adult human retina determined that the relative percent of 22:6n-3 in the macular region (cones) was 15.9% total fatty acids *versus* 22.3% in the peripheral retina (rods) (van Kujik 1992). Furthermore, in the macular region, there were higher amounts of 16:0 (18.8%), 18:1 (18.0%) and 18:0 (17.2%) than 22:6n-3. Frogs also differ from humans in the distribution of 22:6n-3 in the retina, with frog outer segments having ~20% of all retinal 22:6n-3, while humans have ~6% of all retinal 22:6n-3 in the outer segments.

Although species differences may be important, information on the metabolism of 22:6n-3 in the frog retina is frequently used to describe 22:6n-3 metabolism in the human infant retina. The lipid composition of the nonprimate vertebrate (frog) photoreceptor rod outer segment is 80 to 90% PL and 8-10% cholesterol (Daemen 1973), which results in a highly fluid environment (Fliesler & Anderson 1983, Poo & Cone 1974). Frog and bovine photoreceptor PL have the highest level of 22:6n-3 of all tissue PL, making up to 50% of total fatty acids, in the disk membranes of the photoreceptor rod outer segments (Fliesler & Anderson 1983, Boesze-Battaglia & Albert 1989). The high amounts of 22:6n-3 in the rod outer segment may be necessary for normal functioning of the visual system. While most fatty acids in the disk membranes of the rod outer segment exchange freely among disks, 22:6n-3-rich PL is retained within the same disk (Gordon & Bazan 1990). Furthermore, disk membranes are shed on a daily basis, representing up to 10% of rod outer segment mass and phagocytosed by the retinal pigment epithelium which acts as a blood-eye barrier. It is difficult to decrease the levels of 22:6n-3 in the rat rod outer segment, even when the rat has no dietary source of n-3 fatty acids (Wiegand et al 1991). An explanation for this is that 22:6n-3 is retained by the retinal pigment epithelium and recycled back to new disk membranes, thus maintaining a constant source of 22:6n-3 for the rod outer segment (Stinson et al 1991). The retinal pigment epithelium can also provide a source of 22:6n-3 through the conversion and elongation of 18:3n-3 (Wang & Anderson 1993). The finding that the interphotoreceptor retinoid-

binding protein, which facilitates the transfer of retinol from the retinol pigment epithelium to the rod outer segment, has higher affinity for 22:6n-3 than retinol further supports the role of the retinol pigment epithelium as a source of 22:6n-3 (Chen et al 1993). Through both conservation and synthesis, it seems that the retinal pigment epithelium ensures there is a relatively constant supply of 22:6n-3 for the rod outer segment.

In addition to having the highest proportion of 22:6n-3 in tissue PL, the distribution of 22:6n-3 in the rod outer segment PL differs from that of PL in other tissues. Most tissue PL have 22:6n-3 predominantly in the *sn*-2 position, with a saturated or monounsaturated fatty acid in the *sn*-1 position. The frog rod outer segment PL, however, has approximately 25% 22:6n-3 in the *sn*-1 position (Wiegand & Anderson 1983, Wiegand et al 1991, Choe & Anderson 1990). The physiological significance of this is not known.

Animal work investigating the role of dietary n-3 and n-6 fatty acids and their influence on the fatty acid composition and function of the retina have primarily utilized nonhuman primates (rhesus monkey) and rats. Feeding an n-3 fatty acid-deficient diet, with < 0.3% of fatty acids as 18:3n-3, to non-human primates through gestation and into infancy has been shown to result in a progressive decrease in 22:6n-3 in the retina and reduced retinal function, as measured by rod and cone electroretinogram (a measure of electrical activity in the retina) and a decrease in preferential looking acuity (Neuringer et al 1984, Neuringer et al 1986). Similar biochemical findings of reduced 22:6n-3 in the retina have been confirmed in piglets (Hrboticky et al 1991) and rats fed an 18:3n-3-deficient diet (Bourre et al 1989, Bourre et al 1989a). The differences in visual function between n-3 fatty acid deficient and n-3 fatty acid adequate nonhuman primates were only apparent at 4mo. It should be noted that these studies utilized diets that were deficient in all n-3 fatty acids and were not designed to address the issue of the essentiality of dietary 22:6n-3 to the normal functioning of the visual system.

In contrast to the animal studies, infant studies should provide a diet that is adequate in 18:3n-3, as defined by the appropriate regulatory agencies. Thus, infant studies that attempt to determine the role of n-6 and n-3 fatty acids in CNS development should all have an adequate source of 18:3n-3 and 18:2n-6 in the diet. However, knowledge on the minimum, maximum and optimal levels of 18:2n-6 and 18:3n-3 for the human is incomplete. It is well known that infants fed formula without LCPUFA have lower plasma and erythrocyte PL levels of 22:6n-3 and 20:4n-6 than breast-fed infants (Innis 1992, Ponder et al 1992). The one study that has assessed the fatty acid composition of the retina (post-mortem) in breast-fed infants and infants fed formula reported that there was no significant differences in the levels of the retinal fatty acids between the breast-fed and formula-fed infants, despite the absence of 22:6n-3 in the formula (Makrides et al 1994). In order to assess the influence of dietary n-3 and n-6 fatty acids on the infant retina, functional tests of the visual system are used. Some of the early work on n-3 and n-6 fatty acids and retinal function involved very-low-birth-weight neonates who were breast-fed with pooled preterm human milk or randomized to one of three preterm formulae with 1) 24% 18:2n-6 + 0.5% 18:3n-3, 2) 21% 18:2n-6+2.7% 18:3n-3; 3) 20% 18:2n-6+1.4% 18:3n-3 + 0.65% 20:5n-3 + 0.35% 22:6n-3 (Uauy et al 1990). Of note, the breast-fed infants received an average of 75% of their intake as human milk and the other 25% was provided as formula # three with 20:5n-3 and 22:6n-3. Electroretinograms, to assess rod and cone function, were performed at 36 wk postconception, with no difference being found for cone function but with significant differences in rod function due to diet. The infants fed the formula with 0.5% 18:3n-3 but not the infants fed the formula with 2.7% 18:3n-3, and no LCPUFA had rod electroretinogram responses that were significantly different from the infants fed the LCPUFA-supplemented formula and the breast-fed infants. These differences disappeared by 57 wk post-conception (equivalent to 17 wk post-birth), when retinal development is nearly complete. There was no significant difference in cone function at either 36 or 57 wk postconception

(Birch et al 1992). Many studies on the influence of dietary n-6 and n-3 fatty acids on visual acuity with the term infants have been reported. A summary of these studies is in **Table 1.1**.

Table 1.1 *– Summary of term infant studies done at a single site assessing visual acuity of formula-fed and breast-fed infants.*

Reference	Subjects	Diets	Methods	Results	Comments
Auestad et al (1997)	197 term infants from three sites: 1.Kansas city-75 2.Portland-79 3.Seattle-43	Human milk: (n=63) Avg of one site 20:4n-6 0.48±0.1 22:6n-3 0.15±0.09 Control formula: (n=45) % total fatty acids 18:2n-6 21.9 18:3n-3 2.2 18:2/18:3 ratio 10:1 20:4+22:6: (n=46) 18:2n-6 21.7 18:3n-3 1.9 20:4n-6 0.43 22:6n-3 0.12 18:2/18:3 ratio 8.8:1 22:6n-3: (n=43) 18:2n-6 20.7 18:3n-3 1.9 20:5n-3 0.07 22:6n-3 0.23 18:2/18:3 ratio 11.2:1	Prospective multicentre study. Formula-fed infants randomly assigned to one of three formulae plus a non-random human milk group. Visual acuity (Teller acuity cards and pattern sweep VEP) measured in 120 infants at one, two, four, six, 9 and 12 mo	No significant difference in visual acuity at any age assessed by either Teller Acuity Card or pattern sweep VEP.	Human milk group exclusively breast-fed to three mo. Source of LCPUFA egg PL (20:4n-6 +22:6n-3) and fish oil (22:6n-3 formula). Not all infants at all sites had both acuity tests done

Reference	Subjects	Diets	Methods	Results	Comments
Birch et al (1992)	49 term infants	Human milk: (n=35, 20Γ, 15E) % total fatty acids 18:2n-6 12.7 18:3n-3 0.8 18:2/18:3 ratio 15.9:1 Formula: (n=14, 9Γ, 5E) % total fatty acids 18:2n-6 29.4 18:3n-3 0.8 18:2/18:3 ratio 36:1	Prospective study FPL and sVEP acuity at 57 and 66 wk postconception (number of infants not reported).	At 57 wk , but not 66 wk, FPL and sVEP significantly different (level of significance not reported).	Infants exclusively breast-fed for first two mo No information on SES characteristics of infants
Birch et al (1993)	Sample 1: 30 term infant Sample 2: 43 term infants	Sample 1: Corn-oil formula Human milk Sample2: Corn-oil formula Human milk	Prospective study Visual acuity assessed Sample 1: 57wk FPL and sVEP Sample 2: 36mo -operant PL acuity -operant stereo acuity -color vision -picture naming	Sample 1: FPL and sVEP acuity higher in breast fed infants. Sample2: Operant PL not different between breast-fed and formula-fed infants. Operant PL stereo acuity and letter matching significantly better in breast-fed.	No control for confounding variables

Reference	Subjects	Diets	Methods	Results	Comments
Birch et al (1998)	108 term infants	Human milk:(n=29) % total fatty acids 18:2n-6 12.7 18:3n-3 0.8 20:4n-6 0.56 22:6n-3 0.29 18:2/18:3 ratio 15.9:1 Formula: (n=26) % total fatty acids 18:2n-6 14.6 18:3n-3 1.49 18:2/18:3 ratio 9.9:1 Formula + 22:6n-3: (n=26) 18:2n-6 15.1 18:3n-3 1.54 22:6n-3 0.35 18:2/18:3 ratio 9.7:1 Formula+20:4+22:6: (n=27) 18:2n-6 14.9 18:3n-3 1.54 20:4n-6 0.72 22:6n-3 0.36	Prospective study with infants either randomized to a formula group or in a self-selected breast-fed group. FPL and sVEP done at six, 17, 26 and 52 wk	Lower sVEP in formula group compared with formula+22:6 and formula+22:6+20:4 and breast-fed at six, 17, 52, but not 26 wk. No differences in acuity between formula+22:6 and formula+22:6+20:4 and breast-fed.	Breast-fed infants exclusively breast-fed for 17 wk. Human milk fatty acids is the average of 10 samples at six wk post-partum. Source of LCPUFA was single cell oils. Data graphed with no means±SD provided. Demographic characteristics provided, but no analysis for differences between groups. Potential confounders not controlled in analyses.

Reference	Subjects	Diets	Methods	Results	Comments
Carlson et al (1996)	94 term infants enrolled, 58 in study at four mo	Human milk: (n=19) % total fatty acids 18:2n-6 15.8 18:3n-3 0.8 20:4n-6 0.6 22:6n-3 0.1 18:2/18:3 ratio 19.8:1 Conventional formula: (n=20) % total fatty acids 18:2n-6 21.9 18:3n-3 2.2 18:2/18:3 ratio 10:1 Formula+22:6+20:4: (n=19) % total fatty acids 18:2n-6 21.8 18:3n-3 2.0 20:4n-6 0.43 22:6n-3 0.1 18:2/18:3 10.9:1	Prospective study Randomized double blind assignment to formulae, self-selected breast-fed group. Visual acuity (Teller Acuity Cards) at two, four, six and 12 mo.	No significant main effect of diet between any of the groups. Post-hoc analysis found breast-fed infants and infants fed formula with 22:6n-3 and 20:4n-6 had better visual acuity at two mo than infants fed conventional formula. No statistically significant differences in visual acuity found at four, six, 9 and 12 mo.	LCPUFA source is egg PL. Human milk fatty acids from previous unrelated study. Statistically significant differences in the ethnic and educational background of the diet groups. Differences not controlled in statistical analyses. Despite no significant main diet effect in statistical analyses, post-hoc analysis was done.

Reference	Subjects	Diets	Methods	Results	Comments
Courage et al (1998)	105 term infants recruited.	Conventional formula: (n=30) % total fatty acids 18:2n-6 30.5 18:3n-3 4.9 18:2/18:3 6.2:1 Evaporated milk (n=30) % total fatty acids 18:2n-6 2.3 18:3n-3 0.8 18:2/18:3 2.9:1 Human milk (n=30)	Prospective study Non-random diet group assignment. Infants exclusively fed formula or breastmilk to three mo. Visual acuity (Teller Acuity Cards) assessed at three (n=100) and six mo (n=90) for all infants. Visual acuity (Teller Acuity Cards) assessed at 18 mo for the breast-fed and evaporated milk fed groups.	Breast-fed infants had significantly higher visual acuity at three and six mo than evaporated milk fed infants, but were not different to the formula-fed infants. No significant differences in visual acuity at 18 mo between breast-fed and infants fed evaporated milk.	30 infants were needed, per group, to give sufficient power to detect significant difference. Socioeconomic status of parents assessed with the Blishen scale. Differences in SES between groups was controlled in the statistical analyses
Innis et al (1994)	35 term infants	Formula: (n=18) % total fatty acids 18:2n-6 17.9 18:3n-3 2.1 18:2/18:3 8.5:1 Human milk: (n=17) % total fatty acids 18:2n-6 13.4 18:3n-3 1.5 20:4n-6 0.5 22:6n-3 0.2 18:2/18:3 ratio 8.9:1	Prospective study Visual acuity (Teller Acuity Cards) assessed at 14d and 3mo	No statistically significant differences in visual acuity between the formula and breast-fed infants.	Breast-fed exclusively to three mo. Supplemented with vitamins ADC. 18 infants per group were required to detect a difference of 0.5 octaves in preferential looking acuity.

Reference	Subjects	Diets	Methods	Results	Comments
Innis et al (1996)	433 term infants	Subject groups: never breast-fed-34Γ, 34E breast-fed <1mo-21Γ, 19E breast-fed 1-3mo-24Γ, 26E breast-fed 4-6mo-57Γ,38E breast-fed 7-8mo-28Γ, 21E breast-fed >8mo-43Γ, 49E 38 infants fed both human milk and formula. Commercial formulae: % total fatty acids 18:2n-6 14.0-34.0 18:3n-3 1.5-4.7	Cross-sectional study Visual acuity (Teller acuity cards). Breast-feeding history assessed at 39±1wk of age. Duration of breast-feeding determined by questionnaire. Breast-feeding defined as intake of cow's milk or formula< 12 oz/wk.	No significant differences between infant groups fed for varying durations of breast-feeding in visual acuity.	Demographic variables noted but not considered in analysis. Participants highly educated.
Innis et al (1997)	238 term infants enrolled from seven centers. Center 1 (n=24) Centre 2 (n=39) Centre 3 (n=10) Centre 4 (n=43) Centre 5 (n=54) Centre 6 (n=33) Centre 7 (n=35) 191 infants completed.	Human milk (90 d): (n=75) %total fatty acids 18:2n-6 14.6 18:3n-3 1.2 20:4n-6 0.5 22:6n-3 0.2 18:2/18:3 ratio 12.2:1 Formula 1: (n=59) % total fatty acids 18:2n-6 18.0 18:3n-3 1.9 18:2/18:3 ratio 9.5:1 Formula 2: (n=57) % total fatty acids 18:2n-6 34.2 18:3n-3 4.7 18:2/18:3 ratio 7.3:1	Multicentre, prospective study. Formula groups were randomly assigned to study formulae. Visual acuity (Teller acuity card test) at 14 d and 90 d.	At 90 d, no significant difference in visual acuity between the formula-fed and breast-fed infants.	Breast-fed infants received no formula for the first 30 d and could have no more than six oz of formula daily. Study had sufficient power to detect a difference of 0.5 octaves with an α=0.05.

Reference	Subjects	Diets	Methods	Results	Comments
Jensen et al (1997)	99 term infants	Human milk: (n=19, 8Γ, 11E) -not analyzed Formula 1: (n=17; 10Γ, 7E) % total fatty acids 18:2n-6 17.6 18:3n-3 0.4 18:2/18:3 ratio 44:1 Formula 2: (n=17; 10Γ, 7E) % total fatty acids 18:2n-6 17.3 18:3n-3 0.95 18:2/18:3 ratio 18.2:1 Formula 3: (n=16; 6Γ, 10E) % total fatty acids 18:2n-6 16.5 18:3n-3 1.7 18:2/18:3 ratio 9.7:1 Formula 4: (n=13; 6Γ, 7E) % total fatty acids 18:2n-6 15.6 18:3n-3 3.2 18:2/18:3 ratio 4.8:1	Transient VEP at 120 and 240 d of age.	No significant differences in transient VEP latency or amplitude between formula and breast-fed groups at 120 and 240 d of age	Infants exclusively breast-fed or fed formula for 120 d. Ethnic background of breast-fed group significantly different from formula-fed group. No socioeconomic data given.

Reference	Subjects	Diets	Methods	Results	Comments
Jorgensen et al (1996)	33 term infants	Human milk at 2mo: (n=17) % total fatty acids 18:2n-6 10.97 18:3n-3 1.39 20:4n-6 0.47 22:6n-3 0.43 18:2/18:3 ratio 7.9:1 Formula: (n=16) % total fatty acids 18:2n-6 14.4 18:3n-3 1.7 18:2/18:3 ratio 8.5:1	Prospective study Visual acuity (Teller acuity Cards) assessed at one, two and four mo.	Significant increase in visual acuity with age in all infants. Greater increase in acuity with breast-fed infants between two and four mo of age.	14 of 17 infants had visual acuity assessed. Environmental or genetic confounds not controlled for in analysis.

Reference	Subjects	Diets	Methods	Results	Notes
Jorgensen et al (1998)	54 term infants	Human milk: (n=25) % total fatty acids 18:2n-6 10.85 18:3n-3 1.05 20:4n-6 0.40 22:6n-3 0.38 18:2/18:3 ratio 10.3:1 Formula1: (n=14) % total fatty acids 18:2n-6 12.67 18:3n-3 0.54 18:3n-6 1.17 20:4n-6 0.06 22:6n-3 0.32 18:2/18:3 ratio 10.8 Formula2: (n=12) % total fatty acids 18:2n-6 11.95 18:3n-3 1.20 18:3n-6 trace 20:4n-6 0.06 22:6n-3 0.32 18:2/18:3 ratio 10.0 Standard formula: (n=11) % total fatty acids 18:2n-6 12.01 18:3n-3 1.20 18:3n-6 trace 20:4n-6 trace 22:6n-3 trace 18:2/18:3 ratio 10.0	Prospective study Formula-fed infants were randomized to one of three formulae Non-random breast-fed group Visual acuity assessed by sVEP at four mo of age.	Breast-fed infants had a significantly higher visual acuity than conventional formula-fed infants. No difference in visual acuity between breast-fed infants and infants fed formulae one or 2. No significant difference in visual acuity between formulae one or two and conventional formula. A significant association between birth weight and visual acuity.	Source of LCPUFA was fish oil. Formula-fed infants could be breast-fed for up to one month. Formula one and formula 2 group collapsed for acuity assessments as there was no difference in erythrocyte 22:6n-3. 26 of 37 formula-fed infants successfully tested by sVEP. Environmental, genetic and anthropometric differences not controlled in analyses

Reference	Subjects	Diets	Methods	Results	Comments
Makrides et al (1993)	16 term infants	Human milk: (n=8) Formulae: (n=8) % total fatty acids 18:2n-6 12.0-15.0 18:3n-3 1.0-1.7 18:2/18:3 ratio 9.4-11.3:1	VEP acuity at five mo.	Breast-fed infants had better visual acuity than formula-fed infants.	Confounding environmental or genetic variables not controlled in analysis. Insufficient power
Makrides et al (1995)	79 term infants	Human milk: (n=23, 9Γ,E14) % total fatty acids 18:2n-6 13.92 18:3n-3 0.94 20:4n-6 0.4 22:6n-3 0.21 18:2/18:3 14.8:1 Placebo formula: (n=19, 8Γ, 11E) % total fatty acids 18:2n-6 16.79 18:3n-3 1.6 18:2/18:3 10.7:1 LCPUFA formula:(n=13, 9Γ,4E) % total fatty acids 18:2n-6 17.4 18:3n-3 1.5 18:3n-6 0.27 20:4n-6 0.1 22:6n-3 0.36 20:5n-3 0.58 18:2/18:3 ratio 11.6:1	Infants received the formula for 30 wk. Breast-fed infants exclusively breast-fed to 16 wk of age, or were partially breast-fed and supplemented with conventional formula. VEP acuity measured at: 16 wk human milk (n=28) conventional formula (n=18) LCPUFA formula (n=8) 30 wk human milk (n=18) conventional formula (n=17) LCPUFA formula (n=9)	VEP acuities significantly higher at 16 and 30 wk of age in human milk fed and LCPUFA formula-fed infants than the conventional formula-fed infants.	

Possibly, the reduced indices of visual function in some (Birch et al 1993, Makrides et al 1995, Carlson et al 1996, Birch et al 1998), but not all studies (Auestad et al 1997, Jensen et al 1997, Courage et al 1998), are explained by differences in amounts of 18:3n-3, 18:2n-6 to 18:3n-3 ratios, other differences in the formula, the level of n-6 and n-3 fatty acids available to the breast-fed group, 22:6n-3 stores and status of the infants at birth, or variations in methodologies used to measure visual function.

1.2.5.2. Fatty acids and cognitive function

The n-3 LCPUFA are low in the human fetal brain prior to the third trimester and rapidly accumulate during the brain growth spurt (Sinclair & Crawford 1972, Clandinin 1980, Clandinin 1980a, Sastry 1985, Martinez 1992). The PL of the CNS contains high amounts of LCPUFA, particularly 20:4n-6 and 22:6n-3, that are integral to the structure of cellular membranes. There are also significant amounts of 16:0, 18:0 and 18:1, but the proportions of the individual fatty acids vary with PL species. There are particularly high concentrations of LCPUFA in the cerebral cortex phosphatidylethenolamine (PE), phosphatidylserine (PS) and phosphatidylinositol (PI). Docosahexaenoic acid (22:6n-3) is predominantly found in PE and PS, whereas 20:4n-6 is more predominant in the PI fraction (Fliesler & Anderson 1983, Sastry 1985, Farquharson et al 1995, Martinez & Mougan 1998). Myelin accounts for a major portion of brain lipids, with the myelin membranes containing 70-75% lipid (dry weight) (Norton 1984). Whereas the mature myelin membrane consists mainly of long-chain saturated (16:0 and 18:0) and monounsaturated (18:1n-9 and 24:1n-9) fatty acids (Sastry 1985), the immature membrane has a higher content of 20:4n-6 and 22:6n-3 (Norton & Cramer 1973).

Many animal studies have demonstrated the influence of a deficiency of dietary n-6 and n-3 fatty acids during development on CNS membrane-bound receptors and fluidity (Murphy 1990), as well as membrane-associated enzymes (Delion et al 1994). Feeding a diet deficient in n-3 fatty

acids has also been shown to influence the fatty acid composition of the developing CNS. Rats fed a diet for 60 d from birth with 0.2% of fatty acids as 18:3n-3 had a 33% reduction in brain unsaturated fatty acids when compared with fed a diet with 6.5% 18:3n-3 (Bourre et al 1984). The proportion of 22:6n-3 in synaptosomes was reduced from 12.5% to 3.4%, in myelin from 1.4% to 0.2%, in oligodendrocytes from 5.1% to 0.1% and in astrocytes from 12.1% to 5.7% in the 18:3n-3-sufficient rats compared with the 18:3n-3-deficient rats, respectively. Nonhuman primates fed a diet with 0.3% of fatty acids as 18:3n-3 had significantly reduced levels of 22:6n-3 at 22 mo of age, with the occipital cortex having 5.8% 22:6n-3 and the frontal cortex having 3.8% 22:6n-3, compared to 34.0% and 22.3% in the control animals, respectively. (Neuringer et al 1986). Similarly, piglets fed a diet containing 0.7% of fatty acids as 18:3n-3 had significantly lower brain 22:6n-3 than piglets fed a diet with 3.9% 18:3n-3, (8.8% *versus* 10.6%), respectively (Arbuckle et al 1992). In the latter studies, the levels of 22:6n-3 in the synaptic plasma membrane and retina of piglets fed a diet with 3.9% 18:3n-3 were not different from the levels in sow-fed piglets (Arbuckle et al 1992). These results suggest that diets providing adequate amounts (in the latter case 3.9% fatty acids or about 2% energy) of 18:3n-3 seem to support accretion of 22:6n-3 in the CNS in similar amounts to milk with preformed 22:6n-3.

Many studies have provided evidence that alterations of LCPUFA levels in the CNS can influence animal behaviour (Bourre et al 1989a, Yamamoto et al 1987, Wainright 1992). Studies on rats fed diets deficient in n-3 fatty acids have shown behavioral effects that include delayed learning of tasks, delay in reflex development and delay in response to stimuli. Only a few published studies have considered the possible effect of dietary fatty acid composition on cognitive development in infants. A summary of studies on dietary fatty acids and cognitive development in term and preterm infants is presented in **Table 1.2**. The two studies with preterm infants are

included in the summary table because these studies used the Fagan Test of Infant Intelligence, which was used in Study I and III of this thesis.

Table 1.2 *– Summary of studies on the role of dietary n-6 and n-3 fatty acids in cognitive development of preterm and term infants*

Reference	Subjects	Diets	Methods	Results	Comments
Agostoni et al (1995)	90 term infants	Human milk: (n=30, 13Γ,17E) % total fatty acids 18:2n-6 6.9-16.4 18:3n-3 0.7-1.3 20:4n-6 0.2-1.2 22:6n-3 0.1-0.6 Formula 1: (n=29, 15Γ, 14E) % total fatty acids 18:2n-6 10.8 18:3n-6 0.3 18:3n-3 0.73 20:4n-6 0.44 22:6n-3 0.3 18:2/18:3 ratio 14.8:1 Formula 2: (n=32, 16Γ,15E) % total fatty acids 18:2n-6 11.1 18:3n-6 ---- 18:3n-3 0.70 20:4n-6 ---- 22:6n-3 ---- 18:2/18:3 ratio 15.9:1	Prospective study Brunet-Lézine Neurodevelopment Scale used at four mo. Assesses global neurodevelopment.	Breast-fed infants and infants fed the LCPUFA supplemented formula scored significantly higher than infants fed the unsupplemented formula.	Breastmilk fatty acids are a range for European human milk. No difference between groups in SES; data not shown. Egg lipids used as LCPUFA.
Agostoni et al (1996)	Follow-up of subjects in Agostoni (1995)	Same as Agostoni (1996)	Brunet-Lézine Neurodevelopment Scale used at 24 mo.	No significant differences in scores between groups.	Information presented in abstract form only.

Reference	Subjects	Diets	Methods	Results	Comments
Carlson et al (1996a)	59 **preterm** infants	Control formula: (n=12) % total fatty acids 18:2n-6 21.2 18:3n-3 2.4 22:6n-3 ----- 22:6n-3 supp. formula: (n=15) % total fatty acids 18:2n-6 21.2 18:3n-3 2.4 22:6n-3 0.2	Infants randomly assigned to formulae. Fagan Test of Infant Intelligence at 12mo post-term.	No significant difference novelty preference between two formula groups. Significant differences in look duration (shorter) with the 22:6n-3 supp. formula than the control formula.	LCPUFA from marine oil. Infants fed assigned formula to two mo. Non-supplemented formula after 2mo. No demographic/SES information
Innis et al (1996)	433 term infants	Subject groups: never breast-fed-34Γ, 34E breast-fed <1mo-21Γ, 19E breast-fed 1-3mo-24Γ, 26E breast-fed 4-6mo-57Γ,38E breast-fed 7-8mo-28Γ, 21E breast-fed >8mo-43Γ, 49E 38 infants classified as fed both human milk and formula. Formulae: % total fatty acids 18:2n-6 14.0-34.0 18:3n-3 1.5-4.7	Cross-sectional study Novelty preference (Fagan test), looking behaviors during the novelty preference test. Breast-feeding history assessed at 39±1wk of age. Duration of breast-feeding determined by questionnaire. Breast-feeding defined as intake of cow's milk or formula< 12 oz/wk.	No significant differences between groups fed for varying durations of breast-feeding in novelty preference or looking behaviors during novelty preference testing.	Demographic variables noted but not considered in analysis. Study participants highly educated.

Reference	Subjects	Diets	Methods	Results	Comments
Scott et al (1998)	197 term infants from three sites: 1.Kansas city-75 2.Portland-79 3.Seattle-43	Formula-fed infants randomly assigned to one of three formulae plus a non-random human milk group. Control formula: (n=45) % total fatty acids 18:2n-6 21.9 18:3n-3 2.2 18:2/18:3 ratio 10:1 20:4+22:6: (n=46) 18:2n-6 21.7 18:3n-3 1.9 20:4n-6 0.43 22:6n-3 0.12 18:2/18:3 ratio 8.8:1 22:6n-3: (n=43) 18:2n-6 20.7 18:3n-3 1.9 20:5n-3 0.07 22:6n-3 0.23 18:2/18:3 ratio 11.2:1 Human milk: (n=63) Avg of one site 20:4n-6 0.48±0.1 22:6n-3 0.15±0.09	Prospective multicentre study. Bayley Scales of Infant Development administered at 12 mo. MacArthur Communicative Development Inventories at 14 mo.	No significant difference in the Bayley Scales of Infant Development between any diet groups. Vocabulary comprehension and production significantly lower for infants fed formula with 22:6n-3 compared with breast-fed infants. Infants fed the formula with 22:6n-3 + 20:4n-6 and infants fed the control formula not different from breast-fed infants.	Human milk group breast-fed to three mo. Source of LCPUFA egg PL (20:4n-6 +22:6n-3) and fish oil (22:6n-3 formula). Demographic characteristics not reported.

Reference	Subjects	Diets	Methods	Results	Comments
Werkman et al (1996)	67**preterm** infants	Control preterm formula: (n=34, 12Γ, 22E) % total fatty acids 18:2n-6 19.1 18:3n-3 3.0 22:n-3 ---- 18:2/18:3 ratio 6.4:1 Control term formula: % total fatty acids 18:2n-6 33.2 18:3n-3 4.8 22:n-3 ---- 18:2/18:3 ratio 6.9:1 22:6n-3 preterm formula: (n=33, 13Γ, 20E) % total fatty acids 18:2n-6 18.7 18:3n-3 3.1 22:6n-3 0.2 18:2/18:3 ratio 6.0:1 22:6n-3 term formula: % total fatty acids 18:2n-6 32.6 18:3n-3 4.9 22:6n-3 0.2 18:2/18:3 ratio 6.7	Prospective study. Infants were randomized to formula groups. Infants fed preterm formula until hospital discharge, then on term formula to 9 mo. Fagan Test at 6.5, 9 and 12 mo.	No significant difference in percent novelty at any age, for any of the diets. 22:6n-3 supplemented infants had significantly higher number of looks to the stimuli, shorter average look duration and shorter average look duration to the novel stimuli.	Demographic information unavailable.

Reference	Subjects	Diets	Methods	Results	Comments
Willatts et al (1998)	58 term infants enrolled at birth 40 infants completed the assessments at 9mo	Control formula: (n=20,7Γ,13E) % total fatty acids 18:2n-6 11.4 18:3n-3 0.7 20:4n-6 <0.1 22:6n-3 ---- 18:2/18:3 ratio 16.3 LCPUFA formula: (n=20, 8Γ, 12E) % total fatty acids 18:2n-6 11.5-12.8 18:3n-3 0.6-0.65 20:4n-6 0.3-0.4 22:6n-3 0.15-0.25 18:2/18:3 ratio 19.2-19.7	Prospective study. Infants randomized to formula group. Assessed at nine mo with a problem-solving paradigm.	At 3mo, infants classified as late peak-fixation or early peak-fixation using habituation techniques. Using ANCOVA, with covariates of birth weight and gestation, number of solutions to problem reduced in late peak-fixation infants' fed the control formula. Late peak fixation infants fed the LCPUFA formula not different from infants fed the control formula who were early peak-fixators.	No breast-fed control group. Infants fed formula to four mo. LCPUFA was egg lipids. All infants had birth weights between 2500-4000 g. Some of the infants were determined to have reduced growth parameters at birth using a ratio of head circumference and mid-arm circumference. SES variables included as covariates in regression analysis.
Willatts et al (1998a)	93 term infants enrolled at birth. 44 infants assessed at 10 mo	Same formula and subjects as Willatts (1998)	Prospective study. Infants randomized to formula groups. Assessed at 10 mo with a means-end problem-solving test.	Infants fed the LCPUFA formula had more intentional solutions than the infants fed the control formula.	No significant differences in demographic characteristics.

Studies on the influence of dietary essential fatty acids on visual function and the cognitive development of infants have generated conflicting results. The differences in results among different studies could be due to inadequate variations in the ratios of n-6 and n-3 fatty acids in the formulae, differences in the infants themselves, in the study design including the number of infants, age of testing and in the developmental tests used.

1.2.6. Role of the environment in CNS development and function

Studies on animals have demonstrated that the growth of connections between neurons is influenced by external experiences, supporting the concept of "sensitive periods" in development. A sensitive period is a select point in development when external experiences can influence the structural development of the CNS. Two types of experience processes have been suggested by Greenough et al (1987). The first is "experience-expectant" which refers to stimuli in the species' environment that is common to all members. An example of this is the overproduction and culling of synaptic connections, due to experiential input, that occurs in a similar manner among members of a species. The second process is "experience-dependent" which is an experience process that is individual to the member. In infants, this would be best illustrated by the differences in cognitive outcome due to varying home environments.

1.2.6.1. Environment and visual development

The development of the visual system appears to have sensitive periods when experience can affect the resulting quality of the visual response. When the visual system of a cat is blocked by a mask or eyelid suturing from birth, the visual cortex and acuity scores are significantly reduced in comparison to normal cats (reviewed by Zernicki 1991). Animals raised with selective aspects of the visual environment missing (eg. only vertical lines in the visual environment) respond only to the stimuli that were in the rearing environment (Greenough et al 1987). Research done in infants

with reduced visual acuity, due to a reversible visual occlusion, has shown that there appears to be a sensitive/critical period in the development of the human visual system from four mo to three yr of age (Billson et al 1985). If deprived of pattern stimuli during this time, there is a rapid reduction of visual acuity. Occlusions occurring prior to or after these periods do not seem to influence visual acuity.

1.2.6.2. Environment and cognitive development

Genetic potential and elements in the home environment contribute to the cognitive development of infants, although the significance of the contribution is not well understood. Socio-economic status, which is a measure of parental education level, occupational status and economic measures, is an important confounder in developmental studies. Maternal IQ is a confounder that can have both genetic and environmental influences on infant development. The home environment has also been found to be related to childhood cognitive ability (Bradley & Caldwell 1980). Studies of identical twins reared together have found IQ scores with correlations of r=0.86, whereas when reared apart, the r=0.72 (Bouchard & McGue 1981). Siblings reared together have a correlation between their IQs of r=0.47. Adoption studies have shown that the correlation of IQ scores for adopted children to biological parents is r=0.22, whereas the correlation in IQ between parents and biological offspring living in the same household is usually in the order of 0.4. Although adoption studies provide a convenient "natural" experiment to determine the contribution of genetics *versus* environment to IQ, they must be cautiously interpreted due to the potential influence of selective placement of children and genotype-environment interactions (Turkheimer 1991) that may confound these studies. Modelling of the data from adoption studies and twin adoption studies have resulted in heritability estimates of approximately 0.5 at 12 mo and 0.65 at 24 mo (Cardon et al 1992). These association levels

indicate that environment may play as important a role as the genetic ability in the development of intelligence.

Healthy, term breast-fed infants are reported to have better cognitive development than bottle-fed infants in some (Morrow-Tlucak et al 1988, Florey et al 1995, Pollock 1994) but not all studies (Richards et al 1998, Malloy & Berendes 1998) of cognitive performance. Some of the variability in study findings may be explained by the large potential for confounding variables in an infant's environment. Studies evaluating environment and IQ associations have found that social factors such as minority status, head of household occupation, mother's education and family income are highly correlated to child IQ (Sameroff et al 1993, Duncan et al 1994, Kramer et al 1995). Birth order has been shown to relate to early language development, with second born children having more advanced language skills than first-born (Oshima-Takane et al 1996). Further confounding the situation, it is known that mothers who choose to breast-feed are different from mothers who choose to bottle-feed their infants. For example, US and UK studies have found that women who bottle-feed their infants are more likely to smoke, have a low income and a lower assessed IQ, while women who breast-feed are more likely to have higher levels of education and to be older and married (Arango 1984, Smith 1985, Morrow-Tlucak et al 1988). Ideally, confounding variables are controlled through the use of randomized assignment to study group. However, for ethical reasons, infants cannot be randomized to a breast-feeding and formula-feeding cohort, resulting in a large potential for group bias in many non-nutrition characteristics such as family background, parental education and income. Prospective, randomized studies comparing different types of bottle (formula) feedings may also be compromised if groups are of insufficient size to control for confounding variables, or if influential variables are not characterized for the developmental test utilized. It has been estimated that studies of infant development would

require 288 infants in total (144 per group) in order to detect a difference of one-third SD in tests of visual or cognitive function with 80% power (Morley 1998).

In summary, it is known that the environment the infant is raised in can have a significant impact on their developmental outcomes. Furthermore, the environment of a breast-fed infant is often more enriched than that seen for a formula-fed infant. These variables need to be considered in studies of the development of breast-fed *versus* formula-fed infants.

1.3. Lipids of human milk and milk substitutes

1.3.1. Synthesis of human milk fatty acids and incorporation into milk triacylglycerols

The synthesis of human milk TG has recently been reviewed by Neville (Neville 1997) and Barber (Barber et al 1997). There are three potential sources of fatty acids used in the synthesis of human milk TG: *de novo* synthesis in the mammary gland, dietary fatty acids and fatty acids from adipose tissue, liver and other tissues. The magnitude of contribution from each source depends on many factors, including the percentage of energy from carbohydrate and fat in the diet and the time elapsed from the last meal.

De novo synthesis of fatty acids from glucose or acetate in the mammary gland results in the formation of medium-chain fatty acids (C8-C14), which make up approximately 10-35% of human-milk fatty acids (Jensen 1996). Glucose enters the mammary alveolar cell from plasma, as described in Section 1.1.2, and is metabolized through the tricarboxylic acid pathway resulting in the production of malonyl-CoA and subsequently, a fatty acyl chain (Figure 1.3, Section 1.1.2) (Smith 1994). Fatty acid synthesis in the mammary gland differs from the liver in that the cytosol of the mammary epithelial cells contain a medium-chain acylthioester hydrolase, thioester II, which stops fatty acid synthesis after the addition of 8-14 carbons, resulting in medium-chain fatty acids.

Dietary fat and tissue lipids provide a source of saturated, monounsaturated and LCPUFA for human milk. After digestion and absorption of dietary fatty acids, they are incorporated into chylomicrons and delivered to tissues such as the mammary gland, or are transported as free fatty acids (FFA) bound to albumin. When plasma TG are low and FFA are high, as in the fasted state, plasma FFA are used directly in large quantities for milk TG synthesis (Nielsen 1994). Dietary fatty acids are released from chylomicron or VLDL-TG to the mammary gland by lipoprotein lipase (Neville 1997). It is not known how long-chain fatty acids cross the capillary endothelium and interstitial space to reach the alveolar cell, but it may involve a fatty acid binding protein (Glatz et al 1995). Mammary fatty acid-binding proteins are thought to be responsible for maintaining a readily available fatty acid pool in the mammary gland for TG synthesis (Bansal & Medina 1993).

During lactation, the metabolic balance is skewed towards mobilization of stored fatty acids rather than accumulation of fat stores. Very little research has addressed the role of adipose tissue in supporting milk TG synthesis. Some recent studies have shown there is a dependence between adipose tissue 18:2n-6 and the 18:2n-6 content of human milk from delivery to 30 d post-partum, but the same relation is not found for 18:3n-3 (Martin et al 1991, Martin et al 1993a). However, a significant correlation between the ratio of 18:3n-3 to 18:2n-6 in adipose tissue and that in human milk has been reported (Martin et al 1991).

The phosphatidic acid pathway is the major pathway of milk TG synthesis in the mammary gland rather than the monoacylglycerol pathway (Kinsella 1973). Synthesis of milk TG is covered in detail in Section 1.3.1. After TG are synthesized, they are incorporated into microlipid droplets that combine into larger cytoplasmic lipid droplets (Valivullah 1988). The droplets progress toward the apical membrane of the mammary epithelial cell where they are secreted. Following secretion, the lipid droplets become enveloped in a membrane that is partly derived from the apical surface of the mammary epithelial cell and ultimately bud off from the cell. The enveloping membrane is known

as the milk fat globule membrane (Barber et al 1997) (**Figure 1.6**). The milk fat globule membrane is composed of proteins, PL and CE and it allows the dispersion of lipids in the aqueous milk environment.

The average total lipid content of human milk, including TG, cholesterol, CE, PL and FFA, is about four g/100 mL of the total volume (Jensen et al 1995). Approximately 98% of milk lipid is TG. The milk lipid usually accounts for 40 to 50% of the total energy content of human milk (Jensen 1996). The concentration of TG in milk is influenced by several factors including: duration of nursing with the percent of milk lipid increasing in a single nursing from about 2 g/100 mL (foremilk) to four g/100 mL (hindmilk) of the total milk volume; postpartum stage, with an increase in the lipid content of milk increasing from approximately 1.2 g/100 mL at day one postpartum to 2.4 g/100 mL by postpartum day five; parity, where primiparous Gambian women have been shown to have 25% higher milk fat content than women with a parity >four. Milk fat content has also been reported to vary among women from 1.8 g/100 mL to 8.9 g/100 mL, with an average (50^{th} percentile) of four g/100 mL (reviewed by Jensen 1995, Nommsen et al 1991).

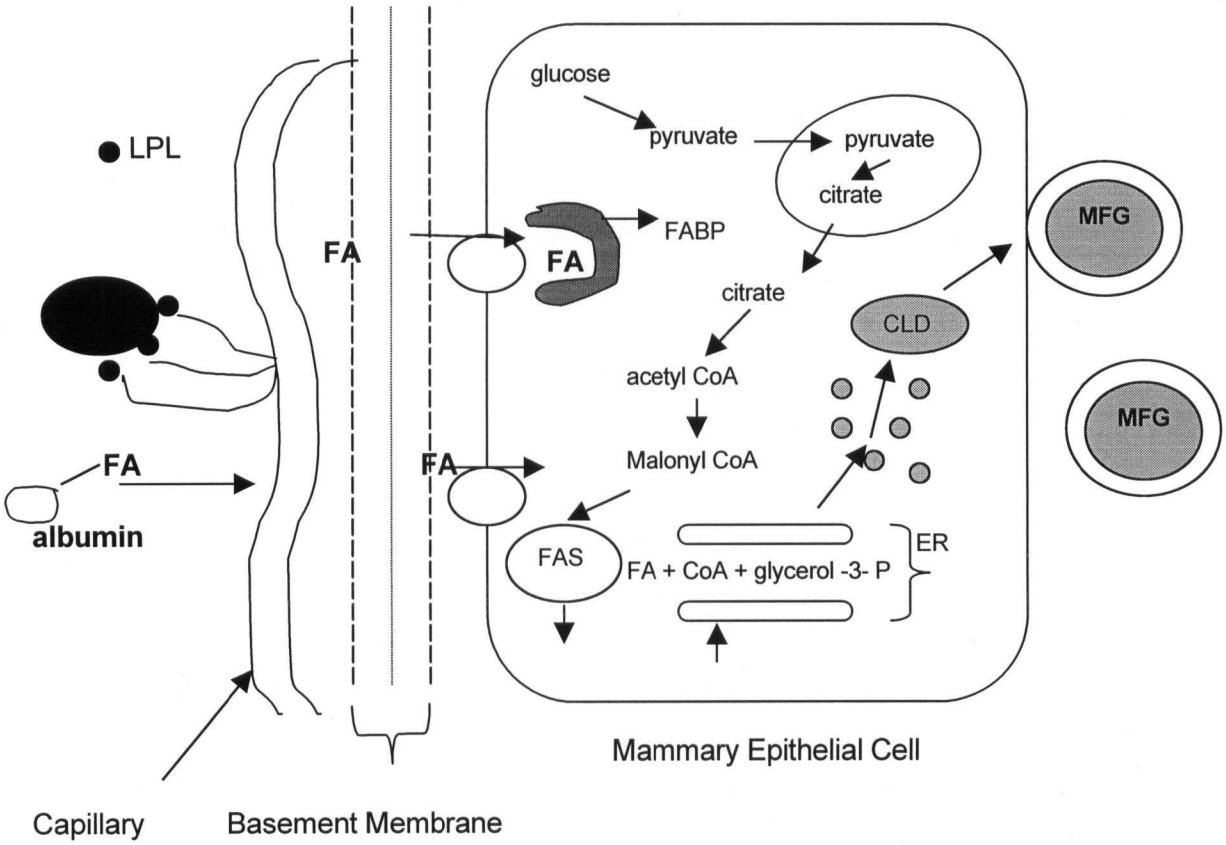


Figure 1.6 - Synthesis and secretion of the milk fat droplet. Fatty acids (FA) enter from albumin or from hydrolysis of chylomicron or VLDL-TG by lipoprotein lipase (LPL). Fatty acids may also be synthesized by fatty acid synthetase (FAS). The fatty acids are bound to fatty acid-binding proteins (FABP) in the cytoplasm or activated with acetyl-CoA and used to synthesize TG. Microlipid droplets synthesized in the endoplasmic reticulum (ER) fuse to form cytoplasmic lipid droplets (CLD) that are enveloped by the apical membrane to form the milk fat globule (MFG) that is secreted in a membrane-bound form into milk. Adapted from Neville 1997.

1.3.2. The composition and distribution of fatty acids in human milk triacylglycerol

1.3.2.1. Human milk fatty acid composition

The fatty acid composition of human milk is influenced by the fatty acid composition of the mother's diet (Koletzko et al 1992). A study by Insull et al (1958), showed that increasing unsaturated fatty acids in the diet resulted in an increase in unsaturated fatty acids in the milk. A low fat, calorie-restricted diet resulted in the milk fatty acid composition reflecting more the adipose tissue fatty acids and when the women consumed a low fat, isocaloric (ie high carbohydrate) diet, the levels of medium-chain fatty acids (MCFA) increased significantly. In a typical North American diet with 25 to 40% of energy from fat, MCFA usually represent about 10-12% of the milk fatty acids (Jensen 1996). Nigerian women, who typically consume a low fat-high carbohydrate diet, can have up to 18% MCFA in their milk (Koletzko et al 1991). Thus, there are distinct regional patterns of fatty acids in human milk among different countries and ethnic groups. Oleic acid (18:1) is the predominant fatty acid in human milk, typically representing 31-38% of total milk fatty acids. Countries such as Spain, however, have average values of milk 18:1 as high as 44%, reflecting the use of olive oil as the liquid vegetable of choice in the diet (Koletzko et al 1992). The long chain saturated fatty acid 16:0 is usually the most prevalent milk saturated fatty acid, with average levels of 20-24% of total milk fatty acids. Total milk saturated fatty acids, including MCFA, usually range from 40 to 50%, with lower amounts found in Spain (39%) and higher amounts found in Africa (50%), reflecting the higher synthesis of milk MCFA due to the low fat-high carbohydrate diet in African women (Koletzko et al 1992). The level of 18:2n-6 in human milk is dependent on the proportion of 18:2n-6 in the diet fat and currently usually averages 14-20% of total fatty acids in Europe and North America women (Koletzko et al 1992). Diets with high amounts of corn oil, containing significant amounts of 18:2n-6, resulted in an increase in milk 18:2n-6 levels from 17% to 44% (Insull et al 1958). The other essential fatty acid, 18:3n-3, usually represents between 0.3% and 1.8% of milk total fatty acids. The long-chain unsaturated n-6 and n-3 fatty acids 20:4n-6 and

22:6n-3 usually represent, on average, 0.4 to 0.7% and 0.1 to 0.4% of milk fatty acid, respectively. A study by Sanders et al (1992) showed that women with intakes of 20:4n-6 that varied from 0 to 0.15 gm/d showed no difference in the level of 20:4n-6 in their milk. This suggests that human milk 20:4n-6 may not depend on dietary intake, but rather is derived either from maternal stores or desaturation and elongation of the 18:2n-6 precursor. However, it is possible that the reported intake of 20:4n-6 did not vary enough to result in statistical differences in the milk 20:4n-6. Levels of 22:6n-3, however, are extremely variable and can be easily altered through changes in the maternal intake of 22:6n-3. This was demonstrated when lactating mothers consumed fish oil and increased their milk 22:6n-3 from 0.1 % to 1.9% (Harris et al 1984). In general, increasing consumption of fish or other seafood in lactating women tends to increase the 22:6n-3 in their milk.

1.3.2.2. The distribution of fatty acids in human milk triacylglycerols

The fatty acids in human milk TG are not randomly distributed on the glycerol backbone. Rather, unsaturated fatty acids are found predominantly in the *sn*-1,3 positions and saturated fatty acids are found in the *sn*-2 position. While the source of some of the fatty acids in milk TG includes diet and adipose tissue, the arrangement of fatty acids is significantly different from that found in either the diet, adipose tissue or any other human tissue (Martin et al 1993a). This difference in fatty acid distribution in precursor TG (diet/plasma/adipose) and TG formed in the mammary tissue suggests that the positioning of fatty acids in the TG of human milk is tightly regulated by the mammary gland. However, little work has been done to elucidate the pathways in the human mammary gland that regulate placement of fatty acids on the glycerol molecule. Rather, much of the work that has been done to elucidate the pathways involved in the synthesis of milk lipids has utilized rat and bovine mammary tissue, although it is not known how representative these animal models are to human mammary tissue lipid metabolism. Askew et al (1971) first demonstrated that

there were varying esterification rates in bovine mammary tissue for fatty acids, dependent on the chain length. The observed rates of fatty acid esterification of mammary glycero-3-phosphate, in both rat and bovine mammary cells, was 8:0 < 10:0 < 12:0 < 14:0 < 16:0 ≅ 18:1 > 18:0 > 18:2n-6 (Askew et al 1971, Tanioka et al 1974). Linoleic acid was poorly esterified and inhibited the esterification of other fatty acids. Palmitic acid (16:0) had the fastest esterification rate, in agreement with previous reports showing that 16:0 is rapidly esterified upon entering bovine mammary cells (Kinsella 1970). The rat mammary microsomal acyltransferases responsible for the biosynthesis of phosphatidic acid from glycero-3-phosphate show marked preference at positions 1 and 2 for palmitoyl-CoA as a substrate for acylation and palmitoyl-CoA is 18 times more effective as an acyl donor than decanoyl-CoA. (Tanioka et al 1974). Furthermore, diacylglycerol acyltransferases, which place acyl groups at the *sn*-3 position prefer palmitoyl-CoA only twice that of decanoyl-CoA. The result of this preference is that medium-chain fatty acids are more likely to be esterified to the *sn*-3 position than other fatty acids (Lin et al 1976).

Kinetic studies of the rat mammary acytransferases suggest that the initial acylation of glycerol-3-phosphate, catalyzed by acyl-CoA-*sn*-glycerol 3-phosphate acyltransferase, occurs at the 1 position, rather than the 2 position. The acyl specificity of acyl-CoA-*sn*-glycerol 3-phosphate acyltransferase appears to be for 18:1 (Cooper & Grigor 1980). The 2 position is quickly acylated by acyl-CoA-2-monoacyl-*sn*-glycerol 3-phosphate acyltransferase, which has specificity for 16:0. The diacylglycerol acyltransferases also have specificity for the type of diacylglycerol acceptor. That is, these acyltransferases prefer *sn*-1,2 diacylglycerols to *sn*-2,3 or *sn*-1,3 diacylglycerols (Lin et al 1976).

It is not clear if a change in maternal diet influences the structure of the milk TG. In an attempt to alter the specific distribution of milk TG fatty acids, two generations of rats were fed diets

high in n-3 PUFA (Jensen 1996). Although the milk fatty acid content was altered, the fatty acid positional distribution was not affected. In contrast, a study of lactating women consuming lacto-ovo, vegan or omniverous diets found that the distribution of milk fatty acids on the TG was sensitive to diet (Jensen et al 1988). It should be noted that this study only had three to four people per group, and likely did not have sufficient power to confidently report differences. Although diet may influence the distribution of the milk TG fatty acids, the duration of the lactation does not seem to be important. Martin et al (1993) reported that there was no difference in the distribution of fatty acids in the TG between colostrum and mature milk (**Table 1.3**). In mature human milk, the majority of 16:0 is at the *sn*-2 position while the majority of 18:1 and 18:2n-6 is located at the *sn*-1, 3 position and 20:4n-6 is primarily located at *sn*-2, 3. About half of 18:3n-3 is located at the *sn*-3 position and about half of 22:6n-3 is at the *sn*-2 position.

Table 1.3 *- Stereospecific analysis of colostrum and mature human milk triacylglycerol*

Fatty Acids	Colostrum			Mature Milk		
	sn-1(%)	*sn*-2(%)	*sn*-3(%)	*sn*-1(%)	*sn*-2(%)	*sn*-3(%)
10:0	nd	0.4±0.3	0.8±0.4	0.4±0.9	0.6±0.7	2.3±1.3
12:0	1.4±1.1	3.8±1.3	9.1±3.2	2.3±4.4	7.8±4.3	13.9±4.0
14:0	4.8±1.9	11.1±2.2	9.7±2.9	3.5±2.2	12.5±3.6	10.7±4.0
16:0	12.6±3.4	53.5±3.2	11.2±3.3	12.4±4.1	51.2±1.5	11.7±6.3
18:0	11.4±1.3	1.7±0.4	4.7±1.1	15.2±2.1	1.5±0.5	5.2±1.3
18:1	48.4±5.5	13.8±1.6	36.6±3.4	46.4±5.5	11.5±5.2	31.8±7.3
18:2	14.1±3.7	8.4±2.2	17.3±3.9	14.6±4.2	8.5±1.0	16.7±1.4
20:2	0.6±0.2	0.3±0.1	0.7±0.2	0.4±0.2	0.2±0.3	0.3±0.1
20:3	0.4±0.2	0.3±0.1	0.5±0.2	0.2±0.0	0.1±0.0	0.2±0.0
20:4	0.2±0.2	0.7±0.2	0.7±0.2	0.1±0.1	0.4±0.1	0.4±0.1
18:3	0.7±0.2	0.4±0.1	1.0±0.2	0.9±0.7	0.8±0.8	1.4±0.9
20:5	nd	0.1±0.1	0.1±0.2	nd	nd	nd
22:5	nd	0.4±0.2	0.2±0.1	nd	0.2±0.0	nd
22:6	0.1±0.1	0.7±0.7	0.6±0.6	nd	0.3±0.1	0.1±0.1

Adapted from Martin et al 1993; values are means±SD; nd=not detected.

1.3.3. The fatty acid composition of infant formula

A blend of several oils is generally used to achieve a mixture of saturated, monounsaturated and polyunsaturated fatty acids in formula which are suitable for infant feeding. The most common oils used in infant formulae are usually corn, soybean, high oleic safflower and sunflower, coconut and palm oils. The oil blend in some infant formulae, however, has been criticized for using saturated "tropical" oils, which may be hypercholesterolemic in adults (Horn 1987, Thomas 1988). Coconut oil is used in infant formulae as a source of lauric acid (12:0) and myristic acid (14:0), which are present in human milk and are more readily absorbed than longer chain saturated fatty acids due to their relatively higher solubility in water. Palm oil or its high oleic fraction palm-olein is attractive because it is high in 16:0 (40-44%) and 18:1n-9 (40-44%), similar to human milk. The availability of the 16:0 to the infant, however, has been questioned (Section 1.4.1). Levels of 18:1n-9 in human milk fatty acids is high, usually 20-40% of fatty acids (Table 1.5). High oleic varieties of safflower and sunflower oil contain approximately 80% 18:1n-9. 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. Prior to the mid 1990s, many powdered infant formulae contained corn oil rather than soybean oil because of the susceptibility of the large amounts of 18:3n-3 in soybean oil to oxidative damage. Canola oils are not an option for infant formulae as they have not been approved for use in infant formulae in the United States. At the current time, no commercial infant formulae in Canada or the USA contain the long chain fatty acids 20:4n-6 or 22:6n-3.

1.3.3.1. The distribution of fatty acids in triacylglycerols of infant formula

In contrast to human milk, the vegetable oils and non-milk fats used in infant formula have 16:0 predominantly esterified at the 1,3-position of the TG (Bracco 1994, Carnielli et al 1996). The *sn*-2 position is predominantly esterified with 18:1 and 18:2n-6 (**Table 1.4**).

Table 1.4 *– Positional distribution of fatty acids in triacylglycerol of term infant formula*

	Total	2 position	% of fatty acid in the 2 position
12:0	12.1	13.9	38.3
14:0	5.3	3.4	21.3
16:0	19.9	7.5	12.5
18:0	3.6	1.0	9.3
18:1	38.3	50.1	43.6
18:2n-6	12.1	19.8	54.5
18:3n-3	1.8	3.1	57.4

Adapted from Carnielli et al 1996; values are percent of total fatty acids

1.4. Digestion and Absorption of Dietary Fat in Infancy

As 50% of the energy supplied by human milk and infant formulae is from its fat content, it is important that the fat is readily digested and absorbed. However, it has been known since the early 1960s that infants fed formula may not absorb the formula fat as readily as fat is absorbed from human milk by infants (Widdowson 1965, Van de Kamer & Weijers 1961). The reason for the difference in the amount of fat absorbed between infants fed formula and breast-fed infants is not completely understood. The digestion of dietary lipid consists of three sequential steps: 1) enzymatic hydrolysis, 2) emulsification of hydrolysis products, and 3) absorption of emulsified products. However, there are important differences in these physiological processes between breast-fed infants and infants fed formula.

1.4.1. Enzymatic hydrolysis of dietary triacylglycerol

The initial stages of dietary TG digestion involve hydrolysis of the *sn*-1,3 position fatty acids by gastric and pancreatic lipases. Gastric lipase hydrolyzes the *sn*-3 position twice as fast as the *sn*-1 position (Staggers et al 1981, Jensen et al 1982). The products of the hydrolysis are free fatty acids released from the 1,3 position and monoacylglycerols (MG) (Small 1991). Milk has an additional enzyme called bile salt-stimulated lipase (BSSL) that is believed to be involved in the digestion of dietary lipids in the breast-fed infant. This lipase is present in the aqueous fraction of the milk emulsion and does not hydrolyze TG until it is activated in the intestine by the bile salts cholate and chenodeoxycholate (Manson et al 1997). While the role of BSSL is not completely understood, it is thought that its presence in human milk is one reason that breast-fed infants have a more efficient absorption of dietary TG than infants fed an infant formula (Hernell et al 1994). It has been hypothesized that BSSL completes the digestion of the MG to FFA and glycerol and that under conditions of low intraluminal levels of bile salts, such as is seen with preterm infants, FFA are more efficiently absorbed than MG (Bernback et al 1990). However, unesterified 16:0 has a

melting point above body temperature (63.1°C) and at the alkaline pH of the intestine readily forms soaps with divalent cations such as calcium. These actions may explain the lower coefficient of absorption of 16:0 (of 76%), compared with 18:1 (92%) or 18:2n-6 (94.5%) (Filer et al 1969, Jensen et al 1986, Tomarelli et al 1968). However, *in vitro* kinetic studies have shown that while BSSL was efficient at removing short-chain fatty acids and LCPUFA, *sn*-2-monopalmitoylglycerol and *sn*-2 monooleoylglycerols were relatively resistant to the action of BSSL (Wang et al 1983). Studies with rats fed a formula with 16:0 selectively esterified to the *sn*-2 position of the TG showed that there was a decrease in the excretion of lipids and 16:0, specifically, when compared with rats fed a standard formula with 16:0 located at the *sn*-1,3 position of the TG (Lien et al 1993, de Fouw et al 1994, Aoyama et al 1996, Lien et al 1997).

As previously described (Section 1.3.2 and 1.3.3) there are significant differences in the TG stereochemistry between human milk and infant formula TG. Consequently, the fatty acids released by the process of lipolysis in the formula-fed infant differ from those that are found in the breast-fed infant. In breast-fed infants, the fatty acids released from the *sn*-1,3 position by pancreatic lipase and gastric lipase are usually 18:1 and 18:2n-6 (Rogalska et al 1990, Martin et al 1993) and the resulting MG is predominantly monopalmitin (**Figure 1.7**)

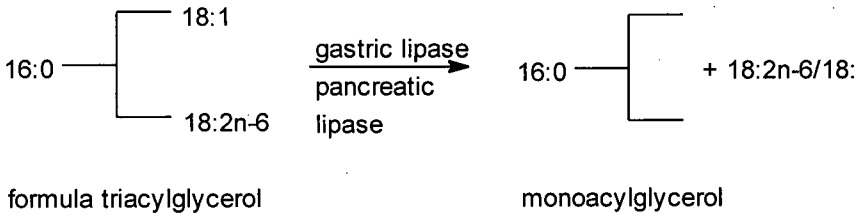


Figure 1.7 – Schematic representation of digestion of human milk TG.

Digestion of lipids in infants fed formula is similar to that seen in breast-fed infants (**Figure 1.8**). Pancreatic lipase removes the fatty acid from the *sn*-1 and *sn*-3 positions and gastric lipase

removes the fatty acids from the *sn*-3 position, but there is no BSSL. As the distribution of fatty acids within the vegetable oil TG used in infant formula is different from that of the human milk TG, fatty acids released from the 1,3 position of standard infant formula TG are predominantly 16:0 while the resulting MG contains 18:1 and 18:2n-6.

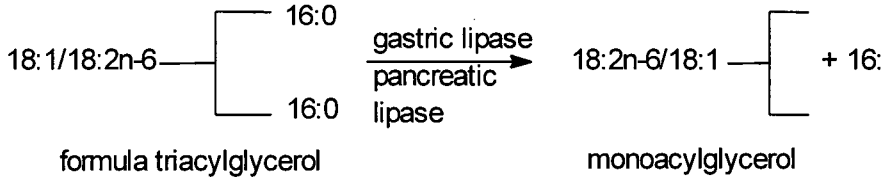


Figure 1.8 – Schematic representation of digestion of vegetable oil/formula TG.

Early work in infants assessing the absorption of dietary fatty acids utilized lard, which has 71% of 16:0 preferentially esterified at the 2 position (Tomarelli et al 1968, Filer et al 1969). Infants fed a formula with lard excreted less lipid than infants fed a standard vegetable oil formula with 13-18% of 16:0 at the 2 position, or a butterfat-based formula with 43% of 16:0 at the 2 position (Tomarelli et al 1968). To determine if it was the non-random distribution of fatty acids in the TG that resulted in the decrease in fatty acid excretion, or some other aspect of the lard, infants were fed a natural lard-based formula, with 85% of 16:0 at the 2 position and a formula with lard that had fatty acids randomly distributed on the TG, resulting in 33% of 16:0 at the 2 position (Filer et al 1969). The infants fed the natural lard absorbed the fat better than infants fed a formula with lard whose fatty acids distribution had been randomized across the TG.

1.4.2. Emulsification, absorption and resynthesis of lipids

As milk lipids are digested, the lipid products become solubilized in the aqueous portion of the intestine by the bile salts, which then form mixed micelles with the MG, FFA and glycerol backbones (Thomson et al 1993, reviewed in Black 1995). The resultant small globule, with its

polar hydrophilic surface then undergoes absorption. The mixed micelle is then exposed to the brush border of the small intestine, and FFA and MG diffuse into the mucosal cell. An exception to this is that the medium-chain fatty acids (C8, C10 and C12) can be transported through the portal vein due to increased solubility in an aqueous environment (Bach et al 1982). When the products of TG digestion enter the mucosa, they cross the cytoplasm, possibly via a fatty acid-binding protein, to the endoplasmic reticulum where the resynthesis of TG occurs (Tso et al 1986). It is thought that MG cross the cytoplasm by simple diffusion. When in the endoplasmic reticulum, TG are resynthesized via either the 2-MG or the *de novo* 3-glycerophosphate pathway (**Figure 1.9**). The 2-MG pathway in which fatty acids are re-esterified, with little or no specificity to the glycerol 1 and 3 positions, generally predominates in the fed state (Breckenridge et al 1975, Tso et al 1991). Studies in rats using *in situ* isolated intestine suggest that about 70% of enterocyte TG synthesis in the fed state proceeds via the 2-MG pathway, with the remaining TG synthesis occurring via the *de novo* 3-glycerophosphate pathway (Breckenridge et al 1975, Paris et al 1968). The predominance of the 2-MG pathway is probably explained by the presence of high amounts of MG in the enterocyte, which inhibit the 3-glycerophosphate pathway (Tso et al 1991). When the supply of 2-MG is low, as during fasting, the 3-glycerophosphate pathway predominates, using substrates derived from glucose metabolism and endogenous fatty acids (Shiau et al 1985, Gangl & Ockner 1975). The assembly of chylomicrons occurs in the intestinal epithelial cells. Chylomicron apolipoprotein A-1, A-II, A-IV, A-V and B-48 are all made by the enterocyte (Thomson et al 1993). After the TG, PL, CE, apolipoproteins are synthesized in the endoplasmic reticulum of the enterocyte, they are transported to the Golgi body, which packages them in to a chylomicron particle. Apolipoprotein B-48 is essential for the synthesis of chylomicron. Lipoprotein metabolism is discussed in more detail in Section 1.4.3.

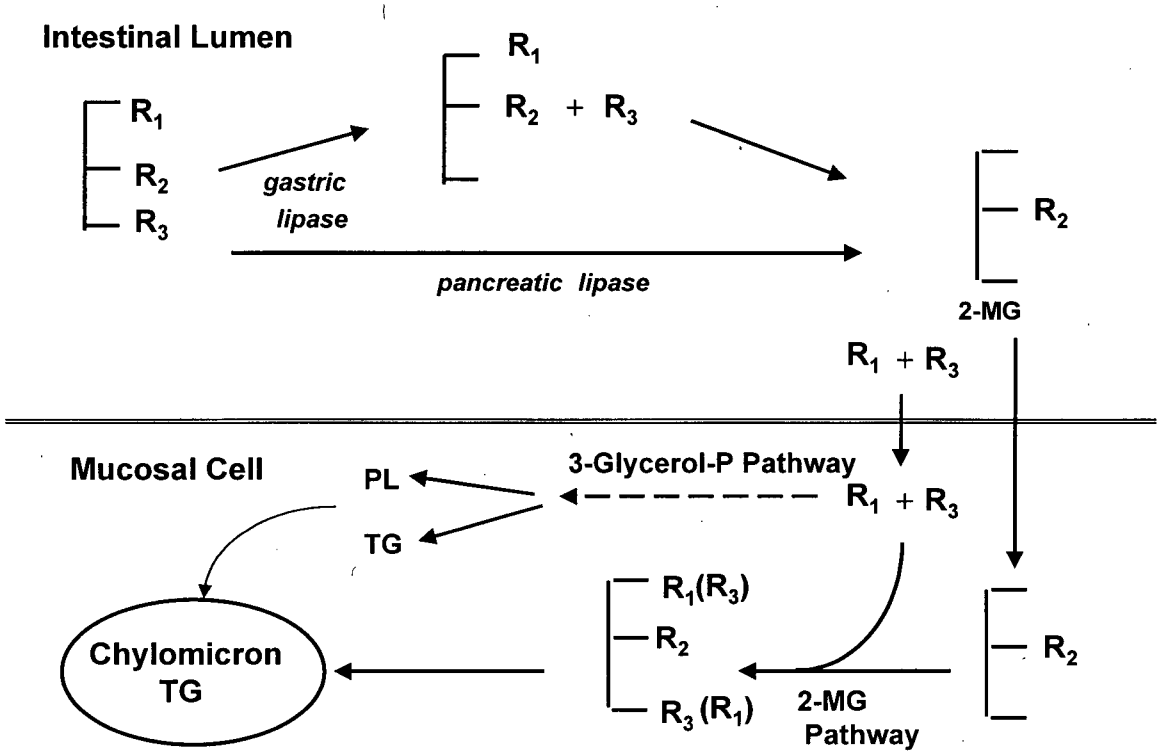


Figure 1.9 – Schematic representation of dietary triacylglycerol digestion and resynthesis of triacylglycerol by the 2-monoacylglycerol (2-MG) pathway or the 3-glycerophosphate pathway (3-glycero-P pathway).

An early study with rats examining the influence of dietary TG fatty acid distribution on the post-absorption (plasma) TG fatty acid distribution estimated that 85% of the 16:0 in the *sn*-2 position of the dietary TG was conserved through digestion, absorption, reesterification to TG and secretion into lymph (Mattson & Volpenheim 1962). Recent work with piglets fed formula with a fatty acid distribution similar to human milk (with about 79% of all 16:0 preferentially esterified at the *sn*-2 position) estimated 44% of the 16:0 found esterified at the plasma TG *sn*-2 position, indicating retention of about 56% of 16:0 at the *sn*-2 position of the diet TG (Innis et al 1995). Piglets fed a standard formula, similar in TG composition to infant formula, with 5% of all 16:0 at the *sn*-2 position, in contrast, had only 15% of all plasma TG 16:0 in the *sn*-2 position, suggesting that absorbed 2-MG and FFA, and the chylomicron TG fatty acid distribution, may differ between infants fed human milk and infants fed formula. In contrast to this suggestion, the hypothesis that the milk enzyme, bile salt-stimulated lipase completes the hydrolysis of the milk TG to FFA and glycerol (Bernback 1990) infers that the positional distribution of fatty acids in human milk or formula TG has no significance to plasma TG fatty acids in the breast-fed infant. Elucidation of this controversy, i.e. 16:0 in human milk as a 2-MG and incorporated into chylomicron TG 2-position fatty acids, is one of the aims of this thesis.

1.4.3. Transport and uptake of fatty acids into the CNS

Absorbed and endogenously synthesized fatty acids require transport between various tissues and organs for utilization and storage. However, with the exception of the medium-chain fatty acids, fatty acids are insoluble in the aqueous plasma environment. The insolubility problem is overcome through the association of nonpolar lipids (TG and CE) with amphipathic lipids (PL and cholesterol) and proteins to make a more water-soluble lipoprotein, or through using albumin as a carrier of lysoPL and FFA.

1.4.3.1. Transport of fatty acids

Lipoproteins are spherical particles that consist of lipid and protein particles (**Table 1.5**). The general structure of lipoproteins includes a monolayer surface of PL and free cholesterol surrounding a hydrophobic core of TG and CE. Lipoproteins also have apolipoproteins, that are specific to the lipoprotein, embedded in their surface (Table 1.5). The apolipoproteins provide structural integrity to the lipoprotein particle and are important in the metabolic fate of the lipoprotein. Two of the common systems for classification of lipoproteins are based on hydration density and electrophoretic mobility. Lipoproteins can be separated into five broad classes based on hydration density: chylomicrons (CM), d<0.94 g/dL; very low-density lipoproteins (VLDL), d=0.95-1.006 g/dL; low density lipoprotein (LDL), d=1.007-1.019 g/dL; intermediate density lipoproteins (IDL), d=1.020-1.063 g/dL; and high-density lipoprotein (HDL), d=1.064-1.21g/dL. When classified on the basis of electrophoretic mobility, plasma lipoproteins are separated and designated as α (HDL), pre-β (VLDL) and β (LDL)-lipoproteins. Chylomicrons and VLDL predominantly transport TG, while LDL and HDL carry PL, cholesterol and CE (reviewed by Shepherd 1994, Ginsberg 1994).

Table 1.5 *-Composition of human plasma lipoproteins*

Lipoprotein	Site of synthesis	Density (g/dL)	Apos	Function	Composition (%wt)[1]					
					Protein (%)	Total Lipid (%)	% total lipid			
							TG	PL	CE	FC
chylomicron	Intestine	<0.94	A-I A-II A-IV B-48 C-I C-II C-III E	LCAT cofactor Hepatic Lipase cofactor ? (satiety) CM secretion LCAT cofactor LPL cofactor LPL activation/inhibition CM-remnant binding to receptor	1-2	98-99	85-95	3-8	2-4	1-3
VLDL	Liver, intestine	0.95-1.006	B-100 C-I C-II C-III E	VLDL secretion LCAT cofactor LPL cofactor LPL activation/inhibition Ligand for receptor	7-10	90-93	50-65	15-20	16-22	4-8
LDL	From VLDL	1.019-1.063	B-100	LDL structure Ligand for LDL receptor	18-22	78-82	4-13	18-28	45-50	6-10
HDL	Liver, intestine	1.064-1.21	A-I A-II A-IV C-I C-II C-III D E	LCAT cofactor Hepatic Lipase cofactor ? (satiety) LCAT cofactor LPL cofactor LPL activation/inhibition ? ligand for receptor	33-55	45-67	2-16	26-43	15-31	3-10

[1]Table adapted from Mayes 1996a and Genest 1990 .

Dietary TG is hydrolyzed and the FFA and MG absorbed by the intestinal enterocyte, predominantly re-esterified into TG and packaged into chylomicrons (see Section 1.4.1). Chylomicrons are secreted into the circulation resulting in the milky appearance that plasma can have in the post-absorptive state. Chylomicrons are secreted with apolipoprotein A-I, A-II, A-IV, B-48 and apolipoprotein C-II, C-III and E are acquired from HDL after release to plasma. The acquisition of apolipoprotein CII is important for later metabolism, as it is an essential cofactor for lipoprotein lipase. Lipoprotein lipase (EC 3.1.1.34) in the vascular endothelium of extrahepatic tissues hydrolyzes chylomicron-TG, resulting in the formation of a cholesterol-rich chylomicron remnant particle (Redgrave et al 1970). Apolipoprotein A-II is transferred from the chylomicron to HDL. Ultimately, the chylomicron remnant is removed from the circulation by the liver via a receptor specific for apolipoprotein E (Mahley 1989). After the chylomicron remnant has been taken up by the liver, it undergoes lysosomal digestion.

Lipids synthesized in the liver have three possible fates: secretion in VLDL, temporary storage as cytoplasmic oil droplets and cholesterol and PL can be secreted in bile (Dietschy & Wilson 1970). VLDL is the major lipoprotein produced by the liver from TG and cholesterol; the rate of VLDL synthesis and secretion seems to be related to the availability of fatty acids and the quantity of synthesized TG in the liver (Dixon et al 1991). Recent work by Yang et al (1996) disputes a previously held belief that VLDL TG is produced via the 3-glycerol-phosphate pathway. Instead, it appears that in rat hepatic tissue the MG pathway is active and is responsible for up to 70% of the TG synthesized and incorporated into VLDL. The presence of apolipoprotein B-100 on VLDL is essential to its secretion from the liver (Janero et al 1984). After release into the circulation, VLDL acquires CE from HDL along with apolipoprotein CII, CIII and E catalyzed by cholesterol ester transfer protein (CETP) and the HDL in turn receives TG from the VLDL. The presence of C-II allows lipoprotein lipase to hydrolyze the VLDL-TG. In addition to converting

chylomicrons to chylomicron remnants, lipoprotein lipase is crucial in the conversion of VLDL to IDL and then to LDL, as demonstrated in cynomologous monkeys which did not produce normal LDL when lipoprotein lipase was inhibited (Goldberg et al 1988). The importance of lipoprotein lipase and apolipoprotein CII to the metabolism of TG-rich lipoproteins is also demonstrated in the rare genetic disorders with lipoprotein lipase deficiency or apolipoprotein CII deficiency, which are characterized by severe hypertriglyceridemia and elevated total cholesterol. Apolipoprotein E is essential to the metabolism of VLDL, for both the conversion to LDL and the direct uptake by the liver (Mahley 1988).

Plasma LDL contains apolipoprotein B-100 as the exclusive apolipoprotein and CE as the major lipid. In humans, LDL is the major transporter of plasma cholesterol. The lifetime of LDL in the circulation seems to be dependent on the availability of LDL receptors, although in general the half-time of disappearance of LDL appears to be 2 d (Brown & Goldstein 1984). Approximately 60% of all LDL are cleared through the liver, with the remaining particles being cleared through other organs, such as the adrenal glands.

As mentioned earlier, one function of HDL is to act as a repository for apolipoprotein C-II and E that are required in the metabolism of chylomicrons and VLDL. HDL is secreted from both the liver and the intestine. When nascent HDL is secreted, lecithin:cholesterol acyl transferase (LCAT) converts surface phosphatidylcholine and free cholesterol into CE and lysoPL (Kostner et al 1987). The CE moves into the core of the HDL and the lysoPL are transferred to plasma albumin. Formation of CE allows more free cholesterol to be taken up by the HDL particle and the LCAT reaction continues until the HDL particle is spherical. Thus, the LCAT system is involved in the removal of unesterified cholesterol from tissues and lipoproteins. This is referred to as "reverse cholesterol transport". The cholesterol-enriched HDL exchanges its CE for TG from VLDL, via the cholesterol-ester transport protein (Tall 1993).

Albumin is a carrier for many compounds including FFA and lysoPL and given that albumin can traverse the blood-brain barrier via adsorptive endocytosis mechanisms (Juurlink & Devon 1990, reviewed in Irie & Tavassoli 1991), it may provide a potential route for delivery of FFA and lysoPL to the brain. Although albumin carries relatively small amounts of the LCPUFA compared with plasma lipoproteins, its rapid turnover could account for a significant transfer of LCPUFA to the CNS (reviewed in Dhopeshwarkar & Mead 1973). LysoPL represent 5-20% of total PL in mammalian plasma and are the second most prevalent PL in rat plasma (Nelson 1967). The lysoPL formed from the LCAT reaction usually contain a saturated fatty acid. However, when the *sn*-2 position of the PL contains an unsaturated fatty acid with more than 18 carbons, human LCAT demonstrates specificity for the fatty acid in the *sn*-1 position, rather than its usual specificity for the *sn*-2 position (Subbaiah et al 1992).

1.4.3.2. The uptake of fatty acids into the developing central nervous system

Despite great interest in the role of the n-6 and n-3 fatty acids and CNS development in the infant, little is known about the mechanisms involved in the transport, uptake and metabolism of fatty acids required for the developing brain. Although some fatty acids can be synthesized *de novo* in the CNS (16:0, 18:1), this is not true of 18:2n-6 and 18:3n-3. There are three potential sources of LCPUFA for the developing brain: preformed dietary LCPUFA; LCPUFA formed from hepatic or intestinal desaturation and elongation of dietary 18:3n-3 and 18:2n-6; and neural tissue desaturation and elongation of dietary-derived 18:3n-3 and 18:2n-6.

1.4.3.2.1. De novo synthesis of lipids in the CNS

Early *in vivo* experiments by Dhopeshwarkar et al (1969, 1970, 1971, 1971a) on the uptake of orally and intravenously administered [^{14}C]-16:0, 18:1, 18:2n-6 and 18:3n-3 by the adult rat brain determined that these four fatty acids were readily taken up across the blood-brain barrier. In

all experiments, effects from plasma contamination of the brain tissue and the possibility of oxidation of labeled fatty acids to acetate and resynthesis within the brain were accounted for in the final analysis. These results were confirmed in a subsequent experiment using the young rat (Anderson & Connor 1988). However, recent work using perdeuterated fatty acids incorporated into the diet, rather than radiolabelled fatty acids has produced conflicting results. Marbois et al (1992) and Edmond et al (1998) have shown in rat pups that perdeuterated dietary 16:0, 18:0 and 18:1 do not enter the brain, as 18:2n-6 and 18:3n-3 do, but rather these fatty acids are synthesized within the brain. Other work has shown that perdeuterated 20:4n-6 also enters the brain (Kulmacz et al 1986). The explanation for the difference in the findings is that using perdeuterated fatty acids and fast atom bombardment that it is possible to find the stable isotopic form of the fatty acid selectively and unambiguously (Marbois 1992). Perdeuterated studies have not been done with 22:6n-3, but studies in rats with [^{14}C]-22:6n-3, given both orally and intravenously (Scott & Bazan 1989, Li & Wetzel 1992) and with intravenous [^{3}H]-22:6n-3 (Martin 1994) have shown that radiolabelled 22:6n-3 crosses into the brain. Given that fatty acids such as 18:2n-6, 18:3n-3, 20:4n-6 and 22:6n-3 are taken up by the brain, but 16:0 and 18:1 are not, it seems probable that there are selective mechanisms for the uptake of specific fatty acids from plasma into the developing brain. Recent studies have identified specific fatty acid binding and transport proteins in brain tissue (Utsonomiya et al 1997, Xu et al 1996)

1.4.3.2.2. Mechanisms of transport and uptake of LCPUFA to the developing brain

Due to specificity of the acyltransferases (Lands et al 1982, Iritani et al 1984), LCPUFA are found in the highest amounts in PL and are predominantly transported in plasma as part of the lipoproteins. Quantitatively the highest amounts of PL are found in HDL and LDL (Table 1.7), suggesting that the PL of these lipoproteins could be a significant source of LCPUFA for the CNS.

Further supporting this hypothesis, LDL receptors have been detected by immunochemical methods in rat and monkey astrocytes and capillary endothelium (Pitas et al 1987, Dehouck et al 1994) and in white matter of humans (Rebeck et al 1993). However, the presence of receptors for LDL is not necessarily evidence of LDL uptake via receptor-mediated endocytosis. It is possible that the LDL particle, *in vitro*, is transported across brain capillary endothelial cells without significant degradation, despite the presence of functional lysosomal cells (Dehouck 1997). It is apparent that the pathway used to transcytose the LDL particle is different from the hepatic LDL pathway. The exact function and fate of the LDL particle in the brain is not known.

The low-density lipoprotein receptor-related protein (LRP), normally found in hepatic tissue, has also been detected in the CNS. Using *in situ* hybridization, LRP was found throughout rat neurons, but not astrocytes, at amounts equivalent to those found in liver (Bu 1994), while expression of LRP mRNA has been found in post-mortem human astrocytes (Moestrup 1992). Whether or not these proteins are involved in intracellular trafficking of cholesterol or other lipids in the brain, or in the uptake of lipids from plasma is not clear.

Chylomicrons have also been suggested to serve as carriers of 22:6n-3 to the brain (Scott & Bazan 1989). Hepatectomized rats injected with chylomicrons containing [^{14}C]-22:6n-3 showed a significant accumulation of [^{14}C]-22:6n-3 in the brain. However, it is possible that the [^{14}C] label was derived during [^{14}C]-22:6n-3 synthesis in brain itself, rather than the liver. This research, however, suggests a possible role for chylomicrons in the delivery of n-3 fatty acids to the brain (Anderson et al 1994). Lipoprotein lipase activity has been detected in the brains of the rat (Ben-Zeev et al 1990, Bessesen et al 1993, Nunez et al 1995) and guinea pig (Vilaro 1990), suggesting lipoprotein lipase-mediated hydrolysis of TG could provide fatty acids to the brain. However, if this

were a significant pathway for the delivery of 22:6n-3 to the brain, CNS problems should be evident in patients with lipoprotein lipase or apolipoprotein CII deficiency which has not been reported.

Evidence for the uptake of FFA into the CNS includes early work by Dhopeshwarkar et al (1973a), who demonstrated that when unesterified albumin-bound [^{14}C]-16:0 was injected into the carotid artery, ensuring first pass through the brain before the liver, or injected into rats that had been hepatectomized to prevent synthesis of lipoproteins, the specific radioactivity of the brain total lipids was 6x higher than that found for intact animals (control group). Later experiments using squirrel monkeys demonstrated that intravenously administered [^{14}C]-1-palmitoyl-lysophosphatidylcholine was rapidly taken up and metabolized by the brain (Illingworth 1972). This work was later criticized for the high concentration of lysoPL used and the questionable generalization to other species (Thiès et al 1992). More recent work utilizing 20 d old rats that were intravenously infused with physiological amounts of radiolabeled 2-arachidonyl-lysophosphatidylcholine, 2-palmitoyl-lysophosphatidylcholine, 2-oleoyl-lysophosphatidylcholine and 2-linoleoyl-lysophosphatidylcholine found that little of the labeled 16:0 appeared in the brain, consistent with Edmond et al (1991), but 18:1, 18:2n-6 and 20:4n-6 appeared in the rat brain within 10 minutes of injection (Thiès et al 1992). Oleic acid (18:1), 18:2n-6 and 20:4n-6 in lysophospholipids were more readily taken up by the brain, as compared with the unesterified form of the fatty acid (Thies et al 1994).

Similar research has occurred to determine the uptake of albumin-bound 22:6n-3-lysoPL in the brain. Young rats (20 d old) were perfused with unesterified [^{3}H]-22:6n-3 or *sn*-2-^{3}H-22:6n-3-lysoPL, bound to albumin (Thies et al 1994). Four to five percent of the injected radioactivity from the *sn*-2-[^{3}H]-22:6n-3 lysoPL was recovered in the brain in comparison to 0.3-0.4% recovered of

the unesterified form of [^{3}H]-22:6n-3. Further confirming these findings, a study using an *in vitro* model of the blood-brain barrier found preferential uptake of lysoPL-22:6n-3 over the FFA-22:6n-3 (Bernoud et al 1999). More recent efforts to characterize the metabolism of 22:6n-3 in the rat used oral [^{13}C]-22:6n-3 given as a single bolus, instead of an intravenous delivery (Brossard et al 1996). There was a significantly higher level of tracer resulting in plasma lysoPL-22:6n-3 than FFA-22:6n-3 and while the [^{13}C]-22:6n-3 accumulated progressively in brain PL without evidence of saturation over the 72h of the study, only 0.18% of the total label fed to the rats appeared in the brain. Very few studies on the transport of LCPUFA in lysoPL and FFA have been done in humans. One study of adult males (n=3) that were given an oral dose of [^{13}C]-22:6n-3 reported that the total [^{13}C]-22:6n-3 on albumin was 1.6 times less than that measured in the chylomicron/VLDL fraction and very little of the [^{13}C]-22:6n-3 was transferred to LDL or HDL during the course of the experiment (Brossard et al 1997). Furthermore, it was found that the albumin-bound unesterified fatty acid-[^{13}C]-22:6n-3 was utilized predominantly by platelets/leukocytes, possibly allowing for the albumin bound lysoPL-[^{13}C]-22:6n-3 to provide a source of LCPUFA for the brain (Brossard et al 1997). There is no data have been published on the relative amounts of 22:6n-3 in plasma FFA compared with lysoPL in human infants. Analysis of plasma to provide this information is one of the aims of this thesis.

1.4.3.2.3. The role of the CNS in providing a source of LCPUFA through the desaturation and elongation of dietary 18:2n-6 and 18:3n-3

The liver is generally considered to be the primary site of desaturation and elongation of the essential fatty acids 18:2n-6 and 18:3n-3 to 20:4n-6 and 22:6n-3, respectively (Naughton 1981, Scott & Bazan 1989). The developing CNS is also capable of desaturating and elongating 18:2n-6 and 18:3n-3 (Brenner 1981, Holman 1986). Specifically, the capillary endothelial cells and

astrocytes of the blood-brain barrier have been shown to be capable of desaturating and elongating 18:2n-6 and 18:3n-3 to 20:4n-6 and 22:6n-3, respectively. Using a culture of postnatal mouse capillary endothelial cells, Moore et al (1990) reported that the desaturation and elongation of 18:2n-6 to 20:4n-6 occurred readily and 18:3n-3 was readily desaturated and elongated to the primary product of 20:5n-3, but 22:6n-3 was not formed in the capillary endothelial cell culture. Subsequent research by Moore et al (1991) showed that 18:2n-6 and 18:3n-3 were desaturated and elongated to 20:4n-6 and 22:6n-3, respectively, by rat astrocytes. Thus, the blood-brain barrier, consisting of capillary endothelial cells and astrocytes, is capable of desaturating and elongating 18:2n-6 and 18:3n-3 to 20:4n-6 and 22:6n-3, respectively and potentially providing a source of these LCPUFA for the CNS.

In an effort to be more physiological, *in vitro* cultures of capillary endothelial cells and astrocytes have been developed that have a high correlation to *in vivo* values for interaction of substances with the blood-brain barrier (Dehouck et al 1992). Co-cultures of bovine capillary endothelium and rat astrocytes incubated with 18:2n-6 and 18:3n-3 resulted in significant production of 20:4n-6 and 22:6n-3 in the astrocyte (Bernoud et al 1998). These findings provide further evidence that the blood-brain barrier is capable of providing a source of LCPUFA for the developing brain. An *in vivo* experiment with fetal rats injected intracranially with 18:2n-6 and 18:3n-3 found a significant increase in 20:4n-6 in brain phosphatidylinositol and 22:6n-3 in brain phosphatidylethanolamine (Green & Yavin 1993). Although intracranial injections are not a physiologically relevant method of delivery of fatty acids, these findings further support the idea that the blood-brain barrier can potentially provide a source of the LCPUFA through desaturation and elongation of 18:2n-6 and 18:3n-3. The possibility that albumin-bound FFA are significant sources of 18:2n-6 and 18:3n-3 for the developing brain is, therefore, of considerable interest.

In summary, the CNS requires a source of LCPUFA during growth and development. Given the absolute requirement the CNS has for LCPUFA, it is not likely that there is only one mechanism available to ensure adequate CNS levels of LCPUFA. Published research indicates that LCPUFA can potentially be provided as a preformed source from human-milk, transported in lipoprotein PL or on albumin. For infants who are fed a formula without a source of LCPUFA, the essential fatty acids 18:2n-6 and 18:3n-3 can be metabolized in the liver to the LCPUFA and then transported to the brain as part of lipoprotein PL or on albumin as FFA or lysoPL. Finally, 18:2n-6 and 18:3n-3 may be also desaturated and elongated by the blood-brain barrier to 20:4n-6 and 22:6n-3, respectively, and taken up into the CNS.

1.5. Significance of Study

At the current time in Canada, 95% of women are exclusively breast-feeding at discharge from the hospital. However, by one-month post-partum, the incidence of breast-feeding has decreased to 60% and by four mo, a time point when many mothers return to work, the incidence of breast-feeding has been further reduced to 30%. This decline in breast-feeding over the first four mo is not an unusual finding, with the United Kingdom also having a prevalence of breastfeeding at four mo of only 27% (Foster et al 1995). Furthermore, cows' milk is not recommended for babies under the age of 12 mo due to the increased renal solute load and low iron content of cow's milk compared to human milk or formula (Canadian Paediatric Society et al 1998). This means that at one mo and four mo, approximately 30% and 70% of infants are relying on formula as a significant, if not sole source of energy. Research into the safety and efficacy of fatty acids is important and will advance the science of early fatty acid requirements and brain development.

Concerns have arisen over the fatty acid composition of infant formulae and their ability to support normal growth and development of the CNS. These concerns have stemmed from reports showing that animals fed a diet deficient in all n-3 and n-6 fatty acids have decreased cognitive and visual processes. Also, there are many reports showing that breast-fed infants tend to perform better on tests of cognitive function in childhood than bottle-fed infants. Thus, these studies are important in determining whether infants fed formula with theoretically adequate amounts of 18:2n-6 and 18:3n-3 have visual or cognitive behaviours that are different to the breast-fed infant. Additionally, these studies are important to contributing to a better understanding of the role the infant's environment plays in influencing their development.

Human milk and formulas in Europe, South America and Asia contain LCPUFA, whereas infant formula from North America does not. Also, the distribution of the fatty acids in human milk is

different from that of formula TG. Despite the importance of n-3 and n-6 fatty acids in the normal development of the CNS, little is understood about the distribution of these fatty acids in the plasma lipoproteins of the human infant. Furthermore, it is not known whether the presence of LCPUFA and the unusual TG fatty acid distribution in human milk results in a distribution of fatty acids in plasma lipoproteins of breast-fed infants that differs from that seen with formula-fed infants. The present studies will provide new information on how n-6 and n-3 fatty acids are transported in plasma of breast-fed and formula-fed term infants. Current approaches to determine the adequacy of dietary n-6 and n-3 fatty acids have tended to focus on the composition of plasma or erythrocyte PL. Whether or not these are valid measures of the adequacy of the dietary n-6 and n-3 fatty acids during growth and development is not clear. Information on differences in lipoprotein fatty acid distribution may give an indication of the distribution that must be achieved in formula-fed infants. Detailed information on the distribution of n-6 and n-3 fatty acids in blood lipids is also useful to guide studies on how these fatty acids are transported to the developing brain.

1.6. Rationale

In the last 30 years, research has suggested a role for n-3 and n-6 fatty acids in CNS development and subsequent infant behavior. As the central nervous system develops, there is significant accretion of the long-chain fatty acids 20:4n-6 and 22:6n-3. Breastmilk contains preformed LCPUFA, providing a dietary source for breast-fed infants. Formulae in North America do not contain a source of LCPUFA and it has been suggested that the fatty acid composition is not adequate to meet the needs of infants for normal membrane LCPUFA accretion (Carlson et al 1986). Some of the evidence offered for this are the reduced visual function in non-human primates (Neuringer et al 1986) and altered behavioral patterns in rats (Wainright 1992, Bourre et al 1989a) fed diets without a source of n-3 or n-6 fatty acids; lower LCPUFA in plasma and erythrocyte PL of infants fed formula rather than human milk (Innis 1992, Clark 1992, Ponder 1992); and reduced

visual acuity and cognitive performance in some studies of formula-fed infants (Birch et al 1993, Makrides et al 1995) but not all (Auestad et al 1995, Jensen et al 1997). Thus, it has been suggested that LCPUFA are essential dietary nutrients for the healthy term infant. The inconsistency in studies in term infant visual and behavioral development, general lack of control for significant environmental differences, and differences in the composition used in different studies make it difficult to draw conclusions on the essentiality of LCPUFA in the term infant's diet. The research in Study I was carried out to determine if there was a significant difference in preferential looking acuity and novelty preference between breast-fed term infants and infants fed formula considered to meet n-6 and n-3 fatty acid requirements from 18:2n-6 and 18:3n-3, but without 20:4n-6 and 22:6n-3. During Study I, however, it was apparent that environmental variables could potentially influence the infant's performance, and that data on this with respect to the tests used were not available. Therefore, Study III was designed to allow for collection of information on infant feeding history, demographic variables and visual and cognitive performance data with sufficient power (>400 infants) to consider the influence of some genetic and environmental variables on test performance.

Plasma and erythrocyte PL are routinely used biochemical measures of the adequacy of fatty acids in the infant's diet and indicators of the CNS n-6 and n-3 fatty acids (Innis 1992, Clark et al 1992, Ponder et al 1992, Carlson et al 1996, Clandinin 1997, Birch 1998). Information is not available on the distribution of n-6 and n-3 in the plasma lipoproteins of the term infant. Furthermore, it is not known if fatty acids within separated lipoproteins differ between the breast-fed and formula-fed infant, and data on infant plasma lysophospholipid and unesterified fatty acids which are potentially important sources of fatty acids for the CNS are not available.

It is known that human milk differs from infant formula not only in the presence of LCPUFA, but also in the distribution of fatty acids on the TG (Martin 1993). It is believed that the unusual

distribution of human milk TG fatty acids are the reason for the enhanced absorption of TG found for the breast-fed infant, in comparison to the infant fed a vegetable oil-based formula (Filer 1969). It is not known if the unusual TG configuration of breast-milk influences the distribution of fatty acids in the plasma lipoproteins. Study II, therefore, was designed to determine the influence of the fatty acid distribution of the human milk and formula TG on fatty acid distribution of lipoprotein lipids, lysophospholipid and unesterified fatty acids.

1.7. Research Hypotheses

1) Despite differences in dietary fatty acid intake and plasma and erythrocyte phospholipid 20:4n-6 and 22:6n-3, visual acuity and cognitive development is similar in infants fed formula with 1% 18:3n-3 to that of breast-fed infants.

2) The fatty acid distribution in human milk and formula triacylglycerol influences the distribution of fatty acids in chylomicron triacylglycerols, but is of no significance to the distribution of 20:4n-6 and 22:6n-3 in the chylomicron, LDL or HDL.

3) The long-chain n-6 and n-3 fatty acids, 20:4n-6 and 22:6n-3, are predominantly distributed in high-density lipoprotein phospholipid.

1.8. Thesis Objectives

This thesis had three broad objectives

A. Study the effect of human milk and formula fatty acid composition on plasma and erythrocyte phospholipid fatty acids and on visual and cognitive development in breast-fed infants and infants fed formula.

B. Study the implication of the unusual distribution of fatty acids in human milk triacylglycerol on the distribution of unsaturated and saturated fatty acids in plasma lipids and lipoproteins of breast-fed infants and infants fed formula.

C. Study the relative distribution of n-6 and n-3 fatty acids in lipoproteins and other plasma lipids from breast-fed infants and infants fed formula.

1.9. Specific Aims of the Thesis

1) To determine the relation between measures of visual and cognitive development and plasma n-6 and n-3 fatty acids among breast-fed infants and infants fed formula.

2) To determine if the position of the fatty acids in triacylglycerols of human milk and formula influence the distribution of 16:0, or the n-6 and n-3 fatty acids in triacylglycerol and phospholipid in plasma lipids of infants.

3) To determine where the dietary 18, 20 and 22 carbon chain n-6 and n-3 fatty acids are carried in plasma, specifically the distribution in plasma lysophospholipids and free fatty acids and distribution in phospholipid, triacylglycerol and cholesterol esters of separated lipoproteins.

2. METHODS AND MATERIALS

2.1. Chemicals

Enzymatic kits for assays of total cholesterol and triacylglycerols were obtained from Diagnostics Chemicals, Charlottetown, PE, Canada and the kit for unesterified cholesterol was obtained from Boehringer Mannheim, Dorval, QC, Canada. The kit for measurement of unesterified fatty acids was obtained from Wako Chemicals USA, Inc, Richmond, VA, USA. Kits for assays of Apolipoprotein A-1 and B were purchased from Incstar Corp., Stillwater, MN, USA. All other chemicals were reagent grade and purchased from Sigma Chemical Co., St. Louis, MI and BDH Chemicals Canada Ltd., Vancouver, British Columbia. Authentic lipid standards for thin-layer chromatography (TLC), gas-liquid chromatography (GLC), porcine pancreatic lipase and phospholipase A_2 for positional analysis were purchased from Sigma Chemical Co., St. Louis, MI and NuChek Prep, Elysian, MI.

2.2. Equipment

Low-speed centrifugation (<3000 *g*) was performed in a J-6B low-speed centrifuge with a swinging basket rotor from Beckman Instruments (Canada), Inc., Mississauga, ON. Centrifugation at 435 000 *g* was done in an ultracentrifuge from Beckman Instruments (Canada), Inc., (model no. TL100) using a fixed angle rotor (T100.2). Fatty acid methyl esters were separated and quantified by GLC using a 3400 gas liquid chromatograph from Varian Canada, Inc., Mississauga, ON, equipped with flame ionization detection and an IBM computer system using Varian "Star" software. Glass capillary SP 2330 columns (30 m x 0.2 mm) were from Supelco Canada Ltd., Oakville ON, were used. Spectrophotometric analysis of assay products was done with a Beckman DU 640 Spectrophotometer (Beckman Instruments, Inc, Fullerton CA). Preferential looking acuity was assessed using the Teller Acuity Card system from Vistech, Inc., Dayton, Ohio. Novelty

preference was assessed with the Fagan test system from Infantest Corporation, Cleveland Heights, Ohio.

2.3. Study I - Effect of dietary fatty acids on plasma and erythrocyte fatty acids and development of visual acuity in term infants

2.3.1. Study Design

This study was a prospective, non-randomized trial with breast-fed infants and infants fed formula. The study was unblinded because randomization to breast-feeding and formula feeding is ethically impossible. Blood lipid fatty acids and preferential looking acuity were compared between a group of infants fed a ready-to-feed liquid formula (**Table 2.1**) (Mead Johnson Nutritionals, Evansville, IN) and a group of infants breast fed to 120 d of age. Novelty preference and mental and motor development with the Bayley Scales of Infant Development was also measured but was not part of the primary hypothesis for infants in the study.

2.3.2. Recruitment and characteristics of study population

All infants were recruited from BC Women's Hospital and Health Centre Society, Vancouver, B.C. Eligible study participants were healthy male or female infants of term gestation between two and 16 d of age, with a birth weight of 2500 g to 4500 g and exclusively fed formula for at least the 24 h prior to enrolment (formula-fed group), or exclusively breast-fed from the day of enrollment (breast-fed group). All parents who agreed to participate in the study were required to sign a consent form.

Infants were not eligible for the study if they had a history of underlying disease or congenital malformation that could interfere with the study or showed evidence of feeding intolerance and/or poor milk or formula intake. Infants born less than 2500 g or over 4500 g weight, who were premature (<37 wk gestation) or of multiple births were also ineligible. Infants were

excluded from the study if they were not fed specifically with the study formula or human milk from enrollment to 120 d, or if the parents decided to withdraw from the study after giving consent.

The protocol and its procedures were approved by The University of British Columbia Screening Committee for Research Involving Human Subjects and by the Research Screening Committee of the British Columbia Children's Hospital.

2.3.3. Study procedure

Mothers of eligible, healthy, term, formula-fed or breast-fed infants were contacted while in B.C. Women's Hospital and Health Centre Society, Vancouver, B.C. within 12 to 48 h after giving birth. A requirement of the study was that the mother's medical chart had noted the mother's intent to breast-feed or feed formula before the mother could be approached about participating in the study. The study was explained to mothers with eligible infants and if they agreed, written informed consent was obtained. Formula-fed infants could consume any marketed formula until the day of enrollment, at which time they were provided with the study formula. Parents of infants fed formula were required to feed the study formula as the sole diet until the infant was 90 d of age and as the only formula to 120 d of age with the frequency and amount of feeding at the discretion of the parent. The study formula feeding period, in total, was from 14±2 d to 120±4 d of age. Mothers of breast-fed infants were required to intend to continue to breast-feed exclusively for at least the first 90 d. Breast-fed infants could receive no more than 175 mL formula/day after 30 d of age. Solid foods or juice could not be fed for the first 90 d after birth.

Infants were seen at 14±2, 30±3, 60±3 and 90±4 and 120±4 d of age, and then at eight, 10, 12 and 18 mo of age. Preferential looking acuity assessments were made at 14±2, 90±4 d of age, prior to blood sampling and at four, eight and 18 mo of age, using the Teller Acuity Card procedure as described in Section 2.8.1. The Fagan Test of Infant Intelligence was done at eight, 10 and 12

mo of age as described in Section 2.8.2. Bayley Scales of Infant Development (1969) were done at four, eight and 18 mo, using standard procedures, in the Neonatal Follow-up Clinic at British Columbia Children's Hospital. The tests at four and eight mo were administered by a trained occupational therapist and a psychologist administered the 18 mo test, using standard clinic procedures. Anthropometric measures including weight, length and head circumference were collected (as described in Section 2.7) at each age except 10 and 12 mo.

Venous blood (3.0 mL) was drawn from the arm for analyses of blood lipids at 14±2 and 90±4 d of age. Ethylenediamine tetraacetic acid (EDTA) was used as the anti-coagulant. All infants had been fed within three hours of the blood draw. The blood was held on ice and quickly transferred to the Research Centre for separation of plasma and erythrocytes. The plasma and erythrocytes were frozen at -80°C for later analysis of PL fatty acids.

2.3.4. Fatty acid composition and distribution in the triacylglycerol of human milk and formula

The fatty acid composition of the human milk collected in these studies (**Table 2.1**) was similar to that reported by other North American and European studies (Innis 1992). Linoleic acid (18:2n-6) and 18:3n-3 represented (mean±SEM as % of total fatty acids) 13.4±0.8% and 1.5±0.1%, respectively. The infant formula used in these studies had 17.9% 18:2n-6 and 2.1% 18:3n-3 with 0.5±0.03% 20:4n-6 and 0.2±0.02% 22:6n-3 with no detectable 20 or 22 carbon n-6 or n-3 fatty acids (Table 2.1). Thus, the absence of a source of 20:4n-6 and 22:6n-3 in the formula in this study, with levels of 18:2n-6 and 18:3n-3 which meet current dietary recommended daily intakes, allowed consideration of the effects of the breastmilk and formula feeding on plasma and erythrocyte fatty acids and preferential looking acuity in term infants. The infant formula contained a similar amount of 16:0 (22.3%) as the human milk. However, the fatty acids at the 2 position of the human milk TG had 54.2% 16:0, indicating that over 80% of the milk total 16:0 was esterified to the

TG 2 position. The formula had only 4.8% of 16:0 in the fatty acids esterified at the 2 position of the formula fat. In contrast to 16:0, only about 14% of the total 18:1 and 20% of the total 18:2n-6 in the milk fat was esterified to the TG 2 position (Table 2.1). The longer chain n-6 and n-3 fatty acids, 20:4n-6 and 22:6n-3, were approximately equally distributed between the *sn*-2 and the *sn*-1/3 positions, with about 46% of the 20:4n-6 and 66% of the 22:6n-3 being recovered at the TG 2 position (Table 2.1). In contrast, the formula had approximately 53% of the 18:1 and 51% of 18:2n-6 esterified to the TG 2 position. These differences in positional distribution of fatty acids in the milk and formula TG allowed for study of the potential effects of the dietary TG distribution on the distribution of fatty acids in the infant plasma TG.

Table 2.1 *– Human milk and infant formula fat total and sn-2 position fatty acids*

	Human milk		Formula	
	Total	TG 2 Position	Total	TG 2 Position
Fatty acid		g/100 g		
12:0	4.1±0.4	2.5±0.4	8.9	4.5
14:0	5.5±0.4	6.2±0.8	4.7	1.0
16:0	21.0±0.5	54.2±1.5	22.3	4.8
18:0	7.1±0.3	2.9±0.4	5.1	1.3
16:1	3.1±0.2	3.5±0.3	0.2	0.2
18:1	40.2±0.7	17.1±0.8	37.1	58.8
18:2n-6	13.4±0.8	8.1±0.7	17.9	27.1
18:3n-3	1.5±0.1	0.9±0.1	2.1	1.8
20:2n-6	0.4±0.0	0.1±0.0	nd	nd
20:3n-6	0.4±0.0	0.2±0.0	nd	nd
20:4n-6	0.5±0.0	0.7±0.1	nd	nd
20:5n-3	0.1±0.0	0.1±0.0	nd	nd
22:4n-6	0.1±0.0	0.2±0.0	nd	nd
22:5n-3	0.2±0.0	0.3±0.0	nd	nd
22:6n-3	0.2±0.0	0.4±0.0	nd	nd

Results for human milk represent mean ± SEM of n=17 mid-feed milks collected when the infants were three mo old. The formula fat blend was (% by vol) 20% coconut oil, 45% palm olein oil, 20% soy oil and 15% high oleic sunflower oil. Values for formula are the average of three separate analyses; values<0.1 indicates value >0.00 and <0.0.5%; nd, not detected.

2.4. Study II – Effect of human milk and infant formula triacylglycerol fatty acid distribution on plasma lipids and lipoprotein fatty acid distribution in term infants

2.4.1. Study Design

This study was a controlled, randomized, prospective study using two different ready-to-feed formulae. Breast-fed infants were studied as an unblinded, non-randomized, but parallel and prospective group. Blood lipids were compared among groups of infants fed one of two ready-to-feed liquid formulae or breast-fed at 30 and 120 d of age.

2.4.2. Recruitment and characteristics of study population

All infants were recruited from the B.C. Women's Hospital and Health Centre Society, Burnaby General Hospital or Surrey Memorial Hospital. Procedures for enrolling the infants, inclusion and exclusion criteria were identical to Study I (see Section 2.3.2). Briefly, eligible study participants were full-term infants (37-42 wk gestation), birthweight, 2500-4500 g, who were less than 72 h old and either exclusively breast-fed or fed formula. Infants were considered ineligible for the study if they were <37 or >42 wk gestation, <2500 g or >4500 g, or had any suspected or known metabolic or physical problems which could interfere with feeding or normal metabolism. Mothers of breast-fed infants were requested to exclusively feed their own breast milk until the infant was at least 120 d old. Infants who were exclusively fed with formula were randomized at enrollment to one of the two study formulae and then fed exclusively with the assigned formula from 72 h until 120 d of age. Infants in the formula groups were randomized to the formulae using a random number system and the formulae were coded so that the identity of the product was not known.

The protocol and procedures for this study were approved by the University of British Columbia Screening Committee for Research Involving Human Subjects and by the Research Screening Committee of the BC Children's Hospital and the Ethics Committee of Surrey Memorial Hospital and Burnaby General Hospital.

2.4.3. Study procedure

Infants were seen at 7±2, 30±2, 60±2 and 120±2 d of age. Weight, length and head circumference was measured at each study visit as described in Section 2.7. Preferential looking acuity was assessed at 120±2 d of age, prior to blood sampling, as outlined in Section 2.8.1. In addition, the parents recorded the infant's dietary intake and stool pattern records for the three d prior to each scheduled study visit. This information was used to assess dietary compliance of each infant.

Venous blood (3.0 mL) was drawn, using EDTA as the anti-coagulant, from the infant's arm for analysis of blood lipid fatty acids at 30±2 d and 120±2 d of age. All infants were in an unfasted state, not having gone longer than three hours since their last feed. Plasma and erythrocytes were separated as described in Section 2.6.1. The plasma was used for the separation of lipoprotein lipids, for assessment of the positional distribution of fatty acids in TG and PL and for quantitation of plasma lipids. The erythrocytes were used for separation and analysis of phosphatidylcholine and phosphatidylethanolamine fatty acids as described in Section 2.6.4.1.

2.4.4. Fatty acid composition and distribution in the triacylglycerols of human milk and infant formula

The two formulae differed only in the composition of the oil blend and distribution of the fatty acids in the TG (**Table 2.2**). The standard formula contained palm-olein, soy, coconut oil and high-oleic sunflower oil (Ross Laboratories, Columbus, OH). The synthesized TG formula contained

Betapol-2® (Loders Croklaan, Wormerveer, The Netherlands), which is a synthesized TG.

Table 2.2 *- Fatty acids in human milk and formula TG and in the 2 position of the TG*[1]

	Total TG			TG 2 position[4]		
Fatty acid	Human Milk (n=25)	Standard Formula[2]	Synthesized TG formula[3]	Human milk (n=15)	Standard formula	Synthesized TG formula
	(g/100 g)					
14:0	7.2±0.3	3.2	3.7	7.5±0.9	0.9	1.6
16:0	23.1±0.6	27.2	24.8	56.4±1.1	5.0	29.1
18:0	7.4±0.2	5.3	5.2	1.9±0.1	0.8	2.2
18:1	43.8±1.8	41.1	39.5	15.3±0.8	56.8	34.6
18:2n-6	14.2±0.5	22.3[3]	23.4	9.4±0.9	32.7	28.4
20:2n-6	0.4±0.0[2]	nd	nd	0.2±0.0	nd	nd
20:3n-6	0.3±0.0	nd	nd	0.2±0.0	nd	nd
20:4n-6	0.5±0.0	nd	nd	0.8±0.1	0.1	0.1
22:4n-6	0.1±0.0	nd	nd	0.3±0.0	nd	nd
22:5n-6	0.1±0.0	nd	nd	0.1±0.0	nd	nd
18:3n-3	1.9±0.1	2.2	2.6	1.3±0.2	2.0	2.6
20:5n-3	0.1±0.0	nd	nd	nd	nd	nd
22:5n-3	0.2±0.0	nd	nd	0.3±0.0	nd	nd
22:6n-3	0.3±0.0	nd	nd	0.6±0.1	nd	nd

[1]Values for human milk are means±SEM and for formula are the means of three separate analyses;
[2]The standard formula had, by vol fat, 48% high oleic palm oil, 26 % soybean oil, 14 % high oleic sunflower oil and 12% coconut oil; 0.0 indicates value ≥0.01 - < 0.05; nd, not detected.
[3] The synthesized TG formula had Betapol-2®, a synthesized TG; both formulae contained 37- 37.5 g of fat per 100 mL formula..
[4]The human milk, standard formula and synthesized TG formula had 81%, 6% and 39% of the total 16:0 esterified in the TG 2 position, respectively.

2.5. Study III - Influence of environmental and dietary factors on measures of novelty preference and visual acuity at nine mo in term infants

2.5.1. Study Design

This study was a cross-sectional study of infants at 39 ± 1 wk (9 mo) of age.

2.5.2. Recruitment and characteristics of study population

All full-term (39-41 wk gestation) infants with birth weights of 2500 to 4500 g born between January 1 and March 2, 1993, or between June 4 and August 7, 1993, to parents resident in Vancouver were eligible to participate in this study. A sample of infants was systematically identified for participation in the study using birth and death lists provided by the City of Vancouver Public Health Department. A letter was sent to parents/guardians of infants identified as eligible, inviting them to participate in this study by attending a clinic offering assessment of their infant's iron status. The letters were mailed to the parents about three wk in advance of clinics, which coincided with the time the infant would be 39 ± 1 wk of age. The letter was followed by a telephone call about one wk later to describe the study and determine if parents/guardians were interested in participating in the study. Three to six attempts were made to contact the parent by telephone, at varying times through the day. If the parent/guardian agreed to participate in the study, an appointment was made to attend a clinic at a time suitable for the parent/guardian. Informed, written consent was obtained from a parent for all infants who participated. The protocol and its procedures were approved by the University of British Columbia Screening Committee for Research Involving Human Subjects.

2.5.3. Study procedures

The clinics were held at Public Health Units, Community Centres and Neighbourhood Houses in the north, south, east and west sections of the City of Vancouver. Clinics were

scheduled to run in the morning, afternoon or evening on weekdays or during the morning on Saturdays. All the clinics provided an opportunity for interested parents to obtain nutrition counselling after completion of the questionnaires. Refreshments were available for the study participants and toys and video entertainment were usually available to help occupy older siblings.

Preferential looking acuity was assessed using the Teller Acuity Card procedure with a test distance of 55 cm as outlined in Section 2.8.1 and novelty preference was measured with the Fagan Test of Infant Intelligence (FTII) during the clinic appointment as described in Section 2.8.2, using the 79 wk (post-conception age) test (version 4.1) appropriate for infants age 39±1 wk of age. Each infant's weight, length and head circumference was measured using methods outlined in Section 2.7.

2.5.4. Study questionnaire

Parents were given two questionnaires to complete - one on information regarding their family background and the other on their infant's nutritional history. In developing the questionnaires, several steps were taken to ensure a high credibility of the instrument. Consultation was made with Ruth Milner, then Head of the Research Support Group at B.C. Research Institute for Child and Family Health and with Vancouver Health Department Public Health Nutritionists to address the content validity of the questionnaires. The questionnaires were pilot-tested to address their face validity, as well as their content validity, with a group of eight mothers from average income homes who had completed a high school education. The mothers were asked to complete the questionnaires, and then give constructive criticism on the wording of the questions and comment on the content, how comfortable they felt answering the questions and if anything should be added. Revisions to the questionnaires were then made, such as adding reasons for why mothers stopped breast-feeding. The revised questionnaires were then taken for comments from the City of Vancouver Public Health Nutritionists and Ruth Milner and final revisions were made.

The questionnaires were translated into Cantonese in order to allow for greater participation of Chinese persons and to obtain a better representation of the lower mainland population. These questionnaires were translated back into English by an independent, second individual to assure accuracy of the translation. Clinics that occurred in a community with a high population density of Chinese people had nutritionists available that could speak Chinese.

The diet history questionnaire was designed to collect current and retrospective information that would allow for the identification of feeding practices associated with risk for iron-deficiency anaemia, specifically breast-feeding and feeding with iron-fortified formula, low iron formula or cows' milk. Thus, the diet history included questions on: duration of breast-feeding, age of introduction of formula, type of formula used and brand name, age of introduction and types of cows milk used, as well as age of introduction of solid foods including cereals, vegetables, fruits, dairy and other animal products, fruit juices and vitamin/mineral supplements (**Appendix 6**). For parents who had chosen to formula-feed their infants, accurate recall of specific formula brands was fostered by having different infant formulae available. Infants were considered to be breast-fed as long as the intake of cows' milk or formula did not exceed more than two 177 mL bottles/wk. An infant was considered as never having been breast-fed if the infant was fed formula with no breast-feeding by seven d of age. Infants who were both breast-fed and fed formula were grouped as mixed feeders and were not analyzed further. Parents were instructed in the method for completion of the questionnaires and assisted by a trained nutritionist.

A separate questionnaire was used to assess the family's background and some aspects of the home environment. The questionnaire asked for information on family income, highest level of maternal and paternal education, infant gender, marital status and language spoken in the home, ethnic background and number of siblings (Appendix 6).

The research nurse or nutritionists working on the project instructed the parents/guardians in the correct method of completing the questionnaire. These personnel were readily available to help or answer queries if needed. The information on family background was collected on a separate questionnaire to ensure confidentiality. The infant's study number was identified on all the forms, but no names or addresses were on the nutritional history or family background forms. A nutritionist reviewed the nutritional history to ensure completeness of the questions. Any problems or inconsistencies in the information recorded were then discussed with the parent/guardian and corrections made if necessary.

2.6. Sample Preparation and Laboratory Analyses

2.6.1. Plasma and erythrocyte separation

Plasma was separated from erythrocytes immediately after collection by centrifugation (3,000 *g* for five min at 4°C). Erythrocytes were washed three times with an equal volume of 0.9% (wt/v) NaCl with 15% (wt/v) EDTA to remove remaining plasma. Aliquots of plasma and erythrocytes were stored at -70°C until further analysis.

2.6.2. Lipoprotein separation

A 0.5 mL aliquot of plasma was utilized for the separation of lipoproteins, within 1h of blood collection, as described by David (1986). Lipoproteins were isolated using sequential ultracentrifugation (Beckman TL100 Tabletop Ultracentrifuge, Beckman Instruments, Inc. Palo Alto, CA) at 436,000 x g, 15°C, with the plasma chylomicrons collected at two h, d=1.006 g/dL, LDL after a further 2.5 h, d=1.063 g/dL and HDL at a further 3h, d=1.21 g/dL. The small band representing the VLDL was not recovered. Accuracy of the lipoprotein separation using the methodology was confirmed using gel electrophoresis (Corning Universal, Palo Alto, CA) and immunoprecipitation

analysis (Incstar Corp. Stillwater, MN) to demonstrate the absence of apolipoprotein B in the HDL and of apolipoprotein A-1 in the LDL fractions.

2.6.3. Analysis of plasma lipids

For all enzymatic kit assays, standards were provided to allow for construction of a standard curve. Quality control samples were included with the kits and were run with each set of assays. Total and free cholesterol and TG were measured using 20 μl aliquots of plasma, made up to 50 μl with distilled water, with cholesterol and glycerol, respectively, as standards. Unesterified fatty acids were analyzed using 50μl plasma and apolipoprotein A-1 and B were determined by immunoprecipitation with 20μl plasma, following the respective kit instructions. HDL cholesterol was determined after precipitation of the apolipoprotein B-containing lipoproteins using the method of Gidez (1982) modified for small samples, utilizing a 0.1 ml aliquot of fresh plasma, 0.5 mL heparin (10,000 units/mL), one mL $MnCl_2$ (2.0 M) and 0.5 mL NaCl (0.15 M). The inter-and intra-assay coefficients of variation for all assays were determined by analyzing piglet plasma in five replicates and over two d (**Table 2.3**).

Table 2.3 *– Inter and intra-assay coefficients of variation (%) for plasma lipid assays.*

	Inter-assay	Intra-assay
Total cholesterol	2.1	1.6
Free cholesterol	1.9	1.1
Triacylglycerol	3.2	1.9
HDL cholesterol	3.7	2.5
Unesterified fatty acids	1.8	1.5
Apolipoprotein A1	4.3	3.2
Apolipoprotein B	4.8	3.7

2.6.4. Lipid extraction, thin-layer chromatography and gas-liquid chromatography for analysis of fatty acids

2.6.4.1. Lipid extraction

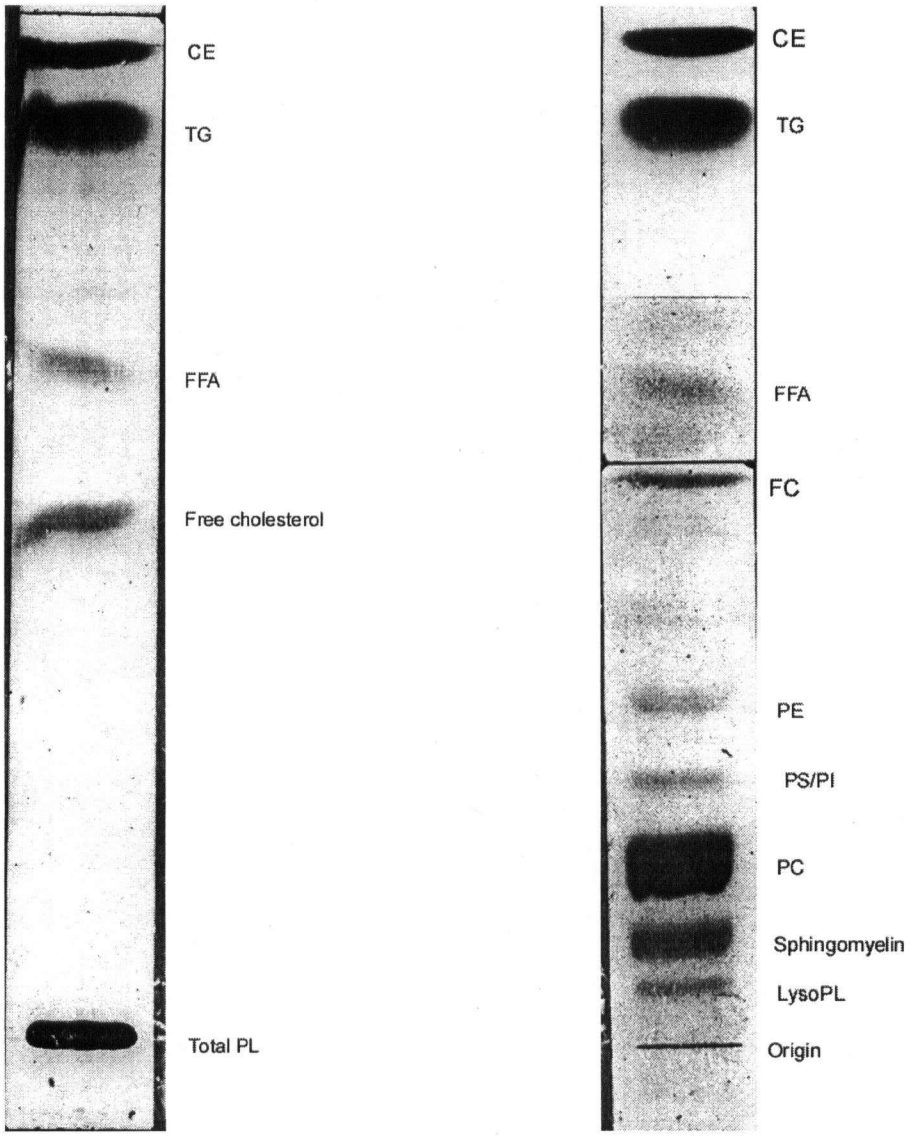
Total lipids were extracted using the Folch method (1957) for all samples with the exception of erythrocyte lipids, which were extracted by the procedure of Rose and Oklander (1965). This method uses chloroform/isopropanol to avoid attracting heme pigments. The total lipid extracts from plasma and erythrocytes were dissolved in chloroform-methanol (2/1, v/v), for separation of lipid classes by thin-layer chromatography (TLC).

2.6.4.2. Plasma and erythrocyte lipid class separation by thin-layer chromatography

Total PL, TG and CE were separated by TLC using silica plates (Whatman PK6F Silica Gel 60 Å, 20x20cm, 500μm) with a solvent system of petroleum ether-diethyl ether-glacial acetic acid (85/15/3, v/v/v) (Hrboticky 1990). Bands corresponding to TG and CE were visualized under ultraviolet light after spraying with 2'7'-dichlorofluoroscein (**Figure 2.1a**). All lipid classes were eluted from the silica with chloroform-methanol (2/1, v/v), TG fatty acids were transmethylated at 100°C for 30 min in one mL BF_3-benzene-methanol (25/20/55, v/v/v) and CE were transmethylated at 100°C for 45 min in one mL BF_3-benzene-methanol (35/30/35, v/v/v). The plasma PL band was transmethylated in one mL methanol-HCl (5/1, v/v) for five min at 100°C.

Plasma lysophospholipids (lysoPL) were isolated using a two solvent TLC system. Total PL was separated from all other lipid fractions by TLC with the petroleum-ether-diethyl ether-glacial acetic acid solvent system as outlined above (Figure 2.1a). The silica plate was allowed to dry thoroughly before being placed back in the same solvent system. The second run allowed all lipid fractions, except PL, to run higher on the silica plate. The plate was again dried thoroughly and

then a third run was done using chloroform-methanol-acetic acid-water (75/5/25/2, v/v/v/v) as the solvent system (Skipski 1964). This last solvent system allowed for separation of the lysoPL from other PL fractions (Figure 2.1b).



a) separation from 1st solvent system

b) separation from 2nd solvent sysytem

Figure 2.1 – Thin-layer chromatography plates with a) initial separation of plasma lipid classes with petroleum ether-diethyl ether-glacial acetic acid; and b) subsequent separation of PL classes with chloroform-methanol-acetic acid-water system.

The erythrocyte PC and PE were separated from the erythrocyte total lipids by TLC on silica gel plates using chloroform-methanol-acetic acid-water (25/15/4/2, v/v/v/v) as the solvent system (Skipski 1964). The bands containing the erythrocyte PE and PC were eluted from the silica and transmethylated in one mL BF_3 for 10 min at 100°C. All samples were stored at -70°C until analysis of fatty acids using gas liquid chromatography (GLC).

2.6.4.3. Milk and formula total fatty acids

Frozen milk samples were thawed in cold water, with lipase activity prevented by rapidly heating to and maintaining at 80°C for one min (Bitman 1983). A 0.1 mL aliquot of human milk, equivalent to about 600 µg of lipid and 500 µg of C17:0 internal standard, was methylated with two mL methanol-benzene (4/1, v/v) by a modification of the direct transesterification method of Lepage and Roy (1986). While vortexing, 200 µl acetyl-chloride was added, and then the tubes capped tightly and heated at 100°C for 1h. During this time, the tubes were vortexed at 15 min intervals. The samples were neutralized by the addition of five mL of 6% (wt/v) K_2CO_3 then recapped and vortexed for three min. Methyl esters were recovered by two extractions with four mL pentane. The pooled pentane layers were dried under nitrogen gas and stored at -70°C until GLC analysis.

2.6.4.4. Analysis of fatty acids by gas-liquid chromatography

Fatty acid methyl esters were separated using gas liquid chromatography. Separation was achieved on 30m x 0.25 mm ID, 0.20 µm film nonbonded, fused silica capillary SP 2330 columns (Supelco, Belefonte PA). Helium was used as the carrier gas, at a column flow of one 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 with the oven temperature programmed to remain at 80°C for two min, then increase to 170°C at 20°C/min, held for 25 min, then rise to 195°C at 20°C/min and hold for 18 min. The column was then heated to 245°C at 20°C/min and held for 20 min before subsequent

analyses. The injectors and detectors were set at 240°C 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, for 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 16:1, 18:1, 20:1, 22:1, and the saturated fatty acids 10:0, 12:0, 14:0, 16:0, 18:0, 20:0, 22:0. Values for 18:1 fatty acids given are for total 18:1 isomers. Fatty acid peak areas were computed by a chromatography data system (Varian 3400 with an IBM computer system using Varian "STAR" software, Guelph, Ont). The response of the detector to equivalent weights of fatty acids across the range of C16 to C22 fatty acids was similar. The consistency of the flame ionization detector response and the GLC column resolution was checked monthly by injecting a calibration mixture containing known amounts of authentic fatty acid methyl ester standard mixtures.

2.6.5. Positional fatty acid analysis of triacylglycerols and phospholipids

The fatty acid composition of the 2 position of the TG was analyzed by a modified method of Kuksis (1984). Briefly, TG was dissolved in 200 µl of petroleum ether and vortexed. After the addition of 600 µl of 1M TRIS buffer (pH 8.0), 100 µl of 1M $CaCl_2$ 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 *sn*-3 positions of the TG. The lipids were extracted from the reaction mixture with two mL diethyl ether and purified by TLC on pre-cleaned PK6F Whatman hard plates. The plates were developed in hexane-diethyl ether-acetic acid (60/40/1.5, /v/v/v). The 2-MGs were identified, using a mono-olein lipid standard. Following addition of 17:0 as an internal standard, the 2-MG was methylated with two mL methanol-benzene (4/1, v/v) by a modification of the direct transesterification method of Lepage and Roy (1986), as described for milk total lipid (Section 2.6.4.3).

2.7. Anthropometric Measure

Measurements of body weight, length and head circumference were obtained using the same standardized procedures in all the studies. No infants were below the 5th percentile for weight, or weight for length, with reference to the National Center for Health Statistics growth percentiles (Hamill 1979).

2.7.1. Body weight

The weights of the infants were measured using an electronic balance (Digital Baby Scale model 727, Lux and Zwingenberger Ltd, Lakeshore, Toronto, Ontario) accurate to five g. The infant's weight was recorded immediately on an anthropometric data form. Two weight measures, without clothing, were taken for Study I and II. When there was greater than a 20 g difference between the two measures, a third measure was done. In Study III, the nine mo old infants were weighed once, with only a diaper.

2.7.2. Length

Crown to heel length was measured in the recumbent position using a pediatric length board (Ellard Instrumentation Ltd, Seattle WA) accurate to the nearest millimetre. A parent held the infant's head in contact with the fixed headboard, while the examiner held the infant's feet, toes pointing directly upward while applying gentle traction. The movable footboard was brought to rest firmly against the infant's heels and the length was read from the base of the footboard. The infants in Study I and II were measured twice. A third measure was done if the difference between the first two measures was greater than three cm. Only one measure was taken for the nine mo old infants in Study III.

2.7.3. Head circumference

Head circumference was measured to the nearest mm using a disposable paper tape (Mead Johnson, Evansville IN) placed over the part of the head that gives the maximum circumference (Gibson 1990). The measure was done twice for the infants in Studies I and II and if the difference in the measures w greater than two cm, then a 3rd measure was done. Head circumference was only measured once for each infant in Study III.

2.8. Developmental Measures

2.8.1. Teller Acuity Card Test

Binocular preferential looking (visual) acuity was tested by using the acuity-card procedure (McDonald MA 1985) with Acuity Cards from Vistech Inc (Dayton, OH) essentially as outlined in the *Teller Acuity Card Handbook*. Furthermore, training was provided by one of the original developers of the Teller Acuity Card test, Velma Dobson, at The Seattle Children's Hospital, Seattle Washington.

The Teller Acuity Card test is a behavioural measure of preferential looking acuity that relies on the inherent tendency of infants to gaze at a discernable visual stimulus. The Teller Acuity Cards consist of a series of cards, each containing a grating (stripes) imbedded in a luminance matched gray background placed on one side of the card. The testers, who are blind to the left or right position of the stripes, watch the infant's looking behaviour through a small central hole in the card. The cards are presented to the infants in stepwise sequence from the coarsest to finest grating, commencing with the age-appropriate start card, as stipulated in the *Teller Acuity Card Handbook and* progressing in 0.5-octave steps. Acuity was assessed by presenting the card to the infant with the stripe position (left or right) unknown. The infant's response, including eye, head and body movement was noted and then the card position was reversed. Again the infant's behaviour was noted. Finally, a third switch was done of the card. If the infant's behaviour

consistently switched with the change in the card position, then the tester made a guess as to the positioning of the stripes. If the guess was correct, then it was assumed the infant could see the stripes on the card. The tester would then progress to the next card and repeat the procedure. If the infant's behaviour was not consistent with the card switches, then it was possible that the infant was not paying attention to the cards. The infant's interest in the cards would then be reinforced with a card with large stripes, and then the tester would again present the original card. Acuity was recorded as the finest grating that the infant could reliably and repeatedly resolve. The infants were tested at an age-appropriate distance under controlled lighting conditions by trained testers. Each infant underwent two separate tests, each by a different tester. Acuity measures were transformed to $\log_{10}$ cycles/degree (cyc/deg), and then separate values for each infant were averaged for each age and the mean and SD for each group was calculated. Then, the antilog of the mean acuity score in $\log_{10}$ was taken to give the mean visual acuity as cycles per degree of visual angle. The SD was divided by 0.301 to convert the value to octaves. These procedures for calculation of means and SD of acuity scores are described in the *Teller Acuity Card Handbook*.

2.8.2. Fagan Test of Infant Intelligence

Cognitive development was assessed by using the Fagan Test of Infant Intelligence, version 4.1 (Infantest Corporation, Cleveland Heights, OH). The 78, 87 wk and 92 wk versions of the test were used, representing the ages eight, 10 and 12 mo. This test is a standardized assessment of recognition memory that uses a paired-comparison technique (Fagan 1984). This test was used because it is relatively quick (~15 min) and, at least in high-risk infants, novelty preference test scores for infants aged <24 mo seem to correlate well with later performance in standardized psychomotor tests of development (Rose 1989). The Fagan Test includes a training video that was used to develop proficiency with the test.

The test was given following the procedure outlined in the manual. The infant was shown visual stimuli (paired picture of a face) until a fixed duration of looking time was reached (familiarization). One of the original stimuli was then paired with a novel stimulus (different face) and the tester recorded the infant's looking direction and looking time during a fixed presentation time. The recording is made directly into a computer through a small central hole in the stage to which the stimuli were affixed. Each test consisted of 10 novelty problems. Looking time, the number of looks at the novel and familiar stimuli and the amount of time not attending to either stimulus were recorded for each novelty problem. The novelty preference score for each infant was calculated as the percentage of total looking time in the novelty portion of the test that was spent looking at the novel picture.

The inter- and intra-assay variability of the Fagan Test of Infant Intelligence was assessed using the IBM Training Certificate Video designed specifically for this test. The inter-assay variability was 4.2% and the intra-assay variability was 3.0%.

2.8.3. Bayley Scales of Human Development

The Bayley Scales of Infant Development assess the developmental status of children between the age of two mo and 2.5 yr. Two scales used are the Mental Development Indices (MDI) and the Psychomotor Development Indices. The mental scale is designed to sample perception, memory, learning, problem solving and vocalization as well as early verbal communication and abstract thinking. The motor scale primarily measures gross motor abilities and hand and finger manipulation. Each scale has a normalized standard with a mean of 100 and a SD of 26 (Anastasi 1988). Due to the training requirements for the Bayley test, this test was administered at four and eight mo by a trained Occupational Therapist in the Neonatal Follow-up Clinic of BC Children's Hospital. The 18-month test was administered by a Registered Psychologist at BC Children's Hospital, as per the standard procedure for the Neonatal Follow-up Clinic.

2.9. Statistical Analyses

2.9.1. Study I – Effect of dietary fatty acids on plasma and erythrocyte fatty acids and development of visual acuity in term infants

Sample size requirements for visual acuity with an $\alpha = 0.05$ determined that 18 infants per group were required to have a 90% probability of detecting a difference of 0.5 octaves in visual acuity. Plasma and erythrocyte fatty acids, visual acuity and growth were analyzed to determine the effects of age and diet by using two-way ANOVA with diet and age as the main factors. Formal tests of differences between diets at each age and between ages within a diet group were based on least-squares means and SEM calculated from ANOVA. The potential relations among the milk and formula contents of 22:6n-3, preferential looking acuity and plasma and erythrocyte were examined within and between the breast-fed and the formula-fed infants using regression analysis. Data for the Teller Acuity Card test were log-transformed before statistical evaluation as per convention (Weistheimer 1979).

Formal tests of differences between breast-fed infants and infants fed formula at each age and between ages within the group of breast-fed and formula-fed infants were based on least squares means and standard errors calculated from ANOVA. An unbalanced repeated measures model was used to examine the individual and possible joint effects of diet, time and maternal characteristics on preferential looking acuity. Similar analyses were used for analysis of the scores for the Bayley Scales of Infant Development. Normal probability plots and studentized residuals were used to assess any violations to the underlying model. The calculations were performed using the Biomedical Data Program (BMDP, University of California Press, Los Angeles CA, version PC90).

The Fagan Test of Infant Intelligence used subsets of the test appropriate for infants of different ages. Therefore, repeated measure ANOVA was not used for analyses of novelty preference or looking behaviour results. Instead, the results from the Fagan Test of Infant Intelligence used a multivariate ANOVA to assess diet differences within ages. Underlying assumptions for the ANOVA test of normally distributed data and independent variables were assessed. Data with skewed distributions were normalized through mathematical transformations. Calculations were performed with the Statistical Package for the Social Sciences (SPSS, version 6.1.3, Chicago, IL).

2.9.2. Study II – Effect of human milk and infant formula triacylglycerol fatty acid distribution on plasma lipids and lipoprotein fatty acid distribution in term infants

2.9.2.1. Influence of formula and human milk TG fatty acid distribution on chylomicron fatty acid composition

The influence of dietary TG fatty acid distribution on plasma lipids and chylomicron fatty acid distribution at 30 and 120 d of age, between infants who were breast-fed or fed the standard formula or with synthesized TG, were analyzed using a multivariate ANOVA procedure with *a priori* contrasts. The independent variables were diet and age and an interaction term, diet and age. The *a priori* contrasts included 1) comparison of fatty acid levels at 30 and 120 d within a diet group, 2) comparison of fatty acid levels between the two formula-groups within an age group, and 3) comparison of fatty acid levels between each formula-fed group and the breast-fed group within an age group.

2.9.2.2. Distribution of n-6 and n-3 fatty acids across lipoproteins and in lysophospholipids and unesterified fatty acids

The differences in fatty acid levels among individual lipoproteins (chylomicron, LDL and HDL) in the breast-fed infants, the infants fed standard formula and the infants fed the synthesized

TG formula and between 30 d and 120 d of age and their interaction (lipoprotein and age) were determined using a multivariate ANOVA with *a priori* contrasts. Differences were considered significant at $P < 0.05$. The relative abundance of 18:2n-6, 18:3n-3, 20:4n-6 and 22:6n-3 in the PL, TG and CE of the individual lipoproteins compared with plasma were analyzed using one-way ANOVA. Post-hoc analyses used Dunnett's test with the plasma fatty acid values as the control variable. Differences were considered significant at $P<0.05$. The differences in lysoPL and unesterified fatty acids levels between the breast-fed infants, the infants fed the standard formula and infants fed the formula with the synthesized TG and between 30 d and 120 d of age and their interaction (diet and age) were determined using a multivariate ANOVA procedure, with *a priori contrasts*. Differences were considered significant at $P<0.05$. All calculations were performed using SPSS, version 7.5.1. Values given in the Tables and Figures are percent means ± SEM.

Potential statistically significant differences in preferential looking acuity, at 120 d of age, between infants who were breast-fed, fed standard formula or fed the synthesized TG formula, were determined by using a one-way ANOVA with the level of significance set at $\alpha=0.05$. Post-hoc power analysis was done to determine if there were a sufficient number of infants to detect potential differences between the groups.

2.9.3. Study III – Influence of environmental and dietary factors on measures of novelty preference and visual acuity at nine mo in term infants.

Potential differences in assessed demographics between infants who completed the Teller Acuity Card test and the Fagan test and those that did not were assessed using the non-parametric Kruskal-Wallis test. The potential difference between testers was assessed by t-test, with a 95% confidence interval.

The association between the *a priori* variables, including duration of breastfeeding and performance on the tests of preferential looking acuity and novelty preference, were assessed by linear regression analysis with significance set at $P<0.05$. In all regressions, categorical variables were coded using dummy variables. Continuous dependent variables that were not normally distributed were transformed to satisfy the requirement for normality. Data for the Teller Acuity Card test were log-transformed before statistical evaluation as per convention (Weistheimer 1979).

Covariates for statistical analysis were specified as part of the design of the study and were selected because of their potential to bias the assessment of the association between duration of breastfeeding and infant development. These included gender, mother's age, mother's education, father's education, family income, marital status, birth order, language spoken at home, ethnic background, if the mother was born in Canada and how many years the mother had lived in Canada.

Correlations between covariates were assessed to eliminate undue influence of highly related variables in the regression analysis. As a result, the regression model included a smaller, but more relevant group of covariates than originally planned. The reduced model excluded covariates that were highly correlated to other covariates. Variables excluded were father's education as it was highly correlated to mother's education and number of years in Canada as it was related to, but a less specific indicator of mother's age. Ethnicity and whether the mother was born in Canada were also eliminated as they were highly correlated to the language spoken at home. All correlations and regressions were done using SPSS, version 7.5.1 (SPSS, Chicago, IL).

Post-hoc power calculations were performed to determine if there was a sufficient sample size to detect the influence of the assessed demographic variables on outcomes in the Teller Acuity test and Fagan test. The power calculations assumed an $\alpha=0.05$. Power calculations were

done using GPower, version 2.0 (Faul F, Erdfelder E. (1992). GPower: a priori, post-hoc and compromise power analysis for MS-DOS (computer program). Bonn FRG: Bonn University, Dept of Psychology).

3. RESULTS

3.1. Study I: Effect of dietary fatty acids on plasma and erythrocyte fatty acids and development of visual acuity in term infants

3.1.1. Characteristics of study population

Seventeen breast-fed infants and 18 formula-fed infants were recruited. At the three mo time point, no infants had withdrawn from the study. Of the 17 infants enrolled as breast-fed, 16 completed the study to 18 mo, with one infant withdrawn because the subject and their family moved. Of the 18 infants enrolled in the formula-fed group, 13 completed the study to 18 mo. Of the five who withdrew, one was due to missed appointments and four were due to the parent's decision not to continue. The gestational ages of the breast-fed and formula-fed infant groups were not significantly different (**Table 3.1**). Socio-economic measures were separated into the mother's and father's characteristics. Overall, the study population was predominantly Caucasian, with approximately 15 yr of formal education, working in a trade or service profession.

Table 3.1 *– Gestational age, gender and family background of term breast-fed infants and infants fed formula*

	Breast *n*=17	Formula *n*=18
Gestational Age (wk, mean±SEM)	39.6±0.3	39.1±0.3
Male/female	9/8	11/6
Ethnic Background		
Mother		
Caucasian	14	10
Asian	2	8
East Indian	1	0
Father		
Caucasian	13	8
Asian	2	10
East Indian	2	0
Education (yr, mean±SEM)		
Mother	15.1±0.7	14.3±0.7
Father	15.4±0.8	14.7±1.3
Job Classification		
Mother		
trade/service	10	7
professional	3	5
unemployed	4	6
Father		
trade/service	9	10
professional	6	7
unemployed	1	1

Values are n except when otherwise indicated.

3.1.2. Anthropometrics of study population

There were no statistically significant ($P>0.05$) differences in weight, length or head circumference between the breast-fed infants and the infants fed formula at any time point in the study (**Table 3.2**). No attempt was made to control for the influence of gender, birthweight, length or head circumference at birth on subsequent weight, length or head circumference due to small numbers of infants in each group.

Table 3.2 *- Weight, length, head circumference of term breast-fed and infants fed formula to 18mo.*

	Weight		Length		Head Circumference	
	Breast	Formula	Breast	Formula	Breast	Formula
	(g)		(cm)		(cm)	
birth	3527.6±121.1 (17)	3496.1±109.4 (18)	51.0±0.5 (15)	51.4±0.5 (15)	35.2±0.5 (15)	34.8±0.5 (15)
14d	3767.5±124.6 (17)	3743.9±101.6 (18)	52.7±0.5 (17)	52.2±0.5 (18)	36.2±0.5 (17)	36.3±0.4 (18)
1mo	4426.3±132.6 (17)	4446.7±115.7 (18)	55.2±0.6 (17)	54.8±0.4 (18)	37.7±0.3 (17)	38.0±0.4 (18)
2mo	5380.3±173.3 (17)	5416.9±127.0 (18)	58.3±0.5 (17)	58.1±0.4 (18)	39.6±0.3 (17)	39.6±0.4 (18)
3mo	6129.4±193.7 (17)	6171.4±168.5 (18)	61.1±0.4 (17)	61.1±0.4 (18)	41.0±0.3 (17)	40.9±0.4 (18)
4mo	6827.9±236.7 (17)	6985.3±204.4 (17)	64.2±0.6 (17)	64.1±0.6 (17)	42.3±0.4 (17)	42.0±0.4 (17)
8mo	8387.6±252.1 (17)	8690.7±219.9 (15)	70.6±0.6 (17)	70.7±0.7 (15)	45.1±0.3 (17)	45.1±0.4 (15)
18mo	11014.7±367.2 (16)	10698.5±157.2 (13)	81.8±0.8 (16)	81.1±0.8 (13)	48.5±0.3 (16)	47.8±0.5 (13)

Values are means±SEM, values in brackets = n. There were no statistically significant differences ($P>0.05$) in anthropometric values between the breast-fed infants and infants fed formula at any age.

3.1.3. Fatty acid composition of infant plasma phospholipid and erythrocyte fatty acids

The results of the plasma PL and erythrocyte PC and PE analyses show marked differences in the major n-6 and n-3 fatty acids of interest (ie 18:2n-6, 20:4n-6, 22:6n-3) between the breast-fed infants and the infants fed formula (**Figure 3.1**). Full results concerning the fatty acids in plasma PL and erythrocyte PC and PE are in the Appendix, **Tables 6.2 and 6.3**. Infants fed the formula had a significantly higher percent 18:2n-6 and lower 20:4n-6 and 22:6n-3 in plasma PL and higher 18:2n-6, 20:4n-6 and 22:6n-3 in erythrocyte PC at both 14 d and three mo of age than infants who were breast-fed. At three mo, the percent 18:2n-6 was higher and 22:6n-3 was lower in the erythrocyte PE of the infants fed formula compared with the breast-fed infants. There were no statistically significant differences in the n-6 or n-3 fatty acid composition of erythrocyte PE between the breast-fed and formula-fed infants at 14 d of age.

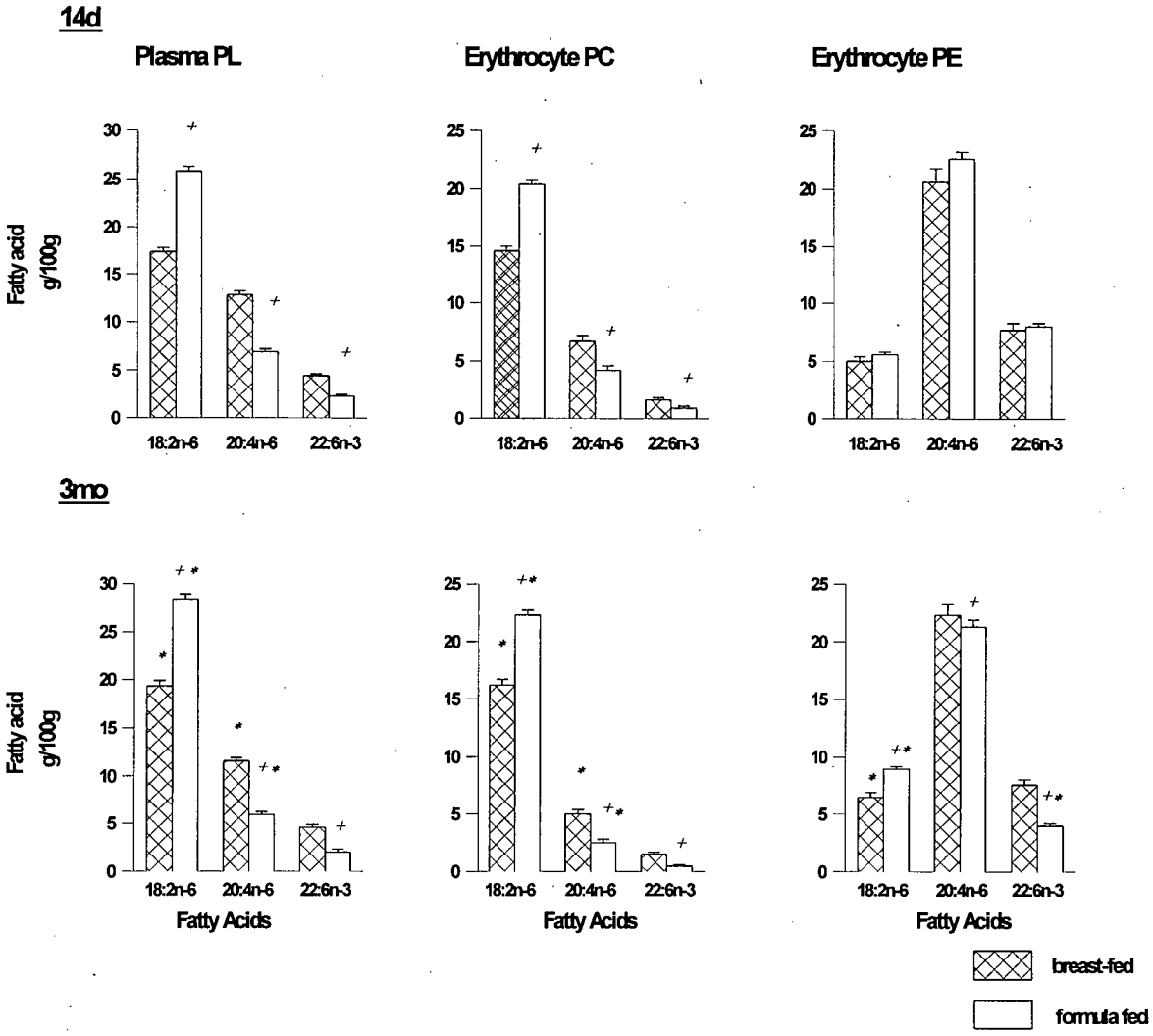


Figure 3.1 – Plasma PL and erythrocyte phosphatidylcholine (PC) and phosphatidylethanolamine (PE) 18:2n-6, 20:4n-6 and 22:6n-3 for breast-fed infants (n=17) and infants fed formula (n=18) at 14 d and three mo of age. The results given are means+SEM; + indicates values significantly different from value for breast-fed infants of the same age, $P<0.05$; * indicates value at three mo significantly different from value at 14 d for infants in a given group, $P<0.05$.

3.1.4. Visual acuity of term infants

No statistically significant differences were found between the first and second tests of preferential looking acuity. Therefore, results of the two tests of acuity were averaged for each infant, converted to $\log_{10}$ cycles/degree and the mean and SD calculated for the group of infants at each age. Preferential looking acuity, measured by the Teller acuity card procedure, increased from a mean value of 0.95±0.51 (cycles/deg)±SD (octaves) to 13.4±0.56 and from 0.91±0.49 to 12.4±0.40 from 14d to 18 mo of age in the breast-fed infants and the infants fed formula, respectively (**Figure 3.2**). The *a priori* calculations of sample size required to detect a difference of 0.5 octaves in the Teller Acuity Card test, with 90% power, indicated that a sample size of 18 infants per group was required. The numbers of infants in the breast-fed and formula-fed groups at 14 d and three mo were n=17 and n=18, respectively, and at four mo the number of breast-fed infants and infants fed formula was each n=17. Due to subject withdrawals, the sample sizes at 18 mo for the breast-fed infants and the infants fed formula were n=16 and n=13, respectively. *Post-hoc* power calculations were done and it was found that there was 90% power to detect a significant difference at each age between the diet groups. The inter-tester reliability in this study calculated from the test results across all ages was 98.4%. The mean preferential looking acuity values of the infants in this study were very similar to preferential looking acuity population norms recently reported for healthy term infants of the same age (Salomao 1995). There were no statistically significant differences (P>0.05) in preferential looking acuity scores between the breast-fed infants and the infants fed formula at 14 d or three mo (**Table 3.3**) or at any of the ages tested to 18 mo (Figure 3.2). There were also no significant differences in preferential looking acuity when compared across gender, mother's job classification, education or language spoken in the home (**Appendix, Table 6.6**). With the possible exception of gender, this study did not have a

sufficient number of infants to assess the possible influence of the family characteristics on visual acuity.

Table 3.3 *- Preferential looking acuity of term infants*[1]

	Age	
	14d	3mo
	(cyc/deg)	
Feeding type		
Human milk	0.9±0.5 (17)[2]	3.9±0.6 (17)
Formula	0.9±0.5 (18)	4.6±0.4 (18)

[1] The results given are mean (cyc/deg)±SD (octaves). No statistically significant differences (P>0.05) were found between the breast-fed infants or the infants fed formula at 14 d or three mo.
[2]Values in brackets are the number of infants per group at the given age.

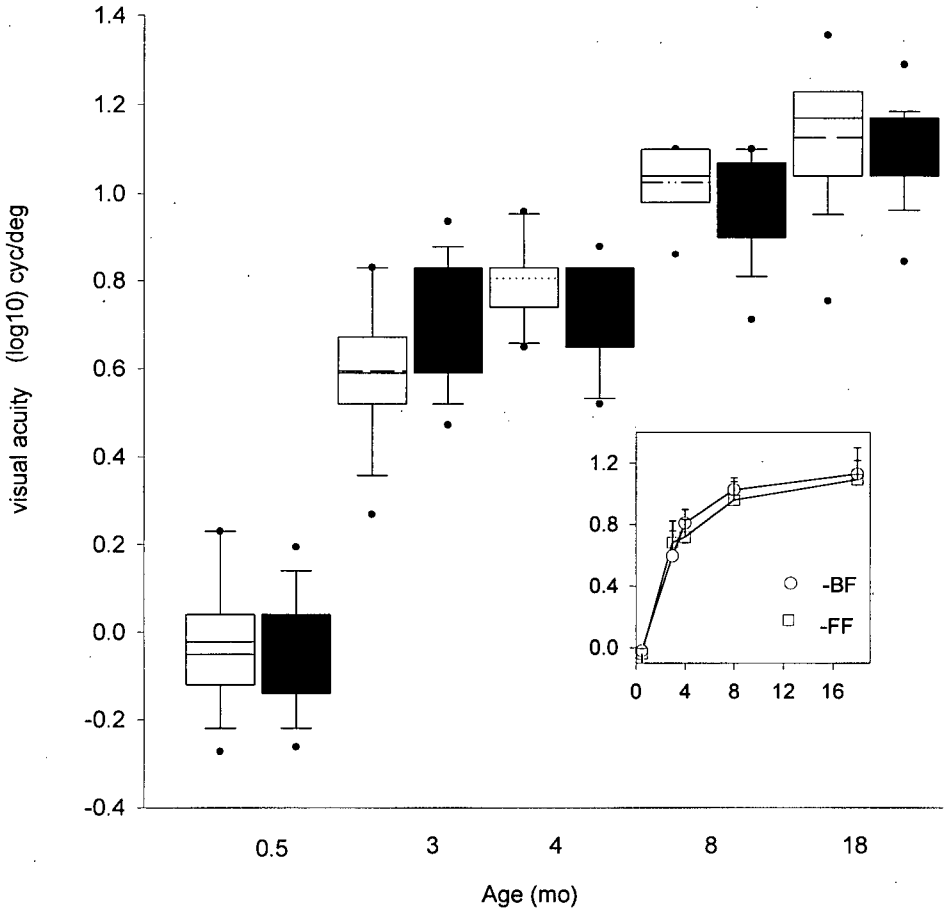


Figure 3.2 – Boxplot and line graph (inset) of the increase in visual acuity of breast-fed infants and infants fed formula at 0.5 (14 d), three, four, 8 and 18 mo of age. The number of breast-fed infants and infants fed formula at each age are, respectively, 14 d, n=17, n=18; three mo n=17, n=18; four mo, n=17, n=17; eight mo n=17, n=15; 18 mo n=16, n=13. For the boxplot graph, the • symbol represents the 5th and 95th range; crosshatch bars represent the 10th and 90th percentile ranges; upper and lower edges of the box represent the 25th and 75th percentile range; the inner solid line represents the 50th percentile range (median); the inner dotted line represents the mean. The plain boxes represent breast-fed infants and the striped boxes represent the infants fed formula.

3.1.5. Scatterplot of acuity scores and dietary and erythrocyte phospholipid 22:6n-3 in term infants

In studies of breast-fed infants and infants fed formula without a source of 20:4n-6 and 22:6n-3, differences in visual acuity, when found, appeared between two and four mo of age. Therefore, the three mo of age is the primary focus of this study. Regression analysis found no significant relation between preferential acuity and the % 22:6n-3 in formula/milk fat or the infant erythrocyte PE (**Figure 3.3**) percent 22:6n-3 at three mo when tested for the entire group of infants, or within the breast-fed infants or the infants fed formula alone.

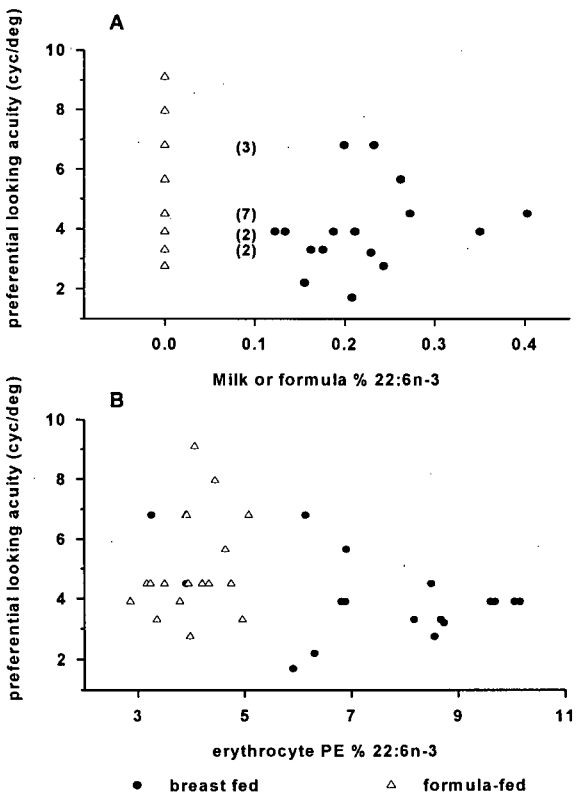


Figure 3.3 – Scatterplot of preferential looking acuity scores and A) human milk or formula % 22:6n-3 and B) erythrocyte phosphatidylethanolamine (PE) % 22:6n-3 for three mo old breast-fed infants (n=17) and infants fed formula (n=18). No statistically significant (P>0.05) relations were found.

3.1.6. Fagan Test of Infant Intelligence in term infants

No significant differences in novelty preference scores were found between the breast-fed infants and infants fed formula at any age using the Fagan Test of Infant Intelligence (**Appendix, Table 6.7**). There were no significant differences in percent novelty between infants when classified by gender, mother's job category, language spoken in the home or mother's education (data not shown). No significant differences were found in looking behaviour, including average number of looks or looking time in either the familiar (**Appendix, Table 6.8**) or novel phases of the test trials (**Appendix, Table 6.9**). Data on looking behaviour for the 87 wk FTII were not analyzed due to the small number of infants within each category. This study did not have sufficient power to detect diet effects, or effects of gender or home environment on novelty preference or looking behaviour. *Post hoc* power calculations indicated that an n of approximately 150 would be required to detect a difference between the diet groups of 2% in novelty preference, with an α=0.05 and 80% power.

Table 3.4 *- Novelty preference (FTII) scores of breast-fed infants and infants fed formula.*

Characteristic	Test type		
	76 wk	87 wk	92wk
	% Novelty Preference		
Feeding type			
Human milk	56.4±2.4 (15)	51.7±3.1 (11)	62.4±1.7 (10)
Formula	55.5±1.9 (15)	54.3±8.1 (4)	61.5±2.4 (7)

Results given are % means±SEM, (n).
There were no statistically significant differences (P>0.05) found at any age between any of the groups.

3.1.7. Bayley Scales of Infant Development of breast-fed infants and infants fed formula

No statistically significant differences ($P>0.05$) were found in the Bayley Scales of Infant Development for either the psychomotor developmental index (PDI) or mental developmental index (MDI) scores between the breast-fed infants and infants fed formula at any age (**Appendix, Table 6.10**) between infants classified by gender, mother's job category, language spoken in the home, or mother's highest education level. This study did not have sufficient power to detect diet or gender effects or effects of the home environment on performance on the PDI or MDI of the Bayley Scales. *Post-hoc* power calculations indicated that an n of approximately 400 would be required to detect a difference in the Bayley test score, due to diet, of five points, with an $\alpha=0.05$ and 80% power.

3.1.8. Influence of human milk and formula triacylglycerol fatty acid distribution on the distribution of fatty acids in plasma triacylglycerol and phospholipid.

There was no significant difference in the plasma total TG percent 16:0 between the infants fed formula and the breast-fed infants. There was, however, a significant three fold, higher percent of 16:0 in the TG 2 position fatty acids of the three mo old breast-fed infants compared with formula-fed infants (23.3 ± 3.3 and 7.4±0.7% 16:0, respectively) (**Table 3.5**). The lower percent 16:0 in the TG 2 position of the infants fed the formula was accompanied by significantly lower TG 2 position levels of 18:1 and 18:2n-6 than in the breast-fed infants. These results provide compelling evidence that 16:0 in the 2 position of human milk and formula TG is absorbed as a 2-MG (MG) by infants and re-esterified to TG, retaining the 16:0 in the 2 position, for secretion into plasma.

The TG total fatty acids of the breast-fed infants had significantly higher levels of 14:0, 18:0, 20:4n-6, 20:5n-3 and 22:5n-6 and lower levels of 18:2n-6 than the infants fed formula. The breast-fed infants also had significantly higher levels of 20:3n-6, 20:4n-6, 20:5n-3, 22:5n-3 and 22:6n-3 in their plasma TG 2 position fatty acids than did the infants fed formula.

Table 3.5 *– Composition of plasma triacylglycerol (TG) total and triacylglycerol 2 position fatty acids in three mo old breast-fed infants and infants fed formula*[1].

Fatty acid	Total TG		TG 2 Position	
	Breast-fed	Formula-fed	Breast-fed	Formula-fed
	(g/100 g)			
14:0	2.9±0.3	2.2±0.2[2]	1.8±0.8	0.7±0.2
16:0	26.0±0.6	26.2±0.6	23.3±3.3	7.4±0.7[3]
18:0	6.6±0.3	5.2±0.2[2]	4.4±0.5	6.0±1.0
18:1	44.0±0.6	42.7±0.6	41.8±2.5	53.3±1.6[3]
18:2n-6	12.8±0.8	17.7±0.4[2]	19.5±1.7	27.4±0.6[3]
18:3n-3	0.7±0.1	0.9±0.1	0.9±0.1	1.1±0.1
20:2n-6	0.3±0.0	0.3±0.0	0.2±0.1	0.2±0.1
20:3n-6	0.3±0.0	0.3±0.1	0.4±0.1	0.2±0.0[3]
20:4n-6	0.8±0.1	0.3±0.0[2]	2.0±0.3	0.5±0.1[3]
20:5n-3	0.1±0.0	0.0±0.0[2]	0.1±0.0	0.0±0.0[3]
22:4n-6	0.2±0.0	0.1±0.0	0.2±0.1	0.1±0.0
22:5n-6	0.1±0.0	0.0±0.0[2]	0.0±0.0	0.0±0.0
22:5n-3	0.2±0.0	0.2±0.1	0.7±0.2	0.1±0.0[3]
22:6n-3	0.3±0.0	0.2±0.0	0.9±0.1	0.1±0.0[3]

[1]Values given are means±SEM for breast-fed infants (n=17) and infants fed formula (n=18).
[2,3]Value for total or 2 position fatty acids, respectively, significantly different from respective values for breast-fed infants; $P<0.05$.

The plasma PL total fatty acids of the breast-fed infants had significantly lower levels of 16:0 and 18:2n-6, but higher levels of 18:0, 20:2n-6, 20:3n-6, 20:4n-6, 20:5n-3, 22:4n-6, 22:5n-3 and 22:6n-3 than the PL of infants who were fed formula (**Table 3.6**). Similarly, the PL 2 position fatty acids of the breast-fed infants had significantly lower levels of 18:2n-6 and 18:3n-3 and higher 20:4n-6, 20:5n-3, 22:5n-3 and 22:6n-3 than the formula-fed infants.

Table 3.6 *– Composition of plasma total phospholipid (PL) and phospholipid 2 position fatty acids of three mo old breast-fed infants and infants fed formula.*[1]

Fatty acid	Total PL		PL 2 Position	
	Breast-fed	Formula-fed	Breast-fed	Formula-fed
		(g/100 g)		
14:0	0.4±0.1	0.4±0.0	1.0±0.3	0.8±0.1
16:0	26.1±0.6	28.4±0.6[2]	13.4±1.5	12.4±1.0
18:0	18.4±0.4	17.7±0.2[2]	10.8±1.3	9.5±0.7
18:1	12.5±0.3	12.6±0.2	14.4±0.6	15.3±0.3
18:2n-6	19.8±0.7	26.4±1.0	25.0±1.4	40.2±1.6[3]
18:3n-3	0.1±0.0	0.2±0.0	0.1±0.0	0.2±0.0[3]
20:2n-6	0.4±0.0	0.3±0.0[2]	0.5±0.0	0.4±0.0
20:3n-6	2.8±0.1	2.3±0.1[2]	4.5±0.3	3.6±0.1[3]
20:4n-6	11.6±0.3	6.9±0.6[2]	17.7±1.4	9.7±0.6[3]
20:5n-3	0.4±0.0	0.2±0.0[2]	0.6±0.1	0.2±0.1[3]
22:4n-6	0.4±0.0	0.3±0.0[2]	0.8±0.0	0.9±0.1
22:5n-6	0.3±0.0	0.3±0.0	0.4±0.1	0.6±0.1
22:5n-3	1.0±0.0	0.5±0.1[2]	1.5±0.1	0.6±0.0[3]
22:6n-3	4.6±0.3	2.1±0.3[2]	5.6±0.6	2.0±0.2[3]

[1]Results given are means±SEM for breast-fed infants (n=17) and infants fed formula (n=18).
[2,3]Value for total or 2 position fatty acids, respectively, significantly different from respective values for breast-fed infants; $P<0.05$.

3.2. Study II: Effect of human milk and infant formula triacylglycerol fatty acid distribution on plasma lipids and lipoprotein fatty acid distribution in term infants

3.2.1. Characteristics of study population

Eighty-seven infants were enrolled into this study, with 47 randomized to be fed one of the two formulae and 40 were enrolled (non-randomized) as breast-fed infants (**Table 3.7**). Of the 47 infants enrolled as formula-fed infants, 22 were fed a standard formula and 25 were fed formula with synthesized TG. Nineteen of the 22 infants fed the standard formula completed the study; three infants were withdrawn because of possible formula intolerance. Seventeen of the 25 infants randomized to the formula with the synthesized TG completed the study, infants were withdrawn because of suspected formula intolerance, n=4; suspected cow's milk allergy, n=1; difficulty in obtaining a blood sample, n=2; and doctor's decision unrelated to the formula, n=1. Eighteen of the 40 breast-fed infants were withdrawn because of formula supplementation, n=6; difficulty in obtaining a blood sample, n=6; parent's decision not to continue, n=4; and subject moved, n=1. There were no statistically significant differences in the birthweight, gestational age, gender distribution or APGAR scores among the groups of infants enrolled to be fed the formula with synthesized TG or standard formula, or as breast-fed infants (Table 3.7).

Table 3.7 *- Demographics of infants randomized to be fed formulae with similar levels, but different positional distribution of fatty acids in the triacylglycerol, or breast-fed*

	Formula		Breast-fed
	Standard	Synthesized TG	
	n=22	n=25	n=40
Birth weight, kg	3.6 ± 0.4	3.5 ± 0.5	3.5 ± 0.3
Birth length, cm	51.1 ± 2.2	52.0 ± 2.5	51.7 ± 2.0
Gestational Age, wk	39.8 ± 1.2	39.5 ± 1.1	39.7 ± 1.1
Apgar, at five min	>9	>7	>7
Gender, M/F	12/10	14/11	23/17
Ethnicity (n)			
Caucasian	16	13	32
Chinese/Other Asian	3	9	3
East Indian/Other	3	3	5

Mean ± SD, unless otherwise indicated; no statistically significant differences (P>0.05) were detected.

3.2.2. Anthropometric measures for study population

There were no significant differences in the weight, length or head circumference between the infants fed the two formulae, or among the infants fed formula and the breast-fed infants, at any age (**Table 3.8**). The weight gain of the infants fed formula and the breast-fed infants in this study were similar to those reported by Guo et al (1991) for normal, healthy term infants (**Table 3.9**).

Table 3.8 *- Weight, length and head circumference of infants fed standard formula or formula with synthesized TG or breast-fed*[1]

	Formula-fed		Breast-fed
	Standard	Synthesized TG	
Weight (kg)	n=22	n=25	n=40
7 d	3.6 ± 0.4 (21)	3.6 ± 0.5 (25)	3.6 ± 0.3 (34)
30 d	4.5 ± 0.4 (19)	4.6 ± 0.6 (21)	4.5 ± 0.4 (29)
60 d	5.5 ± 0.5 (19)	5.6 ± 0.7 (20)	5.5 ± 0.5 (26)
120 d	7.0 ± 0.6 (19)	7.1 ± 0.6 (19)	6.8 ± 0.6 (26)
Length (cm)			
7 d	51.7 ± 1.9	51.9 ± 1.9	51.9 ± 1.9
30 d	54.6 ± 1.7	54.7 ± 2.3	54.9 ± 1.4
60 d	58.6 ± 2.0	59.3 ± 2.2	59.4 ± 1.6
120 d	64.1 ± 1.7	64.4 ± 2.0	63.9 ± 1.8
Head Circumference (cm)			
7 d	36.0 ± 1.2	35.8 ± 1.1	35.8 ± 0.9
30 d	38.1 ± 1.0	38.3 ± 1.2	38.0 ± 0.9
60 d	39.8 ± 1.0	39.9 ± 1.1	39.6 ± 0.8
120 d	42.4 ± 1.2	42.3 ± 1.0	42.0 ± 0.8

[1]Values shown represent the data for all infants who were enrolled and are given as mean ± SD; (n) number of infants for whom weight, length and head circumference were obtained; some infants were enrolled but no measures or incomplete measures were obtained; no significant differences in any of the measures ($P>0.05$) were found.

Table 3.9 *- Weight gain of infants fed standard formula or formula with synthesized triacylglycerol, or breast-fed to 120 d of age*

	Formula-fed		Breast-fed	Reference[1]	
	Standard formula	Synthesized TG formula		Formula-fed	Breast-fed
			(g/d)		
Males					
7 to 30 d	43.4±4.2	44.0±10.2	42.9±8.5	40.4±9.7	39.4±11.9
31 to 60 d	36.8±7.8	35.9±5.8	38.1±7.7	36.8±8.3	35.4±9.0
61 to 120 d	25.3±5.4	23.2±6.3	22.9±6.2	26.9±6.6	23.6±6.2
(n)	(10)	(10-11)	(12-15)		
Females					
7 to 30 d	35.7±5.0	41.6±8.4	31.6±10.6	34.3±8.2	35.1±11.1
31 to 60 d	31.2±6.5	30.4±8.8	29.6±8.0	30.5±7.4	29.3±8.4
61 to 120 d	23.0±4.6	23.6±4.3	21.6±3.8	23.6±5.4	21.5±6.6
(n)	(9)	(9-10)	(13)		

[1]Reference values shown are wt gain (g/d) for 14 to 28 d, 28 to 56 d and 56 to 112 d (Fomon 1995).

3.2.3. Plasma lipids and composition and distribution of plasma fatty acids

3.2.3.1. Plasma lipids

The concentrations of TG and FFA in the plasma of the infants fed the formulae or breast-fed at 30 and 120 d of age are shown in **Table 3.10.** The concentrations of plasma total cholesterol, HDL-cholesterol, apolipoprotein A-1 and apolipoprotein B for the breast-fed infants, infants fed the standard formula and the infants fed the formula with the synthesized TG are in **Figure 3.4.** The infants fed the standard formula and those fed the formula with synthesized TG had significantly lower plasma total cholesterol and apolipoprotein B, but not TG concentrations than the breast-fed infants. Infants fed the formula with synthesized TG also had significantly lower plasma HDL cholesterol and apolipoprotein A-1 concentrations at both 30 and 120 d of age than infants fed the standard formula or the breast-fed infants. Of note, the plasma apolipoprotein B concentration of infants fed the synthesized TG formula was significantly higher than in infants fed the standard formula at both 30 and 120 d of age and was not different from the concentration of apolipoprotein B found in the plasma of the breast-fed infants.

Table 3.10 *– Plasma triacylglycerol and unesterified fatty acid concentrations at 30 and 120 d of age in term infants fed standard formula, formula with synthesized TG or breast-fed to 120 d of age*[1].

	Standard Formula		Synthesized TG Formula		Breast-fed	
	30 d	120 d	30 d	120 d	30 d	120 d
Triacylglycerol (mmol/L)	1.0±0.1 (17)	1.4±0.2 (17)	1.3±0.2 (15)	1.4±0.2 (15)	1.1±0.2 (20)	1.3±0.2 (19)
Unesterified fatty acids (mEq/L)	0.2±0.0 (15)	0.4±0.0[4] (15)	0.3±0.0 (17)	0.5±0.1[4] (13)	0.3±0.0 (15)	0.5±0.1[4] (16)

[1]Values are means±SEM, (number of infants); 0.0 indicates value ≥0.00-< 0.05
[2]Values for infants fed formula with synthesized TG significantly different from value for infants fed standard formula ($P < 0.05$).
[3]Value for infants fed formula significantly different from value for breast-fed infants ($P < 0.05$).
[4]Value for infants at 30 d significantly different from value for infants at 120 d, within the same diet group ($P<0.05$).

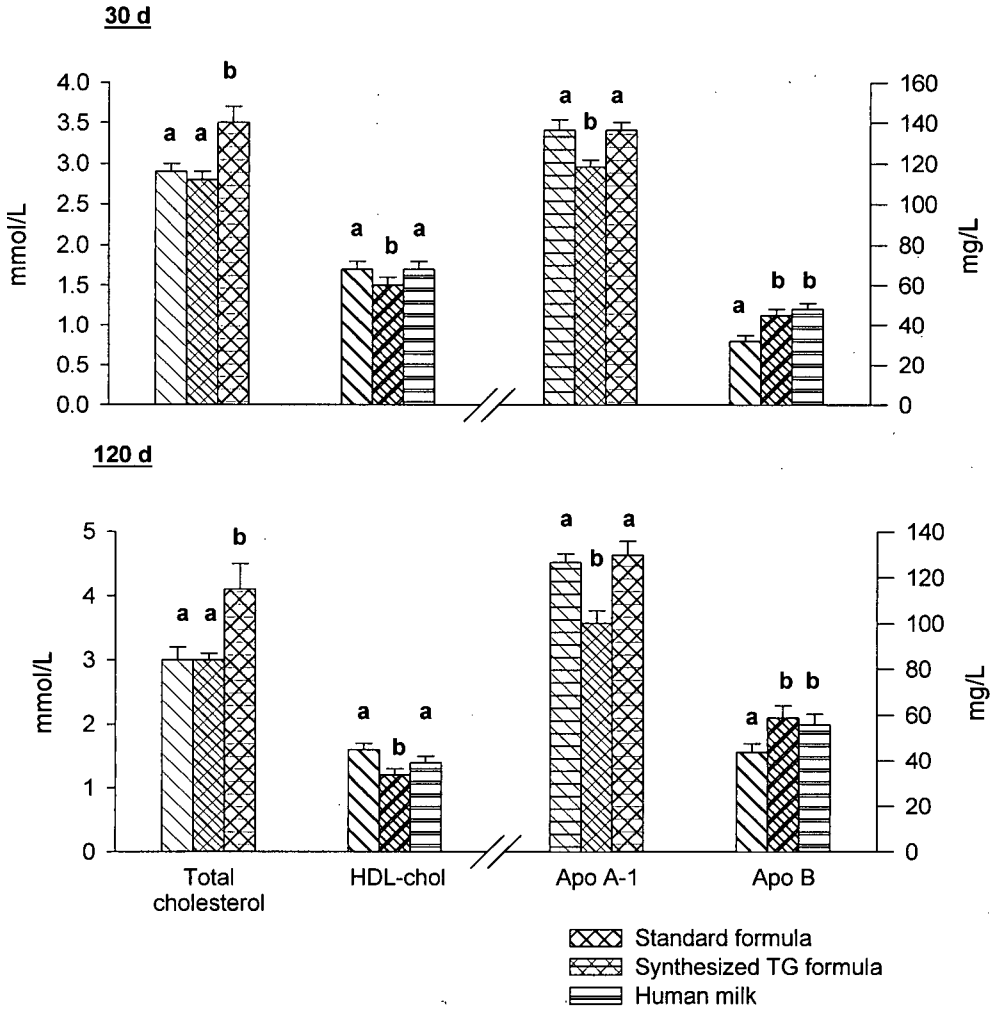


Figure 3.4 –Total cholesterol, HDL-cholesterol, apolipoprotein A-1 and apolipoprotein B levels, at 30 and 120 d of infants fed standard formula, formula with a synthesized TG or breast-fed to 120 d of age. Bars are means+SEM. Statistically significant ($P<0.05$) differences due to diet are indicated by different letters.

3.2.3.2. Plasma TG triacylglycerol fatty acids

There were no statistically significant (P>0.05) age and diet interactions for plasma TG fatty acids for infants fed either of the formulae or the breast-fed infants. There were no significant differences in the plasma total TG percent 16:0, 18:2n-6, 18:3n-3, 20:4n-6, or 22:6n-3 at 30 compared with 120 d of age for any of the diet groups (**Figure 3.5**). Infants fed the synthesized TG formula had significantly lower 18:1 at 120d of age than at 30 d of age. As found in the Study I (Section 3.1.8, Table 3.5), there were no significant differences (P>0.05) in the plasma TG percent 16:0 among the diet groups at either 30 or 120 d of age (Figure 3.5). At 30 d, both groups of formula-fed infants had a significantly higher 18:3n-3 in their plasma TG than the breast-fed infants. Infants fed the synthesized TG formula, however, had significantly higher plasma TG levels of 18:3n-3 than infants fed the standard formula at both 30 and 120 d of age. The two groups of infants fed formula also had a significantly higher percent of 18:2n-6 and lower 20:4n-6 and 22:6n-3 than the breast-fed infants at both 30 and 120 d of age.

3.2.3.3. Plasma triacylglycerol 2 position fatty acids

There were no statistically significant (P>0.05) diet and age interactions for the plasma TG 2 position fatty acids for either the infants fed formula or the breast-fed infants. There were no significant differences in the percent 16:0, 18:1, 18:2n-6, 18:3n-3, 20:4n-6 or 22:6n-3 in the plasma TG 2 position fatty acids at 30 d compared with 120 d of age (Figure 3.5). Infants fed the synthesized TG formula had a significantly higher percent of 16:0 and lower 18:1 in their plasma TG 2 position fatty acids than infants fed the standard formula at both 30 and 120 d of age. At 120 d, but not 30 d, infants fed the synthesized TG formula had significantly higher TG 2 position levels of 20:4n-6 than the infants fed the standard formula.

Both groups of infants fed formula had a significantly lower 16:0, 20:4n-6 and 22:6n-3 and higher 18:2n-6 in their plasma TG 2 position fatty acids than the breast-fed infants at both 30 and 120 d. Although the infants fed the standard formula had a significantly higher percent of 18:1 in their plasma TG 2 position fatty acids than the breast-fed infants at both 30 and 120 d of age,

infants fed the synthesized TG formula had a significantly higher percent of 18:1 at 120 d, but not at 30 d of age than the breast-fed infants.

In summary, the results in Figure 3.5 show that positioning about 29% 16:0 on the TG 2 position of formula results in plasma TG 2 position 16:0 and 18:1 levels closer to those of breast-fed infants (milk TG 2 position 56% 16:0), than infants fed a standard formula (TG 2 position 5% 16:0).

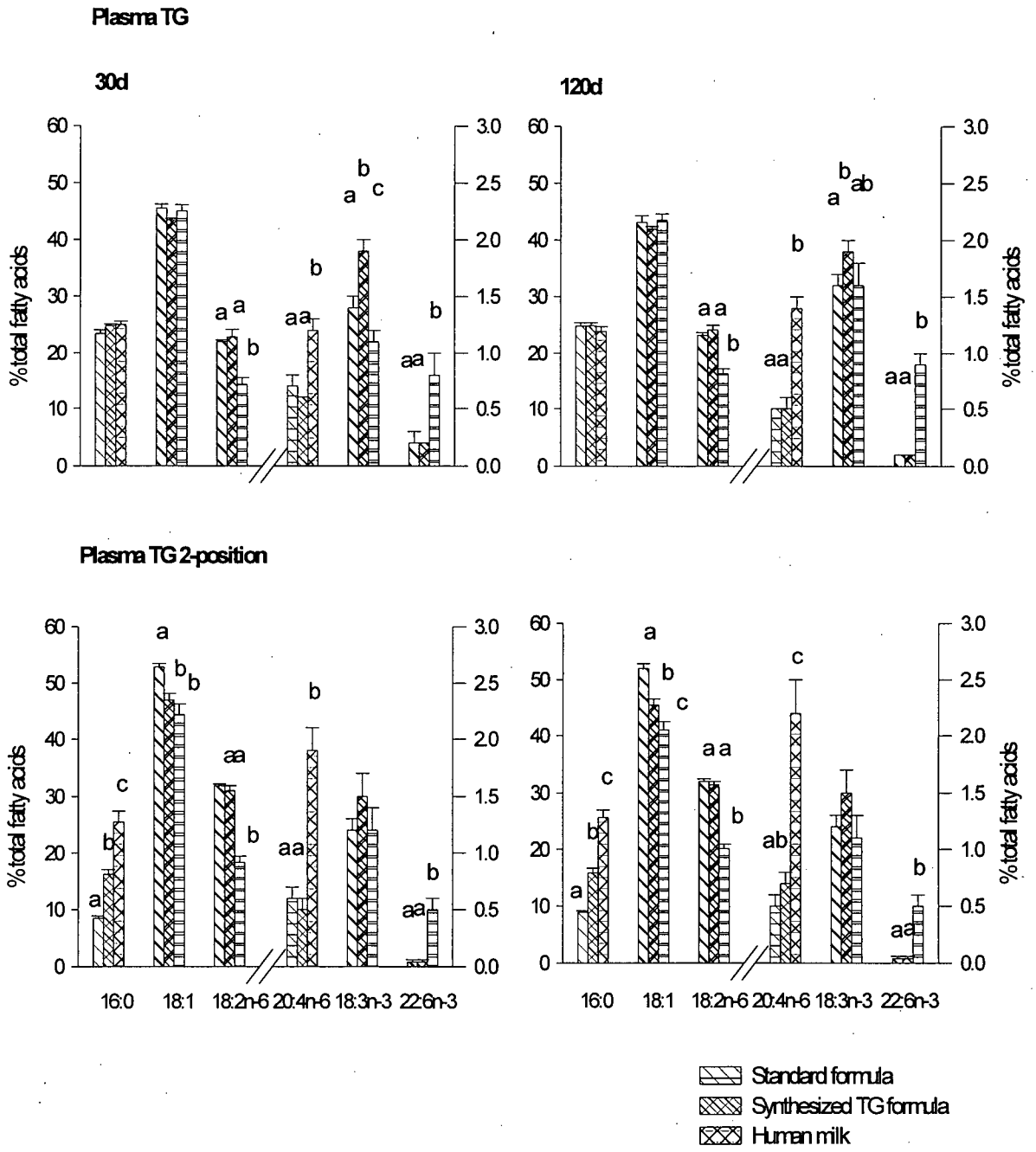


Figure 3.5 – Influence of milk and formula TG fatty acid distribution on infant plasma triacylglycerol and triacylglycerol 2 position fatty acids at 30 and 120 d of age. Bars represent means+SEM. Bars for a given fatty acid with different letters are significantly different ($P<0.05$).

3.2.3.4. Plasma phospholipid total fatty acids

The levels of 16:0, 18:1, 18:2n-6, 20:4n-6, 18:3n-3 and 22:6n-3 in the plasma PL total fatty acids are shown in **Figure 3.6**. There were no statistically significant (P>0.05) diet and age interactions. Age effects, however, were found for infants fed the standard formula and infants fed the synthesized TG formula. Thus, the plasma PL 18:2n-6 was significantly higher and 22:6n-3 was significantly lower at 120 d than at 30 d of age in the infants fed formula. Infants fed the standard formula and the breast-fed infants had a significantly lower plasma PL percent 20:4n-6 at 120 d than at 30 d of age.

The infants fed the synthesized TG formula had a significantly higher plasma PL 18:2n-6 at 30 and 120 d of age than the infants fed the standard formula. At 30 d, but not 120 d, the infants fed the synthesized TG formula had significantly lower plasma PL 20:4n-6 and 22:6n-3 than the infants fed the standard formula. At 30 and 120 d, both groups of infants fed formula had significantly higher 18:2n-6 and lower 20:4n-6 and 22:6n-3 than the breast-fed infants.

3.2.3.5. Plasma phospholipid 2 position fatty acids

There was a significant diet and age interaction for the plasma PL 2 position 18:2n-6, with higher 18:2n-6 at 120 d than at 30 d of age in both the groups of infants fed formula, but not in the breast-fed infants (Figure 3.6). Furthermore, the infants fed the synthesized TG formula, but not the infants fed the standard formula or breast-fed, had significantly lower 18:1 at 120 d than at 30 d of age. The percent 22:6n-3 in the PL 2 position decreased from 30 d to 120 d of age in the infants fed the standard formula, but not in the infants fed the synthesized TG formula or in the breast-fed infants.

Comparisons between the two groups of infants fed formula showed that infants fed the synthesized TG formula had significantly higher plasma PL 2 position 16:0 and lower 18:1 and 18:2n-6 than the infants fed the standard formula at both 30 d and 120d of age. At 30 d, but not 120 d of age, the infants fed the synthesized TG formula also had lower 22:6n-3 in their PL 2

position fatty acids than the infants fed the standard formula. Infants fed the standard formula had lower 16:0 than the breast-fed infants at 30 and 120 d of age. In contrast, the infants fed the synthesized TG formula, but not the infants fed the standard formula, had significantly lower 18:1 at 30 and 120 d of age in the plasma PL 2 position than the breast-fed infants. Both the groups of infants fed formula had higher 18:2n-6, but lower 18:3n-3 and 22:6n-3 in their PL 2 position fatty acids than the breast-fed infants at 30 and 120 d of age.

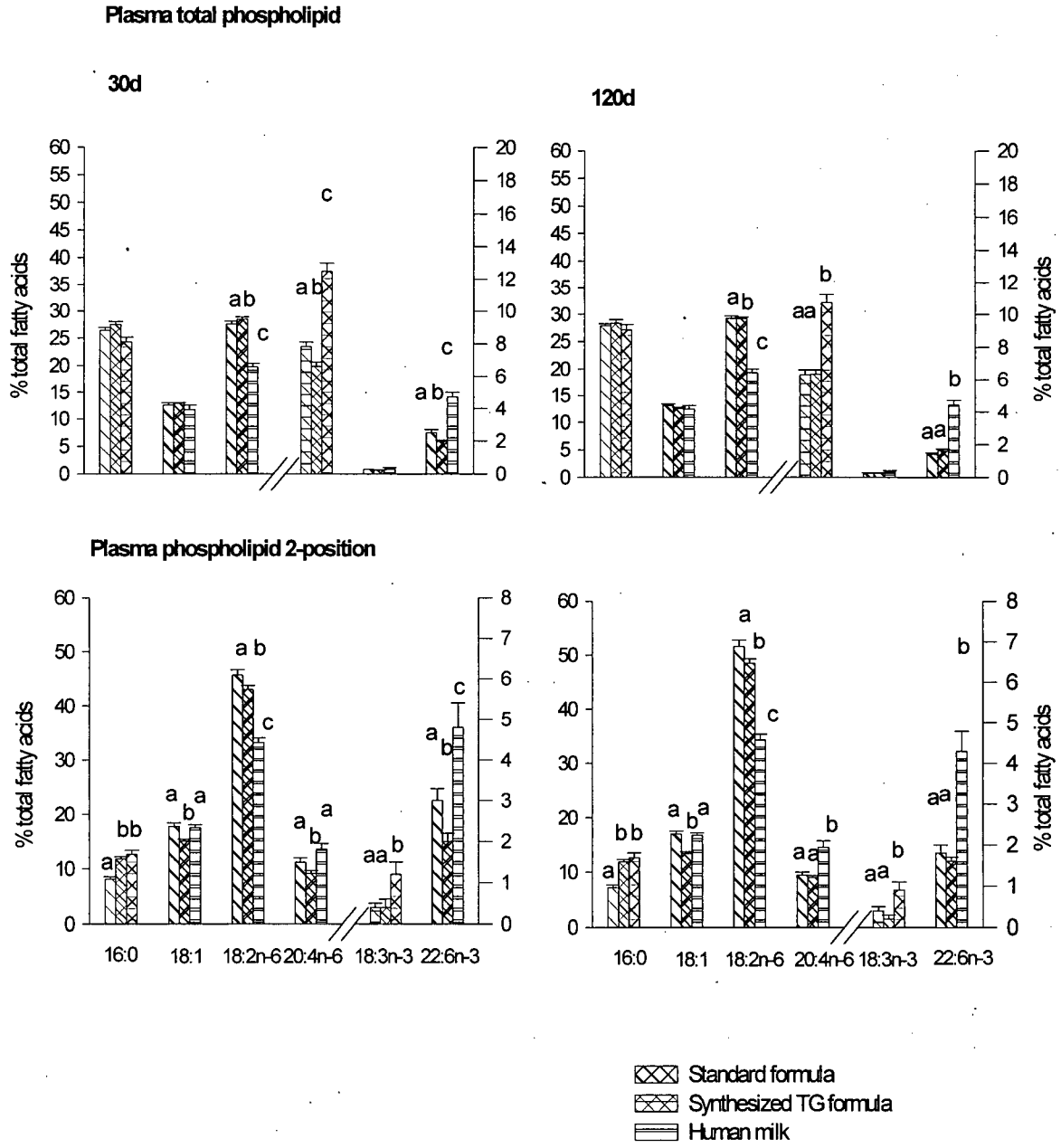


Figure 3.6 – Influence of milk or formula TG fatty acid distribution on infant plasma PL total fatty acids and 2 position fatty acids. Bars are means+SEM for a given fatty acid. Bars with different letters are significantly different (P<0.05).

3.2.4. Influence of formula and human milk TG fatty acid distribution on chylomicron fatty acid composition

3.2.4.1. Chylomicron total TG fatty acids

There were no significant differences ($P>0.05$) in the fatty acid composition of the chylomicron TG total fatty acids within a group of formula-fed or breast-fed infants between 30 and 120 d of age (**Figure 3.7**). Furthermore, no statistically significant differences in the effects of the diet were found at 30 and 120 d (diet and age interactions). No significant differences ($P>0.05$) in the composition of the chylomicron TG fatty acids were found between the infants fed the two formulae, with the exception that 18:3n-3 was higher in the chylomicron TG of infants fed the synthesized TG rather than the standard formula at both ages. Infants fed the standard formula and infants fed the formula with synthesized TG had significantly higher 18:2n-6 in their chylomicron TG than the breast-fed infants. This difference in 18:2n-6 probably reflects the higher amount of 18:2n-6 in the formulae (23% of total fatty acids) than in the human milk (14% of total fatty acids). Both groups of infants fed formula had significantly lower 20:4n-6 and 22:6n-3 in their plasma chylomicron TG than the breast-fed infants. Of note, there were no statistically significant differences in 16:0 or 18:1 in the chylomicron TG total fatty acids between the infants fed formula and the breast-fed infants.

3.2.4.2. Chylomicron TG 2 position fatty acids

The analysis of the 2 position fatty acids of the chylomicron TG, in contrast to the TG total fatty acids, found statistically significant ($P<0.05$) diet and age interactions for 16:0 and 20:4n-6. Thus, the levels of both 16:0 and 20:4n-6 decreased from 30 d to 120 d in the two groups of infants fed formula, but increased in the breast-fed infants. The chylomicron TG 2 position level of 20:4n-6

was significantly lower at 120 d than at 30 d in the infants fed the standard formula and in the breast-fed infants, but not in the infants fed the synthesized TG formula (Figure 3.7).

Infants fed the formula with synthesized TG had higher 16:0 and lower 18:1 in their chylomicron TG 2 position fatty acids than infants fed the standard formula at both 30 d and 120 d. The higher levels of 16:0 and lower 18:1 at the 2 position of chylomicron TG in infants fed the synthesized TG formula than in the infants fed the standard formula can reasonably be explained by higher 16:0 and lower 18:1 in the TG 2 position of the synthesized TG (29% 16:0, 35% 18:1) than standard formula (5% 16:0, 57% 18:1) (Section 2.4.4, Table 2.2). Infants fed the synthesized TG formula had significantly higher 22:6n-3 in the chylomicron TG 2 position fatty acids than the infants fed the standard formula at 120 d.

When compared with the breast-fed infants, infants fed the standard formula or the formula with synthesized TG had significantly higher levels of 18:2n-6, but lower 16:0, 20:4n-6 and 22:6n-3 in their chylomicron TG 2 position fatty acids. There was no difference in the chylomicron TG 2 position fatty acid levels of 18:1 between the breast-fed infants and infants fed the formula with synthesized TG at either 30 d and 120 d. Infants fed the standard formula, in contrast, had significantly higher levels of 18:1 in the chylomicron TG 2 position fatty acids at 120 d than the breast-fed infants. Infants fed the formula with the synthesized TG had significantly higher levels of chylomicron TG 2 position 18:3n-3 than the breast-fed infants at 30 d, but not 120 d. There was no significant difference in the levels of 18:3n-3 between the infants fed the standard formula and those who were breast-fed at 30 d or 120 d of age.

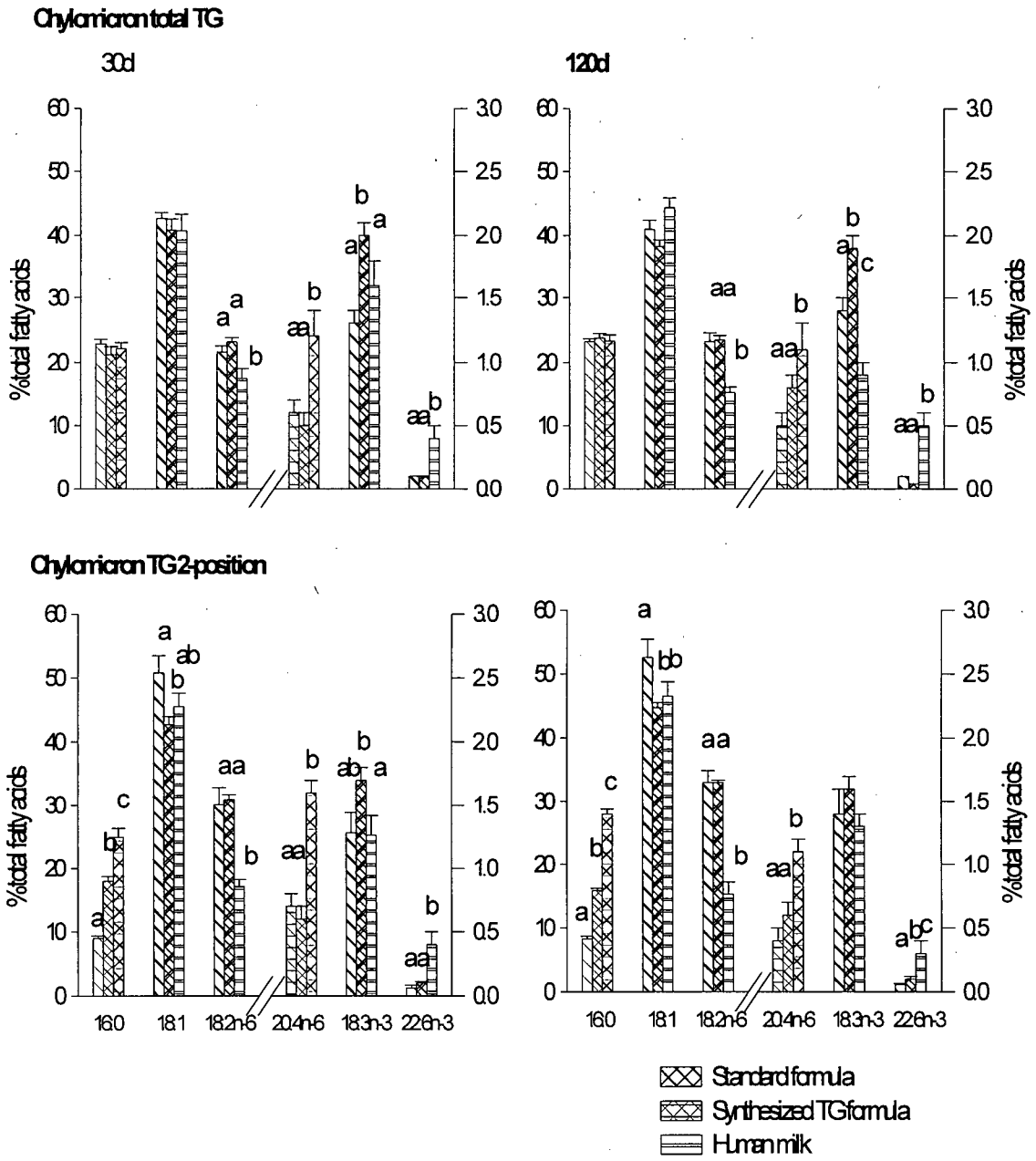


Figure 3.7 – Influence of milk and formula TG fatty acid distribution on chylomicron total TG and TG 2 position fatty acids. Bars are means+SEM. Bars for a given fatty acid with a different letter are significantly different ($P<0.05$).

3.2.4.3. Chylomicron phospholipid fatty acids

There were no statistically significant ($P>0.05$) diet and age interactions for the chylomicron PL fatty acids for the infants fed the formula or breast-fed to 120 d of age (**Figure 3.8**). Both the infants fed the standard formula and the infants fed the formula with the synthesized TG had a significantly higher percent 18:2n-6 in the chylomicron PL at 120 d than at 30 d of age. The breast-fed infants, on the other hand, had a significantly lower chylomicron PL 18:1 at 120 d in comparison to 30 d of age.

There were no significant differences ($P>0.05$) in the fatty acid composition of the chylomicron PL between the infants fed the standard formula and those fed the formula with synthesized TG. Both groups of infants fed formula had significantly ($P<0.05$) lower 20:4n-6 and 22:6n-3 and higher 18:2n-6 in their chylomicron PL than the breast-fed infants. At 120 d, but not 30 d, both groups of infants fed formula had significantly higher chylomicron PL 18:1 than the breast-fed infants. There were no significant differences in levels of 16:0 or 18:3n-3 between any of the diet groups at either age.

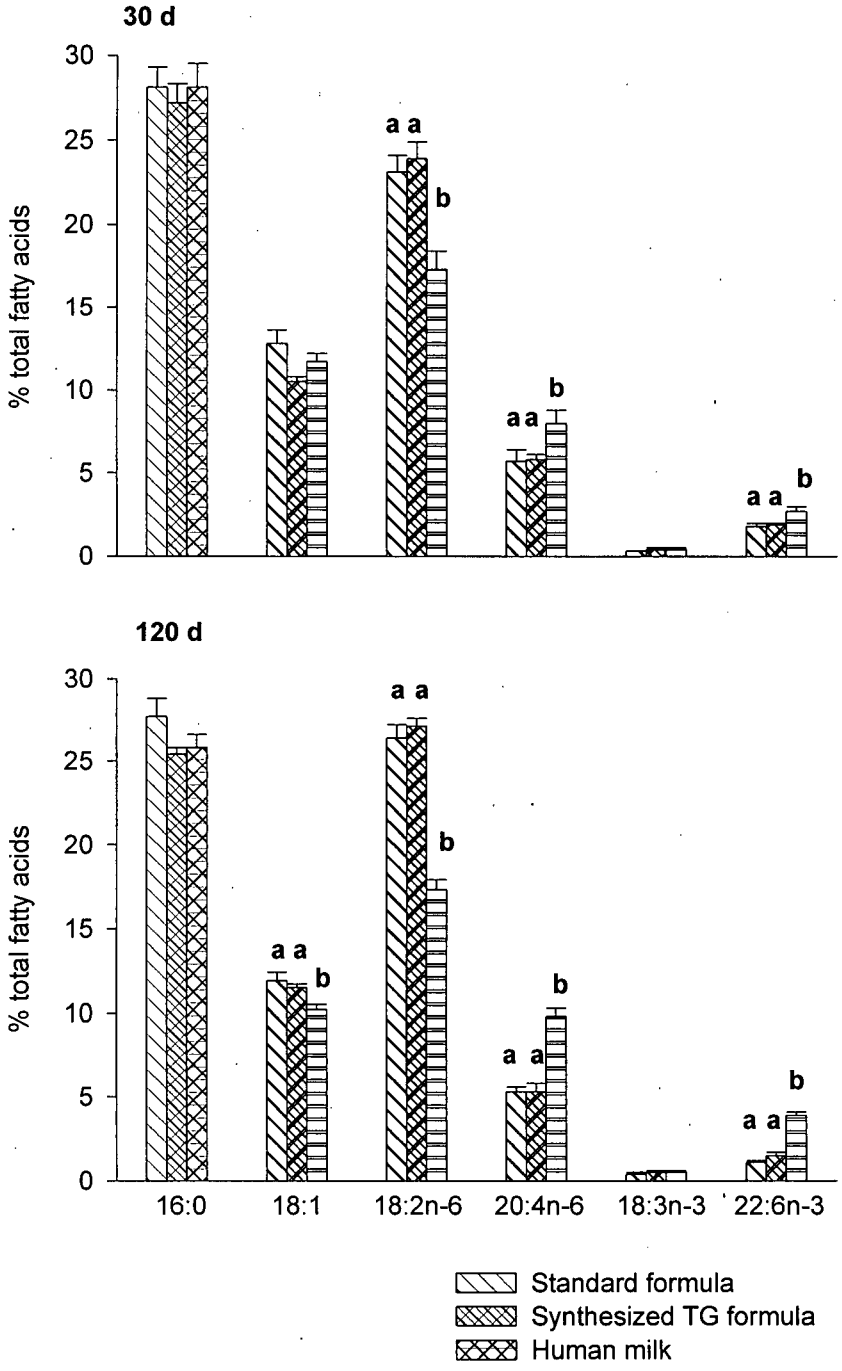


Figure 3.8 – Influence of milk and formula TG fatty acid distribution on chylomicron PL fatty acids. Bars represent means+SEM. Bars with different letters are significantly different ($P<0.05$).

3.2.5. Influence of the formula TG fatty acid distribution on lipoprotein, lysophospholipid and unesterified fatty acid composition

Figure 3.9 provides a summary of the analysis of the lipoprotein, lysoPL and unesterified fatty acids for the infants fed the synthesized TG formula and the infants fed the standard formula at 120 d. This comparison allows specific consideration of whether or not the distribution of fatty acids in formula TG influence the plasma lipid fatty acid composition in the formula-fed infants. These analyses show the distribution of fatty acids in the formula TG did have a significant effect on the levels of 18:1, 18:2n-6, 18:3n-3, 20:4n-6 and 22:6n-3, although the effect depended on the lipoproteins and lipid. There were no significant differences (P>0.05) in the levels of 16:0 or 18:0 in the PL, TG or CE of the chylomicron, LDL and HDL or in lysoPL or unesterified fatty acids between the two groups of formula-fed infants, with the exception that the LDL TG 18:0 and LDL CE 16:0 and 18:0 were higher in infants fed the synthesized TG formula than the infants fed the standard formula. In contrast to 16:0 and 18:0, levels of 18:1 was significantly lower in the LDL and chylomicron CE and lysoPL of the infants fed the synthesized TG than in the infants fed the standard formula. Furthermore, the levels of 18:2n-6 were significantly higher in the unesterified fatty acids and 18:3n-3 was significantly higher in the chylomicron TG and CE, lysoPL and unesterified fatty acids of the infants fed the synthesized TG formula than in the infants fed the standard formula. The levels of 20:4n-6 were significantly higher in the chylomicron TG, but lower in the lysoPL and unesterified fatty acids of the infants fed the synthesized TG than in the infants fed the standard formula. There were no significant differences in 22:6n-3 in the PL, TG, CE of the chylomicrons, LDL or HDL, or in the lysoPL, but the levels of 22:6n-3 were significantly higher in the unesterified fatty acids of the infants fed the synthesized TG formula than in the infants fed the standard formula.

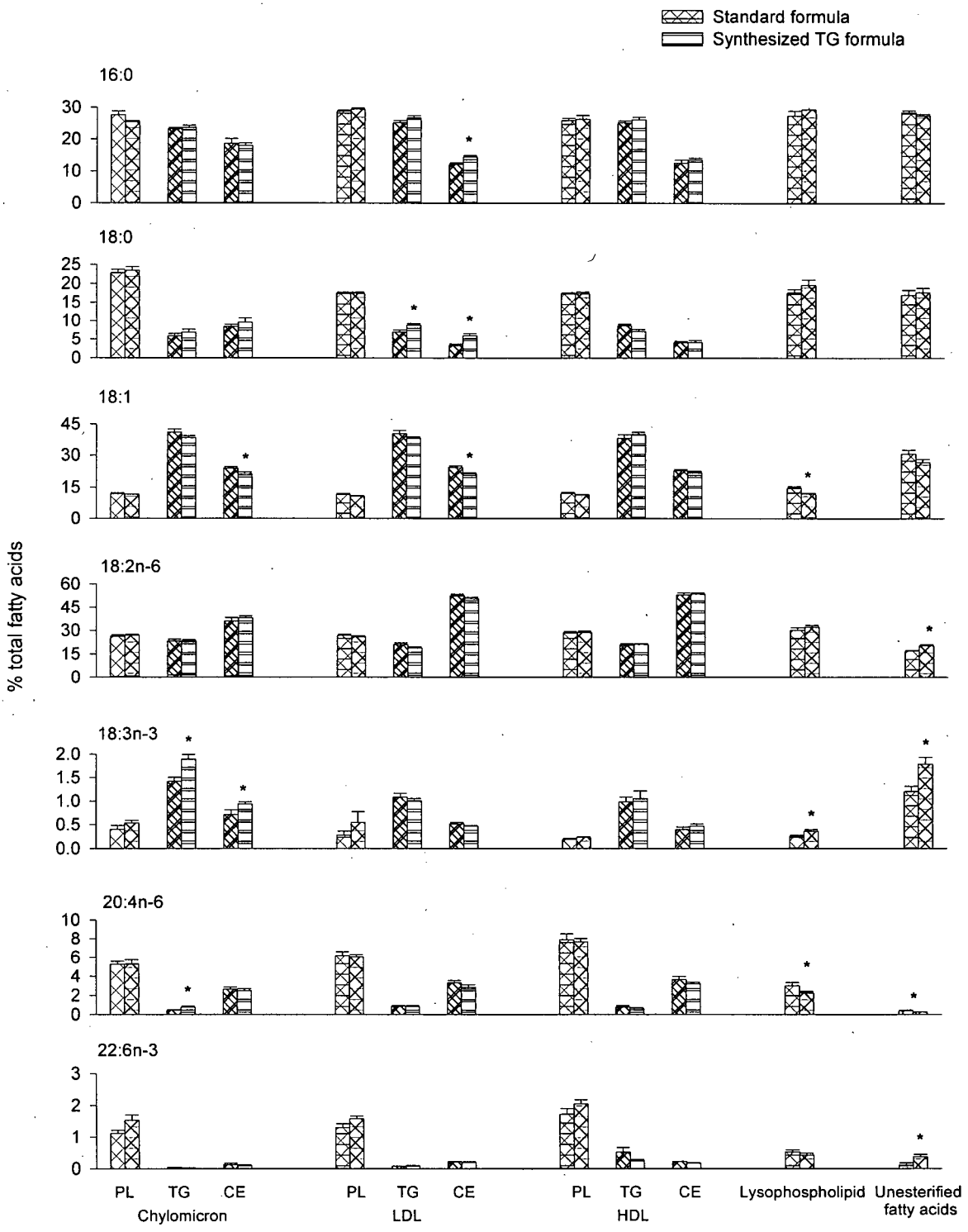


Figure 3.9 – Influence of formula TG fatty acid distribution on infant plasma lipoprotein, lysoPL and unesterified fatty acids. Bars represent means+SEM; *, significantly different (P<0.05) from value for infants fed the standard formula.

3.2.6. Distribution of n-6 and n-3 fatty acids in various lipoprotein fractions

Most of the current interest in n-6 and n-3 fatty acids in infant development has been focused on the potential relation of 20:4n-6 and 22:6n-3 to measures of CNS development and growth (Innis 1994, Auestad 1997, Carlson 1996, Jensen 1997). Therefore, the results concerning 20:4n-6 and 22:6n-3 and their respective precursors 18:2n-6 and 18:3n-3 for breast-fed infants and infants fed formula are the focus of the results presented here.

3.2.6.1. Plasma lipoprotein fatty acids

The distribution of n-6 and n-3 fatty acids in the chylomicron, LDL and HDL PL, TG and CE of the breast-fed infants and of the infants fed formula at 120d of age is shown in **Figure 3.10**. There were no statistically significant (P>0.05) interactions between the levels of the n-6 and n-3 fatty acids in the plasma chylomicron, LDL or HDL PL, TG or CE and age in the breast-fed infants or in the infants fed formula. The results, however, show complex differences in the distribution of the n-6 and n-3 fatty acids among the lipids of chylomicron, LDL and HDL, and differences between the distributions in the breast-fed infants and the infants fed formula.

The breast-fed infants had significantly higher 22:6n-3 in chylomicron, LDL and HDL PL at 120 d of age than at 30d of age (2.7±0.3, 3.5±0.4, 5.0±0.2 % 22:6n-3, respectively, at 30 d). The infants fed formula, on the other hand, had significantly lower 22:6n-3 in chylomicron, LDL and HDL PL at 120d compared to 30d of age (1.8±0.2, 2.2±0.2, 2.8±0.3 %22:6n-3 at 30 d). In contrast to 22:6n-3, there were no significant differences in the levels of 20:4n-6 in the separated lipoprotein PL of the breast-fed infants or formula-fed infants at 120 d when compared to 30 d of age. There were no significant differences in the levels of 20:4n-6 or 22:6n-3 in the lipoprotein TG of the breast-fed infants or the infants fed formula at 120d when compared to 30d of age (Figure 3.10). A significant age effect for LDL CE 22:6n-3 but not 20:4n-6 for the infants fed formula, but not for

those who were breast-fed. In the group of formula-fed infants, the LDL CE levels were significantly lower at 120 d than at 30 d of age (mean±SEM, 0.4±0.01%). There were no other significant differences in the lipoprotein CE levels of 20:4n-6 or 22:6n-3 between the infants at 120 when compared to 30 d of age.

The relative enrichment of 20:4n-6 and 22:6n-3 was significantly different among the lipoproteins. The levels of 20:4n-6 were significantly higher in the HDL than LDL or chylomicron PL of the breast-fed infants (mean±SEM 13.8±0.4, 11.2±0.5, 9.8±0.5 %22:6n-3 for HDL, LDL and chylomicron, respectively) and in the infants fed formula (7.9±0.7, 6.1±0.5, 5.3±0.3 %20:4n-6, respectively). Similarly, the levels of 22:6n-3 were significantly higher in the HDL than LDL, and chylomicron PL (6.0±0.4, 4.6±0.3, 3.9±0.2%) of the breast-fed infants, and higher in the HDL than in the chylomicron PL (1.7±0.2, 1.3±0.1, 1.1±0.1%, respectively) of the formula-fed infants at 120 d of age. In contrast to the PL, the levels of 20:4n-6 were consistently higher in the LDL TG of the 120 d old breast-fed infants and the infants fed formula than in the chylomicron, and not different between the chylomicron and HDL. The level of 20:4n-6 was higher in the HDL and LDL CE than in the chylomicron of breast-fed infants, but were not different among the chylomicron, LDL and HDL CE of the infants fed formula.

The distribution of 22:6n-3 in the lipoprotein TG and CE was significantly different between the breast-fed infants and the infants fed formula. Thus, the level of 22:6n-3 was significantly higher in the LDL than in chylomicron TG or CE, and not different between the LDL and HDL. In contrast, levels of 22:6n-3 were very low (0.1 - 0.2% fatty acids) and not different between the chylomicron, LDL and HDL TG and CE of the infants fed formula, with the exception of a significantly higher 22:6n-3 in the HDL than chylomicron and LDL TG (Figure 3.10).

3.2.6.2. Relative abundance of major n-6 and n-3 fatty acids in separated lipid compared to plasma total phospholipid, triacylglycerols and cholesterol esters of infants

The relative abundance of 18:2n-6, 20:4n-6 18:3n-3 and 22:6n-3 in the PL, TG and CE of chylomicrons, LDL and HDL compared to the same lipid fraction in the plasma of the breast-fed and formula-fed infants is shown in Figure 3.10. Levels of 18:2n-6 and 18:3n-3 are included with 20:4n-6 and 22:6n-3 because the infants fed formula received 18:2n-6 and 18:3n-3, but not 20:4n-6 and 22:6n-3 in the formula. The figure shows that in the 120 d old infants, levels of 18:2n-6 are higher in CE of all the lipoproteins of breast-fed infants than in the infants fed formula (Figure 3.10). Furthermore, the results show plasma lysoPL contain substantial amounts of 20:4n-6 and 22:6n-3, with levels 2-4 fold higher in breast-fed than in infants fed formula, and clearly in the range of the levels found in the plasma total PL. The unesterified fatty acids, on the other hand, contained less than 1% fatty acids as 20:4n-6 and 22:6n-3 in all the infants, but levels of 18:2n-6 and 18:3n-3 were high, with 18:2n-6 and 18:3n-3 representing about 14-17% and 1.2% unesterified fatty acids, respectively, in both the breast-fed infants and the infants fed formula.

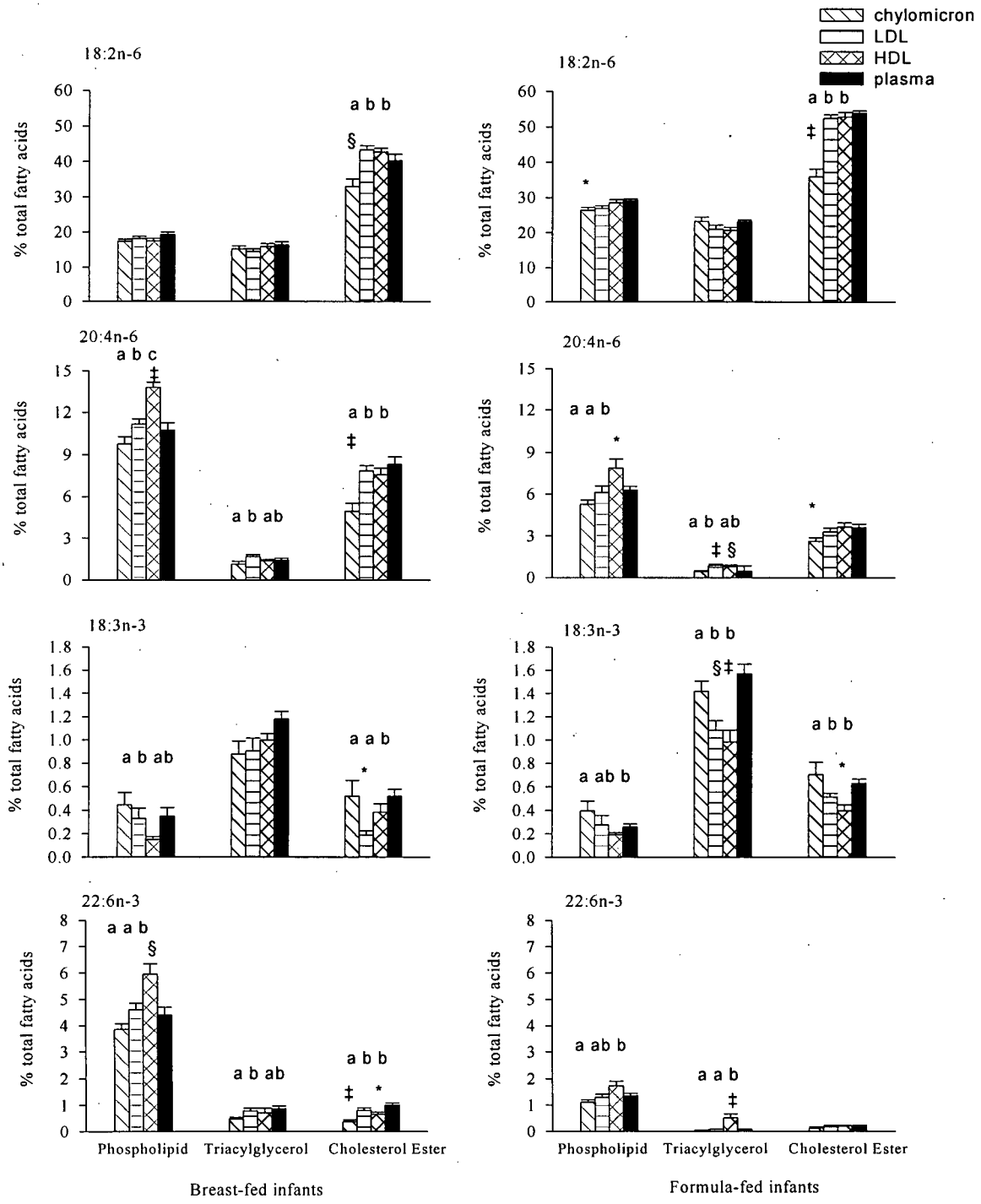


Figure 3.10 – Chylomicrons, LDL, HDL and plasma 18:2n-6, 20:4n-6, 18:3n-3 and 22:6n-3 at 120 d of age of infants fed the standard formula and breast-fed infants. Bars are mean+SEM; * $P<0.05$, § $P<0.01$, ‡ $P<0.001$ between the lipoprotein and the plasma lipid fatty acid. Lipoprotein bars with a different letter are different, $P<0.05$ for a given fatty acid within an age.

Information on the fatty acid composition of lysoPL and unesterified fatty acids of breast-fed infants and infants fed formula does not appear to have been published previously. These plasma lipids, however, are of interest because of the recent studies to show the albumin-bound unesterified and lysoPL fatty acids can enter the brain (Irie 1991, Thies 1994). The results show plasma lysoPL contain substantial amounts of 20:4n-6 and 22:6n-3, with levels 2-4 fold higher in breast-fed than in infants fed formula and clearly in the range of the levels found in the plasma total PL (Table 3.11). The unesterified fatty acids, on the other hand, contained less than 1% fatty acids as 20:4n-6 and 22:6n-3 in all the infants, but levels of 18:2n-6 and 18:3n-3 were high, with 18:2n-6 and 18:3n-3 representing about 14-17% and 1.2% unesterified fatty acids, respectively, in both the breast-fed infants and the infants fed formula.

Table 3.11 *- Plasma lysophospholipid, unesterified fatty acids and phospholipid fatty acids of 120 d old breast-fed infants and infants fed formula*[1]

Fatty acids	Lysophospholipid		Unesterified Fatty Acids		Phospholipid[2]	
	Breast-fed	Formula fed	Breast-fed	Formula fed	Breast-fed	Formula fed
			(g/100g)			
18:2n-6	21.9±1.6	30.2±1.7[4]	13.7±1.5[3+]	17.0±0.6[3+,4]	29.3±0.7	29.2±0.5
20:2n-6	0.5±0.1	0.3±0.1[4]	1.2±0.4	0.6±0.1	0.8±0.1	0.5±0.0
20:3n-6	1.6±0.1[3–]	0.9±0.1[3–,4]	0.8±0.2	0.6±0.2	2.6±0.2	2.1±0.1
20:4n-6	7.3±0.7	3.0±0.3[4]	0.8±0.2	0.4±0.1[4]	10.7±0.5	6.3±0.3
22:4n-6	0.3±0.1	0.3±0.7	0.2±0.1	0.1±0.0	0.3±0.0	0.2±0.0
22:5n-6	0.2±0.0	0.1±0.0	1.3±0.3	0.5±0.2	0.2±0.0	0.3±0.0
18:3n-3	0.3±0.0	0.3±0.0	1.3±0.2[3+]	1.2±0.1	0.4±0.1	0.3±0.0
20:5n-3	0.4±0.1	0.3±0.1	0.3±0.3	0.2±0.1	0.8±0.1	0.2±0.0
22:5n-3	0.5±0.1[3+]	0.2±0.0[4]	0.2±0.1	0.1±0.1	0.8±0.1	0.4±0.0
22:6n-3	2.4±0.2	0.5±0.1[4]	0.9±0.3	0.1±0.0[4]	4.4±0.3	1.4±0.1

[1]Values are means±SEM, values >0.00-<0.05 were rounded to 0.0; breast-fed n=9, formula-fed n=13.
[2]The phospholipid provided as a reference and was not included in the statistical analysis.
[3]Value at 120d significantly (P<0.05) higher (+) or lower (–) from value at 30d of age, within a diet group.
[4]Value for infants fed formula significantly different (P<0.05) from respective value for the breast-fed infants.

3.2.7. Erythrocyte fatty acid composition

3.2.7.1. Phosphatidylcholine

Significant ($P<0.05$) diet and age interactions were found for 18:2n-6, 20:2n-6, 20:4n-6, 22:4n-6, 22:5n-6, 22:5n-3 and 22:6n-3 (**Table 3.12**). For all diet groups, there was significantly lower 18:1, 20:3n-6 and higher 18:2n-6 and 22:5n-3 at 120 d compared with 30 d of age. The infants fed formula had significantly lower 22:6n-3 at 120 d than at 30 d of age, while there was no significant difference in the percent 22:6n-3 at 30 d compared with 120 d of age.

There was no significant difference ($P>0.05$) in erythrocyte PC percent 16:0, 18:0, 20:2n-6, 20:3n-6, 22:4n-6 22:5n-6 20:5n-3 or 22:5n-3 between the infants fed the synthesized TG formula and the infants fed the standard formula. The infants fed the synthesized TG formula, however, had significantly lower 18:1 at 30 d and 120 d of age in their erythrocyte PC than the infants fed the standard formula. The infants fed the synthesized TG formula also had higher 18:2n-6 at 30 d and higher 20:4n-6 and 22:6n-3 in erythrocyte PC at 120 d of age than the infants fed the standard formula.

Both groups of infants fed formula had significantly higher 16:0, 18:2n-6, 18:3n-3 and lower 18:0, 18:1, 20:3n-6, 20:4n-6, 20:5n-3 and 22:6n-3 than the breast-fed infants at 30 d and 120 d of age. There were no significant differences in the erythrocyte PC percent 20:2n-6 or 22:4n-6 between the infants fed formula and the breast-fed infants at 30 and 120 d of age. The infants fed formula, however, had a significantly higher erythrocyte PC 22:5n-6 at 120 d, but not 30 d of age, than the breast-fed infants.

Table 3.12 *- Erythrocyte phosphatidylcholine fatty acids of infants fed either standard formula, formula with synthesized TG or breast-fed to 120 d*[1]

Fatty acids	30 d			120 d		
	Standard formula (n=17)	Synthesized TG formula (n=21)	Human milk (n=25)	Standard formula (n=18)	Synthesized TG formula (n=12)	Human milk (n=22)
	(g/100 g)					
16:0[3]	33.6±0.3[c]	33.7±0.5[c]	31.9±0.3	33.7±0.2[c]	34.2±0.2[c]	32.2±0.3
18:0[3]	12.8±0.2[c]	12.6±0.2[c]	13.6±0.2	12.7±0.1[c]	12.7±0.2[c]	14.2±0.2
18:1[2,3]	19.5±0.3[c]	17.8±0.4[bc]	20.6±0.3	18.2±0.1[ac]	16.8±0.2[abc]	19.4±0.3[a]
18:2n-6[2,3,4]	22.4±0.8[c]	24.6±0.5[bc]	17.4±0.5	26.2± 0.3[ac]	26.3±0.3[c]	18.5±0.6
20:2n-6[4]	0.4±0.0	0.4±0.0	0.4±0.0	0.5±0.1	0.5±0.0	0.4±0.0
20:3n-6[2,3]	1.9±0.1[c]	2.0±0.1[c]	2.5±0.1	1.4±0.1[ac]	1.6±0.1[ac]	1.9±0.1[a]
20:4n-6[2,3,4]	5.4±0.2[c]	5.0±0.2[c]	8.1±0.3	4.1±0.2[ac]	4.6±0.2[bc]	7.8±0.3
22:4n-6[3,4]	0.3±0.0	0.3±0.0[c]	0.3±0.0	0.3±0.0[c]	0.3±0.0	0.3±0.0
22:5n-6[2,4]	0.2±0.0	0.2±0.0	0.2±0.1	0.2±0.0[a]	0.2±0.0	0.1±0.0[a]
18:3n-3[3]	0.2±0.0	0.2±0.0[c]	0.1±0.0	0.2±0.0[c]	0.2±0.0[bc]	0.1±0.0
20:5n-3[3]	0.1±0.0[c]	0.1±0.0[c]	0.3±0.0	0.1±0.0[c]	0.1±0.1[c]	0.3±0.0
22:5n-3[2,3,4]	0.2±0.0[c]	0.2±0.0[c]	0.3±0.0	0.3±0.0[ac]	0.3±0.0[ac]	0.5±0.0[a]
22:6n-3[2,3,4]	1.4±0.1[c]	1.2±0.1[c]	2.1±0.1	0.7±0.0[ac]	0.8±0.0[abc]	2.3±0.1

[1]Values are means ± SEM; >0.00-<0.05 were rounded to 0.0.
[2]Significant main effect of age, (P<0.05).
[3]Significant main effect of diet, (P<0.05).
[4]Significant diet and age interaction, (P<0.05).
[a]Value significantly different from value at 30 d within diet group (P<0.05).
[b]Value for infants fed formula with synthesized TG significantly different from value for infants fed standard formula of the same age, (P< 0.05).
[c]Value for infants fed formula significantly different from values for breast fed infants of the same age, (P<0.05).

3.2.7.2. Phosphatidylethanolamine

There were significant ($P<0.05$) interactions between the effects of diet and infant age in the erythrocyte PE levels of 20:2n-6, 20:3n-6, 22:4n-6, 22:5n-6, 20:5n-3, 22:5n-3 and 22:6n-3 (**Table 3.13**). Levels of 16:0, 22:5n-6 and 22:6n-3 were lower and 18:2n-6, 20:2n-6, 20:4n-6, 22:4n-6, 18:3n-3, 20:5n-3 and 22:5n-3 were higher in PE of all the diet groups at 120 d compared with 30 d of age. The breast-fed infants had significantly higher PE 18:0 at 120 d compared with 30 d of age.

There were no significant differences in the PE fatty acid composition between the infants fed the standard formula and the infants fed the formula with the synthesized TG at 30 d of age. At 120 d of age the PE percent 16:0, 18:0, 18:2n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:4n-6, 22:5n-6, 18:3n-3 and 22:6n-3 similarly showed no differences between the infants fed the standard formula and the infants fed the formula with the synthesized TG. Infants fed the synthesized TG formula, however, had significantly lower 18:1 and higher 20:5n-3 and 22:5n-3 in their erythrocyte PE at 120 d than the infants fed the standard formula.

There was no difference in the erythrocyte PE percent 20:3n-6 and 22:5n-6 between the infants fed formula and the breast-fed infants at either 30 d or 120 d of age. At both 30 d and 120 d of age, however, the infants fed formula had significantly ($P<0.05$) lower 18:0, 20:4n-6, 20:5n-3 and 22:6n-3 and higher 18:2n-6, 22:4n-6 and 18:3n-3 in their erythrocyte PE than the breast-fed infants. Infants fed the standard formula had significantly lower 22:5n-3 at 30 d and both the infants fed the standard formula and those fed the synthesized TG formula had lower PE 22:5n-3 at 120 d than the breast-fed infants.

Table 3.13 *- Erythrocyte phosphatidylethanolamine fatty acids, at 30 and 120 d of age, for infants fed either standard formula, formula with synthesized TG or breast-fed to 120 d*[1]

Fatty acids	30 d			120 d		
	Standard formula (n=17)	Synthesized TG formula (n=20)	Human milk (n=24)	Standard Formula (n=18)	Synthesized TG formula (n=12)	Human milk (n=22)
	(g/100 g)					
16:0[2,3]	20.3±0.4	20.6±0.4[c]	19.6±0.3	16.4±0.2[a]	16.7±0.4[a]	15.8±0.3[a]
18:0[2,3]	6.2±0.1[c]	6.2±0.2[c]	6.6±0.2	6.5±0.1[c]	6.7±0.3[c]	7.5±0.2[a]
18:1[3]	22.4±0.3[c]	22.2±0.5[c]	19.5±0.2	22.3±0.2[c]	20.9±0.2[bc]	19.4±0.4
18:2n-6[2,3]	7.1±0.2[c]	7.5±0.3[c]	4.1±0.2	9.4±0.3[ac]	9.3±0.3[ac]	5.4±0.3[a]
20:2n-6[2,3,4]	0.3±0.0	0.3±0.0	0.2±0.0	0.6±0.0[ac]	0.6±0.0[ac]	0.3±0.0[a]
20:3n-6[4]	1.5±0.1	1.7±0.1	1.7±0.1	1.9±0.1	2.0±0.2	1.5±0.1
20:4n-6[2,3]	21.6±0.4[c]	21.4±0.5[c]	25.0±0.3	24.2±0.3[ac]	24.3±0.4[ac]	26.9±0.4[a]
22:4n-6[2,3,4]	7.4±0.2[c]	7.0±0.2[c]	7.8±0.2	8.4± 0.2[ac]	8.5±0.3[ac]	7.0±0.0[a]
22:5n-6[2,4]	1.6±0.1	1.5±0.1	1.6± 0.1	1.2 ± 0.0[a]	1.2 ± 0.0[a]	0.9±0.1[a]
18:3n-3[2,3]	0.2±0.0[c]	0.2±0.1[c]	0.1±0.0	0.2±0.0[ac]	0.3±0.0[ac]	0.2±0.0[a]
20:5n-3[2,3,4]	0.3±0.0[c]	0.3±0.0[c]	0.5±0.0	0.4±0.0[ac]	0.4±0.0[abc]	1.0±0.1[a]
22:5n-3[2,3,4]	1.3±0.1[c]	1.4±0.1	1.6±0.1	2.5±0.1[ac]	2.9±0.1[abc]	3.7±0.2[a]
22:6n-3[2,3,4]	8.0±0.4[c]	8.0±0.4[c]	9.6±0.2	4.4±0.2[ac]	4.6±0.1[ac]	8.7±0.4[a]

[1]Values are means ± SEM; >0.00-<0.05 were rounded to 0.0.
[2]Significant main effect of age, (P<0.05).
[3]Significant main effect of diet, (P<0.05).
[4]Significant diet and age interaction, (P<0.05).
[a]Value significantly different from value at 30 d within diet group (P<0.05).
[b]Value for infants fed formula with synthesized TG significantly different from value for infants fed standard formula of the same age, (P< 0.05).
[c]Value for infants fed formula significantly different from values for breast fed infants of the same age, (P<0.05).

3.3. Study III - Influence of environmental and dietary factors on measures of novelty preference and visual acuity at nine mo in term infants

3.3.1. Clinic Attendance

The birth list obtained from the city of Vancouver Public Health Department for infants born between January 1st and March 3rd, 1993 and June 4th and Aug 7th, 1993 had 3722 births. From this list, 1813 (48.7%) infants met the eligibility criteria of a gestational age ≥ 37- ≤42 wk, birth weight of 2500-4500 g with an address in the city of Vancouver. Infants on the birth list who had either no address or an incomplete address were not selected for potential participation in the study.

Letters were mailed to 1813 of the eligible infants' parents and a telephone call was attempted to all the parents/guardians. Telephone contact was made with 1461 of the 1813 parents. However, 183 of these could not speak English sufficiently to allow adequate description of the study protocol. From this group, a subgroup of 25 Cantonese speaking families with an eligible infant was selected in order to ensure representation of infants from families of Chinese background in the study. These Chinese families were then telephoned by an assistant fluent in Cantonese. A total of 1278 (70.4%) of the 1813 parents of eligible infants were successfully contacted by telephone. Of these 1278, 592 (46.3%) parents agreed to participate and booked an appointment to attend a clinic coinciding with their infant being 39±1 wk of age. Four hundred and twenty-seven (427) of the parents who booked an appointment attended a clinic (72.1%). In addition, eight infants who met the eligibility criteria attended the clinics without a scheduled appointment. Four hundred and thirty five eligible infants were seen. Data for one infant was later excluded due to prenatal substance abuse which was identified during the clinic appointment. The final study sample size was 434 infants.

3.3.2. Description of the study population

The study population comprised a broad spectrum of racial and ethnic backgrounds, but the infants were predominantly from higher education, higher income two-adult families (**Table 3.14**). No incentives were given to encourage participation in this study. Thus, the parents who did participate were likely to be relatively highly motivated with respect to their infant's well being.

18.7% of the study infants were fed formula from birth, with the remaining 81.3% of the infants breast-fed at least one week or longer. Approximately 60% of the study infants were breast-fed longer than six mo. Of the infants seen, 54% were male and 46% were female. The majority (61.4%) of the infants were Caucasian; Chinese infants comprised 17.6% and 8.7% of the infants were of other Asian backgrounds (eg Japanese, Philipino). The largest proportion (39.1%) of parents reported that they earned over $50,000 per yr, 27.9% earned between $30,000 and $50,000 per yr, 16.2 % indicated that their income was between $20,000 and $29,000 and 16.8% reported their annual income of less than $20,000 per yr. About half of the infants in the study were first born (44.2%), 34.6% were second born and 21.1% were born third or later. Almost all of the parents who participated in the study were married or living common-law (92.7%); only 7.3 % of the people classified themselves as single, divorced or widowed. Only 27 (7.6%) of the mothers and 27 (8.1%) of the father's reported that they had not completed high school. Results for these infants were grouped with those infants whose mothers (20.1%) and fathers (23.2%) had completed highschool. Thirty-four percent of the mothers and 31.5% of the fathers completed college or vocational training, about 45% of both the mothers and fathers had completed university.

Table 3.14 *- Description of study population*

	n	% total
Duration of breastfeeding		
never	73	18.7
to three mo	84	21.5
to six mo	88	22.5
to nine mo	146	37.3
Gender		
male	211	54
female	180	46
Ethnicity[1]		
Caucasian	240	61.4
Chinese	69	17.6
Other Asian	34	8.7
East Indian	33	8.4
Other	15	3.8
Language spoken at home		
English	290	74.2
Chinese	45	11.5
Punjabi	20	5.1
Other	36	9.2
Income (x $1000)		
10 - 19	57	16.8
20 - 29	55	16.2
30 - 50	95	27.9
>50	133	39.1
Birthorder		
first	157	44.2
second	123	34.6
third or later	75	21.1
Marital Status		
Single/sep'd/divor'd	28	7.3
Married/common-law	353	92.7
Mother's education		
high-school	72	20.1
college	121	33.8
university	165	46.1
Father's education		
high-school	78	23.2
college	106	31.5
university	152	45.2
Mother's age (yr)		
20-24	33	8.6
25-29	96	25.1
30-34	155	40.5
35+	99	25.8

[1]Infants of mixed heritage with one Chinese or Asian parent were included in the Chinese or Asian category as appropriate. This decision was due to the prevalence of with-the-rule astigmatism in Asian people compared with against-the-rule astigmatism for Caucasian people that has been suggested to influence acuity scores (Edwards 1991).
[2]Highest level of education attained.

3.3.3. Infant anthropometrics

In the current study, there was significantly (P<0.001) higher weight (9151.1±63.0, 8536.8±68.3, respectively) and longer length (72.5±0.2, 70.8±0.2) and larger head circumference (46.1±0.1, 44.8±0.1) for the boys than the female infants, as expected. Thus, comparisons for the influence of duration of breast-feeding and family characteristics on weight, length and head circumference were done separately for boys (**Table 3.15**) and girls (**Table 3.16**). There was a significant difference for the male infants between ethnic groups in head circumference, with East Indian (45.5±0.2 cm), other Asian (45.6±0.3 cm) having a smaller head circumference than the Caucasian (46.3±0.1 cm) and the "other" (46.8±0.8 cm) group and Chinese (46.0±0.2) not being different to any of the groups. There were no differences in any of the growth parameters due to duration of breast-feeding, mother's or father's education, income or birthorder for the male or female infants.

Table 3.15 *- Infant anthropometric measures for male infants grouped duration of breast-feeding and family variables.*

	n	Weight	Length	Head Circumference
		(g)	(cm)	(cm)
Breast-fed				
Never[1]	70	8994.7±153.5	72.0±0.4	45.9±0.2
≤ 3m	87	9406.9±124.7	73.2±0.3	45.9±0.2
>3m - 6 mo	88	9125.0±125.6	72.2±0.4	46.3±0.2
>6 mo - 9 mo	146	9067.7±108.4	72.6±0.3	46.3±0.2
Mother's education				
high school	72	9053.7±154.7	72.6±0.4	46.0±0.2
college	121	8983.0±97.9	72.3±0.3	46.1±0.1
university	165	9269.5±102.1	72.6±0.3	46.3±0.1
Father's education				
high school	78	9115.0±149.0	72.3±0.4	46.0±0.2
college	106	9220.2±107.0	72.7±0.3	46.2±0.2
university	152	9216.0±102.2	72.6±0.3	46.3±0.1
Gender				
male	211	9151.0±63.0[a]	72.5±0.2[a]	45.4±0.7[a]
female	180	8536.8±68.3[b]	70.5±0.4[b]	44.8±0.1[b]
Ethnicity				
Caucasian	240	9182.3±77.0	72.6±0.2	46.3±0.1[a]
Chinese	69	8976.8±163.0	72.1±0.5	46.0±0.2[ab]
Other Asian	34	8709.7±242.0	72.1±0.7	45.6±0.3[b]
East Indian	33	9504.3±165.9	73.2±0.5	45.5±0.2[b]
Other	15	9476.7±475.4	71.9±0.7	46.8±0.8[a]
Income ($)				
10,000-19,000	57	9177.8±166.1	72.5±0.4	46.0±0.2
20,000-29,000	55	9146.3±187.5	72.6±0.5	46.1±0.2
30,000-50,000	95	9251.6±135.0	72.5±0.3	46.2±0.2
>50,000	133	9248.6±97.2	72.7±0.3	46.2±0.2
Birthorder				
1	157	9311.9±97.4	72.9±0.3	46.4±0.1
2	123	9046.0±112.9	72.2±0.3	45.9±0.2
≥3	75	9036.4±153.1	72.0±0.3	45.9±0.2

[1]The classification of "never breast-fed" includes infants breast-fed for <7d.
[ab]Different superscripts indicate statistically significantly different (P<0.05) from other categories.

Table 3.16 - Infant anthropometric measures for female infants grouped by duration of breast-feeding and family characteristics.

	n	Weight	Length	Head Circumference
		(g)	(cm)	(cm)
Breast-fed				
Never[1]	70	8371.4±133.6	70.9±0.3	44.5±0.2
≤ 3m	87	8707.2±175.8	71.6±0.4	44.9±0.2
>3m - 6 mo	88	8360.4±118.5	70.6±0.4	45.0±0.2
>6 mo - 9 mo	146	8625.5±115.0	70.6±0.3	44.9±0.1
Mother's education				
high school	72	8617.4±173.3	71.2±0.4	44.6±0.2
college	121	8363.3±151.1	70.6±0.3	44.5±0.2
university	165	8639.1±81.4	71.1±0.3	45.2±0.1
Father's education				
high school	78	8652.4±142.1	70.6±0.3	44.7±0.2
college	106	8520.7±130.6	71.3±0.3	44.7±0.2
university	152	8543.0±121.8	70.9±0.3	45.0±0.2
Gender				
male	211	9151.0±63.0[a]	72.5±0.2[a]	45.4±0.7[a]
female	180	8536.8±68.3[b]	70.5±0.4[b]	44.8±0.1[b]
Ethnicity				
Caucasian	240	8649.9±81.3	70.9±0.2	45.2±0.1
Chinese	69	8360.6±141.1	70.8±0.3	44.3±0.2
Other Asian	34	7860.3±201.8	69.6±0.7	43.7±0.3
East Indian	33	8954.2±349.1	72.6±0.6	45.0±0.3
Other	15	8578.3±375.4	70.7±0.9	45.1±0.4
Income ($)				
10,000-19,000	57	8425.2±200.4	71.3±0.5	44.8±0.3
20,000-29,000	55	8583.2±140.4	71.1±0.4	45.0±0.2
30,000-50,000	95	8539.9±165.3	70.7±0.4	44.5±0.2
>50,000	133	8585.5±108.8	70.8±0.3	45.0±0.1
Birthorder				
1	157	8632.3±96.8	71.2±0.2	45.0±0.1
2	123	8399.6±118.5	70.9±0.4	44.7±0.2
≥3	75	8568.9±173.8	70.3±0.4	44.8±0.2

[1]The classification of "never breast-fed" includes infants breast-fed for <7d.
[ab]Different superscripts indicate statistically significantly different (P<0.05) from other categories.

3.3.4. Visual and FTII scores

Preferential looking acuity was determined using the Teller Acuity Card procedure for 428/433 infants. Five infants were not tested due to time constraints. Novelty preference was measured using the FTII for 401/433 infants, with 31 infants not tested due to time constraints of the parents and two test results lost due to computer failure. Of the remaining results, 351 were completed with high confidence and 50 tests were excluded from the overall results for novelty preference due to excessive fussiness, sleepiness or crying during testing. The tests for these infants' results were classified as "low confidence" by the tester, without knowledge of the score achieved (**Table 3.17**).

Table 3.17 *– Summary of infants tested with the FTII*

	Total number of infants	Number tested	Number not tested[1]	Test confidence[2]		Tests not completed
				High	Low	
n	434	401	31	351	50	2
%		(92.4)	(7.1)	(87.5)	(12.5)	(0.5)

[1]Infants were not tested because the parents did not have sufficient time to have test administered

[2]Low test confidence indicated that the tester felt that the infant did not attend to the test in a reliable way due to fussiness, inattentiveness or tiredness.

Potential differences in demographic backgrounds for those infants who were tested with the FTII and those who were not tested were explored. The only statistically significant difference in demographic characteristics was birthorder (P=0.005), with infants of higher birthorder (3 or higher) being more likely not to be tested than infants of lower birthorder (1 or 2). Possibly, it was more difficult for families with other children to give sufficient time to complete the nutritional and personal data questions and testing procedures. There were no statistically significant differences ($P>0.05$) in scores between the two testers who administered the FTII.

Because visual acuity is scored on a logarithmic scale, acuity data do not exhibit a normal distribution. To normalize the acuity data, all values were converted to $\log_{10}$ for all calculations. The

preferential looking acuity mean was 8.7 cyc/deg, with a standard deviation of 0.4 octaves. Preferential looking acuity and novelty preference scores for the infants grouped by gender, duration of breast-feeding and selected environmental variables are presented in **Table 3.18**. There was no significant difference (P>0.05) in preferential looking acuity among the infants when grouped by duration of breast-feeding, mother's education, father's education, ethnicity, language spoken in the home or family income. There was a significant difference (P=0.021) in preferential looking acuity between males and females, with males having higher acuity (9.1±0.4cyc/deg) than females (8.4±0.4 cyc/deg). Birthorder also had a significant effect on preferential looking acuity (P<0.036), but post-hoc analyses within the infants grouped by birthorder of 1st born, 2nd born and 3rd or later born (Tukey test) found no significant group differences.

The novelty preference scores from the FTII were normally distributed, with a mean of 60.4%, standard deviation of 6.5 and a range of 41.2%-81.2%. With the FTII, gender was also the only factor with a significant difference between groups (Table 3.18). Females had higher % novelty than males (61.6±0.5 and 59.6±0.5, respectively). There was no significant difference in % novelty due to duration of breast-feeding, mother's education, father's education, ethnicity, language spoken in the home, income or birthorder. There were no significant differences on any of the looking behaviour measures during the Familiarization phase (**Table 3.19**) or Novel phase of the Fagan test (**Table 3.20**) among the infants grouped by gender, duration of breast-feeding or selected environmental variables.

Table 3.18 *-Visual acuity and FTII scores for infants grouped by duration of breast feeding, gender and selected environmental factors*[1,2]

Factor	n	Visual Acuity (cyc/deg)	*P*	n	FTII (%)	*P*
Breast-fed						
never	52	8.0±0.5	0.830	45	61.2±1.0	0.235
≤ 3m	78	8.7±0.4		65	60.5±0.8	
>3m - 6 mo	70	8.5±0.4		56	62.1±0.8	
>6 mo - 9 mo	117	8.8±0.4		94	60.1±0.7	
Mother's education						
high school	71	8.2±0.5	0.147	58	61.0±0.8	0.268
college	120	9.1±0.4		102	60.3±0.6	
university	163	8.8±0.4		133	60.4±0.6	
Father's education						
high school	78	8.7±0.4	0.173	59	59.0±0.8	0.160
college	103	8.9±0.4		88	61.4±0.6	
university	150	8.8±0.4		122	60.4±0.6	
Gender						
male	209	9.1±0.4[a]	0.021	174	59.6±0.5[a]	0.009
female	172	8.4±0.4[b]		144	61.6±0.5[b]	
Ethnicity						
Caucasian	235	8.9±0.4	0.391	194	60.5±0.5	0.370
Chinese	67	8.3±0.5		55	61.0±0.9	
Other Asian	32	8.9±0.4		29	61.1±1.1	
East Indian	33	8.2±0.4		27	60.3±1.2	
Other	14	9.6±0.5		13	58.1±1.5	
Language at home						
English	283	8.9±0.4	0.412	241	60.5±0.4	0.503
Chinese	44	7.8±0.5		32	60.6±1.2	
Punjabi	20	8.9±0.4		16	61.5±1.4	
Other	34	9.1±0.4		29	60.4±1.1	
Income ($)						
10,000-19,000	55	8.7±0.4	0.927	44	60.2±0.9	0.876
20,000-29,000	55	8.3±0.4		45	60.7±1.1	
30,000-50,000	93	8.6±0.4		75	59.8±0.7	
>50,000	131	8.9±0.4		109	60.9±0.6	
Birthorder						
1	155	8.7±0.4	0.036	130	60.4±0.5	0.152
2	123	8.6±0.4		106	60.1±0.7	
≥3	73	9.4±0.4		51	61.2±0.9	

[1]Values for the visual acuity are cyc/deg±SD in octaves; values for the FTII are means±SEM.

[2]Significant differences ($P<0.05$) indicated by different superscripts.

Table 3.19 *- Looking behaviour during the familiarization phase of FTII for infants grouped by gender, duration of breast-feeding and environmental factors*[1]

	n	# looks left	# looks right	total # looks	R. look time(s)	L. look time(s)	Total look time (s)	Ratio	Individual L look (s)	Individual R look (s)	Dur'n of looks (s)
Breast-fed											
never	53	33.8±0.9	31.2±0.9	26.1±0.8	27.9±0.7	28.1±0.7	126.4±5.5	1.3±0.1	0.9±0.0	0.9±0.0	0.9±0.0
≤ 3m	75	32.4±0.9	30.4±0.9	27.0±0.8	28.0±0.8	28.0±0.8	122.1±8.2	1.2±0.1	0.9±0.0	1.0±0.0	0.9±0.0
>3m- 6m	72	30.6±0.8	28.2±0.9	24.8±0.7	27.8±0.7	28.2±0.7	126.8±7.8	1.3±0.1	1.0±0.0	1.0±0.2	1.0±0.0
>6m-9m	117	32.2±0.8	30.8±0.8	25.9±0.7	28.4±0.5	27.6±0.5	123.4±4.6	1.2±0.1	0.9±0.0	1.0±0.0	0.9±0.0
Mother's ed'n[3]											
high school	57	33.1±1.3	30.1±1.1	26.4±1.0	27.2±0.8	28.8±0.8	120.1±4.8	1.1±0.1	1.0±0.1	0.9±0.0	1.0±0.0
college	102	31.0±0.7	29.3±0.7	25.1±0.7	28.6±0.5	27.4±0.5	128.9±6.0	1.3±0.1	0.9±0.0	1.0±0.0	1.0±0.0
university	133	32.6±0.7	31.0±0.7	26.4±0.6	28.1±0.5	27.9±0.5	122.9±4.5	1.2±0.1	0.9±0.0	1.0±0.0	0.9±0.0
Father's ed'n[3]											
high school	59	33.2±1.0	30.8±1.0	25.3±0.9	27.9±0.7	28.1±0.7	121.4±4.8	1.2±0.1	0.9±0.0	0.9±0.0	0.9±0.0
college	88	32.7±0.9	30.3±0.9	26.9±0.7	28.2±0.6	27.8±0.6	120.0±5.6	1.1±0.1	0.9±0.0	1.0±0.0	0.9±0.0
university	121	31.8±0.7	30.3±0.7	25.9±0.6	28.2±0.5	27.8±0.5	125.2±5.3	1.2±0.1	0.9±0.0	1.0±0.0	0.9±0.0
Gender											
male	174	32.1±0.6	29.9±0.6	25.7±0.5	27.8±0.4	28.2±0.4	126.8±4.5	1.3±0.1	0.9±0.0	1.0±0.0	0.9±0.0
female	143	32.3±0.7	30.5±0.7	26.1±0.6	28.4±0.5	27.6±0.5	121.4±3.8	1.2±0.1	0.9±0.0	1.0±0.0	0.9±0.0
Ethnicity[4]											
Caucasian	194	31.5±0.5	29.9±0.6	25.1±0.5	28.3±0.4	27.7±0.4	128.9±4.2	1.3±0.1	0.9±0.0	1.0±0.0	1.0±0.0
Chinese	54	34.2±1.2	31.8±1.0	28.0±1.1	28.4±0.7	27.6±0.7	117.6±4.4	1.1±0.1	0.9±0.1	0.9±0.0	0.9±0.0
Other Asian	29	33.2±1.6	30.3±1.3	27.8±1.4	26.3±1.0	29.7±1.0	118.0±10.7	1.1±0.2	1.0±0.1	0.9±0.0	0.9±0.0
East Indian	27	32.0±1.6	29.9±1.2	25.1±0.8	28.0±0.9	28.0±0.9	125.6±9.8	1.2±0.2	0.9±0.0	1.0±0.0	0.9±0.0
Other	13	31.0±1.8	28.4±1.4	26.0±1.3	28.0±1.2	28.0±1.2	96.6±5.6	0.7±0.1	0.9±0.1	1.0±0.1	1.0±0.0
Income ($)											
10,000-19,000	44	31.7±1.2	30.7±1.0	26.6±0.8	28.3±0.8	27.7±0.8	114.5±4.8	1.0±0.1	0.9±0.0	1.0±0.0	0.9±0.0
20,000-29,000	45	35.0±1.2	30.8±1.4	27.4±1.1	27.2±0.8	28.8±0.8	119.7±6.5	1.1±0.1	0.9±0.0	0.9±0.0	0.9±0.0
30,000-50,000	74	31.7±1.0	29.4±0.9	25.8±0.8	28.2±0.6	27.8±0.6	119.6±5.8	1.1±0.1	0.9±0.0	1.0±0.0	1.0±0.0
>50,000	109	31.9±0.8	30.8±0.7	26.2±0.4	28.5±0.5	27.5±0.5	125.6±5.5	1.2±0.1	0.9±0.0	1.0±0.0	0.9±0.0
Birthorder											
1	129	32.2±0.8	29.6±0.8	26.5±0.8	27.8±0.4	28.2±0.4	126.9±5.2	1.3±0.1	0.9±0.0	1.0±0.0	1.0±0.0
2	106	32.0±0.8	30.8±0.8	25.3±0.7	28.2±0.5	27.8±0.5	124.6±4.8	1.2±0.1	0.9±0.0	1.0±0.0	0.9±0.0
≥3	51	31.7±1.1	29.8±0.9	25.9±0.9	28.3±0.6	27.7±0.6	120.6±7.3	1.2±0.1	0.9±0.0	1.0±0.0	1.0±0.0

[1]Values are means±SEM; R, right; L, left

[2]No statistically significant differences (P>0.05) were found.

[3]Highest level of education attained.

[4]Infants of mixed heritage with one Chinese or Asian parent were included in the Chinese or Asian category as appropriate. This decision was due to the prevalence of with-the-rule astigmatism in Asian people compared with against-the-rule astigmatism for Caucasian people that has been suggested to influence acuity scores (Edwards 1991).

Table 3.20 - Infant looking behaviour during the novel phase of the FTII for infants grouped by gender, duration of breast-feeding and environmental factors[1,2].

	n	# looks to novel stimuli	Attn to novel stimuli (s)	# looks to familiar stimuli	look time to familiar stimuli	total # looks	total look time	Ratio[3]	overall time/look	time/look at novel stimuli	time/look at familiar stimuli
Breast-fed											
never	53	36.9±1.0	31.0±0.6	65.0±1.6	16.1±0.4	63.0±1.7	47.1±0.7	1.0±0.0	0.8±0.0	0.9±0.0	0.7±0.0
≤ 3m	75	38.0±1.1	30.5±0.5	62.8±1.6	16.3±0.4	65.0±1.8	46.8±0.6	1.1±0.0	0.8±0.0	0.8±0.0	0.7±0.0
>3m- 6m	72	35.5±1.0	31.3±0.5	58.8±1.4	16.8±0.4	60.1±1.7	48.0±0.6	1.0±0.0	0.8±0.0	0.9±0.0	0.7±0.0
>6m-9m	117	36.9±0.9	30.7±0.4	63.0±1.4	16.0±0.3	62.8±1.5	46.7±0.5	1.1±0.0	0.8±0.0	0.9±0.0	0.7±0.0
Mother's ed'n[4]											
high school	57	36.7±1.1	30.4±0.6	63.2±2.1	15.8±0.5	63.0±2.0	46.2±0.7	1.1±0.0	0.8±0.0	0.9±0.0	0.7±0.0
college	102	36.1±0.9	31.3±0.4	60.3±1.2	16.6±0.3	61.2±1.5	47.8±0.5	1.0±0.0	0.8±0.0	0.9±0.0	0.7±0.0
university	133	37.4±0.8	30.5±0.4	63.6±1.3	16.3±0.3	63.8±1.3	46.9±0.5	1.1±0.0	0.8±0.0	0.9±0.0	0.7±0.0
Father's ed'n[4]											
high school	59	35.9±1.2	29.7±0.6	64.0±1.8	16.6±0.5	61.2±2.0	46.3±0.7	1.1±0.0	0.8±0.0	0.9±0.0	0.7±0.0
college	88	38.4±1.0	31.0±0.5	63.0±1.6	15.8±0.3	65.3±1.7	46.9±0.6	1.0±0.0	0.8±0.0	0.9±0.0	0.6±0.0
university	121	36.9±0.8	30.8±0.4	62.1±1.3	16.5±0.4	62.8±1.4	47.3±0.5	1.1±0.0	0.8±0.0	0.9±0.0	0.7±0.0
Gender											
male	174	36.0±0.7	30.5±0.3	62.0±1.0	16.7±0.3	61.7±1.1	47.2±0.4	1.1±0.0	0.8±0.0	0.9±0.0	0.7±0.0
female	143	37.9±0.8	31.2±0.4	62.8±1.2	15.7±0.3	64.0±1.3	46.9±0.5	1.0±0.0	0.8±0.0	0.9±0.0	0.6±.0.0
Ethnicity 5											
Caucasian	194	36.3±0.6	30.9±0.3	61.4±1.0	16.3±0.3	61.4±1.1	47.2±0.4	1.1±0.0	0.9±0.0	0.9±0.0	0.7±0.0
Chinese	54	39.1±1.4	30.9±0.7	65.9±2.0	16.1±0.4	67.1±2.3	46.9±0.8	1.1±0.1	0.7±0.0	0.8±0.0	0.6±0.0
Other Asian	29	38.5±1.8	30.2±0.7	63.5±2.8	15.5±0.6	66.3±3.0	45.7±0.8	1.1±0.1	0.7±0.0	0.8±0.0	0.6±0.0
East Indian	27	35.0±1.2	30.4±0.9	61.9±2.0	16.0±0.6	60.1±1.9	46.4±0.9	1.1±0.1	0.8±0.0	0.9±0.0	0.7±0.0
Other	13	36.0±2.0	31.7±0.9	59.4±2.6	18.8±0.8	62.0±3.0	50.5±0.9	1.0±0.1	0.8±0.1	0.9±0.1	0.8±0.1
Income ($)											
10,000-19,000	44	37.1±1.3	30.6±0.5	62.3±1.7	16.2±0.5	63.7±1.9	46.8±0.7	1.1±0.0	0.8±0.0	0.9±0.0	0.6±0.0
20,000-29,000	45	38.8±1.4	30.3±0.6	65.7±2.4	16.2±0.6	66.2±2.4	46.5±0.7	1.1±0.5	0.7±0.0	0.8±0.0	0.6±0.0
30,000-50,000	74	36.6±1.0	30.7±0.5	61.1±1.7	16.4±0.4	62.3±1.7	47.1±0.6	1.1±0.0	0.8±0.0	0.9±0.0	0.7±0.0
>50,000	109	36.8±0.9	31.1±0.5	62.7±1.3	16.3±0.3	62.5±1.5	47.4±0.6	1.0±0.0	0.8±0.0	0.9±0.0	0.7±0.0
Birthorder											
1	129	37.7±0.8	30.6±0.4	61.8±1.1	16.2±0.3	64.1±1.4	46.9±0.5	1.1±0.0	0.8±0.0	0.9±0.0	0.7±0.0
2	106	35.6±0.8	30.7±0.4	62.8±1.5	16.6±0.4	60.8±1.4	47.3±0.5	1.1±0.0	0.8±0.0	0.9±0.0	0.7±0.0
≥3	51	36.9±1.3	30.7±0.6	61.5±1.7	15.9±0.5	62.8±2.1	46.6±0.7	1.1±0.0	0.8±0.0	0.9±0.0	0.7±0.0

[1]Values are means±SEM

[2]No statistically significant differences ($P>0.05$) were found.

[3]Calculation for the ratio based on Colombo et al 1991

[4]Highest level of education attained.

[5]Infants of mixed heritage with one Chinese or Asian parent were included in the Chinese or Asian category as appropriate. This decision was due to the prevalence of with-the-rule astigmatism in Asian people compared with against-the-rule astigmatism for Caucasian people that has been suggested to influence acuity scores (Edwards 1991).

Multiple regression analyses were done to determine if gender, duration of breast-feeding or selected environmental variables were predictive of preferential looking acuity, or scores in the FTII. **Table 3.21** shows the regression coefficient (β) for acuity, % novelty preference score on the FTII and measures from the FTII that have been reported to reflect more specific looking behaviours of infants. Similar to the results obtained by ANOVA (Table 3.40), gender was found to be predictive of preferential looking acuity (r^2=0.023, *P*=0.03) and percent novelty in the FTII (r^2=0.023, *P*=0.01) (**Table 3.22, Figure 3.11**). The regression coefficient indicates that, if all other variables were held constant, the predictive value of gender would be higher by β=0.15 for every 1% difference in the FTII score. Gender was also significantly predictive of the time spent looking at the familiar stimuli (r^2=0.021, *P*=0.02) and the duration of looks (r^2=0.026, *P*=0.034) at the familiar stimuli during the paired comparison portion of the FTII. Boys spent more overall time looking at the familiar stimuli in the paired comparison (16.7±0.3 s) and had longer look duration (0.66±0.1 s) at the familiar stimuli than girls (15.7±0.3 s and 0.61±0.01 s, respectively).

Infants' visual attention has been shown to be predicted from the total time required to reach the preset looking time required during the first three familiarization tests in the FTII (Rose 1987). The same calculation of infant attention was used in the FTII for the current study (Table 3.21). In the current study, the infant's visual attention was predicted by the marital status of the mother (r^2=0.033, *P*=0.018) (Table 3.21). Infants of single parent families took longer to reach the familiarization criterion (82.5±7.5s) than infants of dual parent families (68.7±2.3s).

The language spoken in the infant's home was found to be predictive of the number of looks and duration of looks the infant had during the familiarization phase of the FTII. The infants classified as "Other" (not English, Chinese or Punjabi) language had more looks, but a shorter look duration than the English, Chinese or Punjabi language groups (**Table 3.23**).

Factors that were not predictive of preferential looking acuity included duration of breast-feeding, birthorder, language spoken at home, maternal age, maternal education, income and

marital status. Factors that were not significant in predicting performance on the FTII were the duration of breastfeeding, birthorder, maternal age, maternal education and income.

Table 3.21 *- Multiple linear regression of visual acuity and looking behaviour during FTII against selected demographic predictors for infants at 39±1 week of age*[1]

	Predictors							
	Breastfed	Gender	Birthorder	Language at Home	Maternal Age	Maternal Education	Income	Marital Status
Visual Acuity	0.051	-0.12*	0.11	0.007	-0.041	0.076	0.046	-0.75
FTII Score	-.035	0.151*	0.034	-0.011	0.012	-0.035	0.029	0.083
Familiarization								
Total # looks	-0.071	0.009	-0.04	0.14*	0.014	0.092	-0.031	-0.028
Time to criterion	0.013	0.015	0.016	0.093	0.036	0.001	-0.025	0.11
Look duration	0.071	-0.009	0.04	-0.14*	-0.014	-0.092	0.031	0.028
Visual attention[2]	0.051	0.005	0.018	0.11	0.072	-0.04	-0.027	0.14*
Ratio[3]	-0.016	-0.013	-0.025	-0.094	-0.031	0.001	0.019	-0.11
Paired comparison								
Looks to novel	-0.068	0.11	-0.091	0.12	0.031	0.076	-0.062	-0.004
Time to novel	-0.01	0.085	0.019	-0.045	-0.033	-0.015	0.029	0.022
Novel look duration	0.057	-0.049	0.095	-0.14	-0.046	-0.078	0.072	0.016
Looks to familiar	-0.065	0.024	-0.082	0.11	0.022	0.065	-0.09	0.007
Time to familiar	-0.005	-0.15*	-0.017	-0.001	0.015	0.053	0.028	-0.053
Familiar look duration	0.055	-0.12*	0.05	-0.087	-0.005	-0.033	0.11	-0.034
Total # looks	-0.073	0.075	-0.091	0.13	0.026	0.077	-0.083	0.000
Total time	-0.004	-0.025	0.007	-0.042	-0.018	0.013	0.054	-0.012
Look duration	0.064	-0.081	0.088	-0.13	-0.034	-0.064	0.096	-0.006
Ratio[2]	0.009	-0.085	-0.018	0.045	0.033	0.015	-0.029	-0.022

[1]Values are standardized partial regression coefficients (β); * $P<0.05$.
[2]Calculation for visual attention from Rose et al 1987, is the total amount of time required to reach the test defined required looking time during the familirization phase of the FTII.
[3]Calculation for the ratio from Colombo et al1991

Table 3.22 *– Summary of the association of visual acuity and looking behaviour during the Fagan test with gender for infants at 39 ± 1 wk of age*[1].

	Gender		r^2	*P*
	male	female		
Visual Acuity (cyc/deg)	9.1±0.4 (n=209)	8.4±0.4 (n=172)	0.023	0.03
FTII Score (%)	59.7±0.5 (n=174)	61.6±0.5 (n=144)	0.023	0.01
Familiarization				
Time/look (s)	0.66±0.02	0.61±0.02	0.026	0.03
Novel				
Time at familiar stimuli (s)	16.7±0.3	15.7±0.3	0.021	0.02

[1]Values for visual acuity are means±SD (octaves); for FTII score, values are means±SEM.

Table 3.23 *– Summary of the association between looking behavior during the Fagan test and language spoken at home for infants at 39 ± 1wk*[1]

	Language at spoken at home				r^2	*P*
	English	Chinese	Punjabi	Other		
	n=240	n=32	n=16	n=29		
Total number of looks during familiarization	60.0±1.0	62.5±1.1	60.7±1.1	66.7±1.0	0.018	0.037
Time/look during familiarization (s)	0.93±0.01	0.90±0.02	0.92±0.02	0.84±0.02	0.018	0.037

[1]Values are means ± SEM.

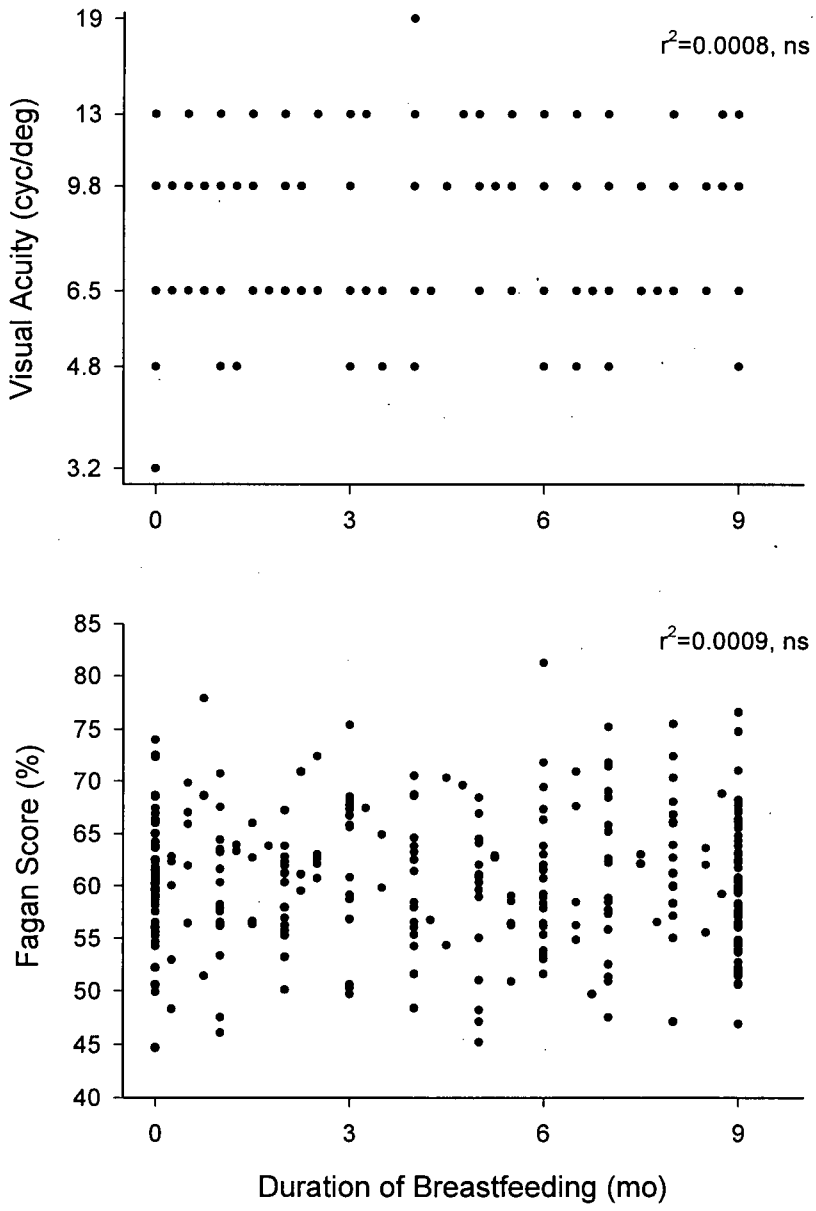


Figure 3.11 - Scatterplot for visual acuity (log_{10} scale) and Fagan score (linear scale) against duration of breast-feeding .No statistically significant (P>0.05) relationship measured.

4. DISCUSSION

4.1. Relation between milk and formula and n-6 and n-3 fatty acids and measures of visual and CNS development

Consistent with previous studies (Innis 1991, Ponder et al 1992, Putnam et al 1982), the plasma and erythrocyte PL percent 22:6n-3 of the infants fed formula with about 1% of energy as 18:3n-3 and no 22:6n-3 were significantly lower than in the breast-fed infants. However, despite the differences in 22:6n-3 in the plasma and erythrocytes, no significant difference in the preferential looking acuity were found between the breast-fed infants and the infants fed formula.

The lower percent 22:6n-3 in plasma PL and erythrocyte PC of the infants fed formula was evident by 14 d of age, but the decrease in erythrocyte PE 22:6n-3 occurred more slowly and was significant by three mo. The slower response of the erythrocyte PE than the erythrocyte PC and plasma PL to the absence of a dietary supply of 22:6n-3 may be due to acyltransferase specificities involved in esterification of 22:6n-3 in PE as well as the differential positioning of PE and PC in the erythrocyte membrane. Phosphatidylethanolamine is preferentially distributed on the cytosolic side and PC on the plasma side of the erythrocyte membrane (Innis 1992a). Phosphatidylcholine on the outer membrane of the erythrocyte is thus able to exchange with the plasma PC.

Several studies have shown that adding 20:4n-6 and 22:6n-3 to infant formula results in increased 20:4n-6 and 22:6n-3 in the blood lipids of infants fed formula (Makrides et al 1995, Carlson et al 1996, Innis et al 1996, Birch et al 1998). Given that the fatty acid composition of plasma and erythrocyte PL respond quickly to the dietary fat composition and the brain does not, it has been argued previously that plasma and erythrocyte 22:6n-3 concentrations are not a consistently reliable index of CNS 22:6n-3 (Innis 1991). Plasma PL fatty acids are a mixture of PL from a heterogeneous plasma lipoprotein pool that includes chylomicrons, VLDL, LDL and HDL.

Each of these lipoproteins has a different amount of PL, varying from chylomicrons with 4% (by weight) to HDL with 50% (by weight) PL and have different sites of synthesis and metabolic fates (Ginsberg 1994). Furthermore there are important differences in fatty acid metabolism between erythrocytes and the CNS. Erythrocytes have a single membrane and lack the enzymes for *de novo* fatty acid synthesis, desaturation or elongation, as well as PL synthesis (Innis 1992a). Brain and retina, on the other hand, are able to desaturate and elongate 18:2n-6 and 18:3n-3 to 20:4n-6 and 22:6n-3, respectively (Moore et al 1990, Moore et al 1991). Brain also has pathways for *de novo* fatty acid and cholesterol synthesis from acetyl CoA, synthesis of PL and numerous other lipids, and has several lipoprotein receptors and fatty acid binding and transport proteins which most likely regulate fatty acid uptake. Relatively rapid changes occur in the erythrocyte membrane fatty acid composition after their release to the vascular system in response to changes in the dietary fatty acid composition (Farquhar & Ahrens 1963, Hill et al 1965, Popp-Snijder et al 1986, Periago et al 1990). In contrast, there is no specific information on which lipoproteins or lipids (PL, TG, CE, lysoPL or FFA) are important in the transfer of n-6 or n-3 fatty acids to the CNS. Thus, analyses of plasma total PL fatty acids, or erythrocyte PC or PE, may not accurately reflect the pool of n-6 and n-3 fatty acids available to the CNS of the breast-fed and formula-fed infants, or provide sensitive and specific markers of differences in tissue n-6 and n-3 fatty acid status.

The primary behavioral outcome measure of Study I was the assessment of preferential looking acuity to three mo using the Teller Acuity Card test. There was no difference in preferential looking acuity between the healthy, full-term gestation infants fed formula with ~1% of energy as 18:3n-3 and breast-fed infants at either 14 d or three mo of age. Post-hoc analysis showed this study had 95% power to detect a difference in visual acuity of 0.5 octaves at these two ages. Furthermore, there continued to be no difference in preferential looking acuity to 18 mo of age between the infants fed formula and the breast-fed infants. Despite a decrease in sample size at

the later ages, there was still >90% power to detect a difference of 0.5 octaves to 18 mo. This result provides no evidence to suggest that 22:6n-3 is an essential dietary nutrient for the early development of preferential looking acuity in formula-fed infants when studied by the methods used in this study. The reason for the lack of a difference in preferential looking acuity between the breast-fed infants and the infants fed formula in this study may be that the formula had an adequate supply of 18:3n-3 and an appropriate ratio of 18:2n-6 and 18:3n-3 to support development of preferential looking acuity similar to that of the breast-fed infants. Conversely, it is possible that the level of 22:6n-3 in the human milk was insufficient to support the development of visual acuity and that the breast-fed infants visual acuity was lower than would have been found at a higher level of human milk 22:6n-3. It is also possible that the Teller Acuity Card method is not sensitive enough to detect the influence of feeding a formula with no source of 20:4n-6 and 22:6n-3 on visual function. Finally, it is not known if a difference in visual acuity would have been found if the assessments had been done at different ages.

The findings of this study showing no difference in preferential looking acuity between breast-fed infants and infants fed formula are consistent with the results of a recent large multicentre study of healthy term gestation breast-fed infants and infants fed formula (Auestad et al 1997). In the study by Auestad et al (1997), preferential looking acuity and visual evoked potential (VEP) acuity scores were similar from birth to 14 mo of age between breast-fed infants and infants fed formula with 9% energy from 18:2n-6 and 1% of energy from 18:3n-3 but without 20:4n-6 and 22:6n-3, or formula with 0.23% 22:6n-3 and infants fed formula with 0.12% 22:6n-3 and 0.43% 20:4n-6 (Auestad et al 1997). Another recent study with term gestation infants found no significant differences in transient VEP acuity at either 120 or 240 d of age among infants fed one of four different formulae with 18:3n-3 levels ranging from 0.2 % to 1.5 % of energy (Jensen et al 1997). In contrast to these studies, however, others have found lower VEP and preferential looking acuity in

infants at ages to 4mo of age, and lower operant preferential looking grating and random dot stereoacuities in three yr old children who had been fed a formula with 0.3-0.6% of energy as 18:3n-3 rather than breast-fed (Birch et al 1992, Birch et al 1993). A more recent study by Birch et al (1998) found lower sweep VEP acuity at six, 17 and 52 wk, but not at 26 wk of age in term gestation infants fed a conventional formula with about ~0.7 % of energy as 18:3n-3 than in breast-fed infants. In the same study (Birch et al 1998), infants fed a formula with 0.35% 22:6n-3 and infants fed formula with 0.72% 20:4n-6 and 0.36% 22:6n-3 had sweep VEP acuities not different from the breast-fed infants. In contrast to earlier work by Birch et al (1993), there was no difference in forced-choice preferential looking acuity between infants fed the formulas with and without 22:6n-3 and the breast-fed infants (Birch et al 1998). Another study with term gestation infants fed a formula with about 1% of energy from 18:3n-3 and no 20:4n-6 or 22:6n-3 reported lower preferential looking acuity at two mo, but not at later ages, than in breast-fed infants (Carlson et al 1996). However, the analysis of variance indicated no diet effects on visual acuity; significant differences at two mo between the breast-fed infants and the infants fed formula were based on *post-hoc* t-tests. A study of infants at approximately five mo of age in Australia found lower VEP acuity in infants who were predominantly fed with a formula with ~6-8% of energy as 18:2n-6 and 0.5-0.8% of energy as 18:3n-3 than in breast-fed infants (Makrides et al 1993). In a subsequent study, the same group found a lower VEP acuity at 16 and 30 wk of age in infants fed a formula with about 16.8% of total fatty acids as 18:2n-6 and 1.6% 18:3n-3 than in breast-fed infants (Makrides et al 1995).

Several reasons could explain the apparent discrepancies in the results of studies which do and those which do not find differences in visual acuity between breast-fed and formula-fed infants. These include lower amounts of 18:3n-3 and/or higher ratios of 18:2n-6 to 18:3n-3 in the formula, resulting in deficiency in n-3 fatty acids in some studies. Recent expert panels have suggested a

minimum level of 18:3n-3 in formula of 1.75% fatty acids (LSRO 1998), whereas the Canadian RDNI (1993) recommend a minimum of 1% energy (about 2% fatty acids) as 18:3n-3 for infants with no dietary source of 22:6n-3. The differences in methodologies used to measure visual acuity as well as the ages of testing may also explain some of the differences in results. It is known that VEP acuity is higher and matures much more rapidly than does visual acuity assessed by preferential looking methods. Also, VEP is a measure of sensory development while preferential looking is a behavioral response requiring an integration of visual, motor and cognitive functions (Salapatek 1975). Furthermore, differences in VEP acuity are not necessarily accompanied by changes in the ability to see (Dobson 1978). This may be explained by signals in the cortical responses to visual stimuli that are not expressed, or have no significance to the ability to see. Whether such signals have any relevance to functional vision is not known. Whether measures of acuity using VEP methods would have revealed differences in the cortical responses to visual stimulation between the breast-fed infants and infants fed formula in the study here, despite the lack of evidence of differences in ability to resolve black and white gratings, is unknown. Not all studies using VEP techniques have reported differences in acuity between infants fed formula with or without 22:6n-3 and breast-fed infants (Auestad et al 1997). In this case, it is possible that the discrepancy in the results could be due to differences in the VEP methodologies, although this is unknown.

The age of testing may also be a significant consideration when trying to understand the variability in test results of studies on dietary n-3 fatty acids and visual function. Differences in visual acuity using preferential looking methods have only detected a difference in visual acuity between breast-fed infants and infants fed formula up to four mo of age (Birch et al 1992, Carlson et al 1996, Jorgensen et al 1996). The foveal area of the retina, which is responsible for visual acuity, has a rapid rate of development and does not mature in humans until approximately four mo

of age (Abramov 1982). Differences in infant visual acuity that are detected before four mo of age may be due to differences in the rate of foveal maturation and possibly these differences may disappear after four mo between breast-fed infants and infants fed formula.

Clinical studies on the adequacy of human milk and infant formula n-6 and n-3 fatty acids for CNS development have frequently relied on measures plasma or erythrocyte PL fatty acids and the relation of these blood lipid measures to results of tests of CNS function (Makrides et al 1993, Carlson et al 1996, Birch et al 1998, Auestad et al 1997, Jensen et al 1997). Prior to the start of the studies here, some studies had reported significant associations (by linear regression) between visual acuity and 22:6n-3 in erythrocyte PE (Birch et al 1993). The study here found no relation between plasma PL, erythrocyte PC or PE or dietary 22:6n-3 and visual acuity at three mo of age within or among breast-fed and formula-fed infants. Carlson et al (1996) found a significant difference in visual acuity at two mo between breast-fed infants and infants fed formula, but found no differences in erythrocyte PE 22:6n-3 or plasma PC 22:6n-3 between the breast-fed infants and the infants fed formula at two mo. The study by Auestad et al (1997) found 40% higher erythrocyte PL 22:6n-3 at four mo of age, but no differences in visual acuity at one, two, four, six, nine and 12 mo of age in infants fed a formula with 22:6n-3 or with 20:4n-6 and 22:6n-3 compared with infants fed formula with no source of 20:4n-6 or 22:6n-3. This suggests that the lower plasma and erythrocyte PL 22:6n-3 found in infants fed formula without 22:6n-3 than in breast-fed infants reflects the difference in dietary intake of 22:6n-3 and not necessarily a change in 22:6n-3 in the CNS of significant magnitude to alter measures of acuity.

Although the primary purpose of Study I was the assessment of visual acuity, measures of novelty preference with the Fagan Test of Infant Intelligence (FTII) and cognitive and motor development with the Bayley Scales of Infant Development (BSID) were also done. No significant differences were detected in the scores on these tests between breast-fed infants and the infants

fed formula. However, it is important to note that Study I had insufficient power to detect a difference of five points for the Bayley test and 2% novelty preference for the FTII between the breast-fed infants and the infants fed formula. The five points for the Bayley test and the 2% for the FTII represent the standard deviation that was found for these measures in the current study. The effect of low power is an increased probability of making a Type 2 error, ie. the study fails to detect a true effect. The scores achieved by the breast-fed infants and the infants fed formula on the BSID, however, were well within the score classification "within normal limits" of 85-114 (Bayley 1969). The FTII does not have a standard set of age-related norms for healthy term infants (Benasich 1992). At the time that this study was started (1992-1994), studies of CNS development in term breast-fed infants and infants fed formula with respect to dietary fatty acids had sample sizes as few as eight per group to about 20-23 per group (Birch et al 1992, Makrides et al 1993, Agostoni et al 1994, Carlson et al 1996). It is now appreciated that studies of cognitive development of breast-fed infants compared with infants fed formula require a sample size of at least 144 subjects per group (for a two group study) to detect a difference of 0.3 SD, with 80% power at the 5% level of significance (Morley 1998).

Only a few studies have considered the possible effect of dietary fatty acid composition on cognitive development in infants. A recent study found no significant differences in scores on the Bayley Scales of Infant Development (n=197) or in tests of language development (MacArthur Communicative Index Device, n=169) between infants who had been breast-fed and infants who had been fed formula with no 22:6n-3 (Auestad 1997, Scott 1998). However, infants fed a formula with 0.2% 22:6n-3 from fish oil had significantly lower language comprehension scores at 14 mo of age than breast-fed infants. Furthermore, a significant negative association between erythrocyte 22:6n-3 and vocabulary production and comprehension was found (Scott 1998). It is not clear if some oil sources of 22:6n-3 or changes in n-6 fatty acid metabolism which might result from 22:6n-

3 supplementation without 20:4n-6 can have negative effects on the developing CNS. A study from Italy that used the Brunet-Lézine Neurodevelopment Scale found lower developmental quotients in four mo old infants fed a formula with (in total fatty acids) 10.8% 18:2n-6 and 0.7% 18:3n-3 than in breast-fed infants (Agostoni et al 1995). In the same study, infants fed formula with 0.44% 20:4n-6, 0.05% 20:5n-3 and 0.3% 22:6n-3 had significantly higher developmental quotients than infants fed the formula without 22:6n-3. When reassessed at 24 mo with the Brunet-Lézine Neurodevelopment Scale, no significant differences were found among any of the groups (Agostoni 1997). The conflicting results of different studies could again be due to variations in levels of n-6 and n-3 fatty acids in the formulae, differences in the tests used, differences in the infants themselves or inadequate samples sizes of the studies.

Lower novelty preference scores in high-risk infants are predictive of lower subsequent cognitive ability, based on lower scores on later tests of cognitive ability (McCall & Carriger 1993). Two studies have found lower novelty preference test scores with premature infants fed formula supplemented with (in total fatty acids) 0.06% 20:5n-3 and 0.2% 22:6n-3, or 0.3% 20:5n-3 and 0.2% 22:6n-3 than in premature infants fed formula without 22:6n-3 (Carlson et al 1996, Werkman et al 1996). In these studies, infants fed the formulae with 22:6n-3 also had more looks and shorter average looking intervals during novelty testing, (a portion of the test in which total time for looking at the stimuli is pre-set, test-controlled), but not during the familiarization phase (when time looking at the stimuli is dependent on the infant, infant-controlled) (Carlson et al 1996, Werkman et al 1996). Studies on visual attention and its relation to later intelligence have found that shorter look duration in infant-controlled procedures are related to higher (not lower) scores on subsequent novelty tests (Colombo et al 1991). In the current study, no significant differences in looking time or behaviour were found between the infants who were breast-fed and those who were fed formula. It

should be noted, however, that the Fagan Test of Infant Intelligence was not developed nor has it been validated as a measure of infant looking behaviour as it may reflect information processing.

A role for the lipids present in breast milk and absent from formula is commonly cited as a determinant of the differences in cognitive development between breast-fed infants and infants fed formula. However, factors such as the breast-feeding process, behavioral characteristics that are unique or more common among breast-feeding mothers, or some effect of breast-feeding on the infant behavior may also explain differences in cognitive development between breast-fed infants and bottle-fed infants (Rogan 1993). Studies of breast-fed infants compared with infants fed formula are non-random studies for ethical reasons. Thus, differences unrelated to diet, such as variation in socioeconomic status could possibly exist between the breast-fed infants and the infants fed formula. While some attempts were made in Study I to control for variability in demographic factors between the breast-fed infants and the infants fed formula through the use of covariates in the statistical design, it is recognized that Study I and many similar studies at the time did not have sufficient numbers of infants to control for confounders.

In summary, Study I provides evidence that at least for the infant population studied, providing a formula with at least 1% of energy as 18:3n-3 and an 18:2n-6 to 18:3n-3 ratio of 9:1 results in preferential looking acuity that is not different from that of breast-fed infants to 18 mo of age. Furthermore, the results show no relation between blood lipid 22:6n-3 and measures of visual acuity. Thus, it is possible that the infants fed a formula with 1% energy as 18:3n-3, with an 18:2n-6/18:3n-3 ratio of 9:1 are able to make sufficient 22:6n-3 either in liver or brain to support normal brain and retina 22:6n-3 accretion. Alternatively, the tests may have had insufficient sensitivity, or have been given at the wrong age to detect physiologically meaningful differences. A final possibility is that the n-3 fatty acid status of the breast-fed infant group was sub-optimal, thus masking any deficiency in the infants fed formula.

As previously indicated, the majority of the published studies examining the influence of n-3 fatty acids in young infants have focused on visual function. An inherent assumption in these studies seems to be that measures of visual function are not influenced by potential confounding variables such as demographic factors. In part, this is because the importance of confounding variables in measures of visual function is not well understood. In an effort to determine the influence of environment on the visual and cognitive development of term infants, studies were done examining the relation between family characteristics, including infant feeding (breast *versus* bottle-feeding) and some aspects of CNS development as measured by visual acuity and novelty preference in a large group of over 400 infants. This study found no association between duration of breastfeeding and healthy term infants' scores on the Teller Acuity Card test, FTII and looking behavior during the FTII at nine mo of age. However, independent variables that were predictive of test performance for visual acuity included infant gender and for the FTII included infant gender, parental marital status and the language spoken in the home. The results of this thesis, therefore, suggest that future studies on the relation of diet to visual acuity or performance in the FTII should consider gender and aspects of the home environment as potential confounding variables.

Several studies have examined the issue of differences in CNS function from a more global perspective in breast-fed and formula-fed infants. Again, the results for these studies are inconsistent. Studies of infant development that attempt to attribute developmental outcomes to one specific variable in the infant's rearing environment, such as breast-feeding, are problematic due to potential differences between a population of breast-fed infants *versus* infants fed formula. An important confounder in these studies on breast-feeding compared with bottle-feeding is socio-economic status, especially given that it has been shown to have a 50% correlation with IQ in school (McCall 1981). Some studies of cognitive development attempt to control for obvious and easily measured differences such as maternal age, parity, ethnicity,

marital status, gender, birth order and total number of children in the home. Eighteen mo old breast-fed infants (n=228) were found to have higher scores on the Mental Development Index of the Bayley Scales of Infant Development than infants fed formula (n=354), after controlling for mother's age, education, social class, alcohol consumption and smoking during the pregnancy (Florey et al 1995). A study of 18 to 29 mo old Spanish infants found that infants breast-fed for more than three mo from birth had higher scores on the Mental Development Index, but not the Psychomotor Development Index, than bottle-fed infants (Temboury et al 1994). This study also showed that an excessive number of tantrums, birthorder and mother's education level influenced the Mental Development Index, whereas social class was related to performance on the PDI. Other studies examining cognitive performance of children who were either breast- or bottle-fed as infants have also reported a slight advantage in the performance of breast-fed children. A study of socially advantaged children, either breast- (n=321) or bottle-fed (n=3183) to three mo of age, utilized on tests of vocabulary ability at five and 10 yr of age (Pollock 1994). After controlling for factors determined to be predictive of feeding group, such as education of mother, smoking during pregnancy and attendance at birth preparation classes, more breast-fed children had above average vocabulary scores than the bottle-fed children. A study by Niemelä et al (1996) assessed cognitive, visual-motor integration and vocabulary ability of 163 children at 56 mo of age classified as breast-fed for less than or more than five mo. Regression analyses determined that maternal education and marital status correlated with performance on all three tests; sex of the child and breastfeeding duration correlated with visual-motor integration; number of siblings correlated with vocabulary scores. A recent study by Malloy et al (1998) assessed the performance of nine and 10 yr old children who had been exclusively breast-fed (n=342) or fed formula (n=176) as infants on the Weschler Intelligence Scale for Children-revised (WISC-r). Regression analysis, controlling for parent's education and annual family income, measured no significant association between feeding type and performance on

the WISC-r. Eight yr old children who had been either breast- or bottle-fed as infants were assessed for vocabulary and reading skills, with regression analysis variables of breastfeeding, social class, maternal education, maternal IQ and attendance at nursery school (Richards et al 1998). Attendance in nursery school and mother's IQ were the only variables that were predictive of vocabulary and reading skills. An Australian study assessed cognitive performance of breast-fed (n=144) and bottle-fed infants (n=199) at two, four, seven and 11-13 yr of age using the Bayley Scales, WISC and McCarthy Scales (Wigg et al 1998). Regression analysis controlling for maternal IQ, SES status, parental smoking and home environment found no statistically significant relation between diet and test outcome.

Although these studies have attempted to control for well-known confounding variables, it may be that subtler and more difficult to measure factors such as parenting skills or quality of the home environment are responsible for cognitive benefits seen in some, but not all, studies of breast-fed infants compared with bottle-fed infants (Golding et al 1997, Jacobson & Jacobson 1992). Other reasons for the disparity in study findings may include the use of different tests of visual and cognitive ability, variation in the type and number of covariates, variation in the definition and duration of breastfeeding, sample size differences and discrepancy in the age of the infants/children across studies.

In Study III, preferential looking acuity assessed by the Teller Acuity Card test was predicted by gender, a finding not previously reported. Contrast sensitivity function, assessed with forced-choice preferential looking methods, has been shown to be higher in female infants than male infants at six mo, but not at four or eight mo (Peterzell et al 1995). Female infants have an earlier age of onset of stereopsis and binocular rivalry than male infants (Gwiazda et al 1989). Infants in Study III also had had mean novelty preference scores and looking behavior on the FTII that could be predicted from gender, language spoken in the home and parental marital status. Fagan et al (1992) have reported that cultural difference, gender, birthorder and socio-economic

level do not influence scores on the FTII. In contrast, a recent study of small-for-gestational age infants found a significant correlation between FTII scores and the quality of the home environment (Andersson et al 1997). Gender differences in cognitive performance across all ages have been frequently reported in the scientific literature. A recent prospective study of four yr old children showed that female children had higher hearing and speech abilities and eye-hand-co-ordination than male children (Nordberg 1996). Infant females have been shown to be superior to infant males on concurrent discrimination tasks, while male infants are better in learning object reversal (Overman et al 1996). The home environment, including the quality of parenting, influences cognitive development in children (Sameroff et al 1993, Duncan et al 1994, Kramer et al 1995). Home and parental factors shown to have the most influence on children's achievement at six to eight yr of age include presence of the father in the home, mother's education, self-esteem, income and family size (Baharudin & Luster 1998).

Look duration in infants has been found to correlate with later measures of speed of processing (Jacobson et al 1992) while novelty preference correlates with later memory and language performance (Rose et al 1991, Thompson et al 1991). There was no difference due to diet (duration of breast-feeding) in percent novelty, number of looks or look duration during the familiar or novel testing of the FTII in Study III. Previous studies with low-birth weight infants have found no difference in the number of looks or average look duration during the familiarization phase of the FTII between the infants fed a formula with added LCPUFA (3.0 % 18:3n-3, 0.2% 22:6n-3, 0.3% 20:5n-3) and a standard formula (3.1% 18:3n-3) at 12 mo (Carlson et al 1996a). Similar results were obtained in looking behavior for low-birth weight infants fed a formula with 3.1% 18:3n-3, 0.2% 22:6n-3 and 0.06% 20:5n-3 and infants fed a standard formula (3.0% 18:3n-3) at six and a half, nine and 12 mo of age (Werkman et al 1996). Furthermore, infants fed a formula with 0.2 % 22:6n-3 had a greater number of looks and had a shorter look duration to the novel stimulus at six and a half and nine mo than the infants

fed the formula with no added LCPUFA. There was no difference in the novelty preference score between infants fed the standard formula and infants fed the formula without LCPUFA at six and a half and nine mo, but infants fed formula with LCPUFA had lower percent novelty scores than the infants fed the formula without LCPUFA at 12 mo. Thus, the infants with shorter look durations, which may be predictive of adult cognitive processing speeds, one component of intelligence, had lower novelty preference, which is thought to be predictive of later lower intelligence. It is possible that look duration is governed by a different processing mechanism than novelty preference and that the CNS structures involved in the speed of processing are more sensitive to the influences of n-6 and n-3 fatty acids than the structures involved in memory.

Studies using a habituation paradigm have found that there was no difference in total fixation or average fixation for term infants fed a standard formula (11.4% 18:2n-6, 0.7% 18:3n-3) or formula with added LCPUFA (approximately 12% 18:2n-6, 0.62 18:3n-3, 0.35% 20:4n-6 and 0.2% 22:6n-3) or breast-fed infants (Willatts et al 1998). Post-hoc analyses, however, found that for a selected subset of infants who did achieve a peak fixation on the first looking trial, those infants that received formula with LCPUFA had shorter total, average and peak fixation times (Willatts et al 1998). This subset of infants was also found to be smaller at birth. Whether this variation in birth size is an indication that these infants were less mature or developed than full-term infants is not known.

In summary, gender and home environment can significantly influence outcomes in tests of CNS competence. In the current study, preferential looking acuity was found to be related to gender and novelty preference was related to infant gender, the parent's marital status and the language spoken in the home. In non-randomized studies comparing visual and cognitive development of breast-fed infants and infants fed formula, there are potential significant population differences that can influence outcomes. It is important that breast-fed and formula-

fed groups are closely matched on variables such as gender and socioeconomic status when selecting participants for these studies. Furthermore, studies should have sufficient numbers of participants to allow for covariates to be included in the statistical analyses.

4.2. Influence of dietary TG fatty acid distribution on plasma and lipoprotein TG fatty acid distribution.

The studies reported here provide *in vivo* data to show that the distribution of saturated and unsaturated fatty acids in human milk and infant formula does influence the fatty acid distribution of infant plasma TG and PL. Specifically, the results of this study provide convincing evidence to suggest that 16:0 esterified to the *sn*-2 position of human milk is conserved through digestion and absorption, reassembly to TG in the enterocyte and secretion into plasma as lipoprotein TG. *In vitro* studies have shown that the milk lipase, bile-salt-stimulated lipase, can complete the digestion of *sn*-2 monoacylglycerol (MG) formed by gastric lipase and pancreatic lipase hydrolysis of TG to give unesterified fatty acids and glycerol (Tomarelli et al 1968, Hernell et al 1989). Information on the extent of hydrolysis of *sn*-2 MG with 16:0 *in vivo* in young infants has not been published. Strong circumstantial evidence that bile salt-stimulated lipase does not quantitatively (completely) hydrolyze 16:0 from the 2 position of human milk fat is provided by the results of this study. The current study found approximately 26% of 16:0 in fatty acids esterified to the plasma TG 2 position of breast-fed infants compared with only about 7.4% 16:0 in the plasma TG 2 position of infants fed a formula containing comparable amounts of 16:0 to the milk, but predominantly esterified to the glycerol *sn*-1,3 positions. These results support the hypothesis that the preferential esterification of 16:0 to the *sn*-2 position of human milk TG is one of the reasons for the high coefficient of absorption of human milk fat by breast-fed infants (Fomon et al 1970). The study reported here indicates that bile salt-stimulated lipase does not quantitatively complete the hydrolysis of milk TG

to free fatty acids and glycerol in infants *in vivo*. Other recent studies have suggested a possible role for bile salt-stimulated lipase in the hydrolysis of milk long chain n-6 and n-3 fatty acids. The positioning of 20:4n-6 and 22:6n-3 in human milk at the TG 3 position (Martin et al 1993) is believed to render the TG relatively resistant to hydrolysis by pancreatic lipase, which is specific for the TG 1 and 3 positions (Bottino et al 1967, Chen et al 1989). An *in vitro* study of the hydrolysis of chylomicron TG containing either 20:4n-6 or 22:6n-3 found improved hydrolysis of 20:4n-6 and 22:6n-3 by pancreatic lipase and bile salt-stimulated lipase when compared with pancreatic lipase alone (Hernell et al 1993, Chen et al 1994).

The analyses reported here show that, in addition to total plasma PL fatty acids, the *sn*-2 position of plasma PL from infants fed formula had lower 20:4n-6 and 22:6n-3 levels, but much higher 18:2n-6 levels than in breast-fed infants (mean±SEM 18:2n-6, 40.2±1.6 and 25.0±1.4%; 20:4n-6, 9.7±0.6 and 17.7±1.4%; 22:6n-3, 2.0 ±0.2 and 5.6±0.6%; for the infants fed formula and breast-fed infants, respectively). The higher levels of 18:2n-6 at the *sn*-2 position of plasma PL in the infants fed formula compared with breast-fed infants may be due to higher levels of 18:2n-6 in the formula TG 2 position compared with the human milk fat (27.1 and 8.1% 18:2n-6 for the formula and human milk, respectively).

The influence of the specific positioning of 16:0 in milk and formula TG was further assessed by analysis of individual lipoprotein lipids. These analyses show that infants fed formula with 16:0 enriched at the 2 position of the TG had higher levels of 16:0 at the 2 position of plasma chylomicron TG than infants fed formula with a similar total amount of 16:0 but with < 5% 16:0 in the TG 2 position fatty acids. The higher levels of 16:0 in the chylomicron TG 2 position of the breast-fed infants also confirms and extend the above findings showed relatively high levels (about 27% total fatty acids) of 16:0 in the 2 position of plasma TG of breast-fed infants. Chylomicron analysis showed 28% 16:0 in the fatty acids at the 2 position of chylomicron TG of the breast-fed

infants, representing about 40% of the total 16:0 in the chylomicron TG total fatty acids. Analysis of the human milk received by the breast-fed infants found 23% 16:0 in the 2 position fatty acids, representing about 56% of the TG total 16:0 in the 2 position fatty acids. These results again suggest that up to about 50% of the 2-MG with 16:0 were absorbed, conserved through the process of TG reassembly and subsequent release into plasma. The relatively similar results for the analysis of chylomicron and plasma total TG suggests that in the postprandial state (blood was drawn 2-3 hr after feeding) most of the plasma TG was in chylomicron rather than LDL and HDL. The analysis of plasma from the infants fed the formula with the synthesized TG also demonstrated that about 50% of the 16:0 in the formula TG 2 position was conserved through digestion, reabsorption and TG reassembly. Thus, the chylomicron TG 2 position fatty acids of the infants fed the synthesized TG formula had 15.8±0.4% 16:0 compared with 29% 16:0 in the TG 2 position of the formula. These results for breast-fed infants and the infants fed the formula with the synthesized TG, each retaining similar (about 50%) of the dietary TG 2 position 16:0, suggest that bile salt-stimulated lipase does not hydrolyze 16:0 from the milk TG 2 position in breast-fed infants.

A study using *in situ* isolated rat intestine estimated that 70% of the 2-monopalmitin perfusate was absorbed into the mucosal cells, with 18% of the absorbed monopalmitin subsequently hydrolyzed in the mucosal cells and 52% re-esterified. (Paris et al 1968). Overall, Paris et al (1968) found that 85% of the mucosal TG synthesis was via the monoglyceride pathway. A similar study with rats using *in situ* isolated intestine suggested that about 70% of TG synthesis in the intestinal mucosa proceeds via the 2-MG pathway, with the remaining TG synthesis occurring via the *de novo* 3-glycerophosphate pathway (Breckenridge et al 1975). Breckenridge et al (1975a) also reported that diacylglycerols from everted sacs of rat intestine contained 55 to 90% of the original 2-MG, suggesting that there may be a greater hydrolysis of MG and a greater contribution of the 3-glycerophosphate pathway to intestinal TG synthesis. A recent

study with adult men fed a diet with 31% energy from synthesized TG, similar to the synthesized TG used in the study reported here, however, also recovered about 70% of the dietary 2 position 16:0 in the 2 position of chylomicron TG in the post-absorptive state (Nestel et al 1995). The range of values found for the re-esterification of 2-MGs via the MG pathway suggests that absorption of 2-MG with 56% 16:0 (i.e. as in human milk) should, theoretically result in about 30 to 50% 16:0 in the chylomicron TG 2 position fatty acids of breast-fed infants, similar to the 28% 16:0 found in the studies here. A possible explanation for the results of this study consistent with the similar relative retention of the dietary 2 position 16:0 in the breast-fed infants and infants fed formula is that some hydrolysis of 2-MGs absorbed from the intestine occurs in the enterocyte with re-esterification via the 2-MG pathway accounting for about 50% of intestinal TG synthesis in the human infant.

Recent studies with premature infants have found significantly higher levels of 16:0 in plasma total TG of infants fed formula with synthesized TG (58% 16:0 in the *sn*-2 position) than in infants fed a conventional premature formula with about 10% 16:0 in the *sn*-2 position (29.3±0.4% compared with 24.9±0.6% 16:0 in plasma TG, respectively, $P<0.05$) (Carnielli 1995). This effect of the position of 16:0 in the formula TG on the plasma total TG or the chylomicron total TG levels of 16:0 was not evident in the term infants in the studies here. Possibly, the higher percent 16:0 in the 2 position of the formula TG in the study by Carnielli et al (1995) than used in Study II could account for these differences. However, the amount of 16:0 in the 2 position in the formula TG used by Carnielli et al (1995) was similar to that in human milk. No difference was found in the plasma or chylomicron total TG 16:0 between the breast-fed infants and infants fed formula in either Study I or Study II here. Perhaps the higher plasma TG 16:0 levels in premature infants fed TG with 16:0 in the *sn*-2 rather than the *sn*-1,3 positions reflects greater immaturity in fat digestion and absorption pathways in the premature than term gestation infant.

The physiological significance of the specific positioning of 16:0 at the 2 position of human milk TG and its apparent retention through digestion and absorption has not been explored. Early studies by Tomarelli et al (1968) found that as the percent of 16:0 in the 2 position of the formula TG increased from 23% to 94%, the percent absorption of 16:0 increased from 67% to 95% in infants. Similarly, Filer et al (1969) found that infants fed formula or human milk with approximately 85% of 16:0 at the TG *sn*-2 position had an average absorption of 16:0 of 94% compared with 58% absorption of 16:0 in infants fed formula with 34% of the 16:0 in the TG *sn*-2 position. Thus, it was hypothesized that the specific positioning of 16:0 in milk TG may be the reason for the high absorption of fat found in breast-fed infants. Many studies have shown that unesterified 16:0 and 18:0 are less well absorbed than 8:0, 10:0, 12:0, 18:1 and 18:2n-6 (Tomarelli et al 1968, Barnes et al 1974, Jensen et al 1986), and that unesterified 16:0 readily forms insoluble soaps with divalent cations such as calcium at the pH of the intestine. Therefore, dietary energy and minerals that are important for skeletal growth are potentially lost. A recent study of term infants found that the stools of infants fed formula had significantly higher calcium, phosphorous, magnesium, total lipid, 12:0, 14:0, 16:0, 18:0, 18:1 and 18:2n-6 than those of breast-fed infants (Quinlan et al 1995). These differences in stool composition were suggested to be beyond that expected given the known differences in nutrient composition of human milk and infant formula. Recent studies by others have considered the effects of using formula with a synthesized TG to provide 16:0 on fat, fatty acid and mineral absorption in term and preterm infants (Carnielli et al 1995, Carnielli et al 1995a, Carnielli et al 1996, Lucas et al 1997). In a study with term infants, the absorption of total fat, 16:0, 18:0 and calcium was significantly higher in infants fed a formula with a synthesized TG (23.9% 16:0, 47.1% in the TG *sn*-2 position) than in infants fed a conventional formula (19.9% 16:0, 7.5% in the *sn*-2 position) (Carnielli et al 1996). Studies with preterm infants have also found significantly higher fecal 16:0 and 18:0 excretion (Carnielli et al 1995), higher 16:0 and 18:0 absorption and significantly lower calcium excretion (58.8 ± 7.8 compared with 82.0 ± 9.9 mg/kg/d) in infants fed

formula with synthesized TG (58% 16:0 in the *sn*-2 position) than in infants fed a standard formula (Carnielli et al 1995a). Both the studies with term and preterm infants found a significant, positive correlation between fecal calcium and fecal 16:0 excretion (r=0.84 and 0.98, respectively) (Carnielli et al 1995a, Carnielli et al 1996). A study with preterm infants using [^{44}Ca] also found significantly higher 16:0 absorption, significantly lower lipid excretion and significantly higher calcium absorption in infants fed formula with synthesized TG (23.9 % 16:0 in TG, 73.9% 16:0 in the *sn*-2 position) than in infants fed a low 16:0 formula (14.7% 16:0 in TG, 8.4% 16:0 in the *sn*-2 -position) or a formula with randomized fatty acids (23.9% 16:0 in TG, 27.8% in the *sn*-2 position) (Lucas et al 1997). This suggests that significant improvements in calcium and fatty acid absorption can be achieved for infants fed formula through the use of formulas with TG fatty acid distributions in which 16:0 is directed towards the *sn*-2 rather than the *sn*-1,3 positions in a manner similar to human milk. The increased absorption of Ca and fat seen in the studies by Carnielli et al (1995, 1995a, 1996) is consistent with the results of Study I and II that a significant portion of 16:0 from milk is located as 2-MG, thus preventing or reducing the availability of unesterified 16:0 in the intestine..

As seen for the plasma TG 2 position fatty acids, the enrichment of 16:0 at the *sn*-2 position of the infant plasma chylomicron TG was accompanied by a decrease in levels of 18:1 in both the breast-fed infants and the infants fed the synthesized TG formula. These differences in chylomicron TG 2 position 18:1 levels reflect the distribution of 18:1 in the milk and formula TG. Similar results have been previously reported for piglets fed formulae with a fatty acid composition and distribution similar to those used in this study (Innis et al 1997).

The distribution of fatty acids in the formula TG had no significant effect on the levels of 20:4n-6 or 22:6n-3 in the plasma chylomicron lipids of the infants fed formula in this study. In contrast, studies with piglets reported lower 20:4n-6 and 22:6n-3 in chylomicron TG of piglets fed

formula with synthesized TG in which about 32% of the formula 16:0 was at the 2 position of the TG (Innis et al 1997). Studies with adults fed a diet with 40% of energy from synthesized TG with 54% 16:0 at the 2 position, however, found higher 20:4n-6 in plasma TG, but lower 20:4n-6 in CE than when the diet contained palm-olein (Zock et al 1996). Possibly, the discrepancies among the results of the different studies involve the differences in age, species and in the nature of the diet and duration of feeding, compared with the formula fed to infants for 120d in the studies here.

Infants fed the formula with synthesized TG in the study here had higher levels of 18:3n-3 in their plasma chylomicron TG than infants fed the standard formula. In contrast, no difference in chylomicron TG 18:3n-3 levels were found in piglets fed formula with synthesized TG formula with approximately 32% 16:0 at the 2 position rather than a standard formula (Innis et al 1997). A similar increase in 18:3n-3 in plasma TG, however, was found in adults fed a diet with synthesized TG with 54% 16:0 at the 2 position of the TG (Zock et al 1996). As for 20:4n-6 and 22:6n-3, the discrepancies could involve species differences and/or experimental differences in the composition of the synthesized TG fed and the plasma lipids (whole plasma and isolated chylomicrons) analyzed. However, despite the discrepancies among the studies, information for both infants (Carnielli et al 1995) and piglets (Innis et al 1997) suggests that the distribution of dietary saturated fatty acids on the TG can influence plasma n-6 and n-3 fatty acids. This suggests that the amount, or pathway of absorption may influence fatty acid metabolism with secondary effects on the clearance, oxidation and/or subsequent acylation or desaturation of dietary 18:2n-6 and 18:3n-3. Furthermore, more specific studies on the interaction between dietary saturated fatty acids and the metabolism of unsaturated fatty acids may be worthwhile.

The studies with infants here found no effect of the position of 16:0 in the formula TG on the levels of 20:4n-6 or 22:6n-3 in the chylomicron PL of the formula-fed infants. The breast-fed infants, however, had significantly higher levels of 20:4n-6 and 22:6n-3 in chylomicron PL than the groups

of infants fed formula, similar to the finding for the plasma total PL reported in many other studies (Putnam et al 1982, Ponder et al 1992, Innis 1991, Carlson et al 1996, Jensen et al 1997, Birch et al 1998). Carnielli et al (1995a) also found no influence of the position of 16:0 in the formula TG on the plasma PL 20:4n-6 or 22:6n-3 of preterm infants. Studies with piglets fed a co-randomized formula with 21% 16:0 and with 31% of 16:0 in the 2 position, however, found that the levels of 20:4n-6 and 22:6n-3 were reduced, whereas 18:2n-6 was higher in the chylomicron PL than piglets fed a standard formula (20.9% 16:0, with 8.3% 16:0 in the 2 position) (Innis et al 1997a). Whether this was due to inhibition of synthesis of 204n-6 and 22:6n-3 or through competition for acylation with 18:2n-6 is not known.

The findings of lower HDL cholesterol and apolipoprotein A-1, but higher apolipoprotein B in infants fed a formula with synthesized TG than in infants fed a standard formula provide the first information to suggest dietary TG fatty acid distribution influences lipoprotein metabolism in young infants. Levels of cholesterol, on the other hand, were not different between infants fed the standard formula and infants fed the formula with synthesized TG. Both groups of infants fed formula had lower plasma cholesterol levels than infants who were breast-fed. This would suggest that although the formula TG fatty acid distribution influenced apolipoprotein B metabolism in the formula-fed infants, there were still important differences in cholesterol metabolism between the breast-fed infants and the infants fed formula in the studies reported here. Possibly, some of the differences may be explained by the absence of cholesterol, long chain n-6 and n-3 fatty acids or other bioactive components of human milk not in formula. The reason why infants fed the formula with synthesized TG had higher levels of plasma apolipoprotein B than infants fed the standard formula is not known. However, studies in rats have shown that increasing the amounts of 18:0 in the 2 position of TG significantly slows the removal of plasma chylomicron remnants (Redgrave 1988). The delayed chylomicron removal does not appear to be explained by decreased lipolysis of

the chylomicron TG (Mortimer et al 1988), although this point is controversial (Pufal et al 1995). Rather, it may be explained by an effect on subsequent uptake of the chylomicron remnant into the liver via LDL receptors (Ishibashi et al 1996). Possibly, this is due to down-regulation of LDL-receptors (Stucchi et al 1995) or an alteration in the ability of the chylomicron remnant to interact with receptors (Boyle et al 1996). Infants fed the formula with synthesized TG had 15.8% 16:0 in the 2 position of plasma chylomicron TG compared with 8.3%, in infants fed the standard formula. Whether delayed removal of chylomicron remnants can explain the higher levels of apolipoprotein B, without a change in plasma total cholesterol in infants fed the formula with synthesized TG when compared with infants fed the standard formula may be worth considering.

The results of the study here show that the infants fed the formula with synthesized TG had lower plasma levels of apolipoprotein A-I than the infants fed a standard infant formula. Levels of HDL cholesterol were also lower in the infants fed the formula with synthesized TG than in those fed the standard formula, suggesting HDL levels were decreased. The explanation for the apparent effects of the formula TG fatty acid distribution on plasma HDL cholesterol and apolipoprotein A-I levels in young infants is not known. Apolipoprotein A-I is secreted not only by the liver, but also by the intestine loosely associated with chylomicron particles. It is not known if the distribution of fatty acids in chylomicron TG influences apolipoprotein A-I secretion by the intestine. However, studies with fasted men have reported a significant decrease in HDL cholesterol and apolipoprotein A-I coincident with the peak in HDL TG and chylomicron remnants following a fat load test with cream (De Bruin et al 1991). In the current studies, infants fed the formula with synthesized TG had significantly higher plasma apolipoprotein B levels than infants fed the standard formula, although plasma TG levels were not different. This suggests a possible difference in the number of remnant particles and the possibility that changes in chylomicron or chylomicron remnant metabolism are causally related to the effects of the formula with synthesized

TG on apolipoprotein A-I and HDL or LDL cholesterol in the infant. Future studies might address this by more specific measures of the lipoprotein apolipoprotein and lipid concentrations and turnover.

Another possible explanation for the lower HDL cholesterol levels in infants fed the formula with the synthesized TG is lower LCAT activity, since this enzyme is pivotal in the synthesis of plasma CE (Jonas 1991). Nascent HDL particles are converted to spherical lipoproteins via LCAT which hydrolyzes 2 position fatty acids from PC to esterify cholesterol located on the outer surface of the HDL particle (Jonas 1991). Decreased levels of apolipoprotein A-I are associated with lower LCAT activity, decreased cholesterol esterification and uptake of cholesterol in HDL (Yokoyama 1978). This would result in a reduction in the number of circulating mature HDL particles through reduced esterification of free cholesterol and would impact on the HDL particle size. The activity of LCAT is known to be influenced by the composition of the fatty acids at the 2 position of the PC. Of relevance to the studies reported here, *in vitro* studies have reported that human LCAT activity is substantially lower with phosphatidylcholines with 16:0 rather than 18:2n-6 at the 2 position (Subbaiah et al 1994). In the studies reported here, infants fed the formula with synthesized TG had higher amounts of 16:0 in the 2 position of plasma HDL PL than infants fed the standard formula (mean±SEM, 22.3±2.1% and 17.2±1.2% 16:0, respectively). Whether these differences in HDL PL fatty acids are sufficient to influence LCAT activity *in vivo* is not known, but could further explain the reduced HDL-cholesterol levels seen in the infants fed the formula with a synthesized TG in the current study.

In contrast to the studies here, piglets fed formula with 70% 16:0 in the TG 2 position fatty acids had higher plasma HDL cholesterol levels than in piglets fed a standard formula (Innis et al 1993a), similar to the standard formula fed to infants here. In humans, HDL CE are exchanged for TG from VLDL via the cholesterol ester transfer protein (CETP) (Tall 1993). The absence of

significant CETP activity in the pig (Ha 1982) could reasonably be expected to alter the effects of dietary fat on CE metabolism in pigs compared with humans. Studies with adult men fed a diet with 40% dietary energy from fat, and 28% energy from synthesized TG with 54% or 6% 16:0 in the dietary TG 2 position, however, found no differences in HDL cholesterol levels in fasting plasma (Zock et al 1995). The studies with piglets (Innis 1993) and adult men (Zock et al 1995) used synthesized TG with about two fold higher levels of 16:0 in the 2 position than in the formula fed to infants here. The proportion of dietary energy from fat consumed by the adult men (Zock et al 1995) was also substantially lower (28% dietary energy) than the amount provided by the infant formula (about 48% energy from fat). The study with adult men also analyzed blood after an overnight fast, whereas plasma collected from the infants 2-3h post-prandial was used here. Whether these experimental variables, age and/or species differences, explain the differences in the results concerning synthesized TG and HDL cholesterol is not known.

In summary, these studies have shown that about 50% of the 16:0 at the 2 position of human milk and formula with synthesized TG are transferred to the chylomicron TG 2 position of young infants. Furthermore, these studies have shown that the effect of dietary triglyceride fatty acid distribution extends beyond facilitating the absorption of long-chain saturated fatty acids (Bracco 1994, Tomarelli et al 1968, Filer 1969) to the plasma lipoprotein lipid fatty acid distribution and apolipoprotein levels, and possibly metabolism. Further studies are needed to consider in more detail the implications of human milk and infant formula triglyceride patterns to lipid metabolism and tissue fatty acid delivery in young infants.

4.2.1. Distribution of 20:4n-6 and 22:6n-3 in plasma lipoproteins of breast-fed infants and infants fed formula.

The results of this study show that the n-6 and n-3 fatty acids, 20:4n-6 and 22:6n-3 are distributed differently in PL, TG and CE among the different plasma lipoproteins. These studies

also provide results to show that the plasma lysoPL of breast-fed infants contain relatively large proportions of 20:n-6 and 22:6n-3, whereas 18:2n-6 and 18:3n-3 are found in the albumin-bound unesterified fatty acids. The studies reported here show that the higher 22:6n-3 and 20:4n-6 in plasma total PL of breast-fed infants than in infants fed formula, as shown in previous studies (Innis 1991, Ponder et al 1992, Carlson et al 1996), is due to higher levels of 22:6n-3 and 20:4n-6 in all of the lipoproteins, HDL, LDL and chylomicrons, as well as the lysoPL of infants who are breast-fed when compared with infants who are fed formula. The chylomicron, LDL and HDL PL, but not the lysoPL or unesterified fatty acid levels of 22:6n-3, decreased by about 40% from 30 to 120 d of age in the infants fed formula, but not in the breast-fed infants who received about 0.3% milk fatty acids as 22:6n-3. The plasma lipoprotein PL, TG and CE, lysoPL and unesterified fatty acid levels of 20:4n-6, in contrast to 22:6n-3, did not change between 30 d and 120 d of age in either the breast-fed infants or the infants fed formula. These findings suggest that circulating levels of 22:6n-3 are more sensitive to differences in dietary intake of 22:6n-3 than for 20:4n-6. Alternatively, the equilibration to a change in dietary intake could be slower for 22:6n-3 than for 20:4n-6.

Higher levels of 20:4n-6 and 22:6n-3 in the chylomicron of the breast-fed infants than in the infants fed formula can reasonably be expected based on the difference in dietary intake; i.e. about 0.5% 20:4n-6 and 0.3% 22:6n-3 in the human milk and no 20:4n-6 or 22:6n-3 in the formula fatty acids. However, the levels of 20:4n-6 and 22:6n-3 were as high, or higher, in the LDL and HDL PL, TG and CE of the breast-fed infants than in the respective chylomicron lipid. This suggests that dietary 20:4n-6 and 22:6n-3 are delivered to the liver of the breast-fed infant in chylomicron remnants and resecreted in VLDL and HDL.

Previous studies with suckling rats and human adults have reported higher levels of 20:4n-6 and 22:6n-3 in the HDL PL and total HDL fatty acids than in chylomicron and LDL (Nouvelot et al 1986, Levy et al 1987). The study reported here similarly found higher levels of 20:4n-6 and 22:6n-

3 in the HDL PL than chylomicron or LDL PL of both breast-fed infants and infants fed formula. The reason for the higher amounts of 20:4n-6 and 22:6n-3 in HDL than in chylomicron or LDL PL is not known. Differences in the origin of chylomicron, LDL and HDL PL, or possibly differences in intravascular metabolism, such as turnover or clearance rates, could be involved. The demonstration of higher 20:4n-6 and 22:6n-3 in HDL than in LDL or chylomicron PL is of interest, however, because low-density lipoprotein-related receptors, purported to take up HDL, at least in the liver, have been detected in human astrocyte tissue (Moestrup et al 1992). Furthermore, recent *in vitro* studies have shown that HDL_3 transports PE to the brain capillary endothelial cells (Magret et al 1996). These enzymes convert the HDL_3 PE to LCPUFA-rich PC through the methylation pathway and subsequently selectively release LCPUFA from PC. Possibly, the unesterified LCPUFA could then be taken up through coordination of the recently identified lipid-binding protein in developing brain tissue which has a strong preference for 22:6n-3 but not 16:0 (Xu et al 1996) and fatty acid transport proteins for which mRNA have also been detected in developing rat brain (Utsunomiya et al 1997, Schaap et al 1997).

With respect to HDL, it is also notable that the HDL TG of the infants fed formula had higher levels of 20:4n-6 and 22:6n-3 than the chylomicron or plasma total TG. Possibly, this is derived from hepatic desaturation and elongation of 18:2n-6 and 18:3n-3 provided in the formula, followed by secretion to plasma for transport to developing brain and other organs in HDL. It is known that lipoprotein lipase, which is responsible for the conversion of VLDL to LDL through the hydrolysis of TG, has limited action against 20:4n-6 and 22:6n-3. The higher 20:4n-6 and 22:6n-3 in the LDL and HDL TG, compared with chylomicron, could also involve selective retention of TG with 20:4n-6 and 22:6n-3 due to restrictive lipoprotein lipase hydrolysis. Although the contribution of HDL TG 22:6n-3 to the plasma total 22:6n-3 pool is likely to be small because HDL has only about 7% TG by weight (Rosseneu et al 1983), it remains possible that HDL TG are a physiologically important pool of 20:4n-6 and 22:6n-3.

Lysophospholipid and FFA are known to be able to deliver n-6 and n-3 fatty acids to brain and albumin is known to cross the blood-brain barrier via adsorptive endocytosis mechanisms that resemble saturable receptor-mediated transport (Dhopeshwarkar et al 1971, Dhopeshwarkar et al 1971a, Irie et al 1991, Thiès et al 1992, Thiès et al 1994). Lysophospholipid represents 5-20% of total PL in mammalian plasma and is the second most prevalent PL in plasma, at least in the rat (Nelson 1967). The unesterified fatty acids usually comprise about 1-5% of plasma fatty acids (Dhopeshwarkar et al 1973). Levels of 20:4n-6 were seven fold higher and 22:6n-3 was 3-5 fold higher in the plasma lysoPL than in the unesterified fatty acids of the breast-fed infants and the infants fed formula. Recent studies utilizing young (20 d old) rats found that 18:2n-6 and 20:4n-6 but not 16:0 from intravenously infused physiological amounts of radiolabeled 2-arachidonyl-lysoPC, 2-palmitoyl-lysoPC and 2-linoleoyl-lysoPC rapidly appeared in the brain (Thiès et al 1992). Furthermore, other studies have found the uptake of 18:2n-6, 20:4n-6 and 22:6n-3 from lysoPL occured more readily from lysoPL than from unesterified fatty acids (Thiès 1994, Bernoud 1999). The plasma lysoPL of the breast-fed infants had levels of 20:4n-6 and 22:6n-3 that were about 50% of the levels in HDL PL and 90% of the levels in plasma PL, consistent with the possibility that lysoPL might be a significant source of these n-6 and n-3 fatty acids. Study III showed that the plasma albumin-bound FFA of the infants fed formula had levels of 18:3n-3 which were not different and higher levels of 18:2n-6 than the breast-fed infants. Several studies have shown that that unesterified n-6 and n-3 fatty acids can enter brain (Dhopeshwarkar et al 1971, 1971a). Furthermore, brain capillary endothelium and astrocytes are able to desaturate and elongate 18:2n-6 and 18:3n-3 to 20:4n-6 and 22:6n-3 respectively (Moore et al 1990, Moore et al 1991).

In summary, Study III shows that plasma PL, TG and CE are a heterogeneous pool of chylomicron, LDL and HDL each with a different enrichment of n-6 and n-3 fatty acids. Furthermore, lysoPL and unesterified fatty acids in the plasma of young infants contain n-6 and n-3

fatty acids. These variations in 20:4n-6 and 22:6n-3 among different lipoproteins and lipids suggest total plasma PL analyses may not have sufficient sensitivity or specificity as biochemical measures of n-6 and n-3 fatty acid status, or reflect the plasma pool of n-6 and n-3 fatty acids available to the brain. Furthermore, it is not clear if PL, rather than TG or CE, and which lipoproteins, lysoPL or FFA, are involved in the transfer of fatty acids to the developing brain. Thus, further work on the physiological significance of n-6 and n-3 fatty acids in different plasma lipid pools is important in the development of more specific and sensitive biochemical measures of n-6 and n-3 fatty acid status for progress on the role of early infants diet and neurodevelopment.

4.2.2. Influence of the formula TG fatty acid distribution on lipoprotein, lysoPL and unesterified fatty acid composition.

The results of this study show that the distribution of fatty acids in the formula TG does influence the levels of fatty acids in the lipoprotein TG and CE, but not PL, as well as the lysoPL and FFA. Specifically, there were significantly higher levels of 16:0 and lower 18:1 in the LDL CE, as well as lower 18:1 in the chylomicron CE in the infants fed the synthesized TG formula when compared with infants fed the standard formula. Earlier studies with piglets fed a formula with ~70% 16:0 in the formula TG 2 position fatty acids resulted in enrichment of plasma CE with 16:0 along with a decrease in 18:1 (Innis et al 1993a, Innis et al 1995). Similarly, recent studies with adult men and women fed a synthesized TG with 70 % of all 16:0 in the TG 2 position (Zock et al 1996) found higher plasma CE 16:0 and decreased CE 18:1. More recent work by Innis & Dyer (1997), however, did not find a difference in plasma CE 16:0 in piglets fed a formula with lower level of synthesized TG (~32% 16:0 in the TG 2 position fatty acids) compared with piglets fed a conventional formula (<5% 16:0 in the TG 2 position), similar to the formulas used in Study III. Although the levels of 16:0 in the LDL CE were higher, there were no differences in levels of 16:0 in the plasma total CE between the infants fed the synthesized TG formula with 29% 16:0 in the 2

position (representing 39% of all 16:0) of the formula TG and the infants fed the standard formula with 5% 16:0 in the TG 2 position (**Appendix, Table 6.15**). Thus, differences in the CE 16:0 seem to be apparent in individual lipoproteins, i.e. LDL, but not total plasma CE at lower levels of 16:0 in the dietary TG 2 position. At higher levels of 16:0 in the dietary TG 2 position, i.e. 70% of 16:0 in the 2 position, elevated CE 16:0 is apparent in total plasma CE. This suggests a dose-response, with the effect primarily on the LDL fraction, and becoming evident in total plasma CE at high intakes. The pathways by which the dietary TG fatty acid distribution influences the composition of plasma CE are not known. Fatty acids esterified to the 2 position of HDL PL are considered a major source of fatty acids for the CE formed by LCAT (Glomset 1979). Cholesterol esters in LDL are derived, at least in part, from transfer of CE from HDL and in part from the CE secreted with the VLDL, and formed via the acyl-CoA:cholesterol acyltransferase (ACAT) reaction (Suckling & Stange 1985). The activity of ACAT is lower with 16:0 than 18:1 (reviewed in Suckling & Stange 1985, Rumsey et al 1995). Whether the higher 16:0, or conversely lower 18:1, in the 2 position of the synthesized TG formula than in the standard formula influences ACAT activity, or the composition of fatty acids used by ACAT is not known. The significance of these findings to cholesterol metabolism in infants fed formula with 16:0 selectively esterified in the 2 position is not known. However, infants fed the synthesized TG formula had lower plasma HDL cholesterol and higher apolipoprotein B concentrations than the infants fed the standard formula suggesting possible important differences in lipoprotein metabolism may be present.

In addition to the differences in CE 16:0, infants fed the synthesized TG formula had higher 18:0 in the LDL TG and CE than the infants fed the standard formula. The synthesized TG formula had 14% of all 18:0 located in the 2 position of the formula compared with 5% in the standard formula. Although 18:0 is absorbed better when present in the 2 position rather than the 1,3 position of the TG as with 16:0, it is not known if these higher levels of 18:0 in the LDL TG and CE

are reflective of this difference in positioning of 18:0 in the formula. Alternatively, it is possible that the higher 18:0 is explained by elongation of 16:0 in the liver. This seems unlikely because this pathway is considered to be minor, contributing less than 6% of the 18:0 pool in adult males (Rhee et al 1997)

A potentially important finding is the significantly higher levels of 18:3n-3 in the chylomicron TG and CE, as well as the lysoPL and FFA in the infants fed the synthesized TG formula than in the infants fed the standard formula. Of note, the formulae had similar amounts of 18:3n-3 (about 2% with about 30% of the 18:3n-3 distributed in the 2 position of the TG of both formulae). The plasma TG, as well as chylomicron TG levels of 18:3n-3 were also higher in infants fed the synthesized TG formula, suggesting the possibility of higher absorption. In possible contrast to the work here, Carnielli et al (1995) found lower 18:3n-3 in the plasma PL of preterm infants fed a formula with 76% of all 16:0 in the TG 2 position than in infants fed a standard formula. However, as found here, there was higher 18:3n-3 found in the plasma total TG and TG 2 position fatty acids, as well as in the CE of adult men and women fed a diet with ~70% of 16:0 in the dietary TG 2 position (Zock et al 1996). The significance of the higher 18:3n-3 in the lipoproteins and plasma lipids of term infants and adults fed a diet fat with 16:0 selectively esterified to the 2 position is not known. It is possible that the different distribution of fatty acids in the synthesized TG influences 18:3n-3 absorption or β-oxidation of 18:3n-3 for energy. Since 18:3n-3 is of importance as a precursor for 22:6n-3 in both liver and brain (Moore et al 1990, Moore et al 1991, Sprecher et al 1995) further understanding of this is warranted. Furthermore, if the lysoPL and FFA pools are an important source of 18:3n-3 between infants fed formulae varying in the TG 16:0 could be of functional importance.

The levels of 20:4n-6 were about 40% times higher in the chylomicron TG, but 23% lower in the lysoPL and 50% lower in FFA of infants fed the synthesized TG formula than in the infants fed the standard formula. In contrast, piglets fed formula with a synthesized TG similar to the formula in this study, about 32% 16:0 in the TG 2 position fatty acids, had lower, not higher, 20:4n-6 in the chylomicron TG (Innis & Dyer 1997). Whether or not this reflects species diffferences in fatty acid metabolism between the piglet and human, or some other difference between the formulae used here and those fed to piglets is not known.

In the current study, there was no difference in levels of 22:6n-3 in the lipoprotein lipid fractions or the lysoPL between the infants fed the synthesized TG formula and infants fed the standard formula. However, the infants fed the synthesized TG formula had 3 fold higher levels of 22:6n-3 in the unesterified fatty acids than the infants fed the standard formula. The significance of this is not unknown, but as discussed previously, the unesterified n-6 and n-3 fatty acids can enter brain. However, although the difference in 22:6n-3 in the FFA was statistically significant, the levels of 22:6n-3 were low, about 0.4±0.1% and 0.1±0.0% of fatty acids in infants fed the synthesized TG and standard formula, respectively. Furthermore, studies by Bernoud et al (1999) have found 22:6n-3 in lysoPI is taken up more rapidly by brain than 22:6n-3 from FFA.

In summary, the specific positioning of 16:0 to the 2 position of the formula TG resulted in alterations in the lipoprotein lipid fatty acids as well as the lysoPL and FFA. Specifically, the positioning of 16:0 in the *sn*-2 position of the formula TG resulted in an increase in 18:3n-3 over the levels found for the infants fed the standard formula. The significance of this higher 18:3n-3 to the developing CNS is not known.

4.3. Concluding remarks

The major findings from these studies are: 1) there is no difference in preferential looking acuity between breast-fed infants and infants fed a formula with 2% energy from 18:3n-3; 2) gender is a major potential confounder in studies using the Teller Acuity Card test and the Fagan Test of Infant Intelligence; 3) the distribution of 16:0 in milk and formula TG influences the distribution of 16:0 in the plasma and chylomicron TG 2 position; 4) the long chain fatty acids 20:4n-6 and 22:6n-3 are predominantly carried in the HDL PL in breast-fed infants, but not infants fed formula with 1% energy from 18:3n-3; 5) the lysoPL and unesterified fatty acids are a significant carrier of 20:4n-6 and 22:6n-3 and 18:2n-6 and 18:3n-3, respectively and 6) the specific positioning of 16:0 in the formula TG 2 position influences the levels of 18:3n-3, 20:4n-6 and 22:6n-3 in plasma lipoprotein lipids.

These studies suggest that feeding a formula with about 1% of energy as 18:3n-3 and an 18:2n-6 and 18:3n-3 ratio of 9:1 can support preferential looking acuity and cognitive development in healthy term infants similar to that seen in breast-fed infants. Furthermore, demographic variables, such as cultural practices and characteristics of the infant, such as gender, can influence measures of visual ability and cognitive development. These variables need to be considered and controlled for in the design of studies of CNS development.

The finding that 2 position 16:0 in milk and formula is conserved through the process of digestion and absorption and secretion into chylomicron TG in breast-fed infants and infants fed formula suggests that bile salt-stimulated lipase does not complete the hydrolysis of human milk TG. Instead, the 2 position fatty acids are likely taken up into enterocytes as MGs, which are then re-esterified to TG. Positioning 16:0 in the 2 position of the formula TG influences the distribution of the n-6 and n-3 fatty acids in the plasma lipoproteins, lysoPL and unesterified fatty acids. The metabolic significance of this finding is not known. However, these studies show that human milk is

a complex entity and that attempting to match single aspects of its composition may not result in a physiological state that is similar between breast-fed infants and infants fed a modified formula.

4.4. Future Directions

The findings of the studies reported here of no difference in preferential looking acuity or novelty preference between breast-fed infants and infants fed formula with 1% energy from 18:3n-3 suggests that this level of 18:3n-3 is adequate to support normal development of the visual system of infants fed formula. However, the results of the studies here cannot be taken as definitive evidence that a diet with 1% energy as 18:3n-3 supports normal retina and CNS development. Future studies are still needed to clarify the essential needs of the developing infant. Such studies could include:

1. Assessing visual acuity at more frequent intervals from birth to four mo of age, and with different techniques, combined with other measures of sensory development.

Although no difference was found in preferential looking acuity between breast-fed infants and infants fed formula at three mo and later in these studies, it is possible that assessments done at earlier ages, ie two mo, might have detected an influence of formula-feeding on acuity. Inclusion of other measures of acuity, such as VEP, or of visual function with contrast sensitivity or color would provide further information on the role of n-3 fatty acids on the development of the visual system. Measures of the developing systems might help elucidate if effects are specific to the retina or if they involve more global effects, for example, due to changes in membrane fluidity. Measures of auditory evoked potentials, cross-modal transfer and taste perception are examples of other tests that could provide additional information on the developing CNS. Further exploration of specific neurotransmitter relations could involve measures of sleep patterns, temperament and body temperature regulation.

2. Assessing cognitive development using other developmental tools

The current studies found no difference in novelty preference between breast-fed infants and the infants fed formula, and although this information is important, novelty preference is only one small aspect of cognitive function. Other forms of cognitive testing are required to determine if dietary n-3 fatty acids influence other aspects of infant cognitive functioning. Thus, further work is required using alternative developmental measures, such as other forms of memory, language comprehension and language production. The study by Scott et al (1998) found significantly lower language production and comprehension in infants at 14 mo of age that had been randomized to receive a formula with 0.23% 22:6n-3 than in infants fed formula with no 20:4n-6 and 22:6n-3, infants fed a formula with 0.12% 22:6n-3 and 0.43% 20:4n-6 or the breast-fed infants. It is not understood how 22:6n-3 influences language systems, but illustrates the need for studies to examine other aspects of cognitive development.

3. Follow-up studies to determine long-term significance

None of the studies examining the influence of n-6 and n-3 fatty acids on infant CNS development to date have attempted to assess the infants past the age of 24 mo. Cognitive functioning in childhood has been shown to be more predictive of functioning in later life (Wilson 1983). Therefore, assessment at later ages could be important to provide information on the long-term implications of human milk and formula n-6 and n-3 fatty acids for CNS development. Second, if differences in cognitive development have been identified in infancy between breast-fed infants and infants fed different formula, long-term follow-up studies that can determine if the effects are transient in nature. Thus it seems critically important to undertake long term follow-up can identify if these effects were transient in nature or if they persist into childhood. It is recognized that these studies will be very difficult to do because of the large numbers needed and the multiple confounding variables. Clearly, the only way to show a relation between a specific nutrient, eg n-3

fatty acids, and development is a randomized intervention where this is the only variable. These studies are very expensive and difficult to do.

4. Determine the significance of varying levels of 22:6n-3 in human milk on CNS development

One possible explanation for the lack of a difference in the preferential looking acuity in the breast-fed infants and the infants fed formula in the current studies is that the levels of 22:6n-3 in the human milk in these studies (about 0.3% of the total fatty acids) may have been at a sub-optimal level. It is known that the level of 22:6n-3 in human milk is a reflection of the maternal diet 22:6n-3 (Harris et al 1984) and that there is a large degree of variability in milk 22:6n-3. Thus, if dietary 22:6n-3 is indeed important for optimal infant CNS development, then it should be possible to demonstrate difference in visual and cognitive development among breast-fed infants with milks which contain different amounts of 22:6n-3. For example, vegetarians have no dietary intake of 22:6n-3 and thus have very low levels of 22:6n-3 in their milk (about 0.1% milk fatty acids). Women with high intakes of fish, on the other hand, have milk levels of 22:6n-3 which may be as high as 0.8%. It seems surprising that this "natural" experiment is not part of the literature on the potential role of dietary n-3 fatty acids in infant development.

The current studies found that 16:0 in the 2 position of human milk or formula TG was partially conserved and secreted in chylomicron TG. However, little is known about the absorption of monoacylglycerol or subsequent metabolism of lipoproteins particles in breast-fed infants or infants fed a formula with 16:0 esterified to the 2 position. Studies which might address these issues are:

1. Stable isotope studies of 16:0 in formula TG

Stable isotope studies with human infants would allow a better understanding of the metabolism of 16:0 and how it differs in infants when fed in the formula TG 2 position compared

with the TG 1,3 position. This study would provide information on absorption and the turnover of fatty acids in chylomicrons. For example, such studies could elucidate if 16:0 in the 2 position of the chylomicron TG results in a slower removal of the chylomicron from the circulation

2. Stable isotope studies of 18:3n-3 in a synthesized and standard TG formula

The current studies found, unexpectedly, that feeding infants formula with 16:0 located in the 2 position resulted in increased free fatty acids and TG levels of 18:3n-3. The reason for this finding is not known. Feeding infants a formula with labelled 18:3n-3 would allow a better understanding of whether the differences are explained by changes in absorption or in oxidation of 18:3n-3 between infants fed a standard formula or a formula with 16:0 selectively esterified to the 2 position. This is potentially of considerable interest because 18:3n-3 is the precursor for 22:6n-3.

Finally, it is very important that further work be done to elucidate the pathways by which the developing brain obtains n-6 and n-3 fatty acids. It is only with this information that biochemical studies can be developed to provide sensitive and specific markers of infants likely to be at risk for reduced brain 20:4n-6 and 22:6n-3 assimilation.

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6. APPENDIX

Table 6.1 *- Fatty acid nomenclature and composition of selected oils and lipid sources*

Name	Fatty Acid	Butter	Coconut oil	Palm oil	Olive oil	Canola oil	Corn oil	Soybean oil	Egg yolk PL[1]
butyric	4:0	2.6							nd
capoic	6:0	1.6	0.6						nd
caprylic acid	8:0	0.9	7.5						nd
capric acid	10:0	2.0	6.0						nd
lauric acid	12:0	2.3	44.6	0.1					nd
myristic acid	14:0	8.2	16.8	1.0				0.1	0.2
palmitic acid	16:0	21.3	8.2	43.5	11.0	4.8	10.9	10.3	29.3
stearic acid	18:0	9.8	2.8	4.3	2.2	1.5	1.8	3.8	15.7
oleic acid	18:1n-9	20.4	5.8	36.6	72.5	53.2	24.2	22.8	27.5
linoleic acid	18:2n-6	1.8	1.8	9.1	7.9	22.2	58.0	51.0	15.3
α-linolenic acid	18:3n-3	1.2		0.2	0.6	11.0	0.7	6.8	0.1
dihomo-γ-linolenic acid	20:3n-6								0.5
arachidonic acid	20:4n-6								5.6
docosapentaenoic acid	22:5n-6								1.5
eicosapentaenoic acid	20:5n-3								nd
docosapentaenoic acid	22:5n-3								0.2
docosahexaenoic acid	22:6n-3								1.8

Values are % of total fatty acids. Values for oils, except egg PL from Laposata 1995.
[1]unpublished data from candidate's lab; nd = not detected.

6.1. Study I – Effect of dietary fatty acids on plasma and erythrocyte fatty acids and development of visual acuity in term infants.

Table 6.2 *- Plasma PL fatty acids of term breast-fed infants and infants fed formula at 14d and three mo of age*[1]

Fatty acid	14d		3mo	
	Breast-fed	Formula-fed	Breast-fed	Formula-fed
	(g/100 g)			
18:1	13.2±0.3	13.8±0.4	12.4±0.3[2]	12.8±0.2[2]
18:2n-6	17.4±0.4	25.8±0.5[3]	19.3±0.6[2]	28.3±0.6[2,3]
20:2n-6	0.5±0.1	0.4±0.0[3]	0.4±0.0	0.3±0.0
20:3n-6	3.2±0.1	2.9±0.1[3]	2.7±0.1[2]	2.2±0.1[2,3]
20:4n-6	12.8±0.4	6.9±0.3[3]	11.6±0.3[2]	5.9±0.3[2,3]
22:4n-6	0.4±0.0	0.2±0.0[3]	0.4±0.0	0.2±0.0
22:5n-6	0.4±0.0	0.3±0.0	0.3±0.0	0.3±0.0
18:3n-3	0.2±0.0	0.1±0.0	0.2±0.0	0.2±0.0
20:5n-3	0.5±0.0	0.2±0.0[3]	0.5±0.0	0.2±0.0[3]
22:5n-3	0.6±0.1	0.3±0.0[3]	0.9±0.0[2]	0.4±0.0[3]
22:6n-3	4.4±0.2	2.3±0.2[3]	4.6±0.3	2.0±0.3[3]

[1]Results given as mean±SEM; n=17 breast-fed infants and 18 infants fed formula.
[2]Significantly different from breast-fed infants of the same age, $P<0.05$.
[3]Significantly different from 14d old infants in same diet group, $P<0.05$.

Table 6.3 *- Erythrocyte phosphatidylcholine (PC) and phosphatidylethanolamine (PE) fatty acids of breast-fed infants and infants fed formula*[1]

Fatty acid	14d		3mo	
	Breast-fed	Formula-fed	Breast-fed	Formula-fed
	(g/100 g)			
PC				
18:1	21.4±0.4	19.6±0.4	21.0±0.5	19.7±0.3
18:2n-6	14.6±0.4	20.4±0.4	16.2±0.5[2]	22.3±0.4[2,3]
20:2n-6	0.4±0.0	0.3±0.0	0.5±0.0	0.4±0.0[3]
20:3n-6	2.2±0.1	2.0±0.1	1.6±0.1[2]	1.1±0.1[2,3]
20:4n-6	6.7±0.5	4.2±0.4	5.0±0.4[2]	2.5±0.3[2,3]
22:4n-6	0.5±0.1	0.3±0.0	0.5±0.1	0.3±0.1[3]
22:5n-6	0.2±0.0	0.1±0.0	0.1±0.0[2]	0.1±0.0
18:3n-3	0.5±0.1	0.3±0.0	0.6±0.1	0.5±0.1
20:5n-3	0.2±0.0	0.2±0.0	0.2±0.1	0.1±0.0
22:5n-3	0.2±0.1	0.1±0.0	0.3±0.0	0.1±0.0[3]
22:6n-3	1.6±0.2	0.9±0.2	1.5±0.2	0.5±0.1[3]
PE				
18:1	19.7±0.6	18.8±0.4	20.7±0.6	21.2±0.5[2]
18:2n-6	5.0±0.4	5.6±0.2	6.5±0.4[2]	9.0±0.2[2,3]
20:2n-6	0.3±0.0	0.3±0.0	0.4±0.1[2]	0.5±0.0[2]
20:3n-6	1.6±0.1	1.8±0.1	1.4±0.1	1.9±0.2[3]
20:4n-6	20.6±1.2	22.6±0.6	22.3±0.9	21.6±0.6
22:4n-6	5.9±0.3	6.4±0.2	5.4±0.2	6.8±0.3[3]
22:5n-6	1.4±0.1	1.6±0.1	0.8±0.0[2]	1.2±0.1[2,3]
18:3n-3	0.4±0.0	0.4±0.0	0.5±0.0	0.5±0.0[2]
20:5n-3	0.3±0.0	0.2±0.0	0.7±0.1[2]	0.3±0.0[3]
22:5n-3	1.1±0.1	1.0±0.0	3.2±0.2[2]	2.0±0.1[2,3]
22:6n-3	7.7±0.6	8.0±0.3	7.6±0.5	4.0±0.2[2,3]

[1]Results given as mean±SEM; n=17 breast-fed infants and 18 infants fed formula; 0.0 refers to values >0.0-<0.05.
[2]Significantly different from breast-fed infants of the same age, $P<0.05$.
[3]Significantly different from 14d old infants in same group, $P<0.05$.

Table 6.4 *– Plasma cholesterol ester fatty acids of three mo old breast-fed infants and infants fed formula.*[1]

Fatty acid	Breast-fed	Formula-fed
	(g/100 g)	
14:0	1.1±0.0	0.8±0.0
16:0	17.2±0.5	15.0±0.62[2]
18:0	3.1±0.3	3.2±0.2
18:1	25.4±0.6	27.1±0.7
18:2n-6	40.0±0.7	45.8±0.8[2]
18:3n-3	0.4±0.0	0.5±0.0[2]
20:2n-6	0.1±0.0	0.1±0.0
20:3n-6	0.7±0.0	0.5±0.1
20:4n-6	6.3±0.3	2.3±0.2[2]
20:5n-3	0.2±0.0	0.1±0.0[2]
22:4n-6	0.3±0.1	0.5±0.1
22:5n-6	0.0±.0	0.1±0.0
22:5n-3	0.1±0.0	0.1±0.0
22:6n-3	0.6±0.1	0.2±0.0[2]

[1]Results given are means±SEM for 17 breast-fed infants and 18 infants fed formula.
[2]Values significantly different from respective values for breast-fed infants; $P<0.05$.

Table 6.5 *- Preferential looking acuity of term infants*[1]

	Age				
	14d	3mo	4 mo	8mo	18mo
			(cyc/deg)		
Feeding type					
Breast	0.9±0.5 (15)[2]	3.9±0.6 (16)	6.3±0.3 (16)	10.7±0.3 (16)	13.5±0.6 (16)
Formula	0.9±0.5 (17)	4.6±0.4 (17)	5.1±0.4 (17)	9.2±0.4 (16)	12.6±0.4 (14)

[1] The results given are acuity mean (cyc/deg)±SD (octaves). No statistically significant differences ($P>0.05$) were found between the breast-fed infants or the infants fed formula at any age.
[2]Values in brackets are the number of infants per group at the given age.

***Table 6.6** - Preferential looking acuity of term infants in relation to infant and family characteristics*[1]

	Age				
	14d	3mo	4 mo	8mo	18mo
			(cyc/deg)		
Gender					
Female	0.9±0.4	4.0±0.5	5.2±0.3	9.6±0.3	11.2±0.6
	(14)	(14)	(14)	(14)	(13)
Male	1.0±0.6	4.5±0.5	6.0±0.4	10.1±0.4	14.6±0.3
	(18)	(19)	(19)	(18)	(17)
Mother's Job					
Service	0.9±0.6	3.9±0.2	5.8±0.1	10.4±0.3	14.3±0.7
	(10)	(11)	(11)	(11)	(11)
Trade	0.9±0.5	4.5±0.6	5.6±0.5	8.9±0.7	11.5±0.5
	(5)	(5)	(5)	(5)	(5)
Professional	0.9±0.5	4.6±0.6	6.0±0.2	9.1±0.3	12.4±0.4
	(7)	(7)	(7)	(6)	(6)
Unemployed	0.8±0.8	4.0±0.3	4.6±0.5	11.7±0.3	13.8±0.1
	(3)	(3)	(3)	(3)	(2)
Unknown	1.0±0.4	4.7±0.3	5.5±0.4	9.9±0.3	12.5±0.2
	(7)	(7)	(7)	(7)	(6)
Language					
English	1.0±0.5	4.3±0.5	5.7±0.3	10.3±0.3	13.0±0.5
	(24)	(25)	(25)	(24)	(24)
Chinese	0.9±0.4	4.8±0.4	5.2±0.5	8.6±0.6	11.9±0.3
	(6)	(6)	(6)	(6)	(4)
Other	0.6±0.2	3.1±0.7	6.0±0.6	9.5±0.0	15.9±0.1
	(2)	(2)	(2)	(2)	(2)
Mother's Ed'n					
< Highschool	1.2±0.2	4.4±0.3	5.8±0.5	11.5±0.2	13.4±0.4
	(3)	(4)	(4)	(4)	(3)
Highschool	1.0±0.6	4.0±0.3	3.9±0.2	9.3±0.4	11.1±0.2
	(3)	(3)	(3)	(3)	(3)
College	0.8±0.6	3.7±0.5	5.6±0.3	10.2±0.3	13.1±0.7
	(12)	(12)	(12)	(12)	(12)
University	1.0±0.5	4.2±0.6	6.0±0.6	8.4±0.6	11.6±0.3
	(6)	(6)	(6)	(5)	(6)
Graduate	0.9±0.2	5.9±0.4	6.1±0.2	9.8±0.3	14.1±0.3
	(4)	(4)	(4)	(4)	(3)
Unknown	0.9±0.6	5.2±0.4	6.1±1.2	10.1±0.5	16.9±0.4
	(4)	(4)	(4)	(4)	(3)

[1] The results given are acuity mean (cyc/deg)±SD (octaves). No statistically significant differences ($P>0.05$) were found between the groups at any age for any characteristic.
[2] Values in brackets are the number of infants per group at the given age.

Table 6.7 *- Novelty preference (FTII) scores of breast-fed infants and infants fed formula.*

Characteristic	Age		
	76 wk	87 wk	92wk
	% Novelty Preference		
Feeding type			
Human milk	56.4±2.4 (15)	51.7±3.1 (11)	62.4±1.7 (10)
Formula	55.5±1.9 (15)	54.3±8.1 (4)	61.5±2.4 (7)
Gender			
Female	52.4±1.7 (12)	50.0±3.9 (8)	64.7±2.5 (7)
Male	58.3±2.1 (18)	55.2±4.6 (7)	60.2±1.3 (10)
Mother's Job			
Service	57.2±3.1 (10)	56.3±4.8 (6)	63.8±2.3 (5)
Trade	57.6±2.7 (4)	40.3±10.2 (2)	59.4±1.2 (4)
Professional	54.5±4.0 (6)	54.5±4.1 (3)	60.4±3.7 (4)
Unemployed	51.9±4.1 (3)	(0)	56.1 (1)
Unknown	56.3±3.0 (7)	51.2±6.0 (4)	66.5±3.2 (3)
Language			
English	55.3±1.8 (22)	51.3±3.0 (14)	63.5±1.5 (13)
Chinese	60.5±3.0 (6)	(0)	57.2±4.0 (2)
Other	50.0±6.0 (2)	67.5 (1)	57.5±1.4 (2)
Mother's Ed'n			
< Highschool	56.6±2.7 (4)	53.3±8.5 (2)	70.2±2.4 (2)
Highschool	49.2±2.8 (3)	63.9 (1)	60.1 (1)
College	55.0±2.6 (10)	48.8±4.8 (7)	61.2±1.5 (9)
University	55.4±3.4 (3.4)	52.8±5.8 (3)	64.7±2.4 (3)
Graduate	55.0±5.1 (4)	46.5 (1)	54.5±1.3 (2)
Unknown	64.5±4.7 (4)	69.3 (1)	(0)

Results given are % means±SEM, (n).
There were no statistically significant differences (P>0.05) found at any age between any of the groups.

Table 6.8 *- Looking behaviour of term breast-fed infants and infants fed formula during the familiarization phase of the Fagan test*

	76 Wk[1]		92 Wk[2]	
	Breast-fed	Formula	Breast-fed	Formula
	n=15	n=15	n=10	n=7
Total Looks (#)	79.9◯10.3	77.4◯6.4	62.8◯4.3	54.1◯3.8
Looks Left (#)	41.1◯5.5	38.2◯3.6	35.4◯2.7	27.9◯2.9
Looks Right (#)	38.9◯4.9	39.2◯3.1	27.4◯2.3	26.3◯2.2
Time to Criteria[3] (s)	166.0◯7.3	155.4◯8.6	104.9◯10.1	88.2◯6.7
Off Time[4]	0.76◯0.09	0.63◯0.1	1.1◯0.2	0.76◯0.13
Time Left (s)	52.0◯2.2	48.1◯1.5	27.0◯1.9	26.0◯2.6
Time Right (s)	42.5◯2.2	47.8◯1.6	23.0◯1.9	24.0◯2.6
Time/Look (s)	1.38◯0.13	1.34◯0.09	0.83◯0.05	0.95◯0.08
Time/Look Left (s)	1.52◯0.18	1.39◯0.11	0.79◯0.07	0.96◯0.08
Time/Look Right (s)	1.25◯0.11	1.32◯0.10	0.86◯0.06	0.93◯0.10

Values are mean±SEM. No significant differences between diet groups were found, $P<0.05$.
[1]Fagan test version FaganAF
[2]Fagan test version 4.1
[3]Total time to achieve criteria fixation time of 94.3±0.81 s at 76 wk or 50 s at 92 wk of familiar stimuli.
[4]Off time=(total elapsed time-fixation time/fixation time) (Colombo 1988).

Table 6.9 *- Looking behaviour during novelty phase of the Fagan test for term breast-fed infants and infants fed formula*

	76 Wk[1]		92 Wk[2]	
	Breast-fed	Formula	Breast-fed	Formula
	n=15	n=15	n=10	n=7
Total Looks (#)	73.8±7.6	81.4±7.3	85.4±6.0	79.7±5.3
Looks to Novel (#)	44.0±6.4	43.5±4.0	50.0±3.3	46.4±3.2
Looks to Familiar (#)	35.0±3.4	37.9±3.4	35.4±2.9	33.3±2.2
Total Looking Time (s)	67.9±2.6	62.8±2.1	41.8±1.6	43.9±1.3
Time to Novel (s)	39.1±3.1	35.8±2.0	27.9±1.3	29.1±2.0
Time to Familiar (s)	28.8±1.5	27.0±1.6	13.9±0.8	14.8±0.8
Off Time[3]	0.59±0.05	0.71±0.05	0.50±0.06	0.42±0.04
Time/Look (s)	1.04±0.1	0.88±0.09	0.51±0.04	0.57±0.04
Time/Novel Look (s)	1.11±0.14	0.95±0.11	0.58±0.04	0.65±0.07
Time/Familiar Look (s)	0.90±0.07	0.80±0.09	0.42±0.04	0.45±0.04

Values are mean±SEM. No significant differences between diet groups were found, $P>0.05$.
[1]Fagan test version FaganAF
[2]Fagan test version 4.1
[3]Off time=(total elapsed time-fixation time/fixation time)(Colombo 1988).

Table 6.10 *- Bayley Scales of Infant Development scores of breast-fed infants and infants fed formula*[1].

	Psychomotor Development Index			Motor Development Index	
	4mo	8mo	18 mo	8mo	18mo
Feeding type					
Human milk	108.6±2.5 (15)	101.6±2.7 (16)	104.8±3.3 (15)	106.1±2.5 (16)	117.4±3.8 (15)
Formula	109.2±1.9 (17)	100.5±2.1 (17)	101.6±1.6 (14)	100.5±2.1 (17)	110.8±3.8 (14)
Gender					
Female	107.8±2.0 (14)	102.0±3.0 (14)	103.3±3.9 (12)	106.2±2.3 (14)	113.6±3.6 (12)
Male	109.8±2.2 (18)	100.4±2.0 (19)	103.2±1.7 (17)	101.0±2.3 (19)	114.7±4.0 (17)
Mother's Job					
Service	107.5±3.4 (11)	96.5±3.0 (11)	98.8±2.1 (10)	104.8±3.7 (11)	114.9±5.3 (10)
Trade	112.6±3.8 (5)	113.2±2.9 (5)	103.0±2.6 (5)	104.8±4.2 (5)	108.2±4.5 (5)
Professional	108.5±2.1 (6)	98.7±2.9 (7)	106.7±3.6 (6)	102.4±2.6 (7)	120.2±5.7 (6)
Unemployed	113.3±5.2 (3)	102.7±1.7 (7)	103.5±9.5 (2)	96.7±5.0 (3)	99.5±9.5 (2)
Unknown	107.1±2.5 (7)	101.1±3.0 (7)	107.3±6.7 (6)	103.0±3.5 (7)	117.0±6.7 (6)
Language					
English	108.2±1.8 (24)	100.8±2.0 (25)	103.2±2.2 (23)	105.6±1.9 (25)	115.7±3.2 (23)
Chinese	109.8±3.9 (6)	101.8±4.5 (6)	103.3±2.4 (4)	96.5±1.9 (6)	104.5±4.5 (4)
Other	115.5±1.5 (2)	101.5±4.5 (2)	103.5±9.5 (2)	93.5±1.5 (2)	116.5±7.5 (2)
Mother's Ed'n					
< Highschool	106.8±2.8 (4)	102.5±0.9 (4)	99.7±4.3 (4.3)	110.0±4.0 (4)	112.0±7.0 (3)
Highschool	114.0±3.5 (3)	98.0±5.7 (3)	98.0±2.0 (3)	96.7±5.0 (3)	115.0±18.0 (3)
College	106.8±3.1 (12)	101.1±3.5 (12)	101.2±2.6 (11)	104.6±2.7 (12)	113.1±2.5 (11)
University	112.5±3.2 (6)	107.5±3.6 (6)	113.0±6.0 (6)	104.2±3.3 (6)	121.5±8.1 (6)
Graduate	108.7±3.5 (3)	94.8±0.8 (4)	104.7±2.3 (3)	98.8±1.4 (4)	114.0±4.6 (3)
Unknown	108.8±4.6 (4)	98.5±4.3 (4)	98.7±2.4 (3)	100.0±8.1 (4)	105.3±9.0 (3)

[1]Results given are means±SEM, (n), out of a maximum score of 150 points
No significant differences at any age, within any category, $P>0.05$.

6.2. Study II – Effect of human milk and infant formula triacylglycerol fatty acid distribution on plasma lipids and lipoprotein fatty acid distribution in term infants

Table 6.11 *– Plasma lipid and apolipoprotein concentrations at 30 and 120 d of age in term infants fed standard formula, formula with synthesized TG or breast-fed to 120 d of age.*

	Standard Formula		Synthesized TG Formula		Breast-fed	
	30 d	120 d	30 d	120 d	30 d	120 d
Total Cholesterol (mmol/L)	2.9±0.1[3] (11)	3.0±0.2[3] (11)	2.8±0.1[3] (14)	3.0±0.1[3] (15)	3.5±0.2 (13)	4.1±0.4[4] (10)
HDL cholesterol (mmol/L)	1.7±0.1 (11)	1.6±0.1[4] (12)	1.5±0.1[2,3] (13)	1.2±0.1[2,3,4] (12)	1.7±0.1 (14)	1.4±0.1[4] (10)
Apo A-1 (mg/L)	136.4±5.2 (10)	126.5±3.8[4] (14)	118.4±3.5[2,3] (14)	100.1±5.6[2,3,4] (14)	136.4±3.8 (13)	129.7±6.1[4] (11)
Apo B (mg/L)	32.1±2.7[3] (10)	43.7±3.7[3,4] (11)	45.1±3.1[2] (10)	58.8±5.2[2,4] (15)	48.2±3.0 (14)	55.9±4.6[4] (9)

[1]Values are means±SEM, n=number of infants; 0.0 indicates value ≥0.00-< 0.05

[2]Values for infants fed formula with synthesized TG significantly different from value for infants fed standard formula ($P < 0.05$).

[3]Value for infants fed formula significantly different from value for breast-fed infants ($P < 0.05$).

[4]Value for infants at 30 d significantly different from value for infants at 120 d, within the same diet group ($P<0.05$).

Table 6.12- *Plasma TG fatty acids or term infants fed a standard formula, formula with synthesized TG or breast-fed to 120 d*

	30 d			120 d		
Fatty acids[1]	Standard Formula (n=12)	Synthesized TG formula (n=17)	Human milk (n=12)	Standard Formula (n=13)	Synthesized TG formula (n=13)	Human milk (n=9)
			g/100 g			
16:0	23.4±0.7	24.8±0.3	25.0±0.6	24.8±0.6	24.9±0.5	23.9±0.8
18:0[3,4]	3.5±0.3[c]	3.3±0.2[c]	6.3±0.4	4.2±0.2[c]	3.7±0.5[c]	7.5±0.3[a]
18:1[3]	45.5±0.7	43.4±0.3	45.0±1.1	43.1±1.2	41.9±0.5[a]	43.4±1.1
18:2n-6[3,4]	22.0±0.3[c]	22.8±1.4[c]	14.3±1.2	23.2±0.5[c]	24.2±0.8[c]	16.3±0.9
20:2n-6	0.5±0.0	0.5±0.0	0.6±0.1	0.4±0.1	0.4±0.0	0.5±0.1
20:3n-6[3,4]	0.3±0.0[c]	0.3±0.0[c]	0.5±0.0	0.2±0.0[ac]	0.2±0.0[ac]	0.4±0.0
20:4n-6[4]	0.7±0.1[c]	0.6±0.0[c]	1.2±0.1	0.5±0.0[c]	0.5±0.1[c]	1.4±0.1
22:4n-6[4]	0.1±0.0[c]	0.1±0.0[c]	0.1±0.0	0.1±0.0[c]	0.1±0.0[c]	0.1±0.0
22:5n-6[3,4]	0.1±0.0[c]	0.1±0.0[c]	0.2±0.0	tr[c]	0.1±0.0[ac]	0.1±0.0
18:3n-3[3,4]	1.4±0.1[c]	1.9±0.1[bc]	1.1±0.1	1.6±0.1	1.9±0.1[b]	1.6±0.2
20:5n-3[4]	0.1±0.1	0.1±0.0[c]	0.2±0.0	0.1±0.0[c]	tr[c]	0.2±0.0
22:5n-3[4]	0.1±0.0[c]	0.1±0.0[c]	0.2±0.0	0.1±0.0[c]	0.1±0.0[c]	0.3±0.0
22:6n-3[4]	0.2±0.1[c]	0.2±0.0[c]	0.8±0.2	0.1±0.0[c]	0.1±0.0[c]	0.9±0.1

[1]Values are means ± SEM; tr=trace amount, >0.00-<0.05.
[2]Values >0.00-<0.05 were rounded to 0.0, values ≥0.05-0.09 were rounded to 0.1.
[3]Significant (P<0.05) age difference.
[4]Significant (P<0.05) difference between diets.
[a]Values significantly different (P<0.05) from values at 30 d, within the same diet.
[b]Significant difference (P<0.05) between formulae, within the same age.
[c]Values for infants fed formula significantly different (P<0.05) from values for breast fed infants, within the same age.

Table 6.13 *- Plasma TG 2-position fatty acids of term infants fed a standard formula, formula with synthesized TG or breast-fed to 120 d*

	30 d			120 d		
Fatty acids[1]	Standard Formula (n=11)	Synthesized TG formula (n=14)	Human milk (n=12)	Standard Formula (n=11)	Synthesized TG formula (n=12)	Human milk (n=9)
	(g/100 g)					
16:0[4]	8.5±0.4[c]	16.3±0.8[bc]	25.5±1.9	8.9±0.3[c]	15.9±0.8[bc]	25.6±1.4
18:0[3]	2.0±0.5	1.1±0.5	1.3±0.4	2.8±0.4	2.1±0.4	2.8±0.5
18:1[4]	52.8±0.6[c]	47.0±1.2[b]	44.4±1.9	51.9±0.9[c]	45.5±1.1[bc]	41.0±1.5
18:2n-6[4]	31.9±0.3[c]	31.0±0.9[c]	18.3±1.1	32.0±0.4[c]	31.4±0.6[c]	20.1±0.8
20:2n-6	0.3±0.0	0.3±0.0	0.4±0.1	0.3±0.0	0.3±0.0	0.2±0.0
20:3n-6[4]	0.2±0.1	0.1±0.0	0.2±0.0	0.1±0.0	0.2±0.1	0.2±0.1
20:4n-6[4]	0.6±0.1[c]	0.5±0.1[c]	1.9±0.2	0.5±0.1[c]	0.7±0.1[bc]	2.2±0.3
22:4n-6	tr	tr	tr	tr	tr	tr
22:5n-6[4]	0.1±0.0[c]	0.1±0.0[c]	0.2±0.0	0.1±0.0[c]	0.1±0.0[c]	0.2±0.0
18:3n-3	1.2±0.1	1.5±0.2	1.2±0.2	1.2±0.1	1.5±0.2	1.1±0.2
20:5n-3[4]	tr	tr	0.1±0.0	tr[c]	tr[c]	0.1±0.0
22:5n-3[3,4]	tr[c]	0.1±0.0[c]	0.3±0.1	0.1±0.0[c]	0.2±0.0[ac]	0.6±0.1[a]
22:6n-3[4]	tr[c]	tr[c]	0.5±0.1	tr[c]	tr[c]	0.5±0.1

[1]Values are means ± SEM; tr=trace amounts, >0.00-<0.05
[2]SEM values >0.00-<0.05 were rounded to 0.0, all values ≥0.05-0.09 were rounded to 0.1.
[3]Significant (P<0.05) age difference.
[4]Significant (P<0.05) difference between diets.
[a]Values significantly different (P<0.05) from values at 30 d, within the same diet.
[b]Values for infants fed synthesized TG formula significantly different (P< 0.05) from values for infants fed standard formula, within the same age.
[c]Values for infants fed formula significantly different (P<0.05) from values for breast fed infants, within the same age.

Table 6.14 *- Plasma FFA at 30 and 120 d of term infants fed either standard formula, formula with synthesized TG or breast-fed to 120 d*

	30 d			120 d		
Fatty acids[1]	Standard Formula (n=11)	Synthesized TG formula (n=17)	Human milk (n=12)	Standard Formula (n=13)	Synthesized TG formula (n=13)	Human milk (n=9)
			(g/100 g)			
16:0[3]	30.4±0.6	30.7±0.7	29.8±0.7	28.5±0.6[a]	27.4±0.6[a]	27.7±1.7
18:0[3]	22.4±1.6	22.1±1.5	23.4±1.3	17.0±1.3[a]	17.7±1.2[a]	20.7±1.4
18:1[3]	24.5±2.6	22.8±1.6	26.5±2.1	30.8±1.8[a]	26.7±1.5	27.2±2.6
18:2n-6[3,4]	14.6±0.6[c]	16.8±0.6[bc]	11.0±0.6	17.0±0.6[ac]	20.4±0.6[abc]	13.7±1.5[a]
20:2n-6[4]	0.7±0.2	0.3±0.0[c,b]	0.9±0.1	0.6±0.1	0.6±0.1[a]	1.2±0.4
20:3n-6[4]	1.0±0.4	0.3±0.1	0.8±0.2	0.6±0.2	0.3±0.1[c]	0.8±0.2
20:4n-6[4]	0.5±0.1[c]	0.4±0.1[c]	0.8±0.1	0.4±0.1[c]	0.2±0.0[c]	0.8±0.2
22:4n-6	0.1±0.0	0.2±0.1	0.1±0.0	0.1±0.0	0.3±0.1	0.2±0.1
22:5n-6[4]	0.7±0.3	tr[bc]	1.3±0.5	0.5±0.2[c]	0.1±0.0[c]	1.3±0.3
18:3n-3[3,4]	1.0±0.1	1.3±0.1[c]	0.8±0.1	1.2±0.1	1.8±0.2[abc]	1.3±0.2[a]
20:5n-3	0.3±0.1	0.7±0.1	0.1±0.0	0.2±0.1	0.2±0.1	0.3±0.3
22:5n-3	0.1±0.1	0.3±0.1	0.1±0.0	0.1±0.1	0.2±0.0	0.2±0.1
22:6n-3[4]	0.2±0.0[c]	0.2±0.0[c]	0.5±0.1	0.1±0.0[c]	0.4±0.1[b]	0.9±0.3

[1]Values are means ± SEM; tr=trace amount, >0.00-<0.05.
[2]SEM values >0.00-<0.05 were rounded to 0.0, all values ≥0.05-0.09 were rounded to 0.1.
[3]Significant (P<0.05) main effect of age.
[4]Significant (P<0.05) main effect of diet.
[a]Values significantly different (P<0.05) from values at 30 d, within the same diet.
[b]Values for infants fed synthesized TG formula significantly different (P< 0.05) from values for infants fed standard formula, within the same age.
[c]Values for infants fed formula significantly different (P<0.05) from values for breast fed infants, within the same age.

Table 6.15 *- Plasma CE fatty acids at 30 and 120 d of term infants fed either standard formula, formula with synthesized TG or breast-fed to 120 d*

	30 d			120 d		
Fatty acids[1]	Standard Formula (n=11)	Synthesized TG formula (n=17)	Human milk (n=13)	Standard Formula (n=13)	Synthesized TG formula (n=13)	Human milk (n=9)
			(g/100 g)			
16:0[4]	12.7±0.7[c]	12.1±0.4[c]	15.0±0.7	11.1±0.5[c]	11.4±0.2[c]	15.8±0.5
18:0	2.4±0.4	3.1±0.3	3.0±0.4	2.8±0.2	3.3±0.3	3.2±0.3
18:1[3]	29.7±0.8	27.9±0.8	29.3±1.8	24.8±0.6[a]	23.3±0.9[a]	26.2±1.2
18:2n-6[3,4]	46.1±0.8[c]	48.1±0.9[c]	39.2±1.5	54.0±0.6[ac]	54.5±1.1[ac]	40.4±1.8
20:2n-6[4]	0.3±0.1	0.2±0.0[c]	0.3±0.0	0.2±0.0	0.1±0.0	0.2±0.1
20:3n-6[3,4]	0.7±0.1[c]	0.5±0.0[c]	0.8±0.1	0.4±0.1[ac]	0.5±0.0[c]	0.8±0.1
20:4n-6[4]	4.4±0.4[c]	4.1±0.3[c]	7.5±0.4	3.6±0.2[c]	3.6±0.3[c]	8.3±0.5
22:4n-6[4]	tr	tr[b,c]	tr	tr	tr	tr
22:5n-6	tr	tr	tr	tr	tr	tr
18:3n-3[4]	0.6±0.0	0.7±0.0[c]	0.5±0.0	0.6±0.0	0.8±0.1[b,c]	0.5±0.1
20:5n-3[4]	0.2±0.0[c]	0.2±0.0[c]	0.5±0.1	0.1±0.0[c]	0.1±0.0[c]	0.6±0.1
22:5n-3[3]	tr	tr	tr	tr	tr	tr
22:6n-3[4]	0.4±0.1[c]	0.4±0.0[c]	0.9±0.1	0.2±0.0[c]	0.3±0.0[b,c]	1.0±0.1

[1]Values are means ± SEM

[2]Values >0.00-<0.05 were rounded to 0.0, values ≥0.05-0.09 were rounded to 0.1.

[3]Significant (P<0.05) main effect of age.

[4]Significant (P<0.05) main effect of diet.

[5]Significant (P<0.05) diet x age interaction.

[a]Values significantly different (P<0.05) from values at 30 d, within the same diet.

[b]Values for infants fed synthesized TG formula significantly different (P< 0.05) from values for infants fed standard formula, within the same age.

[c]Values for infants fed formula significantly different (P<0.05) from values for breast fed infants, within the same age.

Table 6.16 *- Plasma PL fatty acids, at 30 and 120 d of age, of infants fed either standard formula, formula with synthesized TG or breast-fed to 120 d of age*

	30 d			120 d		
Fatty acids[1]	Standard formula (n=11)	Synthesized TG formula (n=17)	Human milk (n=13)	Standard formula (n=13)	Synthesized TG formula (n=13)	Human milk (n=9)
			(g/100 g)			
16:0	27.1±0.6	28.3±0.5	24.3±0.9	27.9±0.4	26.8±0.7	27.1±1.0
18:0[4]	17.7±0.4[c]	17.7±0.7[c]	18.9±0.5	17.2±0.4	15.8±0.3[c]	18.2±0.4
18:1	12.9±0.4	13.3±0.2	11.7±0.9	13.1±0.3	11.8±0.2	12.5±0.6
18:2n-6[3,4]	25.7±0.8[c]	26.3±0.6[c]	19.8±0.6	29.2±0.5[ac]	28.7±0.2[ac]	19.3±0.7
20:2n-6[4]	0.5±0.1[c]	0.4±0.0[c]	1.0±0.2	0.5±0.0[c]	0.4±0.0[c]	0.8±0.1
20:3n-6[3,4]	2.6±0.4	2.7±0.1	3.2±0.3	2.1±0.2	2.3±0.1[a]	2.6±0.2
20:4n-6[3,4]	8.0±0.3[c]	6.5±0.3[bc]	12.5±0.5	6.3±0.3[ac]	6.4±0.3[c]	10.7±0.5[a]
22:4n-6	0.2±0.0	0.2±0.0	0.3±0.0	0.2±0.0	0.3±0.0	0.3±0.1
22:5n-6[3,4]	0.4±0.0[c]	0.3±0.0	0.3±0.0	0.3±0.0[c]	0.3±0.0	0.3±0.1
18:3n-3	0.3±0.0	0.2±0.0	0.3±0.1	0.3±0.0	0.3±0.1	0.4±0.1
20:5n-3[4]	0.3±0.1[c]	0.2±0.0[c]	0.6±0.1	0.2±0.0[c]	0.2±0.0[c]	0.8±0.1
22:5n-3[3,4]	0.4±0.0[c]	0.3±0.0[c]	0.6±0.1	0.4±0.0[c]	0.5±0.0[ac]	0.8±0.1
22:6n-3[3,4]	2.5±0.2[c]	1.9±0.1[b,c]	4.7±0.3	1.4±0.1[ac]	1.7±0.1[ac]	4.4±0.3

[1]Values are means ± SEM; tr=trace amounts, >0.00-<0.05.
[2] SEM values >0.00-<0.05 were rounded to 0.0, all values ≥0.05-0.09 were rounded to 0.1.
[3]Significant (P<0.05) main effect of age.
[4]Significant (P<0.05) main effect of diet.
[a]Values significantly different (P<0.05) from values at 30 d, within the same diet.
[b]Values for infants fed synthesized TG formula significantly different (P< 0.05) from values for infants fed standard formula, within the same age.
[c]Values for infants fed formula significantly different (P<0.05) from values for breast fed infants, within the same age.

Table 6.17 *- Plasma lysophospholipid fatty acids, at 30 and 120 d, of infants fed either standard formula, formula with synthesized TG or breast-fed to 120 d of age*

	30 d[1,2]			120 d		
Fatty acids	Standard Formula (n=10)	Synthesized TG formula (n=17)	Human milk (n=12)	Standard Formula (n=13)	Synthesized TG formula (n=13)	Human milk (n=9)
			g/100 g			
16:0[4]	26.5±1.3	28.6±1.4[c]	23.0±0.7	27.4±1.6	29.4±1.4	26.5±1.2
18:0[3,4]	13.7±0.8[c]	14.8±0.8	16.2±0.8	17.6±.9[ac]	19.7±1.4[a]	21.5±0.9[a]
18:1[3,4]	16.6±0.9	13.5±0.3[bc]	16.7±0.7	14.7±0.7	12.0±0.3[ab]	13.4±0.6[a]
18:2n-6[3,4]	33.0±1.0[c]	34.1±1.3[c]	25.3±0.9	30.2±1.7[c]	32.3±1.2[c]	21.9±1.6
20:2n-6[4]	0.3±0.1[c]	0.2±0.1[c]	0.6±0.1	0.3±0.1[c]	0.3±0.1[c]	0.5±0.1
20:3n-6[3,4]	1.4±0.2[c]	1.0±0.1[c]	2.4±0.1	0.9±0.1[ac]	1.0±0.1[c]	1.6±0.1[a]
20:4n-6[4]	3.5±0.3[c]	2.8±0.3[c]	7.5±0.7	3.0±0.3[c]	2.3±0.2[c]	7.3±0.7
22:4n-6[4]	0.2±0.0[c]	0.1±0.0[c]	0.3±0.1	0.3±0.1	0.1±0.0[b,c]	0.3±0.1
22:5n-6[4]	0.1±0.0	0.1±0.0[c]	0.1±0.0	0.1±0.0	tr[bc]	0.2±0.0
18:3n-3[3,5]	0.2±0.0	0.2±0.0	0.3±0.0	0.3±0.0	0.4±0.0[a]	0.3±0.0
20:5n-3[4]	0.2±0.0[c]	0.2±0.1[c]	0.4±0.1	0.3±0.1	0.1±0.0[c]	0.4±0.1
22:5n-3[3,4,5]	0.1±0.0[c]	0.1±0.0[c]	0.4±0.1	0.2±0.0[c]	0.1±0.0[c]	0.5±0.1[a]
22:6n-3[4]	0.9±0.1[c]	0.7±0.1[c]	2.2±0.2	0.5±0.1[c]	0.4±0.1[c]	2.4±0.2

[1]Values are means ± SEM; tr=trace amounts, >0.00-<0.05.
[2]SEM values >0.00-<0.05 were rounded to 0.0, all values ≥0.05-0.09 were rounded to 0.1.
[3]Significant (P<0.05) main effect of age.
[4]Significant (P<0.05) main effect of diet.
[5]Significant (P<0.05) diet x age interaction.
[a]Values significantly different (P<0.05) from values at 30 d, within the same diet.
[b]Values for infants fed formula with synthesized TG significantly different (P< 0.05) from values for infants fed standard formula, within the same age.
[c]Values for infants fed formula significantly different (P<0.05) from values for breast fed infants, within the same age.

Table 6.18 *- Plasma PL 2 position fatty acids, at 30 and 120 d, of term infants fed either standard formula, synthesized TG formula or breast-fed to 120 d of age.*

	30 d			120 d		
Fatty acids[1]	Standard Formula (n=10)	Synthesized TG formula (n=16)	Human milk (n=11)	Standard Formula (n=12)	Synthesized TG formula (n=15)	Human milk (n=13)
			(g/100 g)			
16:0[4]	8.1±0.5[c]	11.8±0.4[b]	12.6±0.8	7.2±0.5[c]	11.9±0.4[b]	12.6±0.9
18:0[4]	3.9±0.8	9.5±0.7[b,c]	4.1±0.9	3.5±0.6	6.2±0.6[b]	4.7±0.9
18:1[3,4,5]	17.8±0.6	15.1±0.3[bc]	17.5±0.6	17.0±0.5	13.4±0.3[a,b,c]	16.7±0.5
18:2n-6[3,4,5]	45.7±1.0[c]	43.0±0.7[b,c]	33.2±0.9	51.6±1.2[c]	48.5±0.8[b,c]	34.3±1.1
20:2n-6[4,5]	0.6±0.1[c]	0.5±0.0[c]	0.9±0.1	0.7±0.1	0.6±0.0	0.7±0.1
20:3n-6[3,4]	4.4±0.3	3.9±0.2[c]	4.8±0.3	3.2±0.3[a]	3.1±0.2[a]	3.9±0.4
20:4n-6[4]	11.2±0.8	9.2±0.5[c]	13.6±1.0	9.5±0.5[c]	9.0±0.4[c]	14.5±1.2
22:4n-6[4]	0.9±0.2	0.1±0.0[bc]	0.5±0.1	0.6±0.1	0.4±0.1	1.1±0.3
22:5n-6	0.5±0.1	0.6±0.0	0.6±0.1	0.5±0.1	0.6±0.1	0.5±0.1
18:3n-3[4]	0.4±0.1[c]	0.4±0.2[c]	1.2±0.3	0.4±0.1[c]	0.2±0.1[c]	0.9±0.2
20:5n-3[4]	0.3±0.1	0.1±0.0[c]	0.5±0.1	0.1±0.0[c]	tr[bc]	0.5±0.1
22:5n-3[3,4]	0.6±0.1	0.4±0.0[b]	0.6±0.1	0.7±0.1[c]	0.6±0.0[ac]	1.0±0.1[a]
22:6n-3[3,4]	3.0±0.3[c]	2.0±0.2[bc]	4.8±0.6	1.8±0.2[a,c]	1.6±0.1[c]	4.3±0.5

[1]Values are means ± SEM; tr=trace amounts, >0.00-<0.05.
[2]SEM values >0.00-<0.05 were rounded to 0.0, all values ≥0.05-0.09 were rounded to 0.1.
[3]Significant (P<0.05) main effect of age.
[4]Significant (P<0.05) main effect of diet.
[5] Significant (P<0.05) diet x age interaction.
[a]Values significantly different (P<0.05) from values at 30 d, within the same diet.
[b]Values for infants fed formula with synthesized TG significantly different (P< 0.05) from values for infants fed standard formula, within the same age.
[c]Values for infants fed formula significantly different (P<0.05) from values for breast fed infants, within the same age.

Table 6.19 *- Fatty acids of chylomicron TG, at 30 and 120 d, for term infants fed a standard formula, formula with synthesized TG, or breast-fed to 120 d of age*

	30 d			120 d		
Fatty acid[1]	Standard formula (n=10)	Synthesized TG formula (n=10)	Human milk (n=10)	Standard formula (n=10)	Synthesized TG formula (n=11)	Human milk (n=9)
			(g/100 g)			
16:0	22.8±0.7	21.2±1.2	22.1±0.9	23.2 ± 0.4	23.7 ± 0.7	23.3 ± 0.9
18:0[3]	5.7±0.2	7.1±0.6[b]	8.4±0.6	5.8 ± 0.7[c]	6.8 ± 0.8	7.1 ± 0.5
18:1	42.7±0.9	40.8±1.8	40.7±2.6	41.0 ± 1.4	38.3 ± 1.0	44.4 ± 1.5
18:2n-6[3]	21.6±0.9[c]	23.1±0.6[c]	17.4±1.6	23.2 ± 1.3[c]	23.4 ± 0.7[c]	15.2 ± 0.9
20:2n-6	0.5±0.0	0.4±0.1	0.5±0.1	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
20:3n-6	0.3±0.1	0.3±0.0	0.3±0.0	0.2 ± 0.0	0.2 ± 0.0	0.4 ± 0.1
20:4n-6[3]	0.6±0.1[c]	0.5±0.1[c]	1.2±0.2	0.5 ± 0.1[c]	0.8 ± 0.1[c]	1.1 ± 0.2
22:4n-6[3]	0.1±0.0	tr[b]	0.2±0.1	tr[c]	tr[c]	0.1 ± 0.0
22:5n-6[3]	0.1±0.0	0.1±0.0	0.2±0.1	tr[c]	tr[c]	0.2 ± 0.0
18:3n-3[3]	1.3±0.1	2.0±0.1[bc]	1.6±0.2	1.4 ± 0.1[c]	1.9 ± 0.1[bc]	0.9 ± 0.1
20:5n-3[3]	tr	0.1±0.0	0.2±0.1	tr[c]	tr[c]	0.2 ± 0.1
22:5n-3[3]	tr[c]	0.1±0.0[c]	0.2±0.1	0.1 ± 0.0[c]	0.2 ± 0.1	0.3 ± 0.1
22:6n-3[3]	0.1±0.0[c]	0.1±0.0[c]	0.4±0.1	0.1 ± 0.0[c]	tr[c]	0.5 ± 0.1

[1]Values are means ± SEM, 0.0 indicates values ≥0.01-<0.05; tr=trace amount, >0.00-<0.05.
[2]Significant (P<0.05) main effect of age.
[3]Significant (P<0.05) main effect of diet.
[4]Significant (P<0.05) diet x age interaction
[a]Values at 120 d significantly (P < 0.05) different from values at 30 d, within a diet group.
[b]Values for infants fed formula with synthesized TG significantly (P < 0.05) different from values for infants fed a standard formula.
[c]Values for infants fed formula significantly different from value for breast-fed infants (P < 0.05).

Table 6.20 *- Fatty acids of chylomicron TG 2 position, at 30 and 120 d, for term infants fed a standard formula, formula with synthesized TG, or breast-fed to 120 d of age*

	30 d			120 d		
Fatty acid[1]	Standard formula (n=8)	Synthesized TG formula (n=12)	Human milk (n=11)	Standard formula (n=8)	Synthesized TG formula (n=12)	Human milk (n=11)
			(g/100 g)			
16:0[3,4]	9.0±0.4[c]	17.9±0.8[bc]	24.9±1.4	8.3 ± 0.4[c]	15.8 ± 0.4[bc]	28.0 ± 0.8
18:0[2,3,4]	4.9±0.6	2.5±0.8[b]	1.6±0.6	1.6 ± 0.8[ac]	1.2 ± 0.3	1.9 ± 0.6
18:1[3]	50.8±2.7	42.7±1.2[b]	45.5±2.1	52.6 ± 2.8[c]	44.7 ± 0.8[b]	46.5 ± 2.3
18:2n-6[3]	30.1±2.7[c]	30.9±0.8[c]	17.1±1.1	33.0 ± 1.9[c]	33.0 ± 0.4[c]	15.3 ± 1.9
20:2n-6	0.4±0.1	0.3±0.0	0.6±0.2	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.0
20:3n-6[3]	0.3±0.1	0.2±0.0	0.4±0.1	0.1 ± 0.0[c]	0.1 ± 0.0[bc]	0.5 ± 0.1
20:4n-6[2,3,4]	0.7±0.1[c]	0.6±0.1[c]	1.6±0.1	0.4 ± 0.1[ac]	0.6 ± 0.1[c]	1.1 ± 0.1[a]
22:4n-6	tr	tr	tr	tr	tr	tr
22:5n-6	tr	tr	tr	tr	tr	tr
18:3n-3[3]	1.3±0.2	1.7±0.1[c]	1.3±0.2	1.4 ± 0.2	1.6 ± 0.1	1.3 ± 0.1
20:5n-3[4]	tr	0.1±0.1	0.1±0.0	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0
22:5n-3[3]	tr[c]	0.1±0.0[b]	0.3±0.1	0.1 ± 0.0	0.2 ± 0.0	0.3 ± 0.1
22:6n-3[3]	0.1±0.0[c]	0.1±0.0[c]	0.4±0.1	0.1± 0.0[c]	0.1±0.0[bc]	0.3 ± 0.1

[1]Values are means ± SEM, 0.0 indicates values ≥0.01-<0.05; tr=trace amount, >0.00-<0.05.
[2]Significant (P<0.05) main effect of age.
[3]Significant (P<0.05) main effect of diet.
[4]Significant (P<0.05) diet x age interaction.
[a]Values at 120 d significantly (P < 0.05) different from values at 30 d, within a diet group.
[b]Values for infants fed formula with synthesized TG significantly (P < 0.05) different from values for infants fed a standard formula.
[c]Values for infants fed formula significantly different from value for breast-fed infants (P < 0.05).

Table 6.21 *- Major fatty acids of chylomicron PL, at 30 and 120 d, for term infants fed a standard formula or formula with synthesized TG or breast-fed to 120 d of age*

	30 d			120 d		
Fatty acids[1]	Standard formula (n=8)	Synthesized TG formula (n=10)	Human milk (n=11)	Standard formula (n=9)	Synthesized TG formula (n=10)	Human milk (n=13)
			(g/100 g)			
16:0	28.1±1.2	27.2±1.1	28.1±1.4	27.7±1.1	25.4±0.4	25.8±0.8
18:0[3]	24.1±1.0	23.4±1.1[c]	26.9±1.4	22.8±0.9[c]	23.4±1.0[c]	26.9±0.8
18:1[2,3]	12.8±0.8	10.5±0.3	11.7±0.5	11.9±0.5[c]	11.5±0.2[c]	10.2±0.3[a]
18:2n-6[2,3]	23.1±1.0[c]	23.9±1.0[c]	17.3±1.1	26.4±0.8[ac]	27.1±0.5[ac]	17.3±0.6
20:2n-6	0.7±0.1	0.5±0.1	0.7±0.1	0.6± 0.1	0.5±0.2	0.6±0.1
20:3n-6	1.5±0.2	1.8±0.2	1.9±0.2	1.5±0.2[3]	1.7±0.2[3]	2.1±0.3
20:4n-6[3]	5.7±0.7[c]	5.8±0.3[c]	8.0±0.8	5.3±0.3[c]	5.3±0.5[c]	9.8±0.5
22:4n-6[2]	0.1±0.0	0.1±0.0	0.1±0.0	0.2±0.0[a]	0.2±0.0	0.3±0.1
22:5n-6	0.1±0.1	0.2±0.1	0.2±0.1	0.2±0.0	0.2±0.0	0.3±0.1
18:3n-3	0.3±0.0	0.4±0.1	0.4±0.1	0.4±0.1	0.5±0.1	0.5±0.1
20:5n-3[2,3]	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0[c]	0.1±0.0[c]	0.4±0.1[a]
22:5n-3[2,3]	0.2±0.1	0.3±0.0	0.4±0.1	0.3±0.1[c]	0.4±0.1[c]	0.8±0.1[a]
22:6n-3[3]	1.8±0.2[c]	1.9±0.1[c]	2.7±0.3	1.1±0.1[c]	1.5±0.2[c]	3.9±0.2

[1]Values are means ± SEM, 0.0 indicates values ≥0.01-<0.05; tr=trace amount, >0.00-<0.05.
[2]Significant (P<0.05) main effect of age.
[3]Significant (P<0.05) main effect of diet.
[4]Significant (P<0.05) diet x age interaction.
[a]Values at 120 d significantly (P < 0.05) different from values at 30 d, within a diet group.
[b]Values for infants fed formula with synthesized TG significantly (P < 0.05) different from values for infants fed a standard formula.
[c]Values for infants fed formula significantly different from value for breast-fed infants (P < 0.05).

Table 6.22 *- Fatty acids of chylomicron CE, at 30 and 120 d, for term infants fed a standard formula or formula with synthesized TG, or breast-fed to 120 d of age*

	30 d			120 d		
Fatty Acids[1]	Standard formula (n=9)	Synthesized TG formula (n=10)	Human milk (n=11)	Standard formula (n=9)	Synthesized TG formula (n=12)	Human milk (n=10)
			(g/100 g)			
16:0	17.7±1.0	19.1±1.5	17.7±0.9	18.6±1.6	18.0±0.9	20.6±1.0
18:0	9.8±1.0	9.6±1.2	11.5±1.6	8.4±0.6	9.6±1.2	9.1±1.1
18:1	25.2±0.9	25.0±1.4	26.4±0.8	23.9±0.9	21.3±0.8	21.7±1.1
18:2n-6	35.0±1.5	32.1±1.5	30.5±1.5	36.0±2.2	38.1±1.2	33.0±2.1
20:2n-6	0.5±0.2	0.4±0.1	0.6±0.2	0.3±0.1	0.3±0.1	0.3±0.1
20:3n-6	0.3±0.1	0.4±0.0	0.5±0.0	0.4±0.0[c]	0.5±0.1	0.6±0.1
20:4n-6[3]	2.5±0.4[c]	2.7±0.1[c]	4.5±0.4	2.6±0.3[c]	2.5±0.2[c]	4.9±0.6
22:4n-6[3]	0.1±0.0	tr[c]	tr	tr	tr	0.1±0.0
22:5n-6	tr	tr	tr	tr	tr	tr
18:3n-3[3]	0.8±0.1	1.0±0.1	0.7±0.1	0.7±0.1	0.9±0.0[c]	0.5±0.1
20:5n-3[3]	0.2±0.1	1.0±0.0[c]	0.2±0.0	tr	tr	0.2±0.2
22:5n-3[3]	0.1±0.1	tr	tr	0.1±0.1	tr[c]	0.1±0.0
22:6n-3[3]	0.2±0.1	0.1±0.0[c]	0.3±0.1	0.1±0.1[c]	0.1±0.0[c]	0.4±0.1

[1]Values are means ± SEM, 0.0 indicates values ≥0.01-<0.05; tr=trace amount, >0.00-<0.05.
[2]Significant (P<0.05) main effect of age.
[3]Significant (P<0.05) main effect of diet.
[4]Significant (P<0.05) diet x age interaction.
[a]Values at 120 d significantly (P<0.05) different from values at 30 d, within a diet group.
[b]Values for infants fed formula with synthesized TG significantly (P<0.05) different from values for infants fed a standard formula.
[c]Values for infants fed formula significantly different from value for breast-fed infants (P <0.05).

Table 6.23 *- HDL-PL fatty acids, at 30 and 120 d of age, for term infants fed either standard formula, formula with a synthesized TG or breast-fed to 120 d of age*[1]

	30 d			120 d		
Fatty acids	Standard Formula (n=8)	Synthesized TG formula (n=11)	Human milk (n=9)	Standard Formula (n=7)	Synthesized TG formula (n=10)	Human milk (n=9)
	(g/100 g)					
16:0	26.6±0.5	24.9± 0.7	23.3 ± 1.0	26.0 ± 0.7	26.4 ± 1.2	25.1 ± 0.9
18:0[4]	18.2±0.4	18.9± 0.4	18.8 ± 0.2	17.4 ± 0.3	17.4 ± 0.5[b,c]	18.7 ± 0.3
18:1[3,5]	12.5±0.5	11.9 ± 0.4[c]	13.4± 0.3	12.2 ± 0.5	11.2 ± 0.3	10.7 ± 0.4[a]
18:2n-6[3,4]	25.6±0.6[c]	26.0 ± 0.7[c]	17.4 ± 0.7	28.6 ± 0.9[ac]	28.9 ± 0.7[ac]	17.5 ± 0.7
20:2n-6	0.6±0.1	0.6 ± 0.0	0.7 ± 0.0	0.7 ± 0.1	0.6 ± 0.1	0.5 ± 0.0
20:3n-6[3,4]	2.2±0.3[c]	2.6 ± 0.1[c]	3.1± 0.1	1.9 ± 0.2[c]	2.2 ± 0.2[c]	2.8 ± 0.2
20:4n-6[4]	7.9±0.5[c]	8.5 ± 0.2[c]	13.8 ± 0.7	7.9±0.7[c]	7.6 ± 0.4[c]	13.8 ± 0.4
22:4n-6	0.4±0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0
22:5n-6	0.3±0.1	0.4 ± 0.0	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.0	0.2 ± 0.1
18:3n-3[3,4]	0.1±0.0	0.2±0.0[bc]	0.1 ± 0.0	0.2 ± 0.0[a]	0.2 ± 0.0[c]	0.2 ± 0.0
20:5n-3[4]	0.1±0.0[c]	0.2±0.0[c]	0.4 ± 0.1	0.1 ± 0.0[c]	0.2 ± 0.1[c]	0.4 ± 0.1
22:5n-3[3,4]	0.5±0.1[c]	0.5±0.0[c]	0.9 ± 0.1	0.6 ± 0.0[c]	0.6 ± 0.1[ac]	1.3 ± 0.1[a]
22:6n-3[4,5]	2.8±0.3[c]	2.9± 0.2[c]	5.0 ± 0.2	1.7 ± 0.2[ac]	2.0 ± 0.1[ac]	6.0 ± 0.4

[1]Values are means ± SEM; tr=trace amounts, <0.05
[2]Values >0.00-<0.05 were rounded to 0.0, values ≥0.05-0.09 were rounded to 0.1.
[3]Significant (P<0.05) main effect of age
[4]Significant (P<0.05) main effect of diet.
[5] Significant (P<0.05) diet x age interaction.
[a]Values significantly different (P<0.05) from values at 30 d, within the same diet.
[b]Values for infants fed formula with synthesized TG significantly different (P< 0.05) from values for infants fed standard formula, within the same age.
[c]Values for infants fed formula significantly different (P<0.05) from values for breast fed infants, within the same age.

Table 6.24 *- HDL-PL 2 position fatty acids, at 30 and 120 d of age, for infants fed either standard formula, synthesized TG formula or breast-fed to 120 d of age*

Fatty acids[1]	30 d			120 d		
	Standard Formula (n=8)	Synthesized TG formula (n=10)	Human milk (n=11)	Standard Formula (n=7)	Synthesized TG formula (n=6)	Human milk (n=9)
	(g/100 g)					
16:0	17.2 ± 1.2	22.3 ± 2.1	18.5 ± 1.6	19.4 ± 2.1	23.7 ± 3.8	21.9 ± 2.2
18:0	12.8 ± 1.7	14.4 ± 1.6	12.2 ± 1.1	15.3 ± 3.2	17.0 ± 1.4	13.7 ± 1.7
18:1[3]	14.7 ± 0.6	12.6 ± 0.8	14.8 ± 0.5	12.0 ± 1.2[a]	11.4 ± 0.9	12.1 ± 1.0[a]
18:2n-6[4]	34.4 ± 1.8[c]	31.2 ± 3.1	27.4 ± 1.5	31.9.± 3.5[c]	31.3 ± 4.1[c]	23.3 ± 1.6
20:2n-6	0.3 ± 0.1	0.7 ± 0.2	0.6 ± 0.1	0.5 ± 0.2	0.2 ± 0.1	0.5 ± 0.1
20:3n-6[4]	2.8 ± 0.5	2.4 ± 0.3[c]	3.7 ± 0.2	2.6 ± 0.6	1.9 ± 0.4[c]	3.2 ± 0.3
20:4n-6[4]	7.2 ± 0.7[c]	5.1 ± 1.0[c]	12.2 ± 1.0	7.2 ± 1.8[c]	5.0 ± 0.1[c]	10.9 ± 1.4
22:4n-6	0.6 ± 0.2	0.8 ± 0.3	0.4 ± 0.1	0.4 ± 0.2	0.3 ± 0.2	0.9 ± 0.2
22:5n-6	1.3 ± 0.2	1.7 ± 0.4	1.0 ± 0.1	1.2 ± 0.2	1.4 ± 0.4	1.5 ± 0.3
18:3n-3	0.6 ± 0.2	0.6 ± 0.3	0.2 ± 0.2	0.4 ± 0.2	0.2 ± 0.1	0.4 ± 0.3
20:5n-3[4]	0.1 ± 0.0[c]	0.1 ± 0.0[c]	0.2 ± 0.1	0.2 ± 0.0	tr	0.3 ± 0.2
22:5n-3[3,4]	0.2 ± 0.1[c]	0.1 ± 0.0[c]	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.6 ± 0.2
22:6n-3[4]	1.5 ± 0.3[c]	0.9 ± 0.3[c]	2.8 ± 0.4	1.2 ± 0.4[c]	0.7 ± 0.1[c]	2.6 ± 0.5

[1]Values are means ± SEM; tr=trace amounts, >0.00 <0.05.
[2]Values >0.00-<0.05 were rounded to 0.0, values ≥0.05-0.09 were rounded to 0.1.
[3]Significant (P<0.05) main effect of age.
[4]Significant (P<0.05) main effect of diet.
[a]Values significantly different (P<0.05) from values at 30 d, within the same diet.
[b]Values for infants fed formula with synthesized TG significantly different (P< 0.05) from values for infants fed standard formula, within the same age.
[c]Values for infants fed formula significantly different (P<0.05) from values for breast fed infants, within the same age.

Table 6.25 *- HDL-TG fatty acids of term infants at 30 and 120 d of age fed either standard formula, synthesized TG formula or breast-fed to 120 d of age*

	30 d			120 d		
Fatty acids	Standard[1,2] Formula (n=8)	Synthesized TG formula (n=12)	Human milk (n=10)	Standard Formula (n=8)	Synthesized TG formula (n=12)	Human milk (n=10)
			(g/100 g)			
16:0	24.5±0.8	25.4±0.4	25.2±0.6	25.2±0.6	26.2±0.6	27.0±0.9
18:0[4]	8.4±0.5	9.3±0.7[b,c]	8.0±0.4	8.7±0.4[c]	7.1±0.6[c]	9.8±0.7
18:1[4]	39.2±1.5	39.3±0.9[b]	44.9±1.1	38.2±1.6	40.0±1.1[c]	37.9±2.0
18:2n-6[3]	19.1±0.8[c]	19.9±0.6[bc]	12.9±0.7	20.7±0.9[ac]	21.2±0.5[c]	15.9±0.9[a]
20:2n-6[4]	1.3±0.3	0.8±0.1	1.2±0.3	1.0±0.2	0.6±0.1[c]	1.5±0.2
20:3n-6	0.5±0.1	0.3±0.0	0.4±0.0	0.3±0.0	0.3±0.0	0.5±0.1
20:4n-6[4]	0.9±0.2[c]	0.8±0.1	1.4±0.1	0.8±0.1[c]	0.6±0.1[c]	1.4±0.1
22:4n-6	tr	tr	tr	tr	tr	tr
22:5n-6[4]	tr	0.1±0.1[b]	0.3±0.0	tr	0.1±0.0[c]	tr
18:3n-3[4]	1.2±0.3	1.1±0.1	0.8±0.2	1.0±0.1	1.1±0.2	1.0±0.1
20:5n-3	tr	tr	tr	tr	tr	tr
22:5n-3	tr	0.1±0.0	0.2±0.0	0.2±0.1	0.1±0.0	0.2±0.1
22:6n-3[4]	0.5±0.1	0.3±0.1[c]	0.5±0.1	0.5±0.2	0.3±0.0[c]	0.7±0.2

[1]Values are means ± SEM; tr=trace amounts, >0.00-<0.05.
[2]SEM values >0.00-<0.05 were rounded to 0.0, all values ≥0.05-0.09 were rounded to 0.1.
[3]Significant (P<0.05) main effect of age.
[4]Significant (P<0.05) main effect of diet.
[5]Significant (P<0.05) diet x age interaction.
[a]Values significantly different (P<0.05) from values at 30 d, within the same diet.
[b]Values for infants fed formula with synthesized TG significantly different (P< 0.05) from values for infants fed standard formula, within the same age.
[c]Values for infants fed formula significantly different (P<0.05) from values for breast fed infants, within the same age.

Table 6.26 *- HDL-CE fatty acids of infants at 30 and 120 d of age fed either standard formula, synthesized TG formula or breast-fed to 120 d of age*

	30 d			120 d		
Fatty acids[1]	Standard formula (n=8)	Synthesized TG formula (n=12)	Human milk (n=11)	Standard formula (n=7)	Synthesized TG formula (n=12)	Human milk (n=9)
			(g/100 g)			
16:0[3,4]	14.9±0.6[c]	15.0±0.7[c]	16.3±0.5	12.7±0.9[c]	13.6±0.6[c]	16.4±0.8
18:0	4.6±0.3	5.2±0.6	5.7±0.4	4.1±0.4	4.2±0.5	4.9±0.3
18:1[3]	25.3±0.9	25.7±0.8	28.3±0.9	22.9±0.6	21.8±0.7[a]	22.7±0.7[a]
18:2n-6[3,4]	47.1±0.7[c]	45.8±0.9[c]	37.3±1.1	52.9±1.3[ac]	53.7±0.8[ac]	42.8±1.1[a]
20:2n-6	0.2±0.0	0.3±0.1	0.3±0.1	0.3±0.0	0.2± 0.1	0.2±0.0
20:3n-6[4]	0.5±0.1[c]	0.6±0.0	0.8±0.1	0.4±0.1[c]	0.5±0.1	0.7±0.1
20:4n-6[4]	4.0±0.5[c]	3.8±0.3[c]	7.1±0.8	3.7±0.3[c]	3.2±0.2[c]	7.9±0.4
22:4n-6	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
22:5n-6[4]	0.3±0.1	0.1±0.0	0.2±0.0	0.2±0.1	0.1±0.0[c]	0.3±0.1
18:3n-3[3,4]	0.2±0.0	0.4±0.1[bc]	0.2±0.0	0.4±0.1[ac]	0.5±0.1[c]	0.2±0.0
20:5n-3[4]	0.1±0.0[c]	0.1±0.0[c]	0.2±0.0	0.1±0.0[c]	0.1±0.0[c]	0.2±0.1
22:5n-3	tr	tr	tr	tr	tr	tr
22:6n-3[4]	0.3±0.0[c]	0.2±0.0[bc]	0.5±0.1	0.2±0.0[c]	0.2±0.1[c]	0.8±0.1

[1]Values are means ± SEM; tr=trace amounts <0.05.
[2]Values >0.00-<0.05 were rounded to 0.0, values ≥0.05-0.09 were rounded to 0.1.
[3]Significant (P<0.05) main effect of age
[4]Significant (P<0.05) main effect of diet
[a]Values significantly different (P<0.05) from values at 30 d, within the same diet.
[b]Values for infants fed formula with synthesized TG significantly different (P< 0.05) from values for infants fed standard formula, within the same age.
[c]Values for infants fed formula significantly different (P<0.05) from values for breast fed infants, within the same age.

Table 6.27 *- LDL -PL fatty acids, at 30 and 120 d of age, for infants fed either standard formula, formula with a synthesized TG formula or breast-fed to 120 d of age*

	30 d			120 d		
Fatty acids[1]	Standard Formula (n=8)	Synthesized TG formula (n=14)	Human milk (n=11)	Standard Formula (n=9)	Synthesized TG formula (n=10)	Human milk (n=9)
			(g/100 g)			
16:0[4]	28.6±0.5[c]	28.9±0.7[c]	25.4±0.4	28.8±0.3[c]	29.4±0.3[c]	26.4±0.7
18:0[4]	17.0±0.3	16.42±0.4[c]	19.2±0.3	17.6±0.2[c]	17.5±0.3[c]	18.4±0.3
18:1[3,4]	12.6±0.5	11.6±0.2[c]	12.8±0.4	11.6±0.5	10.7±0.3	11.4±0.3[a]
18:2n-6[4]	25.1±0.4[c]	25.3±0.7[c]	18.3±0.7	26.9±0.8[c]	26.5± 0.7[c]	18.1±0.6
20:2n-6[3,4]	0.5±0.0[c]	0.5±0.0[c]	0.5±0.0	0.4±0.0	0.4±0.0	0.4±0.0[a]
20:3n-6[3,4]	2.2±0.4[c]	2.5±0.1[c]	3.0±0.2	1.8±0.1[c]	2.0±0.2[c]	2.9±0.2
20:4n-6[3,4]	6.8±0.6[c]	7.2±0.3[c]	11.2±0.6	6.1±0.5[c]	6.0±0.2[c]	11.2±0.4
22:4n-6	0.3±0.0	0.3±0.0	0.4±0.0	0.4±0.0	0.4±0.0	0.4±0.0
22:5n-6	0.3±0.1	0.4±0.0	0.4±0.1	0.3±0.0	0.3±0.1	0.4±0.1
18:3n-3	0.3±0.1	0.3±0.1	0.2±0.0	0.3±0.1	0.5±0.2	0.3±0.1
20:5n-3[3]	0.8±0.1	0.7±0.1	0.7±0.1	0.8±0.1[a]	0.9±0.1	1.0±0.1
22:5n-3[3,4]	0.6±0.1[c]	0.6±0.0[c]	0.8±0.1	0.7±0.1[c]	0.7±0.1[c]	1.2±0.1[a]
22:6n-3[4,5]	2.2±0.2[c]	2.3±0.1[c]	3.3±0.4	1.3±0.1[c]	1.6±0.1[c]	4.6±0.2

[1]Values are means ± SEM; tr=trace amount, >0.00-<0.05.
[2]SEM values >0.00-<0.05 were rounded to 0.0, values ≥0.05-0.09 were rounded to 0.1.
[3]Significant (P<0.05) main effect of age.
[4]Significant (P<0.05) main effect of diet.
[5]Significant (P<0.05) diet x age interaction.
[a]Values significantly different (P<0.05) from values at 30 d, within the same diet.
[b]Significant difference (P<0.05) between formulae, within the same age.
[c]Values for infants fed formula significantly different (P<0.05) from values for breast fed infants, within the same age.

Table 6.28 *- LDL-TG fatty acids, at 30 and 120 d of age, for infants fed either standard formula, synthesized TG formula or human milk to 120 d*

	30 d			120 d		
Fatty acids[1]	Standard Formula (n=8)	Synthesized TG formula (n=14)	Human milk (n=10)	Standard Formula (n=9)	Synthesized TG formula (n=11)	Human milk (n=8)
			(g/100 g)			
16:0[4]	24.4±1.0	24.6±0.5	24.3±1.0	25.3 ± 0.7	26.7± 0.6[c]	24.6±0.6
18:0	7.9±0.6	8.5±0.4	9.0±0.5	7.0± 0.6	8.9± 0.4	8.3±0.4
18:1[4]	40.8±1.4	40.0±0.5[c]	43.7±1.5	40.3 ±1.5	38.0 ±0.7[b,c]	42.6±1.1
18:2n-6[4]	19.7±1.1[c]	19.4±0.4[c]	13.6±0.5	21.0 ±1.1[c]	18.9 ± 0.5[b,c]	14.5±0.7
20:2n-6[4]	0.5±0.1	0.5±0.0	0.5±0.0	0.5 ± 0.1	0.4 ± 0.0[b,c]	0.6±0.1
20:3n-6	0.6±0.1	0.5±0.0	0.5±0.0	0.4 ± 0.0	0.5 ± 0.0	0.6±0.1
20:4n-6[3,4]	1.2±0.2[c]	1.1±0.1[c]	1.9±0.1	0.9 ± 0.1[c]	0.8 ± 0.1[c]	1.7±0.1
22:4n-6[4]	0.1±0.0[c]	0.1±0.0[c]	0.2±0.0	0.1± 0.0[c]	0.1 ± 0.0[c]	0.2±0.0
22:5n-6[4]	0.1±0.0[c]	0.3±0.0	0.3±0.0	0.1± 0.0	0.3 ± 0.1	0.2±0.0
18:3n-3[4]	1.0±0.1	1.2±0.1[c]	0.8±0.1	1.1 ± 0.1	1.0 ± 0.1	0.9± 0.1
20:5n-3	0.3±0.1	0.4±0.0	0.4±0.0	0.3 ± 0.1	0.6 ± 0.1	0.5±0.1
22:5n-3[3,4]	0.2±0.1[c]	0.3±0.0[c]	0.4±0.0	0.3± 0.0[c]	0.4±0.1[a,b,c]	0.6±0.1[a]
22:6n-3[4]	0.2±0.1[c]	0.2±0.0[c]	0.6±0.1	0.1 ± 0.0[c]	0.1 ± 0.0[c]	0.8±0.1

[1]Values are means ± SEM; tr=trace amounts, >0.00, <0.05.
[2]Values >0.00-<0.05 were rounded to 0.0, values ≥0.05-0.09 were rounded to 0.1.
[3]Significant (P<0.05) main effect of age.
[4]Significant (P<0.05) main effect of diet
[5]Significant (P<0.05) diet x age interaction.
[a]Values significantly different (P<0.05) from values at 30 d, within the same diet.
[b]Significant (P<0.05) difference between formulae, within the same age group.
[c]Significant (P<0.05) difference between formula and breast-fed within the same age group.

Table 6.29 *- LDL-CE fatty acids, at 30 and 120 d of age, for infants fed either standard formula, formula with a synthesized TG or breast-fed to 120 d of age*

Fatty acids[1]	30 d			120 d		
	Standard formula (n=8)	Synthesized TG formula (n=14)	Human milk (n=11)	Standard formula (n=9)	Synthesized TG formula (n=10)	Human milk (n=9)
	(g/100 g)					
16:0[3,4]	13.6± 0.2[c]	14.8±0.3	15.5±0.4	12.2±0.4[ac]	14.5±0.4[b,c]	14.9±0.2
18:0[4]	4.3±0.6	4.8±0.4	4.1±0.3	3.3±0.4	6.0±0.6[b,c]	3.4±0.6
18:1[3,4]	28.0±1.2	27.6±0.8[c]	30.5±1.1	24.4±0.7[a]	21.3±0.5[a,b,c]	24.7±0.8[a]
18:2n-6[3,4]	44.8±0.9[c]	43.4±0.7[c]	36.6±1.5	52.5±1.0[ac]	50.5±1.1[ac]	43.4±1.2[a]
20:2n-6[3]	0.3±0.0	0.3±0.1	0.3±0.0	0.2±0.0	0.2±0.0	0.2±0.0
20:3n-6[4]	0.7±0.1[c]	0.7±0.1[c]	0.9±0.1	0.6±0.0[c]	0.7±0.1[c]	0.9±0.1
20:4n-6[4]	4.1±0.7[c]	3.6±0.2[c]	6.8±0.6	3.3±0.3[c]	2.8±0.3[c]	7.6±0.5
22:4n-6	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	tr	tr
22:5n-6[3]	tr	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
18:3n-3[4]	0.5±0.0[c]	0.5±0.0[c]	0.4±0.0	0.5±0.0[c]	0.5±0.0[c]	0.4±0.0
20:5n-3[4]	0.3±0.0[c]	0.3±0.0[c]	0.4±0.0	0.2±0.0[c]	0.3±0.0[c]	0.4±0.0
22:5n-3[4]	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.2±0.0[b,c]	0.1±0.0
22:6n-3[4]	0.4±0.1	0.4±0.0	0.5±0.1	0.2±0.0[c]	0.2±0.0[c]	0.7±0.1

[1]Values are means ± SEM; tr=trace amounts, >0.00<0.05.
[2]Values >0.00-<0.05 were rounded to 0.0, values ≥0.05-0.09 were rounded to 0.1.
[3]Significant (P<0.05) main effect of age.
[4]Significant (P<0.05) main effect of diet.
[5]Significant (P<0.05) diet x age interaction
[a]Values significantly different (P<0.05) from values at 30 d, within the same diet.
[b]Significant difference (P<0.05) between formulae, within the same age.
[c]Values for infants fed formula significantly different (P<0.05) from values for breast fed infants, within the same age.

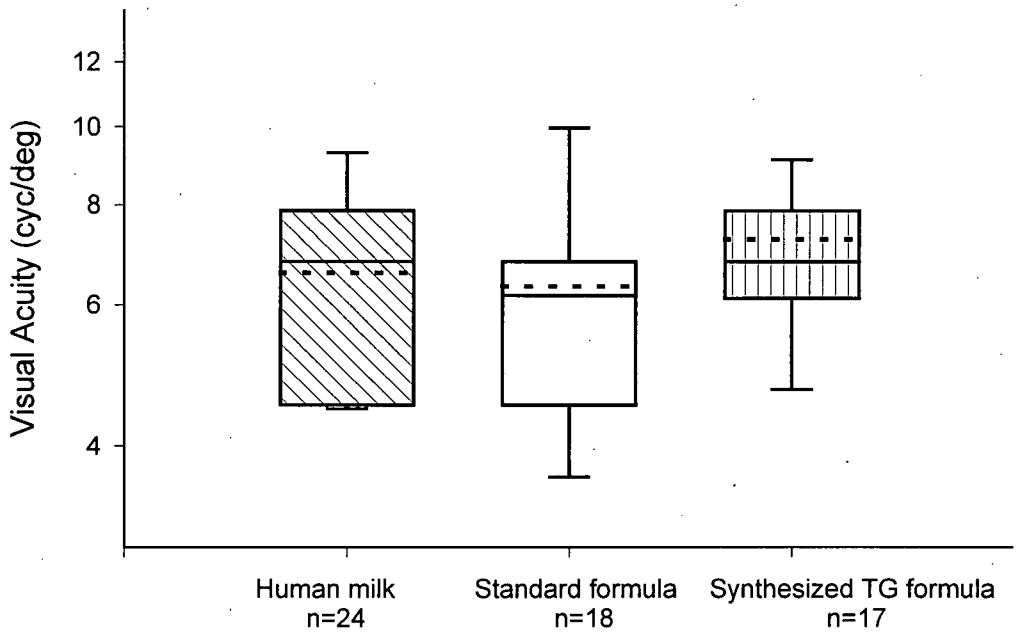

Figure 6.1 – Boxplots of term infant visual acuity at four mo of age for breast-fed infants, infants fed the standard formula and infants fed the synthesized TG formula. Visual acuity is plotted on a logarithmic (base 10) scale. In the boxplot, the dotted line represents the mean, the solid line is the median. The crosshatch bars are the 10^{th} and 90^{th} percentiles.

6.3. Study III - Influence of environment and breast-feeding *versus* formula feeding on measures of novelty preference and visual acuity in nine mo old infants

Table 6.30 *– FTII results categorized by iron status of infants at 39±1 wk of age*

Iron status	n	Mean score±SD
Iron deficient anaemia	23	62.1±5.4
Low iron status	91	60.4±6.8
Non-anaemic iron sufficient	196	60.4±6.3
Uncertain	41	59.5±7.2

The mean score represents the average test score for all infants for tests rated with high confidence within each iron status group.

6.3.1. Demographic and Diet Questionnaire

QUESTIONAIRE: Form 1

CONFIDENTIAL

NUMBER: (4 digit 0001-9,999) ☐☐☐☐

CLINIC DATE: Day ☐☐ Month ☐☐ Year ☐☐

CLINIC SITE: (2 digits) ☐☐

BABY'S BIRTH DATE: Day ☐☐ Month ☐☐ Year ☐☐

BABY'S SEX: Male ☐

Female ☐

Please Check the Appropriate Response

1. I am the baby's:

   1) Mother ☐
   2) Father ☐
   3) Relative ☐
   4) Nanny ☐
   5) Other ☐ (specify)_____

2. Baby's mothers age is: Baby's fathers age is:

1) < 20 years	☐	☐
2) 20-24 years	☐	☐
3) 25-29 years	☐	☐
4) 30-34 years	☐	☐
5) 35+ years	☐	☐

3. What is your marital status?

1) Single	☐
2) Married/Common-law	☐
3) Separated/Divorced	☐
4) Widow/Widower	☐

4. How many other children live in the household? ☐☐

	Mother Yes	Father Yes
5. Did you complete high school?	☐	☐
Did you go to college or vocational training?	☐	☐
Did you go to University?	☐	☐

6. Which of the following describes your family income per year?

1) Less than $10,000 ☐
2) $10,000 - $19,000 ☐
3) $20,000 - $29,000 ☐
4) $30,000 - $50,000 ☐
5) over $50,000 ☐

THANK YOU

QUESTIONNAIRE: Form 2
(completed with nutritionist/nurse)

NUMBER: (4 digit 0001-9,999) ☐☐☐☐

CLINIC DATE: Day ☐☐ Month ☐☐ Year ☐☐

CLINIC SITE: (2 digits) ☐☐

BABY'S BIRTH DATE: Day ☐☐ Month ☐☐ Year ☐☐

BABY'S SEX: Male ☐
Female ☐

---

1. To which ethnic background(s) do you belong?

1) Canadian	☐	12) French	☐
2) American (USA)	☐	13) Italian	☐
3) Chinese	☐	14) Dutch	☐
4) Japanese	☐	15) Scottish	☐
5) East Indian	☐	16) Ukrainian	☐
6) North American Indian	☐	17) Polish	☐
7) English	☐	18) Irish	☐
8) German	☐	19) Greek	☐
9) Vietnamese	☐	20) Jewish	☐
10) Korean	☐	21) Hispanic	☐
11) African	☐	22) other	☐ (specify)_____

1b). How many years have you lived in Canada? ☐☐

1c). Main language spoken at home?

a) English ☐

b) Other ☐ specify _____

2. Your family diet includes which of the following?

	Yes	No
1) Red meat (beef, pork)	☐	☐
2) Fish	☐	☐
3) Poultry	☐	☐
4) Dairy products	☐	☐
5) Eggs	☐	☐
6) Legumes (beans, peas)	☐	☐
7) Nuts	☐	☐
8) Fruit and vegetables	☐	☐
9) Breads/Cereals	☐	☐
10) Pasta/Rice	☐	☐

11) Special/Medical/Allergy related diet (specify)

_____

_____

3. Was the baby breast-fed?

Yes ☐ (complete Q 4 to 6)

No ☐ (go to Q 7)

4. How long was the baby **exclusively** breast-fed?
(can include fruit juices &/or up to one 8oz bottle of formula/cow's milk/wk)

☐ Months ☐ Weeks or ☐☐ Weeks

4b). How much fruit juice/day? _____

5. Is the baby still breast-feeding?

Yes ☐ **(go to Q 6)**

No ☐

5b). At what age was breast feeding completely stopped?

☐ Months ☐ Weeks or ☐☐ Weeks

6. Breast feeding was replaced/supplemented with formula/milk:

1) Infant formula (powdered/concentrate	Check for used	Age started		Changed/ stopped		Brand &/or label colour/or specify
		mths	wks	mths	wks	
a) regular formula (low iron)	☐	☐	☐	☐	☐	_____
b) formula with iron (fortified)	☐	☐	☐	☐	☐	_____
c) soybased formula	☐	☐	☐	☐	☐	_____
d) other	☐	☐	☐	☐	☐	_____
						_____
2) Cow's milk	☐	☐	☐	☐	☐	_____
3) Home made formula (evaporated milk)	☐	☐	☐	☐	☐	_____
4) other	☐	☐	☐	☐	☐	_____

Comments: _____

_____

6b). Which of the following influenced your choice to breast-feed?

1) Public health clinic or nurse's advice ☐
2) Pediatrician ☐
3) Family Doctor ☐
4) Family advice ☐
5) Friend's or other mother's advice ☐
6) Availability ☐
7) Cost ☐
8) Advertising ☐
9) Dietitian ☐
10) In hospital support ☐
11) Prenatal class ☐
12) Personal choice ☐
13) Books ☐
14) Previous experience ☐
15) Other ☐
specify _____

● **SKIP TO Q.8**

7. What type(s) of formula do you feed your infant?
NOTE: ***(baby's fed formula from birth only)***

1) Infant formula (powdered/concentrate	Check for used	Age started		Changed/ stopped		Brand &/or label colour/or specify
		mths	wks	mths	wks	
a) regular formula (low iron)	☐	☐	☐	☐	☐	_____
b) formula with iron (fortified)	☐	☐	☐	☐	☐	_____
c) soybased formula	☐	☐	☐	☐	☐	_____

d) other	☐	☐	☐	☐	☐	_____
2) Cow's milk	☐	☐	☐	☐	☐	_____
3) Home made formula (evaporated milk)	☐	☐	☐	☐	☐	_____
4) other	☐	☐	☐	☐	☐	_____

Comments: _____

_____

7b). Which of the following influenced your choice to feed formula/cow's milk?

	Formula	Cow's milk
1) Public health clinic or nurse's advice	☐	☐
2) Pediatrician	☐	☐
3) Family Doctor	☐	☐
4) Family advice	☐	☐
5) Friend or other mother's advice	☐	☐
6) Availability	☐	☐
7) Cost	☐	☐
8) Advertising	☐	☐
9) Dietitian	☐	☐
10) Used in hospital	☐	☐
11) Prenatal class	☐	☐
12) Personal choice	☐	☐
13) Books	☐	☐
14) Personal experience	☐	☐
15) Other	☐	

(specify)_____

8. ***(all infants receiving formula/cow's milk)***
Which of the following influenced your choice of formula or choice to use cow's milk ? (i.e. Why did you select a particular product)

	Formula	Cow's milk
1) Public health clinic or nurse's advice	☐	☐
2) Pediatrician	☐	☐
3) Family Doctor	☐	☐
4) Family advice	☐	☐
5) Friend or mother's advice	☐	☐
6) Availability	☐	☐
7) Infant tolerance/allergy	☐	☐
8) Cost	☐	☐
9) Advertising	☐	☐
10) Dietitian	☐	☐
11) Used in hospital	☐	☐
12) Prenatal class	☐	☐
13) Personal choice	☐	☐
14) Books	☐	☐
15) Previous experience	☐	☐
16) Other (specify)_____	☐	

9. At what age did you start to introduce cereal foods?

☐ Months ☐ Weeks or ☐ ☐ Weeks

10. What type of cereals did you first use?

	Yes	No
1) Commercial infant cereals	☐	☐
2) Cooked rice	☐	☐
3) Bread	☐	☐
4) Crackers	☐	☐
5) Breakfast cereals(hot or cold) (specify if yes)_____	☐	☐
6) Other ☐ specify _____		

11. Which of the following influenced your choice of cereal to use?

1) Baby did not like it ☐
2) Family advice ☐
3) Pediatrician ☐
4) Friend or other mother's advice ☐
5) Availability ☐
6) Cost ☐
7) Public health clinic or nurse's advice ☐
8) Books/magazines ☐
9) Advertising ☐
10) Infant tolerance (allergy) ☐
11) Family Doctor ☐
12) Previous experience ☐
13) Personal choice ☐
14) Dietitian ☐
15) Other ☐
(specify)_____

12. Does the baby eat any of the following foods? (for all **yes** response indicate at what age these foods were introduced).

	Yes	No	Age
1) fruit juices	☐	☐	_____
2) meat/beef	☐	☐	_____
3) egg yolk	☐	☐	_____
4) chicken	☐	☐	_____
5) fish	☐	☐	_____
6) vegetables	☐	☐	_____
7) fruits	☐	☐	_____
8) legumes (beans/peas/dal)	☐	☐	_____
9) tofu	☐	☐	_____

13. Does your baby currently drink cow's milk?

Yes ☐

No ☐ **go to Q14**

13b). If yes, how much cow's milk/day?

_____cup(s).

13c). What type of cow's milk?

1) Whole milk ☐

2) 2% milk ☐

3) 1% milk ☐

4) Skim ☐

14. Does the baby :

	Yes	No
1) eat from a spoon?	☐	☐
2) eat finger foods?	☐	☐

15. Do you give any vitamin or other nutritional supplements to the baby?

yes ☐ (specify) _____

no ☐

16. How many of the following prepare the baby's food? **(from 6 months)**

1) Mother	☐	5) Nanny/baby sitter	☐
2) Father	☐	6) Daycare	☐
3) Grandmother	☐	7) Other	☐
4) Grandfather	☐	specify _____	

Note: (next question to be answered by all mothers who breast fed)

17. Your choice to start using formula/cow's milk was influenced by which of the following?

1) Returned to work ☐
2) lack of support ☐
3) Illness of mother ☐
4) Illness of baby ☐
5) Concerned about baby's nutrition, adequate milk ☐
6) Uncomfortable with breast feeding ☐
7) Painful ☐
8) Baby biting the breast ☐
9) Not enough time ☐
10) Personal choice ☐
11) Other ☐
specify_____

Name of interviewer: _____