The role of docosahexaenoic acid in brain development and fetal alcohol syndrome
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The essential nature of \( n-3 \) polyunsaturated fatty acids in neurological development

Neural membrane aminophospholipids are characterized by high concentrations of \( n-3 \) polyunsaturated fatty acids (PUFA), particularly docosahexaenoic acid (C22:6n-3). For example, adult human cerebral cortex phosphatidylethanolamine (PE) contains 30.5% C22:6n-3 while C22:6n-3 in the retina accounts for 22.2% of PE fatty acids (reviewed in [1,2]). The presence of high concentrations of C22:6n-3 in neural membranes suggests an important role in the function of the central nervous system (CNS). Present understanding of the significance of \( n-3 \) PUFA in neurological development and subsequent function is derived primarily from the effects of restricted \( n-3 \) essential fatty acid (EFA) availability either due to maternal dietary deprivation in animal models, or due to preterm birth in human infants. The results of these studies indicate that optimal neurological development and subsequent function are critically dependent upon adequate accumulation of C22:6n-3 into phospholipids during specific periods of neural maturation. In humans, the major phase of C22:6n-3 accretion into the CNS is prolonged and includes both prenatal (between about 16 weeks gestation and term) and early postnatal developmental periods [3,4]. In fetal guinea pig brain, the period of maximal C22:6n-3 assimilation is associated temporally with the principal phase of neuritogenesis [5]. This suggests that accumulation of C22:6n-3 may play an important role in the development of brain structure. Feeding pregnant monkeys [6], rats and guinea pigs [7] a diet lacking \( n-3 \) PUFA resulted in significantly reduced brain and retinal PE and phosphatidylserine C22:6n-3 concentration in the offspring. The major physiological effects of

Abbreviations used: C22:6n-3, docosahexaenoic acid; PUFA, polyunsaturated fatty acids; PE, phosphatidylethanolamine; CNS, central nervous system; EFA, essential fatty acid; PC, phosphatidylcholine; FAS, fetal alcohol syndrome.

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decreased C22:6n-3 appeared to be on the visual pathway; these were characterized by impaired electoretinogram responses and decreased visual acuity [5,6]. However, it is difficult from these analyses to define the absolute effects of n-3 EFA deficiency on the retina and associated brain functions. In addition, these studies have indicated that C22:6n-3 accumulation into the CNS is restricted to a limited period of development, after which deficits in C22:6n-3 assimilation cannot be reversed [8]. Comparable changes to visual function and decreased erythrocyte PE C22:6n-3 content have been reported in human infants whose placental transfer of n-3 PUFA had been interrupted by preterm birth and who had then been maintained on a diet lacking n-3 PUFA [9,10]. The effect of decreased availability of C22:6n-3 on brain development and subsequent function remains controversial. Monkeys deprived of n-3 PUFA both pre- and/or post-natally, but not prenatally alone, showed marked polydipsia suggesting altered neurological function associated with a substantial deficit in C22:6n-3 accretion [11]. Since development of the CNS in humans is a complex, prolonged process with potential compensatory mechanisms, it is perhaps not surprising that understanding of the effects of n-3 PUFA deprivation on intellectual function in humans is limited. Impaired C22:6n-3 assimilation has also been implicated causally, but has not been substantiated, in both decreased IQ at 8 years of age in children born preterm [12] and persistence of neonatal neurological dysfunction in late childhood [13]. Furthermore, a number of neuropathologies exhibit altered phospholipid fatty acid composition. Brain phospholipids from patients with neurodegenerative diseases including Zellweger's syndrome [14] and 'kinky hair disease' [15] show decreased C22:6n-3 concentrations. These studies support the view that C22:6n-3 is important for normal brain function, although it is uncertain whether this alteration to phospholipid composition is a primary or secondary effect.

The function of C22:6n-3 in neural membranes is not clear. In cell membranes, C22:6n-3 is associated primarily with phospholipids, linked by an acyl, alkyl or alkenyl bond at the sn-2 position in combination with another fatty acid at the sn-1 position, usually saturated (palmitate; C16:0, or stearate; C18:0) or monounsaturated (oleate; C18:1n-9). Highly unsaturated diC22:6n-3 PE is also found in the retina, and to a lesser extent in the brain. The combination of fatty acids and linkage to glycerol, together with the type of hydrophilic head group, determines the net biophysical properties of C22:6n-3 in the membrane. Therefore, in order to understand the functional consequences of changes the C22:6n-3 content of membrane bilayers, it is necessary to consider the biology of this fatty acid in the context of whole phospholipid molecular species rather than in isolation.

No clear mechanism has been identified by which the C22:6n-3 content of membrane phospholipids may modify neural development and function, and it is likely that more than one process is involved. Many biophysical parameters of cell membranes may be modified by C22:6n-3 content. Analysis of the effect of C22:6n-3 on the packing of phospholipid molecules in bilayers indicates that increasing the proportion of C22:6n-3 increased the fluidity of the membrane [16]. One possible effect of high C22:6n-3 in nerve cells may be to facilitate changes in membrane structure such as membrane thinning associated with variations in membrane potential [16]. Alterations to membrane C22:6n-3 content, therefore, may modify the capacity of a nerve cell for electrical conduction. Cross-linking experiments have indicated preferential association of membrane proteins with phosphatidylserine 18:0/22:6 [16]. This suggests that the function of these proteins may be dependent upon a precise physical environment. Phospholipids are key substrates for phospholipase-mediated signal transduction processes which produce diacylglycerol and phosphatidic acid by the respective activities of phospholipases C and D. The molecular composition of diacylglycerol and phosphatidic acid second messenger pools appears to be an important factor in the extent of protein kinase C activation [17]. It is possible, therefore, that the composition of phospholipid substrate pools may modulate cellular responses to agonists. In this context, membrane C22:6n-3 concentration may be crucial in determining the activities of neural signalling events such as responses to neurotrophic agents or neurotransmitters.

The effect of brain maturation upon membrane phospholipid composition

Since the molecular composition of phospholipids is probably one critical factor in determining the biophysical properties of cell membranes, the effect of increasing gestational age upon the molecular species content of developing guinea pig brain phosphatidylcholine (PC) and PE has
been studied. Fetuses (n = 6 per gestational age) were delivered from timed-pregnant guinea pigs at 25, 35, 40 and 68 (term) days gestation. The major brain phospholipid classes PC and PE were isolated from total lipid extracts [18] of fetal brain by solid-phase extraction using 100 mg aminopropyl silica cartridges [19,20]. PC and PE molecular species compositions were determined by reversed-phase HPLC and quantified by post-column derivatization with 1,6-diphenyl-1,3,5-hexatriene with on-line fluorescence detection [21,22]. PC contained mainly saturated or monounsaturated molecular species, principally PC16:0/16:0 and PC16:0/18:1 which approximately doubled in concentration between day 25 and term, together with PC16:0/18:0 and PC18:0/18:1 which increased in late gestation [22]. This was consistent with reports of the fatty acid content of human [23] and rat [24] brain PC. In contrast, PE was composed almost entirely of PUFA-containing species. Analysis of brain PE content showed that during early gestation (days 25–40) C22:6n-3 was accumulated principally into sn-1 C16:0 species, while assimilation into sn-1 C18:0 and C18:1 species occurred mainly in late (days 40–68) gestation (Figure 1). This implies that C22:6n-3 assimilation into brain PE is closely regulated during development and may reflect changes to the specificity of PE synthesis. Information about the precise biochemical function of individual phospholipid molecular species is limited. However, in both rat [25] and fetal guinea pig [26] liver PE16:0/22:6 is turned over more rapidly than PE18:0/22:6, which suggests that sn-1 C18:0 species may represent a more metabolically stable pool. Since neurite formation in fetal guinea pig brain occurs principally between day 25 and day 40 [5,27], initial accumulation of C22:6n-3 into a short-lived pool during neurite outgrowth may support continual changes in cell structure, while subsequent assimilation into a more stable pool may confer some resistance to C22:6n-3 depletion once inter-neuronal connections have been established.

**The role of docosahexaenoic acid in fetal alcohol syndrome**

Chronic maternal ethanol consumption during pregnancy may result in severe developmental abnormalities: fetal alcohol syndrome (FAS). In particular, infants born with FAS show abnormal neural function including impaired intelligence and motor function, and hyperactivity [28–31].

![Figure 1](image-url)

**Figure 1**

Composition of C22:6n-3-containing PE molecular species in developing fetal guinea pig brain

Values are mean ± S.D. concentrations of fetal brain PE molecular species (n = 6 fetuses per gestational age). *Indicates values which differed significantly from gestational age 25 days (P < 0.05). The peak eluted from the HPLC corresponding to PE18:0/22:6# contained both PE18:0P/22:6 and PE18:0P/22:4.

A, sn-1 alkyl-linked fatty acids; P, sn-1 alkenyl-linked fatty acids.
The biochemical mechanisms by which prenatal ethanol exposure results in damage to the CNS are unknown. Since adequate assimilation of C22:6n-3 in neural tissues appears to be crucial for subsequent optimal function, one hypothesis to account for the harmful effects of alcohol on fetal brain development is that ethanol impairs the accumulation of C22:6n-3 into brain phospholipids. A number of studies have investigated the effects of ethanol exposure on adult brain phospholipid composition using adult laboratory animals; these have demonstrated either decreased C22:6n-3 content [32–35] or no effect [36–38]. One possible explanation for these observations is inconsistencies in the method of ethanol administration either as liquid diet [32,36–38], in drinking water [39] or by inhalation [33–35]. The effects of ethanol on brain development have also been studied, which produced conflicting results also, ranging from decreased C22:6n-3 content [40], to increased C22:6n-3 concentration [41] or no effect [42]. One consideration is the choice of animal model. Rats and mice are probably inappropriate models of prenatal ethanol exposure because the majority of brain development occurs during the early postnatal period. As brain development in the guinea pig is almost complete at birth [5,27], and placental fatty acid transport is comparable with that in humans [43], the fetal guinea pig has been used as a model of human prenatal ethanol exposure. Adult female guinea pigs were fed 6 g/kg ethanol/water (1:1 v/v) per day as two bolus doses for 14 days before mating and throughout pregnancy. Fetuses were delivered by Caesarian section at term [44]. HPLC analysis of PC molecular species showed increased concentrations of PC16:0/16:0, PC16:0/18:0 and PC18:0/18:1 accompanied by decreased content of C22:6n-3-containing PC species in ethanol-exposed fetal brains (n = 6 fetuses per group) [44]. The effects of ethanol exposure on brain PE were more dramatic. All species containing C22:6n-3 and C20:4n-4 were decreased in ethanol-exposed brain: PE18:0-alkyl/22:6-acyl was completely absent, and there was appearance of PE16:0/18:1 (Figure 2). The ratio of total PC to PE was also increased in these fetuses indicating changes to phospholipid biosynthesis and/or turnover. These results demonstrate that ethanol exposure induced profound changes to fetal guinea pig brain phospholipid composition. However, the differential changes to the concentrations of individual molecular species suggest that impaired supply of C22:6n-3 to the fetus was probably not a major mechanism for the adverse effects of ethanol. This was supported by analysis of maternal guinea pig liver and plasma PC compositions which indicated that C22:6n-3 supply to the fetus may have been spared from

### Figure 2

**PE molecular species composition of control and ethanol-exposed term fetal guinea pig brain**

Values are mean± S.D. concentrations of term brain PE molecular species (n = six fetuses per dietary group). *Indicates values which differed significantly from controls (P <0.01).

<table>
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<tr>
<th>PE molecular species</th>
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<tbody>
<tr>
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Concentration (nmol/g)

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the adverse effects of ethanol on other molecular species [45]. One possible explanation for reduced C22:6n-3 content in fetal guinea pig brain, therefore, is changes in the specificity of PE synthesis indicated by the appearance of PE16:0/18:1, and/or increased turnover of C22:6n-3-containing species. Preliminary neurological assessment of neonatal guinea pigs exposed to ethanol showed absence of righting reflex and marked hind limb paresis which suggests that such changes to brain phospholipid composition may be linked causally to abnormal neural function.

**Effects of C22:6n-3 supplementation on the phospholipid composition of ethanol-exposed fetal guinea pig brain**

The observation that ethanol exposure decreased fetal brain C22:6n-3 content presented the possibility that the severity of such effects could be reduced by increased C22:6n-3 availability to the fetus. Adult female guinea pigs were fed either chow, or chow plus ethanol (6 g/kg per day), ethanol and tuna oil (0.5 g/day) or tuna oil alone. The tuna oil (Callanish Ltd, Isle of Lewis, Scotland) was enriched to 26% C22:6n-3 and 0.5 g provided 130 mg C22:6n-3/day [46]. Animals (n = 5 per group) were fed these diets for 14 days before mating and throughout pregnancy. Fetuses (mean data from two fetuses/pregnancy were used for statistical analysis) were delivered at term as before and the fractional concentrations of fatty acid methyl esters prepared from brain PC and PE were determined by gas chromatography [46]. Prenatal ethanol exposure decreased total brain PC C22:6n-3 content by 56.7%. However, feeding mothers both ethanol and tuna oil resulted in increased C22:6n-3 concentration (66.7%) compared with the control, and feeding tuna oil alone did not change brain PC C22:6n-3 content [46]. Similarly, total brain PE C22:6n-3 was decreased (26.6%) in ethanol-exposed fetuses, but was significantly greater than in the controls (40.2% in offspring of mothers) fed both ethanol and tuna oil (Figure 3). Again, tuna oil feeding alone did not significantly alter brain PE C22:6n-3 content (Figure 3). Furthermore, although ethanol exposure increased the ratio of PC/PE in the brain compared with the control (36.9%), the relative amounts of brain PC and PE in fetuses from mothers fed either tuna oil and ethanol or tuna oil alone did not differ significantly from controls [46]. These data indicate that increasing maternal dietary C22:6n-3 intake was able to abolish both the ethanol-induced deficit in fetal brain C22:6n-3 accumulation and the abnormally high...
brain PC/PE ratio associated with ethanol-exposure. Preliminary neurological assessment of neonatal pups showed that these tuna oil feeding-related changes to brain phospholipid composition were accompanied by demonstrable righting reflex and some reduction in the extent of hind limb paresis.

Together, these results suggest that alterations to the metabolism of PC and PE C22:6n-3 represent an important mechanism for the harmful effects of ethanol upon fetal brain development. The precise metabolic changes induced by ethanol exposure have not been characterized. It is possible that increased turnover and degradation of C22:6n-3, possibly due to oxidation, may explain partly the ethanol-induced decrease in brain C22:6n-3 content. However, the complex changes to individual PC and PE molecular species composition and the 'over incorporation' of C22:6n-3 in the ethanol- and tuna-oil-fed group suggest that alterations to the specificity of phospholipid synthesis are probably also important in determining the overall effects of ethanol on neural cell membrane phospholipid composition. Recent studies in guinea pigs have indicated that increased retinal C22:6n-3 content above optimal levels resulted in decreased retinal activity [47]. One implication, therefore, is that over accumulation of C22:6n-3 into fetal brain PC and PE may also be harmful and could contribute to persistence of impaired motor function.

The observation that maternal supplementation with C22:6n-3-enriched tuna oil prevented ethanol-induced decreases in C22:6n-3 content and partially ameliorated the harmful effects on motor function provides the possibility of a nutritional intervention to reduce the adverse effects of ethanol in human FAS. Although maternal supplementation may be difficult, the prolonged period during which the human brain develops after birth may allow evaluation of C22:6n-3 supplementation in the postnatal period.

Do long-chain polyunsaturated fatty acids influence infant cognitive behaviour?

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Observational studies have indicated that children who were breast-fed are advantaged in cognitive and intellectual development compared with children who were fed formula milk [1–3]. The mechanisms which may underlie this advantage remain uncertain. Various constituents of human milk including hormones, growth promoting factors and nutrients, have been postulated as factors which may positively influence neural development. Currently, one hypothesis involves the role of long-chain polyunsaturated fatty acids (LCPUFA), especially arachidonic acid (AA) and docosahexaenoic acid (DHA) which are preferentially accreted by the infant brain during the last trimester of pregnancy and the first months of life; due to their contribution to the structure and function of cellular membranes they are considered to influence vital physiological and metabolic processes during infancy and beyond [4,5].

Abbreviations used: LCPUFA, long-chain polyunsaturated fatty acids; AA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

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Breast milk contains LCPUFA in sufficient amounts to meet infant requirements for tissue accretion, even if the infant is born prematurely [6]. However, in contrast with human milk, commercial infant formulas have not been supplemented with LCPUFA because it was assumed that infants would be able to synthesize LCPUFA from precursor fatty acids [7]. Several studies have now reported that term, and particularly preterm, infants who are fed formulas containing linoleic acid and α-linolenic acid but devoid of LCPUFA, develop a relative LCPUFA depletion in structural lipids, as measured by plasma and erythrocyte membrane concentrations [8–11].

Studies assessing the relationship between brain fatty acids and infant diet have demonstrated that breast-fed infants have higher concentrations of DHA in their cerebral cortex compared with infants fed formula milk [10,12]. Furthermore, the accumulation of DHA in the cerebral cortex is dependent on the duration of breast feeding [10]. In contrast there were no differences in the level of cortical AA between breast- and formula-fed infants, suggesting that