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Fetal brain and liver phospholipid fatty acid composition in a guinea pig model of fetal alcohol syndrome: Effect of maternal supplementation with tuna oil

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Chronic prenatal ethanol exposure may impair neurological development and function severely. We have shown previously that feeding ethanol to guinea pigs throughout pregnancy leads to decreased docosahexaenoic acid 22:6ω3) content in brain phospholipids, accompanied by impaired motor function comparable to some features of human fetal alcohol syndrome. We have tested the hypothesis that dietary supplementation of pregnant guinea pigs with 22:6ω3-enriched tuna oil may reduce the ethanol-induced deficit in 22:6ω3 accumulation into fetal brain. Guinea pigs (n = 5/group) were maintained on chow diet either alone or supplemented with 6 gm ethanol/kg/day, ethanol, and tuna oil (0.5 gm/day; 130 mg 22:6ω3/day), or tuna oil alone both before and throughout pregnancy. Fetuses were assessed at term for brain and liver phospholipid fatty acid composition. Prenatal ethanol exposure significantly decreased fetal brain phosphatidylcholine (PC) and phosphatidylethanolamine (PE) 22:6ω3 contents and increased the PC/PE ratio in fetal brain. Feeding both tuna oil and ethanol increased brain PC and PE 22:6ω3 content above control values, whereas the PC/PE ratio was similar to control fetuses. Feeding tuna oil alone did not alter significantly the polyunsaturated content of fetal brain phospholipids. These feeding regimens induced markedly different changes to fetal liver phospholipid compositions compared with brain, which suggests that the effects of ethanol and/or tuna oil were tissue-specific. These results show that increased 22:6ω3 availability modified the effect of ethanol on developing fetal brain phospholipid composition. Such alterations to brain fatty acid content may potentially reduce some aspects of the harmful effects of maternal ethanol consumption on prenatal neurological development. (J. Nutr. Biochem. 8:438–444, 1997) © *Elsevier Science Inc. 1997*

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Introduction

Chronic ethanol consumption during pregnancy may result in severe irreversible impairment of neurological development and function; fetal alcohol syndrome (FAS).[1,2] The developing central nervous system is particularly sensitive to ethanol exposure such that even moderate ethanol intake by pregnant women has been associated with reduced intelligence in children at 4 years.[3] Infants born to mothers who chronically consume large quantities of ethanol during gestation show severely impaired intelligence, hyperactivity, low attention span, and abnormal motor function.[1,2,4,5] At present the only therapeutic approaches to reducing the severity of FAS are abstinence from ethanol consumption or

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a substantial decrease in alcohol intake. However, in pregnant women with alcoholism, the addiction frequently means that it is impossible to reduce ethanol intake successfully and it is conceivable that side effects of alcohol withdrawal could have adverse effects on the pregnancy.

The biochemical mechanism by which ethanol exposure leads to impaired neurological development in the fetus is unclear. Whereas ethanol exerts direct toxic effects on neural tissue, some of its harmful actions in development may be mediated by alterations to nutrition and metabolism in utero. During normal development of the fetal nervous system, there is a specific program of modifications to membrane phospholipid molecular species compositions that are related temporally to the maturation of cellular function.[6] In this context, adequate accumulation of docosahexaenoic acid (22:6ω3) into aminophospholipids of the developing brain and retina is critical for optimal development and function. Failure to incorporate sufficient 22:6ω3 into neural membranes in utero as a consequence of preterm delivery has been implicated both in impaired retinal function in neonates[7] and in reduced intelligence in children at 8 years of age.[9] Consequently, it is possible that decreased accumulation of 22:6ω3 into fetal brain may be one contributory mechanism to the pathogenesis of FAS.

We have described previously a guinea pig model for evaluating the interaction between lipid nutrition and ethanol consumption on parameters of brain composition and function.[10] The guinea pig is a useful model for the study of the effects of lipid nutrition on prenatal human brain development, because for both animal species 22:6ω3 accumulation into developing neural tissue occurs principally before birth,[11–13] and kinetics of placental fatty acid transport are equivalent.[14] In this model, oral administration of ethanol to guinea pigs before and throughout pregnancy significantly reduced accumulation of 22:6ω3 into defined molecular species of fetal brain PC and PE.[10] Ethanol feeding also decreased brain mass at 40/68 days gestation (term = 68 days) and increased the relative concentration of PC to PE in fetal brain at term. These biochemical changes were consistent with abnormal cellular differentiation and were associated with marked abnormalities in motor function in newborn guinea pig pups.

These previous results are consistent with the hypothesis that failure to accumulate sufficient 22:6ω3 into neuronal phospholipid may be one important mechanism in ethanol-induced impairment of fetal brain function. In the present study we have used this guinea pig model of FAS to test the hypothesis that maternal dietary supplementation with 22:6ω3-enriched tuna oil may reduce the negative effect of ethanol on 22:6ω3 accumulation into fetal brain phospholipids.

Methods and materials

Materials

High performance liquid chromatography (HPLC) grade methanol was obtained from Rathburn, Ltd. (Walkerburn, Scotland). All other solvents were from Merck, Ltd. (Poole, Dorset, UK). All other reagents were purchased from Sigma Chemical Co. (Poole, Dorset, UK).

Table 1 Fatty acid composition of chow and tuna oil

Fatty acid	Chow*	Tuna oil*
14:0	6.0	3.1
16:0	13.1	17.9
18:0	2.8	5.0
16:1ω7	6.4	5.6
18:1ω9	24.7	16.7
18:2ω6	23.7	2.3
18:3ω3	17.0	0.0
20:4ω6	6.7	0.0
20:5ω3	0.0	6.0
22:6ω3	0.0	26.1

*Analyses provided by manufacturers.

Animal procedures

Virgin female Dunkin-Hartley guinea pigs (body mass about 700 gm) from our own colony were randomized to control and three experimental dietary groups (n = 5 adults/group). All animals were fed a nutritionally complete chow diet (FD1, Special Diet Services, Witham, Essex, UK)[12] and had free access to water. The composition of the chow diet is described in *Table 1*. One group were fed ethanol alone (6 g ethanol (50% v/v)/kg body mass/day; equivalent to 42 kcal/kg/day) in two bolus doses,[10] which produced similar blood ethanol concentrations 30 min after oral dose in both the ethanol- (258 ± 32 mg/100 mL) and ethanol and tuna oil- (266 ± 23 mg/100 mL) fed mothers. The ethanol dose used was approximately equivalent to human consumption of 22U (176 g)/day. This ethanol feeding regimen reproducibly produced significant alterations to parameters of fetal guinea pig brain development.[10] A second group were fed ethanol (6 g/kg/day) combined with tuna oil (0.5 g/day). The third experimental group were fed tuna oil (0.5 g/day) alone. The tuna oil contained 97% triacylglycerol composed of (w/w) 26.1% 22:6ω3, 6.0% eicosapentaenoate acid (20:5ω3), 2.3% linoleate (18:2ω6), and the remainder were monounsaturates and saturates (*Table 1*). This ethanol feeding regimen provided approximately 130 mg 22:6ω3/day with a 20:5ω3/22:6ω3 ratio of 0.2. Maternal energy intakes were similar for control mothers and those fed tuna oil alone, but were significantly greater ($P < 0.05$) for animals fed ethanol either alone (54.5% increase) or in combination with tuna oil (65.5% increase) (*Table 2*). Animals were maintained on these diets for 14 days before mating and throughout pregnancy.

Fetal guinea pigs were delivered at 68 days gestation (term) by Caesarian section and sacrificed by intraperitoneal injection of pentobarbitone.[10] There was no incidence of spontaneous abortion in any of the dietary groups. Two fetuses were selected at random from each pregnancy. Litters all contained either two or three pups.

Table 2 Energy consumption

Feeding group	Energy/day (kcal) (n = 5/group)
Chow only	55 ± 10
Chow + ethanol	85 ± 9*
Chow + ethanol + tuna oil	91 ± 13*
Chow + tuna oil	57 ± 17

The calorie content of the chow diet was based upon the manufacturers estimate of available metabolisable energy.

Table 3 Neonatal guinea pig brain, liver, and carcass weights

	Brain (g)	Liver (g)	Carcass (g)
Control (n = 5)	2.2 ± 0.2	4.6 ± 1.1	85.1 ± 13.8
Ethanol (n = 5)	2.4 ± 0.7	4.5 ± 2.3	84.7 ± 9.7
Ethanol & tuna oil (n = 5)	2.6 ± 0.6	4.8 ± 1.5	87.3 ± 14.9
Tuna oil (n = 5)	2.2 ± 0.2	4.5 ± 1.2	83.2 ± 11.2

Values are mean ± SD. There were no significant differences in tissue and carcass weights between maternal feeding regimens.

Analysis of phospholipid fatty acid composition

Fetal brain and liver were removed immediately and frozen in liquid N_2. Tissues (about 100 mg) were homogenized in 0.9% (w/v) NaCl and extracted with chloroform/methanol (1:1 v/v).[15] The organic phase was dried under N_2 at 40°C, dissolved in 1 mL chloroform and applied to disposable 100 mg aminopropyl cartridges (Jones Chromatography, Hengoed, Glamorgan, UK). PC and PE were isolated from the organic phase by sequential elution with chloroform/methanol (60:40, v/v) and methanol, respectively,[16] and dried under N_2 at 40°C. The fatty acid composition of each phospholipid class was determined by preparation of methyl esters using sodium methoxide. Briefly, purified PC and PE were dissolved in chloroform/methanol (2:1, v/v) (100 μL) and 20 μL transferred to an amber vial. Sodium methoxide (150 mM in methanol) (20 μL) was added, the tube flushed with N_2, sealed, and incubated at room temperature for 30 min. The reaction was terminated by addition of acetylchloride in methanol (1:19, v/v) (20 μL). Methyl esters were isolated by extraction with hexane, stored at −20°C, and analyzed within 5 hr. Fatty acid methyl esters were resolved by gas chromatography using a fused silica capillary column (30m × 0.25 mm, DB225; Jones Chromatography) and detected by flame ionisation.[12] Peak areas were determined by on-line integration. Methyl esters were identified by comparison of retention time relative to known standards.

Analysis of the phospholipid classes

Relative concentrations of PC and PE in fetal brain were measured by normal phase HPLC combined with fluorescence detection.[17] Briefly, the organic phases of total lipid extracts[15] of homogenized fetal brain and liver (about 100 mg) were dried under nitrogen at 40°C. PC and PE were resolved on a 25 cm × 4.6 mm aminopropylsilica column (Jones Chromatography) at 50°C using acetonitrile/methanol/water (1460:500:30, v/v/v), containing 151 mg/L^{-1} methylphosphonic acid pH 6.3, as the mobile phase at a flow rate of 1 mL/min^{-1}. The mass of eluted phospholipids was determined by postcolumn derivatization with 1,6-diphenyl-1,3,5-hexatriene followed by fluorescence detection at excitation wavelength λ = 340nm and emission wavelength λ = 460 nm.[18] Phospholipid classes were identified by comparison with retention times of standards.

Statistical analysis

Statistical analysis was carried out by one-way analysis of variance using the Bonferroni post-test correction for multiple comparisons between experimental groups. For each pregnancy, the mean values for two fetuses were calculated such that one pregnancy represented n = 1 for statistical comparisons between dietary groups.

Results

Fetal growth

Whole carcass weights were determined at birth, and brain and liver wet weights were measured immediately after removal. There were no significant differences in these parameters between maternal feeding regimens (*Table 3*).

Fatty acid composition of neonatal brain phospholipids

The three experimental diets caused significant changes to fetal brain phospholipid fatty acid compositions, which differed considerably between PC (*Table 4*) and PE (*Table 5*). Ethanol feeding had a relatively modest effect on neonatal brain PC; contents of both stearic (18:0, 33.9%) and 22:6ω3 (56.7%) were decreased, but those of all other fatty acids remained unchanged (*Table 4*). Feeding tuna oil in combination with ethanol restored the deficit in 18:0, and increased the 22:6ω6 content significantly above that of

Table 4 Fetal guinea pig brain phosphatidylcholine composition

	Fatty acid content (%)			
		Phosphatidylcholine		
Fatty acid	Control (n = 5)	Ethanol (n = 5)	Ethanol & tuna (n = 5)	Tuna (n = 5)
14:0	2.2 ± 1.0	2.1 ± 1.2	2.1 ± 0.2	2.4 ± 0.9
16:0	59.8 ± 2.4	63.6 ± 5.2	54.1 ± 2.4	58.5 ± 6.6
18:0	12.4 ± 3.7	8.2 ± 1.6†	13.4 ± 1.7‡	9.8 ± 2.9
16:1ω7	1.2 ± 1.2	2.3 ± 1.1	1.6 ± 0.7	2.4 ± 0.9
18:1ω9	17.8 ± 7.7	18.8 ± 3.0	18.6 ± 1.5	17.4 ± 4.3
18:2ω6	1.2 ± 0.5	0.9 ± 0.3	1.3 ± 0.5	1.3 ± 1.1
20:3ω9	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1†, ‡, *
20:4ω6	2.4 ± 0.8	2.8 ± 0.7	3.9 ± 0.5	4.4 ± 1.4†, ‡
20:5ω3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1†, ‡, *
22:5ω6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.1†, ‡, *
22:6ω3	3.0 ± 0.8	1.3 ± 0.3†	5.0 ± 0.5†, ‡	3.1 ± 1.5‡

Values are mean ± SD (n = number of pregnancies) fractional concentrations of individual fatty acids in brain phospholipids from term fetal guinea pigs. †, ‡ and *indicate values that were significantly different ($P < 0.01$) from control, ethanol-exposed, and ethanol-exposed with tuna oil supplementation groups, respectively.

Table 5 Fetal guinea pig brain phosphatidylethanolamine composition

Fatty acid	Fatty acid content (%)			
	Phosphatidylethanolamine			
	Control (n = 5)	Ethanol (n = 5)	Ethanol & tuna (n = 5)	Tuna (n = 5)
14:0	0.6 ± 0.1	1.2 ± 0.4†	0.3 ± 0.1‡	0.9 ± 0.2†, *
16:0	21.1 ± 5.7	24.6 ± 4.4	17.6 ± 1.6†, ‡	26.5 ± 2.2*
18:0	37.8 ± 5.2	27.9 ± 8.0†	25.0 ± 1.5†	27.7 ± 1.2†
16:1ω7	0.6 ± 0.2	1.9 ± 1.1†	0.2 ± 0.1†, ‡	0.8 ± 0.1†, ‡
18:1ω9	10.9 ± 3.9	12.5 ± 1.2†	13.2 ± 2.4†	10.8 ± 0.4‡
18:2ω6	0.8 ± 0.2	1.1 ± 0.5	1.3 ± 1.2	0.8 ± 0.1
18:3ω3	0.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
18:3ω6	0.0 ± 0.2	0.3 ± 0.5	0.7 ± 0.2†	0.0 ± 0.0
20:3ω9	0.0 ± 0.0	1.6 ± 3.4	0.3 ± 0.1	0.0 ± 0.0
20:4ω6	9.8 ± 1.4	8.5 ± 1.7	11.4 ± 1.7	11.4 ± 2.9
20:5ω3	0.0 ± 0.0	2.8 ± 0.4†	1.9 ± 0.4†	0.3 ± 0.1†, ‡, *
22:5ω6	0.0 ± 0.0	4.1 ± 1.5†	2.3 ± 1.0†, ‡	2.9 ± 0.6†, ‡
22:6ω3	18.4 ± 1.9	13.5 ± 2.1†	25.8 ± 1.5†, ‡	17.9 ± 5.9‡, *

Values are mean ± SD (n = number of pregnancies) fractional concentrations of individual fatty acids in brain phospholipids from term fetal guinea pigs. †, ‡ and *indicate values that were significantly different ($P < 0.01$) from control, ethanol-exposed, and ethanol-exposed with tuna oil supplementation groups, respectively.

both control (66.7%) and of ethanol-exposed (284.6%) neonates (*Table 4*). Feeding tuna oil alone did not produce major alterations to neonatal brain PC, with the exception of arachidonic acid, which was elevated by 83.3% compared with control pups (*Table 4*).

Fetal guinea pig brain PE was characterized by a substantially greater content of polyunsaturated fatty acids (*Table 5*) compared with brain PC (*Table 4*). Feeding pregnant guinea pigs ethanol and/or tuna oil produced marked changes to the fatty acid content of neonatal brain PE (*Table 5*), which differed from those that occurred in the equivalent PC fractions (*Table 4*). Ethanol exposure again significantly decreased brain PE 18:0 (26.2%) and 22:6ω3 (26.6%) contents, but was associated with increased fractional concentrations of 14:0 (50.0%), 16:1ω7 (216.7%), 18:1ω9 (14.7%), 20:5ω3, and 22:5ω6 (*Table 5*). Although the fractional concentrations of the mead acid (20:3ω9) and γ-linolenate (18:3ω6) were increased in brain PE of ethanol-exposed neonates, this failed to reach statistical significance because of the wide variation in the contents of these fatty acids (*Table 5*). Fetal brain PE 20:4ω6 content was not altered significantly by ethanol exposure (*Table 5*).

Feeding pregnant guinea pigs tuna oil simultaneously with ethanol resulted in an increase in the fractional concentration of 22:6ω3 compared with both controls (40.2%) and with ethanol-exposed (91.1%) neonates (*Table 5*). In addition, fetal brain PE from mothers fed both ethanol and tuna oil also showed increased contents of 18:1ω9 (21.1%), 18:3ω6, 20:5ω3, and 22:5ω6 and decreased amounts of 18:0 (33.9%) compared with controls (*Table 5*).

Comparison of fetal brain PE between the ethanol and tuna oil and the ethanol alone feeding groups showed decreased contents of 14:0 (75.0%), 16:0 (28.5%), 16:1ω7 (89.5%) and 22:5ω6 (43.9%), although only 14:0 reached a level similar to controls (*Table 5*). Feeding tuna oil alone did not significantly alter the 22:6ω3 content of neonatal brain PE compared with controls (*Table 5*). However, this feeding regimen resulted in decreased 18:0 (39.9%) concentration compared with controls. Both brain PE 20:5ω3 and 22:5ω6 concentrations were elevated in neonates born to mothers fed tuna oil alone compared with controls, but were significantly lower than the offspring of mothers fed ethanol (20:5ω3, 89.3%; 22:5ω6, 29.3%). Brain PE 20:5ω3 concentration was less (84.2%) in the group fed tuna oil alone compared with pups in the ethanol and tuna oil feeding group (*Table 5*).

Neonatal brain phosphatidylcholine and phosphatidylethanolamine contents

Prenatal ethanol exposure significantly ($P < 0.01$) increased (36.9%) the ratio of PC/PE in fetal guinea pig brain (2.98 ± 0.51) compared with controls (1.88 ± 0.20). However, brain PC/PE ratios of fetuses from mothers fed either ethanol and tuna oil (1.99 ± 0.13) or tuna oil alone (1.66 ± 0.97) were not significantly different from controls, but were significantly lower than pups from the ethanol-alone group.

Neonatal liver phospholipid composition

Prenatal ethanol exposure had considerably less effect on the composition of neonatal liver PC composition than on neonatal brain PC, only changing the contribution of minor components 18:3ω3 and 22:5ω3 (*Table 6*). In contrast, feeding tuna oil in pregnancy exerted considerable effects on neonatal liver PC composition, which were essentially comparable whether administered in combination with ethanol or alone. These changes were characterized by a significant reduction to 18:2ω6 (29.6% and 14.4%), and significant increases to 18:0 (30.3% and 27.9%) and 22:6ω3 (107.0% and 95.3%). Additionally, in animals fed both tuna and ethanol, the content of 18:1ω9 (39.5%) in liver PC was also decreased compared with control neonates.

Changes to neonatal liver PE composition were more extensive, both in response to ethanol and to tuna oil

Table 6 Fetal guinea pig liver phosphatidylcholine composition

Fatty acid	Fatty acid content (%)			
		Phosphatidylcholine		
	Control (n = 5)	Ethanol (n = 5)	Ethanol & tuna (n = 5)	Tuna (n = 5)
16:0	30.3 ± 1.4	29.9 ± 1.4	28.6 ± 4.5	29.6 ± 2.6
18:0	21.8 ± 2.1	22.2 ± 1.1	28.4 ± 4.9†	25.3 ± 1.6†, ‡
16:1ω7	0.6 ± 0.4	0.6 ± 0.1	1.2 ± 1.9	0.4 ± 0.1
18:1ω9	12.4 ± 1.7	10.7 ± 0.8	7.5 ± 1.9†, ‡	9.5 ± 0.8
18:2ω6	25.7 ± 1.6	27.5 ± 1.2	18.1 ± 2.4†, ‡	22.0 ± 2.3†, ‡, *
18:3ω3	0.0 ± 0.0	1.4 ± 0.3†	0.5 ± 1.3†, ‡	0.0 ± 0.0‡, *
18:3ω6	0.0 ± 0.0	0.1 ± 0.1	0.2 ± 0.4	0.8 ± 0.0
20:3ω9	0.8 ± 0.6	0.8 ± 1.2	0.3 ± 0.5	0.0 ± 0.0†
20:4ω6	3.6 ± 0.4	2.4 ± 0.7	3.7 ± 0.6	3.7 ± 0.9
20:5ω3	0.5 ± 0.3	0.4 ± 0.2	2.3 ± 3.3	0.2 ± 0.2
22:5ω6	0.0 ± 0.0	0.6 ± 0.4†	0.3 ± 0.2	0.3 ± 0.1
22:6ω3	4.3 ± 0.7	3.1 ± 0.9	8.9 ± 2.1†, ‡	8.4 ± 2.1†, ‡

Values are mean ± SD (n = number of pregnancies) fractional concentrations of individual fatty acids in brain phospholipids from term fetal guinea pigs. †, ‡ and *indicate values that were significantly different ($P < 0.01$) from control, ethanol-exposed, and ethanol-exposed with tuna oil supplementation animals, respectively.

administration (*Table 7*). Ethanol significantly decreased the content of 22:6ω3 (38.2%) and increased those of the ω6 fatty acids 18:3ω6, 20:4ω6 (26.6%) and 22:5ω6 (350.0%). All these ethanol-induced alterations to neonatal liver PE composition were reversed by feeding tuna oil in combination with ethanol. The response to feeding tuna oil alone was very similar, with decreased 20:4ω6 (34.0%) and increased 22:6ω3 (20.1%) in neonatal liver PE compared with control animals.

Discussion

The results of the present study demonstrate that feeding ethanol to pregnant guinea pigs resulted in complex changes to fetal brain phospholipid fatty acid content, particularly of polyunsaturated fatty acids. Although such compositional changes were restricted to relatively minor components in the PC fraction, including 22:6ω3, ethanol-exposure induced marked alterations to the fatty acid content of fetal brain PE. Ethanol-exposure was associated with decreased content of brain PE 22:6ω3, accompanied by increased amounts of 20:5ω3 and 22:5ω6. Increased brain phospholipid 22:5ω6 content has been reported previously in the offspring of mothers fed a ω3-deficient diet,[8] which may reflect a physiological attempt to maintain the degree of unsaturation of membrane phospholipids. Because 22:6ω3 is required for normal neurological development[7,8] and 20:5ω3 and 22:5ω6 confer substantially different biophysical properties in phospholipid membranes, such alterations to the PUFA content of brain PE are consistent with a role

Table 7 Fetal guinea pig liver phosphatidylethanolamine composition

Fatty acid	Fatty acid content (%)			
		Phosphatidylethanolamine		
	Control (n = 5)	Ethanol (n = 5)	Ethanol & tuna (n = 5)	Tuna (n = 5)
14:0	0.7 ± 0.1	0.4 ± 0.4	0.6 ± 0.4	0.3 ± 0.2
16:0	20.3 ± 1.2	24.9 ± 5.5	21.4 ± 1.0	26.0 ± 7.8
18:0	29.3 ± 1.6	22.8 ± 5.3	26.4 ± 3.2	28.5 ± 3.5
16:1ω7	0.6 ± 0.1	0.4 ± 0.2	0.4 ± 0.4	0.2 ± 0.1
18:1ω9	10.1 ± 2.1	8.2 ± 1.9	10.6 ± 2.3	7.2 ± 1.0
18:2ω6	13.2 ± 3.6	17.3 ± 0.7	8.9 ± 0.7	11.3 ± 2.0
18:3ω3	0.0 ± 0.0	0.5 ± 1.0†	0.1 ± 0.2‡	0.0 ± 0.0‡
18:3ω6	0.0 ± 0.0	1.2 ± 0.8†	0.2 ± 0.2‡	0.0 ± 0.0‡
20:3ω9	0.6 ± 0.5	1.5 ± 4.1	1.3 ± 2.0	0.0 ± 0.0
20:4ω6	9.4 ± 2.1	11.9 ± 2.3†	7.6 ± 1.6‡	6.2 ± 0.9†, ‡
20:5ω3	1.0 ± 0.7	0.2 ± 0.2	0.0 ± 0.0	2.5 ± 2.2‡, *
22:5ω6	0.4 ± 0.1	1.8 ± 0.7†	0.3 ± 0.3‡	0.5 ± 0.2‡
22:6ω3	14.4 ± 1.1	8.9 ± 1.7†	22.2 ± 1.1†, ‡	17.3 ± 4.2‡, *

Values are mean ± SD (n = number of pregnancies) fractional concentrations of individual fatty acids in brain phospholipids from term fetal guinea pigs. †, ‡ and *indicate values that were significantly different ($P < 0.01$) from control, ethanol-exposed, and ethanol-exposed with tuna oil supplementation animals respectively.

in abnormal nerve function in ethanol-exposed neonates.[19] Accumulation of 22:6ω3 into developing brain PE is associated both in the human[13] and guinea pig[11] fetus with the period of maximal neurite and synapse formation. Therefore, impaired 22:6ω3 assimilation into fetal brain phospholipids as a consequence of chronic ethanol exposure may result in abnormal neurological function caused by reduced formation of connections among neurons. Chronic alcohol consumption in adult animal models has been shown to result in decreased 22:6ω3 content in brain phospholipids, which may protect against the fluidizing effects of ethanol on cell membranes.[20] In the fetus, however, the presence of these proposed protective processes would probably be inappropriate because they seem to inhibit the normal programmed changes to developing membrane phospholipid composition, including 22:6ω3 accumulation,[10] resulting in abnormal brain development. The changes in the fractional concentration of saturated and monounsaturated fatty acids are complex and difficult to interpret, because in molecular species terms these classes of fatty acid can be found at either the *sn*-1 or *sn*-2 positions. Fetal guinea pig brain PE, for example, contains molecular species in which 18:1ω9 is located at both *sn*-1 and *sn*-2 positions (PE18:1 alkyl/18:1 acyl), or at the *sn*-1 position alone (PE18:1/22:6).[6] This is complicated further by the differential effects of ethanol in individual molecular species. For example in term fetal guinea pig brain, ethanol exposure resulted in decreased in PE18:1/22:6 concentration but did not affect PE18:0/18:1 content significantly.[10]

Feeding tuna oil enriched in 22:6ω3 in combination with ethanol prevented the ethanol-induced decrease in the 22:6ω3 content of fetal brain phospholipids, and resulted in a significantly higher 22:6ω3 concentration than pups born to mothers fed either chow or tuna oil alone. Although the daily calorie intake of the mothers fed either tuna oil and ethanol, or ethanol alone was significantly greater than those fed chow or tuna oil alone, the specific alterations to membrane phospholipid essential fatty acid content cannot simply be attributed to variations in energy intake between experimental groups. In addition, because there was no significant variation in fetal carcass, brain or liver mass between dietary groups, it would appear that the differences in calorie intake had a minimal effect on the growth and development of the pups. A simpler explanation for the observed effects of tuna oil feeding on brain phospholipid composition is that the maternal dietary supplementation regimen may have overcome the ethanol-induced deficit in 22:6ω3 accumulation into fetal brain by substantially increasing the amount of 22:6ω3 available for uptake into developing neurones. This is consistent with our recent observation that ethanol exposure does not adversely affect programmed modifications to maternal hepatic and plasma PC molecular species composition, which may be associated with regulating 22:6ω3 supply to the fetus and supports the view that ethanol may impair mechanisms involved in assimilation of 22:6ω3 into fetal brain phospholipids.[21] The low level of 20:5ω3 incorporation into brain phospholipids together with the absence of changes to the 20:4ω6 content of brain PC or PE in ethanol and tuna oil exposed fetuses suggests that alterations to prostaglandin-mediated signalling processes reported previously[22] are unlikely to be the principle mechanisms for the beneficial effect of tuna oil on ethanol-induced fetal brain damage.

A previous study that examined the effect of prenatal ethanol exposure and ω3 essential fatty acid supplementation in mice failed to demonstrate an ethanol-induced 22:6ω3 deficit or an overaccumulation of 22:6ω3 into brain phospholipids after feeding with a combination of tuna oil and ethanol.[23] The differences between the results of this study and the present report may be explained as follows. Accumulation of 22:6ω3 into developing guinea pig brain is initiated early in gestation, which is similar to human fetal brain, leading to substantial incorporation into membrane phospholipids at term,[6,11,12] whereas assimilation into mouse brain is restricted to the late prenatal and early postnatal periods. However, as ethanol exposure was limited to d7-17/19 in the mouse study, the perinatal period was excluded, important ethanol-sensitive developmental stages may have been missed. Feeding ethanol to pregnant guinea pigs throughout gestation ensured that the animals were exposed throughout the major developmental stages. Consistent ethanol exposure also resembles the human situation more closely than the feeding regimen in the mouse study. In addition, feeding ethanol to pregnant mice early in gestation followed by analysis of brain phospholipid fatty acid composition on postnatal days 3 and 10 may have allowed a recovery period that was absent from the current study.[23]

Surprisingly, accumulation of 22:6ω3 both into brain PC and PE of fetuses from mothers fed both ethanol and tuna oil was significantly greater than controls. However, the 22:6ω3 content of these phospholipids did not differ significantly between control fetuses and those of mothers fed tuna oil alone. This is consistent with a direct effect of ethanol on processes that regulate the specificity of phospholipid biosynthesis in developing fetal brain, which is probably distinct from ethanol-induced alterations to the physical properties of cell membranes.[10] Weisinger et al.[24] have demonstrated recently that increasing guinea pig retinal phospholipid 22:6ω3 content above an optimum concentration resulted in a progressive decrease in electroretinogram response. In this context, application of maternal dietary supplementation with 22:6ω3 to pregnant women would require careful titration of dose to prevent overaccumulation of 22:6ω3 into fetal brain.

Ethanol feeding caused changes to fetal guinea pig liver phospholipid fatty acid composition, which differed substantially from those observed in developing brain. For example, ethanol-exposure decreased the 22:6ω3 content of fetal brain PC and PE, and liver PE, but not of liver PC. However, feeding both ethanol and tuna oil increased the PC and PE 22:6ω3 contents of both tissues above control levels. In contrast, although fetal brain PE 20:4ω6 content was not affected by the experimental dietary regimens, hepatic PE 20:4ω6 content was increased in ethanol-exposed fetuses and decreased in fetuses from mothers fed tuna oil alone. Together these data suggest that both the effects of ethanol-exposure and tuna oil supplementation are, to some extent, tissue specific. The biochemical basis for such differences is unclear.

One potential implication of the results of this study is that maternal ingestion of large amounts of 22:6ω3 may be

useful in reducing the severity of ethanol-induced damage to the developing nervous system. However, both clear understanding of the potential harmful effect of elevated phospholipid 22:6ω3 concentrations both in developing nervous and other organ systems and detailed neurophysiological studies on the effect of 22:6ω3 on the severity of prenatal ethanol exposure on fetal brain function are required before clinical trials using dietary supplementation with 22:6ω3-enriched fish oil of pregnant women who chronically consume ethanol or their newborn infants could be initiated.

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