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Prenatal Ethanol Exposure Increases Brain Cholesterol Content in Adult Rats

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Abstract

Fetal alcohol syndrome is the most severe expression of the fetal alcohol spectrum disorders (FASD). Although alterations in fetal and neonate brain fatty acid composition and cholesterol content is known to change in animal models of FASD, the persistence of these alterations into adulthood is unknown. To address this question, we determined the effect of prenatal ethanol exposure on individual phospholipid class fatty acid composition, individual phospholipid class mass, and cholesterol mass in brains from 25-week-old rats that were exposed to ethanol during gestation beginning at gestational day 2. While total phospholipid mass was unaffected, phosphatidylinositol and cardiolipin mass was decreased 14 and 43%, respectively. Exposure to prenatal ethanol modestly altered brain phospholipid fatty acid composition, and the most consistent change was a significant 1.1-fold increase in total PUFA, in the n-3/n-6 ratio, and in the 22:6 n-3 content in ethanolamine glycerophospholipids and in phosphatidylserine. In contrast, prenatal ethanol consumption significantly increased brain cholesterol mass 1.4-fold and the phospholipid to cholesterol ratio was significantly increased 1.3-fold. These results indicate that brain cholesterol mass was significantly increased in adult rats exposed prenatally to ethanol, but changes in phospholipid mass and phospholipid fatty acid composition were extremely limited. Importantly, suppression of post-natal ethanol consumption was not sufficient to reverse the large increase in cholesterol observed in the adult rats.

Keywords

fetal alcohol syndrome; cholesterol; phospholipid fatty acid composition; brain; phospholipid

Introduction

Fetal alcohol spectrum disorders (FASD) is a term that includes a wide spectrum of fetal anomalies related to maternal consumption of ethanol during pregnancy and may affect up to 1% of the U.S. population [1]. The most severe form of FASD is fetal alcohol syndrome (FAS), characterized by a pattern of facial anomalies, growth retardation, central nervous system (CNS) functional and structural abnormalities [2-4]. Within the CNS, prenatal ethanol exposure decreases dendritic elaborations resulting in a reduction in dendrite length

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and arborization [5]. In addition, ethanol impairs proper development of the brain via increased apoptosis [6-9] through a number of different pathways including increased caspase-3 activity [6, 8], suppression of survival kinase activity such as phosphatase and tensin homolog deleted in chromosome 10 (PTEN), extracellular signal regulated kinases (Erk1/2), and protein kinase B (Akt) [7, 10], while increasing c-Jun N-terminal kinase (JNK)-induced apoptosis [7]. Signaling of neurotrophic factors critical for CNS development are reduced including a reduction in brain derived neurotrophic factor (BDNF) and in the expression of its receptor [8]. Further, insulin signaling is attenuated, resulting in a significant reduction in its ability to promote neuronal survival [10]. Thus, ethanol consumption during pregnancy results in profound alterations in CNS development that have lasting effects, yet despite this widespread knowledge regarding the negative impact of ethanol consumption during pregnancy, nearly 10 percent of women still use ethanol during pregnancy [11].

Ethanol consumption broadly impacts brain lipid metabolism. Ethanol rapidly partitions into the lipid portion of membranes, resulting in altered structure and consequently affecting the proper cell function [12-14], including an increase in membrane fluidity *in vitro* [12]. However, ethanol consumption also alters brain phospholipid and fatty acid composition in adults consuming ethanol [15-20]. In adult cats, chronic ethanol consumption results in a reduction in liver docosahexaenoic acid (DHA, 22:6n-3) levels, but also a reduction in 22:6n-3 plasma levels and a net 20% reduction in 22:6n-3 brain levels [16]. In fact, the effect of prenatal ethanol exposure on membrane lipid composition during rodent embryonic development [17, 21-23] or during the early postnatal period [4, 9, 22, 24] has been extensively studied. Although exposure of developing chicks *in ovo* to increasing levels of ethanol does not alter total brain phospholipid levels, it does cause a dose-dependent decrease in brain choline glycerophospholipids (ChoGpl) and sphingomyelin (CerPCho), while increasing ethanolamine glycerophospholipids (EtnGpl) 2-3 fold [21]. While some investigators have observed inconsistent changes in 22:6n-3 [4], others have observed that prenatal and postnatal ethanol exposure results in a 14% reduction in phosphatidylserine (PtdSer) as well as a reduction in PtdSer docosahexaenoic acid (DHA, 22:6n-3) containing molecular species [9, 24]. This slight reduction in 22:6n-3 is important as it results in a membrane change that promotes apoptosis [9, 25, 26], consistent with the enhanced apoptosis that occurs during prenatal and postnatal ethanol exposure. Collectively, these studies have observed a limited change in total brain phospholipid, but often demonstrate a reduction in 22:6n-3 in PtdSer and changed in individual phospholipid classes in young animals (up to 25 days old), suggesting a derangement of 22:6n-3 metabolism following either prenatal and/or postnatal ethanol exposure. However, the persistence of these changes in brain lipid composition into adulthood following prenatal exposure of ethanol is unknown. Although many of these studies have focused on the impact of prenatal and/or postnatal ethanol consumption on brain phospholipid and phospholipid acyl chain composition, there may be a role for altered cholesterol metabolism in this process [27, 28]. In fact, changes in brain cholesterol homeostasis are thought to be a lipid change that persists into adulthood [27], although evidence is lacking. Prenatal ethanol exposure increases brain cholesteryl ester levels [29], but others have demonstrated that exposure to ethanol *in utero* reduces cerebellar total cholesterol by 50% and reduces cerebellar neuronal cholesterol content by 20% when cultured cerebellar neurons are treated with ethanol [30]. None the less, these results suggest a derangement of normal cholesterol metabolism and trafficking. Exposure to ethanol *in vivo* or exposure of fetal astrocytes results in an increase in ATP-binding cassette-1 (ABCA1) levels [31]. This is important because ABCA1 is required for normal CNS cholesterol trafficking [32] and interacts with apolipoprotein E (ApoE) to move cholesterol from the astrocyte to neurons [32]. Trafficking of cholesterol to neurons is essential for proper neurite extension [33], a critical issue in FAS [5]. Hence, the observed changes in ABCA1 and ApoE levels following prenatal exposure to ethanol and

modelling of this change in astrocytes exposed to ethanol, results in increased cholesterol export from astrocytes via increased coupling of ABCA1 and apolipoprotein E (ApoE) trafficking of cholesterol from the cell [31]. The elevation of ApoE levels under these conditions combined with elevated ABCA1 levels results in a net reduction in astrocyte cholesterol levels [31]. This derangement of cholesterol metabolism is potentially profound and critical as the brain is a cholesterol privileged tissue and relies on its' cholesterol metabolism to support CNS function and maintenance during development and during adulthood [34]. However, whether or not cholesterol is changed in adult animals following prenatal exposure to ethanol is unknown.

Herein we address the question whether the changes observed in brain lipid composition observed following prenatal exposure to ethanol persist into adulthood. To address this question, we fed pregnant dams an ethanol diet during pregnancy and then fed the offspring by surrogate mothers not exposed to ethanol. Once weaned, the offspring received the control diet consisting of PUFA-sufficient rat chow and water *ad libitum*. This model better reflects the situation in humans, in which after parturition the infant does not receive more ethanol. Our results demonstrate for the first time there were only slight changes in phospholipid fatty acid composition and a slight reduction in the mass of individual phospholipid, indicating that these changes observed by others do not persist into adulthood. In contrast we observed a profound, significant increase in brain cholesterol levels into adulthood in rats having prenatal exposure to ethanol.

Material and Methods

Rat Model for Prenatal Ethanol Exposure

The experimental protocol used in this study was approved by the IACUC at the University of North Dakota. Nulliparous female Sprague-Dawley rats were obtained from an in-house colony and mated with males overnight. The presence of a vaginal plug the following morning indicated successful mating and was designated gestational day 1 (GD1). The pregnant rats were randomly divided into two groups on GD2 and were introduced to a liquid diet (F1258SP ethanol diet and F1259SP control diet, BioServ Inc., Frenchtown, NJ) with or without ethanol (355 kcal/L) through GD20 [35]. This liquid diet has been used extensively and proved to be nutritionally complete [35-37]. The average daily diet consumption was 51.3 +/- 0.2 ml/day and produced blood alcohol concentrations of 24.4 +/- 6.6 mg/dl in dams [35]. The ethanol-containing diet had 36% of the calories from carbohydrates (such as maltose-dextrin) isocalorically replaced by ethanol. As in most chronic ethanol studies an isocaloric pair-feeding regimen was used to eliminate the possibility of nutritional deficits [35, 37]. In this instance, non-ethanol-consuming animals were offered the same quantity of diet that the ethanol-consuming animals drank the previous day. The amount of diet consumed was measured daily and animal body weights were measured weekly. Both male and female offspring of the ethanol and control groups were housed in a temperature-controlled room under a 12-h light/dark illumination cycle and allowed access to standard rat chow and tap water *ad libitum*. The pups were surrogate fed by mothers that were not exposed to ethanol in the diet and litter sizes were culled after birth to maintain litter sizes of approximately 6 pups per litter from 3-5 litters. There were no differences in the weight of the offspring between groups [35]. The male offspring were used for this study at approximately 25 weeks of age and the rats were killed using ketamine/xylazine sedation (5:3, 1.32 mg/kg, i.p.) [35] and the brains rapidly removed and flash frozen in liquid nitrogen.

Lipid Extraction

Brain tissues were pulverized under liquid nitrogen temperatures to a fine homogeneous powder and lipids were extracted using *n*-hexane/2-propanol (3:2 vol/vol) [38, 39]. Tissue extracts were centrifuged at 1,000x *g* to pellet debris. The lipid containing liquid phase was decanted and stored under nitrogen at -80°C until analysis.

Thin Layer Chromatography

Individual phospholipid classes were separated by thin layer chromatography. Whatman silica gel-60 plates (20 × 20 cm, 250 μm) were heat-activated at 110°C for 1 hour and samples streaked onto plates. Phospholipids were separated using chloroform:methanol:acetic acid:water (55:37.5:3:2 by vol) [40]. This method separates all major phospholipids including phosphatidic acid (PtdOH), cardiolipin (Ptd₂Gro), phosphatidylinositol (PtdIns), PtdSer, EtnGpl, ChoGpl, and CerPCho. Lipid fractions were identified using authentic standards (Doosan-Serdary, Englewood Cliffs, NJ and NuChek Prep, Elysian, MN). Phospholipid mass was determined by assaying for lipid phosphorus content of individual lipid classes [41].

Plasmalogen Analysis

The plasmalogen mass was determined following separation of individual phospholipid classes in brain lipid extracts by high performance liquid chromatography [42]. One half of the ChoGpl and of the EtnGpl fractions were dried under nitrogen and subjected to mild acidic hydrolysis followed by separation using HPLC [43]. The proportion of plasmalogen determined following this separation was used to calculate plasmalogen mass using the EtnGpl and ChoGpl masses determined following separation by TLC as reported above.

Phospholipid Acyl Chain Analysis

The ChoGpl, EtnGpl, phosphatidylinositol (PtdIns) and, phosphatidylserine (PtdSer) fractions were subjected to base-catalyzed transesterification, converting the phospholipid acyl chains to fatty acid methyl esters (FAME). To each fraction, 2 mL of 0.5 M KOH dissolved in anhydrous methanol were added [44]. FAME were extracted from the methanol using 3 mL of *n*-hexane and the *n*-hexane phase containing the FAME was removed. The lower phase was re-extracted two more times with 3 mL of *n*-hexane and these washes were combined with the original aliquot.

Individual fatty acids were separated by gas liquid chromatography (GLC) using an SP-2330 column (0.32 mm ID × 30 m length) and a Trace GLC (ThermoElectron, Austin, TX) equipped with dual autosamplers and dual flame ionization detectors (FID). Fatty acids were quantified using a standard curve from commercially purchased standards (NuChek Prep, Elysian, MN) and 17:0 was used as the internal standard.

Neutral Lipid Analysis

The neutral lipid fraction was separated from the phospholipid fraction using silicic acid column chromatography (Clarkson Chemical Company, Inc., Williamsport, PA, USA) [45]. The individual neutral lipid classes were separated by HPLC using a binary solvent system consisting of *n*-hexane:2-propanol:acetic acid (98.7:1.2:0.1, v/v) and *n*-hexane [46]. The HPLC system consisted of a Selectosil column (5 μm, 4.6 × 250 mm, Phenomenex) and Beckman 114 M pumps controlled by a 421 A controller and an evaporative light scattering detector (Model 301, ESA Inc., Chelmsford, MA). Cholesterol mass and cholesteryl ester (CE) mass were determined using a standard curve from commercially purchased standards (NuChek Prep.). CE mass was converted from grams to moles using the molecular weight of stearoyl cholesterol.

Statistical Analysis

Statistical analysis was done using InStat 2 from GraphPad (San Diego, CA). Statistical significance was assessed using **two-tailed**, unpaired Student's *t*-test, with a $p < 0.05$ considered to be statistically significant.

Results

Prenatal ethanol exposure reduces brain PtdIns and cardiolipin mass in adult rat brain

Total phospholipid mass was not altered as a result of the ethanol treatment; however, there were small, but significant change in several individual phospholipid classes (Table 1,2). In rats receiving ethanol during gestation, brain cardiolipin (Ptd₂Gro) mass was decreased 43% and PtdIns mass was decreased 14%. Thus, we observed a significant reduction in brain cardiolipin mass, along with a small but significant change in PtdIns.

Prenatal ethanol exposure does not alter plasmalogen mass in adult rat brain

Because the brain contains considerable amounts of ethanolamine plasmalogen (PlsEtn) and choline plasmalogens (PlsCho) that could be affected by ethanol [15, 47, 48], we determined the impact of prenatal ethanol exposure on the mass of these two phospholipids. No significant differences in brain plasmalogen mass were observed between groups (Table 2). Altogether these results indicate that gestational ethanol exposure has a moderate effect on individual phospholipid mass classes and that it did not affect plasmalogen mass in adult rat brain.

Prenatal ethanol exposure increases 22:6n-3 levels in EtnGpl and PtdSer in adult rat brain

Brain fatty acid composition is affected by prenatal ethanol exposure and in ethanol exposure in adults [9, 17-20, 24]. In EtnGpl, the proportion of 22:6n-3 was significantly increased 1.1-fold in ethanol treated rats as compared to control (Table 3). This increase resulted in a concomitant increase in total n-3 fatty acids, polyunsaturated fatty acids (PUFA), and in the n-3/n-6 ratio. Although in ChoGpl, the fatty acid composition of the control and the ethanol group was identical, there was a slight, significant increase in the n-3/n-6 ratio (Table 4), similar to what was observed in EtnGpl. The most abundant changes observed were in the acyl chain composition of PtdSer, where the proportions of 16:0, 18:0 and 20:1n-9 were decreased 13%, 5%, and 18%, respectively (Table 5). However, similar to EtnGpl, the proportion of 22:6n-3 was increased 1.1-fold, resulting in a net increase in total n-3 fatty acids and in the n-3/n-6 ratio. In PtdIns, similar to PtdSer there was a decrease in the proportion of saturated fatty acids, with 16:0 and 18:0 decreased 13% and 10% respectively, in the ethanol treated group as compared to control (Table 6). Thus in our results, the most consistent change observed was a slight increase in the proportion of 22:6n-3 in brain phospholipids from adult rats subjected to prenatal ethanol exposure, indicating that the reported reductions in 22:6n-3 levels by others in young animals subjected to prenatal ethanol exposure do not persist into adulthood.

Prenatal ethanol exposure increases brain cholesterol mass in adult rat brain

Cholesterol is an important component of the membranes and its content changes in animals consuming ethanol [20, 22, 49, 50], however a similar effect in adult rats subjected to prenatal ethanol exposure is unknown. We observed a 1.4-fold increase in cholesterol in the ethanol treated group. Because the total phospholipid content did not increase, the cholesterol to phospholipid ratio was increased 1.3-fold. The mass of cholesteryl esters were not different between groups. These results indicate that brain cholesterol mass was significantly affected by maternal ethanol consumption and that this effect persists in adult rats.

Discussion

Because of the social impact of ethanol consumption, there has been a substantial effort to understand how ethanol exerts its neurotoxic effects; however there is still not a clear mechanism that explains its teratogenicity during *in utero* exposure. It is well known that ethanol exerts its effects at the level of the central nervous system, altering the fine structure of the neuronal membranes. Because of its chemical properties, ethanol can alter membrane biophysical properties by easily inserting into the membrane [12]. Biophysical studies *in vitro* show that ethanol disorders and alters membrane structure [51] and affects activity of membrane associated proteins [52, 53]. In addition, ethanol alters fatty acid metabolism [54, 55] and therefore its consumption can affect the membrane fatty acid composition. Thus, it is generally considered that the alteration in membrane lipid composition and its biophysical properties has a role in the neurotoxic effects of ethanol.

There is a considerable amount of literature covering the effects of chronic ethanol consumption during the critical period of fetal development or in adult animals. Within the FAS field, most of the studies are focused on assessing changes in the offspring due to ethanol exposure during embryonic development and during the early postnatal period. However, to our knowledge this is the first study addressing the long-term effects of prenatal ethanol exposure on brain lipid composition, using a model that better simulates the situation of a newborn infant exposed to ethanol only during the mother's pregnancy.

In this study, we showed that brain lipid composition is altered in adult rats exposed to ethanol during embryonic development. While there were slight changes in the mass of individual phospholipid classes and fatty acid composition, there was a robust increase in the brain cholesterol mass in the ethanol group. Our results indicated that the total phospholipid mass in adult rats is not affected by the ethanol treatment. Similarly, in newborn and 21-day-old rats exposed to ethanol during pregnancy, cholesterol mass is increased with no accompanying change in phospholipid mass observed [22], although others show a reduction in phospholipid mass, in 20-day-old rats exposed to ethanol for the same period [56]. Within the individual phospholipid classes we showed a significant decrease in PtdIns and Ptd₂Gro. Although these two phospholipids are considered minor phospholipids, they are critical for cell signaling and mitochondrial function, respectively. In addition, PtdIns is a critical component of the PTEN and Akt signaling pathways that are deranged following prenatal ethanol exposure [7, 10], suggesting a potentially persistent attenuation of this pathway into adulthood. Both PtdIns and Ptd₂Gro confer ethanol tolerance in liver microsomes [57], suggesting that their reduction might have additional importance. Previous FAS studies indirectly showed changes in ChoGpl and EtnGpl mass, but these values were measured in guinea pigs exposed to ethanol during gestation and killed at term [23]. However, hippocampal PtdSer and ChoGpl mass is reduced in young rats subjected to prenatal ethanol exposure [24]. Using our experimental paradigm, we did not observe changes in either of these phospholipids.

An important and distinctive component in brain membranes are the plasmalogens [47], which are putative signaling molecules [58-60]. Plasmalogens are especially enriched in myelin where ethanolamine plasmalogen account for 70-80% of total EtnGpl and for 12% of the total myelin phospholipid [61]. Because delayed myelination and altered ultrastructure of the myelin sheath are observed after ethanol administration [62] and chronic ethanol consumption in adults enhances plasmalogen synthesis [15], we felt it necessary to determine if brain ethanolamine and choline plasmalogens were altered in our model system. However, no differences were observed in brain plasmalogen mass, suggesting any impact that may have occurred did not persist into adulthood.

Because ethanol affects membrane rigidity *in vitro* [12], it is important to consider that changes in fatty acid composition can be a mechanism to compensate for this alteration. Thus, it would be expected that a decrease in PUFA content would occur to counteract the fluidizing effects of ethanol. Although a large number of studies focused their analysis on phospholipid fatty acid composition to prove this hypothesis *in vivo*, the reported effects of ethanol on brain PUFA content are highly variable. For example, in guinea pigs fed ethanol during gestation and killed at parturition, DHA is decreased in EtnGpl and ChoGpl (27 and 57%, respectively) [17]. In 7-week-old rats receiving ethanol for 21 days, PUFA content in synaptic membranes is decreased 10% [18] and myelin DHA content is decreased 16% in PtdSer [19]. Finally, no changes were observed in brain PUFA composition in 5-week-old rats fed ethanol intraperitoneally for 23 days [20]. However, prenatal exposure to ethanol reduces 22:6n-3 content of PtdSer [9]. Additional studies demonstrate that this ethanol exposure reduces PtdSer 18:0/22:6n-3 molecular species with a concomitant increase in the 18:0/22:4n-6 species [24]. In fact, exposure of Neuro2A cells to ethanol *in vitro* shows a similar effect on PtdSer 22:6n-3 levels, suggesting a potential significant impact of ethanol on neuronal PtdSer metabolism [9]. Ethanol treatment of C-6 glioma cells results in a reduction in PtdSer mass and reduces 22:6n-3 targeting to PtdSer, indicating that exposure of cells results in a derangement of normal PtdSer biosynthesis [63].

However, our study showed only subtle changes in fatty acid composition, with the most consistent change a 1.1-fold increase in the ethanol group in 22:6n-3 content in PtdSer and EtnGpl, both well-known 22:6n-3-enriched phospholipids. Both groups of rats were fed the same n-3 and n-6 adequate standard rat chow diet; suggests that if there were prenatal changes in DHA in the ethanol exposed group that these changes were normalized by consumption of a PUFA sufficient diet that the rats received once they were weaned. It is well established that brain fatty acid composition is sensitive to changes in dietary lipids, requiring short times to observe changes with initiation of a new diet [64]. Therefore, it is possible that the diet helped normalize any changes in brain fatty acid composition that have been documented to occur by others following prenatal exposure to ethanol. Nonetheless, our study demonstrates normalization of PUFA content and in fact a small, but significant increase in DHA and n-3 fatty acid content in some phospholipids.

Membrane unesterified cholesterol contents determines its fluidity and permeability [13]. Thus, cholesterol content is tightly regulated because changes in membrane cholesterol content affect the function of integral membrane proteins [65]. In addition cholesterol plays a vital role during brain development because of its high concentration in myelin [66]. In our study, brain cholesterol mass was significantly increased 1.4-fold in rats receiving ethanol treatment. Similar results are seen in erythrocyte membranes and synaptic membranes from adult DBA/2J mice fed ethanol chronically for 8 days [49]. In addition, brain cholesterol increased 1.4-fold as well in newborn and in 21-day-old rats subjected to prenatal and postnatal ethanol exposure [22], although cerebellar cholesterol is reduced by 50% in rats exposed to prenatal ethanol [30]. The reduction observed in whole brain cholesterol suggests that cholesterol synthesis was enhanced by maternal ethanol consumption to compensate for the deleterious effects of ethanol [22]. Of course this suggestion is supported, in part, by studies demonstrating that prenatal ethanol exposure enhances astrocyte cholesterol export and trafficking via enhanced ABCA1 and ApoE levels [29, 31]. However, no changes in brain cholesterol content are observed in adult rats drinking ethanol for five months or receiving the ethanol intraperitoneally during 23 days [20, 50], suggesting that in the adult animal ethanol consumption does not alter brain cholesterol metabolism. Our results demonstrate a profound alteration in cholesterol mass in rats that had not received ethanol during adulthood, but only during gestation. This indicates that there was a permanent alteration in the pup brain cholesterol content that persisted into adulthood. This is consistent with the impact of ethanol on cholesterol trafficking in the brain which could

result in a persistent increase in astrocyte cholesterol synthesis in response to the increased export of cholesterol from the astrocytes [29, 31]. In fact, alteration in brain cholesterol metabolism is considered as a potential mechanism underlying important neurological disorders such as Alzheimer's disease [67] and Smith-Lemli-Opitz syndrome [28]. However, in our model system, more studies are needed to fully understand the role and the consequences of this increase in cholesterol mass as well as the underlying metabolic changes eliciting this effect following prenatal ethanol exposure.

In summary, we showed that in adult animals exposed to ethanol during gestation that PtdIns and Ptd₂Gro mass were reduced. While there were changes in phospholipid acyl chain composition, the most consistent change was a slight, but significant increase (1.1-fold) in 22:6n-3 proportions. This observation demonstrates that the observed reduction in 22:6n-3 in young animals subjected to prenatal ethanol exposure do not persist into adulthood [9, 17, 24]. However, similar to the increase in brain cholesterol mass previously reported in mice and rats exposed to ethanol during gestation [22, 49], we observed a similar 1.4-fold increase in brain cholesterol content in adult rats exposed to ethanol during gestation. This alteration in brain cholesterol mass might account for the CNS effects that *in utero* exposure of ethanol has in adult individuals.

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Abbreviations

ABCA1	ATP-binding cassette-1
Akt	protein kinase B
ApoE	apolipoprotein E
BDNF	brain derived neurotrophic factor
CerPCho	sphingomyelin
ChoGpl	choline glycerophospholipids
CNS	central nervous system
EtnGpl	ethanolamine glycerophospholipids
Erk1/2	extracellular signal regulated kinases
FASD	fetal alcohol syndrome disorders
HPLC	high-performance liquid chromatography
JNK	c-Jun N-terminal kinase
PakCho	1- <i>O</i> -alkyl-2-acyl- <i>sn</i> -glycero-3-phosphocholine
PakEtn	1- <i>O</i> -alkyl-2-acyl- <i>sn</i> -glycero-3-phosphoethanolamine
PlsCho	1- <i>O</i> -alkyl-1'-enyl-2-acyl- <i>sn</i> -glycero-3-phosphocholine
PlsEtn	1- <i>O</i> -alkyl-1'-enyl-2-acyl- <i>sn</i> -glycero-3-phosphoethanolamine
PtdSer	phosphatidylserine
PtdIns	phosphatidylinositol

PtdOH	phosphatidic acid
Ptd₂Gro	cardiolipin PTEN: phosphatase and tensin homolog deleted in chromosome 10 <i>sn</i> , stereospecifically numbered
PUFA	polyunsaturated fatty acids
TLC	thin layer chromatography

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Table 1

Brain phospholipid mass in control and ethanol exposed rats

	Control group nmol/g ww		Ethanol group nmol/g ww	
	mean	SD	mean	SD
CerPCho	7240	478	6952	504
ChoGpl	24202	1209	23882	1754
PtdSer	9423	1097	9098	927
PtdIns	4006	366	3455	243 *
EtnGpl	22287	4002	24873	2088
PtdOH	2081	418	2241	377
Ptd ₂ Gro	1588	203	907	153 *
Total	70827	5437	71409	4436

Values are expressed as mass (nmol/g ww) and represent mean $\pm$ SD, n=6-7.

The (*) indicates a significant effect of the ethanol treatment as compared to control (p<0.05).

Table 2

Brain plasmalogen mass in control and ethanol exposed rats

	Control group nmol/g ww		Ethanol group nmol/g ww	
	mean	SD	mean	SD
PakEtn+PtdEtn	10640	1940	12807	1922
PlsEtn	11647	2143	12949	2037
PakCho+PtdChol	22410	1144	23118	1959
PlsCho	1791	608	1519	275

Values are expressed as mass (nmol/g ww) and represent mean $\pm$ SD, n = 7-8, for both groups. There were no significant differences between groups ($p < 0.05$). PakEtn: 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphoethanolamine; PlsEtn: 1-*O*-alkyl-1'-enyl-2-acyl-*sn*-glycero-3-phosphoethanolamine; PakCho: 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphocholine; and PlsCho: 1-*O*-alkyl-1'-enyl-2-acyl-*sn*-glycero-3-phosphocholine.

Table 3

Brain EtnGpl acyl chain composition in control and ethanol exposed rats

Total fatty acid %	Control group		Ethanol group	
	mean	SD	mean	SD
16:0	7.6	0.2	7.5	0.2
16:1	0.3	0.02	0.4	0.1
18:0	22.7	0.7	22.2	0.5
18:1 n-9	18.4	0.9	18.5	0.6
18:1 n-7	3.8	0.2	3.6	0.1
18:2 n-6	0.4	0.03	0.4	0.03
20:1 n-9	3.1	0.3	2.8	0.2
20:3 n-6	0.4	0.04	0.4	0.02
20:4 n-6	17.7	0.9	17.1	0.7
22:4 n-6	5.6	0.2	5.2	0.4
22: 5 n-6	0.4	0.06	0.3	0.1
22:5 n-3	0.4	0.08	0.5	0.2
22:6 n-3	19.3	0.9	21.1	0.6 [*]
sat	30.3	0.7	29.7	0.5
MUFA	25.6	1.4	25.2	0.9
PUFA	44.1	0.9	45.0	0.5 [*]
n-3	19.7	1.0	21.6	0.7 [*]
n-6	24.4	1.0	23.5	1.1
n-3/n6	0.81	0.07	0.92	0.07 [*]

Values are expressed as percentage of total fatty acid (mole %) and represent mean $\pm$ SD, n=7-8.

The asterisk (*) indicates a significant effect of the prenatal ethanol treatment as compared to control (p<0.05).

Table 4

Brain ChoGpl acyl chain composition in control and ethanol exposed rats

Total fatty acid%	Control group		Ethanol group	
	mean	SD	mean	SD
16:0	47.8	0.8	47.3	1.1
16:1	0.5	0.03	0.6	0.1
18:0	12.6	1.5	13.2	0.5
18:1 n-9	21.5	0.7	21.6	0.3
18:1 n-7	6.0	0.1	5.8	0.1
18:2 n-6	0.8	0.07	0.8	0.1
20:1 n-9	0.8	0.04	0.8	0.1
20:3 n-6	0.2	0.03	0.2	0.1
20:4 n-6	6.0	0.3	5.9	0.3
22:3 n-3	0.2	0.02	0.2	0.03
22:4 n-6	0.5	0.03	0.4	0.04
22: 5 n-6	0.1	0.01	0.1	0.02
22:5 n-3	0.1	0.06	0.1	0.02
22:6 n-3	2.9	0.2	3.1	0.2
Sat	60.4	1.0	60.5	0.8
MUFA	28.9	0.8	28.8	0.4
PUFA	10.7	0.3	10.7	0.5
n-3	3.1	0.2	3.3	0.3
n-6	7.6	0.3	7.4	0.4
n-3/n6	0.41	0.04	0.45	0.03 [*]

Values are expressed as percentage of total fatty acid (mole %) and represent mean $\pm$ SD, n=7-8.

The asterisk (*) indicates a significant effect of the ethanol treatment as compared to control (p<0.05).

Table 5

Brain PtdSer acyl chain composition in control and ethanol exposed rats

Total fatty acid%	Control group		Ethanol group	
	mean	SD	mean	SD
16:0	1.5	0.1	1.3	0.1 *
18:0	49.3	1.9	47.0	0.9 *
18:1 n-9	16.8	0.9	16.8	0.7
18:1 n-7	2.4	0.1	2.3	0.1
20:0	0.4	0.04	0.4	0.04
20:1 n-9	1.6	0.2	1.3	0.2 *
20:4 n-6	4.1	0.4	4.1	0.2
22:1 n-9	0.4	0.04	0.4	0.04
22:3 n-3	0.4	0.04	0.5	0.03
22:4 n-6	2.3	0.1	2.3	0.2
22:5 n-6	1.4	0.2	1.4	0.4
22:5 n-3	0.3	0.1	0.4	0.03
22:6 n-3	19.2	1.8	21.8	1.1 *
sat	51.2	1.9	48.7	1.0 *
MUFA	21.2	1.2	20.8	0.9
PUFA	27.7	1.6	30.4	1.2 *
n-3	20.0	1.8	22.6	1.1 *
n-6	7.7	0.4	7.8	0.3
n-3/n6	2.60	0.34	2.91	0.16 *

^aValues are expressed as percentage of total fatty acid (mole %) and represent mean $\pm$ SD, n=7-8.

The asterisk (*) indicates a significant effect of the ethanol treatment as compared to control (p<0.05).

Table 6

Brain PtdIns acyl chain composition in control and ethanol exposed rats

FAME	Control group		Ethanol group	
	mean	SD	mean	SD
16:0	7.5	0.5	6.5	0.5 *
18:0	47.4	4.0	42.8	0.7 *
18:1 n-9	4.0	0.3	4.0	0.2
18:1 n-7	1.8	0.2	1.8	0.2
20:4 n-6	32.0	3.1	34.7	1.9
22:4 n-6	1.0	0.1	0.9	0.3
22: 5 n-6	2.7	1.5	4.8	2.3
22:6 n-3	4.4	1.5	4.4	2.0
sat	54.9	4.1	49.4	1.1 *
MUFA	5.8	0.4	5.8	0.3
PUFA	39.3	4.0	44.8	1.2 *
n-3	4.4	1.5	4.4	2.0
n-6	34.9	4.2	40.4	2.1 *
n-3/n6	0.13	0.05	0.11	0.05

Values are expressed as percentage of total fatty acid (mole %) and represent mean $\pm$ SD, n=7-8.

The asterisk (*) indicates a significant effect of the ethanol treatment as compared to control (p<0.05).

Table 7

Brain cholesterol and total phospholipid mass in control and exposed rats

	Control group		Ethanol group	
	mean	sd	mean	sd
Cholesterol $\mu\text{mol/g ww}$	24.8	4.7	34.1	3.4 *
Phospholipid $\mu\text{mol/g ww}$	70.8	5.4	72.7	5.2
Cholesteryl ester $\mu\text{mol/g ww}$	2.6	0.2	2.7	0.3
Chol/P ratio	0.35	0.08	0.45	0.06 *

Values are expressed as mass ($\mu\text{mol/g ww}$) and represent mean $\pm$ SD, n = 6-8, for both groups.

The asterisk (*) indicates a significant effect of the prenatal ethanol treatment as compared with the control ($p < 0.05$).