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Altered maternal plasma fatty acid composition by alcohol consumption and smoking during pregnancy and associations with Fetal Alcohol Spectrum Disorders

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Abstract

Objective: Polyunsaturated fatty acids are vital for optimal fetal neuronal development. The relationship between maternal alcohol consumption and smoking with third trimester plasma fatty acids were examined and their association with Fetal Alcohol Spectrum Disorders (FASD). Methods: Moderate to heavy alcohol-using and low/unexposed comparison women were recruited during mid-pregnancy from two prenatal clinics in Ukraine. The participants' infants underwent physical and neurobehavioral exams prior to one-year of age and classified as having FASD by maternal alcohol consumption and neurobehavioral scores. A subset of mother-child pairs was selected representing three groups of cases and controls: Alcohol-Exposed with FASD (AE-FASD, n=30), Alcohol-Exposed Normally Developing (AE-ND, n=33), or Controls (n=46). Third trimester maternal plasma samples were analyzed for fatty acids and levels were compared across groups.

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Results: The percent of C18:0 ($p<0.001$), arachidonic acid (AA, C20:4n-6, $p=0.017$) and C22:5n-6 ($p=0.001$) were significantly higher in AE-FASD women than controls or AE-ND women. Alcohol-exposed women who smoked had lower C22:5n-3 ($p=0.029$) and docosahexaenoic acid (DHA, C22:6n-3, $p=0.005$) and higher C22:5n-6 ($p=0.013$) than women consuming alcohol alone or abstainers.

Conclusion: Alterations in fatty acid profiles were observed in moderate to heavy alcohol-consuming mothers with infants classified with FASD compared to alcohol-exposed normally developing infants or controls.

Keywords

fatty acids; alcohol; cigarettes; fetal alcohol spectrum disorder; DHA

Introduction

Alcohol use during pregnancy can result in a range of birth defects, including pre- and postnatal growth retardation, developmental and cognitive delay, and facial and cardiac anomalies [1, 2]. Fetal alcohol spectrum disorders (FASD) encompass all alcohol-related birth defects including fetal alcohol syndrome and alcohol-related neurodevelopmental disorders. Since alcohol consumption can aggravate nutrient deficiencies, it has been suggested that maternal nutritional status plays a role in the risk for FASD [3–5]. In addition, rates of cigarette smoking during pregnancy are often higher among women who consume alcohol creating additional consequences to nutritional status, as well as increasing odd ratios for preterm birth, low birth weight, and growth restriction beyond either alcohol or cigarette use alone [6–11].

Early in pregnancy, insulin sensitivity is increased to promote lipid storage in maternal adipose tissue, which in the third trimester is switched to a more catabolic and insulin resistant state with increased maternal lipolysis and reduced adipose storage [12]. As a result, maternal fat stores are mobilized for placental transfer of fatty acids required for fetal development. Long chain omega-3 and omega-6 polyunsaturated fatty acids (PUFA) are essential for normal neurological and cognitive development with rapid fetal accretion of these fatty acids occurring in the third trimester [13, 14]. Epidemiological studies have linked omega-3 and omega-6 status to immune, visual, and cognitive function in the offspring [13, 15]. In addition, supplementation of omega-3 fatty acids or DHA alone in experimental animal studies have been shown to ameliorate or prevent deficits caused by prenatal alcohol exposure [16–18], as well as reductions in oxidative stress and increases in the oxidant defense capability of brain tissue [19].

Alcohol has been shown to alter fatty acid metabolism in both humans and animal models. In rhesus monkeys, alcohol consumption lowered DHA and arachidonic acid (AA, C20:4n-6) levels in plasma, erythrocytes, and liver tissue [20]. Although the exact mechanism(s) through which alcohol exerts these effects are unclear, long-term alcohol consumption can lead to an increase in lipid peroxidation of the essential fatty acids that may have negative consequences for the conceptus [19, 20]. Consistent with experimental animal findings, low concentrations of DHA in plasma and erythrocytes at 24 weeks’

gestation were observed in a population of pregnant women consuming alcohol [21], while interestingly, levels of DHA in cord blood samples of alcohol-exposed neonates have been reported to be elevated compared to control neonates [22–24].

As with most known human teratogens, not all children with risky prenatal alcohol exposure demonstrate measurable physical or neurodevelopmental effects. This suggests that there are factors that moderate prenatal alcohol-related risk. For this reason, a goal of the current study was to explore relationships between alcohol consumption during pregnancy and maternal plasma fatty acids, as well as to determine if specific fatty acid profiles might confer risk or protection against some prenatal alcohol effects.

Method

Study Design

Subjects were selected from a larger prospective cohort study investigating a variety of risk and protective factors for FASD, including whether prenatal nutritional supplements might mitigate the effects of prenatal alcohol exposure. In the parent study, between 2008 and 2012, pregnant women were enrolled during an antenatal appointment at one of two western Ukrainian sites, Rivne Provincial Medical Diagnostic Center or the Khmelnytsky Perinatal Center. This study was done as part of the Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD). The methods and scope of CIFASD studies have been described in more detail elsewhere [25–27].

Those women who agreed to participate signed an informed consent document approved through institutional review boards at the University of California, San Diego and Lviv Medical University in Ukraine. The study participants completed a maternal interview upon enrollment that captured information about demographics and pregnancy history as well as day-by-day alcohol consumption for a typical week around conception and the most recent two weeks prior to enrollment. Women were classified as Alcohol Exposed (AE) if they reported weekly binge-drinking episodes (5+ drinks), five episodes of 3–4 drinks, or at least 10 episodes of 1–2 drinks either in the month around conception or during the most recent month. A classification of low level or non-drinking (Controls) was given if they reported no binge episodes, minimal or no alcohol in the month around conception, and no drinking in the most recent month.

At enrollment, participants were randomized into one of three groups to receive: 1) standard of care in Ukraine (women are advised to take prenatal supplements, but they are not provided free of charge), 2) provision of a daily multivitamin mineral (MVM) supplement or 3) the same daily MVM supplement plus an additional 750 mg choline supplement. Mothers provided a blood sample at the time of enrollment and again in the third trimester. This trial was registered with clinicaltrials.gov as NCT03782935. More information on recruitment can be found in [28].

Infant Outcomes

Infant birth size and gestational age at delivery were obtained from medical records. Infants underwent standard blinded physical examinations for physical features of FASD and

growth using a standard checklist. The Bayley Scales of Infant Development II (BSID-II) [29] assessments were performed at approximately six and/or twelve months of age. The BSID-II measures mental and psychomotor development leading to standardized scores (Mental Development Index: MDI; and Psychomotor Development Index: PDI).

An FASD classification was given to a child if there was reported moderate to heavy maternal alcohol consumption as defined for the alcohol-exposed group and at least one BSID-II score below 85 at approximately six or twelve months. A child may have been classified with FASD with or without physical features of FASD [26, 30]. As children progress through childhood, research reclassification may be required, as it is needed in clinical practice. Those infants born to alcohol-exposed women with no FASD classification, i.e., Bayley score <85, were considered to have normal development (ND).

Sample Selection

Mother-infant pairs from the overall sample were selected to be included in the nested case-control analysis based on the following criteria; 1) Low/no alcohol-exposure (Controls, n=46) which included maternal subjects with low/no alcohol consumption regardless of infant BSID-II scores to represent the normal population, 2) Alcohol-exposed, normal development (AE-ND, n=33) which included maternal subjects with moderate to high alcohol consumption who had a normally developing infant (no FASD classification); and 3) Alcohol-exposed, FASD (AE-FASD, n=30) which included maternal subjects with moderate to high alcohol consumption who had an infant classified with FASD (as described in *Infant Outcomes* section). In the Controls group, 19 out of 46 infants had at least one BSID-II score <85. Plasma samples taken in the third trimester (average 31.5 weeks gestation) were used for this analysis due to the rapid fetal accretion of fatty acids occurring during this time and their importance for neurological development. Maternal and fetal fatty acid levels are generally thought to be well correlated [31].

Plasma Fatty Acids

A 25 ml sample of maternal blood was collected from participants into EDTA-treated tubes. The sample was centrifuged at 1500g, for 10 min, at 4^{o}C, and the plasma was aliquoted into tubes and frozen at −80^{o}C until shipped to the U.S., where it remained frozen at −80 ^{o}C until analyzed. Plasma fatty acid profiles were analyzed by OmegaQuant, LLC (Sioux Falls, South Dakota) using gas chromatography (GC) with flame ionization detection per the manufacturer's protocol. Plasma was transferred to a screw-cap glass vial, which contained 1,2diheptadecanoyl-sn- glycero-3- phosphocholine as in internal standard (di-C17:0 PL) (Avanti Polar Lipids, USA) and the methylation reagent (methanol containing 14% boron trifluoride, toluene, methanol; 35:30:35 v/v/v; Sigma-Aldrich, St. Louis, MO) was added. The vial was briefly vortexed and heated in a hot bath at 100°C for 45 min. After cooling, hexane (EMD Chemicals, USA) and HPLC grade water were added, the tubes were recapped, vortexed and centrifuged to separate layers. An aliquot of the hexane layer was transferred to a GC vial. GC was carried out using a GC-2010 Gas Chromatograph (Shimadzu Corporation, Columbia, MD) with flame ionized detection equipped with a SP-2560, 100-m fused silica capillary column (0.25 mm internal diameter, 0.2 um film thickness; Supelco, Bellefonte, PA). Fatty acids were identified by comparison with a

standard mixture of fatty acids (GLC OQ-A, NuCheck Prep, Elysian, MN), which was also used to determine individual fatty acid calibration curves. The di-C17:0 PL was used to calculate recovery efficiency of the assay and applied to all fatty acids. The following 24 fatty acids (by class) were identified: saturated (14:0, 16:0, 18:0, 20:0, 22:0 24:0); cis monounsaturated (16:1, 18:1, 20:1, 24:1); cis n-6 polyunsaturated (18:2, 18:3, 20:2, 20:3, 20:4, 22:4, 22:5); and cis n-3 polyunsaturated (18:3, 20:5, 22:5, 22:6). Fatty acid composition is expressed as a percent of total identified fatty acids.

Statistical Analysis

Frequencies and percentages were used to describe characteristics of the participants by group. Comparisons between the three groups were performed by chi-square tests for independence for categorical variables and general linear models for continuous variables adjusted with Tukey's or Games-Howell post-hoc analysis for multiple comparisons.

Fatty acid measurements were expressed as composition (percent of total fatty acids) and were log transformed as necessary for normalization. Spearman correlation was used to assess the relationship between each third trimester fatty acid concentration/composition and maternal alcohol consumption data captured from the maternal interview. Alcohol exposure in the week around conception and the most recent two weeks of pregnancy was summarized across types of alcohol, quantity, frequency and pattern of use into two variables: average absolute ounces of alcohol per day (ozAA/day) and average absolute ounces of alcohol per drinking day (ozAA/drinking day). An absolute ounce of alcohol is equivalent to approximately two standard drinks. A control for multiple comparisons was not done due to the exploratory nature of this analysis.

Plasma fatty acid composition and fatty acid ratios were compared among the three groups (AE-FASD, AE-ND, and Controls) by analysis of covariance (ANCOVA) with Bonferroni post-hoc comparisons. Confounding variables were selected based on scientific knowledge of their association with fatty acid levels during pregnancy.

Secondary analyses using the same methods described above examined the associations of alcohol combined with cigarette use or MVM supplementation group (intent to treat analysis) with maternal plasma fatty acids. Women were considered smokers and drinkers (AE+Smoker, n=19) if they met the criteria for moderate to heavy alcohol exposure and had self-reported continued cigarette use at study enrollment. Drinkers only (n=44) were women who met criteria for moderate to heavy alcohol exposure but who were not current smokers at enrollment. Two women from the control group who reported current smoking were removed from this analysis.

Missing values for covariates resulted in exclusion of subjects on a case-by-case basis in each analysis. A two-sided p-value <0.05 was considered to be statistically significant. All analyses were conducted using IBM SPSS Statistic Version 25.

Results

Subject Characteristics

There were no significant differences in the gestational week of blood draw, maternal age and education, or randomization into supplement intervention groups (Table 1). Control mothers enrolled into the study significantly earlier in their pregnancy and had lower pre-pregnancy BMI than AE-ND mothers. Average alcohol consumption was similar at conception and enrollment for both alcohol groups and, by design, significantly higher than Controls at both time points. The majority of subjects in the AE-ND group were from Rivne, whereas Control and AE-FASD women had equal distributions between study sites. At enrollment, women in the AE-FASD group smoked more cigarettes per day than AE-ND and Control women. Paternal age was significantly different between groups, with a trend for significance between AE-FASD and control women (p=0.051). AE-FASD women had a lower SES score than Controls, but not significantly different from AE-ND women.

A total of 99 infants were evaluated at approximately six months (Controls: n=46, AE-ND: n=27, and AE-FASD: n=26) and 97 infants at approximately twelve months of age (Controls: n=46, AE-ND: n=28, and AE-FASD: n=23). Infants born to AE-FASD mothers were delivered earlier than Control infants and were smaller in both birth weight and length compared to Control and AE-ND infants. As specified in the definition of group membership, infants classified with FASD had lower BSID-II scores, mental development index (MDI) and psychomotor development index (PDI) at six and twelve months compared to Control and AE-ND infants. MDI and PDI scores were significantly higher in AE-ND infants than Controls. However, it is important to note the differential in inclusionary criteria between the groups, as a MDI and PDI score ≥ 85 was inclusionary for AE-ND infants and not for control infants, which represented the general population.

Relationships between third trimester maternal plasma fatty acids, smoking, and alcohol intake

Table 2 presents the Spearman's rank correlation coefficients between the mean plasma fatty acid composition (% of total fatty acids) in the third trimester and alcohol intake at conception and at enrollment. In all participants, alcohol consumption (absolute ounces of alcohol per drinking day) at conception was positively correlated with stearic acid (C18:0) and oleic acid (C18:1n-9), as well as C20:1n-9, and C22:0. In contrast, alcohol intake at conception (ozAA/day and ozAA/drinking day) was negatively associated with the omega-6 products γ-linolenic acid (GLA, C18:3n-6), dihomo-GLA (C20:3n-6), and docosatetraenoic acid (C22:4n-6). Alcohol intake at enrollment (ozAA/day and ozAA/drinking day) was positively associated with circulating levels of palmitic acid (C16:0) and palmitoleic acid (C16:1n-7), and was not significant for C18:0 and C18:1n9. Docosahexaenoic acid (DHA, C22:6n-3), in addition to C20:3n-6, was negatively associated with alcohol intake during this time.

Beyond alcohol intake, we examined the potential influence of cigarette smoking. The number of cigarettes smoked per day at enrollment was positively correlated with the percent fatty acid concentration of C16:1n-7 and n-6 docosapentaenoic acid (C22:5n-6) and

negatively correlated with eicosapentaenoic acid (EPA, C20:5n-3), n-3 docosapentaenoic acid (C22:5n-3), and DHA in third trimester maternal plasma (Table 2).

Alcohol consumption at conception was positively correlated with percent C20:0 and negatively with AA in women with children classified with FASD. Alcohol-exposed women with normally developing children had a positive correlation of C16:0 and negative correlation of C16:1n-7 and C18:3n-6 with alcohol consumption at conception. In women with children classified as FASD, there was a positive correlation for percent circulating levels of C16:0, C16:1n-7, C18:3n-6, and alpha linolenic acid (ALA, C18:3n-3) with alcohol intake at enrollment. In these same individuals, a negative association was observed with linoleic acid (LA, C18:2n-6, Table 2).

For those women who consumed moderate to heavy amounts of alcohol, but did not have children classified with FASD, negative correlations were observed with third trimester myristic acid (C14:0), ALA, C20:3n-6, and EPA with alcohol intake at enrollment (ozAA/drinking day). There were no significant correlations for alcohol intake at enrollment and third trimester fatty acids within the AE-ND group.

Third trimester plasma fatty acid composition by MVM supplement intervention

In contrast to expectations, no differences were observed in the fatty acid profiles of women randomized into the different MVM supplementation groups after controlling for site, pre-pregnancy BMI, cigarettes smoked per day at enrollment, and gestational age at blood draw (Supplemental Table 1).

Third trimester plasma fatty acid composition by maternal alcohol intake and its association with FASD

Differences in mean plasma fatty acid composition (% of total fatty acids) was examined by analysis of covariance after controlling for site, SES, pre-pregnancy BMI, cigarettes smoked per day at enrollment, and gestational age at blood draw (Table 3). The saturated fatty acid (SFA) stearic acid (C18:0) was significantly increased in the AE-FASD and AE-ND groups, compared to Control. A significant decrease in the product substrate ratio C18:ln-9/C18:0, an estimate of stearoyl-CoA desaturase (SCD), was observed in the AE-FASD relative to the AE-ND group, with increased C20:1n-9 in the AE-ND group relative to Control.

Distinct between-group differences in plasma fatty acid composition were observed for omega-6 PUFA, but not for omega-3 PUFA. While there were no significant differences in percent LA ($p=0.141$), a significant decrease in C18:3n-6 and delta-6 desaturase (C18:3n-6/C18:2n-6) was observed in the AE-ND group relative to Control. AE-FASD women had significantly lower C20:3n-6 relative to Control, and an increase in delta-5 desaturase (20:4n6/20:3n6) relative to Control and AE-ND, translating to an increase in the circulating levels of AA in the AE-FASD group compared to Controls and AE-ND women. Omega-6 elongation to C22:4n-6 (C22:4n-6/C20:4n-6) and percent C20:4n6 was significantly reduced in AE-ND women compared to Controls and AE-FASD, respectively. Finally, a significantly higher C22:5n-6/C22:4n-6 ratio, a general index of elongase, delta-6 desaturase and beta-oxidation, was observed in the AE-FASD group only, similarly C22:5n-6 was significantly increased in the AE-FASD group. Fatty acid ratio of omega-6 and omega-3 end products

(22:5n-6/22:6n-3) was also significantly higher in the AE-FASD group compared to Controls and AE-ND, however, C22:6n3 was not different between groups. No significant between group differences were noted for the LA/ALA or for omega-6/omega-3 ratios.

Third trimester plasma fatty acid composition by maternal alcohol intake and smoking status

An examination of third trimester plasma fatty acids for those with combined current smoking and alcohol exposure, alcohol but no continued smoking, or controls revealed distinct differences in circulating fatty acid composition (Table 4). Palmitic acid levels (C16:0) were significantly elevated for those who smoked and consumed alcohol compared to those who consumed alcohol only, as well as, an increase in C18:1n9 compared to Control. With regard to PUFA, those that continued to smoke and consume alcohol had significantly lower LA levels compared to those who drank alcohol only, and higher C22:5n-6 compared to the alcohol only and Control groups. Most notable were the reductions in omega-3 fatty acids, EPA, C22:5n-3, and DHA, in the smoking and alcohol group compared to alcohol only. In addition, DHA was significantly higher in Controls than the smoking plus alcohol group. Smoking and alcohol intake resulted in increased ratios of C20:4n-6/C20:5n-3 and C22:5n-6/C22:6n-3 compared to alcohol only (Table 4). The above data supports the concept that concurrent smoking and alcohol use can perturb omega-3 and omega-6 metabolism.

Discussion

In this sample of Ukrainian women, distinctive differences in SFA, MUFA and omega-6 PUFA metabolism were observed between those with and without moderate to heavy alcohol exposure, as well as between AE-FASD and AE-ND groups. While considerable attention has been given to PUFA metabolism in fetal development, in the context of FASD it is important to appreciate the distinct changes in SFA/MUFA that occur with ethanol intake, and the potential for significant interactive effects between SFA/MUFA and PUFA. Specifically in this study, an increase in C18:0 was observed in the AE-FASD group, but not in the AE-ND group. Instead, the AE-ND group had increased C20:1n9 relative to the Control group. There were no significant between-group differences in third trimester omega-3 fatty acids; however, the AE-FASD group had higher AA and C22:5n-6 relative to Control and AE-ND groups. The aforementioned differences occurred apart from any significant between-group differences in circulating levels of omega-6 LA and omega-3 ALA. The increase in AA, but not omega-3 levels, is particularly noteworthy, and may represent an increase in first trimester maternal adipose AA stores derived directly from the diet, a reduction in placental transfer of LC-PUFA to the fetal circulation, as well as endogenous synthesis plus utilization through the eicosanoid pathway in the third trimester.

Ethanol intake increases de novo lipogenesis, resulting in increased C16:1n-7 production [32]. In the current dataset, we observed a positive correlation between alcohol intake and C16:1n-7 production in the AE-FASD group, along with a significant increase in C18:0 that was most likely due to a significant increase in elongase activity. PUFA are known to regulate transcription factors peroxisome proliferator-activated receptor (PPAR) and sterol

regulatory element binding protein (SREBP) [33], with ethanol modulating both in animal models [34, 35]. Appreciably, SFA could also be a factor as elongation of very long chain fatty acids (ELOVL)-6, responsible for the production of C18:0, and is regulated by the transcription factor SREBP-1C [36]. SREBP-1C has been related to insulin sensitivity[37], and is modulated by the endogenous production of C18:0 and C18:1n9 [38].

We, along with others, have previously reported that alcohol-consuming women with FASD children selected from the same parent cohort have a higher ratio of pro-inflammatory to anti-inflammatory cytokines compared to alcohol-consuming women with normally developing children [27, 39, 40]. Specifically, an increase in third trimester tumor necrosis factor-α (TNF-α) relative to interleukin (IL)-10 was associated with increased risk of FASD in alcohol-consuming women [27]. Eicosanoids and docosanoids are produced from 20C and 22C PUFA, respectively, and regulate the immune response and labor induction [41]. For example, prostaglandin (PG) E2 produced from AA is considered anti-inflammatory as it downregulates macrophage TNF production through increased IL-10 production [41], a function that also is considered key in the regulation of parturition [42, 43]. Ethanol production of PGE1 from C20:3n-6 has been observed in platelets[44], with PGE1 production interfering with AA metabolism [45]. In this study, C20:3n-6 was reduced with alcohol intake that was coupled with a significant increase in AA in the AE-FASD group. Although not measured in the current study, eicosanoid dysregulation would be of considerable interest as a reduction in PGE1 and C20:3n-6 secondary to reduced delta-6 desaturase activity has been postulated as a contributing factor for FASD [46, 47].

Alcohol is known to increase the concentrations of free radicals and oxidative stress produced via alcohol metabolism, as well as alcohol's negative effects on the oxidant defense system ultimately leading to an increase in lipid peroxidation and a reduction in PUFA levels [48, 49]. Similarly, current and prior cigarette smokers have reported higher levels of lipid peroxidation products and reduced antioxidant enzyme activities [50, 51]. PUFAs are more susceptible to free radical damage due to the presence of methylene-interrupted double bonds, thus an alcohol and smoking-induced increase in oxidative stress and lipid peroxidation may contribute to a reduction of select essential fatty acids.

Stark et al. (2005) reported that as the amount and frequency of alcohol consumption increased at conception, maternal plasma levels of LA, DHA, and AA decreased at 24 weeks' gestation and at delivery in moderate drinkers [21]. In the current study, the maternal fatty acid profile of women who drank moderate to heavy amounts of alcohol was characterized by significantly lower levels of omega-6 fatty acids C18:3n-6 and C22:4n-6 compared to low or unexposed women who did not continue smoking. In contrast, maternal fatty acid profiles of women who both drank at moderate to high levels and continued smoking had lower levels of omega 6 LA and omega 3 EPA, C22:5n-3, and DHA, suggesting a combined effect of alcohol and cigarette smoking on free fatty acid profiles. Moreover, the number of cigarettes smoked per day at enrollment was positively correlated with omega 6 fatty acids, while negatively correlated with plasma omega-3 fatty acids measured in the third trimester. Stark et al. (21) also reported an increase in the number of cigarettes smoked per day in alcohol-consuming pregnant women compared to abstainers; however, the data were not separated based on current smoking and drinking behaviors,

therefore, we are unable to directly compare their results to our findings. Nonetheless, the current dataset suggests that cigarette smoking may exacerbate an already aberrant fatty acid profile with ethanol intake.

To our knowledge, this is the first study to investigate the association between maternal fatty acids and FASD in humans. Strengths of this study include the collection of detailed information on quantity and frequency of alcohol in pregnancy as well as a wide range of other covariates in a prospective intervention study with infant dsymorphology and neurobehavioral assessments. This study is not without limitations. The sample consisted of a homogeneous population of Caucasian women in Ukraine thus may not translate to other race or ethnic groups. The study did not collect dietary intake data and relied on maternal report of alcohol and tobacco use, although careful questioning techniques were used to elicit precise alcohol information. Timing of blood sampling within the postprandial or fasted state was not standardized. In addition, the classification of neurodevelopmental delay based on a Bayley score measured in young infants may not be predictive of future performance of these children.

In the parent cohort, our group has reported a positive association between the supplement intervention (MVM alone and MVM+choline) and MDI scores of alcohol-exposed infants at 6 months of age [52] as well as improved neurophysiological encoding and memory in alcohol-exposed infants when the addition of choline was included with regular MVM use [53]. In contrast to our expectations, alterations in fatty acid profile was not directly correlated with MVM supplement use. However, future studies should determine if there are associations with specific micronutrients.

In summary, increasing amounts of reported alcohol intakes throughout pregnancy were associated with differences in maternal plasma fatty acid profiles in the third trimester between AE-ND and AE-FASD mothers. Alterations in fatty acid profiles, in particular increases in C18:0, AA, and C22:5n-6 were observed in moderate to heavy alcohol-consuming mothers with infants classified with FASD, compared to infants exposed to similar amounts of alcohol in utero but with normal development. These results support the concept that maternal alcohol use and smoking can significantly modify plasma fatty acids and further investigation is warranted in determining the potential role of these alterations in the development of FASD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used:

FASD
Fetal Alcohol Spectrum Disorder

MVM
multiple vitamin and mineral supplement

BSID-II
Bayley Scale of Infant Development II

AE-ND
alcohol exposed women and child with normal development

AE-FASD
alcohol exposed women and child with FASD

ozAA
ounces of absolute alcohol

SES
socioeconomic status

MDI
mental development index

PDI
psychomotor development index

SCD
stearoyl-CoA desaturase

GLA
γ-linolenic acid, C18:3n-6

dihomo-γ-linolenic acid
C20:3n-6

docosatetraenoic acid
C22:4n-6

n-3 docosapentaenoic acid
C22:5n-3

n-6 docosapentaenoic acid
C22:5n-6

LA
linoleic acid, C18:2n-6

ALA
alpha linolenic acid, C18:8n-3

ELOVL
elongase of very long chain fatty acid protein

β-ox
beta-oxidation

SREBP
sterol regulatory element binding protein

PG
prostaglandin

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Table 1.

Characteristics of the sample by alcohol use and child neurodevelopment status[1]

	Controls N=46	Alcohol-Exposed mother: Child with Normal Development AE-ND N=33	Alcohol-Exposed mother: Child with FASD AE-FASD N=30	Sig
Maternal/Paternal Characteristics				
Gestational week at enrollment	17.41±0.61[a]	21.14±1.08[b]	19.35±1.32[a,b]	0.020
Gestational week at blood draw	31.65±0.29	30.73±0.84	32.50±0.70	0.148
Site, n (%)				
Rivne	21 (45.7)	30 (90.9)	16 (53.3)	<0.001
Khmelnytsky	25 (54.3)	3 (9.1)	14 (46.7)	
Maternal age (years)	26.09±0.68	24.45±0.87	26.57±1.07	0.218
Maternal educational level, n (%)				
Less than 9 years	0 (0)	1 (3.0)	0 (0)	
9 years (uncompleted high school diploma)	1 (2.2)	2 (6.1)	4 (13.3)	
High school diploma /vocational or trade school;	20 (43.5)	18 (54.5)	20 (66.7)	0.065
College degree or unfinished university education	6 (13.0)	5 (15.2)	1 (3.3)	
University graduate	19 (41.3)	7 (21.2)	5 (16.7)	
Paternal age (years)	27.46±0.75	27.88±0.84	32.21±1.802	0.007
Pre-pregnancy BMI	20.83±0.50[b]	23.28±0.86[a]	21.49±0.70[a,b]	0.030
Socioeconomic Status[2]	39.22±1.50[a]	34.82±1.90[a,b]	30.28±2.35[b]	0.004
MVM Intervention, n (%)				
Standard of care group	22 (47.8)	20 (60.6)	19 (63.3)	
Prenatal MVM group	15 (32.6)	8 (24.2)	4 (13.3)	0.374
Prenatal MVM + choline group	9 (19.6)	5 (15.2)	7 (23.3)	
Maternal Alcohol Use				
Conception				
ozAA/per day	0.00±0.00[a]	0.60±0.08[b]	0.62±0.10[b]	<0.001
ozAA/per drinking day	0.00±0.00[a]	1.56±0.14[b]	1.57±0.22[b]	<0.001
Enrollment				
ozAA/per day	0.00±0.00[a]	0.05±0.01[b]	0.12±0.04[b]	<0.001
ozAA/per drinking day	0.00±0.00[a]	0.31±0.08[b]	0.53±0.13[b]	<0.001
Maternal Smoking				
Smoking status				
Never smoked	40 (87.0)	10 (30.3)	5 (16.7)	
Past smoker (quit before pregnancy)	3 (6.5)	5 (15.2)	2 (6.7)	
Past smoker (quit after pregnant)	1 (2.2)	13 (39.4)	9 (30.0)	<0.001
Current smoker	2 (4.3)	5 (15.2)	14 (46.7)	
Cigarettes per day at enrollment				
All women	0.15±0.10[a]	0.42±0.20[a]	3.97±1.18[b]	<0.001
Smokers only	3.50±0.50	2.80±0.66	8.50±1.93	0.184

	Controls	Alcohol-Exposed mother: Child with Normal Development AE-ND	Alcohol-Exposed mother: Child with FASD AE-FASD	Sig
	N=46	N=33	N=30	
Child Birth Information				
Child's sex, n (%)				
Male	21 (45.7)	14 (42.4)	13 (43.3)	0.956
Female	25 (54.3)	19 (57.6)	17 (56.7)	
Gestational age at birth (weeks)	39.81±0.19[a]	38.85±0.36[a,b]	37.97±0.62[b]	0.003
Birth Weight (g)	3373.70±69.20[a]	3312.81±99.19[a]	2850.43±138.95[b]	0.001
Birth Length (cm)	51.96±0.33[a]	51.63±0.54[a]	48.80±0.98[b]	0.001
Bayley Scale of Infant Development-II				
6 Months MDI	92.15±1.32[b]	96.67±0.92[a]	80.31±2.72[c]	<0.001
PDI	90.39±1.74[b]	99.04±1.65[a]	79.00±2.82[c]	<0.001
Number of infants tested, n (%)	46 (100)	27 (81.8)	26 (86.7)	
12 Months MDI	91.11±1.60[a]	96.89±1.65[a]	80.57±2.54[b]	<0.001
PDI	98.28±1.63[a]	103.82±2.21[a]	89.43±2.92[b]	<0.001
Number of infants tested, n (%)	46 (100)	28 (84.8)	23 (76.7)	

[1] Means in a row with different superscript letters [a,b,c] differ wth a p value <0.05 for post-hoc analysis. For example, a group with a superscript [a] is not statistically different than a different group with [a], but is statistically different than a group with [b] or [c].

[2] Socioeconomic status: based on Hollingshead score calculated from maternal and paternal education and occupation[54].

MVM: multiple vitamin/mineral supplement; ozAA: ounces of absolute alcohol; MDI: mental development index; PDI: psychomotor development index. Missing values are as follows for each group: 1 in the AE-FASD group and 1 in AE-ND group for paternal age; 1 in the AE-FASD group for socioeconomic status; 1 in AE-ND group for pre-pregnancy BMI, and 1 in the AE-ND group for both birth weight and length.

Table 2.

Spearman correlation coefficients of fatty acid composition (% of total fatty acid) and maternal alcohol exposure during pregnancy in all subjects and by alcohol-exposure group.[1]

	14:0	16:0	16:1 n7	18:0	18:1 n9	18:2 n6	20:0	18:3 n6	20:1 n9	18:3 n3	20:2 n6	22:0	20:3 n6	20:4 n6	24:0	20:5 n3	24:1 n9	22:4 n6	22:5 n6	22:5 n3	22:6 n3
All Subjects (n=109)																					
Conception ozAA/day	−0.042	0.064	0.004	0.167	.241*	−0.118	0.096	−.233*	.230*	0.069	−0.099	0.150	−.232*	−0.108	−0.138	−0.059	−0.009	−.277*	−0.097	−0.065	−0.176
Conception ozAA/drinking day	−0.086	−0.006	−0.075	.189*	.194*	−0.080	0.148	−.261*	.249*	0.051	−0.068	.233*	−.231*	−0.055	−0.074	0.017	0.059	−.259*	−0.088	0.000	−0.091
Enrollment ozAA/day	−0.058	.207*	.234*	0.075	0.154	−0.184	−0.030	0.023	0.109	−0.007	−0.129	0.042	−.250*	0.057	−0.104	−0.155	0.019	−0.027	0.142	−0.121	−.219*
Enrollment ozAA/drinking day	−0.099	.202*	.229*	0.060	0.179	−0.184	−0.033	−0.011	0.140	−0.028	−0.113	0.025	−.273*	0.033	−0.124	−0.159	0.042	−0.063	0.114	−0.134	−.219*
Cigarettes/day	−0.017	0.067	.323*	0.055	0.102	−0.131	−0.129	0.087	0.108	0.045	0.176	−0.017	−0.003	0.119	0.019	−.243*	0.134	0.134	.234*	−.257*	−.326*
AE-FASD (n=30)																					
Conception ozAA/day	0.309	0.314	0.118	0.081	0.011	−0.314	0.323	0.125	−0.135	0.221	−0.043	0.042	0.095	−.410*	0.167	−0.207	−0.149	−0.262	−0.308	−0.062	−0.304
Conception ozAA/drinking day	0.271	0.143	−0.021	0.150	0.087	−0.208	.534*	0.021	0.064	0.046	0.200	0.254	0.223	−0.262	0.296	−0.219	−0.072	−0.031	−0.130	−0.138	−0.162
Enrollment ozAA/day	0.299	.413*	.397*	−0.104	0.061	−0.360	−0.073	.412*	−0.069	.370*	−0.243	−0.187	−0.094	−0.023	−0.092	−0.080	−0.189	0.014	−0.011	0.016	−0.266
Enrollment ozAA/drinking day	0.230	.396*	.421*	−0.195	0.194	−.373*	−0.124	.393*	0.074	.388*	−0.171	−0.249	−0.112	−0.106	−0.124	−0.094	−0.124	−0.132	−0.090	−0.073	−0.272
AE-ND (n=33)																					

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	14:0	16:0	16:1 n7	18:0	18:1 n9	18:2 n6	20:0	18:3 n6	20:1 n9	18:3 n3	20:2 n6	22:0	20:3 n6	20:4 n6	24:0	20:5 n3	24:1 n9	22:4 n6	22:5 n6	22:5 n3	22:6 n3
Conception																					
ozAA/day	0.094	.358*	0.144	0.122	0.048	−0.185	0.017	−.446*	0.104	−0.118	−0.046	−0.042	−0.268	−0.324	−0.335	−0.263	−0.204	−0.081	−0.179	−0.119	−0.255
ozAA/drinking day	−0.131	0.030	−.375*	0.216	−0.276	0.045	0.234	−.489*	−0.001	0.043	−0.111	0.280	−0.249	−0.158	0.024	0.202	0.082	−0.222	−0.250	0.307	0.053
Enrollment																					
ozAA/day	−0.245	0.296	0.247	0.116	−0.031	−0.100	−0.038	−0.072	0.050	−0.284	−0.054	0.028	−0.257	0.061	0.006	−.353*	0.184	0.227	0.331	−0.223	−0.275
ozAA/drinking day	−.367*	0.292	0.229	0.121	0.025	−0.112	−0.031	−0.211	0.111	−.361*	−0.077	0.028	−.358*	0.042	−0.058	−.352*	0.247	0.162	0.257	−0.220	−0.256

[1] Results presented as Spearman rank order correlation coefficient (ρ). ozAA: ounces of absolute alcohol

* indicates a p-value <0.05; green indicates a significant positive correlation and red indicates a significant negative correlation.

Table 3.

Plasma fatty acid composition and product to substrate ratios in the third trimester by maternal alcohol exposure and FASD classification controlled for maternal smoking.

	Controls N=46	AE-ND N=32	AE-FASD N=29	Sig		Controls N=46	AE-ND N=32	AE-FASD N=30	Sig
Saturated					Desaturation				
C14:0	1.09±0.07	0.97±0.09	1.04±0.09	0.633	16:1n7/16:0	0.05±0.00	0.05±0.00	0.05±0.00	0.614
C16:0	25.93±0.39	25.02±0.47	26.26±0.50	0.197	18:1n9/18:0	3.03±0.07^{a,b}	3.26±0.09^{a}	2.80±0.10^{b}	0.005
C18:0	6.16±0.11^{a}	6.20±0.14^{b}	6.91±0.15^{b}	<0.001	18:3n6/18:2n6	0.005±0.00	0.004±0.00	0.005±0.01	0.039
C20:0	0.12±0.01	0.12±0.01	0.13±0.01	0.604	20:4n6/20:3n6	3.01±0.18^{a}	3.45±0.22^{a}	4.34±0.23^{b}	<0.001
C22:0	0.19±0.01	0.20±0.01	0.21±0.01	0.487	Elongation				
C24:0	0.16±0.01	0.15±0.01	0.15±0.01	0.714	18:0/16:0	0.24±0.01	0.25±0.01	0.27±0.01	0.067
Monounsaturated					22:4n6/20:4n6	0.053±0.00^{a}	0.044±0.00^{b}	0.045±0.00^{a,b}	0.021
C16:1n7	1.36±0.09	1.20±0.11	1.40±0.12	0.442	22:5n3/20:5n3	1.49±0.11	1.27±0.13	1.41±0.14	0.459
C18:1n9	18.44±0.37	19.89±0.46	19.11±0.49	0.061	Fatty Acid Ratios				
C20:1n9	0.21±0.01^{a}	0.26±0.01^{b}	0.21±0.01^{a,b}	0.028	16:0/18:2n6	0.78±0.03	0.73±0.03	0.83±0.04	0.140
C24:1n9	0.26±0.02	0.21±0.03	0.22±0.03	0.433	18:2n6/18:3n3	190.15±11.76	181.96±14.46	190.90±15.48	0.894
Omega-6					20:4n6/20:5n3	32.29±3.37	26.77±4.14	39.93±4.43	0.122
C18:2n6	34.68±0.71	35.06±0.87	32.60±0.93	0.141	22:5n6/22:6n3	0.11±0.01^{a}	0.09±0.01^{a}	0.17±0.02^{b}	0.002
C18:3n6	0.16±0.01^{a}	0.13±0.01^{b}	0.15±0.01^{a,b}	0.034	22:5n6/22:4n6	0.82±0.04^{a}	0.82±0.05^{a}	1.15±0.05^{b}	<0.001
C20:2n6	0.44±0.03	0.39±0.03	0.35±0.03	0.109	22:6n3/22:5n3	8.48±0.30	7.84±0.37	8.59±0.40	0.333
C20:3n6	1.63±0.07^{a}	1.41±0.09^{a,b}	1.29±0.10^{b}	0.022	Omega 6:Omega 3	17.38±0.89	16.98±1.09	17.99±1.17	0.833
C20:4n6	4.59±0.16^{a}	4.50±0.20^{a}	5.30±0.21^{b}	0.017					
C22:4n6	0.24±0.01^{a}	0.20±0.01^{b}	0.23±0.01^{a,b}	0.024					
C22:5n6	0.20±0.01^{a}	0.16±0.02^{a}	0.27±0.02^{b}	0.001					
Omega-3									
C18:3n3	0.22±0.01	0.21±0.02	0.19±0.02	0.637					
C20:5n3	0.21±0.04	0.32±0.05	0.27±0.05	0.250					
C22:5n3	0.24±0.01	0.26±0.02	0.22±0.02	0.380					
C22:6n3	1.95±0.09	1.99±0.11	1.85±0.12	0.717					
Total Fatty Acids (μg/ml)	4753.96±147.06	4685.80±180.76	4419.61±193.49	0.411					

Estimated marginal means ± standard error of mean (SEM) by general linear model. Individual plasma fatty acids are presented as a percent of total fatty acids. Means in a row with different superscript letters differ with a p-value < 0.05. Models were adjusted for SES, site, pre-pregnancy BMI, cigarettes smoked per day at enrollment, and gestational age at blood draw. One subject in the AE-ND group had a missing variable for pre-pregnancy BMI and one subject in the AE-FASD group had a missing variable for SES thus were not included in this analysis.

Table 4.

Plasma fatty acid composition and product to substrate ratios in the third trimester by maternal alcohol and smoking status[1]

	Control N=44	AE-only N=44	AE+Smoker N=17	Sig		Control N=44	AE-only N=44	AE+Smoker N=17	Sig
Saturated					Desaturation				
C14:0	1.11±0.08	0.99±0.08	1.05±0.12	0.554	16:1n7/16:0	0.05±0.00[a,b]	0.04±0.00[a]	0.07±0.00[b]	<0.001
C16:0	26.04±0.38[a,b]	25.13±0.38[a]	27.05±0.59[b]	0.023	18:1n9/18:0	3.00±0.08	3.00±0.08	3.19±0.12	0.380
C18:0	6.23±0.12	6.53±0.12	6.47±0.19	0.244	18:3n6/18:2n6	0.005±0.00[a,b]	0.004±0.00[a]	0.006±0.00[b]	0.006
C20:0	0.13±0.01	0.12±0.01	0.12±0.01	0.779	20:4n6/20:3n6	2.98±0.18[a]	3.94±0.18[b]	3.69±0.28[a,b]	0.002
C22:0	0.19±0.01	0.21±0.01	0.19±0.02	0.589	Elongation				
C24:0	0.16±0.01	0.15±0.01	0.15±0.01	0.787	18:0/16:0	0.24±0.01	0.26±0.01	0.24±0.01	0.092
Monounsaturated					22:4n6/20:4n6	0.05±0.00[a]	0.04±0.00[b]	0.05±0.00[a,b]	0.005
C16:1n7	1.30±0.09[b]	1.11±0.09[b]	1.92±0.14[a]	<0.001	22:5n3/20:5n3	1.49±0.11[a,b]	1.20±0.11[a]	1.70±0.17[b]	0.029
C18:1n9	18.53±0.37[a]	19.21±0.37[a,b]	20.32±0.58[b]	0.040	Fatty Acid Ratios				
C20:1n9	0.21±0.01	0.23±0.01	0.23±0.02	0.309	16:0/18:2n6	0.78±0.03[a,b]	0.74±0.03[a]	0.89±0.04[b]	0.008
C24:1n9	0.26±0.02	0.21±0.02	0.25±0.04	0.415	18:2n6/18:3n3	193.46±11.71	191.41±11.60	169.28±18.20	0.511
Omega-6					20:4n6/20:5n3	32.20±3.37[a,b]	27.91±3.34[a]	46.97±5.24[b]	0.011
C18:2n6	34.39±0.70[a,b]	34.75±0.69[a]	31.46±1.09[b]	0.035	22:5n6/22:6n3	0.11±0.01[a]	0.11±0.01[a]	0.19±0.02[b]	<0.001
C18:3n6	0.16±0.01[a,b]	0.13±0.01[a]	0.17±0.01[b]	0.014	22:5n6/22:4n6	0.82±0.05[a]	0.93±0.04[a,b]	1.01±0.07[b]	0.006
C20:2n6	0.44±0.03	0.35±0.03	0.41±0.04	0.084	22:6n3/22:5n3	8.42±0.31	8.14±0.31	8.38±0.48	0.810
C20:3n6	1.64±0.07[a]	1.29±0.07[b]	1.55±0.11[a,b]	0.006	Omega 6:Omega 3	17.13±0.89	16.63±0.88	19.92±1.39	0.130
C20:4n6	4.60±0.17	4.87±0.17	4.93±0.27	0.461					
C22:4n6	0.24±0.01[a]	0.20±0.01[b]	0.24±0.02[a,b]	0.023					
C22:5n6	0.19±0.02[a]	0.19±0.02[a]	0.27±0.02[b]	0.013					
Omega-3									
C18:3n3	0.20±0.01	0.20±0.01	0.21±0.02	0.993					
C20:5n3	0.22±0.04[a,b]	0.35±0.04[a]	0.14±0.06[b]	0.010					
C22:5n3	0.24±0.01[a,b]	0.26±0.01[a]	0.19±0.02[b]	0.029					
C22:6n3	1.95±0.09[a]	2.06±0.09[a]	1.51±0.14[b]	0.005					
Total Fatty Acids (μg/ml)	4670.39±144.92	4599.47±146.61	4505.06±255.17	0.828					

[1] Estimated marginal means ± standard error of mean (SEM) by general linear model. Individual plasma fatty acids are presented as a percent of total fatty acids. Means in a row with different superscript letters differ with a p value < 0.05. Models were adjusted for SES, site, pre-pregnancy BMI, and gestational age at blood draw. Two subjects in the AE+Smoker group had a missing variable for pre-pregnancy BMI thus were not included in this analysis.