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Alcohol Consumption in Pregnant, Black Women Is Associated With Decreased Plasma and Erythrocyte Docosahexaenoic Acid

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Background: Inner-city, black women are among those groups that are at higher risk for having infants with fetal alcohol spectrum disorders that can include life-long neurobehavioral and cognitive impairments. Chronic alcohol consumption can decrease amounts of docosahexaenoic acid (DHA), a fatty acid that is essential for optimal infant neural and retinal development in a variety of tissues.

Methods: Black women who presented at an inner-city antenatal clinic for their first prenatal visit were recruited into a longitudinal, observational study. Alcohol intake was determined by a structured interview. Participants provided blood specimens and completed food frequency surveys at 24 weeks of gestation, infant delivery, and 3 months postpartum. Fatty acid composition analyses were completed on 307, 260, and 243 for plasma and 278, 261, and 242 erythrocyte specimens at 24 weeks of gestation, delivery, and 3 months postpartum, respectively.

Results: Proportion of drinking days at the first prenatal visit was associated with decreased DHA in plasma and erythrocytes throughout the study. This association was the strongest at 24 weeks of gestation. In addition, an interaction between proportion of drinking days at the time of conception and ounces of absolute alcohol per drinking day at the time of conception was detected and demonstrated that, in daily drinkers, high intakes of alcohol are associated with decreased DHA and arachidonic acid (AA) concentrations in plasma.

Conclusions: Frequent and high intakes of alcohol that have been previously associated with fetal alcohol spectrum disorders are also associated with decreased maternal DHA and AA plasma concentrations. The present findings indicate that maternal DHA deficiency is associated with high-risk drinking and may contribute to the mechanism(s) of alcohol-related neurodevelopmental disorders.

Key Words: Fatty Acids, Fetal Alcohol Syndrome, Prenatal Alcohol, Arachidonic Acid, Docosahexaenoic Acid, Black Women.

THE INCIDENCE OF fetal alcohol spectrum disorders (FASD) has been liberally estimated to be almost 1 in 100 births in the United States (Sampson et al., 1997). Recent estimates by the Centers for Disease Control and Prevention (CDC) put the rate of fetal alcohol syndrome (FAS), the most severe expression of FASD, between 0.2 and 1.5 per 1000 live births (CDC, 2002) and emphasize that the variable rate reflects higher sensitivities in certain populations, including urban, rural, and low socioeconomic status groups (CDC, 2002). It was estimated recently that ~30% of low-income black women consume alcohol postconception (O'Connor and Whaley, 2003). The effects and prevention challenges of FASD and the identification of risk drinking during pregnancy have been reviewed (Hannigan and Armant, 2000; Sokol et al., 2003). Briefly, children who are exposed prenatally to alcohol via maternal risk drinking, particularly averaging more than one drink per day or more than five drinks per drinking episode, are subject to a variety of neurobehavioral and cognitive impairments. There are several proposed mechanisms for the effects of prenatal alcohol intake on central nervous system (CNS) maturation leading to neurobehavioral and cognitive deficits (Michaelis, 1990; Randall et al., 1990; Schenker et al., 1990) and include possible alcohol-mediated alterations in polyunsaturated fatty acid (PUFA) metabolism and PUFA deposition into the fetal CNS (Denkins et al., 2000).

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Docosahexaenoic acid (DHA; 22:6n-3) and arachidonic acid (AA; 20:4n-6) comprise a significant proportion of structural brain fatty acids (O'Brien and Sampson, 1965). DHA in particular has been demonstrated to be essential for optimal infant neural and retinal development (Larque et al., 2002; Salem et al., 2001; Uauy et al., 2003). Low consumption of fish and/or n-3 PUFAs are also associated with increased risk for preterm delivery and low infant birth weight (Olsen and Secher, 2002) and with increased risk for postpartum depression (Hibbeln, 2002; Otto et al., 2003). Even in the absence of alcohol, maternal DHA status may be compromised during pregnancy (Al et al., 1995; Wijendran et al., 1999) as a result of the demands of specific placental nutrient transfer (Dutta-Roy, 2000; Haggarty, 2002) and high fetal accretion of DHA during pregnancy (Clandinin et al., 1980).

Alcohol consumption tends to promote the loss of DHA and AA in various tissues in humans and animals (Salem, 1989; Salem and Ward, 1993). There is evidence suggesting that alcohol can inhibit (Nakamura et al., 1994; Narce et al., 2001; Nervi et al., 1980; Wang and Reitz, 1983) and/or stimulate PUFA synthesis (Narce et al., 2001; Pawlosky and Salem, 1999) and that alcohol can enhance PUFA catabolism through increased lipid peroxidation (Ma et al., 1993; Pawlosky and Salem, 1999). Discrepancies in the effects and possible mechanisms can be accounted for by differences in the amount and/or pattern of alcohol consumption (i.e., quantity, duration, frequency, and binging patterns of exposure) and are further complicated by differences in dietary fatty acid intakes. In addition, there is evidence suggesting that sex/hormonal influences may influence alcohol-mediated effects on PUFA status (Kuriki et al., 2003) and metabolism (Ma et al., 1993), suggesting that pregnancy itself can alter PUFA metabolism (Al et al., 1995; Stark et al., 2004).

Because decreased maternal DHA and AA may be involved in the risk for alcohol-related neurodevelopmental disorders, the primary purpose of the present study was to examine the influence of alcohol consumption on plasma and erythrocyte fatty acid composition in pregnant, inner-city, black women. This longitudinal, observational study required participants to provide detailed alcohol consumption information at recruitment and was followed by blood specimen collection and completion of food frequency surveys at 24 weeks of gestation, infant delivery, and 3 months postpartum. The present study is the only study to date to examine the influence of maternal alcohol consumption on specific fatty acids during pregnancy and postpartum while controlling for the corresponding dietary fatty acid intake.

MATERIALS AND METHODS

Participants and Study Design

The Wayne State University Human Investigations Committee approved all procedures and protocols before the commencement of the study, and informed consent was obtained during the initial clinical visit. Pregnant, black women who presented to the antenatal clinic of Wayne State University (Detroit, MI) between February 1999 and January 2001 were candidates for this observational study. A structured interview at the first antenatal visit assessed demographic characteristics, alcohol intake, and smoking exposure and determined eligibility (Jacobson et al., 1993). Exclusions included women with high-risk pregnancies, known fatty acid metabolism disorders, and diabetes and women who developed gestational diabetes. All women who reported intakes of ≥0.5 oz of absolute alcohol per day at the time of conception were recruited into the study, plus a random 8% sample of the remaining patients. This selection strategy oversampled the high-risk drinking women (Jacobson et al., 1991, 1993), and the final enrollment at 24 weeks of gestation was 338 women. Maternal fasting blood samples (15 ml) were collected by venipuncture at 24 weeks of gestation, infant delivery, and 3 months postpartum. Specimens were collected into heparinized tubes, kept cold (4°C) until centrifuged (5 min at 2000*g*) to separate plasma and erythrocytes, and frozen at −75°C until analysis.

Alcohol consumption was determined at the structured antenatal interview by a validated 14-day recall method that generated estimates as ounces of absolute alcohol per day (AAD), ounces of absolute alcohol per drinking day (AADD), and proportion of drinking days (PROPDD). These alcohol intakes were estimated from the time of conception (AAD-0, AADD-0, and PROPDD-0) and at the first prenatal visit (AAD-1, AADD-1, and PROPDD-1). At-risk drinking was also determined by several proven screening tests, including the Michigan Alcoholism Screening Test (MAST) and the Tolerance, Annoyed or Angry, Cut down or quit, Eye opener (T-ACE) questionnaire (Bradley et al., 1998; Ernhart et al., 1988; Russell et al., 1996). Cigarette smoking by the mother around the time of conception and at the first prenatal visit, and smoking by the father were determined by maternal recall of the number of cigarettes smoked per day. A modified Hollingshead index was used to measure socioeconomic status (Hollingshead, 1971). Nutritional intakes at each time point was assessed using a food frequency survey validated for low-income pregnant women (Suitor et al., 1989) and modified to quantify selected dietary fats. Individual nutrient intakes were adjusted for total energy intake to reduce measurement error (Subar et al., 2001) using the nutrient residual model (Willett and Stampfer, 1986).

Laboratory Analyses

Fatty acid composition analyses were completed successfully on 307 plasma and 278 erythrocyte specimens at 24 weeks gestation, 260 plasma and 261 erythrocyte specimens at the time of delivery, and 243 plasma and 242 erythrocyte specimens at 3 months postpartum. Total lipids were extracted from plasma samples (Folch et al., 1957) and from erythrocytes (Reed et al., 1960) with an internal standard (23:0 or 22:3n-3; NuCheck Prep, Elysian, MN). Lipid extracts then were methylated with boron trifluoride in methanol (14% wt/vol; Alltech Assoc., Deerfield, IL) (Morrison and Smith, 1964), and fatty acid methyl esters were collected and analyzed by capillary gas chromatography (Salem et al., 1996) on an Agilent 6890N gas chromatograph (Agilent, Palo Alto, CA) with a 0.25-mm × 30-m DB-FFAP column (J&W Scientific, La Palma, CA).

Statistical Analyses

All statistical analyses were completed with SPSS for Windows statistical software (release 11.5.1; SPPS, Chicago, IL). Multiple linear regression analyses at each time point and linear mixed modeling including all three time points were completed for selected fatty acids and alcohol consumption variables with and without adjusting for potential confounders. When corresponding adjusted dietary fatty acid data were available, they was included as a covariate to control for possible differences in intakes. Categorical analyses were also completed. PROPDD-1 was categorized into three groups: abstainers during pregnancy (PROPDD-1 = 0), occasional drinkers during pregnancy (less than one drinking day per week, PROPDD-1 <0.14) and habitual drinkers during pregnancy (greater than one drinking day per week, PROPDD-1 ≥0.14). The General Linear Model procedure was used with multiple comparisons by Tukey's honestly significant difference (HSD) tests to compare demographic characteristics, dietary fatty acid intakes, and plasma and erythrocyte fatty acid compositions according to PROPDD-1 categories. Also, the proportion of drinking days (PROPDD-0) and ounces of absolute

alcohol per drinking day at the time of conception (AADD-0) were categorized into abstainers (PROPDD-0 = 0), sporadic drinkers (PROPDD-0 <1), and daily drinkers (PROPDD-0 = 1) and low- (AADD-0 <2), medium- (AADD-0 = 2–6), and high- (AADD-0 >6) level alcohol intake groups. Linear mixed modeling was used to examine selected fatty acid concentrations in plasma with PROPDD-0, AADD-0, and time (stage of pregnancy) set as fixed factors with interactions and with corresponding fatty acid intakes as a covariate. Tukey's HSD tests were used to compare individual means when a significant F value was detected. Significance for all tests was set at $p < 0.05$.

RESULTS

Alcohol Consumption Effects on DHA and AA

The influence of alcohol consumption on DHA and AA in plasma and erythrocytes was examined by linear mixed modeling across all time points and by linear regression at individual time points, with and without adjustment for potential confounders. The potential confounding variables included in the analyses were mother's age, education, smoking, body mass index (BMI), total energy intake, and adjusted dietary intake of the corresponding fatty acid. Maternal parity and socioeconomic status were also entered confounders. However, maternal parity was highly correlated to mother's age ($r = 0.60, p < 0.001$), and socioeconomic status was significantly correlated to education ($r = 0.51, p < 0.001$) and resulted in multicollinearity in the model. Parity and socioeconomic status were excluded from the model as mother's age and education were more highly correlated with DHA and AA.

Linear mixed modeling from 24 weeks' gestation to 3 months postpartum for plasma and erythrocyte DHA (Table 1) and AA (Table 2) indicated a negative association with PROPDD-1 and significant associations with several demographic characteristics. Interactions between PROPDD-1 and time were not detected in the analyses. Highly significant associations involving time ($p < 0.001$) were detected for both DHA and AA. Pregnancy (24 weeks' gestation and delivery) were significantly associated with increased DHA status and with decreased weight percentage (wt %) of AA in both plasma and erythrocytes as compared with 3 months postpartum. Elevations in DHA during pregnancy have been observed previously (Al et al., 1995; Makrides and Gibson, 2000; Marangoni et al., 2002; Otto et al., 2001a,b; Wijendran et al., 1999). The effects of pregnancy on DHA and AA status in the

Table 1. Results of Linear Mixed Models to Determine the Association of PROPDD-1 on Plasma and Erythrocyte DHA Throughout Pregnancy to Postpartum[a]

	Plasma DHA (μg/ml)		Plasma DHA (wt %)		Erythrocyte DHA (wt %)	
	β	p	β	p	β	p
Age (years)	0.74	<0.001	0.007	0.011	0.015	<0.001
Education (highest grade)	1.03	0.041	0.037	0.001	0.034	0.048
Father's smoking (cigarettes/day)	−0.25	<0.001	−0.002	0.14	−0.004	0.08
BMI (kg/m^2)	0.18	0.033	0.005	0.014	0.011	<0.001
Total energy (MJ)	−0.62	<0.001	−0.008	0.018	−0.017	0.001
Adjusted dietary DHA (g/day)	46.87	<0.001	0.72	<0.001	0.84	0.004
PROPDD-1	−38.57	0.024	−0.60	0.12	−1.97	0.001
Time						
24 wk gestation	31.80	<0.001	0.36	<0.001	0.74	<0.001
Delivery	23.69	<0.001	0.33	<0.001	0.85	<0.001
3 months postpartum[b]	0	—	0	—	0	—

[a] Based on total lipid extracts. Number of deliveries, socioeconomic status, and mother's smoking were considered as covariates but resulted in multicollinearity as they correlated with mother's age and education and father's smoking, respectively. Mother's age and education and father's smoking were more highly correlated with blood DHA measures.

[b] Reference value set to zero for comparison with other time points.

Table 2. Results of Linear Mixed Models to Determine the Association of PROPDD-1 on Plasma and Erythrocyte AA Throughout Pregnancy to Postpartum[a]

	Plasma AA (μg/mL)		Plasma AA (wt %)		Erythrocyte AA (wt %)	
	β	p	β	p	β	p
Age (years)	3.69	<0.001	0.025	0.012	0.032	<0.001
Education (highest grade)	0.03	0.99	0.081	0.042	−0.069	0.015
Father's smoking (cigarettes/day)	−0.82	0.008	0.001	0.82	0.005	0.20
BMI (kg/m^2)	0.96	0.011	0.027	<0.001	0.007	0.12
Total energy (MJ)	−1.68	0.009	−0.008	0.46	−0.001	0.90
Adjusted dietary AA (g/day)	108.94	0.008	1.80	0.015	1.00	0.06
PROPDD-1	−13.30	0.86	0.072	0.96	−1.74	0.07
Time						
24 wk gestation	21.47	0.004	−1.59	<0.001	−1.44	<0.001
Delivery	−19.58	0.010	−1.84	<0.001	−1.47	<0.001
3 months postpartum[b]	0	—	0	—	0	—

[a] Based on total lipid extracts. Number of deliveries, socioeconomic status, and mother's smoking were considered as covariates but resulted in multicollinearity as they correlated with mother's age and education and father's smoking, respectively. Mother's age and education and father's smoking were more highly correlated with blood AA measures.

[b] Reference value set to zero for comparison with other time points.

present study are presented in detail elsewhere (Stark et al., 2004).

There were significant negative correlations of drinking frequency as PROPDD-1 with DHA concentrations in plasma [$\beta = -38.57$; 95% confidence interval (CI): −72.11 to −5.04] and with the relative percentage of DHA levels in erythrocytes ($\beta = -1.97$; 95% CI: −3.12 to −0.82) but not with the relative percentage of DHA levels in plasma ($\beta = -0.60$; 95% CI: −1.36 to 0.17; see Table 1). Total energy intake was also negatively correlated with DHA in plasma and erythrocytes. Curiously, exposure to tobacco smoke as measured by the father's smoking was significantly and negatively correlated with maternal plasma concentrations of DHA ($\beta = -0.25$; 95% CI: −0.39 to −0.11) and approached significance for the relative percentage of DHA levels in maternal erythrocytes ($\beta = -0.004$; 95% CI: −0.009 to 0.0005). However, mother's smoking was not correlated to DHA blood status. Father's smoking in the present study has been previously demonstrated to be a better predictor of nutrient concentrations (i.e., plasma 5-methyltetrahydrofolic acid) in maternal plasma than mother's smoking (Stark et al., 2005). Maternal age, education attained, BMI, and adjusted dietary DHA intake all were significantly and positively correlated with DHA in plasma (μg/ml and wt %) and in erythrocytes (wt %).

The correlation results for AA (Table 2) were not as consistent in plasma and erythrocytes but shared some similarities with DHA. Although PROPDD-1 levels were not significantly correlated with plasma AA levels, the correlation in erythrocytes approached significance ($\beta = -1.74$; 95% CI: −3.62 to 0.15). Maternal age, BMI, and dietary AA intake all were positively correlated with plasma concentrations and weight percentages of AA. In erythrocytes, maternal age was also positively correlated with weight percentage of AA, whereas dietary AA intake just failed to reach significance ($\beta = 1.00$; 95% CI: −0.02 to 2.03). Total energy intake and father's smoking were negatively correlated with AA concentrations in plasma but were not significantly correlated with AA percentages in plasma and erythrocytes.

The correlations of the various alcohol consumption parameters with DHA and AA were examined at each time point by linear regression. There were significant correlations between PROPDD-1 and DHA wt % in plasma and in erythrocytes at 24 weeks' gestation (Table 3). DHA content in plasma was negatively correlated with PROPDD-1 before ($\beta = -1.00$; 95% CI: −1.97 to −0.03) and after adjusting for potential confounders ($\beta = -1.56$; 95% CI: −2.78 to −0.34). The negative correlation between DHA (wt %) and PROPDD-1 became stronger in erythrocytes with $\beta = -1.81$ (95% CI: −3.52 to −0.09) before and $\beta = -2.81$ (95% CI: −4.49 to −1.12) after adjustment. There were no other consistent and significant correlations (i.e., significant correlation remains after adjusting for confounders) between either DHA or AA with alcohol consumption parameters as determined by linear regression (data not shown).

Maternal Demographics, Clinical Characteristics, and Dietary Intakes

On the basis of these linear regression and linear mixed modeling analyses, maternal demographics, clinical characteristics, and dietary intakes and fatty acid analyses at 24 weeks of gestation were categorized by PROPDD-1 to allow mean comparisons between abstainers during pregnancy (PROPDD-1 = 0), occasional drinkers during pregnancy (PROPDD-1 <0.14), and habitual drinkers during pregnancy (PROPDD-1 >0.14).

Demographics and clinical characteristics of the women in the present study are presented according to PROPDD-1 categories in Table 4. The occasional and habitual drinkers were significantly older and as such also had a greater total number of pregnancies and deliveries. The occasional and habitual drinkers tended to smoke more cigarettes, score higher on at-risk drinking screening tests, and report greater alcohol consumption at the time of conception and at the first prenatal visit. There were no statistical differences in height, body

Table 3. Results of Linear Regression Models Determining the Association of Alcohol Consumption Parameters on Plasma and Erythrocyte DHA at 24 Weeks' Gestation

	Plasma DHA (wt %)		Erythrocyte DHA (wt %)	
	β	95% CI	β	95% CI
Unadjusted				
AAD-0	−0.01	−0.03 to 0.02	−0.02	−0.05 to 0.01
AADD-0	0.001	−0.02 to 0.02	0.002	−0.03 to 0.03
PROPDD-0	−0.09	−0.25 to 0.08	−0.12	−0.36 to 0.12
AAD-1	−0.10	−0.50 to 0.29	−0.43	−1.30 to 0.44
AADD-1	0.002	−0.05 to 0.05	−0.01	−0.09 to 0.08
PROPDD-1	−1.00[b]	−1.97 to −0.03	−1.81[b]	−3.52 to −0.09
Adjusted[a]				
AAD-0	−0.01	−0.03 to 0.02	−0.02	−0.05 to 0.01
AADD-0	0.000	−0.02 to 0.02	−0.002	−0.03 to 0.02
PROPDD-0	−0.10	−0.27 to 0.08	−0.16	−0.41 to 0.08
AAD-1	−0.14	−0.57 to 0.30	−0.87[b]	−1.72 to −0.01
AADD-1	−0.01	−0.06 to 0.04	−0.06	−0.14 to 0.03
PROPDD-1	−1.56[b]	−2.78 to −0.34	−2.81[b]	−4.49 to −1.12

[a] Adjusted for mother's age, education, smoking, BMI, total energy intake, and corresponding dietary fatty acid intake. Number of deliveries and socioeconomic status were considered as covariates but resulted in multicollinearity as they correlated with mother's age and education, respectively. Mother's age and education were more highly correlated with blood DHA measures.

[b] Significance detected at $p < 0.05$.

weight, BMI, education, socioeconomic status, and father's smoking. Energy-adjusted dietary intakes at 24 weeks of gestation broken down by frequency of alcohol consumption categories, according to PROPDD-1, are presented in Table 5. There were no statistical differences with increasing PROPDD-1 except that the intake of stearic acid (18:0) was decreased with increased PROPDD-1 ($p < 0.05$). DHA intakes (estimated marginal means ± SEM) were 68 ± 5 mg/day in the abstainers, 67 ± 12 mg/day in the occasional drinkers, and 90 ± 30 mg/day in the habitual drinkers, whereas AA intakes were 104 ± 4, 110 ± 10, and 130 ± 26 mg/day, respectively. Despite that the mean intakes of DHA and AA seemed to be higher in the habitual drinkers, they were not significantly different; however, there is possibility of a type II statistical error as there are a low number of habitual drinkers in this study ($n = 8$).

Table 4. Characteristics of Women According to the PROPDD-1[a]

	Abstainers PROPDD-1 = 0 (n = 254)	Occasional PROPDD-1 < 0.14 (n = 45)	Habitual PROPDD-1 ≥ 0.14 (n = 8)
Age (years)	24.2 ± 0.3[1]	27.9 ± 0.8[2]	30.5 ± 1.9[2]
Height (m)	1.65 ± 0.01	1.65 ± 0.01	1.65 ± 0.03
Body weight (kg)			
Prepregnancy	73.8 ± 1.4	81.7 ± 3.3	72.6 ± 8.1
First prenatal visit	80.8 ± 1.4	86.2 ± 3.4	72.5 ± 8.3
BMI (kg/m^2)			
Prepregnancy	27.3 ± 0.5	29.9 ± 1.2	26.6 ± 2.9
First prenatal visit	29.7 ± 0.5	31.5 ± 1.2	26.4 ± 2.9
Education (highest grade)	11.7 ± 0.1	11.7 ± 0.2	11.5 ± 0.5
SES (Hollingshead class)	3.9 ± 0.1	4.0 ± 0.2	4.5 ± 0.4
Total number of pregnancies	3.4 ± 0.1[1]	5.0 ± 0.3[2]	7.0 ± 0.8[2]
Total number of deliveries	1.5 ± 0.1[1]	2.2 ± 0.3[2]	4.0 ± 0.6[3]
Smoking (cigarettes/day)			
Prepregnancy	5.5 ± 0.5[1]	9.8 ± 1.3[2]	11.1 ± 3.0[1,2]
First prenatal visit	5.4 ± 0.5[1]	10.0 ± 1.3[2]	11.1 ± 3.0[1,2]
Father's smoking	6.0 ± 0.6	9.5 ± 1.5	9.3 ± 3.6
At-risk drinking screening			
MAST	2.80 ± 0.44[1]	6.22 ± 1.04[2]	8.50 ± 2.46[1,2]
CAGE	0.60 ± 0.07[1]	0.96 ± 0.15[1,2]	1.50 ± 0.37[2]
NET	1.15 ± 0.07[1]	1.84 ± 0.17[2]	2.50 ± 0.41[2]
T-ACE	1.40 ± 0.09[1]	2.40 ± 0.21[2]	2.75 ± 0.51[2]
TWEAK	1.64 ± 0.10[1]	2.69 ± 0.25[2]	2.63 ± 0.59[1,2]
Alcohol consumption			
AAD-0	1.09 ± 0.13[1]	1.30 ± 0.31[1,2]	3.06 ± 0.74[2]
AADD-0[b]	2.42 ± 0.17	2.87 ± 0.40	4.68 ± 0.96
PROPDD-0	0.30 ± 0.02[1]	0.44 ± 0.04[2]	0.70 ± 0.13[2]
AAD-1	0[1]	0.12 ± 0.01[2]	0.52 ± 0.03[3]
AADD-1	0[1]	1.65 ± 0.11[2]	2.59 ± 0.26[3]
PROPDD-1	0[1]	0.07 ± 0.003[2]	0.24 ± 0.008[3]

SES, socioeconomic status.

[a] Values are estimated marginal means ± SEM of total lipid extract. Means with different superscripts are significantly different across PROPDD-1 categories by Tukey's HSD after significance detected by General Linear Model procedure, $p < 0.05$.

[b] Significance detected by General Linear Model procedure, but no differences detected by Tukey's HSD.

MAST, Michigan Alcoholism Screening Test; CAGE, Cut down or quit, Annoyed or Angry, Guilty, Eye opener; NET, Normal, Eye opener, Tolerance; T-ACE, Tolerance, Annoyed or Angry, Cut down or quit, Eye opener; TWEAK, Tolerance, Worried, Eye opener, Amnesia, Cut down.

Fatty Acid Composition of Maternal Plasma and Erythrocytes

The fatty acid percentage compositions at 24 weeks' gestation for the abstainers and occasional and habitual drinkers are shown according to the PROPDD-1 categories in Tables 6 and 7 for plasma and erythrocytes, respectively. Adjusted dietary fatty acid intakes were included as a covariate with the corresponding plasma and erythrocyte fatty acid when available. Plasma DHA levels were significantly lower in the habitual drinkers (1.34 ± 0.15, wt %) as compared with the abstainers (1.73 ± 0.03, wt %) but were not significantly different from the levels in the occasional drinkers (1.70 ± 0.06, wt %). In erythrocytes, the DHA concentrations in the habitual drinkers (3.23 ± 0.25, wt %) were significantly lower than both the abstainers (3.98 ± 0.04, wt %) and the occasional drinkers (3.96 ± 0.09, wt %). AA (wt %) did not differ as a function of alcohol consumption frequency in either plasma or erythrocytes.

Total n-3 PUFAs and total n-3 highly unsaturated fatty acids (≥20 carbons, ≥3 double bonds) in habitual drinkers were significantly lower as compared with the abstainers and occasional drinkers in both plasma (~16% and ~20% lower, respectively) and erythrocytes (~13% and ~16% lower, respectively). In addition, total PUFAs was significantly lower in habitual drinkers as compared with abstainers (~9% lower in plasma and ~4% lower in erythrocytes) but not different from that of occasional drinkers. Total n-6 PUFAs and linoleic acid (LA; 18:2n-6) in plasma were decreased in the habitual drinkers as compared with the abstainers. In erythrocytes, eicosapentaenoic acid (EPA; 20:5n-3) and eicosadienoic acid (20:2n-6) in habitual drinkers were significantly lower than in abstainers but not in occasional drinkers. Also, the occasional and habitual drinkers had lower levels of docosapentaenoic acid n-3 (DPA n-3; 22:5n-3) and gamma-linolenic acid (18:3n-6) as compared with abstainers, but significance was not reached for the habitual drinkers. Total monounsaturated fatty acids and oleic acid (18:1n-9) were increased for both drinking groups in plasma and only significantly so for the habitual drinkers in erythrocytes. Eicosenic acid (20:1n-9) and lignoceric acid (24:0) were also increased in the plasma of the habitual drinkers as compared with the other groups. Total saturated fatty acids and palmitic acid were significantly greater in the erythrocytes of the habitual drinkers compared with the abstainers.

Effects of Increased Alcohol Consumption on Plasma PUFAs

These results indicate that the frequency of alcohol consumption as indicated by PROPDD-1 has an influ-

ence on PUFA levels in pregnant, black women. Significant interactions between AADD-0 and PROPDD-0 at the time of conception were detected by linear mixed modeling analysis across the three time points. Significant AADD-0 × PROPDD-0 interactions were detected for plasma concentrations of DHA ($p = 0.004$), AA ($p = 0.027$), and α-LNA (18:3n-3; $p = 0.030$) but not for concentrations of LA ($p = 0.44$), EPA ($p = 0.19$), and DPA n-6 ($p = 0.24$). There were no significant interactions involving time, although main effects of time were detected for all of the above-mentioned fatty acids. To aid in further interpreting the results, we categorized the proportion of drinking days variable into abstainers (PROPDD-0 = 0), sporadic drinkers (PROPDD-0 <1), and daily drinkers (PROPDD-0 = 1) and further categorized the variable describing the amount of absolute alcohol drunk per drinking day into low (AADD-0 <2), medium (AADD-0 = 2–6), and high groups (AADD-0 >6). In this categorical analysis, the interaction between AADD-0 and PROPDD-0 remained significant only for DHA ($p = 0.028$) and AA ($p = 0.010$) plasma concentrations. Figure 1 illustrates the mean comparisons of the significant AADD-0 × PROPDD-0 interaction. The sporadic drinkers in the high AADD-0 category had significantly higher concentrations of both AA and DHA than the abstainers and the daily drinkers with high AADD-0. In addition, the high daily drinkers had fatty acid concentrations that were significantly lower than most of the other groups. This included the abstainers for DHA but not for AA.

Table 5. Selected Dietary Intakes of Women at 24 Weeks' Gestation According to the PROPDD-1[a]

	Abstainers PROPDD-1 = 0 (n = 254)	Occasional PROPDD-1 < 0.14 (n = 45)	Habitual PROPDD-1 ≥ 0.14 (n = 8)
Protein (g)	71.0 ± 1.2	68.2 ± 2.8	75.5 ± 6.6
Carbohydrates (g)	248 ± 3	243 ± 7	239 ± 18
Fat (g)	98 ± 4	96 ± 10	99 ± 24
Saturated fat (g)	35.7 ± 0.5	35.5 ± 1.1	33.0 ± 2.7
14:0 (g)	3.1 ± 0.1	2.7 ± 0.2	2.5 ± 0.5
16:0 (g)	19.6 ± 0.2	20.0 ± 0.6	18.8 ± 1.4
18:0 (g)	10.0 ± 2.1[1]	9.4 ± 1.7[2]	7.8 ± 1.7[3]
Monounsaturated fat (g)	36.9 ± 0.5	39.0 ± 1.1	38.0 ± 2.7
16:1n-7 (g)	1.72 ± 0.04	1.70 ± 0.09	1.87 ± 0.21
18:1n-9 (g)	34.6 ± 0.5	36.6 ± 1.1	35.4 ± 2.6
20:1n-9 (mg)	144 ± 6	165 ± 15	220 ± 37
Polyunsaturated fat (g)	17.2 ± 0.3	18.1 ± 0.8	17.6 ± 1.8
n-6 PUFA (g)	16.6 ± 0.3	17.5 ± 0.7	16.5 ± 1.6
18:2n-6 (g)	15.2 ± 0.3	16.0 ± 0.7	15.2 ± 1.7
20:4n-6 (mg)	104 ± 4	110 ± 10	131 ± 25
22:5n-6 (mg)	11 ± 1	10 ± 2	11 ± 5
n-6 HUFA (mg)	124 ± 4	132 ± 10	130 ± 26
n-3 PUFA (g)	1.78 ± 0.03	1.71 ± 0.06	1.91 ± 0.16
18:3n-3 (g)	1.54 ± 0.03	1.47 ± 0.06	1.62 ± 0.14
20:5n-3 (mg)	31 ± 3	29 ± 8	52 ± 20
22:6n-3 (mg)	68 ± 5	67 ± 12	90 ± 30
n-3 HUFA (mg)	108 ± 8	106 ± 19	150 ± 49
Total energy (MJ)	9.9 ± 0.3	9.1 ± 0.9	10.3 ± 2.0

[a] Values are estimated marginal means ± SEM of adjusted nutrient intakes. Means with different superscripts are significantly different across PROPDD-1 categories by Tukey's HSD after significance detected by General Linear Model procedure, $p < 0.05$. HUFA, highly unsaturated fatty acids.

Table 6. Categories of PROPDD-1 and Fatty Acid Composition of Maternal Plasma at 24 Weeks' Gestation[a]

	Abstainers PROPDD-1 = 0 (n = 254)	Occasional PROPDD-1 <0.14 (n = 45)	Habitual PROPDD-1 ≥ 0.14 (n = 8)
Saturated fatty acids[b]	31.05 ± 0.14	31.22 ± 0.33	30.99 ± 0.78
14:0[b]	0.92 ± 0.02	0.92 ± 0.05	0.94 ± 0.12
16:0[b]	22.69 ± 0.12	22.86 ± 0.28	23.13 ± 0.66
18:0[b]	6.21 ± 0.05	6.24 ± 0.12	5.59 ± 0.28
20:0	0.22 ± 0.005	0.24 ± 0.012	0.24 ± 0.028
22:0	0.45 ± 0.01	0.44 ± 0.02	0.41 ± 0.04
24:0	0.37 ± 0.01[1]	0.36 ± 0.02[1]	0.49 ± 0.05[2]
MUFA[b]	24.07 ± 0.16[1]	25.24 ± 0.38[2]	26.43 ± 0.90[2]
16:1n-7[b]	2.07 ± 0.05	2.35 ± 0.11	2.49 ± 0.27
18:1n-7	2.25 ± 0.02	2.30 ± 0.05	2.43 ± 0.11
18:1n-9[b]	17.75 ± 0.13[1]	18.56 ± 0.30[2]	19.56 ± 0.71[2]
20:1n-9[b]	0.17 ± 0.004[1]	0.16 ± 0.009[1]	0.24 ± 0.022[2]
22:1n-9	0.05 ± 0.003	0.05 ± 0.007	0.08 ± 0.018
24:1n-9	1.65 ± 0.04	1.69 ± 0.11	1.50 ± 0.25
n-6 PUFA[b]	36.48 ± 0.23[1]	35.31 ± 0.54[1,2]	33.53 ± 1.28[2]
18:2n-6[b]	26.28 ± 0.22[1]	25.35 ± 0.51[1,2]	23.50 ± 1.21[2]
18:3n-6	0.20 ± 0.004	0.19 ± 0.010	0.20 ± 0.025
20:2n-6	0.27 ± 0.003	0.27 ± 0.007	0.26 ± 0.016
20:3n-6	1.45 ± 0.02	1.45 ± 0.04	1.23 ± 0.10
20:4n-6[b]	7.41 ± 0.09	7.25 ± 0.21	7.49 ± 0.49
22:2n-6	0.02 ± 0.002	0.03 ± 0.004	0.04 ± 0.010
22:4n-6	0.33 ± 0.01	0.30 ± 0.01	0.31 ± 0.03
22:5n-6[b]	0.53 ± 0.01	0.48 ± 0.02	0.49 ± 0.06
n-6 HUFA[b]	9.73 ± 0.10	9.51 ± 0.23	9.52 ± 0.55
n-3 PUFA[b]	2.61 ± 0.03[1]	2.56 ± 0.08[1]	2.16 ± 0.18[2]
18:3n-3[b]	0.45 ± 0.01	0.44 ± 0.02	0.38 ± 0.05
20:3n-3	0.02 ± 0.001	0.02 ± 0.001	0.02 ± 0.004
20:5n-3[b]	0.17 ± 0.01	0.17 ± 0.01	0.13 ± 0.03
22:5n-3	0.25 ± 0.01	0.24 ± 0.01	0.22 ± 0.02
22:6n-3[b]	1.73 ± 0.03[1]	1.70 ± 0.06[1,2]	1.34 ± 0.15[2]
n-3 HUFA[b]	2.17 ± 0.03[1]	2.12 ± 0.07[1]	1.71 ± 0.18[2]
Total HUFA[b]	11.89 ± 0.12	11.63 ± 0.28	11.21 ± 0.69
Total PUFA[b]	39.09 ± 0.24[1]	37.89 ± 0.57[1,2]	35.66 ± 1.34[2]
Total fatty acids (μg/ml)	4981 ± 60	5036 ± 142	5330 ± 337

[a] Values are estimated marginal means ± SEM of total lipid extract. Data are % by weight of total fatty acids. Means with different superscripts are significantly different across PROPDD-1 categories by Tukey's HSD after significance detected by General Linear Model procedure, $p < 0.05$.

[b] Corresponding adjusted dietary intakes were included as covariates. MUFA, monounsaturated fatty acids.

The negative effects on plasma PUFAs of increasing AADD-0 in daily drinkers (PROPDD-0 = 1) was examined across the three time points (Fig. 2). Significant effects of AADD-0 were detected for AA, DPA n-6, and DHA ($p = 0.027$, 0.028, and 0.013, respectively). For these three fatty acids, participants with high AADD-0 intakes had significantly lower plasma concentrations than those with medium AADD-0 intakes, with the participants with low AADD-0 intakes intermediate and not statistically different. A significant effect of time was detected by the linear mixed model procedure with AADD-0 and time as fixed factors for LA, α-LNA, EPA, DPA, and DHA, with a general pattern of decreased plasma fatty acid concentrations at 3 months postpartum, except for EPA, which was increased. These differences reflect changes associated with pregnancy itself (Stark et al., 2004).

DISCUSSION

The present study examined the association between alcohol consumption and plasma and erythrocyte fatty acid status in pregnant, inner-city, black women. Detailed alcohol consumption data were collected during a structured interview. This intensive process required participants to estimate the amount (AAD) and pattern of their alcohol consumption (AADD and PROPDD) around the time of conception (AAD-0, AADD-0, and PROPDD-0) and after they knew they were pregnant during the 14 days before their first visit to the antenatal clinic (AAD-1, AADD-1, and PROPDD-1). The present results demonstrate that in pregnant black women, a high frequency of alcohol consumption as indicated by the measures PROPDD-1 and PROPDD-0 is associated with significantly decreased maternal PUFA status, particularly with lower levels of DHA. The results also indicate that greater amounts of alcohol consumed in frequent drinkers is associated with further decreases in DHA and AA.

The primary influence of alcohol on fatty acid metabolism seems to be increased catabolism. It has been proposed that ethanol activates phospholipase A_2, which releases from phospholipids highly unsaturated fatty acids that then can be metabolized to eicosanoids (Narce et al., 2001; Salem and Karanian, 1988). Alcohol has also been demonstrated to increase plasma concentrations of 4-hydroxynonenal, an indicator of increased lipid peroxidation (Meagher et al., 1999; Pawlosky et al., 1997). The mechanisms of increased lipid peroxidation from alcohol intake have been reviewed (Lands et al., 1998). These catabolic processes are believed to stimulate PUFA anabolism in an attempt to maintain tissue PUFA concentrations (Denkins et al., 2000). However, persistent and increased catabolism caused by frequent alcohol consumption is likely to overwhelm this adaptive stimulation of PUFA synthesis, resulting in a net loss of PUFA in tissues over the long term.

In the present study, PROPDD-1 was negatively correlated with the concentration of DHA in plasma and the percentage of DHA in erythrocytes throughout pregnancy and postpartum. This effect was the strongest at 24 weeks of gestation as PROPDD-1 was negatively correlated with and without adjustment for potential confounders, with the percentage of DHA in both plasma ($\beta = -1.00$ and -1.56, respectively) and erythrocytes ($\beta = -1.81$ and -2.81, respectively). This is probably because the blood specimens collected at 24 weeks' gestation best represent the drinking data collected at the first prenatal visit (PROPDD-1, AADD-1, and AAD-1) as patterns of drinking at infant delivery and 3 months postpartum may change (Floyd and Sidhu, 2004).

The majority of the significant differences were observed in the highest PROPDD-1 category (≥0.14, habitual drinkers). The differences in the plasma of the habitual drinkers in the present study (Table 6) reflect those observed in plasma of feline and rhesus monkeys exposed to ethanol (Pawlosky and Salem, 1995, 1999). The similarities include significantly decreased DHA and LA (18:2n-6) and significantly increased 16:1n-7 and 18:1n-9 in all three studies. AA in plasma was decreased in the alcohol-treated groups in both animal studies, although the difference was not significant in the feline study. There was no difference in either plasma or erythrocyte AA status according to PROPDD-1 categories in the present study. It is important to note that liver AA has been shown to be significantly decreased in alcohol-treated animals despite no differences in AA levels in plasma (Pawlosky and Salem, 1995) and erythrocytes (Salem et al., 1996).

There is evidence that the amount of alcohol intake may differentially influence PUFA status. Specifically, low levels of alcohol may stimulate PUFA synthesis, whereas high levels of alcohol may inhibit PUFA synthesis. It has been demonstrated that Δ6 and Δ5 desaturase activities in hepatocytes are stimulated at low ethanol concentrations and inhibited at high ethanol concentrations (Narce et al., 2001). In the present study, there were no consistent effects of the amount of alcohol consumption alone on PUFA levels, but when exam-

Table 7. Categories of PROPDD-1 and Fatty Acid Composition of Maternal Erythrocytes at 24 Weeks' Gestation[a]

	Abstainers PROPDD-1 = 0 (n = 230)	Occasional PROPDD-1 < 0.14 (n = 42)	Habitual PROPDD-1 ≥ 0.14 (n = 6)
Saturated fatty acids[b]	39.19 ± 0.13[1]	39.31 ± 0.30[1,2]	41.17 ± 0.80[2]
14:0[b]	0.77 ± 0.02	0.76 ± 0.04	0.85 ± 0.10
16:0[b]	22.80 ± 0.08[1]	23.03 ± 0.19[1,2]	24.31 ± 0.50[2]
18:0[b]	9.71 ± 0.05	9.57 ± 0.12	9.50 ± 0.32
20:0	0.33 ± 0.004	0.34 ± 0.009	0.37 ± 0.023
22:0	1.59 ± 0.02	1.58 ± 0.04	1.78 ± 0.11
24:0	3.94 ± 0.04	3.98 ± 0.08	4.30 ± 0.22
MUFA[b]	19.87 ± 0.08[1]	20.08 ± 0.19[1,2]	21.13 ± 0.51[2]
16:1n-7[b]	0.43 ± 0.01	0.43 ± 0.01	0.47 ± 0.04
18:1n-7	1.81 ± 0.01	1.80 ± 0.03	1.72 ± 0.08
18:1n-9[b]	10.63 ± 0.05[1]	10.80 ± 0.12[1,2]	11.42 ± 0.30[2]
20:1n-9[b]	0.26 ± 0.005	0.25 ± 0.012	0.24 ± 0.032
22:1n-9	0.09 ± 0.001	0.09 ± 0.003	0.09 ± 0.007
24:1n-9	6.39 ± 0.06	6.47 ± 0.15	6.83 ± 0.39
n-6 PUFA[b]	28.79 ± 0.08	28.39 ± 0.19	28.05 ± 0.49
18:2n-6[b]	9.21 ± 0.06	9.04 ± 0.14	8.96 ± 0.38
18:3n-6	0.05 ± 0.001[1]	0.04 ± 0.001[2]	0.04 ± 0.03[1,2]
20:2n-6	0.31 ± 0.003[1]	0.30 ± 0.006[1,2]	0.27 ± 0.017[2]
20:3n-6	1.30 ± 0.01	1.31 ± 0.03	1.14 ± 0.09
20:4n-6[b]	12.58 ± 0.06	12.50 ± 0.13	12.63 ± 0.36
22:2n-6	0.07 ± 0.001	0.07 ± 0.003	0.07 ± 0.007
22:4n-6	4.22 ± 0.03	4.09 ± 0.07	3.91 ± 0.19
22:5n-6[b]	1.06 ± 0.01	1.03 ± 0.03	1.05 ± 0.09
n-6 HUFA[b]	19.23 ± 0.07	18.99 ± 0.16	18.79 ± 0.43
n-3 PUFA[b]	5.70 ± 0.05[1]	5.58 ± 0.11[1]	4.90 ± 0.28[2]
18:3n-3[b]	0.11 ± 0.002	0.11 ± 0.004	0.10 ± 0.011
20:3n-3	0.04 ± 0.003	0.05 ± 0.01	ND
20:5n-3[b]	0.19 ± 0.01[1]	0.18 ± 0.01[1,2]	0.14 ± 0.02[2]
22:5n-3	1.42 ± 0.01[1]	1.34 ± 0.03[2]	1.31 ± 0.07[1,2]
22:6n-3[b]	3.98 ± 0.04[1]	3.96 ± 0.09[1]	3.23 ± 0.25[2]
n-3 HUFA[b]	5.58 ± 0.05[1]	5.47 ± 0.11[1]	4.65 ± 0.29[2]
Total HUFA[b]	24.82 ± 0.09	24.46 ± 0.21	23.60 ± 0.57
Total PUFA[b]	34.48 ± 0.09[1]	33.98 ± 0.21[1,2]	32.95 ± 0.54[2]

[a] Values are estimated marginal means ± SEM of total lipid extract. Data are % by weight of total fatty acids. Means with different superscripts are significantly different across PROPDD-1 categories by Tukey's HSD after significance detected by General Linear Model procedure, $p < 0.05$.

[b] Corresponding adjusted dietary intakes were included as covariates. ND, not determined.

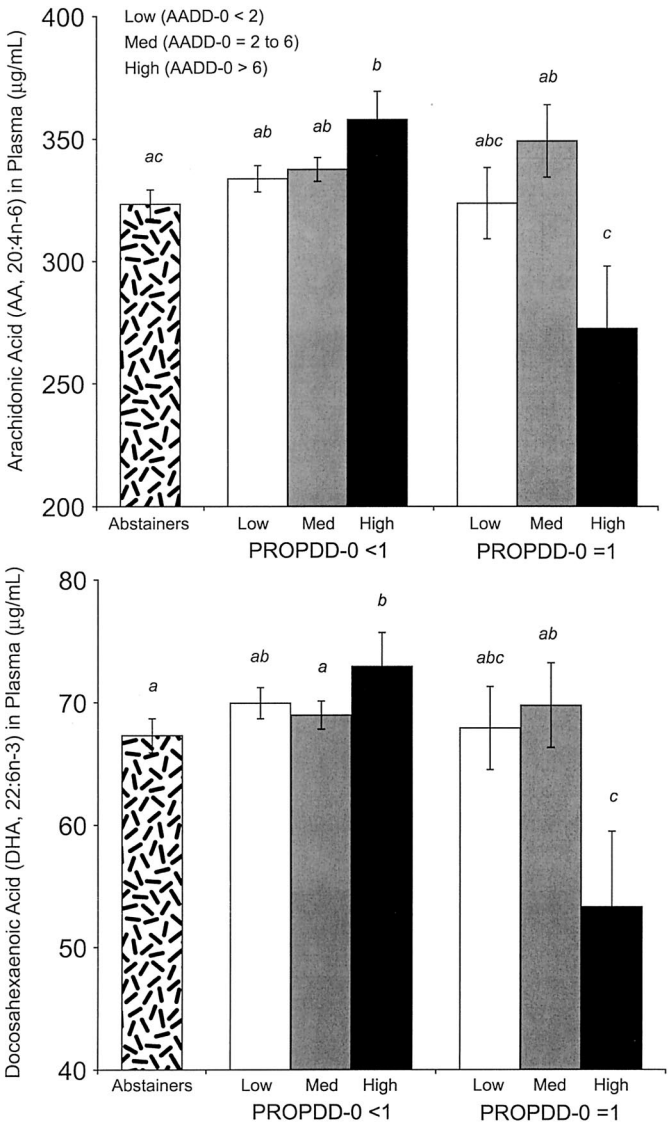

Fig. 1. Influences of increasing AADD-0) and PROPDD-0 on plasma AA and DHA. The Linear Mixed Modeling procedure was used with time and categories of AADD-0 (low, <2; medium, 2–6; high, >6) and PROPDD-0 (abstainers, 0; sporadic drinkers, <1; daily drinkers, 1) set as main effects and the corresponding, adjusted fatty acid intake included as a covariate. Estimated marginal means ± SEM are presented to demonstrate the significant AADD-0 and PROPDD-0 interaction (AA, $p = 0.010$; DHA, $p = 0.028$) as there were no interactions with time. The number of observations for each bar is as follows: abstainers = 190; for PROPDD-0 <1, low = 225, medium = 275, and high = 49; for PROPDD-0 = 1, low = 31, medium = 30, and high = 10. Bars with different alphabetic labels are significantly different by Tukey's HSD, $p < 0.05$.

ined with frequency of consumption (as AADD-0 × PROPDD-0 interaction; Fig. 1), decreased DHA and AA concentrations were observed in women who reported high amounts (>6 absolute ounces of alcohol per drinking day) of alcohol consumed on a daily basis (PROPDD-0 = 1). Also, in women who drank high amounts of alcohol but not on a regular basis, DHA and AA concentrations were increased as compared with abstainers. These observations could explain some of the inconsistencies reported in the literature. The present study also supports the hypothesis that ethanol influences fatty acid metabolism in pregnancy through alterations in both catabolism and biosynthesis (Denkins et al., 2000; Pawlosky and Salem, 1999) by which the net effect of alcohol on fatty acid status depends on the amount and the frequency of alcohol consumed.

One limitation in this study is that the number of drinkers and the amount of alcohol consumed were fairly low for observing changes in fatty acid compositions, even after targeting a population at high risk for FASD and using an oversampling strategy for higher risk drinking (Jacobson et al., 1991, 1993). The low numbers of high-risk drinkers is partly a result of the counseling provided regarding alcohol consumption, a necessary ethical obligation to the participants during pregnancy. Despite these limitations, differences in fatty acid status were significantly associated with alcohol consumption in the present study.

Decreased levels of DHA and AA in tissues of human alcoholics has been observed and reviewed previously (Salem and Olsson, 1997; Salem and Ward, 1993). Interpreting studies involving alcohol consumption and nutrient status is difficult (Lands et al., 1998). Briefly, increased alcohol intake is associated with diets of poorer nutrient quality (Hillers and Massey, 1985) and lower intakes of polyunsaturated fat (Thomson et al., 1988). Plasma levels of DHA are associated with dietary intake of n-3 PUFAs (Bjerve et al., 1993), and in the present study, both dietary DHA and dietary AA were positively correlated with their respective amounts in plasma and erythrocytes (Tables 1 and 2, respectively).

In the present study, nutrient intakes, including specifically DHA and AA intakes, were adjusted for energy intake by the nutrient residual model (Willett and Stampfer, 1986) to control for over- and underreporting associated with food frequency surveys (Livingstone and Black, 2003; Subar et al., 2001). The adjusted mean macronutrient and fatty acid intakes showed no differences according to PROPDD-1 groupings, except for decreased 18:0 intake with more frequent drinking (i.e., increased PROPDD-1; Table 5). Individual fatty acid intakes were included as covariates in fatty acid plasma and erythrocyte statistical analyses to control for possible dietary differences. As participant recruitment for the present study was tightly defined (pregnant, inner-city black women), the variability in lifestyle behaviors and demographic characteristics was minimized (Tables 4). It is reasonable to presume that there would be concomitantly low variability in diet selection and frequency of intake of specific foods. Therefore, in the present study, disparities in DHA and AA levels with alcohol consumption are likely to be attributed to alcohol-related differences in fatty acid metabolism and not dietary intake. Nevertheless, the overall mean intake of DHA in this population was low (71.2 ± 5.2 mg/day), as compared with 571 mg/day for Japanese women (Kuriki et al., 2003) and 160 mg/day for pregnant Canadian women (Innis and Elias, 2003). On the basis of stable isotope studies of 18-carbon essential fatty acids in rhesus monkeys, it seems that alcohol consumption does not decrease absorption of PUFAs (Pawlosky and Salem, 1999).

The habitual drinkers in this study were significantly older,

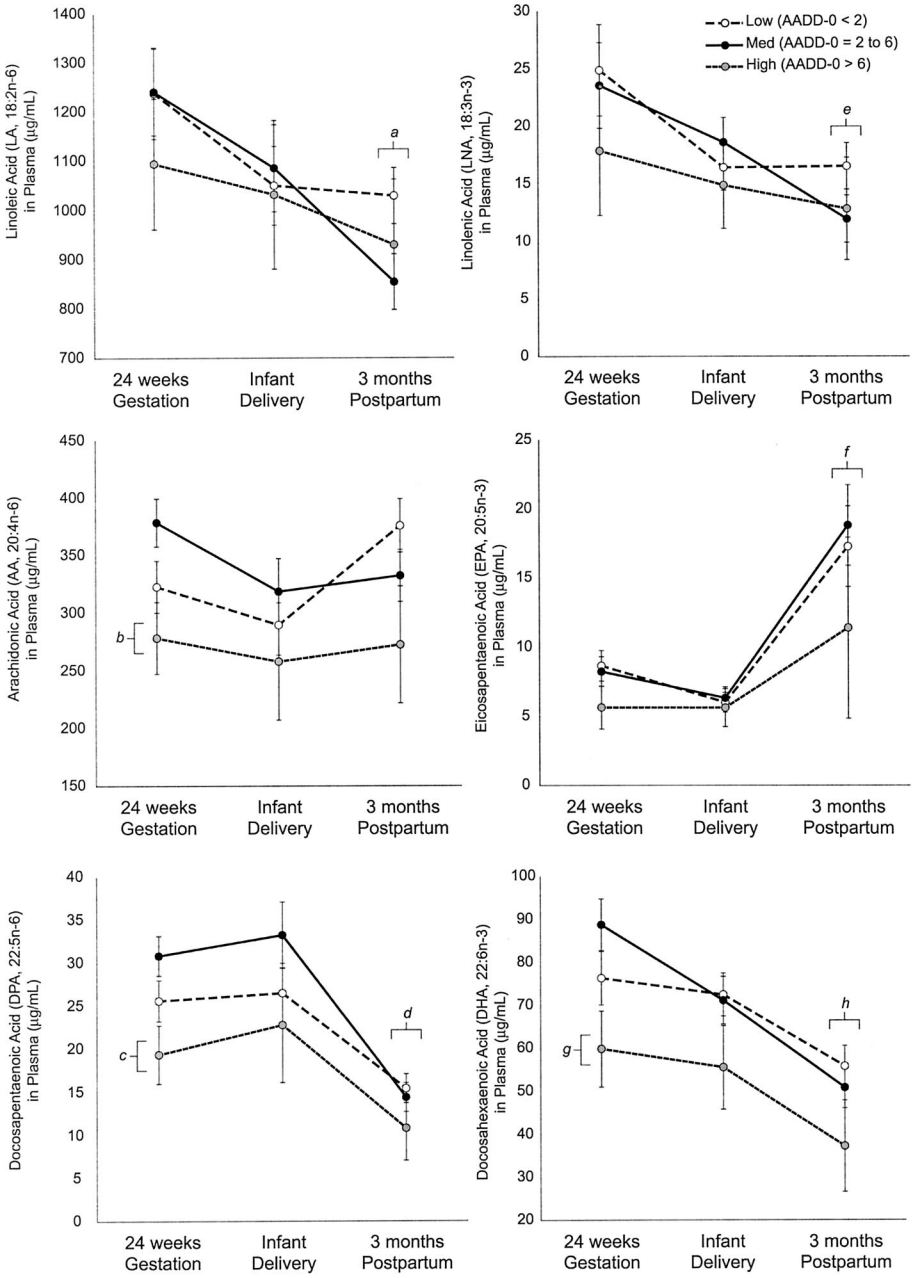

Fig. 2. Selected PUFAs and effects of AADD-0 for participants who reported drinking daily at the time of conception from 24 weeks' gestation to 3 months postpartum. The Linear Mixed Modeling procedure was used with time and alcohol intake category set as main effects and the corresponding adjusted fatty acid intake included as a covariate. Values presented are estimated marginal means ± SEM. For low, medium, and high, respectively: $n = 10$, 11, and 5 at 24 weeks gestation; $n = 11$, 9, and 3 at infant delivery; and $n = 10$, 10, and 2 at 3 months postpartum. Tukey's HSD tests with significance set at $p < 0.05$ were used after a significant F value. [a]Significant main effect of time ($p = 0.001$) detected, with LA significantly lower at 3 months postpartum than at 24 weeks' gestation across alcohol intakes. [b]Significant main effect of alcohol intake ($p = 0.027$) was detected with AA significantly lower with high intake than with medium intake across time. [c, d]Significant main effects of time ($p < 0.001$) and alcohol intake ($p = 0.028$) were detected with DPA n-6 significantly lower with high intake than with medium intake across time, and DPA n-6 at 3 months postpartum was significantly lower than 24 weeks' gestation and infant delivery across alcohol intakes. [e]Significant main effect of time ($p = 0.008$) detected, with alpha-linolenic acid (LNA) significantly lower at 3 months postpartum than at 24 weeks' gestation across alcohol intakes. [f]Significant main effect of time ($p = 0.001$) detected, with EPA significantly higher at 3 months postpartum than at 24 weeks' gestation and infant delivery across alcohol intakes. [g, h]Significant main effects of time ($p < 0.001$) and alcohol intake ($p = 0.013$) were detected with DHA significantly lower with high intake than with medium intake across time, and DHA at 3 months postpartum was significantly lower than at 24 weeks' gestation and at infant delivery across alcohol intakes.

smoked more cigarettes, and had a significantly greater total number of pregnancies and deliveries as compared with the abstainers (Table 4). Plasma concentrations of DHA and AA (cf., Tables 1 and 2) were negatively correlated with exposure to tobacco smoke ($\beta = -0.25$ and -0.82, respectively) and with total energy intake ($\beta = -0.62$ and -1.68, respectively) and positively correlated with maternal age ($\beta = 0.74$ and 13.69, respectively) and BMI ($\beta = 0.18$ and 0.96, respectively). The total number of pregnancies and/or deliveries was not included in the multivariable model as they both were col-

linear with age. Maternal DHA was positively correlated with the total number of pregnancies and deliveries ($r = 0.08$, $p = 0.031$, and $r = 0.09$, $p = 0.016$, respectively). Parity had been associated with decreased maternal DHA status (Hornstra et al., 1995), but further investigation has demonstrated no significant relationship (van den Ham et al., 2001). However, we have reported previously increased DHA status in older women with more pregnancies (Denkins et al., 2000).

The role of maternal DHA deficiency in infant alcohol-related neurodevelopmental disorders remains to be elucidated. Although it seems unlikely that maternal fatty acid status is the primary determinant of the neurobehavioral and cognitive deficits associated with FASD, the present study suggests that mothers who are at the highest risk for infants with FASD (i.e., frequent and heavy consumers of alcohol) do have significantly reduced DHA and AA status. Infants with low DHA intakes have decreased scores in problem-solving tasks (Willatts et al., 1998) and cognitive tests (Agostoni et al., 1995; Birch et al., 2000), and animals that are raised on n-3–deficient diets have decreased brain DHA levels and deficits in various neurobehavioral modalities, including spatial task performance (Frances et al., 1996; Moriguchi et al., 2000; Moriguchi and Salem, 2003; Nakashima et al., 1993; Wainwright et al., 1998) and olfactory discrimination (Catalan et al., 2002; Greiner et al., 2001), all problems associated with prenatal alcohol exposure (Institute of Medicine, 1996). Recommendations and targeted education for pregnant drinking women, including black women, to limit alcohol consumption must continue. Including dietary advice for women who receive counseling for high-risk drinking during pregnancy and programs that encourage prenatal vitamin and nutrient supplementation that includes DHA and AA has the potential to reduce the severity and the number of cases of alcohol-related neurodevelopmental disorders.

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